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PLANT NUTRITION

ISMOM 2008

**5th International Symposium of
Interactions of Soil Minerals with Organic
Components and Microorganisms**



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de la Ciencia del Suelo**

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**Revista de la
CIENCIA DEL SUELO
Y NUTRICIÓN VEGETAL**

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Sociedad Chilena
de la Ciencia del Suelo

Plenary, keynote and abstract papers presented to the
5th ISMOM 2008, Commission 2.5: Soil Physical/Chemical/
Biological Interfacial Reactions of the International Union
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Preface

ISMOM 2008: 5th International Symposium on Interactions of Soil Minerals with Organic Components and Microorganisms

Commission 2.5 Soil Physical/Chemical/Biological Interfacial Reactions of the International Union of Soil Sciences

This special issue of the *Journal of Soil Science and Plant Nutrition* contains the plenary papers, keynote lectures, and abstracts of the oral and poster papers presented at ISMOM2008 in Pucón, Chile (24–28 November 2008).

The objective of the symposium is to bring together environmental chemists, mineralogists, microbiologists, ecologists, toxicologists, and soil scientists to share information, identify gaps in knowledge, and stimulate research on the physicochemical and biological interfacial interactions in the soil environment, with special focus on the soil-root interface (rhizosphere). The five session themes, listed below, reflect this objective.

Four plenary lectures, 11 keynote addresses, 47 oral papers, and 157 posters were presented over five days during the following sessions:

1. Ecological Significance of Interactions among Clay Minerals, Organic Matter and Biota
2. Soil-Root-Microbe Interactions and their Effects on the Transformation and Bioavailability of Nutrients
3. Soil-Root-Microbe Interactions and their Effects on the Biophysical Transformation, Fate, and Toxicity of Metals and Metalloids
4. Dynamics and Transformations of Natural and Xenobiotic Compounds in Soil Environments
5. Environmental Biotechnology: Biochemical and Molecular Mechanisms of Microbe-Plant-Root Interactions and their Genomics and Proteomic Advances pertaining to Restoration of Contaminated Sites

The symposium was attended by over 200 participants from the following countries:


Argentina (5), Australia (6), Austria (2), Brazil (6), Canada (11), Chile (79), China (6), Croatia (1), Dominican Republic (1), France (14), Germany (1), Hong Kong (1), Hungary (3), India (7), Indonesia (2), Iran (6), Ireland (2), Italy (12), Japan (2), Kazakhstan (1), Korea (1), Latvia (2), Malaysia (1), México (11), Netherlands (1), New Zealand (1), Pakistan (3), Paraguay (2), Russian Federation (3), Saudi Arabia (1), Slovenia (1), Spain (6), Sweden (2), Thailand (4), USA (3), Venezuela (8).

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We also thank the Chilean Society of Soil Science for providing space in their official journal, and staff and postgraduate students of the Doctoral Program in Sciences of Natural Resources, La Frontera University, for assistance in organizing this symposium.



María de la Luz Mora Gil
Chairperson
ISMOM2008



Benny K.G. Theng
Guest Editor

Content Index

Plenary Papers

Solute Interactions in Soils in Relation to Bioavailability and Remediation of the Environment.....	1
<i>N.S. Bolan, B.G. Ko, C.W.N. Anderson and I. Vogeler</i>	
Impacts of Metals and Metalloids on Soil Microbial Diversity and Ecosystem Function.....	6
<i>David Crowley</i>	
Enzymes of Significance to the Restoration of Polluted Systems: Traditional and Advanced Approaches.....	12
<i>L. Gianfreda</i>	
Soil Physicochemical and Biological Interfacial Interactions in the Rhizosphere: Impacts on Food Security and Ecosystem Integrity.....	23
<i>P.M. Huang</i>	

Keynote Papers

Interactions of Soil Components and Microorganisms and their Effects on Soil Remediation (K-1).....	28
<i>J.-M. Bollag</i>	
Use of Nanoparticles in Soil-Water Bioremediation Processes (K-2).....	33
<i>Nelson Duran</i>	
Soil-Root-Microbe Interactions in the Rhizosphere - A Key to Understanding and Predicting Nutrient Bioavailability to Plants (K-3).....	39
<i>P. Hinsinger, M.N. Bravin, N. Devau, F. Gérard, E. Le Cadre and B. Jaillard</i>	
Soil Organic Matter in Nano-Composite and Clay Fractions, and Soluble Pools of the Rhizosphere (K-4).....	48
<i>C.M. Monreal* and M. Schnitzer</i>	

Contaminant Fate, Dynamics and Bioavailability: Biochemical and Molecular Mechanism at the Soil: Root interface (K-5).....	56
<i>R. Naidu and K.R. Kim</i>	
Protein Adsorption at Solid Surfaces and Protein Complexation with Humic Acid (K-6).....	64
<i>W. Norde, W. Tan and L. Koopal</i>	
Interaction with Soil Constituents Determines the Environmental Impact of Proteins (K-7).....	75
<i>H. Quiquampoix</i>	
Bioavailability of Phosphorus and Micronutrients in the Soil-Plant-Microbe Continuum (K-8).....	84
<i>Z. Rengel</i>	
The Fate of Anthropogenic Organic Pollutants in SOIL: Adsorption/Desorption of Pesticides Possessing Endocrine Disruptor Activity by Natural Organic Matter 8Humic Substances (K-9).....	92
<i>N. Senesi and E. Loffredo</i>	
Sorption-Desorption Processes of Metals and Metalloids in Soil Environments (K-10).....	95
<i>A. Violante and M. Pigna</i>	

Oral Abstracts

Session 1. Ecological Significance of Interactions Among Clay Minerals, Organic Matter and Biota.....	102
Session 2. Soil-Root-Microbe Interaction & their Effects on the Transformation & Bioavailability of Nutrients.....	111
Session 3. Soil-Root-Microbe Interaction & Their Effects on Biophysical Transformation, Fate, and Toxicity of Metal and Metalloids.....	121
Session 4. Dynamics and Transformations of Natural and Xenobiotic Compounds in Soil Environments.....	132
Session 5. Environmental Biotechnology: Biochemical and Molecular Mechanisms of Microbe-Plant-Root Interactions & Their Genomic & Proteomic Advances Pertaining to Restoration of Contaminated Soils.....	142

Poster Abstracts

Session 1. Ecological Significance of Interactions Among Clay Minerals, Organic Matter and Biota.....	146
Session 2. Soil-Root-Microbe Interaction & their Effects on the Transformation & Bioavailability of Nutrients.....	176
Session 3. Soil-Root-Microbe Interaction & Their Effects on Biophysical Transformation, Fate, and Toxicity of Metal and Metalloids.....	211
Session 4. Dynamics and Transformations of Natural and Xenobiotic Compounds in Soil Environments.....	235
Session 5. Environmental Biotechnology: Biochemical and Molecular Mechanisms of Microbe-Plant-Root Interactions & Their Genomic & Proteomic Advances Pertaining to Restoration of Contaminated Soils.....	268

Authors Index

Achard, R.	S4-O4	Bassirani, N.	S1-O1, S1-P6,
Acevedo, E.	S1-P9		S1-P7, S4-P4
Acevedo, F.	S1-P1	Bastidas, J.	S1-P11
Aditiawati, P.	S5-O1	Becerra, A.G.	S2-P8
Aguilera, P.	S4-P1, S4-P7,	Bech, J.	S1-P27
	S4-P8, S4-P29	Bech, J.R.	S1-P27
Ahmad, H. R.	S3-O2	Benitez, E.	S5-P15
Ahmed, O.H.	S1-P33	Benizri, E.	S2-P27, S2-P28
Ahn, M.Y.	S4-P5	Benzo, D.	S1-P11, S2-P7
Ahumada, L.	S3-P1	Berthelin, J.	S3-O5
Akhmaloka, A.	S5-O1	Beznosikov, V.A.	S4-P20
Alberdi, M.	S3-P10, S3-P13,	Bhowmik, S.N.	S2-P4
	S3-P24	Blackburn, D.M.	S3-P27
Alfaro, M.	S2-P31	Blanco Jarvio, A.	S5-P2
Al-Hawas, I. A.	S1-P2	Boeckx, P.	S1-P34
Almendros, G.	S1-P23	Bolan, N.	Plenary 1
Alsina, I.	S2-P1, S2-P9	Bolaños	
Alvear, M.	S3-P25	-Benavides, M.M.	S2-P39
Anderson, C.W.N.	Plenary 1	Bollag, J.M.	K1, S4-P5
Andrade, M.L.	S3-P2, S3-P8,	Borie, F.	S1-P26, S2-P6,
	S3-P20		S2-P33, S3-P5,
Andreoni, V.	S3-P3, S3-P16		S3-P6, S3-P11,
Andreux, F.	S3-P4		S3-P17
Anil Kumar, K.S.	S1-O3	Borie, G.	S1-P9
Ansevica, A.	S2-P1	Borin, A.	S4-P11
Antilén, M.	S1-P31, S1-P32	Boupassia, C.	S3-P4
Antoun, H.	S1-P15, S4-P16	Bourrié, G.	S3-O1, S2-P35
Arain, S.A.	S2-P2	Brandt, S.A.	S2-P19
Arancibia, N.	S1-P18, S4-P2	Bravin, M.N.	K3
Aravena, S.	S1-P3, S4-P13	Briceño, G.	S4-P1, S4-P6,
Arias, M.	S4-P3		S4-P7, S4-P8,
Armijo, J.	S1-P31, S1-P32		S4-P29, S5-P3
Arriagada, M.	S3-P25	Briceño, M.	S1-P32
Ascar, L.	S3-P1	Briones, R.	S5-O4, S5-P9
Ávila, G.	S3-O7	Burkitbayev, M.	S1-O7, S3-O11
Ávila, M.	S3-P1	Cabidoche, Y.M.	S4-O4
Azcón, R.	S3-P26	Cabirol, N.	S5-P2
Aziz, M. A.	S3-O2	Cáceres, L.	S4-P13
Azócar, L.	S5-O4, S5-P9	Cai, P.	S1-O4, S1-P22,
Bachate, S.	S3-P3		S1-P25
Bacilio-Jiménez, M.	S1-P4, S1-P5	Calabi, M.	S1-P1, S1-P35
Baer, A.J.	S4-O9	Calamai, L.	S4-O10
Bákonyi, N.	S2-P17	Calderini, D.	S2-P34
Balasubramanian, V.	S5-P1	Calderón, R.	S3-P22, S4-P13,
Banerjee, M.R.	S2-P3		S4-P19, S5-P4
Barbosa, A.	S2-P22	Calvaruso, C.	S2-P5
Barea, J. M.	S5-O6, S3-P26	Canales, J.	S1-P32

Candia, M.	S4-P1, S4-P7, S4-P8, S4-P29	Curaqueo, G.	S1-P9
Candia, O.	S4-P1, S4-P7, S4-P8, S4-P29	D'Acqui, L.P.	S4-O10, S1-P23, S2-P23
Canquil, N.	S5-P12	D'Ascoli, R.	S2-P21
Canzi, E.	S3-P3	Daudin, G.	S4-O7
Cárcamo, I.	S1-P19	de La Fuente, P.	S1-P18
Cardoso da Luz, E.	S4-P12	De Neve, S.	S1-P13
Carrasco, M.A.	S3-P1, S4-P9	Dec, J.	S4-P5
Cartes, P.	S2-P16, S2-P30, S3-P23	Déjardin, P.	S4-O7
Castilla-Lozano, L.A.	S2-P39	Del Aguila, J.P.	S2-P40
Castillo, C.	S2-P6	Demanet, R.	S2-P30, S2-P36, S2-P38, S4-P1, S4-P6, S4-P7, S4- P8, S4-P29, S5-P3
Castillo, G.	S4-P9		
Castillon-Baturoni	S1-P4	Dendooven, L.	S4-P21
Casucci, C.	S3-P16	Devau, N.	K3
Cavalca, L.	S3-P3	Dezzeo, N.	S4-P15
Cavaria-Galicia, J.A	S2-P12	Di Meo, V.	S3-P7
Caviedes, M.A.	S4-P9	Diez, M.C.	S4-O1, S1-P1, S4-P33, S4-P36, S5-P14
Cazanga, M.	S3-P22, S4-P13, S4-P19		
Cea, M.	S1-P1, S1-P35, S5-P14	Dominguez, L.S.	S2-P8
Chacón, N.	S1-P11, S2-P7, S2-P11	Drury, C.	S1-O5
Chaer-Borges, A.	S2-O2	Dubova, L.	S2-P1, S2-P9
Chang, A.C.	S3-O12, S3-P14, S4-P25	Durán, N.	K2, S4-P11
Cha-um, S.	S1-O9, S1-P8	Dutra, M.	S2-P29
Chaussod, R.	S3-O4	Dutra-Costa, M.	S2-O2
Chavarría, J.A.	S1-P12	Edou-Minko, A.	S3-P4
Chen, W.	S3-O12, S1-P25, S3-P14, S4-P25	Elsheikh, M.A.	S1-O2
Chevallier, T.	S1-P26	Escudey, M.	S3-O12, S1-P18, S3-P14, S3-P22, S4-P2, S4-P13, S4-P19, S4-P25, S5-P4
Ciampi, L.	S2-P34, S2-P42		
Ciudad, G.	S5-O4, S5-P9	Esparza, Y.	S5-P10, S5-P13
Cliff, J.	S2-O8	Espinosa, E.	S3-P22, S4-P19, S5-P4
Clode, P.	S2-O8		
Cofré, N.M.	S2-P8	Etcheverria, P.	S1-P26
Colombo, M.	S3-P3, S3-P16	Fabris, J.D.	S1-P3, S1-P24
Colombo, P.	S4-P3	Félix de Barros, N.	S2-O2, S2-P29
Contreras, E.	S3-P1, S4-P10	Fernández, N.V.	S2-O5
Cordi, L.	S4-P11	Fiedler, S.	S2-P13
Cornejo, P.	S3-P5, S3-P6, S3-P11, S3-P17	Figueroa-Viramontes, R.	S2-P24
Covarrubias-Robledo, D.	S1-P5	Fisahn, J.	S5-P8
Covelo, E.F.	S3-P2, S3-P8, S3-P20	Flores, C.	S5-O2
Cozzolino, V.	S1-P28, S3-P7, S4-P35	Flores, S.	S1-P11, S2-P7, S2-P11
Crowley, D.	Plenary 2, S5-O5	Flores, V.C.	S2-P10
Cuenca, G.	S2-P10	Fontenla, S.B.	S2-O5, S2-O6
		Förster, J.E.	S1-P18, S4-P2
		Fortis, M.	S1-P12
		Fortis-Hernández, M.	S2-P12

Frey-Klett, P.	S2-P5	Hinsinger, P.	K3
Froehner, S.	S4-P12	Hinzpeter, I.	S5-P11
Fuentes, B.	S1-P10, S5-P5	Hoefgen, R.	S5-P8
Fuentes, R.	S4-P13, S5-P4	Huaiquil, A.	S5-P11
Furukawa, W.	S4-P12	Huang, P.M.	Plenary 4
Gabov, D.N.	S4-P20	Huang, Q.	S1-O4, S1-P25
Gabriels, D.	S1-P13	Huygens, D.	S1-P34
Gaete, H.	S3-O7	Ibrahim, M.	S3-P9
Gajdos, É.	S2-P17	Inostroza	
Gallardo, F.	S2-P36, S2-P38, S4-P33	-Blancheteau, C.	S3-P10, S3-P24
Gallo, L.	S2-O5	Iraira, S.	S2-P31
Gangadharappa, K.R.	S1-O3	Jaillard, B.	K3
García, B.	S1-P11, S2-P7	Jaipheng, R.	S1-O9
García, J.	S1-P9	Janot, J.M.	S4-O7
García, L.	S1-P12	Jara, A.A.	S1-P10, S1-P35, S1-P36, S2-P16, S2-P30, S2-P36, S3-P23
García-Galindo, L.	S2-P12		
García-González, M.T.	S3-P22	Jeon, H.-S.	S1-O6
Garrido, E.	S1-P19, S1-P29	Jones, D.	S2-O8
Garrido, E.G.	S1-P36	Jorquera, M.	S5-O4, S2-P15, S5-P3, S5-P5, S5-P14
Garrido, F.	S3-P22		
Garrido, T.	S3-P12	Kannan, V. R.	S5-P1
Gérard, F.	K3	Karpachevski, L.O.	S2-O7
Ghafoor, A.	S3-O2	Kasim, S.	S1-P33
Gianfreda, L.	Plenary 3, S4-O2, S2-P21, S4-P27	Kazi, T.G.	S2-P2
Giesler, R.	S2-O10, S2-P20	Kelleher, B.P.	S1-P14
Gill, K.S.	S2-P19	Kheyrodin, H.	S1-P15, S1-P16, S4-P16
Godoy, R.	S1-P34		
González, A.	S4-P14	Kies, A.	S2-P5
González, A.F.	S4-P15	Kilburn, M.	S2-O8
González, B.	S4-P30, S5-P6	Kim, B.-G.	S1-O6
González, I.	S3-P6, S3-P15	Kim, J.-E.	S4-P5
González, M.	S3-P21, S5-O2	Kim, K.R.	K5
González, M.A.	S1-P32	Kirdmanee, C.	S1-O9, S1-P8
González-Méndez, B.	S2-P13	Kiss, A.	S2-O4, S2-O9, S4-P17, S4-P18
Graham, R.C.	S1-P24		
Grant, C.A.	S4-P23	Klepsch, S.	S2-O3, S2-P25
Gu, J.D.	S4-O6	Knicker, H.	S4-O8, S1-P19
Guckert, A.	S2-P27, S2-P28	Knight, J.D.	S2-P19
Gutiérrez, M.	S3-P22, S4-P19, S5-P4	Ko, B.G.	Plenary 1
He, Y.	S4-O3, S2-P14	Kollmann, A.	S5-P7
Helassa, N.	S4-O7	Koopal, L.	K6
Henmi, T.	S1-O2	Kraiser, T.	S5-P6
Henry, P.	S3-O1	Kumar, R.	S4-O9
Hernández, J.M.	S2-P13	Kuyanov, Y.	S1-O7
Hernández, M.	S5-O2	Labanowski, J.	S3-O3
Hernández		Lamy, I.	S3-O3, S5-P7
-Hernández, R.M.	S1-P13	Landry, D.	S3-O4
Herrmann, A.	S2-O8	Langer, H.	S5-P14
Hesse, H.	S5-P8	Laurie, A.	S2-P6

Le Cadre, F.	K3	Meier, S.	S3-P6, S3-P11, S3-P17
Leal, J.	S5-O4, S5-P9	Mele, P.	S5-O5
Lebrun, J.	S5-P7	Mendonça, E.S.	S1-P17
Leclerc, E.	S2-P5	Mendoza, J.	S3-P12
Lee, G.-S.	S1-O6	Mestre, M.C.	S2-O6
Lehotay, T.	S4-P17	Mexica, P.	S1-P12
Leitner, D.	S2-O3, S2-P25	Meza, F.	S3-O12
Lejon, D.	S3-O4	Mihailova, A.	S2-P9
Lemke, R.	S4-P22, S4-P23	Millaleo, R.	S3-P13
León, O.	S4-P9	Milloux, M.	S3-O4
Lesueur Jannoyer, M.L.	S4-O4	Minkina, T.M.	S1-P20
Lévai, L.	S2-P17	Miralles, P.	S5-O2
Lévêque, J.	S3-O4, S3-P4	Mohammadzai, U.	S3-P9
Leyton, A.	S5-P13	Molina, M.	S3-O12, S3-P14, S4-P2, S4-P13, S4-P25
Liang, W.	S1-O4, S1-P25	Monreal, C.M.	K4
Libkind, D.	S2-O6	Mora, M.L.	S1-P10, S1-P35, S1-P36, S2-P15, S2-P16, S2-P30, S2-P36, S2-P37, S2-P38, S2-P41, S3-P10, S3-P13, S3-P23, S3-P24, S3-P27, S4-P1, S4-P6, S4-P7, S4- P8, S4-P29, S4-P36, S5-P3, S5-P5, S5- P14
Lima, C.C.	S1-P17	Morales, A.	S1-P9, S2-P33
Lima, J.C.	S2-P29	Morales, C.	S1-P3
Līmane, B.	S2-P9	Mora-Palomino, L.	S4-P26
Lima-Neves, J.	S2-O2	Moreau, P.	S3-O1
Liu, Y.	S4-O6	Moreno, B.	S5-P15
Lodygin, E.D.	S4-P20	Morgante, V.	S5-O2
Loffredo, E.	K9	Mosaleeyanon, K.	S1-O9
López, R.	S2-P32	Motuzova, G.V.	S1-P20
López-Martínez, J.	S2-P18, S2-P24	Mougin, C.	S5-P7
López-Valdez, F.	S4-P21	Muena, V.	S3-P15
Lozano, Z.	S1-P13	Muñoz, C.	S1-P38
Lugo de la Fuente, J.	S2-P40	Murad, E.	S1-P24
Luna-Suárez, S.	S4-P21	Murányi, Z.	S2-O4, S2-O9, S4-P18
Madayanti, F.	S5-O1	Murphy, D.	S2-O8
Majid, N.M.A.	S1-P33	Muter, O.	S2-P9
Malhi, S.S.	S2-P19, S4-P22, S4-P23	Naár, Z.	S2-O4
Manosalva, L.	S4-P34	Naidu, R.	K5
Manquían, K.	S1-P18, S4-P2, S2-P16	Nair, K.M.	S1-O3
Manzano, M.	S5-P6	Natarajan, A.	S1-O3
Marcato, P.D.	S4-P11	Navel, A.	S3-O4
Marchelli, P.	S2-O5		
Maricán, A.	S4-P24		
Marschner, P.	S2-P15		
Martínez, O.	S2-P15		
Martins, J.	S3-O4		
Maruyama, F.	S2-P15		
Marzaioli, R.	S2-P21		
Matsue, N.	S1-O2		
Matus, F.	S1-P19, S1-P29, S1-P30		
Mayor, M.	S3-O1		
Mehreen, F.	S4-P28		
Mei, L. H. I.	S4-P11		

Navia, R.	S5-O4, S4-P14, S5-P9	Priest, N.	S1-O7
Nazarenko, O.G.	S1-P20	Pucci, A.	S4-O10, S1-P23
Neaman, A.	S3-O7, S3-P6, S3-P15	Puschenreiter, M.	S2-O3, S2-P25
Nejati, M.	S4-P31	Qiao, X.	S1-O4
Németh, L.	S4-P17	Quezada, P.	S3-P25
Nogales, R.	S5-P15	Quilodrán, B.	S5-P11
Noinville, S.	S4-O7	Quiquampoix, H.	K7, S4-O7, S1-P26
Norde, W.	K6	Quiroz, A.	S4-P34
Nowak, V.	S3-O4	Ramesh, M.	S1-O3
Núñez, P.	S2-P30	Ramesh, N.	S5-P1
Nziengui, P.	S3-P4	Ramírez, L.	S2-P31
Ochoa, R.	S1-P11	Rangel, M.	S2-P7, S2-P11
Olsson, R.	S2-P20	Ranjard, L.	S3-O4
Ondrasek, G.	S3-O6	Rao, M.	S3-P16
Ovalle, C.	S1-P38	Rao, M.A.	S4-O2, S2-P21, S4-P27
Oyarzún, J.	S2-P42	Rengel, Z.	K8, S3-O6, S2-P37
Palma, C.	S4-P10	Reyes, I.	S2-P22
Palma, G.	S4-P1, S4-P6, S4-P7, S4-P8, S4-P29, S5-P3	Reyes, P.	S1-P35
Panichini, M.	S1-P30	Reyes-Díaz, M.	S3-P10, S3-P13, S3-P24
Pardo, F.	S4-P34	Reynolds, D.	S1-O5
Paredes, C.	S2-P36, S4-P36	Ribeiro da Silva, I.	S2-O2, S2-P29
Park, C.-L.	S1-O6	Ribera, A.	S2-P37, S2-P38
Parker, D.	S5-P4	Richter, P.	S3-P1, S4-P24
Parra, L.	S4-P34	Río-Portilla, F. del	S1-O10
Pascault, N.	S3-O4	Ristori, G.G.	S2-P23
Paskiewicz, I.	S3-O5	Rivas, C.	S4-P3
Pasricha, N.S.	S4-O5, S1-P21	Rivas, J.	S4-P24
Paulino, L.	S1-P38	Rivas, Y.	S1-P19, S1-P34
Perálvarez, M.C.	S3-P26	Riveros, M.	S5-P10, S5-P12
Perelomov, L.	S3-O8, S1-P28	Rodríguez-Rivers, J.	S2-P12
Peres, B.H.	S1-P17	Roig, A.	S1-P17
Pérez-Pantoja, D.	S4-P30	Rojas-Lillo, Y.	S3-P24
Perich, F.	S4-P34	Roldán, A.	S3-P26
Perotti, E.	S1-O8	Romagnoli, C.	S3-P3, S3-P16
Persson, P.	S2-O10, S2-P20	Romic, M.	S3-O6
Perucci, P.	S3-P16	Romic, D.	S3-O6
Pichihuencho, C.	S4-P33	Rong, X.	S1-P25
Pidello, A.	S1-O8	Rosa, C.A.	S2-O6
Piedra Buena, A.	S1-P23	Rosas, A.	S2-P32, S2-P37, S3-P27
Pigna, M.	K10, S1-P28, S3-P7, S4-P35	Rubilar, M.	S5-P10, S5-P11, S5-P12
Pinilla, L.	S3-P27	Rubio, R.	S1-P9, S2-P6
Pinochet, D.	S2-O1, S2-P42	Ruggiero, P.	S3-O9, S3-P16
Piutti, S.	S2-P27, S2-P28	Ruiz-Suárez, L.G.	S2-P13
Pizarro, C.	S1-P3, S1-P24, S3-P22	Rumpel, C.	S1-P19
Pokasombat, Y.	S1-P8	Russo, F.	S4-P27
Poppi, R.J.	S4-P11	Rutigliano, F.	S2-P21
		Saez, J.	S4-P14
		Sagredo, P.	S4-P9

Sahota, T.S.	S2-P19	Singh, H.	S2-O11
Sainju, U.	S2-O11	Siriratpiriya, O.	S5-O3
Salazar, E.	S1-P12	Sleutel, S.	S1-P13
Salazar, F.	S2-P31	Slezack-Deschaumes, S.	S2-P27, S2-P28
Salazar-Sosa, E.	S2-P12, S2-P18, S2-P24	Sokolovska, M.	S1-P27
Salbu, B.	S3-O11	Solans, M.	S2-P26
Salcedo, M.	S4-P3	Spadini, L.	S3-O4
Salman, S.M.	S4-P28	Spagnuolo, M.	S3-O9
Sánchez-González, A.	S1-O10	Spence, A.	S1-P14
Sandaña, P.	S2-P42	Strithundon, S.	S1-O9
Sandoval, Y.	S3-P25	Staunton, S.	S4-O7, S1-P26
Sanhueza, K.	S4-P1, S4-P7, S4-P8, S4-P29	Stegnar, P.	S3-O11
Sanhueza, S.	S1-P1, S1-P35, S4-P14	Steinberga, V.	S2-P1, S2-P9
Sanke Gowda, A.T.	S3-O10	Stockdale, E.	S2-O8
Santander, M.A.	S4-P9	Stohmann, E.	S2-P37
Santero, A.	S3-O9	Stolpe, N.	S1-P38
Santner, J.	S2-O3, S2-P25	Stucki, J.W.	S1-P24
Santoro, A.	S3-P16	Sudhir, K.	S1-O3
Sauvé, S.	S3-O7	Sumathi, C.S.	S5-P1
Scelza, R.	S4-O2, S2-P21	Szováti, K.	S2-O9
Schnepf, A.	S2-O3, S2-P25	Tan, W.	K6
Schnitzer, M.	K4	Terzano, R.	S3-O9, S3-P16
Schoenau, J.J.	S2-P19	Teuber, N.	S2-P31
Scotti, R.	S4-P27	Theng, B.K.G.	S4-O8
Sebastia, J.	S3-O3	Thimme Gowda, H.S.	S3-O10
Seeger, M.	S5-O2, S3-P21	Thimme Gowda, A.	S3-P19
Seguel, A.	S3-P11, S3-P17	Tinoco, P.	S1-P23
Senesi, N.	K9	Tortella, G.R.	S4-O1
Sepúlveda, B.	S4-P24	Trejo-Escareño, H.	S2-P18, S2-P24
Sepúlveda, L.	S4-P10	Trolard, F.	S3-O1, S2-P35
Sepúlveda, N.	S1-P29, S1-P-30	Tume, P.	S1-P27
Serrano, J.	S4-P30	Tunesi, K.	S3-P3
Shahidi, A.	S4-P31	Turpault, M.P.	S2-P5
Shahzad, K.	S3-P18	Uralbekov, B.	S3-O11
Shar, G.Q.	S2-P2	Uroz, S.	S2-P5
Shar, L.A.	S2-P2	Urrutia, C.	S4-P36
Shene, C.	S5-P10, S5-P11, S5-P12, S5-P13	Vaca, P.R.	S2-P40
Shirzadi, A.	S4-O9	Valenzuela, E.	S2-O1
Siddaramappa, R.	S1-O3	Van Cleemput, O.	S1-P34
Siebe, C.	S1-O10, S2-P13, S4-P26	van Oort, F.	S3-O3
Sieverding, E.	S3-P5	Vázquez-Vázquez, C.	S2-P18, S2-P24
Silva, I.R.	S1-P17	Vega, F.A.	S3-P2, S3-P8, S3-P20
Silva, L.H.M.	S1-P17	Véliz, D.	S2-P34
Simpson, A.J.	S4-O9, S4-P32	Vera-Martínez, M.	S1-O10
Simpson, M.J.	S4-O9, S4-P32	Veres, S.	S2-P17
Singh, B.	S2-O11	Violante, A.	K10, S1-P28, S3-P7, S4-P35
Singh, C.S.	S2-P4	Virág, D.	S2-O4, S2-O9, S4-P17, S4-P18
		Vistoso, E.	S2-P41
		Vivas, A.	S5-P15

Vobis, G.	S2-P26
Vogeler, I.	Plenary 1
Vong, P.-C.	S2-P27, S2-P28
Wall, L.G.	S2-P26
Wanichananan, P.	S1-O9
Wenzel, W.W.	S2-O3, S2-P25
Woignier, T.	S1-P26
Wolff, N.	S5-P7
Wulff-Zottele, C.	S5-P8
Xu, J.	S4-O3, S2-P14
Xu, Y.	S4-O9
Xu, Z.	S2-P14
Yang, X.	S1-O5
Yáñez, C.	S3-P21
Yañez, P.	S2-P36
Yesmin, L.	S2-P3
Yohandini, H.	S5-O1
Yoshida, S.	S3-O8
Yusop, K.	S1-P33
Zagal, E.	S1-P38
Zambrano, J. A.	S2-P29
Zambrano-Gonzalez, J.	S2-O2
Zanchi, R.	S3-P3
Zariņa, D.	S2-P9
Zeni, J.	S4-P12
Zentner, R.P.	S2-P19
Zhang, Z.	S1-O5
Zhao, L.	S1-O5
Zhiyanski, M.	S1-P27
Zhu, J.	S1-O4, S1-P28
Zia-ur-Rehman	S3-O2
Zovko, M.	S3-O6
Zubkova, T.A.	S2-O7
Zúñiga, A.	S2-P34

Solute Interactions in Soils in Relation to Bioavailability and Remediation of the Environment

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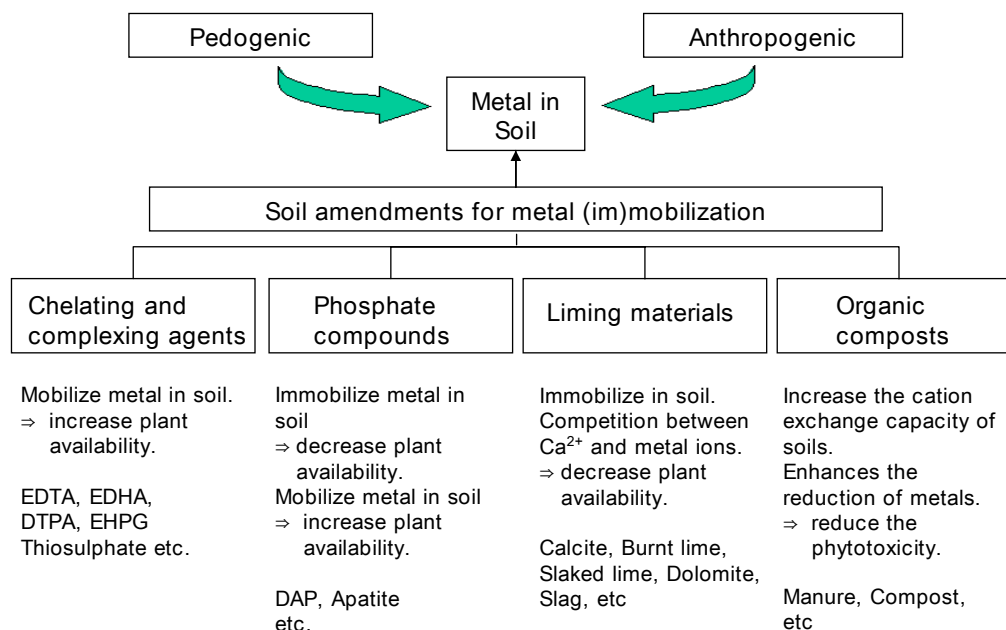
Unlike organic contaminants, most metals do not undergo microbial or chemical degradation and the total concentration of these metals in soils persists for a long time after their introduction (Adriano, 2003). With greater public awareness of the implications of contaminated soils on human and animal health there has been increasing interest amongst the scientific community in the development of technologies to remediate contaminated sites. For diffuse distribution of metals (e.g. fertilizer-derived Cd input in pasture soils), remediation options generally include amelioration of soils to minimize the metal bioavailability. Bioavailability can be minimized through chemical and biological immobilisation of metals using a range of inorganic compounds, such as lime and phosphate (P) compounds (e.g. apatite rocks), and organic compounds, such as 'exceptional quality' biosolid (Figure 1; Bolan and Duraisamy, 2003). Reducing metal availability and maximizing plant growth through inactivation may also prove to be an effective method of *in situ* soil remediation on industrial, urban, smelting, and mining sites.

The more localised metal contamination found in urban environments (e.g. Cr contamination in timber treatment plants) is remediated by metal mobilization processes that include bioremediation (including phytoremediation) and chemical washing. Removal of metals through phytoremediation techniques and the subsequent recovery of the metals (i.e. phytomining) or their safe disposal are attracting research and commercial interests. However, when it is not possible to remove the metals from the contaminated sites by phytoremediation, other viable options, such as *in-situ* immobilisation should be considered as an integral part of risk management.

Since bioavailability is a key factor for remediation technologies *in-situ* (im) mobilization using inorganic and organic compounds that are low in heavy metal content may offer a promising option. In this paper, after a brief introduction of sources of metal inputs and their dynamics in soils, the role of various inorganic and organic soil amendments in the (im) mobilization of metals in soils in relation to managing their bioavailability and remediation, will be discussed.

The bioavailability of a chemical in the soil environment has been defined as the fraction of the total contaminant in the interstitial pore water (i.e., soil solution) and soil particles that is available to the receptor organism (Naidu et al., 2008). Bioavailability of metals in soils can be examined using chemical extraction and bioassay tests. Chemical extraction tests include single extraction and sequential fractionation (Ruby *et al.*, 1996; Basta and Gradwohl, 2000). Bioassay involves plants, animals and microorganisms (Yang *et al.*, 1991).

PLENARY PAPERS



In-situ techniques involving growing of the organisms of interest in the contaminated material and quantifying the uptake of metal into the organism or assessing the toxicological response are being used by many researchers as bioindicators of contamination. Measurements of metal bioavailability and toxicity in soils using soil microorganisms are receiving increasing attention, as microorganisms are more sensitive to heavy-metal stress than plants or soil macrofauna. The methods using microflora and protozoa have the potential to provide a measure of bioavailability of heavy metals in the short-term and even facilitate the measurement of temporal changes. In contrast responses by mesofauna (microarthropods) and macrofauna (enchytridae, invertebrates and earthworms) are cumulative effects.

A number of amendments are used either to mobilise or immobilize heavy metals in soils (Table 1). The basic principle involved in the mobilization technique is to release the metals in to soil solution, which is subsequently removed using higher plants. In contrast, in the case of the immobilization technique the metal concerned is removed from soil solution either through adsorption, complexation and precipitation reactions, thereby rendering the metals unavailable for plant uptake and leaching to groundwater.

Since one of the primary objectives of remediating contaminated sites is to reduce the bioavailability of metals, *in-situ* immobilization using soil amendments that are low in heavy metal content may offer a promising option. However, a major inherent problem associated with immobilisation techniques is that although the heavy metals become less bioavailable, their total concentration in soils remains unchanged. The immobilised heavy metal may become plant available with time through natural weathering process or through breakdown of high molecular weight organic-metal complexes. Although the formation of soluble metal-organic complexes reduces the phytoavailability of metals, the mobility of the metal may be facilitated greatly in soils receiving alkaline-stabilized biosolid because of the reduction of metal adsorption and increased concentration of soluble metal-organic complex in solution.

PLENARY PAPERS

Table 1. Selected references on the potential value of soil amendments in the (im) mobilization of metals in soils.

Amendments [#]	Metal	Observations	References ^{\$}
Chelating /complexing agents			
Thiosulphate	Hg	Shoot Hg accumulation in the presence of thiosulphate salt was dependent upon plant species characteristic and humic acid content	Moreno <i>et al.</i> (2004)
EDTA	Cu	Mobilized and increased plant uptake	Thayalakumaran <i>et al.</i> (2003b)
EDTA	Cu, Fe, Mn, Pb, Zn	EDTA had only a limited effect on metal uptake by plants	Walker <i>et al.</i> (2003)
EDTA	Pb	Plant uptake in roots and shoots increased	Chen <i>et al.</i> (2004)
Humic acid	Cd	Plant uptake enhanced significantly	Evangelou <i>et al.</i> (2004)
EDTA	Cd	Mobilized Cd in soil, but did not increase plant total uptake	Jiang <i>et al.</i> (2003)
CCA EDTA	Pb, Zn	CCA improved bioavailability in soil, enhanced shoot content of Pb but not of Zn	Li <i>et al.</i> (2005)
Phosphate compounds			
KH ₂ PO ₄	Cd	Enhanced immobilization, decreased plant availability	Bolan <i>et al.</i> (2003c)
Apatate, Zeolite, Fe-Oxide	Cd, Pb	Reduced the mobility and uptake by plant	Chlopecka <i>et al.</i> (1997)
KH ₂ PO ₂	Pb, Zn, Cd	Reduced Pb level but not Zn and Cd in earthworms	Pearson <i>et al.</i> (2000)
Phosphate	As	Increasing phosphate supply decreased As uptake. Increasing As supply decreased the P concentration in the root	Wang <i>et al.</i> (2002)
Phosphate	Pb, As	Increased plant uptake of soil As. No effect on soil Pb phytoavailability	Creger <i>et al.</i> (1994)
Liming materials			
Ca(OH) ₂	Cd	Transformed to less mobile fractions, reduced phytoavailability	Bolan <i>et al.</i> (2003d)
Lime, FBA, Bark	Cr	Reduced the availability for plant uptake and leaching ground water	Bolan <i>et al.</i> (2001)
Lime	Cd	Did not reduce uptake and transfer to the kernels of sunflower	Li <i>et al.</i> (1996)
Lime	Cd	Reduced uptake by lettuce	Lehoczhk <i>et al.</i> (2000)
Lime	Pb, Cd	Decreased Cd uptake, but little influence Pb uptake by radish	Han <i>et al.</i> (1996)

PLENARY PAPERS

Ca(OH) ₂ , CaCO ₃	Cd	Ca(OH) ₂ prevented phytotoxicity, but CaCO ₃ was no effective in reducing phytotoxicity	Chaney <i>et al.</i> (1977)
Lime	Cd, Ni, Zn, Cu	Reduced uptake of Cd, Ni, Zn, but did not reduce Cu	Brallier <i>et al.</i> (1993)
Organic matter			
Biosolid	Cd	Reduced the bioavailability	Bolan <i>et al.</i> (2003b)
Biosolid, Manure	Cr	Reduced the phytotoxic effect	Bolan <i>et al.</i> (2003e)
PS, SS	Cd, Zn	Cd uptake increased, but Zn unaffected	Merrington <i>et al.</i> (2000)
Biosolid	Cd	Reduced plant availability	Brown <i>et al.</i> (1998)
Manure	Zn, Mn	Corn and leaf concentrations of Zn and Mn were enhanced	Wallingford <i>et al.</i> (2000)
Biosolid	Zn	Decreased plant availability	Shuman <i>et al.</i> (2000)
Biosolid	Cd	Cl-complexation of Cd increased the phytoavailability of Cd in biosolid amended soil	Weggler-Beaton <i>et al.</i> (2000)
Organic matter	Ni, Cd, Zn	Reduced Ni uptake but not Cd, Zn in rice	Kashem <i>et al.</i> (2000)
Compost, Manure	Cr	Reduced the phytotoxicity	Bolan <i>et al.</i> (2003g)

[#]EDTA, ethylene diaminetetra-acetic acid; CCA, coated chelating agent; FBA, Fluidised bed boiler ash; PS, Papermill sludge; SS, Sewage sludge

^sAdopted from Bolan *et al.* (2008)

A large number of studies have provided conclusive evidence for the potential value of both water-soluble and water-insoluble P compounds to immobilize metals in soils, thereby reducing their bioavailability for plant uptake. However, it should be recognized that, depending on the nature of P compounds and the heavy metal species, application of these materials could cause either mobilization (e.g., As) or immobilization (e.g., Pb) of the metals. While mobilization by certain P compounds enhances the bioavailability of metals, immobilization inhibits their plant uptake and reduces their transport in soils and subsequent ground water contamination. Furthermore, some of these materials contain high levels of metals (e.g., Cd) and can act as an agent of metal introduction to soils. Accordingly, these materials should be scrutinized prior to their large-scale use as immobilizing agent in contaminated sites.

Keywords: Phytoremediation; heavy metals; soil remediation

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Impacts of Metals and Metalloids on Soil Microbial Diversity and Ecosystem Function

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Metal and metalloid concentrations in soil exert an enormous influence on the diversity, composition, and activity of soil microorganisms that carry out essential ecosystem services. At low concentrations, microorganisms can compete for essential trace elements that are required to support their growth and in this manner affect plant nutrition and disease through the production of metal chelators. At high concentrations, the toxic effects of metals result in reduced microbial diversity and altered rates of key biological processes that underlie ecosystem function. The latter is now of great concern as large land areas across the globe have become contaminated with metals from land application of wastes and atmospheric deposition of heavy metals. An understanding of how plants, soils, organic matter, and microorganisms influence metal transformations in the rhizosphere is thus critical for managing soils to assure the long term protection of soil quality, food safety, and ecosystem function.

One of the major difficulties in making practical management decisions with respect to metal pollution for different types of soils has been our inability to derive bioindicators of soil quality that can provide indices of soil quality across the landscape at different scales and over time. Ecosystems respond very differently to acute and chronic metal toxicities depending on their chemical properties and prior exposure histories. Toxicities are particularly influenced by physico-chemical conditions in soils that influence the bioavailability of metals and metalloids to plants and microorganisms. Nonetheless, the dynamic nature of microbial communities, which respond much faster to changes in management practices than do soil physical and chemical properties enables us to use bioindicators as a sensitive tools for predicting possible long term changes in soil properties. From a land management perspective, it is critical that soil microbiologists offer appropriate bioindicators for use in measuring the impacts of soil pollution on soil biological properties and extend knowledge as to how specific bioindicators may reflect long term changes in soil quality that can be used to guide land management practices and remediation of contaminated soils.

Responses of Microbial Communities to Metal Contamination

The microbial community concept is based on the interactions among in all of the various species of bacteria, fungi, protozoa, and microfauna that carry out the various broad level functions of the soil, ranging from nutrient cycling to organic matter formation, and plant disease protection. Differences in the species composition of various soils are linked to changes in soil biological properties that in turn affect the long term chemical and physical properties and ability to support plant growth. Among the most basic functions are respiration and organic matter formation, both of which are affected by metal contamination. Acute responses of microbial populations to metal toxicities include reduction in species diversity,

PLENARY PAPERS

the biomass of microorganisms, and an increase in the respiration rate. As microbial respiration controls the turnover and accumulation of carbon, the measurement of the soil respiration rate per unit of biomass, also known as the metabolic quotient (Anderson and Domsch, 1993), is one of the most sensitive response of microbial communities to metal contamination. As the metabolic quotient increases, carbon is released at a faster rate from the soil, leading to lower organic matter accumulation that over years can affect the soil structure and cation exchange capacity. In turn loss of soil structure affects aeration, bulk density, and root growth which then leads to lower primary productivity. Loss of soil carbon due to metal pollution is also a concern for global climate change as the soil is a major reservoir of carbon and is being managed in national carbon budgets to offset anthropogenic releases of carbon to the atmosphere.

Going beyond the biomass and respiration, one of the principle effects of metal pollution is the loss of biodiversity (vanBeelen and Doelman, 1997). The soil microbial community consists of assemblages of tens of thousands of species of archaea, bacteria, and fungi, all of which occupy different niches in the soil and carry out various biogeochemical processes and ecosystem functions. The large diversity of species as compared to the list of the soils most basic functions has led to the concept of functional redundancy in which many different species operating in guilds are presumed to function as interchangeable members of functional guilds. As long as there are many different species that carry out the same function, i.e. cellulose degradation or plant hormone production, then the process is likely to proceed over the full range of environmental conditions that occur in the soil. On the other hand, the functional diversity of the soil provides an index of the number and types of substrates that are utilized by the predominant microbial populations. The latter can be measured using specially developed assays such as Biolog plates or in-situ substrate additions that can be measured colorimetrically using dehydrogenase activity or by measurement of substrate induced respiration rates.

One of the major concerns arising from studies of metal contamination is the loss of both substrate diversity and functional redundancy that occurs as an effect of metal toxicity. A primary function of soil microbial communities is to degrade all of the natural carbon substrates that enter the soil or that are produced as a result of microbial growth. The chemical diversity of soils is enormous with many thousands of plant and microbial secondary metabolites undergoing transformation and degradation. Many of these substances are potentially toxic to plants when they occur at elevated concentrations, and one of the functions of slow growing, oligotrophic population of bacteria is thought to be the degradation of chemicals that occur at low concentrations. The vast majority of these bacteria are nonculturable under standard laboratory conditions due to their extremely slow growth rates and requirement for special growth media and conditions. Thus it is very difficult to assess the impact of metals on the oligotrophic population. Nonetheless, early work conducted using culture based methods has shown the impact of metals on the functional diversity of the soil bacterial population (Figure 1). In the study illustrated here, the number of species that could grow on selected chemicals was determined for soil bacteria that could grow on agar amended with the chemical in the presence and absence of zinc. In all instances, the metal tolerant population represented a subset of bacteria such that metal contamination reduced the overall functional diversity or numbers of species that could grow on individual chemicals. In some instances, there was a complete lack of metal tolerant species, which if

PLENARY PAPERS

extended to the unculturable population means that the soil may lose the ability to carry out this particular degradative function.

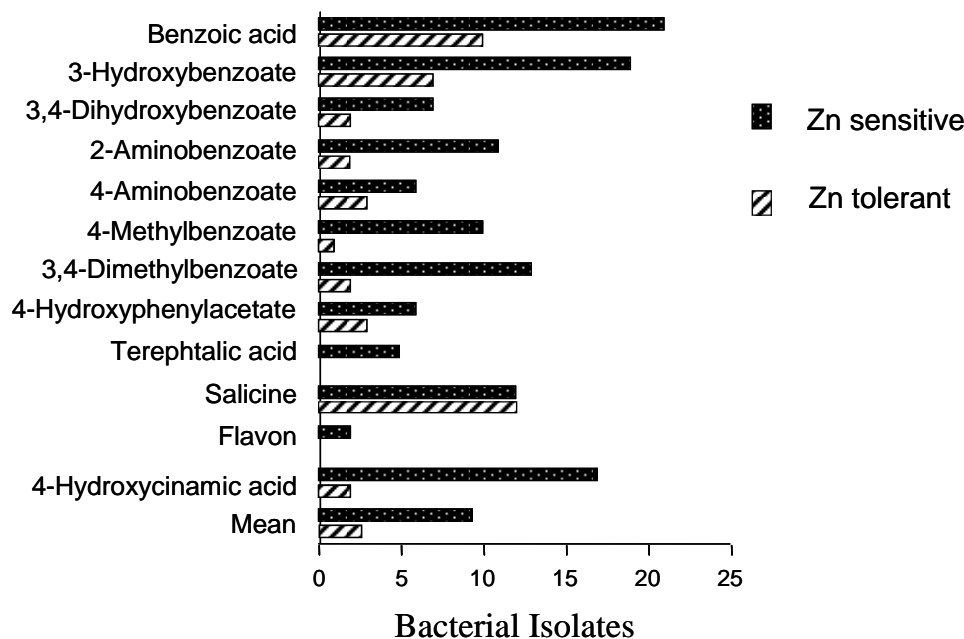


Figure 1. Loss of metabolic diversity in microbial communities exposed to zinc during enrichment culture on selected carbon substrates. Figure adapted from Doelman et al., 1994).

The idea that loss of functional diversity may impair the stress tolerance of the soil bacterial community has been examined in a series of studies in which soils were partially sterilized using heat or were exposed to various stressors such as metal contamination to determine the overall impact on the rate of degradation of various substrates and other measures of microbial activity (Degens et al., 2001; Griffiths et al., 2000, 2001). Here, the primary concern centers on the property of microbial community resilience, which is the ability to recover from various normal shocks to the microflora that occur as a result of normal fluctuations in the environment. These studies have clearly shown that loss of microbial diversity lead to loss of resilience and declines in ecosystem function. The major question that arises from this body of research is to what extent can microbial diversity be reduced before ecosystem functions are affected, and what degree of loss is tolerable from a regulatory perspective (Dahlin et al., 2001).

In addition to broad scale functions that are carried out by guilds that are comprised of tens to hundreds of species, metals may also affect so called keystone microorganisms that carry out very specific functions and that are comprised of narrow phylogenetic groups of species. For example, ammonia oxidation and symbiotic nitrogen fixation are carried out by very specialized bacteria. Agriculture is highly dependent on the use of rhizobium bacteria that fix nitrogen in association with legumes and the efficiency of various strains of symbiotic bacteria in carrying out this process is of concern for optimizing the rates of N fixation. In this case, the bacteria are classified both by their infectivity (ability to colonize plant roots)

PLENARY PAPERS

and affectivity (ability to fix nitrogen). Research by McGrath and coworkers (Castro et al., 2002) have shown that strains with good infectivity may remain, but that these strains may be less or completely ineffective for fixing nitrogen. The level of metal tolerance of effective strains is thus proposed as a measure for setting the upper limits of acceptable metal loading and soil contamination.

Bioindicators of Soil Quality and Metal Contamination

A review of the 52 environmental/soil monitoring programs available world-wide (Winder 2003) shows that the vast majority of programs utilize a minimum dataset to describe soil quality. Of these programs, the vast majority only measures of soil chemical characteristics such as pH, C fractions, NPK, EC, CEC, half use soil physical variables such as moisture, PSA, and penetration resistance and less than one third measured soil biological data, almost exclusively microbial biomass or enzyme activity. Nonetheless, it is now increasingly acknowledged that biological indicators will extend the utility of soil quality assessment in soils that are undergoing more subtle, management-induced changes (Filip, 2002; Neilson and Winding 2002). The ideal tool must also be spatially and temporally defined and account for heterogeneity at a given spatial and temporal scale (Conroy et al., 1996). Most importantly, the information generated by these tools needs to have practical utility at a scale where it is possible to make recommendations on different soil management practices.

Bioindicators used to assess soil microbial quality can include single, holistic measures of the overall microbial community such as biomass, respiration rates, metabolic quotient and enzyme activities or can include measures that have a multiple number of variables to describe the structural and or functional composition of a soil microbial community. Examples of multiparametric measures include phospholipid fatty acid (PLFA) profiles (Baath et al., 1998), 16S rRNA gene methods such as PCR-DGGE and TRLFP, and substrate diversity assays. Single parameters provide an integrative estimate of the entire microbial community and are relatively widely used because they are easy to measure, are relatively inexpensive and they can be incorporated into Analysis Of Variance (AOV) linear models. However, such measures have been increasingly criticized for being inconsistent and demonstrate a high coefficient of variation in response to seasonal climatic changes (Sojka et al., 1999; Gil-Sotres et al., 2005).

Any one method for characterizing microbial community structure and function provides only a limited perspective and are therefore subject to over and under interpretation in assessing soil biological responses to different environmental variables. For example, soil respiration and may reflect the activity of diverse microorganisms on many different substrates, or growth of a few species on a particular substrate such as cellulose. Although this short-coming can be improved by polyphasic measurements in which the investigator looks for consistency across a range of methodologies such as enzymes, respiration, biomass, and others, the interpretation of polyphasic data is often speculative. Alternatively, multiparametric data attempts to describe community structures and functions that use multiple variables within one category. These data can include biochemical, catabolic, and genetic fingerprints of the microbial communities and can be based on 30 to 100 or more variables within the one methodology. Unlike the straightforward AOV models used for single parameter data, multiparametric data are highly dimensional and need to undergo statistical procedures to reduce dimensionality before they can be incorporated into models.

PLENARY PAPERS

They tend therefore to have restricted use. Despite these difficulties, there are now many bioindicators that have potential application for assessing the impacts of heavy metals on soil biological function and for guiding the development of policy to guide land application of metal waste and criteria for restoration of metal contaminated soils (Tandy et al., 2005).

Application of Artificial Neural Networks for Evaluation of Bioindicators

In recent years, one of the most powerful approaches that has emerged for integrating disparate types of qualitative and quantitative data is the use of artificial neural networks (ANN) that employ machine learning to extract and recognize patterns in relationships among descriptive variables (Schultz and Wieland 1997; De la Rosa, Mayol et al. 2004). Although not widely used yet for analysis of soil quality variables, there has been increasing application of ANN for modeling of data sets used to describe individual soil physical, chemical, and biological properties during the past ten years. ANNs are particularly useful for extracting patterns among data sets in which there may be complex nonlinear interactions that cannot be detected with traditional methods for multivariate analyses. Among the range of applications that have been realized, ANNs have been used to classify soil structure and physical properties (Levine, Kimes et al. 1996; Chang and Islam 2000), for soil and vegetation mapping (Tan and Smeins 1996; Deadman and Gimblett 1997), prediction of non-point source pollution (Muleta and Nicklow 2005), for prediction of soil salinization (Patel, Prasher et al. 2002), and for predicting soil organic carbon accumulation under different land use schemes (Somaratne, Seneviratne et al. 2005).

For studies on soil biological data, neural network analysis (ANN) has most recently been used to relate microbial biomass and 16S rRNA gene banding patterns to soil texture and other chemical properties (Lentzsh, Wieland et al. 2005; Ramadan, Hopke et al. 2005). With ANN, multidimensional data can be reduced in dimensionality by a combination of self organizing maps (SOM) which are a type of neural network, and principle components analysis. Results generated from this approach provide a 2- or 3-dimensional map of clusters that reflect relationships between variables and site locations. The maps can be used to explore the influence of environmental variables on biological indicators (Kampichler et al., 2000; Noble et al., 2000; Lentzsh et al., 2005). This information can be used to identify key variables and can be used for forecasting changes in response variables over time. Most importantly the data generated can be presented visually in a way that provides for easy interpretation of complicated datasets. It is likely that such models will have extensive application for understanding the relationships between soil chemical and physical variables on metal bioavailability, transformations, and the effects on soil microbial communities that carry out key ecosystem processes..

Conclusions

Aside from food chain transfer, the impact of metals and metalloids on microbial communities is one of the most serious concerns of heavy metal pollution of soils and can result in long term reduction in soil quality and primary productivity. Microbial communities can adapt to metal pollution, but loss of species diversity results in potential loss of biological functions and reduced soil resilience. The extent to which this occurs depends on soil physico-chemical properties that affect the bioavailability of metals. To this end,

PLENARY PAPERS

bioindicators of metal toxicity provide an integrative measure of the toxicities of metals in different soils. These relationships are only now being explored in detail and are complicated by the many interactions among the different variables that affect metal toxicities and microbial communities. New statistical modeling methods that employ artificial neural networks provide a means to sort out these interactions and develop hypotheses. Such models may also eventually be useful as decision support tools for land management of soils receiving heavy metal waste and for restoration of contaminated soils.

Keywords: Metal and metalloids; soil microbial diversity; contaminated soils.

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Enzymes of Significance to the Restoration of Polluted Systems: Traditional and Advanced Approaches

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In the second part of the twentieth century, intense environmental pollution has been one of the biggest concerns for science. Xenobiotic chemicals are continuously released into the biosphere because of several industrial and/or agricultural activities. In particular, the rapid industrialization of agriculture, expansions in the chemical industry, and the need to generate cheap forms of energy have all resulted in an ever-increasing reliance on anthropogenic organic chemicals and caused the contamination of the atmosphere, the hydrosphere and the soil environment. Examples of xenobiotics very dangerous for the environment and considered very often priority pollutants are shown in Figure 1.

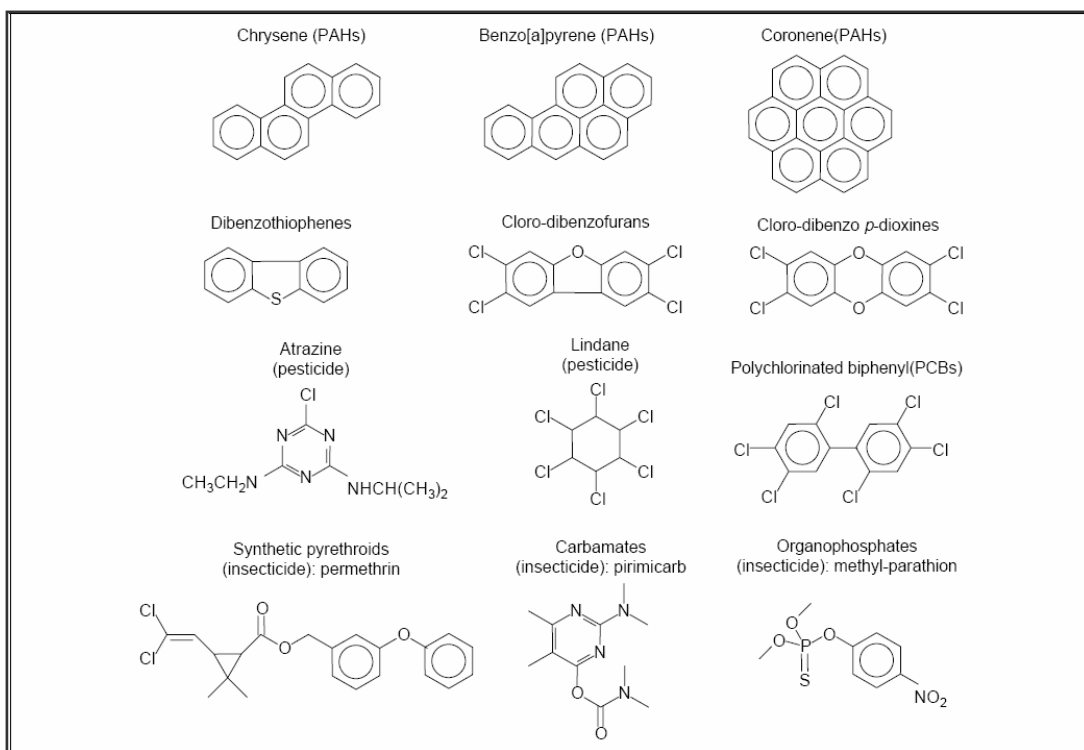


Figure 1. Some examples of dangerous environmental pollutants

PLENARY PAPERS

Highly halogenated monoaromatic compounds, including tetra and penta-halogenated phenols and anilines, are widely distributed pollutants. They are present in the environment either as naturally-occurring or synthetic introduced compounds, being used for a wide range of domestic, agricultural and industrial purposes. Pentachlorophenol (PCP) and the lower chlorinated phenol, tetrachlorophenol (TCP), are still intensively used as fungicides, herbicides, insecticides, and precursors in the synthesis of other pesticides. Their production is currently made in countries such as China and Mexico, although, for instance, PCP production in USA and in Europe has been banned since 1992 and 2000, respectively. Their recalcitrance depends on the number, type and position of the halogen substituents. The removal of halogen atoms from aliphatic and aromatic halogen-carbon-substituted compounds is an essential step in their biochemical transformation and the reaction reduces or eliminates toxicity.

The chemical properties and quantities of xenobiotics determine their toxicity and persistence in the environment. Their interaction with targeted and non-targeted organisms has extensively damaged the ecosystem through entry into the food chains. The net result has been bioaccumulation and biomagnification of these xenobiotics in aquatic and terrestrial organisms. Adverse effects in humans and other organisms such as acute toxicity, carcinogenesis, mutagenesis and teratogenesis, have been observed from the presence of many of these compounds at high concentration or following prolonged exposure. This is the consequence of the general high resistance of these toxic compounds to physical, chemical or biological degradation, thus constituting an environmental burden of considerable magnitude. Also substances not toxic at low concentration may accumulate in the environment and produce adverse effects on environmental health.

Such ecological stress, imposed by massive amounts of xenobiotics, is partially surmounted through natural, mainly microbial, attenuation. While microbial attenuation may be almost infallible, it is not adequate to protect the biosphere from indiscriminate agricultural and industrial activities. This may be because (i) xenobiotics are relatively new to the biosphere (ii) enhanced resistance to attenuation is inherent due to unusual chemical bonds or functional groups in their structure, that do not (or seldom) occur in nature (iii) microbes have not had enough time to evolve suitable metabolic apparatus to deal with incorporated xenobiotics. Therefore, they not have appropriate metabolic pathways for them and (iv) only a few microbes have evolved with the ability to sustain in adverse ecological states.

Therefore, a solely biochemical explanation is not sufficient for explaining the accumulation of such compounds. Efficient degradation involves various factors, such as bioavailability of the substrates, which have to be understood. Furthermore, the substrate has to diffuse or be transported into the cell. Besides these factors, the organism capable of degradation must be present at the site where it is needed and it has to perform under the given or manipulated environmental conditions.

Thus, a combined approach is required to understand the bottlenecks of xenobiotic degradation, to rationally overcome them by different methods, to elucidate the microbial metabolic diversity, and to understand the metabolic and organismic network necessary for activity under environmental conditions.

PLENARY PAPERS

Several strategies may be adopted to efficiently improve the process and help natural biodegradation processes work faster. These biological methodologies, collectively indicated as *bioremediation*, and usually considered environment-friendly treatments, constitute essentially a managed or spontaneous process mediated by living organisms (mainly microorganisms), which degrade or transform contaminants to less toxic or non toxic products, with mitigation or elimination of environmental contamination. The use of plants (phytoremediation) and their associated microorganisms has been also considered as an appealing technology to degrade, contain or render harmless contaminants in soil or groundwater.

Intra-, ecto- and extra-cellular enzymes are, ultimately, the main effectors of pollutant transformation during plant and/or microbial processes. Enzymes perform a wide range of very important functions throughout nature. They are highly specific and efficient, guiding the biochemistry of life with great precision and fidelity. This fidelity is essential in the cells of living organisms, and a multitude of mechanisms have evolved for controlling the activity of these enzymes themselves. Enzymes play a key role in harvesting energy from the sun via photosynthesis, perform a wide range of metabolic functions throughout every living cell in the bodies of plants and animals, and are in fact really the catalysts of all biological processes constituting life on earth.

Enzymes production (mainly of extra-cellular enzymes) is controlled by several factors including at the organism level the pairing of low-level constitutive synthesis with induction-repression pathways, and at the microbial population and community levels the quorum-sensing systems that regulate unique and varied cellular responses as well as protection from competing microbial communities.

The quorum-sensing systems allow bacteria to monitor their environment for the presence of other bacteria and to respond to fluctuations in the number and/or species present by altering particular behaviors. Most quorum-sensing systems are species- or group-specific, which presumably prevents confusion in mixed-species environments. However, some quorum-sensing circuits control behaviors that involve interactions among bacterial species. These quorum-sensing circuits can involve both intra- and interspecies communication mechanisms. Finally, anti-quorum sensing strategies are present in both bacteria and eukaryotes, and these are apparently designed to combat bacteria that rely on cell-cell communication for the successful adaptation to particular niches.

Many enzymes of both microbial or plant origins have been recognized to be able to transform pollutants at a detectable rate and potentially suitable to restore polluted environment. The main enzymatic classes involved in such a process are: hydrolases, dehalogenases, and oxidoreductases.

Amide, ester and peptidic bonds undergo hydrolytic cleavage by amidases, esterases and proteases in several xenobiotic compounds and may lead to products with little or no toxicity. Hydrolases responsible for the cleavage of pesticides are among the best studied groups of enzymes. Most of these hydrolases are extracellular enzymes, except for the cell wall-bound enzymes of *Penicillium* and *Arthrobacter* sp., which hydrolyze barban and prophan, respectively.

Evidences have been provided that lytic enzymes produced by many fungi and bacteria are strictly involved in the microbes' antagonism toward plant pathogens and pests. Exoenzymes possessing chitinolytic, glucanolytic, cellulolytic or proteolytic activities can be used

PLENARY PAPERS

individually or in combination to provide an enzymatic basis for a number of processes, which ultimately lead to a biocontrol effect. Very interesting is the findings that selected strains of *Trichoderma* introduced in rhizosphere soil rapidly catabolised phytotoxic concentrations (10 mM) of cyanide through the action of two cyanide catabolizing enzymes: formamide hydrolyase and rhodanese (Lynch 2002).

As reported above, many xenobiotics contain halide atoms bonded to aliphatic or aromatic carbon. Therefore, dehalogenation reactions are important initial steps in the degradation of such xenobiotics.

The cleavage of carbon-halogen bonds may occur by:

- (a) Enzymatic dehalogenation catalyzed by specific enzymes (dehalogenases);
- (b) A fortuitous reaction catalyzed by enzymes with a broad substrate specificity and acting on halogenated analogs of their natural substrate;
- (c) Spontaneous dehalogenation of unstable intermediate products of unrelated enzymatic reactions.

Although molecular mechanisms of dehalogenation are not fully understood and require further studies, certain reaction types are fairly well known:

1. Reductive dehalogenation, where the halogen is replaced by hydrogen;
2. Oxygenolytic dehalogenation, where one or two atoms are incorporated in the molecule;
3. Hydrolytic dehalogenation, with substitution of the halogen by an hydroxy group derived from a water molecule;
4. Thiolytic dehalogenation by which halogen is removed through a transfer reaction ;
5. Intramolecular substitution, in which the halogen removal involves the formation of an epoxide;
6. Dehydrohalogenation, with elimination of a HCl molecule and formation of a double bond;
7. Hydration, with addition of a water molecule to a double bond and elimination of the halogen

The reactions catalyzed by enzymes bringing about dehalogenation and degradation of both aliphatic and chloroaromatic compounds can be broadly classified as follows (Bhatt et al., 2007):

Table 1. Enzymes involved in the dehalogenation reactions

Reaction	Enzymes
Oxidative dehalogenation	Mono- or dioxygenases
Dehydrohalogenation	Dehydrohalogenases
Substitutive dehalogenation	Halido-hydrolyases
Dechlorination via methyl transfer	Methyltransferases
Reductive dehalogenation	Dehydrohalogenases

Examples of degradation of pollutants by dehalogenases are reported in Table 2.

Table 2. Degradation of pollutants by dehalogenases

Mechanism of dehalogenation	Pollutant	Source
Reductive	3-chlorobenzoate, PCE, 3-Cl-4-hydroxybenzoate	<i>Desulfomonile tiedjei</i> , <i>Flavobacterium</i> ,
Oxygenolytic	PCP, Cloroalkanes, 4-chlorophenylacetate	<i>Burkholderia cepacia</i> , <i>Flavobacterium sp.</i> ,
Hydrolytic	Haloaromatic compounds	<i>Arthrobacter</i> , <i>Pseudomonas</i>
	Haloalkanes	<i>Pseudomonas</i> , <i>Hypomicrobium</i> , <i>Methlobacterium</i>
	Haloacid compounds	<i>Pseudomonas</i> , <i>Alcaligenes</i> ,
Haloalcohol	C-2, C-3 bromo-, chloro-alcohols	<i>Flavobacterium</i> , <i>Pseudomonas</i> , <i>Arthrobacter</i>
Dehydroalogenation	Lindane	<i>Pseudomonas paucimobilis</i>

Another no less important process is reduction of nitro groups carried out by nitroreductases. Nitro-aromatics are produced by incomplete combustion of fossil fuel or nitration reactions and are used as chemical feedstock for synthesis of explosives, pesticides, herbicides, dyes, pharmaceuticals, etc. Their indiscriminate use in the past due to wide applications has resulted in inexorable environmental pollution. The transformation of nitro compounds may occur in both aerobic and anaerobic conditions. For instance the catabolic pathways and enzymes involved in degradation processes in *P. putida* 2NP8. were deciphered and are summarized in Figure 2 (Kulkarni and Chaudhari, 2007)

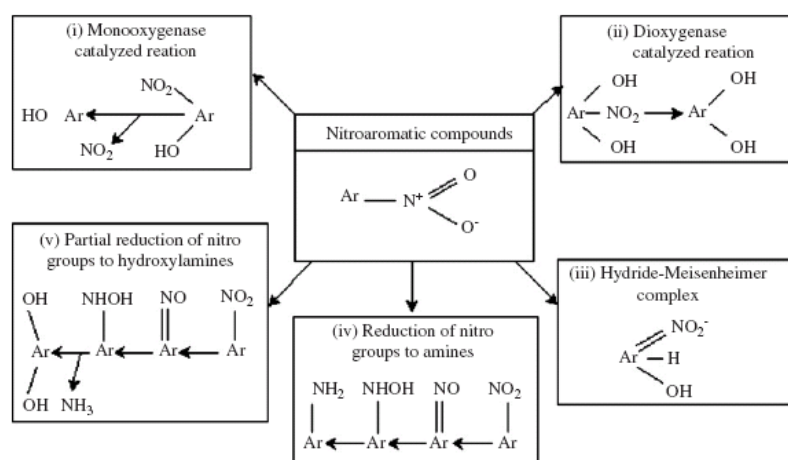


Figure 2. Microbial strategies for remediation of nitro-aromatic compounds by aerobes (i, ii, iii and iv) and anaerobes (v) (after Kulkarni and Chaudhari, 2007).

PLENARY PAPERS

Oxidative processes, carried out by oxido-reductases, are very important in the bioconversion of xenobiotics, especially bioconversions which follow hydrolysis and dehalogenation of the parent compound. In this case, the basic reactions are hydroxylation, followed by cleavage of the aromatic ring, oxidative O- and N-dealkylation, and epoxidation. Certain compounds, e.g. derivatives of aromatic acids, are completely degraded due to the involvement of oxidative enzymes. Moreover, oxido-reductases may catalyze the oxidative coupling of reactive compounds through formation of highly reactive free radicals and production of polymeric products by self-coupling or cross-coupling with other molecules. Dechlorination, demethoxylation and decarboxylation during coupling and polymerization of differently substituted substrates may also occur.

As also seen above, a key role in the transformation of xenobiotics is performed by oxygenases. Mono- and dioxygenases catalyze essential steps of the metabolic pathway of several toxic compounds. Indeed, alkanes, aromatics, chlorophenols and nitrophenols, and PAHs are oxidatively degraded by bacteria and mediated by either specific or non-specific oxygenases.

Another important group of enzymes particularly effective in the transformation of pollutants is formed by the lignin-degrading systems produced by fungi and involved in the transformation of lignin. Lignin-peroxidases, manganese peroxidases and laccases are the main enzyme belonging to this systems and they are responsible of the transformation and removal of a long list of pollutants of different nature and dangerousness (Cameron et al., 2000, Esteve-Nunez et al., 2001, Verdin et al. 2004, Ruggaber et al., 2006, Longoria et al 2008) (Table 3).

These enzymes are typically extra-cellular thus allowing their isolation and purification to be achieved more easily than intracellular enzymes. For instance, a complex system of extra-cellular non-specific peroxidase to degrade a wide range of nitro-aromatics including TNT was evolved by *Phanerochaete chrysosporium* and other fungi (Esteve-Nunez et al., 2001).

Table 3. Pollutants transformed by oxidoreductases.

Pollutant	Enzymes	Source
Kraft, lignin	LiP, MnP	White-rot fungi
Pyrene Benzo(a)pyrene	LiP Laccase and peroxidase	<i>P. chrysosporium</i> <i>Fusarium solani</i> , <i>F. oxysporum</i>
Anthracene Fluorene	Laccase Mn-peroxidase	<i>T. versicolor</i> <i>P. leavis</i>
4-Hydroxybiphenil Delor 106 PCB	Laccase MnP, LiP, Laccase	<i>P. ostreatus</i> <i>T. versicolor</i>
Bromophenol blue Reactive Orange 16	Laccase Mn-peroxidase	<i>P. sanguineis</i> Various with rot-fungi
TNT	Peroxidases	<i>P. chrysosporium</i>
Tetra and pentachlorinated phenols and anilines	Chloroperoxidase	<i>Caldaromyces fumago</i>

PLENARY PAPERS

Plants also harbor highly versatile enzymatic machineries to attack and detoxify pollutants. Similarities to the mammalian detoxification led to the coining of the term green liver for plant xenobiotic metabolism. Important enzyme classes such as cytochrome P450 monooxygenases, glutathione S-transferases, glycosyltransferases and transporters are involved in both kingdoms. The availability of the first whole plant genome sequence of *Arabidopsis thaliana* revealed an unforeseen complexity of these enzyme classes. Genetic and biochemical diversity, by far exceeding at least single microorganisms, seems to exist in plants. In agreement with previous investigations at the enzymatic level both terrestrial and aquatic plants possess an enormous potential for phytoremediation of soil, water and air if limitations due to insufficient uptake into plants can be overcome.

Although this large array of enzymes with a potential for the remediation of polluted environments, several shortcomings still exist limiting the potentiality of enzymes and leading to the prolonged persistence of pollutants in the environment. Indeed, the main drawbacks in the extensive application of enzymes in the *in situ* restoration of polluted environments lie in the limited knowledge of the biochemistry of the degradation processes, in the short catalytic life of enzymatic proteins in harsh environments such soil, in the need of more than an enzyme acting sequentially or of cofactors and coenzymes acting simultaneously to the enzymes. Another, not minor bottleneck restricting the use of enzymes, is the limitation still existing in methods suitable to improve their performance and to actually identify their involvement in the process.

If reference is made to nitroaromatics, microbial transformation of nitro-aromatics in nature occurs at a relatively slow pace despite the presence of several nitroreductases in most organisms. Secondly, enzymes for complete catabolic breakdown of nitro-aromatics are very rare. Specificity of these enzymes renders them yet slower on analogous substrates. Thirdly, a variety of intermediates render them yet slower.

In addition, if cell-free enzymes are selected as the decontaminating agents, other limitations to their efficiency in the transformation of pollutants may arise:

- ✓ Low catalytic efficiency and low reaction yields
- ✓ Complexity of pollution
- ✓ Low stability and survival in harsh environments
- ✓ Necessity of continuously feeding the polluted environment

Two basic approaches can be addressed to improve the efficiency of an enzyme-catalyzed process of pollutant transformation, and their applicability and effectiveness will depend whether isolated enzymes or whole cells are used.

Traditional approaches are based on the improvement of the performance of the selected enzyme by acting on the conditions under which the enzyme displays its catalytic activity. Advanced approaches will improve the enzyme itself. In other words, the intrinsic and inherent catalytic features of the enzyme will be optimized. Indeed, the increasing information on the structure and function of catabolic enzymes and pathways offers further possibilities for their optimization.

PLENARY PAPERS

Examples of traditional approaches, mainly addressed with isolated enzymes, are: a) the use of additional compounds acting as co-substrates or mediators (Figure 3) (Cabana et al., 2007); b) the use of additives as ameliorant of the enzyme activity; c) the modification of enzymes by appropriate molecules, d) the use of more than one enzyme acting simultaneously or in sequence.

An example of enzyme modification is that of an organophosphorus hydrolase (OPH) by the addition of an N-terminal dodecahistidine tag (His₁₂-OPH). The introduction of the His₁₂-tag caused a 30- and 74-fold increase in catalytic efficiency of the enzyme with parathion and methyl parathion, respectively, compared to OPH.

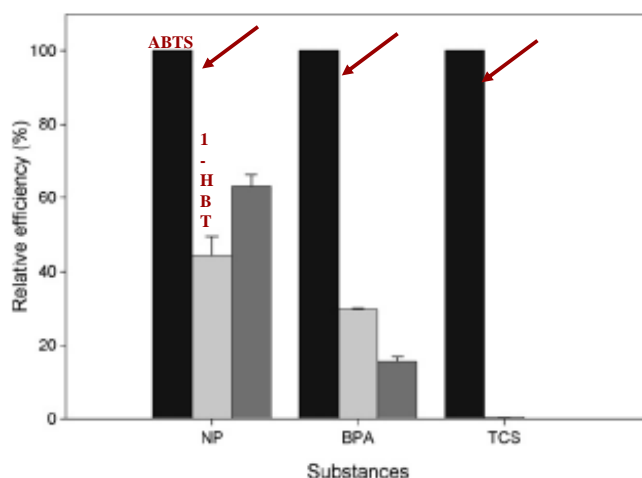


Figure 3. Effect of ABTS and 1-HBT on the removal of NB, BPA and TCS by laccase from *C. polyzona* (after Cabana et al., 2007)

Advanced approaches are based on strategies to take existing catabolic pathway genes and generate optimized enzymes and pathways (Parales and Ditty, 2005). A rational site-directed mutagenesis approach to improving enzyme function is possible if a detailed characterization of a given enzyme and at best the crystal structure is available. Alternatively, sequence alignments can help in identifying residues critical for enzyme activity (usually highly conserved) or substrate specificity (usually visible as differences in the sequences), and a rational design can lead to improved biocatalysts. Besides such site-directed approaches, various DNA-shuffling methods (i.e. the random fragmentation of a population of mutant genes of a certain family followed by random reassembly) have been developed, which allow the creation of a vast range of chimeric proteins and protein variants. Examples of catabolic enzymes with new or improved activities are reported in Table 4. Such techniques can be also used to generate biosensors for the identification and quantification of environmentally relevant chemicals.

PLENARY PAPERS

Table 4. Catabolic enzymes with new or improved activities (after Parales and Ditty, 2005)

Enzyme/source	Mutagenesis method	New enzyme/ Δ aa ^a	Improved activity/new information ^b
BPDO/ <i>Burkholderia xenovorans</i> LB400	Random mutagenesis of region III	T335A/F336M	2,2'-Dichlorobiphenyl is converted to 3,4-dihydro-3,4-dihydroxy-2,2'-dichlorobiphenyl; interactions between chlorine substitutions and active-site amino acids influence substrate orientation
DNTDO/ <i>B. cepacia</i> R34	Random mutagenesis of V350 and expression in <i>E. coli</i> TG1	V350F	Increased activity with <i>o</i> -nitrophenol (47-fold), <i>m</i> -nitrophenol (34-fold) and <i>o</i> -methoxyphenol (174-fold) <i>o</i> -nitrophenol → nitrohydroquinone and 3-nitrocatechol <i>m</i> -nitrophenol → 4-nitrocatechol <i>o</i> - and <i>m</i> -methoxyphenol → methoxyhydroquinone <i>o</i> -cresol → methylhydroquinone and 2-hydroxybenzyl alcohol <i>m</i> -cresol → methylhydroxyquinone Enhanced activity toward naphthalene (10-fold)
		V350M	Increased activity with <i>o</i> - and <i>m</i> -nitrophenol (20- and 162-fold, respectively) <i>o</i> -Nitrophenol → nitrohydroquinone and 3-nitrocatechol <i>o</i> -Methoxyphenol → methoxyhydroquinone <i>o</i> -Cresol → 2-hydroxybenzyl alcohol Enhanced activity toward naphthalene (10-fold)
TecA/ <i>Ralstonia</i> sp. PS12	Site-directed mutagenesis based on naphthalene dioxygenase structure	F366L	Controlled regioselectivity; greatly reduced oxidation of chlorotoluenes
		L272F or L272W	Increased product formation rate with dichlorotoluenes; changed oxidation of trichlorotoluenes
		All mutants	2,4,5-Trichlorotoluene → 1,2-dihydro-1,2-dihydroxy-1-methyl-3,4,6-trichlorohexa-3,5-diene, 3,4-dichloro-6-methylcatechol, 3,6-dichloro-4-methylcatechol, 4,6-dichloro-3-methylcatechol
TOM/ <i>B. cepacia</i> G4	DNA shuffling	TOM-Green/V106A	Increased degradation rate of trichloroethylene, 1,1-dichloroethylene, <i>trans</i> -1,2-dichloroethylene; increased 1-naphthol synthesis from naphthalene (sixfold); expressed in <i>E. coli</i> , oxidized phenanthrene, fluorene and anthracene faster than wild-type enzyme

Another aspect in which enzymes may play an important role is the monitoring of the site under restoration. Mandatory needs to evaluate the effectiveness of the enzymatic transformation of recalcitrant compounds in a contaminated site are: to identify and quantify the product or the products of the reactions, to evaluate their toxicity, to evaluate the quality of the contaminated site upon the enzymatic treatment

Moreover, to claim that a given enzyme or group of enzymes have been involved in the remediation of a polluted site, it is necessary to relate protein presence to the biochemical transformation of the pollutant and to identify the source of organism for specific enzymes.

Several chemical methods are available to evaluate the concentration of a pollutant and its depletion in a contaminated site. They include gas chromatography (GC), GC/mass spectrometry (MS), GC/flame ionization detector (FID), infrared (IR), fluorescence spectroscopy, luminescence techniques, and the method known as SIP that relies on the changes in stable isotope composition of the molecule of interest. Advanced strategies based on a set of different genomic and proteomic approaches (Figure 4) are now available for monitoring the site under bioremediation.

As reported above, one of the main questions remaining after a bioremediation process is to assess that the toxicity at the contaminated site has been lowered and the site has regained its initial biological activity and productivity. Therefore, toxicity tests and biological activity measurements may be used as potential monitoring tools or bioindicators during and after bioremediation of contaminated sites.

PLENARY PAPERS

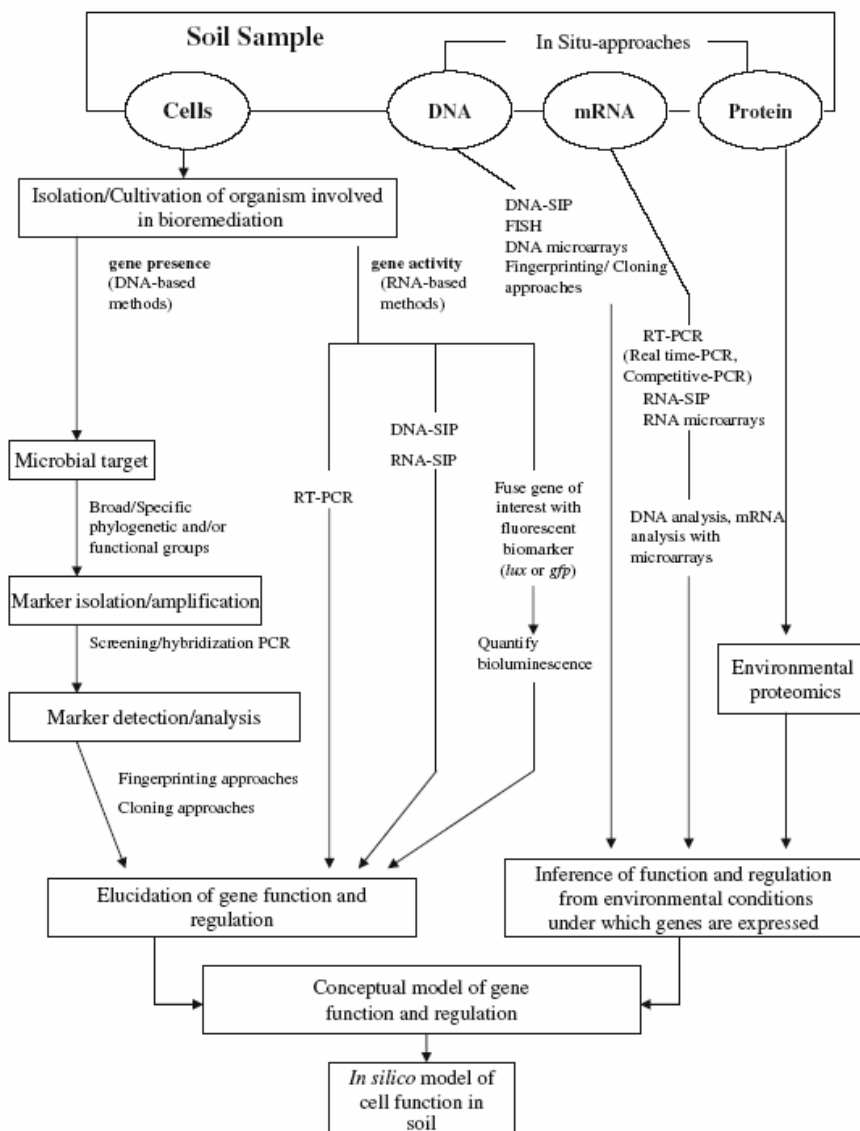


Figure 4. Different genomic approaches available to examine microbial communities of polluted soils (after Andreoni and Gianfreda 2007)

In spite of the possible drawbacks previously cited, in the restoration of polluted environments biological processes have more merits than physico-chemical processes as (i) no harmful intermediates are generated, (ii) complete destruction of the xenobiotic to C1 is feasible, (iii) in situ bioremediation of several xenobiotics is feasible, (iv) the process is eco-friendly and economically sustainable, and (v) it enjoys wide public acceptance (Kulkarni, and Chaudhari, 2007). As claimed by Reiner and Pieper (2000) "It is evident that we are just beginning to understand and, thus, to fully exploit the natural diversity for biodegradation and

PLENARY PAPERS

bioremediation purposes. New genes, enzymes and metabolic routes involved in bacterial xenobiotic degradation have been discovered, and new methods have been developed, which allow the discovery of the broad flexibility of microorganisms.

Besides application of the natural diversity, the artificial evolution of enzymes and pathways will lead, without doubt, to improved biocatalysts and high-throughput methods of screening for the desired phenotypes are becoming available. Strategies to design superior biocatalysts are taking into account more and more the necessity of such organisms to perform in a reliable fashion under environmental conditions. Thus, studies to understand the interaction between xenobiotics and organisms and on the fate, survival and activities of microorganisms in the environment have to intersect with the biochemical and genetic engineering studies. Such a cross feeding will provide the ground for successful interventions into environmental processes and, thereby, lead to optimized strategies for bioremediation.

Keywords: Xenobiotics; enzymes; soil bioremediation.

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Soil Physicochemical and Biological Interfacial Interactions in the Rhizosphere: Impacts on Food Security and Ecosystem Integrity

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Introduction

The soil root interface (the rhizosphere) plays a vital role in sustaining life in the terrestrial ecosystem in the Earth's Critical Zone (CZ). The CZ is defined as the volume extending from the upper limit of vegetation down to the lower limit of groundwater (Anderson et al., 2004).

The CZ is the system of coupled chemical, biological, physical, and geological processes operating together to support life at the Earth's surface (Brantley et al., 2007). While our understanding of this zone has increased over the last hundred years, further advance requires scientists to cross disciplines and scales to integrate understanding of processes in the CZ, ranging in scale from the mineral-organic matter-organism-water-air interfaces at a molecular level to the globe. Soil is the central organizer in the CZ. The rhizosphere is a hot spot of activity within soils.

The rhizosphere is the 'bottle neck' of the supply of vital elements to sustain ecosystem productivity and integrity and food security. The rhizosphere is also the 'bottle neck' of the contamination of the terrestrial food chain by inorganic and organic pollutants to endanger human and animal health.

Physical, Chemical and Biological Interactions at the Soil-Root-Biota Interfaces

Solid-liquid interface character should vary substantially with lithology, climate, vegetation, below-ground biota, landscape position, time, and anthropogenic activities. The rhizosphere should greatly influence solid-liquid interface character and, thus, the nature and properties of their reactive surfaces. In the rhizosphere, the kinds and concentrations of substrates are different from those in the bulk soil because of root exudation. This leads to colonization by different populations of bacteria, fungi, protozoa, and nematodes. The plant-microbe interactions result in intense biological processes in the rhizosphere. These interactions, in turn, affect physicochemical reactions in the rhizosphere. Physicochemical properties that can be different in the rhizosphere include: acidity, concentration of complexing biomolecules, redox potential, ionic strength, nutrient status, enzyme activity, bulk density and porosity, and moisture.

These differences in soil physicochemical properties would, in turn, influence biological processes in the rhizosphere (Huang and Germida, 2002; Huang, 2008). The total rhizosphere environment is governed by an interactive trinity of the soil, the plant, and the organics associated with the roots. The reactions and processes in the rhizosphere can only be understood satisfactorily with interdisciplinary approaches. The impacts of physicochemical and biological interfacial interactions on food security and ecosystem integrity, thus, merit serious attention.

PLENARY PAPERS

Rhizosphere Interfacial Interactions and Food Security

The rhizosphere chemistry, biology, and physics and their interactions at the molecular level are of fundamental and practical importance in sustaining food security. The rhizosphere is the “bottle neck” of the supply of nutrients and the ecotoxicological effect of inorganic and organic contaminants to plants. Therefore, the impacts of the rhizosphere on plant health and crop production is a critical issue in feeding the world population. 30% of farmers in developing countries are food-insecure. Some of the most profound and direct impacts of climate change over the next few decades will be on agricultural and food systems (Brown and Funk, 2008).

Therefore, food insecurity is likely to increase under climate change, unless early warning systems and development programs are used more effectively. Managing world soils for food security and environmental quality (Lal, 2001) is an extremely important mission of soil scientists. Transform agricultural systems through improved seed, cropping system, fertilizer, land use, and governance, and food security may be attained by all (Brown and Funk, 2008; Lobell et al., 2008). In this context, rhizosphere management deserves close attention in sustaining and enhancing soil productivity and crop production.

Impacts of Rhizosphere Interfacial Interactions and Ecosystem Integrity*- Carbon Transformation, Storage, Emission, and Climate Change*

The CO₂ emission from the soil to the atmosphere is the primary mechanism of soil C loss. Agricultural practices contribute about 25% of total anthropogenic CO₂ emission. Soil minerals control carbon storage and turnover (Torn et al., 1997; Davidson and Janssens, 2006). Respiration by plant roots contribute about half of CO₂ emitted from the soil. The rhizosphere significantly controls SOM decomposition (Cheng and Kuzyakov, 2005). Given abundant mineral nutrient supply, soil microbes prefer labile root-derived C to SOM-derived C, resulting in a decreased SOM decomposition in the rhizosphere. If mineral nutrients are in short supply, soil microbes prefer nutrient-rich SOM to root-derived C, resulting in increased SOM decomposition in the rhizosphere. Root effects on the rate of SOM decomposition can range from negative 70% to as high as 33% above the unplanted control.

- Formation and Transformation of Nanoparticles and Ecosystem Health

Nanoparticles are discrete nanometer (10⁻⁹ m) scale assemblies of atoms. A significant fraction of atoms are exposed on surfaces rather than contained in the particle interior of nanoparticles (ca. 1-100 nm). The biogeochemical and ecological impacts of nanomaterials are some of the fastest growing areas of research today, with not only vital scientific but also large environmental, economic, and political consequences (Wigginton et al., 2007). Most biominerals generated by microorganisms are nanominerals. Therefore, microbial processes play an important role in nanoparticle formation and fate. Little is known on nanoparticles in the rhizosphere. Formation, transformation, and fate of nanoparticles in the rhizosphere and the impacts on food security and safety and ecosystem health merit increasing attention.

- Transformation of Metals, Metalloids, and Xenobiotics and Food Safety

The interactions at physical-chemical-biological interfaces govern the kinetics and mechanisms of transformation, speciation, transport, bioavailability, toxicity, and fate of metals and metalloids in soil and related environments (Huang, 2008). Fundamental

PLENARY PAPERS

understanding of soil physical, chemical, and biological interfacial interactions at the atomic and molecular levels is essential to understanding the behavior of metals and metalloids in the pedosphere and restoring terrestrial ecosystem health on the global scale. We are still at the eve of fully understanding the processes controlling the mobility and bioavailability of metals and metalloids at the soil-root interface (Hinsinger and Courchesne, 2008). Unraveling the biogeochemistry of trace elements in the rhizosphere and the impact on food safety and ecosystem health is both a challenge and an opportunity for soil and environmental scientists for years to come. The multiple interactions between microorganisms and soil which take place at the soil-root interface can have a dramatic impact on anthropogenic organic pollutants in the terrestrial environment (Anderson et al., 2002). The characteristics of the rhizosphere allow for a unique environment which encourages biotic and abiotic interactions in greater magnitude and diversity than in bulk nonvegetated soil. These interactions generally resulted in the decrease in the toxicity of organic contaminants through increased degradation and decreased availability of these contaminants. As the importance of bioavailability has increased in regulatory decision making, a greater understanding of the plant, soil, and managing factors affecting the bioavailability of contaminants is warranted.

- Ecotoxicological Problems

Ecotoxicology is defined as “the study of fate and effect of toxic agents in ecosystem”. Ecotoxicology research deals with the interactions among organisms, toxic agents (in this case metals and metalloids), and the environment. Long-term ecological effects of metals and metalloids introduced to soils remains to be investigated, as only a few such experiments exist. Even less is known about the adverse long-term effects of metals and metalloids on soil microorganisms (McGrath et al., 1995; Huang, 2008.)

- Biodiversity

The functioning and stability of the terrestrial ecosystem are determined by plant biodiversity and species composition (Tilman et al., 1996). Above and below ground communities can be powerful mutual drivers, with both positive and negative feedback (Wardle et al., 2004). Above- and belowground components are closely interlinked at the community level, reinforced by a greater degree of specificity between plants and soil organisms. The impact of soil physicochemical and biological interfacial interactions in the rhizosphere on belowground biodiversity remains to be uncovered.

- Geomedical Problem and Human Health

Geomedicine is the science dealing with the environmental factors that influence the geographical distribution of pathological and nutritional problems relating to human and animal health (Låg, 1980). Knowledge of soil science is indispensable for solution to many geomedical problems (Låg, 1994). The effects of soil physical, chemical, and biological interfacial interactions in the rhizosphere on quality of vegetation and the food and feed produced and related geomedical problems warrant in-depth research (Huang, 2008). The transformation and bioavailability of trace elements are profoundly influenced by soil physical, chemical, and biological interfacial interactions in the rhizosphere (Huang and Gobran, 2005). Many trace elements are of concern to animal nutrition and human health. These include Se, Fe, I, Zn, Cu, Mn, Mo, Cr, F, Co, Si, V, Ni, As, and Mg. It is essential to promote research on the relationship between soil physicochemical-biological interfacial interactions, especially in the rhizosphere and the impacts on the transformation, transport,

PLENARY PAPERS

and toxicity, and fate of trace elements in the terrestrial environment. This would facilitate fundamental understanding of the linkage of trace elements in soils with plant-animal-human-environmental systems and related geomedical problems. This is essential to provide practical solutions to their deficiency and toxicity problems.

Significance of Rhizospheric Interfacial Interactions in Risk Assessment and Restoration of Ecosystem Integrity

Regulatory-driven risk assessment and management are essential in restoration of ecosystem integrity. Assessing exposure to contaminants in soil environments includes determination of the pathways to human exposure. The cleanup of soils polluted by hazardous contaminants has become a matter of urgent concern. Physical, chemical, and biological interfacial interactions in the rhizosphere play a significant role in the natural remediation and restoration of the terrestrial ecosystem. Bioremediation, phytoremediation, and chemical remediation have been commonly used in land management practices.

Combined biotic and abiotic remediation would enhance remediation efficiency. The impact of physical, chemical, and biological interfacial interactions in the rhizosphere on risk assessment and management of contaminants and restoration of ecosystem integrity merits increasing attention.

Conclusions and Future Prospects

Soil is the most diverse ecosystem and most important terrestrial resource in sustaining life in the Earth's CZ. Soil physical, chemical, and biological interfacial interactions in the rhizosphere play a vital role in carbon cycling and climate change, the formation and transformation of environmental nanoparticles, the fate of nutrients, metals, metalloids, and xenobiotics, ecotoxicological problems, biodiversity, and geomedicine. Fundamental understanding of soil physical, chemical, and biological interfacial interactions in the rhizosphere at the molecular level is essential for developing innovative strategies for land resource management to sustain food security and ecosystem integrity. Future research on this extremely challenging and important area of science should be stimulated to sustain and enhance ecosystem productivity, services, and integrity in the Earth's CZ and the human welfare.

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Keywords: Rhizosphere; climate change; nanoparticle.

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KEYNOTE PAPERS

K-1**Interactions of Soil Components and Microorganisms and their Effects on Soil Remediation****J.-M. Bollag****Penn State Institutes of Energy and the Environment (PSIEE), The Pennsylvania State University, University Park, PA 16802, USA.. *E-mail: jmbollag@psu.edu*

Minerals, organic matter and microorganisms are intimately associated in soils and closely interact in environmental processes. These interactions are especially important in the soil rhizosphere and the sediment-water interface, where low-molecular weight biochemicals are abundant and microbial activity is intense.

Current research is driven by the widespread contamination of our soils with organic pollutants and the desire to apply the knowledge acquired during earlier basic research to practical applications in the field (Atlas and Philp, 2005). These explorations not only increase our understanding of the interactions between microorganisms and other soil components, but also assist us in establishing and applying methods for waste remediation and the cleanup of contaminated soils. As the importance of bioavailability has been recognized and is being considered in regulatory decision-making, a greater understanding of the factors affecting the bioavailability of contaminants to plants and organisms is necessary. Increased knowledge will provide more realistic information about how to take bioavailability and toxicity into consideration for risk assessment and site remediation.

Human activities and industrialization development generate by-products and waste that must be disposed of in a way that should not affect the environment. Even when such by-products are used on agricultural lands as a resource, e.g. organic waste that may contribute to maintain or increase the organic matter and nutrient content in the soil, there are growing concerns about the fate of undesirable constituents they may contain.

Soil Pollution

The soil has become increasingly subjected to various chemical stresses, not only because of our need for more food and fiber, but also because of ever-increasing industrialization. Various anthropogenic substances, either organic or inorganic in nature, upon entering the soil, may not only adversely affect its productivity potential, but may also compromise the quality of the food chain and groundwater. This situation may require risk assessment and evaluation of remedial techniques in order to restore the quality of the soil so that safe food products and clean groundwater and air may be obtained once again.

A wide variety of naturally occurring toxic and recalcitrant organic compounds exist on earth. In addition, various man-made materials have been dumped on land adjacent to industrial plants in landfills and on unregulated dumping grounds. As a result, the soils at many of these sites contain a complex mixture of contaminants, such as petroleum products, organic solvents, metals, acids, bases, brine, and radionuclides. Over a very long period of time, natural degradation activities may eventually destroy most of these organic contaminants. However, affordable technologies are needed to speed up the natural remediation processes. Furthermore, natural degradation activities would not solve the

KEYNOTE PAPERS

problems of metal contaminants. Therefore, risk management through remediation is essential to reducing health risks and restoring natural balances. The treatments currently used to remove or destroy contaminants include physical, chemical and biological technologies.

Cleanup of the environment

The cleanup of soils polluted by hazardous man-made materials has become a matter of urgent public concern. Traditional methods of waste handling, such as landfilling or incineration, often exchange one problem with another. For instance, landfilling merely confines the pollution while doing little to remove it; certain kinds of garbage are known to remain intact for decades in a landfill. Incineration removes wastes, but disposal of ash or residues remains to be dealt with, and new concerns about air pollution are created. As a result, society has turned to new technologies to devise better methods of disposal; one such method is bioremediation (Atlas and Philp, 2005).

Microorganisms are ultimately responsible for degrading most organic matter to carbon dioxide, minerals and water. Bioremediation utilizes the natural potential of microorganisms to cause transformation, mineralization or complexation by directing those capabilities toward environmental pollutants.

The enhancement of microbial degradation as a means of bringing about the in situ clean up of contaminated soils has spurred much research. The most common methods to stimulate degradation rates include supplying inorganic nutrients and oxygen, but the addition of degradative microbial inocula or enzymes as well as the use of plants (phytoremediation) should also be considered (Dec and Bollag, 2001). Basic research is needed to better understand the biological, chemical, and physical factors affecting transformation pathways and reaction rates.

Over a very long period of time, natural degradation might remove many of the organic contaminants, but may possibly result in an accumulation of heavy metals. Affordable novel technologies are needed to enhance natural remediation processes (natural attenuation) and reduce health risks by restoring natural balances. Therefore, ecosystem restoration can best be achieved through development of innovative management strategies involving interactive processes.

Research into the interactions between microorganisms, humic materials and minerals in the soil continues to impact the fields of environmental remediation and regulation through the development of new techniques and an expanding base of knowledge about the relationships between microorganisms and soil particles. Mixed biotic/abiotic mechanisms by which xenobiotic chemicals are transformed and degraded in soil environments will be an important area of future investigations.

Soil is undoubtedly the most complex of all microbial habitats. Largely because of this complexity, there is insufficient information on how and where most microbial activity occurs in situ and which microorganisms are the most important participants. There is considerable evidence that certain particulate soil components, especially some types of clay minerals, significantly affect microbial life (Stotzky, 1986; Huang, 1990). Clay minerals appear to exert their primary influence by modifying the physicochemical characteristics of microbial habitats; this either enhances or attenuates the growth and metabolism of individual

KEYNOTE PAPERS

microbial populations, which, in turn, influence the growth and activity of other populations. In contrast to these indirect effects of clay minerals, relatively little is known about the mechanisms of direct surface interactions (e.g., adhesion) between clays and microorganisms.

Transformation of Organic Pollutants

The transformation of xenobiotics in a multicomponent system, such as soil, is a result of the combined activity of microorganisms, extracellular enzymes, mineral colloids and humic materials (Huang and Bollag, 1998). Each of these important components of the soil system not only participates in the transformation of xenobiotics but also modifies the activity of the other components. Humic substances associated with mineral colloids add an additional aspect to the process of xenobiotic biodegradation in soil environments. Humic substances are strong adsorbents themselves, and the adsorption of microorganisms, enzymes and xenobiotics on the organic fraction may be difficult to distinguish from that occurring on the mineral surfaces. Humic acids are believed to catalyze certain transformation reactions, but their major impact is their ability to adsorb e.g. microorganisms, enzymes and chemicals or to be adsorbed e.g. on soil minerals. Minerals exhibit mixed functions as well. They transform naturally occurring and xenobiotic substrates abiotically; at the same time, they act as sorbents, thus altering the impact of microorganisms, enzymes, and chemicals. Adsorption and other binding interactions that occur on both mineral and humic surfaces are believed to reduce the bioavailability of xenobiotics.

Fate and Transformation of Metals

The transformation of metals is governed by abiotic and biotic processes in soil and related environments (Huang, 2000; Huang and Germida, 2002). Abiotic processes include solution complexation, adsorption – desorption, precipitation – dissolution, redox reactions, and catalysis.

Redox reactions are important in controlling the chemical speciation and toxicity of a number of contaminant metals, notably As, Se, Cr, Pu, Co, Pb, Ni, and Cu (Sparks, 2002). Redox reactions are also important in controlling the transformation and reactivity of Mn and Fe oxides in soils, which have enormous capacities to adsorb metal pollutants and are the major sinks of these pollutants. Furthermore, reduction of sulfate and sulfide in aerobic environments may also affect metal speciation and solubility. In addition microbial activity has a very important role in influencing the dynamics of metals in the terrestrial ecosystem. Microbes can dissolve minerals by direct or indirect action under aerobic and anaerobic conditions (Kurek, 2002). Indirect dissolution of minerals can be the result of microbial activity connected with the productions of organic and inorganic acids. Volatilization of metals and metalloids or biomethylation of metals and metalloids from the soil into the atmosphere can be a mechanism of detoxification of toxic elements such as Hg, As, and Se.

KEYNOTE PAPERS**Biotic Processes**

Nature has evolved ways of mineralizing many contaminants or xenobiotics without human intervention. Soil organic matter is recycled by a diverse array of soil organisms including bacteria, fungi, actinomycetes, protozoa, earthworms and insects. Microorganisms are usually responsible for mineralizing most organic matter to carbon dioxide, water and inorganic components. However, soil mineral colloids also play a significant role in abiotic transformation of organic compounds through catalysis (Huang, 2000).

Microorganisms are the primary factors that influence the fate of organic xenobiotics in soil. Environmentally, the most desired result of microbial activity is the complete degradation (mineralization) of toxic chemicals of both natural and synthetic origin. Some anthropogenic compounds may be used by microorganisms as a source of energy and nutrients for growth. Another important process of microbial transformation, however, is cometabolism, in which microorganisms transform the xenobiotic molecules, but are unable to use these molecules as a source of carbon or energy. The cause of microbial transformation is the activity of intracellular and extracellular enzymes produced by microorganisms. Xenobiotics having chemical structures similar to organic compounds that occur in nature are usually more susceptible to biodegradation than those whose structures bear little resemblance to natural products. This is because the microbial enzymes that specifically degrade toxic chemicals of natural origin may also be able to degrade structurally analogous xenobiotics. On the other hand, when microorganisms are exposed to structurally different xenobiotics, they usually need a prolonged acclimation period during which they may undergo genetic mutations, resulting in the production of novel degrading enzymes (van der Meer et al., 1992).

Soils contaminated with recalcitrant xenobiotics or environments hostile to indigenous microorganisms may benefit from microbial inoculation. Using classic enrichment techniques or gene transfer technology, large populations of bacteria may be developed, and then inoculated into the contaminated soil.

Our present knowledge of the microbial degradation of xenobiotics has been achieved through numerous *in vitro* experiments in which xenobiotic compounds were exposed to specific microorganisms isolated from soil or other natural environments. Monitoring the transforming or degradative activities of microorganisms under field conditions may, however, generate ambiguous results, because it is difficult to distinguish between physico-chemical and microbial transformation reactions. The ability of microorganisms to transform xenobiotics relies largely on four major processes: oxidation, reduction, hydrolysis, and synthetic reactions, which are all catalyzed by microbial enzymes.

Also of great importance is the fact that the microbial activity in soil is strongly influenced by mineral colloids and humates that bind organic chemicals, inorganic ions and water films to their surfaces (Huang 1990). In addition, extracellular enzymes are rapidly sorbed at clay and organic surfaces. Immobilized through these events, enzymes usually acquire greater stability but may modify their activity. Some immobilized enzymes in soils are associated with the humic fraction by the formation of enzyme-phenolic copolymers during the generation of humic substances. Enzymes are vital biotic catalysts in the transformation of organic pollutants (Bollag, 1992).

KEYNOTE PAPERS

Much research has been conducted on the use of bioremediation as a tool for cleaning many environmental contaminants such as polycyclic aromatic hydrocarbons, explosives, pesticides polychlorinated biphenyls and other organic toxicants (Atlas and Philp, 2005). The application of bioremediation technology to decontaminate polluted sites is still a developing science. The mechanisms driving microbial activity and the degradation pathways of specific pollutants need to be further elucidated before successful and better-controlled site-specific treatments can be applied. Recent advances in biotechnology have enabled the modification of organisms at the molecular level for improved degradative performance. This approach has already contributed new tools for the analysis and monitoring of complex environmental processes.

Abiotic Processes

Abiotic factors, such as the catalytic activity of mineral and organic colloids, may make a substantial contribution to the degradation of organic compounds in soil. To date, however, the reports of abiotic transformation have been limited to include only a relatively narrow group of xenobiotics. The significance of soil mineral-catalyzed abiotic transformation of naturally occurring and xenobiotic compounds in the environment has become widely recognized only in recent years (Huang, 1990). Clay minerals, with their high concentration in soils, their large surface area and relatively high charge density, contribute to the overall xenobiotic transformation at least as much as does the organic matter. Even in soil environments where biological activity is intense, abiotic transformation is of importance.

Keywords: Organic pollutants; biotic and abiotic processes; soil remediation.

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KEYNOTE PAPERS

K-2

Use of Nanoparticles in Soil-Water Bioremediation Processes

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Introduction

As a general definition, nanotechnology is involved with objects on the nano scale, or materials measuring between 1 and 100 nm. It was found a good definition that is practical and unconstrained by any arbitrary size limitations: The design, characterization, production, and application of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale that produces structures, devices, and systems with at least one novel/superior characteristic or property (Bawa et al., 2005). Nanotechnology has contributed to the development of a great diversity of materials as those used in electronic, optoelectronic, biomedical, pharmaceutical, cosmetic, energy, catalytic, and materials applications. In the manufacturing community, the most profitable trail for nanoscale particles and materials have been in the areas of sunscreen, magnetic recording tape, automotive catalyst supports, biolabeling, chemical-mechanical polishing, electroconductive coatings, and optical fibers. However, the emergence of nanotechnology presents a number of potential environmental benefits. This potential impact area could be divided into three categories: treatment and remediation, sensing and detection, and pollution prevention. Some nanoparticles destroy contaminants, for instance, while others sequester them. The specific nanotechnologies that it will be discussed hereafter focus on site remediation and waste water treatment. Besides the applications for soil, groundwater, and wastewater, a number of nanotechnologies for air remediation are also in development. Carbon nanotubes, for example, have been recognized for their ability to adsorb dioxin much more strongly than traditional activated carbon. Smaller particle size enables the development of smaller sensors, which can be deployed more easily into remote locations. The ability of nanotechnology to abate pollution production is in progress and could potentially catalyze the most revolutionary changes in the environmental field (Watlington, 2005).

Some Uses of Nanotechnology

Nano iron and its derivatives

Remediation has grown and evolved, continually developing and adopting new technologies and improving the remediation process. One of the most established systems is that termed “pump-and-treat”. Pump-and-treat systems operate on the basis of removing contaminated groundwater from the ground, downstream of the contamination site, and then treating it before returning it to the ground. This technology it takes a long time to achieve cleanup goals and it is expensive to operate and maintain.

Reducing capabilities of metallic substances, such as zero-valent iron (ZVI), were examined for their ability to treat a wide range of contaminants in hazardous wastewater. The most common use of ZVI has been in the form of permeable reactive barriers (PRBs)

KEYNOTE PAPERS

designed to intercept plumes in the subsurface and subsequently remediate them. However, new technologies are now available to compete with PRBs. Nanoscale iron particles and their derivatives offer more alternatives to many remediation technologies. The small particle size of the nano iron (1-100 nm) facilitates a high level of remedial versatility. This allows a much greater diversity in applications as compared to the traditional ZVI employed in PRBs. Nanoscale Zero Valent Iron (nZVI) and Reactive Nanoscale Iron Product (RNIP) are the most basic form of the nano iron technology (Watlington, 2005). Table 1 lists many of the pollutants potentially remediated by nano iron (Zhang, 2003).

Table 1. Pollutants remediated by nano iron technology

Carbon tetrachloride	Chrysoidine	<i>cis</i> -Dichloroethene
Chloroform	Tropaeolin	<i>trans</i> -Dichloroethene
Dichloromethane	Acid Orange	1,1-Dichloroethene
Chloromethane	Acid Red	Vinyl Chloride
Hexachlorobenzene	Mercury	PCBs
Pentachlorobenzene	Nickel	Dioxins
Tetrachlorobenzenes	Silver	Pentachlorophenol
Trichlorobenzenes	Cadmium	NDMA
Dichlorobenzenes	Bromoform	TNT
Chlorobenzene	Dibromochloromethane	Dichromate
DDT	Dichlorobromomethane	Arsenic
Lindane	Tetrachloroethene	Perchlorate
Orange II	Trichloroethene	Nitrate

Dendrimers

Dendritic polymers, which include random hyperbranched polymers, dendrigraft polymers, dendrons and dendrimers, are relatively monodispersed and highly branched macromolecules with controlled composition and architecture consisting of three components: a core, interior branch cells and terminal branch cell (Savage and Diallo, 2005).

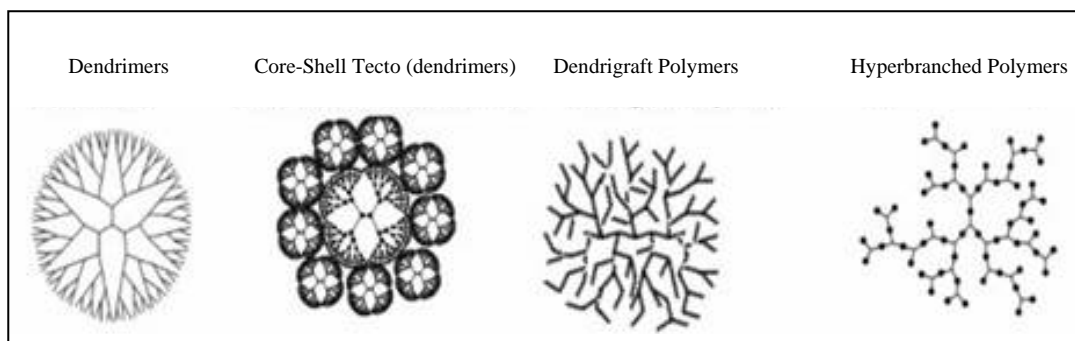


Figure 1. Dendritic polymers

Diallo's group proposed process consisting of a clean water recovery unit and a dendrimer recovery unit can be seen below in Figure 1. Diallo et al. (2005) are currently working on the engineering development of this process with hopes of having pilot-scale demonstration in

KEYNOTE PAPERS

the next years (Figure 2). The development of a method that permits removal of organic pollutants by employing a simple filtration step has been described by Arkas et al. (2006). This was pursued by utilizing titanium dioxide (TiO_2) porous ceramic filters of which the pores were impregnated with an alkylated poly(propylene imine) dendrimer, poly(ethylene imine) hyperbranched polymer or β -cyclodextrin, thus resulting in hybrid organic/inorganic filter modules of high mechanical strength and high surface area (Diallo, 2005).

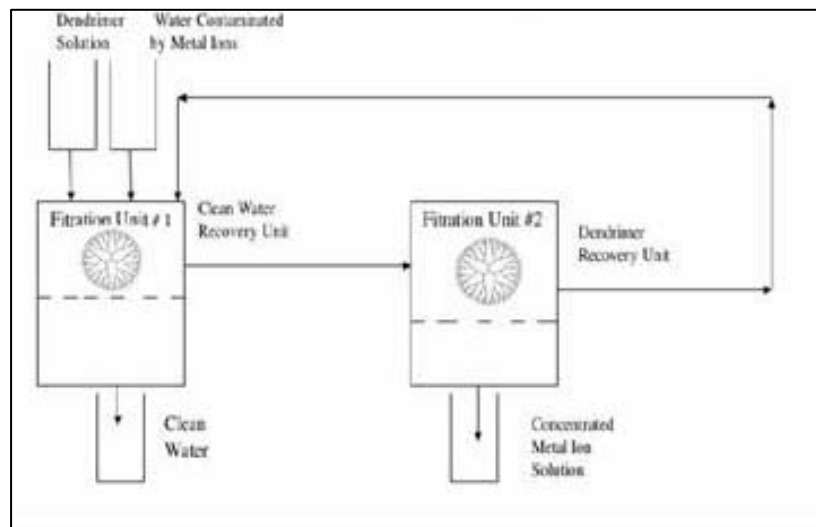


Figure 2. Recovery of metal ions from aqueous solutions by dendrimer-enhanced filtration

Nanoparticles (nanocrystals, carbon nanotubes, etc.)

A variety of nanomaterials are in various stages of research and development, each possessing unique functionalities that is potentially applicable to the remediation of industrial effluents, groundwater, surface water and drinking water (Table 2).

Single-Enzyme Nanoparticles (SENs)

Due to their specificity and targeted effectiveness, enzymes may offer vast capabilities in the areas of chemical conversions, biosensing, and bioremediation (Durán and Esposito 2000). However, the lack of stability and relatively short catalytic lifetimes of enzymes may limit their usefulness as cost-effective alternatives to synthetic catalysts. Although methods such as enzyme immobilization, enzyme modification, and genetic modification have been used frequently to improve the stability and subsequent persistence of enzymes, nanotechnology has provided a new method whereby enzymes can be stabilized in the form of single-enzyme nanoparticles (SENs). The first SENs were assembled by Kim and Grate (2005), using chymotrypsin as a model enzyme. The synthesis of SENs involves enzyme surface modification, vinyl polymer growth from the enzyme surface (Watlington, 2005; Theron et al., 2008). Immobilized enzymes in biopolymers and carbon nanotubes are another strategy for environmental nanobiosensors (Pereira et al., 2008).

KEYNOTE PAPERS

Table 2. Examples of nanoparticles and nanomaterials for use in water remediation (Theron et al., 2008)

Nanoparticle/Nanomaterial	Pollutant
Nanocrystalline zeolites	Toluene, nitrogen dioxide
Carbonaceous nanomaterials	
Activated carbon fibres (ACFs)	Benzene, toluene, xylene, ethylbenzene
CeO ₂ -carbon nanotubes (CNTs)	Heavy metal ions
CNTs functionalized with polymers	<i>p</i> -nitrophenol Benzene, toluene,
CNT functionalized with Fe	dimethylbenzene Heavy metal ions
Single-walled carbon nanotubes	Trihalomethanes (THMs)
Multi-walled carbon nanotubes	Heavy metal ions
	THMs Chlorophenols
	Herbicides
	Microcystin toxins
Self-assembled monolayer on mesoporous supports (SAMMS)	Inorganic ions
Anion-SAMMS	Heavy metal ions
Thiol-SAMMS	Actinides and lanthanides
Self-assembled monolayer on mesoporous supports (SAMMS)	Inorganic ions
Anion-SAMMS	Heavy metal ions
Thiol-SAMMS	Actinides and lanthanides
HOPO-SAMMS	
Biopolymers	Heavy metal ions
Single-enzyme nanoparticles (SENs)	*NT
Zero-valent iron nanoparticles (nZVI)	Polychlorinated biphenyls (PCBs)
	Inorganic ions Chlorinated organic compounds Heavy metal ions
Bimetallic nanoparticles Pd/Fe nanoparticles	PCBs
	Chlorinated ethene
	Chlorinated methanes
Ni/Fe nanoparticles Pd/Au nanoparticles	TCE and PCBs
	Dichlorophenol
	Trichlorobenzene
	Chlorinated ethene
	Brominated organic compounds (BOCs)
	TCE
TiO ₂ photocatalysts	
Nanocrystalline TiO ₂	Heavy metal ions
Nitrogen (N)-doped TiO ₂	Azo dyes
Fe(III)-doped TiO ₂	Phenol
Supported TiO ₂ nanoparticles	Aromatic pollutants
TiO ₂ -based p-n junction nanotubes	Toluene

*NT - Not tested.

KEYNOTE PAPERS**Nanotoxicity of Engineered Nanoparticles and Nanomaterials**

The use of engineered nanoparticles and nanomaterials for environment treatment, as discussed partially in the previous sections, has raised concerns for human exposure which dash from the absence of specific technologies orienting the removal of engineered nanomaterials from the water, and the safety of the new nanoparticles and nanomaterials that may be used by the water industry. These concerns are often based on its large mobility, small sizes, thus implying a greater potential for exposure as they are dispersed over greater distances and their persistence in the environment increases. However, few examples of nanomaterials treatment in the industrial chain of nanoparticles productions are published (Durán et al. 2007, 2008; Benn and Westerhoff, 2008). Some authors as Wiesner et al. (2006) suggested that smaller nanoparticles should not be very mobile, since their relatively large diffusivity would enable them to produce more frequent contacts with the surfaces of porous media such as groundwater aquifers or the sand filters used in potable water treatment. Consequently, Wiesner et al. (2006) concluded that there is no evidence to support the scenario of a new nanoparticulate contaminant that current water treatment infrastructure cannot handle. Many other researchers have contrary data, then, showing that this is still an open area for discussion.

Conclusions

It is clear from the literature that while much attention has been focused on the development and potential benefits of nanomaterials in water treatment processes, concerns have also been raised regarding their potential human and environmental toxicity. However, it is difficult to assess the effect of nanomaterials on health and the environment because the methods and tools for such a task have not been well developed yet (NNCO, 2006; Davies, 2007; Morris and Willis, 2007). It is extremely important that these processes be developed and investigated to ensure that nanomaterials are as safe as possible, while reaching their full potential. Once these information gaps were clear, it is certain that new nanomaterials, in water environment treatment, will play key roles in ensuring sufficient and good quality water and soil to meet the ever-increasing demand for potable water and safe soil in the agriculture (Tungittiplakorn et al., 2004; Mauter and Elimelech, 2008; Theron et al., 2008)

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Keywords: Nanoparticles; bioremediation processes; nanotechnology.

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KEYNOTE PAPERS

K-3

Soil-Root-Microbe Interactions in the Rhizosphere - A Key to Understanding and Predicting Nutrient Bioavailability to Plants

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Introduction – Why shall we care about nutrient bioavailability to plants?

As stressed in the Millennium Ecosystem Assessment, over the last 50 years, human beings have modified the ecosystems to an unprecedented point in humankind history, in order to meet the increasing world demand in food, drinking water, wood, fibers and energy (Tilman 1999). Such changes much contributed to improving humankind well-being, but this was achieved at the expense of a degradation of numerous ecosystem services and increasing poverty of the poorest populations. Prediction models forecast further degradation of ecosystem services in the coming 50 years, *a fortiori* if agroecosystem management strategies are unchanged (Tilman et al. 2001 et 2002). In this context, Millennium Development Goals will hardly be achieved, and especially the very first of these: to eradicate hunger worldwide (Priority 1). The scientific challenge is considerable: how to feed the world in a context of limited changes of land use, i.e. a limited increase in productive arable land surface area?

As pointed out by Vance et al. (2003), by 2030, world population shall have increased by about +33 to +67%, while in the mean time, the potential increase in surface area of arable land will reach at most +20%, and that of irrigated land less than +10%. Given the climate changes which operate and the increasing use of arable land for other uses than food production, the challenge in front of us is even bigger (Tilman et al. 2002). The level in agriculture productivity needs to be maintained or further increased, but this has to be achieved in a sustainable manner, both environmentally and economically, in order to preserve ecosystem services. There is a need for an ecological intensification of agroecosystems, in order to cover global food demand while decreasing agricultural inputs such as fertilisers (Cassman 1999).

The Millennium Ecosystem Assessment underlined that the cycles of nutrients, especially nitrogen (N) and phosphorus (P) were among the most affected ecosystem services, leading to a massive and fast-increasing eutrophication of aquatic ecosystems (Mackenzie et al. 2002) and contamination of groundwaters by nitrate. These phenomena are the direct consequence of the considerable increase in agricultural inputs and the steady decrease of their efficiency (Tilman et al. 2002) : from 1965 to 2000, the doubling of the production of world agriculture concurred with a 3.5- and 6.9-fold increase in the amounts of P and N fertilisers applied, respectively (Tilman 1999). Besides the effects of eutrophication and of nitrate in drinking water, the high and fast-increasing cost of energy and thus that of manufactured N fertilisers, as well as the negative impacts of greenhouse gas emissions invalidate the perspective of pursuing the current, steady increase in N fertiliser application. For P, the fast exhaustion of high grade phosphate ores worldwide within about 90 years at the current rate of consumption of P fertilisers (Herring and Fantel, 1993; Runge-Metzger, 1995 ; Stewart et al. 2005) clearly challenges the sustainability of current P fertilizer use in developed countries.

KEYNOTE PAPERS

Increasing the efficiency of nutrients to plants while decreasing nutrient inputs means that better exploration and exploitation of soil resources must be achieved in agroecosystems. For this purpose, one needs to better know what are the intimate processes and factors that govern soil nutrient bioavailability to plants. The aim of this review is to address this issue, with a particular focus on those soil-root-microbe interactions at play in the rhizosphere.

Revising the concept of bioavailability to plants – a rhizosphere perspective

Bioavailability is a widely used concept, although poorly defined by most of its users, in various scientific communities such as agronomy, ecology, ecotoxicology, food and medical sciences. Initially used for nutrients, this term is nowadays largely used for chemicals, e.g. inorganic or organic pollutants in the context of ecotoxicology. As stressed by Harmsen et al. (2005), many of the definitions are rather loose and do not explicitly state how strongly the bioavailability relies on the target organism and time. For instance, Thornton (1999)'s definition stated that is '*bioavailable that fraction of an element present in soil which is available for intake into plant roots or into soil microorganisms and other flora and fauna*', which is parallel to the concept developed in toxicology or medical science, stating that it corresponds to '*that fraction in the soil, dust or diet that is bio-accessible (e.g. soluble and available for uptake in the gastro-intestinal tract of animal, including farm livestock and humans)*'. Although this definition suggests that the bioavailability of a soil nutrient varies with the target organism, it does not provide any indication of the time frame, as one needs when moving from a conceptual definition towards a more operational definition. At this point of view, one may prefer the following definition: *the bioavailability is the amount of a nutrient in the soil that is present in forms that plants (or other organisms) can take up during the time that they are growing*. In other words, and as stressed by the ISO (2008), bioavailability is best defined as a flux or rate of uptake of a nutrient by the plant.

Harmsen et al. (2005) and the working group which led to the ISO (2008) document on bioavailability also stressed that one should make a clear distinction between availability (or environmental availability) and bioavailability (or environmental bioavailability). Since the end of the nineteenth century, agronomists have been attempting to design chemical tools to assess the availability of nutrients in soils, some of which having been initially designed to mimick what plant roots were doing to acquire plant nutrients. In Europe only, there are for instance 17 soil test procedures that are currently being used for estimating P availability (Tunney et al. 1997), which is a clear indication that none is quite satisfactory to predict the bioavailability of P to crops. Of concern is the fact that many of the designers of these methods and an even larger proportion of their users take the easy (but wrong) shortcut, pretending that they provide a measurement of the bioavailability. They are to be considered at best as a surrogate, a practical way of estimating the bioavailability. But it shall be stressed that chemical methods cannot measure the bioavailability of a nutrient in a soil because by definition, the bioavailability is expected to vary depending on the organism that is targeted. In other words, contrary to the availability for a given soil and a given nutrient, there are different bioavailabilities depending of the target organism. As pointed by Harmsen et al. (2006) and ISO (2008), as a follow up on the former work of Lanno et al. (2004), this is largely due to the fact that for each considered organism, a so-called 'bio-influenced zone' can be defined, which is the portion of the environment with which an organism interact. In this zone, which typically corresponds to the rhizosphere in the case of plants, the

KEYNOTE PAPERS

physiological activities of the organism results in changes of those soil parameters and processes that determine the availability of a nutrient, e.g. a change in pH or the release of an exo-enzyme such as phosphatase for instance. Thus, the bioavailability of nutrients will depend on how much each organism is capable to alter the availability of nutrients in his 'bio-influenced zone'. For the case of plants, one may expect considerable variations in bioavailability of nutrients from one species to another, or even from one genotype to another within a single species, depending on its ability to alter rhizosphere processes and properties (see for example for P, Horst et al. 1996; Hinsinger 2001; Li et al. 2008).

Rhizosphere biophysics

The bioavailability being defined as a flux of nutrients that can be taken up by the roots of a given plant genotype, it is rather straightforward that processes implied in the transfer of nutrients towards the root surface, and ultimately their uptake into root cells must be accounted for. This is well illustrated by the Barber (1995) modeling approach of bioavailability. Transfer processes that play a key role in that respect are mass-flow and diffusion, the relative contribution of each varying from a nutrient to another, depending also on the plant requirements and the availability of the nutrient in the soil, especially its concentration in the soil solution. Actually this was first modelled by Nye and Mariott (1969) in the case of a single root model, assuming a Michaelis-Menten formalism for nutrient absorption across the root surface: beside the concentration of the nutrient in soil solution, they also accounted for the buffering capacity of the soil, i.e. the ability of the soil to replenish soil solution when depleted as a consequence of nutrient uptake. In such models and in the many nutrient uptake models that have been deriving since then, the central hypothesis is that the driving force of nutrient bioavailability is the absorption process which results in a decrease of the nutrient at the surface of the root, leading to a diffusion gradient in the rhizosphere. This is supported by ample of experimental evidence for nutrient depletion occurring in the rhizosphere, and is especially documented for poorly mobile major nutrients such as potassium (K) or P (Kuchenbuch and Jungk 1982; Hendricks et al. 1981; Jungk and Claassen 1986; Hinsinger 1998 and 2001; Jungk 2002). For such nutrients, mass-flow contributes rather little to the transfer towards the root surface as K and P usually occur at rather low concentrations in the soil solution. Mass-flow becomes more important for those nutrients that occur in larger concentrations in the soil solution, typically for calcium (Ca) and magnesium (Mg) among cations: their transfer by mass-flow can even be larger than plant requirements, thereby resulting in an increase in their concentration in the rhizosphere. Mass-flow can also contribute a significant proportion on nitrate transfer towards the root surface, but depletion is expected to occur because of the large N requirements of plants. There has been some reports of more complex patterns of nutrient distribution in the rhizosphere, such as a combination of depletion at the very root surface with nutrient accumulation occurring farther away from the root surface, relative to the bulk soil, as shown for P (Hübel and Beck 1993 ; Hinsinger and Gilkes 1996 ; Hinsinger 1998 and 2001; Hinsinger et al. 2008). Such complex patterns can hardly be accounted for by the classical model of nutrient bioavailability of Barber (1995).

Further refinements of the Nye and Mariott (1969) derived models consisted to upscale from the root segment to the whole plant (root system), which needed to account for root growth (Baldwin et al. 1973 ; Claassen and Barber 1976 ; De Willigen et al. 2002). When

KEYNOTE PAPERS

applied to nutrients such as K and P, such models have generally proved quite efficient at predicting the bioavailability to crops over time scales of days or weeks in the case of soils exhibiting high K or P availability, but they failed in limiting conditions (Brewster et al. 1976; Schenk and Barber 1980; Lu and Miller 1994; Mollier et al. 2008). In soils exhibiting low K or P availability, those models systematically underestimate the actual uptake flux, which suggest other processes than those accounted for by the model are operating, and ultimately driving nutrient bioavailability. Nevertheless, sensitivity analyses conducted with such models (Barber 1995) showed for poorly mobile nutrients such as P (and K) that the major parameters were (i) root elongation rate and (ii) nutrient availability in the soil (concentration and buffer power), while the least important parameters were those describing the nutrient absorption capacities of the roots (Michealis-Menten parameters). Rengel (1993) underlined that the uptake was thus not the limiting step of nutrient acquisition for poorly mobile nutrients such as K and P, contrary to water or the case of more mobile nutrients such as nitrate.

Major challenges are in front of us for improving current models of plant nutrition and upscaling rhizosphere knowledge (Darrach et al. 2005; Dunbabin et al. 2006; Hinsinger et al. 2005 and 2008; Jones and Hinsinger 2008) given that reducing fertiliser inputs needs better predicting the bioavailability of nutrients in nutrient-poor soils. At a biophysical point of view, a better prediction of nutrient bioavailability would need two major improvements. First of all, as long as the bioavailability is defined as a flux, it is needed to better describe what the actual surface of uptake to account for is. So far, most models rely on a very poor description of root growth, and do not explicitly account for root architecture, although root architecture models as those developed by Lynch (Ge et al. 2000; Liao et al. 2001; Lynch et Brown 2001; Rubio et al. 2003) have proved useful for describing situations of heterogeneous distribution of nutrients in the soil profile. Accounting for architecture is especially needed for the most mobile nutrients such as nitrate, as root-root competition and consequent overlapping of nutrient depletion zones is increasing with increasing diffusion coefficient (Ge et al. 2000; Hinsinger et al. 2005). While the initial models of nutrient bioavailability have been improved to account for root hairs which play a prominent role in extending the volume of the depletion zone for poorly mobile nutrients such as P (Gahoonia et al. 2001; Gahoonia et al. 2004 a and b), very little progress has been made to account for the contribution of mycorrhizal hyphae. This contradicts the largely admitted view of the key role of mycorrhizal symbiosis in determining P bioavailability to most plants (Smith et al. 2003), especially in soils exhibiting low P availability. Schnepf and Roose (2006) have recently made a first attempt to account for P depletion zones around mycorrhizal hyphae in a root segment model. This shall be done at the more realistic scale of a root system model of plant nutrition and may have implications for predicting the bioavailability of P and also other nutrients such as N, K, zinc (Zn) or copper (Cu), for which a significant contribution of mycorrhizae has been shown in the literature.

Another major improvement of nutrient bioavailability models shall rely on a better coupling with water dynamics. Doussan and co-workers (Doussan et al. 1998, 1999, 2003 and 2006) have made major progress in modelling water uptake at the rhizosphere up to whole root system scales by explicitly accounting for root architecture and spatial heterogeneity of water uptake capacities (fluxes) along root axes. This, combined with the temporal and especially diurnal, patterns of water uptake generates complicated water content distribution patterns around roots, which should be accounted for as they ultimately govern

KEYNOTE PAPERS

the fluxes of nutrients as well. Finally other biophysical processes shall also be accounted for in a comprehensive model of rhizosphere functioning, such as those processes that are related to mechanical effects of root growth (both axial and radial) or the production of mucilage by either the roots of rhizosphere microorganisms, and the subsequent formation of rhizosheaths (Hinsinger et al. 2008).

Rhizosphere biogeochemistry

Another major drawback of current models of nutrient bioavailability is that they do not account for the many biogeochemical processes that have been shown to occur in the rhizosphere, as a consequence of either root or microbial activities (Marschner 1995; Hinsinger 1998; Hinsinger et al. 2005 and 2008). A few attempts have been made to account for the potential impact of root exudates such as carboxylic anions in the acquisition of P at the root segment scale (Geelhoed et al. 1999; Kirk et al. 1999). Such models hardly account for the many concurrent biogeochemical processes that interact with nutrient bioavailability, especially so for P (Raghothama 1999; Hinsinger 2001; Vance et al. 2003; Raghothama and Karthikeyan 2005) and micronutrients. At best, they only account for a single rhizosphere function (citrate exudation for instance, Geelhoed et al. 1999; Kirk et al. 1999) and its interaction with P availability (competitive desorption of phosphate ions by citrate for instance, Geelhoed et al. 1999). They should *a minima* account for additional rhizosphere processes such as pH changes and complexation which have been shown to play a key role in determining P bioavailability (e.g. Gerke et al. 2000; Hinsinger 2001) and iron (Fe) bioavailability (Marschner 1995; Robin et al. 2008). The role of phytosiderophores in the complexation of Fe and other micronutrients such as Zn and Cu is well known as a major strategy as developed by Graminaceous plant species (Römheld and Marschner, 1985). Rhizosphere microbes are also known to be key players in the bioavailability of Fe, especially via the release of siderophores (Marschner 1995; Robin et al. 2008). The complexation of metals by root or microbial exudates is however depending on the pH, and the potential competition with the many metal cations, of which concentrations can tremendously vary in the rhizosphere. Obviously, only reactive transport, multicomponent models as developed in geochemistry appear as suitable tools to better predict the bioavailability of reactive nutrients such as P and micronutrients in soils (Anoua et al. 1997; Nowack et al. 2006; Devau et al. 2008; Szegedi et al. 2008).

A number of the rhizosphere functions that have been cited above as key players in nutrient bioavailability are regulated by the nutritional status of the plant itself: the root-induced release of protons or the exudation of carboxylic anions as well as enzymes such as phosphatases have been shown to be stimulated under P deficient conditions (Neumann and Römheld 1999; Raghothama 1999; Hinsinger 2001; Richardson et al. 2001; Vance et al. 2003; Tang et al. 2004; Raghothama and Karthikeyan 2005). Such feedback processes would need to be accounted for in future attempts to model nutrient bioavailability. In addition, contrary to the function of nutrient absorption, as for example for P (Rubio et al. 2004), many of those rhizosphere functions are not homogeneously distributed along root axes, which means again that a functional root architecture should be implemented in future models. Many works have shown that apical root zones were responsible for larger fluxes of exudates or protons (Neumann et al. 1999; Hinsinger et al. 2003; Vansuyt et al. 2003; Lambers et al. 2006). This is especially documented for protons in strategy I plant species which exhibit

KEYNOTE PAPERS

enhanced proton efflux behind root apices as a response to Fe deficiency (Marschner 1995; Hinsinger et al. 2003; Vansuyt et al. 2003). The enhanced secretion of phytosiderophores in strategy II plant species (Graminaceous species) as a response to Fe deficiency is also known to have a rather constrained spatial and temporal pattern. They are released at greater flux at the root tips and between 3 and 6 hours after the onset of light in the morning. These patterns shall be accounted for when modelling soil Fe bioavailability to Graminaceous plants, as suggested by Darrah (1991) for other root exudates.

The work of Dunbabin et al. (2006) which accounted for the increased availability of rhizosphere P as a consequence of the exudation of surfactants (phospholipids) has led to major advances in rhizosphere modelling. It showed that it was worth up-scaling at the whole root system scale in order to account for feedback effects of improved P bioavailability on root growth and further extension of the prospected soil volume, ultimately increasing the amount of P acquired. Dunbabin et al. (2006) calculated in a soil exhibiting a high P availability that the extra benefit of the exudation of surfactants on P uptake was rather negligible at the root segment scale (only 4% increase), while it became significant at the whole root system scale (about 13%). In a soil exhibiting a low P availability, the extra benefit was obviously much larger, close to 50% increase in P bioavailability, at the whole root system scale.

Rhizodeposition is a key biogeochemical process occurring in the rhizosphere (Hinsinger et al. 2005 and 2008; Jones et al. 2008). Besides the direct implication of root exudates in the mobilization of specific nutrients, this process can have a considerable impact on the bioavailability of nutrients via the stimulation of microorganisms in the rhizosphere. Raynaud et al. (2006) recently showed that the whole microbial loop could be modelled to better describe the bioavailability of N in the rhizosphere. These authors accounted for the stimulation of microorganisms and also for predation of rhizosphere bacteria by the fauna (protozoa or nematodes) which is known to play a significant role in delivering extra N (Griffiths 1994).

While our knowledge of the various biogeochemical processes that may influence nutrient bioavailability has considerably increased over the last decades, as reviewed by Hinsinger et al. (2008), and is now much more advanced than for biophysical processes, there is still a need to put all this knowledge together.

Conclusions – what is the way forward?

A central question is how to make use of the accumulated knowledge on nutrient bioavailability to face the issue of ecological intensification of agroecosystems, and especially that of improving N and P efficiencies and micronutrient fortification in plants.

The perspectives for further intensification of cropping systems based on a better knowledge and valorization of genetic resources are nevertheless limited in terms of increased productivity, given that the ceiling of yield potential is considered to be close to be reached by now (Wissuwa et al. 2008); progress is to expected rather at the level of an increased stability and sustainability, via increased use efficiency of soil resources such as water, N and P for instance (Tilman et al. 2002). For this purpose, the development of an ecological engineering of agrosystems is promising, which shall take its inspiration from the understanding of natural ecosystems. A major difference between intensive agrosystems and

KEYNOTE PAPERS

natural ecosystems is biodiversity, especially so at the level of plant community. While most natural ecosystems are made of complex assemblages of plant species, agrosystems are characterized by extremely simple plant communities (most often a single species and a single variety in a field). A better nutrient use efficiency shall be expected from more diverse systems, either pluri-specific such as intercropping or agroforestry (Tilman et al. 2002; Li et al. 2007 and 2008). Such complex systems only start being modelled (e.g. Raynaud et al. 2008).

Plant breeding has essentially been conducted in non limiting environments, thereby leading to the selection of highly productive genotypes under conditions of heavy use of fertilisers, while leaving aside rustic genotypes which may be better adapted to low input conditions (Dawson et al. 2008). This has ultimately led to a considerable impoverishment of the genetic diversity in commercial varieties of most crop species (Khush 2001; Rengel and Marschner 2005). It is thus now necessary to fully revise the breeding schemes in order to take into account new criteria such as soil N or P use efficiency in low input growing conditions (Tilman 1999; Rengel and Marschner 2005; Ismail et al. 2007; Lynch 2007; Wissuwa et al. 2008). As pointed out by Lynch (2007), the 'roots of the second Green Revolution' rely on better accounting for root traits and soil-root-microbe interactions that occur in the rhizosphere (Wissuwa 2003 and 2005; Wissuwa et al. 2008).

For N, it is most relevant to better valorize symbiotic N₂ fixation in legumes by giving a more prominent position of legumes in cropping systems, either in rotation, or intercropped with cereals or other crops (Rengel 2002). As far as cereals are concerned, the aim should be to increase soil N use efficiency while minimising losses of N as nitrate (leaching and groundwater pollution) or N oxides (greenhouse gas emission). For such purpose, innovative strategies must be promoted instead of current practices which are mostly based on the assumption that nitrate is the sole pool of soil N which is bioavailable to crops (Giles 2005 ; Subbarao et al. 2006). The ability of plants to mobilise N forms that are produced prior to nitrate along organic matter mineralisation, such as ammonium or even aminoacids would be worth being better exploited (Lipson et al., 1999; Falkengren-Grerup et al., 2000; Glass 2003). Rather than applying synthetic nitrification inhibitors, it seems now possible to mimic natural ecosystems where certain grasses have been reported to produce such compounds (Lata et al. 2004). Subbarao et al. (2007) have adopted such a strategy and shown that exudation of nitrification inhibitors by roots was observed in a whole range of plant species, including crops such as sorghum (*Sorghum bicolor* L.) or peanut (*Arachis hypogea* L.). In addition, better exploiting other rhizosphere processes such as associative N₂ fixation by diazotroph rhizobacteria (e.g. *Azospirillum brasilense*) is another means worth being pursued, in the light of cereal-based systems of Mexico or Brasil, especially sugarcane production (Baldani et al. 2002 ; Boddey et al. 2003).

For P, there are many potential options for increasing acquisition efficiency in crop species (Raghothama 1999 ; Hinsinger 2001 ; Vance et al. 2003; Raghothama et Karthikeyan 2005 ; Lambers et al. 2006 ; Ismail et al. 2007), either based on root traits (root architecture, root hairs ; Ge et al. 2000 ; Gahoonia et al. 2001 ; Lynch and Brown 2001 ; Rubio et al. 2003 ; Wissuwa 2003 and 2005; Gahoonia et Nielsen 2004a et b ; Lynch 2007), or rhizosphere traits related to the physiology of plant roots (exudation of protons, carboxylates or phosphatase enzymes ; Neumann et Römheld 1999 ; Hinsinger 2001 ; Richardson et al. 2001; Vance et al. 2003 ; Wissuwa 2003 and 2005 ; Tang et al. 2004 ; Yan et al. 2004 ;

KEYNOTE PAPERS

Raghothama et Karthikeyan 2005) or the physiology of associated microorganisms, symbiotic such as mycorrhizal fungi (Hettrick et al. 1993 et 1996 ; Zhu et al. 2001), or not such as P-solubilising bacteria and fungi (Marschner et al. 2006).

Keywords: Rhizosphere; soil-root-microbe interactions; nutrient bioavailability.

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KEYNOTE PAPERS

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KEYNOTE PAPERS

K-4

Soil Organic Matter in Nano-Composite and Clay Fractions, and Soluble Pools of the Rhizosphere**C.M. Monreal* and M. Schnitzer***Eastern Center for Oilseed Research Center, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada, K1A 0C6. *E-mail: MONREAL@ARG.GC.CA*

Soil organic matter (SOM) has been studied in physical fractions (i.e., aggregates), chemical components (i.e., humic substances); and biological constituents (i.e., microbial biomass), or represented in kinetic compartments in simulation models (i.e., Century). In soils most organic matter is found as organo-mineral complexes, the interactions between inorganic and organic matter have been studied by Schnitzer and Kodama (1992). The physical domains of organic matter involve hierarchical units of aggregates and particle size fractions (Oades and Waters, 1991). The chemical domains involve chemically extracted humic substances (Schnitzer and Kodama, 1975), or several chemical classes of compounds in whole soil as characterized in mass spectrometry studies (Schnitzer and Schulten, 1992). The biological components and their interactions with inorganic matrices and organics control the flows of energy, carbon, nitrogen and other crop nutrients (Monreal and McGill, 1997). Another important component of organic matter is the soil solution, where chemical, biochemical and physico-chemical reactions occur (Monreal and McGill, 1997). The latter reactions affect supply of crop nutrients, the type of microbial communities, and molecular structures of soil organic matter (Monreal et al., 1997).

In soil microsites organic matter binds to clay colloids and other minerals to form domains of nanocomposites at various arbitrary scales (< 1000 nm). Nano size materials are composed of nanoparticles characterized by a high surface-to-volume ratio. Various workers have offered potential mechanisms for the formation of nanoparticles in solutions, from those involving the classical nucleation and growth by addition of single atoms or ions, to those based on the growth of zeolites templated by organics involving 3 nm cubohectahedral clusters (Banfield and Zhang, 2001; Casey et al., 2001; Furrer et al., 2002; deMoor et al., 1995). In soil niches, nanomaterials are porous and hydrated and as such they control moisture retention, permeability, solute transport, and availability of plant nutrients in soils. These nanomaterials materials also control exchange reactions of dissolved inorganic and organic species between the soil solution and colloidal surfaces. The physico-chemical properties in the surface of nanocomposites provide much of reactivity to soil biological and abiotic processes (Navrotsky, 2004). With respect to organic matter, it is still uncertain how much of it may be found as pure molecular clusters ($< 0.5 - 2$ nm) versus that found complexed with minerals in nanoparticles (2-100 nm), nano-composites (100-1000 nm), or microcomposites (> 1000 nm). On the other hand, bacteria may be considered as single domain microparticles or nanomolecular catalytic assemblies. Living communities of microorganisms in soils produce and utilize diverse nanoparticles during their metabolic reactions of oxidation and reduction of growth and energy synthesis (35). Thus, the microbial-soil-plant interaction taking place at nanosites in rhizospheres may influence the stabilization of organic matter and the availability of nutrients for crop use.

KEYNOTE PAPERS

At present, little is known about the chemistry and dynamics of organic matter in the soil solution of crop rhizospheres and in nanocomposites of the clay fraction. This knowledge is necessary in order to elucidate processes that control the storage of atmospheric carbon in soil organic matter and the supply of nutrients for crop use in the soil rhizosphere. The objective of this article is to describe and characterize the chemistry and kinetics of organic matter in the soil solution of wheat rhizosphere and in nanocomposite and clay fraction of a cultivated soil. These results are to be compared with published information on the nature of organic matter in soil solutions and aggregate fractions.

Materials and Methods

In this article, the characteristics of organic matter in three soil compartments (i.e., soil solution, clay and nanocomposites) were determined in separate studies. A recent study has been initiated to determine the chemical composition of organic molecules in the soil solution of a cultivated Gleysol. A soil pot replicated study involves extracting the solution from the rhizosphere of wheat grown in a loamy sand Gleysol and from unplanted soil pots. The experiment was carried out in the greenhouse under controlled conditions of moisture and temperature until wheat reached maturity. The soil solution from wheat rhizosphere and from unplanted soil pots was obtained once a month. Roots were separated from soil by hand. Soil samples were then passed through a 2 mm sieve. The soluble pool was extracted by shaking soil samples taken from the rhizosphere with dilute KCl (0.01 N) for half an hour. The supernatant was then passed through a micropore (<0.2. μm) fibreglass filter, and stored frozen until chemical analysis.

A separate study involved collecting composite soil samples from the A horizon of a cultivated Black Chernozem in Saskatchewan. The clay fraction of soil samples was obtained after sonication and sedimentation as described by Monreal and Kodama (1997). The clay fraction was shaken and dispersed repeatedly with sodium hexametaphosphate to obtain a nanocomposite fraction. A nanocomposite sample (< 250 nm) was obtained by centrifugation at high speed (4800 rpm) based on the particle sedimentation and centrifugal acceleration scheme of Tanner and Jackson (1947). The chemical characterization of soil organic matter in soil solution was done by Chemical Ionization Electrospray Mass Spectrometry, and in the nanocomposite fraction was done by Pyrolysis Field Ionization Mass Spectrometry (Py-FIMS) as described by Monreal et al. (1997).

The dynamics of organic matter in the clay and nanocomposite fractions were characterized using the kinetic concepts of mean residence time (MRT) and organic carbon turnover as described by Monreal et al. (1997). The age or MRT was determined using radiocarbon dating by the IsoTrace Radiocarbon Laboratory of the University of Toronto in 2007.

Results and Discussion*Organic Molecules in the Soluble Pool of Wheat Rhizosphere*

Figure 1 shows the ESMS spectra for soil solution (extracted with 0.01 N KCl) collected from the rhizosphere of wheat during the late booting stage and from a soil pot after 46 days of incubation in the greenhouse. Noteworthy, the total number of molecular ions (TII) is ten

KEYNOTE PAPERS

times greater (i.e., 28,400) in the solution obtained from the soil rhizosphere of wheat than in the solution extracted from soil alone or non-rhizosphere soil (2,090) (Figure 1, Table 1). The latter was also paralleled by greater diversity of organic molecules in the rhizosphere than in non-rhizosphere soil solutions. The same trends in diversity and number of molecular ions were observed for rhizosphere and non-rhizosphere soil solution during other stages of crop growth (i.e., tillering, flowering and maturity; data not shown).

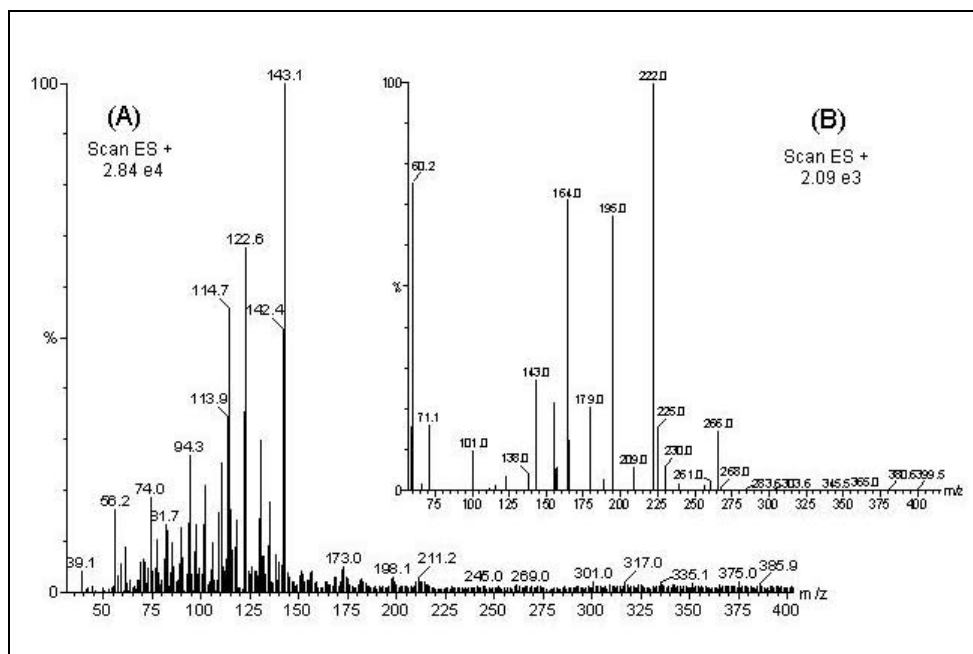
According to Table 1 and Figure 1, the most abundant classes of compounds in the soil solution of the rhizosphere during the booting growth stage of wheat were heterocyclic-N and amino acids followed by carbohydrates and lignin monomers and other aromatics, a nucleotide, ketones and aldehydes. Most heterocyclic compounds involved pyrazole (m/z 68), pyrazoline (m/z 70), pyrazine (m/z 80), and several derivatives of pyrrole (m/z 69, 81, 97, 109, 123, 137), methylpyrazole (m/z 82), hydroxypyrrolidone (m/z 85), methylpyridine (m/z 93), methylpiperidine (m/z 99), pyridineamine (m/z 104), benzimidazole (m/z 118), and quinoline (m/z 129), methylquinoline (m/z 143) and dimethylquinoline (m/z 171). The solution extracted from the rhizosphere contained several amino acids such as glycine (m/z 75), alanine (m/z 89), formylhydroxyglycine (m/z 101), serine (m/z 105), proline (m/z 115), valine (m/z 117), cysteine (m/z 121), hydroxyproline (m/z 130), leucine (m/z 131), asparagine (m/z 132), histidine (m/z 155); propylamide (m/z 73) was also found. Carbohydrates derivatives included pentoses such as hydroxyfuran (m/z 84), dimethylfuran (m/z 96), acetamidofuran (m/z 125); trioses such as glyceraldehyde (m/z 90); furanones like aminomethylfuranone (m/z 113), aminoethylfuranone (m/z 127) and aminopropylfuranone (m/z 141); other carbohydrate like derivatives included methylfurfuraldehyde (m/z 110) and 3-hydroxypenteno-1,4 lactone. Ketone such as butanone (m/z 72), and aldehydes such as hydroxypropanal, hydroxybutanal (m/z 74 and 88) were also found. Nucleotides involved primarily cytosine (m/z 111), uracil (m/z 112) and thymine (m/z 126). All these compounds are important constituents of microbial and plant cell tissues, and may be also associated with root exudates and compounds of metabolism during crop growth or microbial cell lyses.

In comparison, the non-rhizosphere solution presented fewer and less diverse molecular species than the solution obtained from the wheat rhizosphere. According to Table 1 and Figure 1, heterocyclic-N, carbohydrates, and lignin monomers and other aromatics were the most abundant classes of compounds in the solution extracted from the non-rhizosphere soil during the booting growth stage of wheat. Heterocyclic compounds involved pyrazoline (m/z 70), diethylpyrazine (m/z 137), decyl- and undecyl-pyrrole (m/z 207 and 221), other N-compounds (m/z 229, 265), and adenosine (m/z 267). Carbohydrate derivatives included acetamide (m/z 59), and the ketone pentanedione (m/z 100) was also observed. Ketone such as butanone (m/z 72), and aldehydes such as hydroxypropanal (m/z 74), hydroxybutanal (m/z 88), hydroxyketobutanal (m/z 102) and coniferylaldehyde (m/z 178) were also found. Prominent signals of lignin monomers and other aromatics included methylnaphthalene (m/z 142), dimethoxybenzonitrile (m/z 163), ferulic acid (m/z 194), and tridecylbenzene and methyl-sinapyl alcohol (m/z 224).

KEYNOTE PAPERS

Table 1. Major organic matter signals of soil solutions assigned by Electrospray Mass Spectrometry in positive mode. Soil solutions were obtained during the late booting stage of wheat.

Chemical classes of compounds	m/z
<i>Non-rhizosphere soil</i> (THI = 2,090)	
Carbohydrates	59
Lignin monomers, other aromatics	142, 163, 194, 224, 260
Heterocyclic- and other N-compounds	70, 137, 207, 221, 229, 265, 267
Ketone and aldehydes	100, 178
Nucleotides	267
<i>Rhizosphere soil</i> (THI = 28,400)	
Carbohydrates	84, 90, 96, 110, 113, 114, 125, 127, 141
Lignin monomers, other aromatics	92, 108, 122, 124, 140, 142, 156
Organic acids	60
Ketones and aldehydes	72, 74, 88, 102
Heterocyclic-N	68, 69, 70, 80, 81, 82, 85, 93, 97, 99, 104, 109, 123, 129, 137, 139, 143
Amino acids, amides	73, 75, 89, 101, 105, 115, 117, 118, 121, 130, 131, 132, 155, 171
Nucleotides	111, 112, 126

**Figure 1.** Electrospray mass spectra (M+H) for organic matter in the solution obtained from the soil rhizosphere of wheat (A) and from the solution obtained from a soil pot with no wheat planted (B). The soil solution was collected during the late booting stage of wheat before heading (46 days after planting date).

KEYNOTE PAPERS

These results clearly indicate that the presence of crop roots influence the size and diversity of the molecules cycling through soil solutions. The mechanisms controlling the latter are unknown, but they may be associated with the amount and type of organic matter exuded from roots or secreted by active microbial cells, by the amounts adsorbed onto solid surfaces in organo-mineral complexes, and/or associated with the turnover of compounds in the soluble pool. In soils, the number and the amount of roots rapidly increase during the vegetative stages of crop growth and reach a maximum at the heading stage (Inada, 1967). Alberda (1953) indicated that root mats start to develop at the end of the tillering stage and last until the ripening stage. In addition, crop and root growth is accompanied by the exudation of organic materials in the rhizosphere (Rovira, 1956). The chemical compounds in root exudates act as regulators of microbial growth and function, and contain molecules which directly control the rhizosphere processes that enhance soil N mineralization and crop nutrient uptake and assimilation (Dakora and Phillips, 2002). Barber and Gunn (1974) showed that 5-9% of total plant weight was released as root exudates. Barber and Martin (1976) reported that wheat or barley root exudates could comprise 7-13% of the total dry matter in axenic plants growing in soils, and that non-sterile soil root exudates accounted for 19-25% of the dry matter produced. Most root exudation studies have been conducted in hydroponics and axenic sand culture and in the absence of soil (Darwent et al., 2003; Rovira and McDougall, 1967). Root exudates consist of sugars, amino acids, vitamins, organic acids, nucleotides, fungal stimulators and inhibitors, enzymes and other compound classes (Dakora and Phillips, 2002). Further research is warranted to explain the mechanisms and factors inducing the differences in chemical composition between the soil solution obtained from the rhizosphere and non-rhizosphere soil.

Organic Molecules in a Clay and Nanocomposite Fraction

A separate study was also conducted to chemically characterize the organic matter in the clay and nanocomposite fractions of a Black Chernozem. The total number of molecular ions detected by the mass spectrometer was three times higher in the clay fraction (9.38×10^6 counts/mg) than in the nanocomposite fraction (3.36×10^6 counts/mg sample). Figure 2 shows that the m/z signals detected by Py-FIMS were assigned into carbohydrates, lignin monomers and phenols, lignin dimers, lipids (alkanes, alkenes and n-alkyl esters) alkylaromatics, N compounds, peptides, sterols, peptides, suberin and fatty acids. The clay fraction has a relatively higher content of lignin monomers and phenols, lipids, sterols and fatty acids than the nanocomposite fraction. Conversely, the nanocomposite fraction showed higher relative abundance of carbohydrates, heterocyclic-N, peptides and alkylaromatics than the clay fraction. On average, the maximum evolution of pyrolysis products of organic matter in the clay fraction occurred at 360 to 370°C and those in the nanocomposites occurred at 390 to 410°C (data not shown). Carbohydrates, heterocyclic-N and alkylaromatics in nanocomposites were released at higher temperatures than the same compound classes in the clay fraction. This indicates that these components of organic matter are held more strongly in the nanocomposite fraction through preferential interaction with inorganic components. The exact inorganic components in this sample were not determined and mechanisms for these reactions are unknown.

KEYNOTE PAPERS

Age and Molecular Characteristics of SOM

A study was conducted to examine potential age differences between the soil carbon associated with the clay and that associated with the nanocomposite fraction of a Black Chernozem. Radiocarbon dating shows that the average age of organic carbon in the nanocomposite and clay fractions were similar. The average carbon age (before present) was 1200 ± 170 years in the nanocomposite and 1090 ± 130 years in the clay fraction (Table 2). These results indicate that the organic matter in the clay fraction of the Black Chernozem involves the storage of carbon with similar residence time in nanocomposites < 250 nm. In comparison, the age of organic carbon in microaggregates $< 50 \mu\text{m}$ was 275 yr and < 100 yr in macroaggregates $> 250 \mu\text{m}$ (Monreal et al. 1997).

The qualitative nature of organic matter in the clay and nanocomposite fractions is similar to that published for macro and microaggregates of a Gleysol (Monreal et al. 1997). It remains to be determined whether the age and chemical structures turnover of carbon reported for the nanocomposite fraction < 250 nm is similar to that found in smaller nanocomposite fractions (i.e., 50 to 100 nm or 10-50 nm), or in pure molecular clusters (0.5 – 2 nm) if they exist *in-vivo* soils. The hierarchical model of aggregates with associated particle size fractions provides a consistent approach to study soil organic matter dynamics and the mechanisms controlling its stabilization and chemical quality.

Table 2. The age (years) of organic carbon in various soil fractions obtained from two cultivated soils in Canada.

Age ¹	Black Chernozem		Gleysol ²		
	Nanocomposite < 250 nm	Clay < 2 μm	Macroaggregate (> 250 μm)	Microaggregate (50-250 μm)	Microaggregate (< 50 μm)
YBP	1200	1090	< 100	<100	275
Error	170	130	-	-	85

1. YBP = Years before present using the Libby ^{14}C meanlife of 8,033 years. The error represents 68.3 % confidence limits. 2. Data from Monreal et al. 1997.

In summary, results from these two studies and those obtained from earlier published work show that organic matter involves the same ten classes of chemical compounds in nanocomposites, clay, macro- and micro-aggregate fractions in soils with different parent material, pedogenesis and inorganic matrices. Main differences in the nature of soil organic matter in solid fractions appear at the level of individual molecules and the energy of stabilization by inorganic colloids in scales ranging from hundreds of nanometers to hundreds of micrometers. In comparison, the organic matter in soil solution comprises fewer chemical classes and showed to be less diverse than in soil physical fractions ranging from nanometer to micrometer scale.

KEYNOTE PAPERS

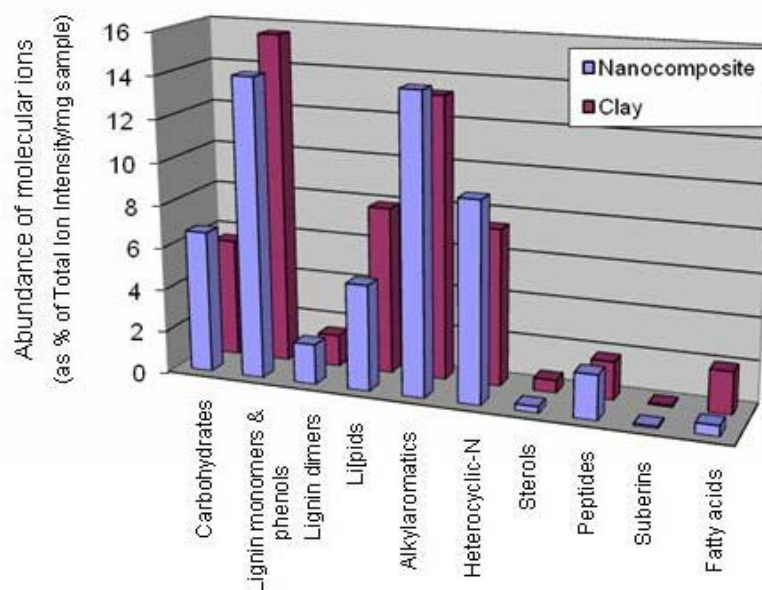


Figure 2. Chemical classes of soil organic matter in the clay and nanocomposite fractions of a cultivated Black Chernozem as characterized by Pyrolysis Field Ionization Mass Spectrometry.

Conclusions

1. The soil solution has from five to seven chemical classes of molecular ions ($m/z < 250$ da) involving heterocyclic-N, amino acids, carbohydrates, lignin monomers, phenols, nucleotides, ketones and aldehydes. The organic matter in soil solutions extracted from wheat rhizosphere is more diverse and abundant than non-rhizosphere soils.
2. The organic matter in the clay and nanocomposite < 250 nm fractions consisted of ten chemical classes of molecular ions ($m/z < 550$ da) involving carbohydrates, lignin monomers and phenols, lignin dimers, lipids, alkylaromatics, heterocyclic-N, sterols, peptides, suberins and fatty acids. The organic matter in the nanocomposite fraction had relatively higher content of carbohydrates, lignin dimers, alkylaromatics and heterocyclic-N.
3. The age of soil organic carbon in the nanocomposite < 250 nm fraction was 1200 years and similar to that in the clay fraction which was 1090 years old. was more stable and The residence time of carbon in clay and nanocomposite fraction was slower than in macro- and micro-aggregates.

Keywords: Soil organic matter; nano-composite; clay fraction.

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KEYNOTE PAPERS

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Contaminant Fate, Dynamics and Bioavailability: Biochemical and Molecular Mechanism at the Soil: Root interface**R. Naidu*** and K.R. Kim*Cooperative Research Centre for Contamination Assessment and Remediation of the Environment, PO Box 486, Salisbury South, SA 5106. Tel.: +61-8-8302-5041; Fax: +61-8-8302-3057.***Email: Ravi.naidu@crccare.com***Introduction**

Environmental contamination from improper disposal of hazardous industrial and municipal wastes has long been recognised as an issue of public concern, regulatory activity, and scientific investigation. Such disposals have resulted in the occurrence of in excess of 80,000 potentially contaminated sites in Australia (Natusch, 1997) and over 3 million such sites in the Asia region. In the United States (US), there are estimated to be about 400,000 waste disposal sites where soil and groundwater contamination is deemed to be of sufficient extent and magnitude that some type of remedial action is warranted to protect public health or to minimize adverse environmental and ecological impacts (Rao et al., 1996; USEPA, 2004, <http://www.epa.gov/superfund/news/30years.htm>).

Remediation of contaminated sites to the extent practicable is expected to cost approximately \$5 to 8 billion in Australia (Powell, 1992; *CMPS&F – Environment Australia*, 1997) alone while it may exceed one trillion dollars in USA (Naidu et al., 1996; USEPA, 2004). While the remediation endpoints required by regulators may be similar, the scope of monitoring programs and the assignment of legal/financial liability for remediation efforts may vary greatly from country to country. The public perception of the relative risks of soil and groundwater contamination (in comparison to other hazards) can influence the regulatory policy, and levels of allowed contamination as well as the expectations of the required clean up of contaminated sites.

The decision to remediate is often based on the total contaminant loading in soil and ground water. This is still the case in many countries including Australia. However, there is increasing evidence that as contaminants persist in the environment, they become increasingly less available for uptake by organisms, for exerting toxic effects, and for biodegradation and bioremediation by micro-organisms. These changes in the impact of contaminants to ecosystem have been attributed to a decline in the labile fraction of contaminant available for causing such adverse impacts. The fraction of contaminant that is mobile and available for uptake by plants, animals and human is often defined as the bioavailable fraction.

The amount of a contaminant in soil that is bioavailable depends on a variety of factors including the properties of both the contaminant and the soil environment (Adriano et al., 2004). Bioavailability defines the relationship between the concentration of a contaminant in soil and the amount of the contaminant that actually enters biological receptors, and thus may cause harm (Reichenberg and Mayer, 2006). Consideration of bioavailability during risk assessment is important given that the mobility and the subsequent availability of a contaminant is inversely related to risk-based cleanup levels. This means that the risk-based

KEYNOTE PAPERS

cleanup level of a contaminant increases with its decreasing bioavailability. In this chapter, following a brief overview, the various biogeochemical processes controlling the fate and dynamics of contaminants in the soil environment and its implications to contaminant bioavailability is presented.

Contaminant interactions in soil

Following input into the soil environment contaminants interact with soil solid phase via a series of sorption-desorption (all ionic and non-ionic solutes), precipitation-dissolution (polar and ionic compounds) reactions including physical migration into subatomic pores and diffusion into solid phase (Figure 1).

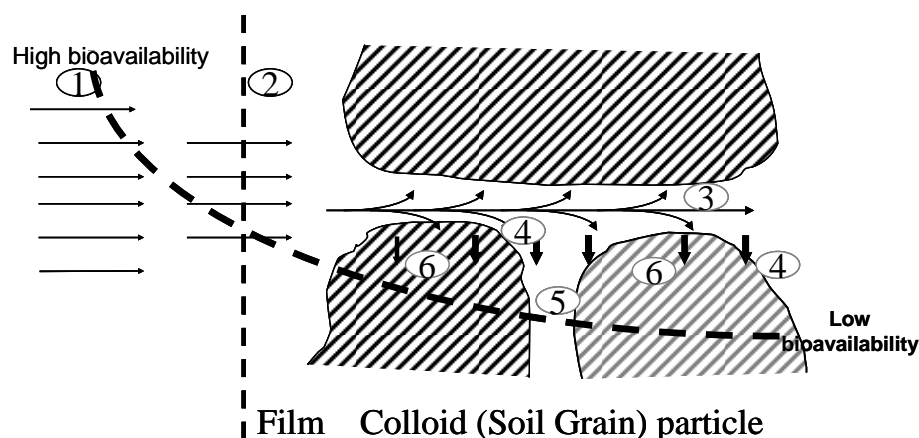


Figure 1. Fate of chemicals in soil environment (modified from Sparks, 1998)

Once the chemical substance is released into soil solution (1) it crosses the solution-solid phase boundary (2) following which it is transported in a liquid filled soil macropores (3). During transport, the chemical substance interacts with colloid surface via either columbic interaction (non-specific sorption) or covalent bonding (specific sorption) (4). This leads to the binding of the chemical to the surface of soil colloids (4). Once bound to the solid surface, the chemical substance redistributes between soil solution and solid mineral and organic phases. This redistribution of the solute as quantified by its distribution constant, K_d is a function of both specific and non-specific sorption (equation 1) with the soil solution concentration being governed by environmental factors (including contaminant loading) all of which influencing the above processes.

$$K_d = f(\text{non-specific})(\text{specific binding})$$

KEYNOTE PAPERS

With ageing, the initially surface sorbed chemicals are slowly redistributed to interior of soil aggregates including the subatomic pores (5, Fischer et al., 1996) with a small proportion diffusing into the solid particles (6, Barrow et al., 1989) from which desorption becomes slow and often difficult and hence contaminants become inaccessible to microorganisms (Karickhoff, 1980). Thus the sorption of a chemical onto soil plays an important role in determining its mobility and bioavailability. Ageing of contaminated soils results in sequestering compounds within the soil particles, resulting in an overall decrease in bioavailability of the sorbed compound (Manilal and Alexander, 1991; Naidu et al., 2003). There is evidence that sequestration of certain organic chemicals with time results in significant decline of their bioavailability. These sequestered organic compounds are known as bound or recalcitrant compounds. For instance, ethylene dibromide, a soil fumigant with relatively high water solubility, volatility, and biodegradability was reported to persist up to 19 years after its last application (Steinberg et al., 2003). Similarly DDT has been shown to persist in soils for a long period (Boul, 1995). While inorganic chemicals such as heavy metal(loid)s often exist in bound form (unless present in concentrations that saturate the binding sites), organic chemicals exist in soils in both free or bound form; “free” indicates that the compound can be extracted from soil without altering chemical structures while “bound” chemicals are those resistant to extraction.

Enzymes and certain minerals can influence oxidation of organic compounds to free radicals, which then results in coupling the contaminant to the organic fraction of soils. For example, enzymes found in soil fungi (e.g. laccases) have been shown to oxidize phenolic compounds and initiate the polymerization process (Hartzinger and Alexander, 1995). This results in the oxidative coupling of the organic contaminant to organic fraction, resulting in the incorporation of these materials (covalently) into humic material (Fahrenhorst, 2006). Studies have shown that certain pesticides become covalently bound to the humic fraction of soils and the bioavailability of these humic bound fragments were practically negligible (Kelsey and Alexander, 1997).

Contaminants present in soil solution (pore water) are the most bioavailable fraction and it is this fraction to which organisms, plants and other species are directly in contact with, thereby having direct access for uptake, and it is this fraction that is mobile, thereby resulting in the leaching through soil to contaminate ground water. Often the soil solution fraction is defined by soil scientists as the “intensity factor”. In long-term contaminated soils, chemicals present in soil solution reach equilibrium with the most labile fraction (i.e chemicals non-specifically bound (exchangeable), equation 2 – ‘capacity factor’). As chemicals present in pore water deplete, they are replenished through sequential release from other pools (specific and non-specifically sorbed fraction, see Figure 2) with the rate of replenishment (buffering) being governed by the partition coefficient (k_d) controlling the equilibrium between the different chemical binding substrates (Figure 2 and Eq. 2).

Metal(loid)s in the solid or sorbed phase are usually unavailable and the uptake by an organism usually occurs when these substances become soluble and reach the solution phase or pore water. Thus, processes influencing the equilibrium between soluble and solid or bound phases for heavy metal(loid)s impact their overall bioavailability. For example, orthophosphate can complex metals such as Pb, Cd, and Zn to form precipitates which are insoluble, relatively stable, and reduce the overall toxicity of the metal contaminants (Bolan et al., 2003). Unless conditions support precipitation, metal(loid)s and ionisable organics

KEYNOTE PAPERS

generally sorb to charged surface such as layer silicate minerals and organic matter (Naidu et al., 1996). In contrast, the non ionic organic compounds are sorbed generally through partitioning into organic matter (Chiou, 1989). In general, low water solubility of non ionic organic compounds and high organic matter content in soils manifest increased sorption.

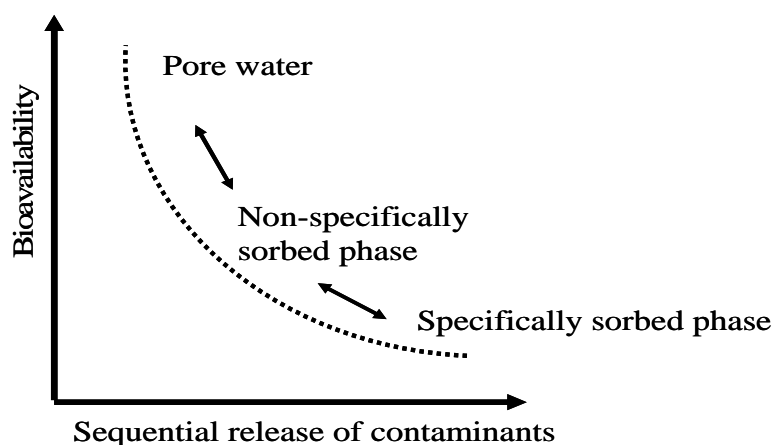


Figure 2. Chemicals are sequentially released as the pore water concentration depletes

Chemicals that persist in the environment can exhibit a wide range of toxicities across different soil types. Bioavailability is impacted not only by soil type, but also contact time between soil and contaminant (i.e. ageing), which reduces the level of contaminant available for degradation/bioremediation (Feng et al., 2000). There are important physical-chemical-biological processes occurring in soil systems which have a significant impact on the bioavailability of organic and inorganic (metal) contaminants. Thus the bioavailable fraction varies with time, the nature of soil types, organisms and the environmental factors. Conceptually the bioavailable fraction may be defined as:

$$\text{Bioavailability } (F_{b,t}) = f(\text{soil properties})(\text{environmental conditions})(\text{plant characteristics})(\text{microbes}).dT \quad 2$$

$$F_{b,t} = F_{b,0} K_I$$

F = fraction bioavailable at time 't'

$$K_I = a [\text{clay}] + b [\text{organic matter}] + \text{etc}$$

Given the dependence of bioavailability on plant type, microbial genre, the definitions currently used to define bioavailability may be limiting. Soil and solution factors influencing chemical bioavailability in the soil environment are briefly discussed in the following sections.

Key soil properties influencing chemical bioavailability in soils

Soil plays a significant role in controlling the potential bioavailability of contaminants in the environment. The primary soil factors influencing the potential bioavailability of

KEYNOTE PAPERS

contaminants are soil pH (Figure 3), cation and anion exchange capacities (available charged sites on soil surfaces), texture (clay content), soil type and soil organic matter. Thus the fate and behavior of contaminants vary significantly with changes in soil type. As shown in figure 4, the ability of metals sorbed to soils varies with soil type considerably demonstrating that the bioavailability of contaminants is likely to vary with partition coefficient (Figure 5).

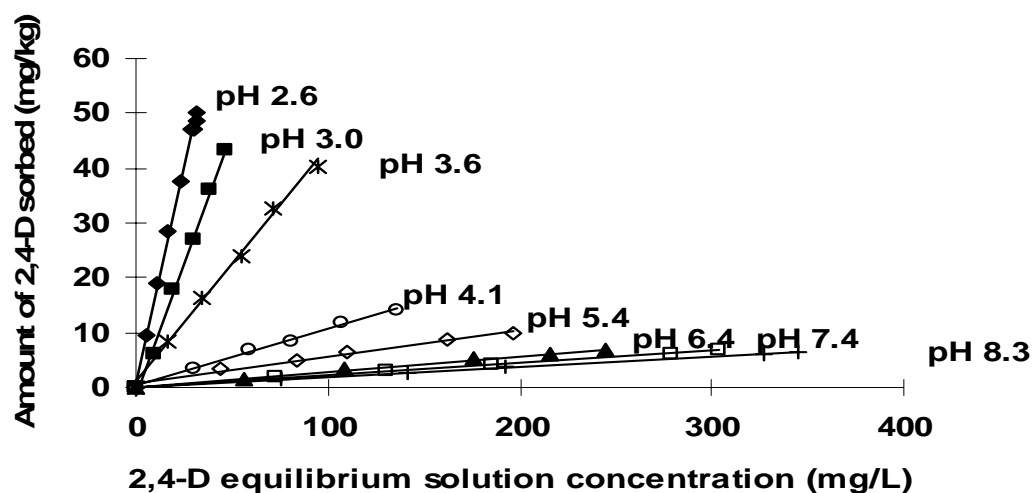


Figure 3. Effect of pH on sorption of 2,4-D by a variable charge soil (R Naidu, unpublished)

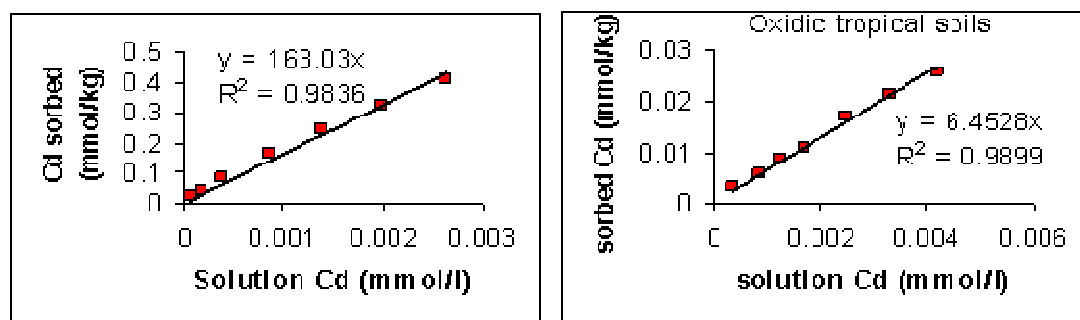


Figure 4. Sorption of Cd varies with soil type. (a) Smectitic soil and (b) Oxidic soils.

KEYNOTE PAPERS

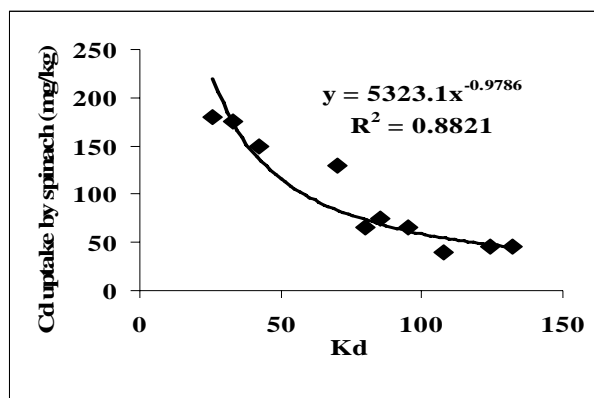


Figure 5: Relationship between Cd uptake and cadmium partition coefficient (K_d) in 10 soils.

Contaminant bioavailability at the root:soil interface

Contaminant bioavailability is influenced at the root:soil interface given the biochemical changes induced by plants in the rhizosphere. The characteristics of the rhizosphere soil is different from that of the bulk soil because of the soil-root interaction including root exudation and associated microbial activities. Many researchers have studied the role of root exudation for the detoxification of heavy metals and found that root exudates reduce the toxicity of hazardous materials through the formation of organo-metal complexes. However, root exudates may play a significant role in hyperaccumulation. Plants can exhibit specific mechanisms when they are exposed to high concentration of heavy metals resulting in changes of soil pH (Figure 6) and DOC/Organic acids concentrations in the rhizosphere ultimately influencing the contaminant solubility and their uptake by plants.

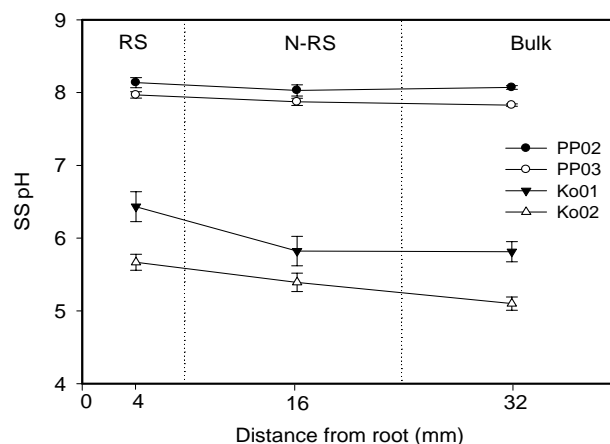


Figure 6. Influence of plant roots on soil solution pH at a distance from root (rhizosphere; RS, near-rhizosphere; N-RS, bulk soil; Bulk) (unpublished).

KEYNOTE PAPERS

Accessibility to contaminants bound to soil determines biodegradation of contaminants

Although bioremediation is considered to be one of the potential remediation options, the lack of contaminant bioavailability in long-term contaminated soils makes it difficult to be successful remediation option. As discussed above, as contaminated soils are aged, contaminants gradually disperse and migrate into subatomic pores (Ogram et al., 1985). While diffusion into subatomic pores is spontaneous (though kinetically slow) the reverse process is difficult and requires significant input of energy. For example, several reports suggest the use of surfactants and cosolvents to increase the solubility and consequent bioavailability of organic contaminants to enhance bioremediation (Muller et al., 2006). However, the use of surfactants can cause soil damage besides being expensive. Kantachote et al. (2000) recently examined the ability of sodium (Na) to increase DDT transformation as Na is known to cause clay dispersion and increase dissolved organic carbon (DOC) content, thereby increasing the DDT bioavailability. This work clearly demonstrated the increased solubility of DDT as shown by an increase in soil solution DDT concentration which is further supported by an increase in bacterial growth in the Na amended long-term DDT contaminated soil.

Conclusion

An overview of the transformation and fate of contaminants in soils as controlled by both the properties of the soil and the nature and type of contaminants will be presented in this lecture. Soil properties, including pH, organic matter and clay contents, surface charge, soil solution composition, and contaminant physicochemical properties, including ionic and oxidation state, molecular structure, polarity, aqueous solubility, and lipophilicity, are responsible for controlling the behaviour of contaminants. These properties in turn affect the fraction of contaminants that may be bioavailable in a given soil. This is further impacted by the duration of contact between contaminants and soils with the bioavailability declining with ageing. The paper also presents the effect of plant roots on plant rhizosphere and the implications of biochemical changes to contaminant bioavailability.

Keywords: Environmental contamination; contaminant bioavailability; root:soil interface.

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KEYNOTE PAPERS

K-6

Protein Adsorption at Solid Surfaces and Protein Complexation with Humic Acids**W. Norde*, W. Tan and L. Koopal***Laboratory of Physical Chemistry and Colloid Science, Wageningen University,
PO Box 8038, 6700 EK Wageningen, The Netherlands. *E-mail: Willem.Norde@wur.nl***Introduction**

Proteins in soils have different origins. Most of them are exudates from animals and plants, and from dead (micro-) organisms. These proteins are substrates for enzymatically active proteins excreted by micro-organisms. Thus, proteins play an important role in biogeochemical cycles and hold potential as indicators of soil quality in ecosystem management (Burns and Dick, 2001).

Proteins have a strong affinity to bind to various soil constituents. Binding may involve changes in the protein structure and structural stability. This would affect the biological functioning, *e.g.*, enzymatic activity, of the protein and it may also influence the availability and susceptibility of proteins for enzymatic degradation. Especially in case of pathogenic proteins this could lead to environmentally hazardous conditions (Schramm et al., 2006).

Binding of proteins to soil minerals and humic substances mediate in aggregation of these substances and therewith influences the soil structure. This is relevant for the penetration of water and air containing nutrients.

Adsorption of proteins to surfaces, including those of (models of) soil minerals has been well documented (Rigou et al., 2006; Quiquampoix and Burns, 2007). At the previous ISMOM conference (ISMOM 2004, Wuhan, China) one of us has reported on the mechanism of the adsorption of globular proteins from aqueous solution to solid surfaces. In particular, the contribution of various types of interaction to the overall adsorption affinity has been discussed. Because of the chemical and structural variation among the almost endless number of (globular) protein species, it is virtually impossible to develop a generic protein adsorption theory. Nevertheless, some principles with respect to the roles of different physical-chemical parameters have emerged, see *e.g.*, (Norde, 1996). This resulted in the qualitative prediction scheme presented in Table 1. Against this background, we discuss, in our contribution to ISMOM 2008, some experimental data on protein adsorption in model systems and examine the influence of various variables.

Besides adsorption to mineral oxides, in soils proteins interact with humic substances. However, protein – humic substance interaction has received only little attention. A few years ago, we started a study on the binding between the protein lysozyme and purified humic acid (Tan et al., 2008a; 2008b). In the second part of this paper we will summarize a few highlights of this research, emphasizing the role of electrostatic interaction in protein – humic acid complexation and in aggregation of the complexes into larger structures.

KEYNOTE PAPERS

Table 1. Predictive scheme of protein adsorption. Conditions at which adsorption is predicted are marked “yes” and predictions of absence of adsorption are marked “no”.

structural stability		electrical charge	sorbent surface			
			hydrophobic		hydrophilic	
			electrical charge		electrical charge	
			+	-	+	-
Protein	stable	+	Yes	yes	no	yes
		-	Yes	yes	yes	no
	unstable	+	Yes	yes	yes	yes
		-	Yes	yes	yes	yes
			hydrophobic dehydration dominates adsorption		electrical interactions or structural changes in proteins dominate adsorption	

Protein adsorption at surfaces of silica and hematite

Silica and hematite are selected since they may be considered as models for mineral oxides in soil. Silica, *i.e.*, silicium dioxide (SiO_2), is the constituent component of quartz, which occurs abundantly in sandy soils. Hematite, *i.e.*, iron(III)oxide ($\alpha\text{Fe}_2\text{O}_3$), is the dominant iron oxide mineral in (red) tropical soils, but it is less common in soils of temperate climates. As both materials are well wetted by water they are characterized as hydrophilic. Due to association or dissociation with protons the surface of SiO_2 and $\alpha\text{Fe}_2\text{O}_3$ acquire positive or negative electrical charge, depending on the pH of the surroundings. Table 2 summarizes some relevant properties of the SiO_2 and the $\alpha\text{Fe}_2\text{O}_3$ samples, that, in our study, are supplied as finely dispersed particles.

Table 2. Some properties of silica (SiO_2) and hematite ($\alpha\text{Fe}_2\text{O}_3$).

	SiO_2	$\alpha\text{Fe}_2\text{O}_3$
nature of charged groups	$-\text{OH}_2^+ \text{ } \overline{\text{R}} - \text{OH}^{\text{R}} - \text{O}^-$	$-\text{OH}_2^+ \text{ } \overline{\text{R}} - \text{OH}^{\text{R}} - \text{O}^-$
isoelectric point (pH units)	2-3	8-9
water wettability	good	good

The proteins, discussed in this paper are, together with some of their properties, listed in Table 3.

KEYNOTE PAPERS

Table 3. Some properties of the proteins used in this study.

protein	source	molar mass (Da)	isoelectric point (pH units)	T _d (°C)	D _d G (J g ⁻¹)
LSZ	hen's egg	14600	11.1	76	4.1
RNase	bovine pancreas	13680	9.4	70	3.6
MGB	sperm whale	17800	7.0		3.0
αLA	bovine milk	14200	4.3	63	1.4
HSA	human blood	69400	4.7	65
αCT	bovine pancreas	25200	8.1	59	2.1
SUB	<i>Bacillus lentus</i>	27000	10.0
CUT	<i>Aspergillus niger orizae</i>	20600	7.8	53
LAM	<i>Pyrococcus furiosis</i>	30085	4.4	109

LSZ: lysozyme; RNase: ribonuclease; MGB: myoglobin; αLA αLactalbumin; HSA: human serum albumin; αCT: αchymotrypsin; SUB: subtilisin; CUT: cutinase; LAM: laminarase

Adsorption data are often presented in adsorption isotherms, where, for constant temperature, the adsorbed amount Γ is plotted against the protein concentration c_p in the adjacent solution, after adsorption. Adsorption isotherms for various proteins on silica and hematite are displayed in the Figures 1 and 2 (Arai and Norde, 1990; Norde and Arai, 1991). By examining the isotherms it is evident that protein adsorption is strongly influenced by electrostatic interaction. For LSZ and RNase on the hydrophilic $\alpha\text{Fe}_2\text{O}_3$ surface electrostatics determine whether or not adsorption occurs. However, MGB and, even more so, αLA adsorb on $\alpha\text{Fe}_2\text{O}_3$ even under electrostatically adverse conditions. Apparently, for these two proteins the unfavorable effects of hydrophilic dehydration and electrostatic repulsion are outweighed by the adsorption promoting contribution related to structural rearrangements in the protein molecules that increase their conformational entropy. Adsorption of these proteins on silica confirms this conclusion. Note that the structures of MGB and αLA are less stable ("soft" proteins) than those of LSZ and RNase ("hard" proteins). Moreover, for the silica surface the initial part of the adsorption isotherms (reflecting the affinity between the protein and the sorbent surface) and the plateau-values of the isotherms scale with electrostatic attraction (Figure 2, left panel); the same applies to the rate of adsorption (Figure 2, right panel).

Many protein adsorption studies included bovine or human serum albumin (BSA and HSA, respectively). The globular structures of BSA and HSA are relatively unstable and, hence, they show a strong adaptability towards structural changes upon adsorption. Therefore, the profile of the plateau-value Γ_p of the adsorption isotherm of these proteins (and many others) as a function of pH is similar for different sorbent surfaces (Morrissey and Stromberg, 1974; Bagchi and Birnbaum, 1981; Haynes and Norde, 1994)

KEYNOTE PAPERS

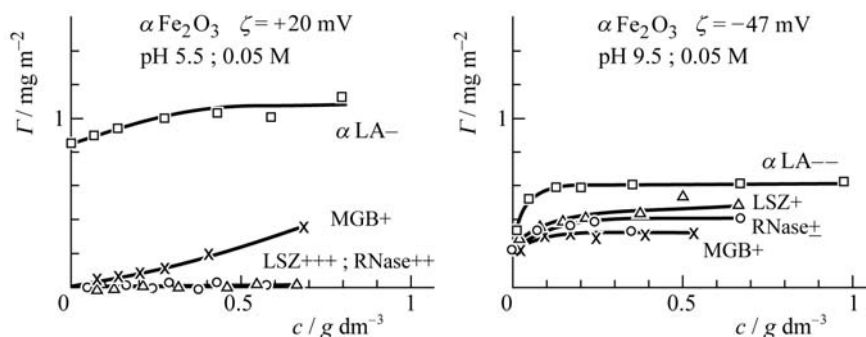


Figure 1. Adsorption isotherms for various proteins at $\alpha\text{Fe}_2\text{O}_3$ surfaces. $T=25^\circ\text{C}$. (Redrawn from reference 8)

It suggests that plateau-adsorption is related to protein characteristics rather than to protein-sorbent interactions. Figure 3 gives $\Gamma_p(\text{pH})$ for HSA on $\alpha\text{Fe}_2\text{O}_3$ at various ionic strengths (Koutsoukos et al., 1983). At all ionic strengths $\Gamma_p(\text{pH})$ passes through a maximum around pH 5.1, which is slightly beyond the isoelectric point (iep) of HSA. Maximum adsorption in the isoelectric region is quite general for proteins. It may be explained by progressive structural rearrangements in the adsorbing molecules when the pH is further away from the iep and stronger intramolecular electrostatic repulsion weakens the protein structure stability. Furthermore, intermolecular repulsion between adsorbed charged protein molecules may contribute to lower adsorbed amounts per unit area of sorbent surface.

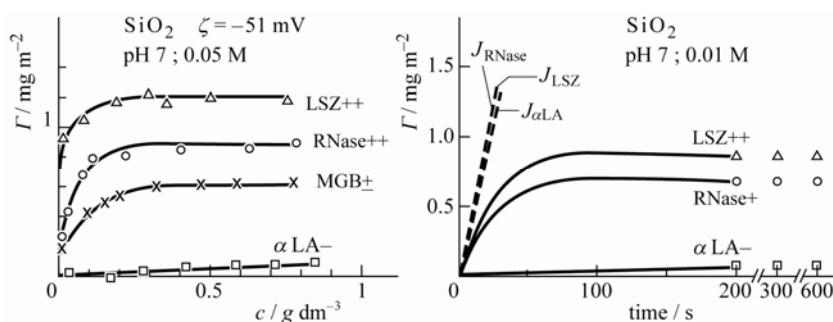


Figure 2. Adsorption isotherms (left) and adsorption kinetics (right) for various proteins at SiO_2 surfaces. $T = 25^\circ\text{C}$. (Redrawn from references 8 and 9)

Increasing the ionic strength reduces intra- and intermolecular electrostatic interaction and, hence, result in a less pronounced $\Gamma_p(\text{pH})$ profile. However, the role of low-molecular-weight electrolyte, *i.e.*, small ions, is more complicated. Simultaneously with protein adsorption ions are incorporated in (or expelled from) the contact region between the protein and the sorbent surface to prevent development of a too high charge density in that non-aqueous, low dielectric, region (van Dulm et al., 1981). The corresponding charge density

KEYNOTE PAPERS

effect $\Delta_{\text{ads}}\sigma$ may be derived from comparing the electrokinetic surface charge densities σ_{ek} of the species before and after adsorption. In Figure 4 $\Delta_{\text{ads}}\sigma$ is plotted for HSA adsorption on $\alpha\text{Fe}_2\text{O}_3$, as a function of surface coverage, Γ/Γ_{p} , and for different pH-values. $\Delta_{\text{ads}}\sigma$ varies linearly with Γ/Γ_{p} , meaning that the charge uptake is proportional to the number of adsorbing protein molecules. The sign of $\Delta_{\text{ads}}\sigma$ is as expected: negative at low pH, where both the protein and the sorbent are positive, and gradually more positive with increasing pH, *i.e.*, when the interacting species become more negatively charged.

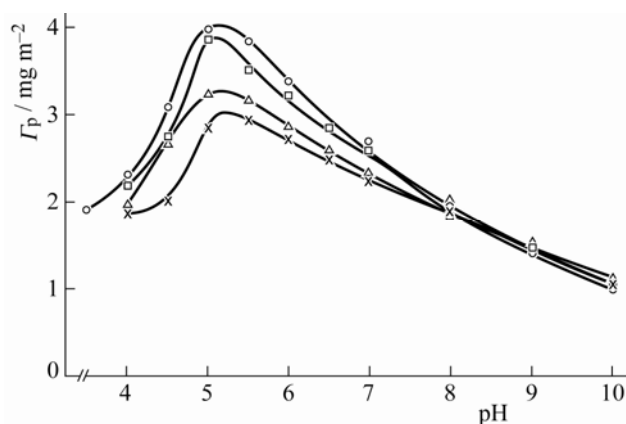


Figure 3. Influence of the pH on the plateau-adsorption of HSA on $\alpha\text{Fe}_2\text{O}_3$. $T = 25^\circ\text{C}$. Ionic strengths (KNO_3) 0.001M (\circ); 0.005M (\square); 0.010M (Δ); 0.015M (\times). (From reference 10, with permission)

At this point a few remarks on the reversibility of protein adsorption are made. (a) Protein adsorption is highly irreversible towards changes in protein concentration in solution. In other words, only a minor fraction, if any at all, of the adsorbed proteins can be removed from the surface by rinsing with solvent. (b) Proteins adsorb semi-reversibly with respect to variation in pH and ionic strength, that is, changing the pH or ionic strength may result in additional adsorption, but it does not induce considerable desorption.

Structural changes in protein molecules, having direct consequences for their biological functioning, is the most intriguing and challenging aspect of protein adsorption, both from a theoretical and practical point of view. In Figure 5 information on the influence of adsorption on the structure and activity of a number of proteins is collected (Norde and Zoungana, 1998; Maste et al., 1995 and 1996; Koutsopoulos et al., 2005). Silica was chosen as the sorbent material because a suspension of ultra-fine particles of this material allows circular dichroism (CD) spectroscopy to be used to probe the secondary structure of adsorbed proteins. This is not possible with $\alpha\text{Fe}_2\text{O}_3$ dispersions. α -chymotrypsin (αCT), subtilisin (SUB) and cutinase (CUT) are mesophilic proteins, *i.e.*, they are biologically active at moderate temperatures. Laminarase (LAM) is a hyperthermophilic protein, being active in hot environments as subterranean geothermally heated biotopes, and in hot outflows from industrial plants. The denaturation temperature T_d of LAM is therefore extraordinary high.

KEYNOTE PAPERS

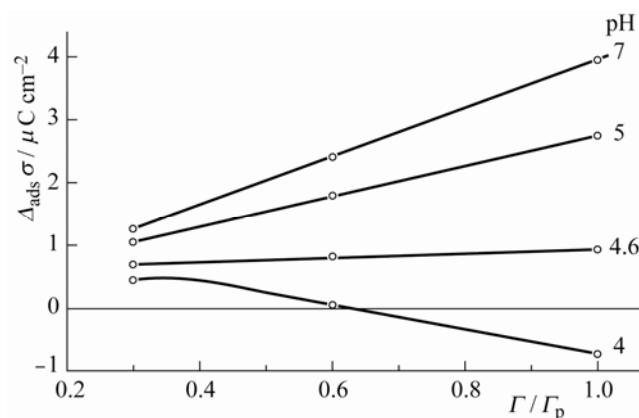


Figure 4. Charge regulation by low-molecular-weight ions during adsorption of HSA at αFe_2O_3 . $T = 25^\circ C$; 0.01M KNO_3 . (Redrawn from reference 10)

At the experimental conditions αCT and SUB are positively charged and, hence, electrostatically attracted to the negatively charged silica surface. The CD spectra of the proteins shift, although not drastically, as a result of adsorption, indicating a change in the ratio ordered/unordered structure along the polypeptide chain of the protein. For both proteins the fraction of ordered structure is lower in the adsorbed state. CUT and LAM are electrostatically repelled from the silica. The ordered structure (mainly α -helix) of cutinase is very strongly suppressed by adsorption. The ensuing gain in conformational entropy supposedly is the driving force for the protein to adsorb at the silica surface. LAM , as a hyperthermophilic protein having peculiar structural features [18, 19], does adsorb at the electrostatically unfavorable, hydrophilic silica surface without detectable change in secondary structure and, hence, without significant increase of its conformational entropy. Therewith, the adsorption behavior of this peculiar protein does not fit in the predictive scheme given in Table 1. In this context, it should be mentioned that the adsorption isotherm reflects very low affinity between LAM and silica.

It is remarkable, especially for those proteins that have undergone adsorption-induced structural rearrangements, that the same denaturation temperatures T_d are detected for the adsorbed and dissolved states. However, the enthalpy of denaturation $\Delta_d H$, expressed per unit mass of protein, is lower for the adsorbed proteins (except for LAM). The reduction of $\Delta_d H$ resulting from adsorption correlates with the extent of structural changes revealed by the CD spectra. A decreased enthalpy change at an unaltered denaturation temperature points to a structurally heterogeneous population of adsorbed protein molecules, *i.e.*, a fraction of the adsorbed molecules exists in a native-like structure (as in solution), whereas the remainder is in one or more adsorption-induced perturbed states (Norde and Zoungrana, 1998).

KEYNOTE PAPERS

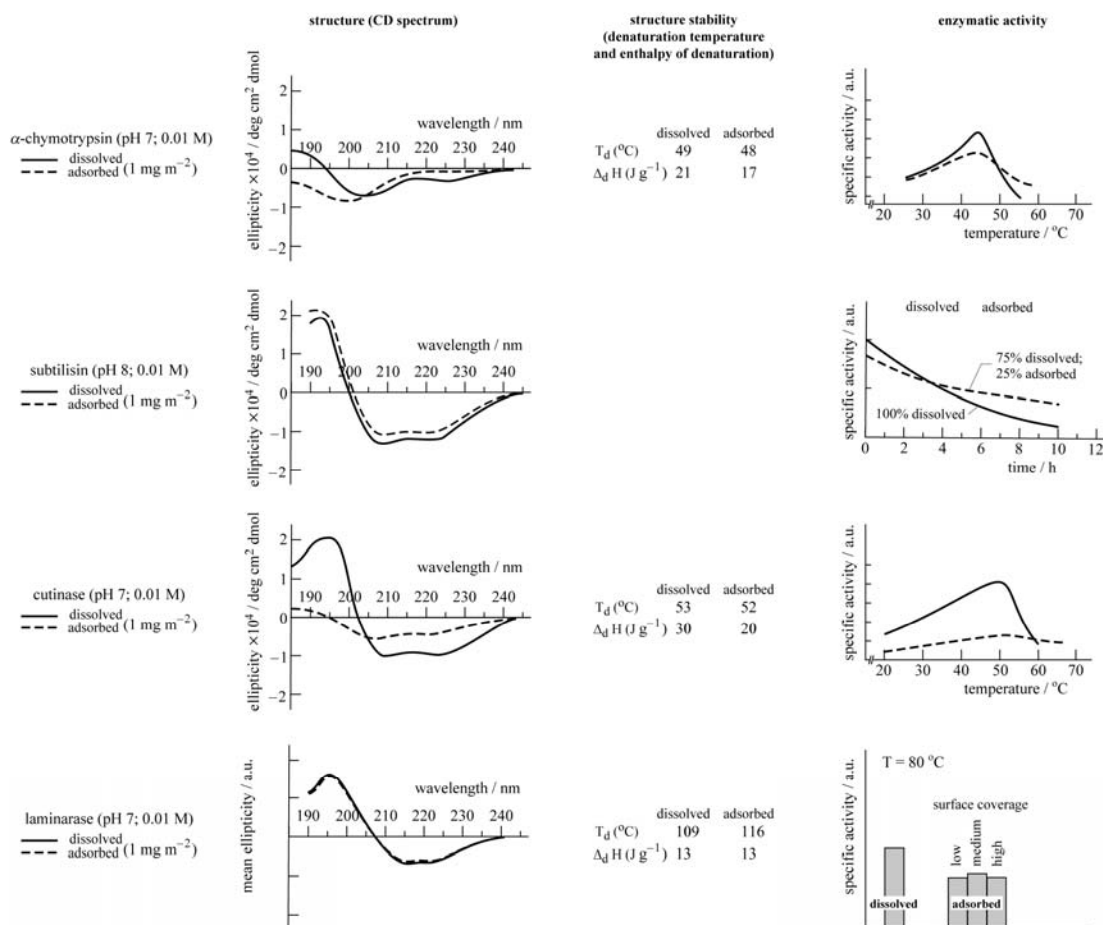


Figure 5. Effect of adsorption on the structure, structural stability and biological activity of various proteins. (Data collected from Norde and Zoungrana, 1998; Maste et al., 1995 and 1996; Koutsopoulos et al., 2005)

The impact of protein adsorption on the activity of these enzymes is more or less in line with their structural alterations. First, it is noted that in the activity assays of α CT, SUB and CUT relatively small substrate molecules were used, whereas the activity of LAM was determined using the polysaccharide laminarin. Hence, substrate inaccessibility of the protein's active site due to unfavorable orientation and crowding of the adsorbed molecules at the sorbent surface is expected to be higher for LAM than for the other proteins. Thus, the activity activity drop of LAM, by about 50%, may be attributed to random orientation of the adsorbed molecules, such that half of the molecules adsorb with their active site down towards the surface rendering them inaccessible for the polymeric substrate. Another interesting feature is observed for α CT and CUT: the specific enzymatic activity is much less sensitive to temperature variation, so that at elevated temperatures ($> 55^\circ\text{C}$) the specific

KEYNOTE PAPERS

activity in the adsorbed state may be higher than in solution. Finally, it is observed for SUB that adsorption protects the enzyme against proteolytic attack, a feature that may apply to other proteins as well.

Protein complexation with humic acids

As a model for humic substances in soil purified Aldrich humic acid (PAHA) is chosen (Vermeer et al., 1998). The binding of ions and surfactants to PAHA has shown to be similar to that to other humic acids (Milne et al., 2003, Ishiguro et al., 2007), but for protein binding such comparisons have not been made yet. The molar mass of PAHA is around 20 kDa. PAHA is an anionic polyelectrolyte, mainly due to dissociation of its carboxyl groups. The chemical structure of humic acid, according to Stevenson (Stevenson, 1994), is given in Figure 6. Lysozyme (LSZ) from hen's egg (see Table 2) was selected for studying binding to PAHA. Over a wide pH range, *i.e.*, $\text{pH} < 10.5$, LSZ is positively charged. The charges of PAHA and LSZ, as a function of pH, are given in Figure 7 for various ionic strengths. Upon adding PAHA and LSZ to each other they form a complex.

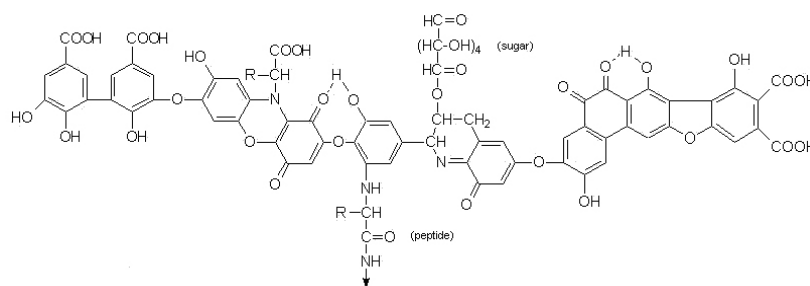


Figure 6. Model of the chemical structure of humic acid.

Complex formation is mainly driven by electrostatic attraction between the oppositely charged components. Figure 8 shows the amounts of PAHA needed to completely neutralize different amounts of LSZ (*i.e.*, forming an isoelectric complex), at pH 5. The required amount of PAHA is larger at higher KCl concentration. This is due to an increased cation (K^+) binding at the negative PAHA. The mass ratio PAHA/LSZ ($\equiv m/m_+$) in the isoelectric complex is reflected by the (constant) slope of the curves in Figure 8. The very small intercept at the PAHA-axis indicates that complexation between PAHA and LSZ occurs with high affinity. The isoelectric complexes are not reversible structures. Their composition depends on the path of formation, *i.e.*, addition of PAHA to LSZ or LSZ to PAHA, as shown in Figure 8 (right panel). The different compositions involve different structural stabilities of the neutral complexes: the complexes resulting from PAHA titrated by LSZ aggregate and precipitate after a few hours, but LSZ titrated by PAHA did not lead to complex aggregation over a prolonged period of time.

KEYNOTE PAPERS

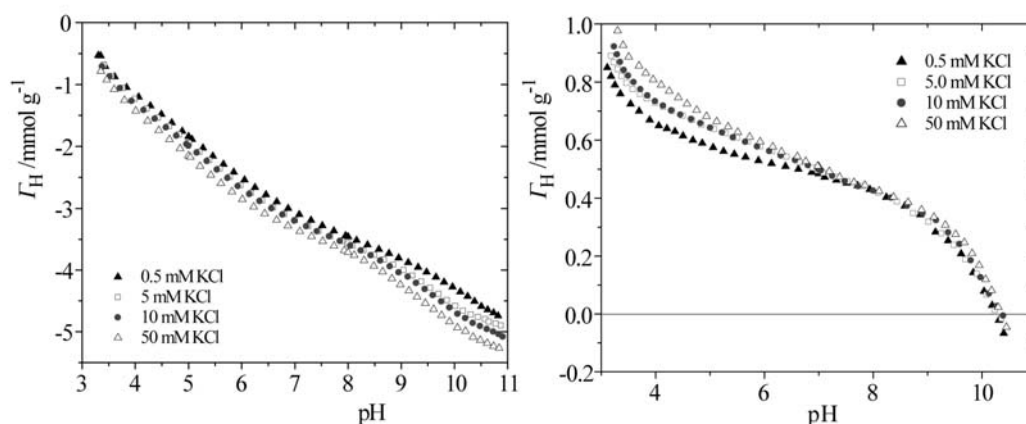


Figure 7. Proton binding on PAHA (left) and on LSZ (right) as a function of pH. (Redrawn from reference 6)

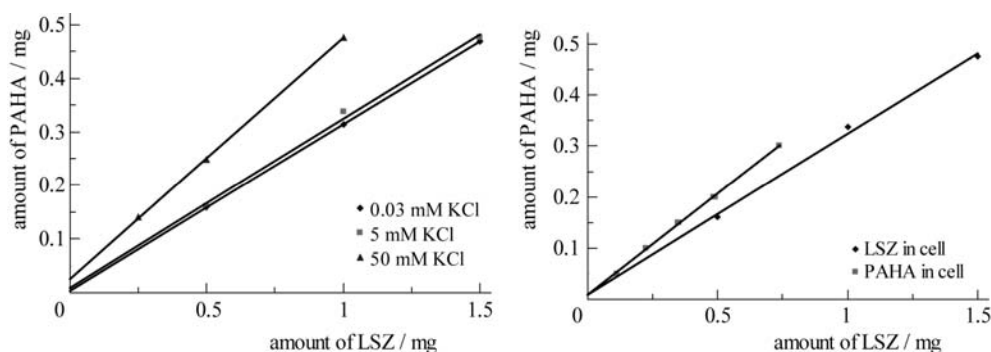


Figure 8. Amount of PAHA required to reach LSZ the isoelectric point of the LSZ-PAHA complex at pH5 at different ionic strengths (left) and for different sequences of adding the components to each other (right). (Redrawn from reference 6)

The size of the complexes was derived from dynamic light scattering, DLS, experiments. Figure 9 displays the hydrodynamic radius R_h of the complex, as a function of m/m_+ , at pH 5 and different ionic strengths. Clearly, complexes aggregate to larger structures when the iep is approached. The m/m_+ dependency of R_h is less pronounced at higher ionic strength, where electrostatic repulsion between the charged complexes is reduced. The a-symmetry of the R_h (m/m_+) curves implies that at equal absolute charge complexes aggregate into clusters of different sizes.

The calorimetrically established enthalpy (\approx energy) of complexation, where, at pH 5, PAHA is added to LSZ is shown in Figure 10. The negative enthalpy change is in line with favorable electrostatic interaction and this is corroborated by the reduced enthalpy change in a medium of higher salt concentration, where electrostatic interactions are screened. The first

KEYNOTE PAPERS

molecules of PAHA added bind to the energetically most favorable sites on LSZ. When approaching the iep of the complex, and beyond, the enthalpy effect is essentially zero. It implies that the binding is driven by an increase in entropy, which may (partly) be due to dehydration of hydrophobic patches of the PAHA and/or LSZ molecules. The R_h -data (Figure 9) show that overcompensation of charge induces fragmentation of the aggregates. This as well increases the entropy of the system. Experimental results for PAHA-LSZ interaction are, *mutatis mutandis*, similar to those reported for pH 5 [6, 7].

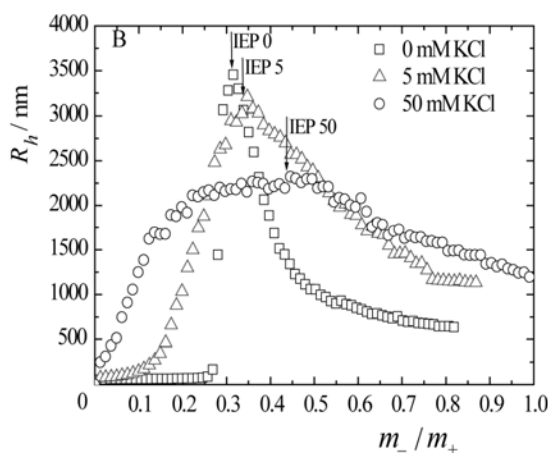


Figure 9. Hydrodynamic radius of PAHA-LSZ complexes at pH 5.

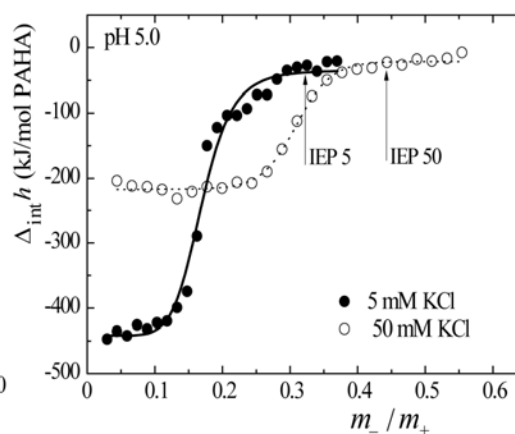


Figure 10. Enthalpy of interaction between PAHA and LSZ at pH 5.

The arrows point to the isoelectric points of the complexes. (Redrawn from reference 7)

In summary, the anionic PAHA and cationic LSZ complex with high affinity and, especially at and around the isoelectric state of the complex aggregates are formed. The features of this system are also expected to occur for other humic acid – cationic proteins. Moreover, since the driving force for the PAHA-LSZ interaction was found to be not only of electrostatic nature, negatively charged humic substances are expected to bind to some extent with negatively charged proteins as well. Complexation with humic substances probably influences the biological activity of the protein. Further, interaction between humic acid-protein complexes and mineral surfaces in the soil may lead to adsorption phenomena different from those observed when only humic acid or protein is present.

Keywords: Proteins; solid surfaces; humic acids.

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KEYNOTE PAPERS

K-7

Interaction with Soil Constituents Determines the Environmental Impact of Proteins**H. Quiquampoix****Biogéochimie du Sol et de la Rhizosphère, INRA, 2 Place Pierre Viala, 34060 Montpellier, France***E-mail: quiquampoix@montpellier.inra.fr*

Proteins are important compounds in soils, made up of monomeric amino acids joined by peptide bonds, and there are several reasons for studying their fate in soil: (i) their amino acids are an important source of soil nitrogen, (ii) enzymes secreted by soil microorganisms, plant roots and soil invertebrate guts can be involved in the biogeochemical cycles of soil organic matter (Quiquampoix, 2000; Quiquampoix and Mousain, 2005), (iii) pathogenic proteins such as prions involved in transmissible spongiform encephalopathies (Brown and Gajdusek, 1991; Revault et al., 2005) or insecticidal toxins expressed in transgenic plants (Tapp and Stotzky, 1998; Pagel-Wieder et al., 2004) represent a growing environmental concern.

Proteins generally have a strong affinity for all types of interfaces found in soil, both the solid-liquid interfaces of soil minerals and organic colloids and the liquid-gas interfaces developed in a microporous system and dependent on the soil pore-water content. The affinity for various types of interfaces originates in the flexibility of the polypeptide chain and in the diversity of the 20 amino acids that can be classified on an electrical scale as positively, neutrally or negatively charged and on a hydrophobic scale from polar to non-polar. These properties give rise to a large variety of interactions with soil surfaces, relationships that may be dominated by enthalpic or entropic contributions to the free energy. The strong and often largely irreversible adsorption of proteins on the mineral phase of the soil has important consequences not only on their mobility, but also on their resistance to breakdown (proteins as N source in soil) and functional activity (catalytic for enzymes, infectious for prions, toxic for Bt proteins).

Extracellular enzymes can be secreted into soil solution by microorganisms. This process makes possible the degradation of the soil organic matter since polymers cannot usually pass through the membranes of the microorganisms and need to be hydrolysed into soluble low molecular weight compounds which can reach membrane permeases specific for monomers (sugars, amino acids) or occasionally oligomers. For this reason, they will play an important role in the biogeochemical cycles of C, N, P and S in soil. A consequence of the adsorption of these extracellular enzymes on mineral surfaces is a shift of the optimal pH of the catalytic activity toward more alkaline values and a general decrease of their activity.

All proteins can make an important contribution as biochemical substrates in proteolysis reactions in the N cycle when released in soil after death of biota and lysis of the membranes since they have an average N content of 16%.

Prion proteins are an infective agent of several transmissible spongiform encephalopathies (TSE). Epidemiological studies of ovine scrapie in Europe and, more recently, the chronic wasting disease of wild cervids (deer, elk and moose) in North America, suggest the possible horizontal transmission from a soil reservoir.

KEYNOTE PAPERS

Insecticidal toxins, such as the Bt toxin, are associated with the beginning of the first large-scale commercial planting of transgenic crops. The use of transgenic crops expressing the Bt toxin suggests the possibility that the toxin could be released, survive and then accumulate in the soil when roots are degraded. This, in turn, may trigger the development of resistance towards targeted, pathogenic soil invertebrates and have inhibitory or even lethal effects on non-target, beneficial invertebrates.

All these different proteins released in soil may exhibit a wide range of types of interactions with the mineral surfaces or the organo-mineral complexes. Nevertheless some general tendencies may be identified.

Influence of pH on adsorption of proteins on clay minerals

Since the pioneering works of Mc Laren et al (1958) it is well established that for most proteins the maximum of adsorption occurs near their isoelectric point (i.e.p.). Early interpretations of this fact included, for $\text{pH} > \text{i.e.p.}$ a repulsive electrostatic interaction between the negatively charged protein and the negatively charged clay surface, and for $\text{pH} < \text{i.e.p.}$, either a competition of the protons of the solution for adsorption sites (Mc Laren et al., 1958), or a decrease in the amount of protein required to satisfy the negative charge of the clay since the net positive charge of the protein increases as pH decreases (Armstrong and Chesters, 1964). Alternatively the possibility of an artefact resulting from protein precipitation, more likely to occur at the i.e.p., has been proposed (Durand, 1964).

More recently the fact that the adsorption of proteins on clay mineral surfaces is accompanied by a release of charge compensating cations (McLaren et al., 1958; Albert and Harter, 1973) has been exploited to obtain both the clay surface coverage and the quantity of protein adsorbed, which has led to a different interpretation (Quiquampoix and Ratcliffe, 1992). The latter study is based on the detection by nuclear magnetic resonance (NMR) spectroscopy of the release of a paramagnetic cation, manganese, on adsorption of bovine serum albumin (BSA) on montmorillonite. The fraction of Mn^{2+} released is assimilated to the fraction of the clay surface covered by the protein. A maximum adsorption occurs near pH 4.7 which is the i.e.p. of BSA, but the quantity of Mn^{2+} released show a different pattern. Above the i.e.p., both the quantity of protein adsorbed and the quantity of Mn^{2+} released decrease in the same proportion when pH increases, indicating a lowering of the surface coverage of the clay surface and confirming the early interpretation of electrostatic repulsions. But below the i.e.p. the quantity of Mn^{2+} released remains constant when the quantity of protein adsorbed decreases, indicating an unfolding which increases the specific interfacial area of the protein as pH decreases.

Structural studies of adsorbed proteins

Studies on the quantity of protein adsorbed on surfaces cannot be separated from the study of the conformation adopted by the proteins on these surfaces. The reason is that a modification towards a more disordered structure contributes to the driving forces of adsorption, since it increases the entropy of the system and thus decreases the Gibbs energy. The modification of conformation also can have an effect on the maximum quantity of

KEYNOTE PAPERS

protein adsorbed, since a modification of conformation may affect the area occupied by each single protein the surface.

The spontaneous adsorption of proteins at constant temperature and pressure leads to a decrease of the Gibbs energy of the system, according to the second law of thermodynamics (Norde and Lyklema, 1991; Haynes and Norde, 1994). The Gibbs energy, G , depends on enthalpy, H , which is a measure of the potential energy (energy that has to be supplied to separate the molecular constituents from one another), and entropy, S , which is related to the disorder of the system.

$$\Delta_{\text{ads}}G = \Delta_{\text{ads}}H - T\Delta_{\text{ads}}S < 0$$

with T being the absolute temperature and α_{ads} being the change in the thermodynamic functions resulting from adsorption.

Some difficulties arise in the analysis of these processes because enthalpic effects, related to intermolecular forces, and entropic effects, related to the spatial arrangements of molecules, are not totally independent. Intermolecular forces influence the distribution of molecules, and the potential energy also is also dependent on the molecular structure of the system.

The entropic contribution to adsorption can result from a modification of the conformation of the protein. This phenomenon is related to an increase of the rotational freedom of the peptide bonds engaged in secondary structures, such α -helices and β -sheets. The ordered secondary structures are an important part of the densely packed hydrophobic core of proteins. After adsorption, internal hydrophobic amino acids can reach more external positions in contact with the surface, since the amino acids remain shielded from contact with the water molecules of the surrounding solvent phase. If a decrease of internal ordered secondary structures accompanies this process, it will result in an increase of conformational entropy. The gain of conformational entropy, S_{conf} , can be calculated from the assumption that four different conformations are possible for peptide units in random structures as compared with only one in α -helices and β -sheets:

$$\Delta_{\text{ads}} S_{\text{conf}} = R \ln 4^n$$

where R is the molar gas constant and n is the number of peptide units involved in the transfer from an ordered secondary structure to a random secondary structure (Norde and Lyklema, 1991; Haynes and Norde, 1994).

No method currently exists which enables the direct measurement of the conformation of proteins in an adsorbed state. Only two methods are suitable for the determination of the tertiary structure of the proteins, and neither can be employed when proteins are adsorbed. One method, X-ray diffraction, necessitates the preparation of protein crystals, which is impossible for adsorbed proteins. The other, NMR spectroscopy, is confined to molecules with a sufficiently high tumbling rate to obtain narrow line widths, a condition not compatible with the adsorption on a surface larger in dimensions than the protein itself. In addition the determination of a tertiary structure has a sense only if all the protein molecules

KEYNOTE PAPERS

have the same structure. In reality it seems probable that the proteins are adsorbed in multiple states (Horbett and Brash, 1987).

For these reasons the secondary structures (α -helices, β -sheets, random parts) are probably the higher order structures on which information can be obtained. Circular dichroism (Kondo et al., 1991) and infrared spectroscopy (Tarasevich et al., 1975; Quiquampoix et al., 1993; Baron et al., 1999; Servagent-Noinville et al., 2000; Noinville et al., 2004) have been applied to the resolution of these structures for proteins adsorbed on mineral surfaces. Circular dichroism can be used only for the study of adsorption on very small mineral particles due to problems arising from light scattering effects. Kondo et al. (1991) have studied by this technique the modification in α -helix content of proteins adsorbed on ultrafine silica particles (diameter of 15 nm) and found a greater decrease for the proteins whose adiabatic compressibility is high, i.e. whose flexibility of the structure is high in water. For example BSA retained only 60 to 80% of its native α -helix content, depending on pH. Infrared spectroscopy does not suffer from light scattering perturbations and has thus been used for the study of the adsorption of BSA on montmorillonite. A decrease in the α -helix content and an increase in intermolecular β -sheet content have been observed for the BSA by FTIR investigations (Servagent-Noinville et al., 2000; Quiquampoix et al., 2002). But the adsorption of α -chymotrypsin on montmorillonite has only a very small effect on the secondary structure of this protein (Baron et al., 1999).

Intercalation of proteins between clay sheets

The suspected protective effect of protein intercalation in clay interlayer position (Loll and Bollag, 1983) has led to a vast number of studies by X-ray diffraction on d (001) spacing of clay-protein complexes (Mc Laren et al., 1958; Armstrong and Chesters, 1964; Albert and Harter, 1973; Harter and Stotzky, 1973). Early interpretations of these results were that the proteins could penetrate between the interlayer spaces by a process of lateral diffusion (McLaren et al., 1958). However such a process is not compatible with the strong, largely irreversible aspect of protein adsorption which implies large activation energy for surface diffusion (Norde, 1986). A more convincing process of intercalation has been proposed (Larsson and Siffert, 1983), where adsorption of proteins occurs on the external surfaces of the clay, but the shear stress induced by the stirring of the suspension may induce an opening of tactoids exposing a new clay surface which is then free to interact with a clay surface already bearing a protein monolayer.

Consequences of electrostatic interactions on enzyme activity

Two main hypotheses have been proposed to explain the shift of the optimal pH of the catalytic activity of enzymes adsorbed on negatively charged surfaces such as clay minerals.

The first hypothesis considers that the pH in the region of the active site of the adsorbed enzyme is lower than the pH in the bulk of the solution which is effectively measured with a glass electrode and this explains the observed shift (Mc Laren and Estermann, 1957; Durand, 1964; Goldstein *et al.*, 1964; Aliev *et al.*, 1976; Douzou and Petsko, 1984). Indeed the negative charge originating in the isomorphic substitution in the crystalline lattice of the clay is compensated by cations, including protons, which make up a diffuse double layer, and thus

KEYNOTE PAPERS

the activity of the protons near the surface is higher than in the bulk. But this hypothesis has three serious drawbacks.

1. The shift in the optimal pH of activity should give rise to a higher rate of catalytic activity for the adsorbed enzyme than for the enzyme in solution in the alkaline range of pH. This has never been observed. When the absolute values of catalytic activity are reported, they invariably show that the values for the adsorbed enzyme are contained in the envelope of the values for the enzyme in solution (Aliev et al. 1976; Quiquampoix, 1987a; Quiquampoix, 1987b; Quiquampoix et al., 1989; Leprince and Quiquampoix, 1996). A misleading presentation of the results which is partly responsible for the popularity of this hypothesis in soil science and biotechnology is the normalization of enzyme activity values to the maximum value attained for each case, free and bound (Mc Laren and Estermann, 1957; Durand, 1964; Goldstein *et al.*, 1964; Douzou and Petsko, 1984). This masks the occurrence of the general decrease in catalytic activity.

2. The hypothesis implies that the conformation of the adsorbed enzyme is similar to the conformation of enzyme in solution and can act as a "molecular pH-meter". Ample evidence to the contrary has been presented above.

3. Finally the basis of this theory is far from being assured since, as some authors have pointed out (Rouxhet, 1990; Rouxhet and Mozes, 1990; Fletcher, 1991), the tendency of a proton to react with the active site of the enzyme is not given by the proton activity but by its molar free enthalpy, namely its electrochemical potential:

$$\mu = \mu_0 + RT \ln [H^+] + F\psi$$

And since the system is in a state of thermodynamical equilibrium, the molar free enthalpy of the proton is the same in the bulk of the solution and at the clay surface.

The second hypothesis is based on evidence of pH dependent modifications of protein conformation. An *Aspergillus niger* β -D-glucosidase exhibits decreasing relative activity, R, defined as the ratio of activity in the adsorbed state and activity of an equal amount in solution, with decreasing pH. The attractive electrostatic interactions between the positively charged protein and the negatively charged clay surface, which lead to the unfolding of the enzyme, play a major role. The fraction of the enzyme which is not adsorbed, F, increases with increasing pH. Again electrostatic forces are the main determinant of this behaviour, but this time between a negatively charged enzyme and the negatively charged clay surface. The *Aspergillus niger* β -D-glucosidase is an example of enzyme for which electrostatic interactions probably completely regulate the interactions with clay surfaces.

Irreversibility of the structural alteration of adsorbed enzymes

Further evidence for the theory of the pH-dependent modification of conformation of adsorbed enzymes which relies purely on catalytic activity measurements, and which is independent of the information obtained by the physical methods presented above, can be obtained. An example is the effect of the adsorption of an enzyme at a given pH followed by the measurement of its catalytic activity on a wider range of pH. It can be observed that, the lower the pH of adsorption on montmorillonite, the lower is the measured catalytic activity of

KEYNOTE PAPERS

the enzyme at a given pH. This result cannot be explained by the surface pH effect since, according to this theory; no lasting effect of the pH at the moment of adsorption should be detected when the catalytic activity is measured at another pH. The change in activity cannot therefore be due to a modification of local pH.

Conversely, a modification of conformation is compatible with this observation, since the higher extent of unfolding at the lower pH values creates a higher number of contact points between the protein and the clay surface. The consequence is that, in order to return to its original conformation, the adsorbed enzyme would require an energy of activation corresponding to the energy of adsorption of all the additional amino acids brought into contact with the solid surface. It is likely that this activation energy is higher than the thermal energy available to the system.

Effect of the hydrophobicity/hydrophilicity of the surfaces on enzyme activity

Adsorption of proteins on artificial organic surfaces is known to involve hydrophobic interactions (Norde, 1986). The occurrence of this type of interaction can also be shown on some mineral surfaces. The destabilizing effect of different surfaces on a sweet almond β -D-glucosidase conformation can be estimated by the effect on its relative activity R. It can be observed that the minimal destabilization of the enzyme structure is obtained with adsorption on goethite in a citrate buffer (Quiquampoix, 1987a). This surface is hydrophilic and with a low electric charge due to the complexation of the citric acid with the hydroxyls of the oxide surface. The occurrence of hydrophobic interactions is shown by the larger destabilizing effect of the uncharged hydrophobic talc surface (Quiquampoix et al., 1989). Compared to the goethite and talc surfaces, the negatively charged surface of montmorillonite confirms the strength of electrostatic interactions since the most important denaturation of the enzyme structure is observed on this surface at pH below 4 when the enzyme bears a net positive charge.

An interesting additional observation is that the effect of an increase of the ionic strength does not suppress the unfolding of the enzyme at pH below 4, as could be expected for an electrostatic interaction. The absence of an effect of the ionic strength on the interaction between a cationic polymer and a negatively charged surface has been observed with the adsorption of ammonium substituted galactomannans (Gu and Doner, 1992) on illite surfaces and has been attributed to a surface charge neutralization of the negative charge of the illite which is independent of the ionic strength. In contrast the anionic carboxyl substituted galactomannans (Gu and Doner, 1992) show an adsorption behavior dependent on the ionic strength, like the adsorption of the sweet almond β -D-glucosidase above its i.e.p. (Quiquampoix, 1987a). Thus attractive and repulsive electrostatic interactions between polymers and clay surfaces seem to show different sensitivities to the ionic strength.

Finally the relative activity of the enzyme on montmorillonite at high ionic strength and high pH is similar to that observed on the hydrophobic talc. The decrease in the repulsive electrostatic interactions and the fact that the hydrophilic nature of the montmorillonite surface is due to the hydration properties of the exchangeable cations, and not to the siloxane surface which is hydrophobic (Chassin et al., 1986; Skipper et al., 1989; Bleam, 1990; Jaynes and Boyd, 1991), are two factors which may explain this convergence. Additional evidence for the occurrence of hydrophobic interactions between proteins and montmorillonite surfaces

KEYNOTE PAPERS

is the increase in adsorption when proteins are methylated (Staunton and Quiquampoix, 1994).

Interfacial competition of adsorption of enzymes on natural clay-humic complexes

The real situation in soil is not represented by the presence of "clean" uncoated mineral surfaces as adsorbent surfaces for enzymes, but by the presence of organo-mineral complexes, and above all clay-humic complexes (Theng, 1979; Fusi et al., 1989; Rao et al, 1996). The heterogeneity of the soil organic matter is such that the study of better defined artificial clay-organic complexes is helpful to understand the mechanisms implied.

The effect of different clay-organic complexes on the relative activity of a sweet almond β -D glucosidase can illustrate the interfacial competition for the mineral surfaces. The lysozyme-montmorillonite complex involves a protein with a high i.e.p. (11.7). For this reason it is strongly held on the clay surface and renders the surface electropositive. As a result there is no evidence of the unfolding observed with the uncoated montmorillonite surface, which was caused by attractive electrostatic interactions. The enzyme appears to unfold on the polyethylene glycol-montmorillonite complex at low pH, as on the uncoated clay surface. This occurs because the adsorption of the positively charged enzyme on the electronegative clay surface is energetically more favourable than that of polyethylene glycol which is thus displaced. As the pH increases the enzyme loses its positive charge and hence its ability to displace the polyethylene glycol. The higher relative activity in this pH range may be explained by a reduction in the destabilizing hydrophobic interactions because a hydrated polymer layer is intercalated between the enzyme and the surface (Quiquampoix, 1987b).

A natural clay-humic fraction (mineral fraction composed of smectite, interstratified minerals, illite and kaolinite; organic fraction composed of 21% fulvic acid, 29% humic acid and 50% humin) shows an intermediate destabilizing effect on the enzyme. It has been proposed that there is a lower energy of interaction for some of the natural humic substances with the clay surfaces, which are thus more easily exchanged than polyethylene glycol (Quiquampoix, 1987b). Thus the interaction of an enzyme with a mixture of several natural clay minerals, coated with poorly defined natural organic matter, can be adequately explained by taking in account of electrostatic interactions, hydrophobic interactions and interfacial competition of adsorption observed on a very simple model such as a polyethylene glycol-montmorillonite complex.

Conclusions

The adsorption of enzymes on mineral surfaces is a complex phenomenon which involves both enthalpic and entropic effects. An important and difficult question is the determination of possible changes of conformation of the adsorbed enzyme. NMR and FTIR spectroscopies are useful tools to answer this question since they respectively give information on the interfacial area of the surface of contact of protein-clay and on the secondary structure of adsorbed proteins. It has been shown that both pH-dependent modification of conformation and pH-dependent orientation of the catalytic site of the enzyme can explain the alkaline pH shift of the enzyme activity on electronegative soil mineral surfaces. Soft proteins, such as

KEYNOTE PAPERS

BSA, are more prone to the first mechanism, whereas hard proteins, such as α -chymotrypsin are more prone to the second. In addition to electrostatic forces, hydrophobic interactions are also implied in the interaction of proteins with clays. An important aspect is the interplay between different driving forces in adsorption. For example, the hydrophobic interactions with clays can result from an electrostatic exchange of the hydrophilic counterions on the clay surface leaving a hydrophobic siloxane surface. The rearrangement of the protein structure on the clay surface subsequently can be facilitated when hydrophobic amino acids come in contact with the hydrophobic siloxane layer and remain shielded from the water molecules of the solution. If this rearrangement is accompanied by a decrease of ordered secondary structures, it would result in an additional increase in conformational entropy, lowering the Gibbs energy of the system. The combination of all these different subprocesses is responsible for the irreversible aspects of the modifications of conformation of enzymes on clay surfaces.

The concepts which have been introduced in this text are more developed in other publications (Quiquampoix, 2000; Quiquampoix et al., 2002; Quiquampoix and Burns, 2007; Quiquampoix, 2008).

Keywords: Proteins; enzymes; clay-humic complexes.

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KEYNOTE PAPERS

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KEYNOTE PAPERS

K-8

Bioavailability of Phosphorus and Micronutrients in the Soil-Plant-Microbe Continuum**Z. Rengel****Soil Science and Plant Nutrition, School of Earth and Geographical Sciences, University of Western Australia, M087, Crawley WA 6009, Australia. *E-mail: Zed.Rengel@uwa.edu.au***Introduction**

An increasing need to produce food for the expanding world population creates significant pressure on suitable land already in production and requires continuous expansion of food-producing ecosystems into less fertile areas. In every such food-producing system, crops and pastures must be provided with sufficient nutrients for vigorous growth and high outputs, putting an emphasis on understanding soil-plant microbe interactions governing nutrient acquisition by plants. This review will summarise the available knowledge on relevant interactions underlying plant acquisition of P and micronutrients (with an emphasis on Mn).

Soils resulting in P and micronutrient deficiency in crops and pastures are abundant in the world, but such nutrient deficiency arises from poor P and micronutrient mobility rather than low total amounts present in soil (Rengel, 2001). Hence, the plant-available nutrient fraction and the concentration in the soil solution may be insufficient to satisfy plant requirements (Jorquera et al., 2008; Rengel and Marschner, 2005).

Around 90% of the total P use in the world today is for food production (Jasinski, 2006). Hence, modern agricultural systems are dependent on continual inputs of P fertilizers processed from phosphate rock. Yet, the world reserves of phosphate rock are becoming increasingly scarce, and estimates are they will be depleted within 50-100 years, with a global peak in usage of P reserves occurring by 2040 (Jasinski, 2006). While the exact timing might be disputed, it is widely accepted that the quality of P rock is decreasing and cost increasing (indeed, the price of phosphate rock has risen 7-fold in the 14 months since Feb 2007) (Cordell, 2008).

The rhizosphere

The rhizosphere (a layer of soil around the root that is influenced by the root) extends up to a few millimetres from the root surface into the surrounding soil. Bioavailability of P and micronutrients in the rhizosphere is controlled by soil properties, plant characteristics, and the interactions of plant roots with microorganisms (Rengel and Marschner, 2005).

The fluxes of organic anions exuded into the rhizosphere by roots were rather small in comparison with the flux of H^+ or OH^-/HCO_3^- (Hinsinger, 2001). The balance between excretion of H^+ and OH^-/HCO_3^- depends on the cation/anion uptake ratio. Greater excretion of H^+ (accompanying greater absorption of cations than anions) results in rhizosphere acidification; the reverse occurs when uptake of anions exceeds that of cations, with excretion of OH^-/HCO_3^- exceeding that of H^+ (Tang and Rengel, 2003). Solubility of micronutrient-

KEYNOTE PAPERS

containing and Ca-P complexes increases with increasing soil acidity. Solubility of Zn increases 100-fold with each unit decrease in pH (Rengel, 2001).

For diffusion-supplied micronutrients, larger amounts are transported toward roots if a large concentration gradient between the root surface and the bulk soil can be maintained by vigorous nutrient uptake at the root surface. However, when a capacity of root cells to take up nutrients exceeds the rate of nutrient replenishment at the root surface, the uptake rate is governed by the nutrient supply rather than by the capacity of plants to take up nutrients (Rengel, 1993). Therefore, an increased capacity of root cells to take up nutrients is expected to be of secondary importance as an efficiency mechanism for diffusion-supplied micronutrients, with a greater effect achieved by increasing the plant capacity to exude chelating and other agents into the rhizosphere, resulting in increased solubilisation and conversion of nutrients into plant-available forms.

Plants exude a variety of organic compounds (carboxylate anions, phenolics, carbohydrates, amino acids, enzymes, other proteins, etc.) and inorganic ions (protons, phosphate and other nutrients, etc.) into the rhizosphere to change chemistry and biology of rhizosphere and enhance adaptation to a particular environment (Crowley and Rengel, 1999). Complete understanding of complex interactions governing the relationship of quantity and differential composition of root exudates with soil properties as well as plant genotype and phenotype is still far away. One of the reasons for such a statement stems from inadequate experimental methods to assess spatial and temporal variability in root exudation as well as to follow the fate of various organic and inorganic compounds exuded from roots in soil (cf. Shen et al., 2003) and their differential effectiveness in increasing availability of soil P and micronutrients (Rengel, 2002). The interactions between microorganisms and plants at the soil-root interface add additional layers of complexity.

Physiological traits important in P-use efficiency

In acidic soils, P is fixed in either Al or Fe complexes, whereas in calcareous soils of higher pH it is Ca phosphate complexes. In addition, there is a large pool of organic P in most soils (can be as high as 80% of the total P) (Hinsinger, 2001). Enhanced acquisition of P from soils relies on morphological, physiological, biochemical and molecular adaptations (eg. Lambers et al., 2006; eg. Liu et al., 2005; Marschner et al., 2006; 2007; Nuruzzaman et al., 2006; Shu et al., 2007b; Wang et al., 2007b). P-use efficiency may be underpinned by increased capacity to 1) transform non-available P forms into plant-available ones, 2) explore a larger soil volume more thoroughly, and/or 3) transport P into the root cells.

Given differences in P fractions in acid vs neutral and alkaline soils, mechanisms allowing plants to access sufficient P for growth might differ among soils. However, for wheat grown in either neutral or acidic soils, P acquisition was dependent on extensive root exploitation and high phosphatase activity in the rhizosphere (especially alkaline phosphatase and diesterase, indicating microbial facilitation of organic P mineralisation) (Marschner et al., 2005b).

KEYNOTE PAPERS

1. Root morphology

Plants growing in P-deficient soil allocate a greater proportion of assimilates to root growth and tend to have fine roots of a small diameter and therefore a large surface area. P-efficient barley (Gahoonia et al., 2001) and cowpea cultivars (Krasilnikoff et al., 2003) have longer root hairs allowing them to take up more P in comparison with P-inefficient genotypes. P-deficient *Lupinus angustifolius* increased the primary root elongation and developed a large number of the cluster-like first-order lateral roots with dense root hairs, thus allowing efficient P acquisition under low P supply (Wang et al., 2008). Fine roots and especially root hairs effectively scavenge P from soils because of a large surface area of contact with the soil.

The shoot P status may regulate the formation of cluster roots, as specialised structures of selected plant species for thorough exploration of the soil volume (Lambers et al., 2006; Shu et al., 2007b). However, the form of P in soils may also regulate cluster root formation (Shu et al., 2007a; Shu et al., 2007b). In addition, the development of cluster roots can respond to a presence of organic matter adjacent to the root (Adams and Pate 1992).

2. Exudation of organic compounds

Under P deficiency, plants exude a wide range of organic compounds (carboxylates, enzymes, phenolics, etc.) to increase mobilisation of P from sparingly soluble sources (eg. Neumann and Römheld, 1999). Typical carboxylates (organic acid anions) found in root exudates of P-deficient plants include citrate, malate, malonate, acetate, fumarate, succinate, lactate and oxalate (see Rengel, 2002). In barley, P-use efficiency may be linked to the capacity of genotypes to increase exudation of citrate as an organic acid anion with a strong capacity to mobilise P (Gahoonia et al., 2000). Interestingly, citrate exudation by *Lupinus albus* roots increased only due to localized Fe-P application, but not when other P sources were applied (Shu et al., 2007a; Shu et al., 2007b).

Carboxylates may be exuded by P-deficient roots at appreciable rates [an average rate of 0.57 nmol citrate cm⁻¹ root h⁻¹ for *Brassica napus* (Hoffland et al., 1989) or 200-400 nmol oxalate g⁻¹ soil h⁻¹ by *Cassia spectabilis*, with rhizosphere soil containing at least 29 µmol oxalate g⁻¹ soil (Radersma and Grierson, 2004)]. Exuded carboxylate anions may have a role in solubilisation of mineral nutrients and as growth substrates for microorganisms. Because carboxylates are excellent substrates for microbial growth, high concentrations of carboxylates may occur only temporarily and only at rapidly growing root apices not yet densely colonised by microorganisms.

Plants and microorganisms increase exudation of P-hydrolysing enzymes under P deficiency. These enzymes break down organic P, thus making P available for uptake. Phytase specifically catalyses the break-down of phytate, the major form of organic P in soils (Rengel and Marschner 2005). Roots excrete little, if any, phytase, whereas microorganisms (eg. *Aspergillus niger*) exude large amounts (Richardson et al., 2001), indirectly enabling plants to utilise phytate (Osborne and Rengel, 2002). Genetically modifying plants to excrete microbial phytase (eg. George et al., 2005) may allow plants to increase P uptake, but effectiveness of phytase is limited by the low phytate availability in soil and binding of phytase to soil particles.

KEYNOTE PAPERS

Exudation of phosphatases increases when plants are P deficient (eg. Radersma and Grierson, 2004). When grown in an acidic P-deficient soil amended with Fe-P, the P-efficient *Triticum aestivum* genotype had a greater acid phosphatase activity in the rhizosphere than the inefficient genotype, with phosphatase activity correlating positively with growth and P uptake (Marschner et al., 2005b; 2006).

3. Rhizosphere microorganisms and nutrient availability

Root exudates are good nutrient source for microorganisms, allowing some microbial species, especially those with high growth rates and relatively high nutrient requirements such as pseudomonads (Marilley and Aragno, 1999), to proliferate rapidly in the rhizosphere. The amount and composition of root exudates affect microbial community composition which in turn will influence nutrient availability.

Plants grown with deficient vs. sufficient nutrient supply often have differential microbial communities in the rhizosphere (eg. Marschner et al., 2004; Marschner et al., 2005b; 2006; 2007). Nutrient deficiency can influence rhizosphere microorganisms either directly (by affecting their nutrition) or indirectly (via altering root morphology and exudation) (Rengel and Marschner, 2005). In addition, rhizosphere soil of different plant species shows differential composition and abundance of microbial populations (eg. Ponnuragan and Gopi, 2006). However, roots may maintain distinct rhizosphere microbial communities even when intermingling with roots of other species (Wang et al. 2007a).

Microbial community composition is influenced by soil properties as well as P addition (Marschner et al. 2006; Solaiman et al., 2007) and other management factors (Marschner et al., 2005b; Steenwerth et al., 2008; Steenwerth et al., 2003), with agricultural intensification resulting in decreased microbial diversity and lowering of ecosystem function (Steenwerth et al., 2005). Differential structure of microbial communities was also noted for different plant genotypes and different growth stages (Marschner et al., 2006; Solaiman et al., 2007). For example, the microbial community composition in the rhizosphere of the native Australian grass *Austrostipa* differed significantly from that of the two wheat genotypes, and was characterised by a high abundance of the fungal fatty acid 18:2 ω 6 (Marschner et al., 2006).

Genotypic differences in the rhizosphere microbial community composition may possibly be due to differences in root exudation (chemical type and the amount). Indeed, it has been shown recently that organic acid anions in the *Lupinus albus* cluster root exudates can affect soil microbial community composition in the rhizosphere (Marschner et al., 2002), with an addition of artificial root exudates also showing an effect (Baudoin et al., 2003).

In contrast to *Poaceae* genotypes grown in a soil with neutral pH where differential microbial composition of the rhizosphere appeared important for differential capacity of genotypes to acquire P (explaining 54% of the variation in plant growth and P uptake, Marschner et al., 2006), in *Brassicaceae* genotypes (grown in the same soil) root length and P mobilisation in the rhizosphere explained differential P acquisition, with microbial communities in the rhizosphere appearing to play only a minor role (Marschner et al., 2007). However, these relationships were dependent on soil properties, especially pH (Solaiman et al., 2007). In the acidic soil, while the microbial community composition in the rhizosphere of wheat differed from that in *Brassicas* (Wang et al., 2007b), the microbial P concentrations were in the same range in the rhizosphere of *Brassicaceae* and *Poaceae*, suggesting that

KEYNOTE PAPERS

glucosinolate release by *Brassicac*s (followed by formation of isothiocyanates) may not necessarily have a negative effect on microbial activity in the rhizosphere (Marschner et al., 2007).

Many microbial species have the capacity to solubilise sparingly soluble P *in vitro* (Rengel and Marschner, 2005; Whitelaw, 2000). Phytate- and phosphate-solubilising bacteria have been identified, with the genus *Pseudomonas* being one of the most studied P-solubilising bacteria (eg. Jorquera et al., 2008; eg. Peix et al., 2003; Peix et al., 2004).

About half of culturable rhizobacteria associated with perennial ryegrass, white clover, oat and wheat were capable of solubilising P-containing compounds. The rhizosphere of pasture plants (perennial ryegrass and white clover) contained predominantly Na-phytate solubilisers, whereas in the rhizosphere of crops (oat and wheat) bacteria solubilising Ca-phosphate were more prevalent than those solubilising Na-phytate (Jorquera et al., 2008).

An effective interaction between P solubilisers and plants depends on (i) high population of P solubilisers maintained in the rhizosphere over long periods, (ii) exudation of carboxylates and protons into the rhizosphere by roots and microorganisms, (iii) low P uptake by microorganisms, and (iv) positive interaction with mycorrhizal fungi or other beneficial microorganisms.

P-solubilising bacteria could potentially be used as biofertilizers (see the references in Deubel and Merbach, 2005; Jorquera et al., 2008; Rengel and Marschner, 2005). However, large-scale inoculation with P solubilisers in farming practice is hampered by several factors that could diminish effectiveness of the introduced microorganisms: (i) most soils already contain P solubilisers, so the effect of inoculation may be small, (ii) introduced strains may have poor survival in the rhizosphere due to low competitiveness against indigenous, well-adapted strains, (iii) microorganisms are selected based on their P solubilisation *in vitro* in conditions ideal for growth and P solubilisation, whereas conditions in the rhizosphere may be far from optimal, and (iv) P solubilised by the microorganisms may be unavailable to plants because microorganisms take it up (Crowley and Rengel, 1999; Rengel and Marschner, 2005). It is of utmost importance that the possible contribution of P-solubilising microorganisms to crop P uptake be evaluated in realistic soil conditions in the field (cf. Jones et al. 2004) because literature abounds in reports on *in vitro* solubilisation of P that could not be repeated in field conditions (see Gyaneshwar et al., 2002).

Manganese availability in the rhizosphere

Yield of crops and pastures on calcareous soils is frequently limited by Mn deficiency caused by low Mn availability, rather than low Mn content in soil (Rengel 2000). The available Mn concentration was up to two orders of magnitude greater in the rhizosphere of three *Banksia* species (*B. attenuata*, *B. ilicifolia* and *B. menziesii*) than in bulk soil (Marschner et al., 2005a). An addition of 500 $\mu\text{g MnO}_2 \text{ g}^{-1}$ soil before incubation doubled the available Mn concentration to 4 $\mu\text{g Mn g}^{-1}$ soil. After 7 days incubation, the concentration of available Mn increased more than 10-fold, indicating active populations of Mn reducers (P. Marschner, unpublished).

Medicago sativa plants exude a variety of carboxylates under Mn deficiency. The amounts of exuded citrate and malonate (and to a lesser extent fumarate, malate, oxalate and lactate)

KEYNOTE PAPERS

under Mn deficiency were positively correlated with Mn efficiency of *M. sativa* genotypes (Gherardi and Rengel, 2003).

Manganese availability is increased in acidic rhizosphere. However, the form of N supplied, and therefore differences in rhizosphere acidification, had no effect on differential expression of Mn efficiency among *Hordeum vulgare* genotypes (see Rengel 2001) grown in calcareous soils. Strong pH buffering capacity of calcareous soils may contribute to preventing differential expression of Mn efficiency (eg. Tong et al., 1997).

Reduction and oxidation of Mn by microorganisms are important components of Mn cycling in soil. Fluorescent pseudomonads are effective Mn reducers, which appear to be more abundant in the rhizosphere of some Mn-efficient compared with Mn-inefficient *Triticum aestivum* genotypes (Rengel et al., 1998).

The bacterial communities in the *Triticum aestivum* rhizosphere were correlated with the concentration of DTPA-extractable Mn in the rhizosphere, shoot dry matter and Mn content (Marschner et al., 2003), suggesting the importance of microorganisms in plant Mn uptake.

Future work

More research into understanding the basis of qualitative and quantitative differences in root exudation is required. Given that exudation of organic compounds represents a big drain of energy and resources, thorough understanding of the regulation of the whole sequence of processes culminating in exudation of organic compounds into the rhizosphere is required before practical applications become feasible. Bioengineering the rhizosphere by adding beneficial microorganisms will require understanding of microbe-microbe and microbe-plant interactions enabling introduced microorganisms to show full activity in the targeted rhizosphere.

Keywords: Phosphorus; micronutrients; rhizosphere.

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KEYNOTE PAPERS

K-9

The Fate of Anthropogenic Organic Pollutants in Soil: Adsorption/Desorption of Pesticides Possessing Endocrine Disruptor Activity by Natural Organic Matter (Humic Substances)**N. Senesi*** and E. Loffredo*Dipartimento di Biologia e Chimica Agroforestale e Ambientale, Università di Bari, Via Amendola 165/A, 70126-Bari, Italy. *E-mail: senesi@agr.uniba.it***Introduction and Objectives**

The purpose of this presentation is to firstly provide a general survey on the various phenomena that anthropogenic organic pollutants of various nature and origin are subjected in soils and on the specific role exerted by humic substances on these processes. The presentation will then focus on adsorption/desorption of two important classes of organic pollutants, that are some representative pesticides possessing endocrine disruptor activity and some properly active endocrine disruptor compounds (EDCs), onto soil humic acids (HAs) that are the most chemically and biochemically active fractions of humic substances and natural soil organic matter.

General Part

Organic pollutants can reach the soil either by purpose, such as pesticides and other agricultural chemicals, or incidentally through a variety of materials commonly used in agricultural practices, e.g., fertilizers and amendments. Once on the soil surface, the organic pollutant can be partially photodecomposed and/or volatilized, and partially enter the soil or be transported to surface aquatic bodies by runoff and/or erosion. When in the soil, the pollutant can be subjected to partial or total chemical decomposition and/or biodegradation. The original pollutant and, possibly, its breakdown products may be adsorbed to soil organic and inorganic constituents, uptaken by plant roots, and/or leached through the unsaturated zone eventually reaching the groundwater. All these processes are controlled by several factors including the physical and chemical properties of the pollutant and its breakdown products, the nature and thickness of the soil, the amount of water applied to the soil, and the type and extent of interactions between the pollutant and soil components.

EDCs are hormone-like substances that are able to interact with the development and functioning of endocrine systems in wildlife and humans by causing alteration, i.e., disruption, of their normal endocrine functions. Compounds proven or suspected to act as EDCs include natural and synthetic estrogens of human origin, several agricultural products such as pesticides, various industrial chemicals, and several products and byproducts of the paper, paint, plastic and pharmaceutical industries. These compounds may enter the soil through current agricultural practices and/or disposal of urban and industrial effluents, sludges and wastes. The response of soil to the estrogenic risk of endocrine disruptors is generally related to their distribution and speciation in the various soil phases, in which adsorption processes play a very important role. Depending on the strength and extent of interactions of EDCs with soil phases, they can either accumulate in the top soil layer or be

KEYNOTE PAPERS

moved down to deeper soil horizons and groundwater. Adsorption/desorption of EDCs onto soil HAs is generally considered one of the most important processes that controls EDCs behavior, performances, and fate in soil, including mobility, transport, accumulation, bioavailability and toxicity, and the assessment of the potential environmental hazard associated with EDCs. Thus, the evaluation of the kinetics and extent of adsorption/desorption processes of EDCs onto/from HAs in different soil horizons is very important to understanding EDCs behavior, performances, and fate in soil, and assessing the potential environmental hazard associated with them.

Experimental part

The pesticides possessing endocrine disruptor activity considered in this work are: atrazine, alachlor and *cis*- and *trans*-chlordane; and the proper EDCs are: bisphenol A (BPA), octylphenol (OP), 17- α -ethynilestradiol (EED) and 17- β -estradiol (17ED). The HA samples used for experiments with pesticides and EDCs were isolated, according to conventional standard procedures: from the surface layer (0-30 cm) of five reference Eurosoils of the first generation (a Vertic Cambisol E1, an Orthic Rendzina E2, a Dystric Cambisol E3, an Orthic Luvisol E4, an Orthic Podzol E5), an alluvial soil (AS), two loamy carbonatic Terra Rossa soils (SO1, SO2) and two sandy soils (UK1, PO1), and the surface (depth 0-30 cm) and deep (depth 30-90 cm) horizons of two sandy soils (P30 and G30, and P90 and G90, respectively). Adsorption kinetics and adsorption/desorption isotherms and coefficients of each pesticide and EDC onto each HA sample were determined using the slurry-type (batch equilibrium) method and the HPLC technique with various detectors, with the exception of *cis*- and *trans*-chlordane that were analysed after preliminary solid phase extraction (SPE) and subsequent GC using an electron capture detector (ECD). Experimental data were fitted in linear and nonlinear, Freundlich and Langmuir equations. The distribution coefficient, K_d , was also calculated from available data.

Results and Discussion

Adsorption of EDCs onto all HAs examined occurs in two phases, a rapid one in the first few hours of contact, which corresponds to more than 90% of total adsorption, and a slow one that needs generally less than 24 h to be completed. Experimental adsorption data were best fitted in a linear isotherm for alachlor, Langmuir isotherm for BPA and 17ED, in a nonlinear Freundlich isotherm, S-shaped for *cis*- and *trans*-chlordane and L-shaped for EED, and either in a linear or a nonlinear, L-shaped Freundlich isotherm for atrazine and OP. Thus, no limiting adsorption is observed for all pesticides and EDCs onto HAs examined over the concentration range tested, with the exception of BPA and 17ED for which a maximum adsorption, i.e., saturation, is reached. In general, the values of the Freundlich constant, K , and of the distribution coefficient, K_d , calculated from the experimental isotherms do not follow the same trend for pesticides, but follow the same trend for EED and OP, which is: HA-G30 > HA-P30 > HA-P90 \geq HA-G90. In general, the adsorption capacity of HAs for chlordane is much larger than that for atrazine and alachlor, which are adsorbed at almost the same extent by HAs. Further, the adsorption capacity for EDCs of HAs from surface horizon soils is much larger than that of HAs from deep horizon soils. The extent of adsorption of

KEYNOTE PAPERS

BPA onto HAs is generally smaller than that of the other three EDCs which are adsorbed at almost the same extent. Adsorption of EED and, especially, BPA onto HAs is generally reversible, and desorption occurs quickly and almost completely after few desorption steps. On the contrary, adsorption of OP is mostly irreversible, a partial desorption occurs slowly, and high amounts of OP are retained by most HAs at the end of the experiment.

Conclusions

In conclusion, the HAs examined in this work are able to adsorb variable amounts of pesticides possessing endocrine disruptor activity and EDCs that tend to accumulate in the soil surface horizon. The EDCs that are desorbed quickly and completely from HAs are expected to move easily down the soil profile, and possibly contaminate groundwater. On the contrary, the EDCs desorbed slowly and only partially are expected to remain mostly adsorbed by HAs, especially on the surface soil layer, with corresponding soil contamination but protection of groundwater. The carboxylic and phenolic hydroxyl group content, the organic free radical concentration, and the aromaticity and humification degrees appear to be the most important chemical properties of HAs affecting atrazine adsorption, whereas it is not clear which compositional, structural and functional features of HAs may affect adsorption of alachlor, chlordane and EDCs.

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Keywords: Organic pollutants; pesticides; organic matter.

KEYNOTE PAPERS

K-10

Sorption-Desorption Processes of Metals and Metalloids in Soil Environments

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Trace elements may be present in solution with positive or negative charges and in different redox state. They occur predominantly in cationic form (Pb, Cu, Zn, Ni, Cd, Hg, Cr(III), Co) but some trace elements are present in anionic form (As, Se, Cr(VI), Mo and B). In soil environments, sorption/desorption reactions on/from inorganic and organic sorbents control the concentration of metals and metalloids in solution and affect their bioavailability, leaching and toxicity. Sorption-desorption processes of elements in cationic form differ greatly from those in anionic form. These reactions are affected by many factors, such as pH, nature of the sorbents, presence and concentration of organic and inorganic ligands, including humic and fulvic acids, root exudates and nutrients. Furthermore, redox reactions, both biotic and abiotic, are of great importance in controlling the oxidation state and thus, the mobility, the phytoavailability and the toxicity of many elements, such as Cr, Se, Co, Pb, As, Ni and Cu (Kabata-Pendias, 2001; Huang and Germida, 2002; Violante et al., 2002; Sparks, 2003; Huang and Gobran, 2005; Violante et al., 2007).

The aim of this presentation is to provide information on the factors which affect the mobility of trace elements. Special attention is devoted to the influence of inorganic and organic ligands, including nutrients and root exudates, on the sorption/desorption processes of trace elements in cationic and anionic forms on/from soil components, soils and selected synthetic minerals (double layered hydroxides, the so called "anionic clays").

The soil components responsible for trace element sorption include, soil humic substances, phyllosilicates, carbonates and variable charge minerals (constituents such as Fe, Al, Mn and Ti oxides, short-range ordered aluminosilicates as well as phyllosilicates coated by OH-Al or OH-Fe species whose charge varies with the pH of the soil solution). Recently, evidence on the sorption of heavy metals on microorganisms have been reported (Jackson, 1998). Soil components differ greatly in their sorption capacities, their cation and anion exchange capacities, and the binding energies of their sorption sites. Trace element sorption kinetics depend on the type of surface and trace element, but generally are rapid. Half times for bivalent cations, such as Pb, Zn, Cu and Cd, sorption on peat range from 5 to 15 seconds.

Except for some noncrystalline minerals that have very high specific surface charge density with highly reactive sites, humic substances appear to have the greatest capacity for sorption of trace elements in cationic form. A body of evidence has demonstrated that humic matter and metal oxides are much more effective scavengers of trace elements in cationic form, than even the most efficient sorbent among phyllosilicates, indicating that specific sorption and other complexation processes are the dominant binding mechanisms (Jackson, 1998; Huang and Germida, 2002; Spark, 2003). Complexation reactions have the following effects: i) metal ions are prevented from being precipitated; ii) complexing agents can act as carriers for trace elements in soil solution; iii) their toxicity is often reduced by complexation.

Trace elements in cationic form are probably not dominantly sorbed on 001 faces of phyllosilicates because they are always vastly outnumbered by other cations with which they

KEYNOTE PAPERS

compete (Jackson, 1998). They may be strongly sorbed only on the edges of the phyllosilicates. However, clay minerals have also an important role as carriers of associated oxides and humic substances forming organo-mineral complexes, which present peculiar sorption capacities different from those of each single soil constituent.

Variable charge minerals selectively sorb polyvalent cations even when their surfaces are positively charged (solution pH values lower than the point of zero charge [PZC] of the sorbent). Most transition cations (Pb, Cu, Cr, Ni, Co, Zn, Al, Fe, Mn) are often sorbed more strongly than alkaline earth cations. Spectroscopic techniques such as electron spin resonance (ESR) and Extended X-ray Absorption Fine Structure Spectroscopy (EXAFS) have been used for the identification of metal complexes at the surfaces of Al, Fe or Mn oxides, silicate clays and soil organic matter (Sparks, 1999). The mechanism of metal ion association with hydrous oxide surfaces involves an ion exchange process in which the sorbed cations replace bound protons. Usually, specifically sorbed cations raise the value of PZC of variable charge minerals. pH affects sorption of trace elements, either by changing the number of sites available for sorption (sorption increases by increasing pH) or by changing the concentration of cation species (Me^{2+} , MeOH^+ , $\text{Me}(\text{OH})_2$).

The nature, crystallinity, size of the crystals, surface charge of metal oxides play an important role in the sorption selectivity of trace elements in cationic form. Sorbed trace element species may range from simple cations to complex polynuclear hydroxides formed by hydrolysis and condensation in solution or by nucleation on the mineral surfaces (Jackson, 1998). In the last decade extensive research has been carried out on the heterogeneous precipitation of trace elements on the surfaces of minerals, using modern spectroscopy techniques such as synchrotron-based X-ray absorption spectroscopy (Sparks, 2003; Borda and Sparks, 2008 and references therein). Reported observations include zinc precipitates on calcite, cobalt precipitates on Al_2O_3 , polynuclear chromium(III) hydroxide structures on silica and goethite, and the formation of mixed nickel-aluminum hydroxides on pyrophyllite (Scheidegger *et al.*, 1996).

Few studies have been carried out on the competition in sorption of trace elements to soil components and soils. Competition in sorption between two or more trace elements is of paramount importance for understanding their relative affinity for a given sorbent (Violante *et al.*, 2003). Many factors such as pH, surface properties of the sorbents, the number of sites available for sorption, the nature and charge of Me-L species in solution influence trace element sorption onto soil inorganic components (phyllosilicates and variable charge minerals) in the presence of inorganic and biological ligands. Inorganic and organic ligands (e.g., organic acids) which form strong complexes with trace element cation usually prevent or reverse their association with negatively charged sorbents, as clay minerals, by forming stable dissolved or dispersed negatively charged complexes with the cations (Violante *et al.*, 2007). In contrast, the presence of certain foreign ligands, occurring naturally in the rhizosphere, such as siderophores produced by microorganisms and phytosiderophores exuded by plants, may promote the formation of positive complexes and, consequently, the sorption of trace elements onto phyllosilicates.

The processes, which affect the sorption of trace element cations onto variable charge minerals in the presence of chelating agents, are particularly complex and are different from those onto phyllosilicates (Violante *et al.*, 2007). Usually, high concentrations of low molecular weight organic acids may promote the formation of negative complexes with metal

KEYNOTE PAPERS

ions and favour their sorption onto variable charge minerals, whereas siderophores may promote the formation of positive complexes, preventing sorption. In conclusion, biological ligands play a very important role on the mobility and then the phytoavailability of trace elements at soil-root interface (Huang and Germida, 2002).

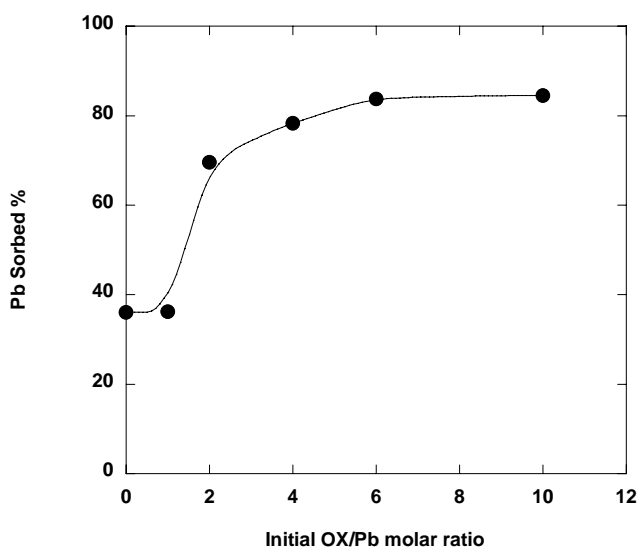


Figure 1. Effect of increasing concentration of oxalate (OX) on the sorption of Pb onto ferrihydrite

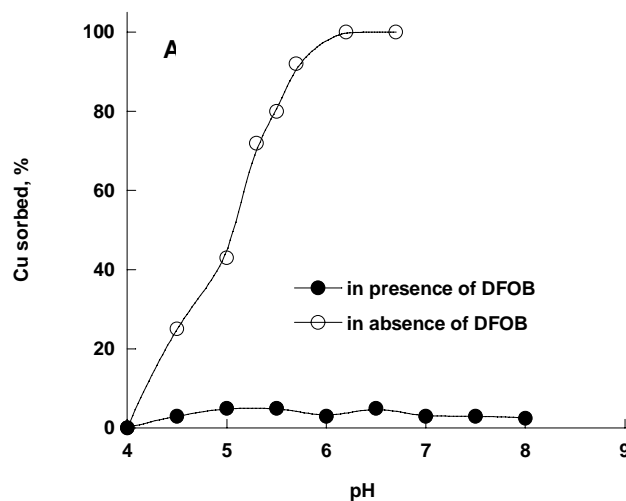


Figure 2. Percent of Cu sorbed onto ferrihydrite without (○) and with (●) 300 mM DFOB

KEYNOTE PAPERS

Trace elements which exist in anionic form are sorbed primarily by chemisorption at reactive sites of metal oxides and allophanes and at the edges of phyllosilicates. Sorption of anions onto variable charge minerals and soils varies with pH. With increasing pH, within a certain range, sorption decreases (due to a decrease of positive charge of minerals) or else increases to a maximum close to the pKa for anions of monoprotic conjugate acids and then decreases. Anions may be specifically or nonspecifically sorbed. Ligands which are specifically sorbed replace OH⁻ or OH₂ groups from the surfaces of variable charge minerals. Specific sorption is also termed *inner-sphere* sorption because it involves direct coordination to the surface metal atom. Nonspecific sorption is also termed *outer-sphere* sorption and is influenced by the ionic strength of the system. Specifically sorbed anions usually lower the PZC of metal oxides, thus the PZC of a particular oxide may give rise to different values depending on the kind and extent of foreign ion sorption (Sparks, 2003).

Table 1. Adsorption (mmol kg⁻¹) of arsenate (AsO₄) and phosphate (PO₄) after 24 h of reaction on metal oxides, phyllosilicates and soils at pH 5.0, when the ligands were added alone or as a mixture (initial PO₄/AsO₄ molar ratio of 1.0). Modified from Violante and Pigna (2002).

<i>Samples</i>	Anion added alone		Anion added as a mixture		
	AsO ₄	PO ₄	AsO ₄	PO ₄	rf*
	pH 5.0				
<i>Birnessite</i>	28.5	25.7	23.8	13.1	1.81
Pyrolusite	26.0	21.0	12.7	9.8	1.30
Smectite (ferr.)	21.4	19.9	17.4	14.9	1.17
Goethite1	175	158	87	78	1.11
<i>Montmorillonite</i>	10.9	11.6	7.8	9.2	0.84
<i>Rhodoxeralf</i>	27.7	29.4	14.9	22.0	0.68
Andisol	644	806	341	738	0.46
Gibbsite1	205	280	82	198	0.41
Kaolinite	10.5	15.2	6.7	14.8	0.45
Allophane	498	917	126	880	0.14

*rf stands for adsorbed arsenate/adsorbed phosphate molar ratio

The presence of organic and inorganic ligands also affects the sorption of trace elements in anionic form by competing for available sorption sites and/or reducing the surface charge of the variable charge minerals and soils (Violante et al., 2005; 2007). The competition

KEYNOTE PAPERS

depends on the affinity of the anions for the surfaces of the sorbents as well as the nature and surface properties of the minerals and soils. Competition may be easily observed at sufficient high surface coverage. It has been demonstrated that competition in sorption between phosphate and arsenate may vary greatly on different soil minerals and on soils characterized by different mineralogy and chemical properties, although it has been established that the chemical behaviour of arsenate is similar to that of phosphate.

Time of reaction and the surface coverage have also a great influence on the competitive sorption between trace elements and organic and inorganic ligands (Violante and Pigna, 2002). Most competitive sorption studies have been carried out adding the ions contemporaneously. In natural environments, however, it is more likely that the ions will come in contact with a sorbent sequentially, i.e., the solid is exposed to one ion first, with the second ion coming in contact with a solid at a later time. The sorption of metals and metalloids is strongly affected by the order of addition of organic and inorganic ligands and trace elements on the sorbents. It has been demonstrated that larger amounts of selected heavy metals (Pb, Cu) were sorbed when chelating organic anions (oxalate, tartrate) were added before or after the metals as referred to the systems where the heavy metals were added as a mixture with the organic ligands or alone (Violante et al., 2003).

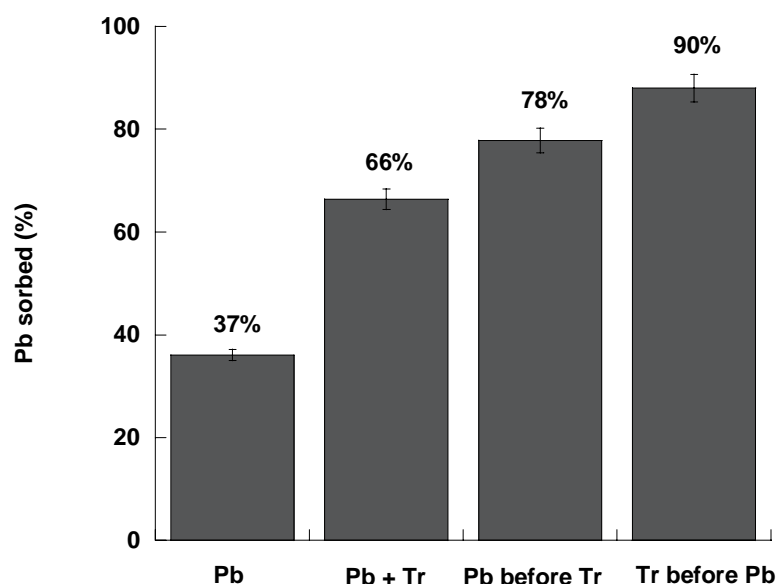


Figure 3. Effect of sequence of addition of Pb and tartrate (Tr) on Pb sorption

In contrast to sorption studies relatively little information is available on the desorption of metals and metalloids from soils or soil components. Presence of inorganic and organic ligands, such as phosphate, sulfate and root exudates, has a significant impact on the desorption of trace elements. Usually, desorption studies have biphasic reaction processes; a fast reaction followed by a slow reaction.

A number of factors, such as the type, mineralogy and crystallinity of the sorbents, the pH, the pe, the surface coverage, the residence time of metals and metalloids on the surfaces of

KEYNOTE PAPERS

soil components may affect the desorption of trace elements. Some studies on the effect of residence time on the desorption of some heavy metals and metalloids have been carried out. Usually, the longer the residence time, the greater the decrease in trace element desorption, because of a rearrangement of surface complexes and/or a conversion of surface complexes into surface precipitates (Scheidegger *et al.*, 1996; Pigna *et al.*, 2006; Violante *et al.*, 2006).

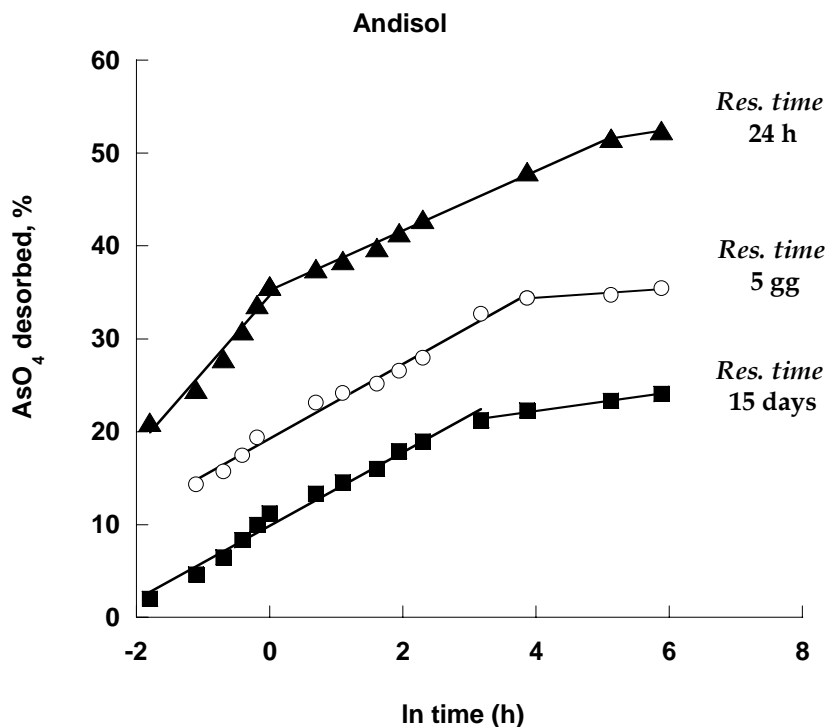


Figure 4. Kinetics of desorption (from 3 hours to 2 months) of AsO_4 by PO_4 from an Italian Andisol, containing 40% of allophonic material, as a function of residence time, by keeping the surface coverage (70%) and pH (6.0) constant.

In soil environments trace elements may be not only sorbed on but also coprecipitated in metal oxides. Coprecipitation of arsenic with iron or aluminum occurs in natural environments and is a remediation technology to remove this toxic metalloid from drinking waters and hydrometallurgical solutions. Violante *et al.* (2006, 2007) studied the nature, mineralogy and reactivity towards phosphate of iron-arsenate or aluminum-arsenate coprecipitates. The mineralogy, surface area and chemical composition of the coprecipitates were affected by pH, arsenic concentration and aging. Less than 20% of arsenate present in the coprecipitates was removed by phosphate and more from the aluminum-arsenate than iron-arsenate samples. These authors also found that more arsenate was desorbed by phosphate from a ferrihydrite or boehmite on which arsenate was added than from an iron-arsenate or aluminum-arsenate coprecipitates, attributed to partial occlusion of some arsenate anions into the framework of the coprecipitate.

KEYNOTE PAPERS

In the last decades many studies have been carried out on the possible use of layered double hydroxides (LDHs) as powerful filter for removing anionic pollutants from polluted waters. LDHs are formed in soil environments (Goh et al., 2008; and references there in).

Keywords: Sorption-desorption processes; metals and metalloids; soil environments.

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S1-O1**Soil Moisture Characteristic in Some Low Land Soils****N. Bassirani****Department of Soil Science, Zabol University, Zabol, I. R. Iran.***E-mail: N_Bassirani@yahoo.com*

A model to predict the moisture characteristic of a Low Land soil from some physical and chemical properties such as soil texture (percent of sand, silt, clay) bulk density and organic matter parameters is presented. Soil moisture content in unsaturated soil is reflected by two important factors of Field Capacity (FC) and Permanent Wilting point (PWP) which affect irrigation scheduling and field management. FC and PWP can be estimated from some of the physical and chemical properties of soil. Pressure plate apparatus is usually used for determination of FC and PWP, but this is a time-consuming and laborious procedure. Besides, the apparatus may not be available in many laboratories. For reporting a model to predict soil moisture characteristic, soil samples were taken from 55 locations in Sistan and Baluchestan province of south east Iran (unsaturated Soils). Soil texture, organic matter, bulk density and cation exchange capacity (CEC) were determined. Soil moisture at FC and PWP of the soils were measured with a pressure plate. Simple and multiple regression analyses were used to study the relationships between FC and PWP and soil texture, soil organic matter and bulk density.

Keywords: characteristic, low land, soil moisture.

S1-O2**Competitive Adsorption of Phosphate and Oxalate on Nano-Ball Allophane with Different Si/Al Ratio****M.A. Elsheikh*, N. Matsue and T. Henmi***Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Japan.***E-mail: abdalla@agr.ehime-u.ac.jp*

The interaction between phosphate and low molecular mass organic acids (LMMOAs) on clay minerals has a great importance for understanding their impacts on minerals dissolution and metal mobility in rhizosphere. The mobility and bioavailability of these ligands depends on characteristics of the mineral surface. Oxalate is a nearly ubiquitous dicarboxylate ligand that occurs in significant concentrations in soils, surface waters, sedimentary basins and chemical processing solutions. Allophane is a short-range ordered hydrous aluminum silicate and a principal material of clay fraction in Andisols and Podzols. Previously, the aluminum silicate had not been believed to have definite morphology and structure, however later on it was proved that allophane, at least separated from volcanic ash soils and weathered pumice grains, have definite hollow spherical morphology (nano-ball with diameter of 3.5 to 5.0 nm). A literature on competitive adsorption between phosphate and LMMOAs in soils showed that previous studies were usually carried out at high concentrations (1-100 mM), and resultant adsorption amounts were much higher than those found in soils. Therefore objective of this study was to investigate competitive adsorption of phosphate and oxalate onto two allophane samples with low and high Si/Al ratio, at low concentration level, using the recent

information about detailed morphology and chemical structure of the wall of the aluminum silicate. In order to attain our study purposes, the experiments were carried out by adding different initial concentrations of oxalate (OX) or phosphate (P) (single system), or both at equimolar (binary system) to allophane suspension at various constant pH. Then the Langmuir and Freundlich adsorption equations were used to describe the collected data. In almost cases, adsorption isotherms fitted to the Langmuir equation rather than the Freundlich equation, indicating strong monolayer adsorption of P and OX on allophane. In the single system, Langmuir X_m value (maximum adsorption) for P was much greater than that for OX, on each allophane sample at all pH values. Langmuir K value related to adsorption strength was also greater for P than for OX in all cases. These indicated that the adsorption affinity toward allophane was higher for P than for OX. The amount of adsorption of P on two allophane samples decreased almost linearly with increasing pH from 4 to 8. The amount of adsorption of OX on allophane with low Si/Al also decreased with increasing pH, but that on allophane with high Si/Al had a maximum at pH 5. This is related to change in chemical species of OX with pK_2 of 4.3. In binary system, the amount of adsorption of P and OX was lower as compared to that in the corresponding single system. In case of allophane with low Si/Al, the sum of adsorption of P and OX in binary system was higher than that of P in single system. This means OX in the binary system can adsorb the site on allophane where P did not adsorb in single system. However, in case of allophane with high Si/Al, the sum of adsorption of P and OX in binary system was almost equal to that of P in single system. This indicates that OX in binary system can adsorb only on the site where P adsorbed in single system. The ratio of OX/P adsorbed was smaller than unity in all cases. The ratio decreased with increasing initial concentration, suggesting increasing affinity of P as compared to OX onto allophane with increasing their concentrations.

Keywords: Phosphate; oxalate; nano-ball allophane

S1-O3

Soil Organic Carbon Stocks - A Land Quality Indicator of Degraded Agro-Eco-Systems

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Soil organic matter (SOM) is the primary sink and source of plant nutrients in natural and managed terrestrial ecosystems. It increases the ion exchange, water holding and infiltration capacity, promotes the formation of soil aggregates and is the major energy substrate for the soil fertilizers, those that do not have toxicity, aggregation or erosion problems or those supplied ample irrigation and other management inputs can support crops without much SOM. These situations, which are basically field nutrient culture, are rare and in the majority of soils, SOM is a prerequisite for ecosystem health and productivity (Lal *et. al.*, 1997). A study was conducted on Characterization and classification of salt affected soils of part of

Bellary taluk using Remote sensing techniques. A detailed assessment of land resources was undertaken. Where drastic changes in land resources and their usage occurred due to the introduction of canal water from Tungabhadra catchment. Using Remote sensing data of IRS-1C, the salt affected soils of Bellary were identified. A total of 15 pedons were excavated to a depth of around 2 meters from agro-eco-systems and characterized for their morphological, physical, chemical and fertility parameters. The study revealed that the area has very deep fine soils with pH and EC ranged from 8.0 to 9.9 and 0.19 to 30.1 d Sm⁻¹ respectively and indicated that soils are moderate to strongly alkaline soil reaction. The soils had high CEC values varied from 29.6 to 82.4 c mol (P⁺) kg⁻¹, AWC (20.9 to 46.6 k Pa), % Base Saturation (63 to 99%) and able to supply major and micro-nutrients to plant growth. There was dominance of monovalent cations on surface adding to alkalinity and sodicity near the canals and increases salinity and calcareousness towards the streams. The soil organic carbon stocks in the degraded ecosystem revealed that total % organic carbon from all the pedons ranged 3.6 to 22.5. The organic carbon content ranged from 0.09 to 0.98 percent decreased with the soil depth in all the pedons indicating less soil microbes in the lower profiles. Soils of pedon 1, 7, 10, 12 and 14 are having good stock of SOC compared to soils of pedon 4, 8, 9, 13 and 15 respectively. Better SOC stock will promote soil-root microbial interaction helping the sustained productivity of the agro-ecosystem. Similarly poor organic carbon stock will have adverse impact on the soil root microbial interaction leading to total degradation of the system making it not useful for agriculture. The pedons 1 to 15 have been classified into Fine mixed calcareous isohyperthermic Sodic Haplusterts, Fine mixed calcareous isohyperthermic Sodic Haplusterts, Loamy mixed calcareous isohyperthermic Typic Haplustepts, Fine mixed calcareous isohyperthermic Haliic Haplusterts, Fine mixed calcareous isohyperthermic Typic Haplusterts, Clayey mixed calcareous isohyperthermic Vertic Haplustepts, Fine mixed calcareous isohyperthermic Halic Haplusterts, Loamy mixed calcareous isohyperthermic Typic Haplustepts, Fine-loamy mixed calcareous isohyperthermic Typic Haplustepts, Fine mixed calcareous isohyperthermic Sodic Haplusterts, Fine mixed calcareous isohyperthermic Vertic Haplustepts, Fine mixed calcareous isohyperthermic Sodic Haplusterts, Fine mixed calcareous isohyperthermic Vertic Haplustepts, Clayey mixed calcareous isohyperthermic Aquic Haplustepts, Fine mixed calcareous isohyperthermic Fluventic Haplustepts respectively based on the physicochemical characteristics of soils. The land use is preferentially getting changed from sorghum and red gram to corn and red gram with a catch crop of coriander in the first instance; then to paddy alone in two seasons and finally some areas are unsuitable for any agricultural purpose.

Keywords: Organic carbon; land quality; degraded agro-eco-systems.

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S1-O4**Conformation, Stability and Activity of Acid Phosphatase during the Interaction with Clay Minerals and Soil Colloids from an Alfisol****Q. Huang***, J. Zhu, X. Qiao, P. Cai and W. Liang*Key Laboratory of Subtropical Agriculture and Environment of Ministry of Agriculture, Huazhong Agricultural University, Wuhan 430070, China. *E-mail: qyhuang@mail.hzau.edu.cn*

Conformation, proteolytic stability and enzymatic activity of acid phosphatase on montmorillonite, kaolinite and soil colloids from an Alfisol were studied. The conformation of native and desorbed phosphatase was tested by fluorescence and circular dichroism (CD) spectroscopy. The proteolytic stability and enzymatic activity of free and immobilized phosphatase were determined by biochemical assay and isothermal titration microcalorimetry (ITC), respectively. Most original conformation of phosphatase was preserved by kaolinite and organic soil clay. However, marked changes in conformation of phosphatase were observed when desorbed from montmorillonite and inorganic soil colloid. Higher percentages of α -helices and β -sheets were formed for the enzyme molecules desorbed from the surface of montmorillonite and inorganic soil clay. Proteolytic stability of immobilized phosphatase on soil colloids and minerals was higher than that of free enzyme. The catalytic heat of phosphatase was declined after immobilization on soil colloids, indicating the depression of enzymatic activity. The enhancement in proteolytic stability and decrease of enzymatic activity for phosphatase bound on montmorillonite and organic soil clay were greater than that on kaolinite and inorganic soil colloid. The results obtained in this study would be helpful for better understanding of the behavior and fate of phosphatase in soil environments, which is fundamental for the evaluation of soil fertility and quality.

Keywords: Acid phosphatase; clay minerals; soil colloids.

S1-O5**Carbon Mineralization in Particle Size Fractions of a Brookston Clay Loam Soil under No-Tillage and Mouldboard Plough Management****X. Yang^{1*}**, Z. Zhang^{1,2}, C. Drury¹, W. Reynolds¹ and L. Zhao²¹*Agriculture and Agri-Food Canada, 2585 County Road 20, Harrow, Ontario, Canada N0R 1G0.*²*Department of Natural Resource & Environ. Science, Jilin Agricultural University, Changchun, China 130118. *E-mail: yangx@agr.gc.ca*

The organo-mineral fractions of specific particle sizes often play a significantly different role in the composition and turnover rate soil organic carbon (SOC). However, no data are available on the contents of SOC in organo-mineral particle-size fractions and its turnover rates for heavy textured soils under different tillage management in Southwest Ontario. Hence, the objectives of this study were to: 1) determine the contents of SOC associated with the sand (53-2000 μm), silt (2-53 μm) and clay (<2 μm) size fractions of a Brookston clay loam soil under no-till (NT) and mouldboard plough (MP); and 2) measure SOC mineralization within the sand, silt and clay fractions. Soil samples (0-10 cm) were collected in early November 2007 from a tillage study which was initiated in 1996 by splitting existing (13-y) MP and NT plots in half. One half of 13-y MP plot was converted into new-NT (N-

NT) while the remaining half was left intact as long-term MP (L-MP). Similarly, one half of 13-y NT plot was converted to new-MP (N-MP) while the remaining half was left intact as long-term NT (L-NT). Bulk soil was dispersed into sand, silt and clay fractions by applying ultrasound energy at 750 J ml^{-1} to a 1:4 soil:water suspensions. The SOC content of the particle size fractions was determined and the size fractions were aerobically incubated at 20°C and 30% moisture (w/w) for 29 days. The C-CO_2 emissions from the samples were measured at day 1, 2, 5, 9, 14, 20 and 29. We found that more SOC was associated with clay than with silt and sand in all tillage treatments. There was more SOC associated with clay (34.2 g C kg^{-1}) and silt (24.1 g C kg^{-1}) under NT than with clay (28.8 g C kg^{-1}) and silt (20.9 g C kg^{-1}) under MP. Similarly, significantly ($P < 0.05$) more SOC was associated with the sand fraction under NT (7.1 g C kg^{-1}) than under MP (3.7 g C kg^{-1}). For all tillage treatments, carbon mineralization decreased with time. Both No-till soils showed significantly higher mineralization rates than two MP soils. The clay size particles emitted significantly greater CO_2 relative to the silt and sand fractions. Hence NT produced more SOC in all three particle size fractions relative to MP in the top 10 cm, and that SOC associated with the silt and clay fractions was more labile under NT than under MP.

Keywords: Soil size fractions, Soil organic carbon (SOC), Mineralization, Tillage.

S1-O6

Artificial Soil Based on Clay Minerals for Use as a Source of Growing Media

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Artificial soil for use as a source of growing media has been made by calcinations of clay minerals under controlling pore space. Calcined clay is a granular agglomerate of clay having large surface area and a myriad of small pores. It has a low holding capacity for easily available water (EAW) and a low water buffering capacity (WBC). This research modified physical properties of calcined clay to increase EAW and WBC for possible use as an ideal substitute for plant growing media. To determine proper sizes of unit particles of calcined clay showing a range of matric potential from 1 to 10 kPa, selected feldspar materials with known dimensions were agglomerated and water suction tests were performed on the product. Based on the proper subunit sizes determined, calcined clay was physically modified using kaolin in a two-step process. The first step involved the consolidation of kaolin by mixing with water, drying it, and classifying into required sizes after crashing of the dehydrated material. The second step involved agglomeration of the material in a spherical form using a pan-type pelletizer and baking the product at 900°C for 2 h. The modified calcined clay (MCC) has improved water holding and buffering characteristics, mainly due to the presence of bimodal pore sizes: small pores having about $1 \mu\text{m}$ diameter and large pores of greater than $10 \mu\text{m}$ diam. Water suction tests verified that MCC has higher EAW and WBC than those of the typical ingredients of root substrates commonly used. The MCC, with granular sizes of 2-4 mm diameter, was mixed with Canadian sphagnum peat in various proportions, and the physical and chemical properties of the mixtures as a new source of growing media were characterized.

Keywords: Artificial soil; clay minerals; growing media.

S1-O7**Soil Contamination with Strontium-90 at the Former Semipalatinsk Nuclear Test Site, Kazakhstan****Y. Kuyanova^{1*}, M. Burkitbayev¹ and N. Priest²**¹*Department of Inorganic Chemistry, Al-Farabi Kazakh National University, Almaty, Kazakhstan.*²*AECL Chalk River Laboratory, Ontario, Canada. *E-mail: k-yelena@mail.ru*

The territory of the former Semipalatinsk nuclear test site (STS) is uniquely placed for the implementation of environmental studies/investigations. Over a period of past 40 years (1949-1989), at the STS more than 450 nuclear explosions, including atmospheric, above ground and underground tests have been conducted at the STS. The study investigated the level of radiostrontium ⁹⁰Sr in soil samples collected from the north region of STS. ⁹⁰Sr is the most biological hazardous fission product and its present in soil and food items grown/produced on contaminated soil may give rise to significant radiation exposure within exposed populations. Soil samples were collected during a 2006 summer expedition to the STS as part of the NATO Science for Piece Project SEMIRAD-2 (SfP 960906) programme. The soil at the area of investigation is of the mollisols type; typical of the dry steppe zone of Kazakhstan. A range of parameters were analyzed for the physical-chemical characterization of the soil samples. These were cation (Mg, K, Na) exchange capacity, exchangeable calcium content, pH value, humus and organic matter content. In addition, X-Ray diffraction analysis has been used for the determination of the mineralogical composition of these soil samples. In order to establish the degree of radiological contamination of the selected area of the STS territory and considering wind transfer to its adjoining territories, strontium contamination was analyzed in the soil samples. This included analysis of its distribution along the soil vertical column and across the soil particle size fraction. Cored soil samples of 15 cm depth were collected and sliced into two centimeter layers. The distribution of ⁹⁰Sr across soil size fraction was analyzed using the top 5 centimeters fraction of the core. Soil samples pre-treatment including drying, sieving and grinding. ⁹⁰Sr concentrations were determined by performing Sr separation followed by counting ⁹⁰Sr by liquid scintillation - in the conventional procedure. The study of vertical distribution of ⁹⁰Sr in core profiles showed that contamination levels decreased exponentially with increasing depth and significant concentrations of the radionuclide were only present down to a depth of 10 cm below the soil surface where most plants roots are present. The content of ⁹⁰Sr associated with particles transferred by wind (less than 50 µm) is about 20%. A special sequential extraction experiment has been undertaken to determine the degree of ⁹⁰Sr binding to different kind of soil minerals. It was found that the water soluble fraction is only 0.4-0.5% and more than 50% of ⁹⁰Sr is on the strong fixed fraction.

Acknowledgement: This work is supported by NATO Science for Piece Program (SEMIRAD-2).

Keywords: Soil contamination; strontium; Mollisols.

S1-O8**Microbial Functions Related to Soil Quality in a No-Tilled Argiudoll****A. Pidello and E. Perotti****Laboratorio de Química Biológica- FCV-Consejo de Investigaciones-Universidad Nacional de Rosario- Bd. O. Lagos y Ruta 33-2170-Casilda, Argentina. *E-mail: eperotti@unr.edu.ar*

Soil quality can be defined according to its capacity to sustain plant and animal productivity, and to maintain or enhance water and air quality. In this way, when new strategic methods of soil conduction are implemented, to improve soil sustainability, the evolution of the soil quality should be studied. Many indicators of soil quality are used, among them the quantity of the soil organic matter soil (SOM) is the indicator most wide used in no-tilled systems. The main microbial population in agricultural soil is heterotrophic. In general, it is in relation to the SOM. In spite of this relationship, the capability of the SOM to support the microbial functionality is not understood that all yet. There are many gaps in relation to the role of the soil organic-C on the microbial functionality, in particular when the oxidizable-C is accumulated on top of stratificated soil, as the SOM stratification observed in the no-tilled Argiudoll studied by us. This stratification increases the organic compounds with C-atoms that have low oxidation states; so it suggests that their oxidation depends on the habitat's oxido-reduction state. The main objective of this work was to address the following question: in no-tilled soil does the accumulated SOM gives rise to a physico-chemical and biotic stratification?. Three different approaches were made to address this question. First, it was to study the distribution of the oxidizable carbon in relation to the expression of some microbial functions of heterotrophic microorganisms and physico-chemical parameters in the soil profile. This study could show the stratification status at 0-5, 5-15 and 15-25 cm of depth. The second approach was to study the impact of the *Pseudomonas fluorescens* inoculation, a soil heterotrophic bacterium, in the soil ecosystem of two soil levels. This strategy was performed to relate level-capacity to support microbial activity depending on oxidizable-C. The third approach, oxidizable-C and microbial respiration of soil' aggregates (> 2000, 2000-1000, 1000-250, 250-50, 50-20, < 20 μm) at the three selected depths were studied. The employed soil was a Vertic Argiudoll (90 years under conventional tilled and 10 years under continued wheat-soybean-corn no-tilled system). In these studies we employed complementary techniques that signed the SOM quantity and the aptitude for its utilization for soil microorganisms. These were evaluated by changes in oxidizable-C, CO_2 and N_2O production measured by gaseous chromatography, reduction of 2,3,5-triphenyl-tetrazolium-chloride (TTC) and total microorganisms number (acridine-orange stain). Taken as a whole, changes in oxidizable-C stratification and the studied biological parameters evolution were conducted both by stable macro-aggregates and by micro-aggregates, which are less predictable on their physical-chemical and biological behaviors. Nature of this "instability" of micro-aggregates should be studied in depth to ensure o guarantee the success of biotechnological practices, as bacteria inoculation, in no-tilled Argiudoll.

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Keywords: Soil quality; organic matter; *Pseudomonas fluorescens*.

S1-O9**Salinity Land Recovering: Effective Technique Using Organic Matter and Calcium Phosphate**

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Global Warming is currently an undeniable fact that has become a critical issue for economics and environment. An increase in the global temperature will accelerate evaporation rate resulting in higher salinity concentration (<http://www.architecture2030.org>). Global Warming will worsen salinity diffusion into the mainland. The salinity areas are spread widely over 397 million hectares in many regions of the world, which is three times larger than agriculture area (FAO, 2000). Phyto-remediation on salinity land is one of the effective strategies to control diffusion of salt and reduction of carbon dioxide to atmosphere leading to less greenhouse effect. However, the lack of salt-tolerant plants and agricultural practice are an important barrier to solve this problem. It is difficult to find out the accurate phenotypic expression and agricultural practice of plant grown under salinity condition because there are many factors related to salinity and environment condition that need to be manipulated and controlled. To overcome the salinity problems in agricultural production, precise, rapid and cheap process of *in vitro* and *ex vitro* environmental control system have been innovated and applied in the plant selection and agriculture practice. This understanding was used to modify the environment in the fields during phytoremediation on salinity land. The availability of organic matter for soil rehabilitation was used and monitored as a key factor. More than 200,000 varieties of tropical forest trees, rises, and sugarcane were identified *in vitro* and grown in 2,000 hectares in four salt mining areas and salt affected areas. From the field experiment, the salinity concentration decreased from 4-10% NaCl to 0.5-0.8% NaCl in 2-4 years. As a reason of land recovery, the diversity of tropical forest tree, algae, insect, reptile, bird and fish increase. The findings have shown that the rice and sugarcane grown under high organic matter and high calcium phosphate delivered twice yield than those grown under low organic matter and low calcium phosphate. The organic matters demonstrated the high binding capacity of salt ion and water in the soil. Now, farmers are able to grow rice, sugarcane and forest tree with yield in the salinity land under recommended conditions and agricultural practices. This research has offset the equivalent of preventing the distribution of salt by 80,000 metric tons and the release of carbon dioxide emissions by 16,000 metric tons, thus contributing mitigation of salinity distribution and greenhouse effect. It helps the farmer to strengthen their competency in managing fragile ecosystems.

Keywords: Salinity land; organic matter; calcium phosphate.

S1-O10**Assessment of Dissolved Organic Matter Quality in Wastewater Irrigated Soils****A. Sánchez-González^{1*}, C. Siebe¹, F. del Río-Portilla² and M. Vera-Martínez³**¹*Instituto de Geología, Universidad Nacional Autónoma de México, México.* ²*Instituto de Química, Universidad Nacional Autónoma de México, México.* ³*Universidad Autónoma Metropolitana, México.*

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The wastewater from Mexico City has been used for agricultural irrigation since more than a century. This has increased the total and soluble organic matter concentrations in the soils. The concentration of potentially mineralizable carbon has increased also, suggesting a change in soil organic matter quality. The heavy metals introduced by irrigation accumulate in the top soil and adsorb to the solid soil organic matter (Siebe, 1994). Nevertheless, a correlation between water extractable organic carbon and diverse metallic ions is observed, which indicates that these metals are partially mobile (Herre *et al.*, 2004) and are absorbed by crops (Siebe, 1994). The aim of this study was to analyze the quantity and quality of dissolved organic matter collected from fields of two representative soil units from the Mezquital Valley, which have been irrigated with wastewater for different periods of time. The sampling was carried out in Leptosol and Vertisol fields from the Mezquital Valley which has been irrigated for 0, 20, 40 and 100 years. In each field, undisturbed soil columns were taken, in three replications, these columns were moved to a greenhouse where the temperature and relative humidity were kept constant (22.3°C and 58%). The dissolved organic matter (DOM) samples were obtained by applying wastewater in a simulated irrigation and collecting the percolates of each column. The E_4/E_6 ratio was determined with a UV spectrometer (Cary 3E) quantifying the absorbance at 465 and 665 nm. For the infrared analyses compound samples of several percolations were condensed 10 times and freeze-dried. The acquisition of infrared spectra was made in an infrared spectrometer (IRFT Bruker Tensor 27) using tablets made of 0.5-1.0 mg of the freeze dried sample and 300 mg of NaBr. The solid state ^{13}C CPMAS were obtained with a NMR spectrum (Bruker Avance II 300) at 75 MHz in a cylindrical rotor of 4 mm with a speed spin of 5kMz, a contact pulse of 1ms, 90° ^1H pulse of 4μs and a 11s recycle delay. The DOM from Leptosols increases its size and molecular weight the longer the soils have been under irrigation. In addition these compounds seem to have a limited reactivity as the E_4/E_6 value and the carboxyl group concentration indicates. On the other hand the structural environment of the carboxylates (COO^-) is the same, and the relative distribution of carbon nuclei is similar in all soils irrespective of the irrigation length. In the DOM from Vertisols the wastewater irrigation produces smaller compounds and increases their reactivity as well as the COOH concentration. The structural environment of these functional groups presents a greater diversity. The relative distribution of different kinds of carbon nuclei in the DOM of Vertisols displays an increase in the nucleus from methoxyl, O-alkyls, anomeric and carbonyls.

Keywords: Organic matter; wastewater; irrigated soils.**References:**

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S2-O1

Changes on Soil Phosphorus Fractions by Phosphorus Solubilising Fungi after Rock Phosphate Addition**D. Pinochet* and E. Valenzuela***Facultad de Ciencias Agrarias, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.***E-mail: dpinoche@uach.cl*

Soils have significant amount of phosphorus which is not immediately available to crops and only a small fraction becomes available during crop season. When rock phosphate is applied to soils a significant P accumulation occurs in the inorganic P fractions extracted with HCl (Figure 1). The aim of this study was to evaluate changes on soil P fractions after rock P applications due to native solubilising fungi collected previously from an Andisol (Valenzuela et al., 2002). Soil samples of an Andisol, Valdivia soil Series, 15.4% OM, pH 5.6 with 8.5 mg kg⁻¹ P-Olsen were incubated, for 90 days to 25°C and 70% of maximum water holding capacity, with 1500 mg P kg⁻¹ applied as North Carolina rock phosphate. The treatments were native fungi *Aspergillus niger* 1 (H1) and *Aspergillus niger* 2 (H2). A *Aspergillus niger* strain CBS was used as a control (HC) and a sample with no fungi addition (RP). Phosphorus fractions were measured in 1 g of soil samples using three replicates by Tiessen and Moir method (Pinochet *et al.*, 2001). Results showed a decrease on inorganic P fractions extracted with HCl both diluted and concentrated extractions, which represents mainly P associated with Calcium, indicating that fungi solubilised rock P in soils. Also, fungi did not decrease labile organic P fraction extracted with NaOH and increased organic P extracted with HCl which represent non labile P associated to fulvic and humic acid. This study shows that *Aspergillus niger* native and CBS control strains solubilise rock P applied to soil increasing labile inorganic without decreasing labile organic P.

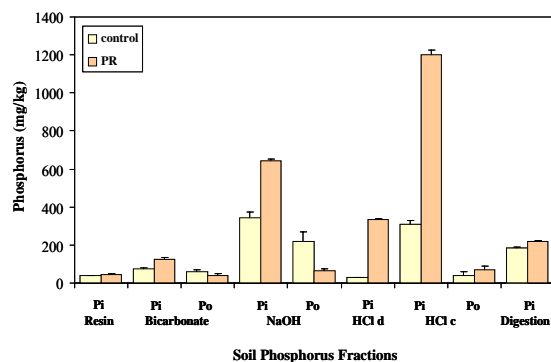


Figure 1. P Distribution after incubation without (control) and rock P addition

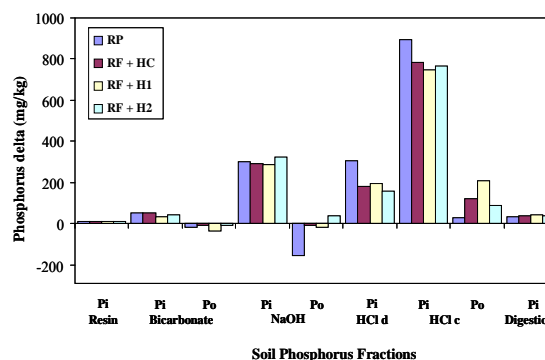


Figure 2. Changes in soil P fractions over non applied P control (delta P).

Keywords: Soil phosphorus fractions; *Aspergillus niger*; rock phosphate.

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S2-O2**Oxalate Crystal Formation in Fine Lateral Roots of *Eucalyptus* sp. Induced by Ectomycorrhizal Fungi**

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The accumulation of calcium oxalate crystals (CaOx) was evaluated in fine lateral roots and ectomycorrhizas of *Eucalyptus* sp. cultivated for 2.5 years in an area with typical topography with concave-convex side in the region of Viçosa, MG. Approximately 2,100 fine lateral roots, mycorrhizal and nonmycorrhizal, randomly collected in the study area, were diaphanized and analyzed by light microscopy for CaOx visualization. Ectomycorrhizal morphotypes were analyzed by scanning electron microscopy for the presence of calcium oxalate crystals on the mantle surface. Seventy percent of the total number of lateral roots observed showed the accumulation of CaOx in the root cortex cells either in the form of druses or grains. The conspicuous presence of CaOx was observed in 56.2% of the ectomycorrhizae and in 17.5% of the nonmycorrhizal lateral fine roots, evidencing the role of the ectomycorrhizal association in the storage of calcium in the roots of *Eucalyptus* sp. In the ectomycorrhizae druses were the predominant CaOx forms, while in nonmycorrhizal roots crystalline grains were most frequent. In the topographical positions studied (top, slope, lowland), ten ectomycorrhizal morphotypes were observed which varied as to the content of CaOx in the root cortex, suggesting distinct capacities of each ectomycorrhizal fungal species to supply calcium to the host plant. The analysis of the mantle surface of the different ectomycorrhizal morphotypes by scanning electron microscopy did not evidence the presence of CaOx in this structure, confirming that under the conditions evaluated, the accumulation of calcium crystals in the association is limited to the root cortex. This the first report on the occurrence of CaOx in the ectomycorrhizae of eucalypts under the prevalent conditions of Brazilian soils, suggesting a role for the association in supplying calcium to the plant in areas with low calcium availability.

Keywords: Oxalate; *Eucalyptus* sp.; ectomycorrhizal fungi.

S2-O3**A Single Root Model for the Impact of Root Exudates on the Fate of Phosphorus in Soils****S. Klepsch^{1,2*}, A. Schnepf¹, D. Leitner¹, J. Santner¹, M. Puschenreiter¹ and W.W. Wenzel¹**¹*University of Natural Resources and Applied Life Sciences, Vienna, Institute of Soil Research, Peter-Jordan Strasse 82, A-1190 Vienna, Austria.* ²*Austrian Research Centers GmbH - ARC, A-2444 Seibersdorf, Austria.* *E-mail: saine.klepsch@boku.ac.at

A mechanistic single root model for rhizosphere processes is proposed, which describes the effect of root exudates on the bioavailability phosphorus (P). The model includes reaction kinetics between root exudates, different forms of P, dissolved organic carbon, metal ions, and the respective sorbed species. Equilibrium and kinetic sorption processes, besides complexation, mineralization, dissolution/precipitation, degradation and decay processes are implemented in the model. Interaction between soil microorganisms, P and exudates will additionally be accounted for. Time-dependent boundary conditions imply exudation of organic ligands, and uptake of phosphate ions at the root surface. All dissolved species are subject to diffusive/dispersive processes resulting in a system of coupled 1-dimensional partial differential equations. The model will be applied to study the P nutrition of oil seed rape as affected by root exudation. It will be parameterized with data from literature and databases, but also from experiments within the scope of a project funded by the "Vienna Science and Technology Fund". Experimental approaches include the selection of oil seed rape cultivars with differing exudation pattern, determination of phosphorus exudate interactions and assessment of the effect of exudation and root architecture on plant phosphorus efficiency. Under certain conditions, plants can express higher amounts of root exudates, leading to an enhanced solute concentration around the root. Thus more appropriate conditions regarding nutrient supply may be created. The proposed model shall help to interpret the complex chemical, physical and biological interactions in the rhizosphere with regard to P and exudate control mechanisms, and lead to a better understanding of the relevant processes involved.

Keywords: Mathematical model, root exudates, phosphorus.

S2-O4**Studies on Modeling Impact of Pesticides and Photodegradation Products on Soil Microbiota by Microbiological Test-Experiments****D. Virág, Z. Naár, A. Kiss* and Z. Murányi***Eszterházy Károly University, Regional Knowledge Center, Leányka str. 6, H-3300, Eger, Hungary.***E-mail: attkiss@ektf.hu*

Pesticides might be regarded as one of the most relevant soil microcontaminants, since the use of agrochemicals has been recently significantly increased. The impact of the most frequently applied pesticides on soil microbes is more or less revealed, however degradation products have not been studied for antimicrobial effect. Thus it is of crucial importance to acquire relevant information of interaction between products formed by pesticides' natural

transformation and the soil microbiota. Our study aimed at acquiring information about the biological effect of pesticides and their degradates produced by UV-treatment on microbiological activity. Five photosensitive pesticides (carbendazim, acetochlor, simazine, chlorpyrifos, EPTC) and six representative soil microbes (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Mycobacterium phlei*, *Fusarium oxysporum*, *Penicillium expansum*, *Trichoderma harzianum*) were applied throughout our model experiments. A special, immerseable UV-light source emitting light of 254 nm effected degradation. Subsequent to the irradiation of the sample the isolation of the degradation products was carried out by column-, and gas-chromatography. Identification of degradation species was implemented by mass spectrometry. The antimicrobial effects of the pesticides and their degradates were assessed by means of applying filter paper disk method. The antimicrobial effect of the degradation products exhibited marked differences in terms of pesticide types, irradiation time, and the test organisms. Acetochlor and its photolytic degradation products were found to be more toxic to bacteria than fungi. All the three bacteria proved to be sensitive to the basic compound and its degradation products as well. The end-product of carbendazim was weakly antibacterial against *P. fluorescens* and *B. subtilis* but strongly antifungal against *T. harzianum*. Chlorpyrifos and its end-product inhibited neither test organisms, but the degradates hindered the growth of four of them. The basic compound of EPTC and the degradates of simazine exhibited significant toxicity to the test bacteria. It might be claimed that the pesticide photodegradation may result in significant changes in soil microbiota, as well as formation of biologically harmful degradates.

Keywords: Pesticides; Photodegradation; soil microbiota.

S2-O5

Ectomycorrhizas ... Does Age Matter?

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Soil microorganisms play a significant role in regulating ecosystem processes, ranging from nutrient cycling to plant health. Studies on the interactions between plants and rhizospheric microbes are important for understanding ecosystem dynamics and because it would be interesting to find out how these microorganisms could be used in agriculture and forestry management. Mycorrhizas are mutualistic associations between soil fungi and plant roots, and form a key component of soil microbial populations. They influence plant growth and nutrient uptake, and provide a greater area for interactions with other soil microbes. In temperate and boreal forests, the establishment, growth and survival of different tree species are usually dependent on colonization by ectomycorrhizas (ECM). *Nothofagus nervosa* (Raulí) is an ecologically and economically important species of South American temperate forests. In Argentina, this species has a reduced natural distribution area, in part due to its overexploitation in the past because of its high wood quality. This critical situation led to the implementation of conservation and domestication programs. Among the different aspects that should be considered in these programs are the ECM associated with this species, which

are of great importance for plant growth and for their subsequent implantation in the field. The general aim of the project is to analyze the abundance and diversity of ECM in *N. nervosa*, to compare them between native and nursery cultivated individuals and finally to select some ECM strains which would have significant application in domestication processes. In this context, the starting point is the quantification and characterization of ECM in *N. nervosa*. Hence, the first step was to analyze the percentage of root tips colonized by ECM in *N. nervosa* individuals and to compare it among plants of different ages belonging to four categories: seedlings, young and old native individuals and young nursery cultivated individuals. During spring 2007, 5 trees of each category were randomly selected from a native forest and from a field trial established in this forest in Patagonia, Argentina. Seedlings with complete root systems were carefully removed with a shovel and the rest of the plants were sampled by taking 3 soil cores per tree. Samples were wrapped in plastic bags and stored at 4°C. Roots were sieved from the soil cores, carefully washed and then ECM were quantified. ECM were observed in every sample. At least 95% of the root tips examined per plant had formed ECM. There were no significant differences in the percentage of root tips colonized by ECM among the four analyzed plant categories or within each of them. These results agree with some authors which have suggested that the development of ECM do not depend on the age of the tree. It might be possible to find different ECM morphotypes in different plant categories, but this fact needs to be further studied. This work constitutes the first description of the influence of tree age on ECM colonization in a forest of Patagonia. Besides, this study constitutes the initial step in the analyses of ECM in *N. nervosa* and provides the basis for further investigations, such as the interactions of ECM with other soil microbes, their influence in plant fitness and their possible application in conservation and domestication programs.

Keywords: Ectomycorrhizas; *Nothofagus nervosa*; plant growth.

S2-O6

Yeasts from Soil, Rhizosphere and Ectomycorrhizosphere of *Nothofagus pumilio* Forest in Northwestern Patagonia, Argentina

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Most of the microbial activity occurs in the soil, mainly in the surface layers and in the rhizosphere. The rhizosphere fraction is mainly influenced by root exudates and symbiotic activities, among which mycorrhiza fungi are one of the predominant microorganisms. These are found inside roots, in the rhizosphere (mycorrhizosphere) itself and in the bulk soil. In such soil fractions they interact with other members of the soil biota. The interactions may be of various types, both beneficial and deleterious. Some author sustain that for many ectomycorrhizal trees in the world (Pinaceae, Fagaceae, Betulaceae) the survival and normal growth is dependent of ectomycorrhizal colonization. The Andean-patagonic forests are dominated by species of genera *Nothofagus* and conifers like *Austrocedrus chilensis* and *Araucaria araucana*. These native forests are characterized by its low antropic impact and minimal atmospheric pollution. High rates of ectomycorrhizal colonization (73-79% of

infected tips) in *Nothofagus* species of the Northwestern region were previously observed. Yeasts are present in soil, but the knowledge of their diversity and function on such complex ecosystem is scarce. There are many unknown aspects of the interactions of yeasts with other biotic soil components, particularly in the mycorrhizosphere. A few reports indicated that yeasts have a probable role as colonization helper for mycorrhizal fungi. Moreover, a broad diversity of yeasts associated to roots and spores of mycorrhiza have been reported. In an attempt to understand yeast ecology of forest soils, the diversity of yeasts inhabiting bulk soil, rhizosphere and ectomycorrhizosphere of a *N. pumilio* forest is being investigated. The sampling site was located at the Southeast slope of Cerro Otto in S.C. Bariloche (Parque Nacional Nahuel Huapi, Argentina). Soil samples were collected from surrounding areas trees and were processed for yeast isolation and counting. Each sample was analyzed for ectomycorrhizal occurrence and specimens of the same ectomycorrhizae morphotype were pooled together for yeast isolation and counting. Pure colonies were obtained for yeast isolates and these were then grouped based on morphological and physiological characteristics. All *N. pumilio* trees presented ectomycorrhizal infection. A total of 134 yeast isolates were obtained: 40 from bulk soil, 49 from rhizosphere and 45 from the six more abundant ectomycorrhizal morphotypes. Yeast counts indicated that *N. pumilio* bulk soil contained an average of 4.35×10^3 viable yeast cells, while similar values were obtained for the rhizosphere fraction. Ectomycorrhizosphere values ranged between 10^2 and 10^3 , depending on the morphotype. The 134 yeast isolates were organized in 24 groups of which more than the 50% corresponded to soil fraction. Molecular studies are being performed in order to obtain species identification.

Keywords: Ectomycorrhizas; *Nothofagus pumilio*; forest soils.

S2-O7

Soil Matrix and Plant Nutrition

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The Maximum quantity of nutritious elements in soil is concentrated in solid particles, and accessible to plants in a soil solution. However the soil solution does not exist in itself, in dry soil it even is not present. It is result of interaction of atmospheric precipitations with a soil matrix. As morphology the soil matrix represents a superficial layer of solid soil particles. Its functioning is shown in the organization particles, molecules, ions around of itself in the certain order. The matrix is characterized by geometrical, chemical and power heterogeneity. The most active elements on it are the active centers. They take up very insignificant part of all surface (0.1-20%), and define almost all its reactionary ability and orientation of substances around of it, and also formation of an absorbed layer. The matrix is characterized by quantity and force of the active centers. Their number, as a rule, is proportional to the general surface, but the factor of proportionality can be distinguished in different soils and horizons. Chernozems have many "very weak" and "weak" acid centers on which nutritious elements are kept. Soddy-podzolic soils are characterized by rather raised maintenance of the "strong" acid centers. The matrix together with an absorptive layer keeps nutritious elements with different force. A part from them can pass in a solution and be absorbed by roots of

plants. The surfaces of a root and a soil matrix have similarities and distinctions in a structure. Similarities are in available active sites. There are active centers on a soil matrix, a root hair on a root surface and compartments in a cellular membrane. All of them occupy an insignificant part of a total surface (0.1-10%). Compartments in a cellular membrane differ of the high specificity. The active centers of a soil matrix are less specific, though differ on the acid-basic and power properties. Potentially accessible volume of nutritious elements in soil is defined by volume of a soil matrix (not of all soil weight) and connected with its sizes, capacity of absorption and an exchange cation capacity. Existence of matrix systems in soil: biological (roots of plants) and abiotic (horizons E, B,C) and a bioinert matrix where microbiota plays a role of the active centers (horizon A), defines special mechanisms of interaction of matrix systems in soil with other elements by means of the active centers and carrying channels. Such mechanisms allow adjusting and organizing more effectively substances in surrounding space. Regulation is shown in buffer properties of a soil matrix and a root surface. Buffer action of a soil matrix is capable to support a solution in a condition necessary for a plant during all vegetation. Potentially accessible nutritious elements are defined by their stocks in a soil matrix. They make the maximal limit of elements accessible to plants.

Keywords: Soil matrix; plant nutrition; podzolic soils.

S2-O8

Isotopic Image Analysis of Soil-Microbe-Root Interactions at the Nano-scale

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Many microbially-mediated processes exhibit high spatial variability across a wide range of scales (nm to cm) and very little is known about the spatial organization of soil microorganisms and its control. Understanding the link between the heterogeneity of the soil's physical/chemical environment and its impact on biological processes is arguably the next major frontier in soil science. Nano-scale secondary ion mass spectrometry (NanoSIMS) is a novel imaging approach that links isotopic analysis at high sensitivity with high resolution microscopy; levels of spatial analytical resolution are better than 50 nm. Here we have used NanoSIMS for soil-microbe-plant studies to trace and image ¹⁵N isotopes into individual bacterial cells and intracellular within root cells. Amino acids are an important source of N for plants and microorganisms and are major factors regulating ecosystem productivity. ¹⁵N- and ¹³C-labelled amino acids are often used to determine the relative competition between plants and microorganisms for dissolved organic N. However, it remains challenging to distinguish between direct and indirect (amino acids first mineralized to NH₄⁺) uptake by roots. Existing methods also lack adequate sensitivity for data collection

at appropriate spatial scales. In this study we combined traditional ^{15}N isotopic tracer techniques with NanoSIMS $^{15}\text{N}/^{14}\text{N}$ imaging approaches to investigate the competition between roots and microorganisms for amino acids in the rhizosphere. Highly enriched ^{15}N -labelled solutions containing N as either NH_4 or amino acid were injected along the zone of root elongation of Wheat (*Triticum aestivum* L.). Rhizocosms were sampled sequentially over a 24 hour period. Subsamples allowed the traditional determination of $^{15}\text{N}/^{14}\text{N}$ isotopic ratios for roots, soluble N pools and residual soil. In addition samples were rapidly fixed and resin embedded so that $^{15}\text{N}/^{14}\text{N}$ isotopic ratio image maps ($10\text{-}30\ \mu\text{m}^2$) of cross-sections of the rhizocosms could be obtained by NanoSIMS. Data indicate differential enrichment of roots cell types and microbes and show clear spatial patterns between the soil physical matrix (assessed as ^{28}Si), soil organic matter (assessed as ^{12}C), microbial cells- ^{15}N and plant roots- ^{15}N within the rhizocosm. NanoSIMS enabled visualization and quantification of nutrient resource capture between competing plant and microbial cells. The ability to measure ^{15}N enrichment within the rhizosphere at this previously unattainable scale provides the first opportunity to simultaneously quantify and image nutrient flow pathways in complex biological systems at a scale appropriate to the size of the competing organisms.

Keywords: Soil-Microbe-Root Interactions; NanoSIMS; *Triticum aestivum*.

S2-O9

Comparative Studies on Revealing Relation between Pesticide Accessibility and Major Soil Parameters

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Accessibility of pesticides for plants is an issue recently coming into the forefront of interest; however most of achieved results of scientific research have not led to direct agrochemical implications and practical applications so far. Relevance of the concerned research fields is emphasized by the pronounced harmful biological and environmental effect of pesticides, as well as the fact that versatile impacts of environmental conditions on plant uptake and bioavailability have not been lately extensively studied. Comprehensive studies including investigation availability of distinctive pesticides from several soil types at different pH values and organic matter content, extending to consideration of microbiological activity of soil samples have not been performed yet. In addition to filling in this gap, extensive comparative studies of diverse extraction methods have also been carried out by us in order to be capable of precise assessment of pesticide residues in distinctive environmental and biological samples. The objective of this study was to model the way and extent of plant uptake of pesticides in - one of the most important agricultural plant - wheat (*Triticum aestivum*) samples, in order to acquire information regarding "biologically incorporated" amounts of examined pesticides. On the other hand impacts of environmental conditions, including major soil types and parameters were also assessed in our studies. Applying 5 diverse extraction methods provided solid basis for proper comparison and selection of the best method, as well as led to authentic estimation of pesticide residues. The bioavailability

and the extent of plant uptake were investigated for 3 different types of soil (sandy, brown forest and alluvial soil) and 5 pesticides (simazine, chloropyrifos, acetochlore, diuron and pirimicarb) at different pH values, in cases of diverse organic matter contents. Pesticide residues in both soil and plant (roots and aerial parts, separately) samples were analyzed by GC-MS technique. The effect of microbiological activity has also been studied pointing out marked differences between extractable amounts of pesticides from sterilized and non sterilized soil samples. According to the statistical analysis the bioavailable amounts of pesticides were largely conditional on their chemical characteristics, but physical and chemical properties of the soil types might also play a considerably role. The available amounts of pesticides in microbiologically inactive soil samples were found to be 20-60% lower than those that could be gained from air-dry soil samples, where 5 germs (Gram+ and Gram-) and 4 different fungi genus were identified. The extracted pesticide amounts were in inverse proportion with the increase of the organic content in cases of all the three soil types. On the average the aqueous solvents proved to be nearly as effective in terms of extracting pesticides as chloroform. In general it might be stated that natural-like extraction methods provided significantly efficient and excellent models for estimation of bioavailability of pesticides. Pesticides were adsorbed in soils having distinctive pH values to different extent in accordance with their chemical characteristics. Pesticides were not equally accumulated in different segments of the plants exhibiting major role of pesticides' chemical feature. Differences in accumulated amounts in terms of the examined soil types were observed.

Keywords: Pesticides; GC-MS; *Triticum aestivum*.

S2-O10

FTIR as a Tool to Study Interactions between Mineral Surfaces and Microorganisms

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Sorption may affect the bioavailability and biodegradation of surface-bound substrates in the soil. The aim of this presentation is to show how attenuated total reflectance Fourier transform Infrared Spectroscopy (ATR-FTIR) can be used as a tool to follow the fate of different surface bound substrates with a molecular-level examination. We are specifically interested in P containing substrates and their bioavailability. We used the P-containing pesticide glyphosate as a model substrate in our first experimental setup since its sorption characteristics are well documented. We followed the degradation of glyphosate added to a forest soil in a bioassay where we stimulated microbial growth by additions of carbon (C), nitrogen (N) and phosphorus (P) in different combinations. Glyphosate was added in sorbed state to goethite or in a free state without goethite. We followed the effect of glyphosate additions by means of microbial CO₂ production and FTIR spectroscopy. Additions of glyphosate, in combination with glucose and N, did not change the respiration rate in

comparison to the same treatment but without glyphosate. In contrast, glyphosate additions combined with glucose and P decreased microbial growth, whereas the combination with goethite counteracted the negative effect. The different treatments were examined using FTIR; the results suggest that glyphosate was decarboxylated in the sorbed state. Stimulating microbial growth by the addition of glucose and nitrogen resulted in further oxidation of glyphosate and only phosphate was detectable after 13 days incubation. Our results show that sorbed glyphosate is microbially degradable and that it retards microbial activity. The study emphasizes the importance of combining quantitative measurements with a molecular-level examination, to better understand biogeochemical processes. We have now refined the FTIR technique so that we can study the effect of bacterial and fungal interactions with surface bound compounds in situ using FTIR-microscope with the novel array detector technology. Goethite is added directly as a thin film on the IR-transparent crystal in combination with the P-containing substrate we want to study. The set-up allows us to follow bacterial-surface interactions temporally by additions of inoculums directly on the crystal or by in-growth of fungal hyphae from the side of the crystal. Here we will present some preliminary results from the novel experimental set-up.

Keywords: FTIR; mineral surfaces; microorganisms.

S2-O11

Cover Crop Composition Affects on Soil Nitrogen Dynamics

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Cover crops are grouped into two broad categories, viz., leguminous and non-leguminous, to differentiate between nitrogen fixers and non-fixers. How the two types of cover crop residues differ in influencing the nitrogen turnover in the soil will be described based on our own research findings as well as those reported in the literature. Factors affecting the rate of mineralization of cover crops and the role of C/N ratio in this process will be discussed. The extent of variation in the nitrogen mineralization rate that can be expected between legumes and non-legumes and among different species within each group will be given. Additionally, several studies in which we incorporated cover crops in the sustainable crop production system to meet full or partial nitrogen needs of the succeeding crops will be narrated. The need for research geared to developing reliable methods of determining the rate of availability of nitrogen from different cover crop species under varying climate, soil, and tillage practices and procedures for predicting the need, required amount, and the time of application of additional nitrogen from commercial sources to achieve optimum plant growth and yield will be expounded. The relationship of biomass and nitrogen concentration of cover crop residue with the soil organic nitrogen content will be deliberated and various reported estimates of soil organic nitrogen changes due to different cover crops will be provided. The possible advantages of using a blend of legume and nonlegume instead of mono-cropping for nitrogen supply to the succeeding crop and improvement of soil organic nitrogen content will be examined.

Keywords: Cover crops; nitrogen dynamics; C/N ratio.

S3-O1**Fourier Transform Analysis of Time Series Applied to *In situ* Measurement of the Composition of Soil Solutions in Irrigated Riziculture****G. Bourrié^{1*}, P. Moreau¹, P. Henry², M. Mayor¹ and F. Trolard¹**¹INRA UR1119, Géochimie des sols et des eaux, Aix-en-Provence, France. ²Collège de France, Université Paul Cézanne, CNRS Cerege, Aix-en-Provence, France. *E-mail: bourrie@aix.inra.fr

Interactions between soil, root and microorganisms directly influence the chemical composition of soil solution. Such changes can easily be studied in irrigated crops by *in situ* measurements. A hourly frequency was chosen to monitor temperature, pH, redox potential and conductivity in irrigated riziculture. Those parameters were continuously monitored during several months, from April to September. Complete chemical analyses were made by sampling weekly soil solution, filtering and analyzing for major elements by ion chromatography and ICP-AES. Samples were collected with a syringe preventing contact of the sample with air. Ammonium, nitrate, Mn, Fe (II), sulfate and sulfide were measured directly in the field. In Camargue (Rhône Delta), soils are silty to clayey, very young and neutral to alkaline due to present calcite sedimentation. The results show temperature variations from 10 to 32°C (Fig. 1), very large pH variations (Fig. 2), with sudden drops from 8 to 3 and Eh variations from +600 to -300 mV (Fig. 3).

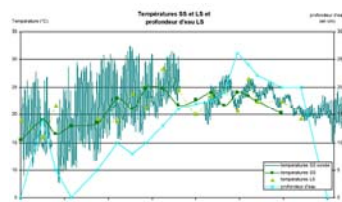


Fig. 1 : t variations

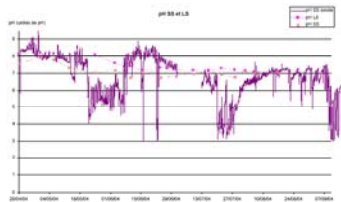


Fig. 2 : pH variations

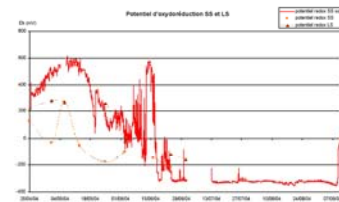


Fig. 3 : Eh variations

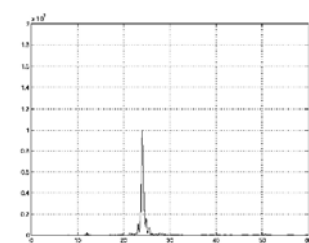


Fig. 4 : F. T. of t

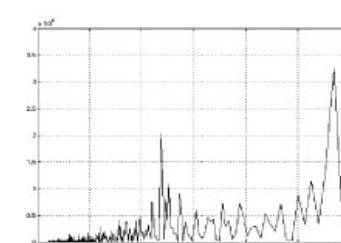


Fig. 4 : F. T. of pH

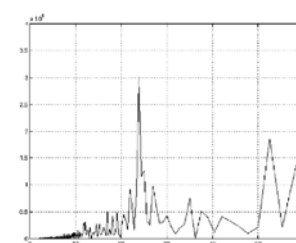


Fig. 4 : F. T. of Eh

Fourier transforms (F.T.) (Fig. 4) show a common period of 24 h (i) with phase opposition for t and pH during all the period of cultivation; (ii) with phase opposition too for t and Eh, after definitive ponding. As Leaf area increases, the shade damps temperature daily variation. Nutrient absorption by roots cause pH lowering, due to proton pump, which explains excursions from the steady value about 8, in equilibrium with calcite and atmospheric CO₂ to low values. After definitive ponding, consumption of O₂ by microflora leads to a decrease of Eh with daily variations due to the nycthemeral variations of the activity of microflora. F.T.

Session 3. Soil-Root-Microbe Interaction & Their Effects on Biophysical Transformation, Fate, and Toxicity of Metal and Metalloids

are thus an appropriate tool to analyze the complex interplay of environmental parameters and biogeochemical cycles in the field.

Keywords: Fourier transforms; soil solutions; irrigated riziculture.

S3-O2

Extractability of Nickel, Zinc and Lead as Affected by Time from a Contaminated Soil Treated with Farm Manure

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Application of farm manure not only affects the fertility properties of soils but also alter the physical and chemical attributes of the soil including the bioavailability of heavy metals. Organic matter may increase or decrease the availability of heavy metals depending upon the nature of decomposition products. A laboratory scale experiment was conducted to evaluate the effect of farm manure application on the availability of Ni, Zn and Pb. Farm manure application initially decreased the availability of Ni and Zn but increased as the organic matter decomposition proceeded. However, Pb availability decreased throughout the period of study. It is concluded that it is very difficult to predict the effect of farm yard manure on Ni, Zn and Pb availability or extractability; as it is affected by the nature of metals, metal contamination level, composition of soil and organic amendment itself.

Keywords: nickel; zinc; lead; farm manure.

S3-O3

Fate of Metals Associated with Particulate Organic Matter in Soils: Unravelling Abiotic versus Biotic Processes

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Particulate organic matter (POM) is operationally defined as 50-2000 µm-sized organic particles in soils. The quantity and quality of this labile organic constituent were shown to be strongly influenced by soil management, but the use of POM as an index for soil quality is still a matter of discussion. Recently, POM was identified as a metal-enriched organic fraction in both metal-contaminated and uncontaminated soils. The mechanisms for such metal-enrichment remain poorly understood, as there is little information on the chemical properties of POM and the fate of metal-associated POM during biodegradation. Abiotic as well as biotic processes may be involved. In this work we report both lab and *in situ* investigations of various polluted or unpolluted soils, aiming to assess the intrinsic - abiotic-reactivity of soil POM fractions and to follow the status of metals in POM during biodegradation. In the lab various POM size fractions were extracted by means of physical fractionation. EDTA was used to extract metals from the isolated organic fractions and metal

concentrations were determined by AAS. Chemical reactivity of each POM fractions was quantified by potentiometric titrations. *In situ*, we collected undisturbed samples for sub-microscopic studies. The nature, morphology and microfabrics of soil constituents were studied on thin sections and elemental mapping was performed by μ -Xray fluorescence on decaying POM. In spite of their relatively low abundance in soil (< 5% of total bulk soil weight), the POM fractions often contain a significant proportion of the total metal stock of the soils, i.e. around 10-20% for Zn and Pb, and up to 20-30% for Cu and Cd. The results show that total metal concentrations increase with decreasing POM size, while metal extractability decreases. Each POM-size fraction provides a buffer capacity due to the presence of reactive sites but affinity for copper was found comparable between the coarse and fine POM fractions. Finer POM fractions were found however to exhibit the highest contents in reactive sites per g of organic carbon. This suggests that with decreasing POM size, mainly due to decomposition, new reactive surface sites are generated. The micromorphological study reveals the occurrence of black opaque parts in decaying large POM fragments, which coincide with hot spots of heavy metals. Combined with microscopic observations, the analytical results suggest that metals are immobilized as organo-metallic complexes on very small POM-derived organic fragments. Such a process would explain the increasing organic C and metal contents frequently observed in the < 20 μ m fractions of metal contaminated soils, and suggest that small POM-derived organic particles may favour long-term metal sequestration in soils. These findings show that POM is undoubtedly an interacting medium and suggest a role of fine POM fractions as a metal sink in soils and of coarser POM fractions as a metal source.

Keywords: Metals; particulate organic matter; abiotic and biotic processes.

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S3-O4

Influence of Soil Organic Status on the Dynamics and Impact of Copper on Microbial Communities in a Vineyard Soil

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The effect of soil organic status (SOS) on copper impact was investigated by means of a microcosm study carried out on a vineyard soil that had been amended with varying types of organic matter during a previous long-term field experiment. Soil microcosms were assessed in the solid matrix by a sequential extraction procedure and in the soil solution by measuring total and free exchangeable copper concentrations. Copper bioavailability was also measured with a whole-cell biosensor. Modifications of microbial communities were assessed by means

Session 3. Soil-Root-Microbe Interaction & Their Effects on Biophysical Transformation, Fate, and Toxicity of Metal and Metalloids

of biomass-C measurements and characterization of genetic structure using ARISA (Automated-Ribosomal-Intergenic-Spacer-Analysis). The results showed that copper distribution, speciation and bioavailability are strongly different between organically amended and non-amended soils. Surprisingly, in solution, bioavailable copper correlated with total-copper but not with free-copper. Similarly the observed differential copper impact on micro-organisms suggested that organic matter controlled copper toxicity. Bacterial-ARISA modifications also correlated with the estimated metal bioavailability and corresponded to the enrichment of the *Actinobacteria*. Contrarily, biomass-C and Fungal-ARISA measurements did not relate trivially to copper speciation and bioavailability suggesting that the specific composition of the indigenous-soil communities controls its sensitivity to this metal.

Keywords: Copper; microbial communities; vineyard soils.

S3-O5

Impact of Maize Roots and Rhizospheric Microorganisms on the Mobility, Redistribution and Availability of Metals Associated to Ferralsol Oxyhydroxides

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In different type of soils, ferric oxy-hydroxides and oxides contain large amounts of trace metals (Mn, Co, Ni, Cr...) which can become available to soil solution and living organisms, depending on the environmental parameters. In New-Caledonia ferralsols, large metal contents and relatively high metal availability were observed in colluvio-alluvial and plain soils. Toxicity was noted in cultivated plants suggesting possible transfer to the food chain. Processes of iron bacterial reduction was proposed to occur in association with the biodegradation of soil organic matter. To progress in knowledge on the processes and parameters involved, in particular with the rhizosphere phenomenons, experiments were performed using soil columns or mixture of ferrasol samples and sand as support of plant culture in controlled conditions. Soil columns were either planted or not in maize in well defined water regime and subjected to different treatments: plant cultivated alone in sterile condition (without microorganism), or in non sterile soil with the whole non symbiotic microbial community and/or addition of autochtonous communities of iron reducing bacteria. Analysis concerned plant growth and metal uptake, soil solutions for pH, Eh, organic matter, metals, nitrates, ... solid phases and in particular microbial communities, sequential extraction of metals, mineral analysis. The results showed that the maize rhizosphere alone (comparatively to non cultivated soils) increased the weathering of Mn and Fe oxy-hydroxides. The amounts of well crystallized oxy-hydroxides (e.g. Fe_d) decreased with a redistribution of Fe and Mn to the ill (non well) crystallized forms (e.g. Fe_o), soluble acids, exchangeable, water soluble compartments. The presence and activity of the whole non symbiotic microbial community and much more of the autochtonous iron reducing bacterial community increased such redistribution processes of the different forms of Fe and Mn. All the metals became more available. The microbial communities increased the transfer factor of metals from soil to plant (maize), the redistribution indexes and the indexes of availability of

Session 3. Soil-Root-Microbe Interaction & Their Effects on Biophysical Transformation, Fate, and Toxicity of Metal and Metalloids

Fe, Mn, Cr, Ni, Co determined by calculation from different analytical results. The behavior of Fe, Cr, Ni are similar and those of Mn and Co are much related. Some parameters such as the dynamic and activity of bacterial communities, the availability of organic compounds originating from soil or roots and the speciation of metals have to be better defined to proposed a more accurate model of rhizosphere functioning.

Keywords: Metals; rhizospheric microorganisms; Ferralsol.

S3-O6

Cadmium Uptake and Distribution in Radish Plants Affected by Rhizosphere Salinization and Metal Contamination

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The influence of a factorial combination of four salinity levels (0, 20, 40 and 60 mM NaCl) in nutrient solution, and three cadmium (Cd) levels (0.3, 2.5 and 5 mg kg⁻¹) in peat growing media on mineral accumulation, vegetative growth and edible hypocotyl yield of radish plants (*Raphanus sativus* L. var. *sativus*) was studied in a greenhouse. After 34 days of exposure to NaCl treatment, salt-stressed plants had a reduced number of fully developed leaves (up to 30%) and total fruit yield (up to 35%) in addition to the progressively increased Na and Cl accumulation as well as significantly lower K concentration in leaf and hypocotyl tissues. Salinity significantly increased the uptake and accumulation of Cd in leaves (up to 20%). In contrast, raising salinity levels did not affect the Cd translocation and deposition into the edible hypocotyls (Cd contents being 4- to 6-fold lower than in leaves), which may indicate that the phloem mobility of Cd in radish plants is relatively poor and unaffected by NaCl exposure. Contamination of growing media by Cd had no effect on any measured parameter, except that accumulation of Cd in radish tissues was increased by one order of magnitude compared with non-contaminated control. Salinity resulted in decreased total organic carbon (TOC) content in rhizosphere solution (up to 21%). Chemical speciation modeling using Visual MINTEQ indicated the predominant role of organic reactive surfaces in Cd chemisorption and complexation processes within the whole range of tested salinity/contamination conditions, emphasizing the importance of organic matter content in restricting Cd phytoavailability and accumulation in edible parts of radish plants.

Keywords: Salinity; total organic carbon; cadmium organo-complexation.

S3-O7**Soil Organic Matter Affects Copper Toxicity to Earthworms in the Avoidance Test****A. Neaman^{1*}, G. Ávila¹, H. Gaete² and S. Sauvé³.**¹*Facultad de Agronomía, Pontificia Universidad Católica de Valparaíso, Quillota, Chile.*²*Departamento de Biología y Ciencias Ambientales, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile.* ³*Département de Chimie, Université de Montréal, Montréal, Québec, Canada.***E-mail: alexander.neaman@ucv.cl*

The earthworm avoidance test has been proposed as a potential tool for quality assessment of contaminated soils. In this test, the earthworms (*Eisenia fetida*) are presented with a choice between the tested contaminated soil and a control soil placed into the same container. Eleven agricultural topsoils (0-20 cm) were sampled at varying distances from copper mining industries in the Aconcagua River basin, central Chile. Total copper concentrations in the tested soils were in the range of 230-850 mg kg⁻¹. A control soil (95 mg kg⁻¹ of total soil copper) was sampled in an area where no mining or other anthropogenic metals sources could be found nearby. All tested soils exhibited near neutral pH, in the range of 6.8-7.8. The earthworms' avoidance was poorly correlated with concentrations of soluble and free (Cu²⁺) copper in the water extracts. The avoidance was best explained in terms of total soil copper and soil organic matter (SOM) concentrations. A tested soil containing less than 20% of the earthworms was considered to be toxic. Tested soils with total copper concentrations above 500 mg kg⁻¹ were toxic for the earthworms, regardless SOM concentrations. However, SOM determined the earthworms' avoidance in the soils with total copper concentrations below 500 mg kg⁻¹. The latter soils with SOM concentrations below 4.5% were toxic for the earthworms. In contrast, soils with total copper concentrations below 500 mg kg⁻¹ and SOM concentrations above 4.5% were not toxic. The kinetic of metal mobilization from the solid phase to the soil solution is known to decrease with the increase of SOM concentrations. The present data suggest that earthworms avoidance depends not only on the copper pool in the soil solution but also on the kinetic of copper mobilization that, in turn, is affected by the SOM concentrations.

Acknowledgments: This study is funded by the FONDECYT projects 1050403, 7070011, and 1085005.

Keywords: Soil organic matter; copper toxicity; earthworms.

Session 3. Soil-Root-Microbe Interaction & Their Effects on Biophysical Transformation, Fate, and Toxicity of Metal and Metalloids

S3-O8

Forms of Rare Earth Element Sorbed by Quartz and Goethite in the Presence of Microorganisms

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Investigation of environmental and biological effects of rare earth elements (REE) or lanthanides has recently attracted much attention because as a result of the development of modern separation and purification techniques, lanthanides have been widely applied in many fields of industry, pharmacy, and agriculture. A great many types of human activity cause REE emissions to soil, air and water. Much of the current research has focused either on the adverse influence of lanthanides on the biota or on the physicochemical interactions between metals and soil minerals, and little investigation has been directed toward the triple interaction between rare earth elements, minerals, and the biota. In our research the effect of *Rhodopseudomonas pallustris* bacterium on the sorption of sixteen isotopes of lanthanides by quartz and goethite at different pH values was studied. The sea quartz sand with particle size 420-840 μm was used. Goethite ($\alpha\text{-FeOOH}$) was precipitated on the sand using a modification of the procedure of Kinniburgh *et al.* (1975). The cell suspension of microorganisms (4.8×10^8 cell ml^{-1}) was added to quartz sand or goethite with mixed solution of lanthanides ($C=15$ ppb) and incubated at 22°C for 7 days. The pH values of experimental solutions were 4, 7 and 9. After incubation period the chemical states (exchangeable or non-exchangeable) of the lanthanides, sorbed by minerals, were determined. Exchangeable state of the elements was extracted by adding 10 ml of 0.5 M NH_4NO_3 . Non-exchangeable lanthanides were recovered by 40 ml of 0.3 M $\text{NH}_2\text{OH-HCl}$ in 1 M HNO_3 after decanting the NH_4NO_3 solution according to Stahl and James (1991). Our study shows that pH of sorption solution and affinity of elements to surface seems to be most important parameters in the interactions between metal ions and surfaces of biological and mineral sorbents. At acidic and neutral conditions these interactions was affected by electrostatic forces; at alkaline conditions the mechanism of lanthanides precipitation was dominant. Microorganisms sufficiently affected on lanthanides sorption by quartz at acidic and neutral conditions, but largest one was at pH 7. They increased sorption of all elements by goethite at pH 4. There was negligible effect of bacteria on the sorption of lanthanides at pH 7 and pH 9 by goethite that demonstrates greater affinity of the elements to goethite surface. Microorganisms increased concentration of lanthanides in the non-exchangeable states on the surfaces of quartz at pH 7 and 9, and on the surface of goethite at pH 7 in comparison to the minerals alone. It may be attributed to formation of low-soluble complexes of lanthanides with organic substances, produced by bacterium.

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Keywords: Quartz; goethite; microorganisms.

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S3-O9**Identification of Mercury Chemical Forms in Polluted Soils by Chemical Assays and Combined Synchrotron X-Ray Techniques****R. Terzano, A. Santero, M. Spagnuolo and P. Ruggiero****Department of Agro-forestal and Environmental Biology and Chemistry, University of Bari, Bari, Italy.***E-mail: pruggier@agr.uniba.it*

Determining mercury (Hg) chemical forms in polluted soils is an indispensable step towards a correct risk assessment and the development of effective remediation strategies. In this research, soil samples were collected inside and outside an industrial polluted area of National environmental interest located in “Val Basento” (Basilicata, Italy) where a chlor-alkali plant was active during the 1960-80's. Hg concentration in these soil samples ranged from 12 up to 240 mg g⁻¹. Mercury speciation in these samples was studied by a combination of sequential extraction procedures, thermal desorption analyses, and different bulk- and micro-analytical techniques exploiting high intensity synchrotron generated X-rays. Bulk XANES (X-ray Absorption Near Edge Structure) and EXAFS (Extended X-ray Absorption Fine Structure) spectra were collected for direct Hg-speciation in soil samples sieved at 2 mm as well as in the clay fraction (< 2µm), where the highest amount of Hg was concentrated. The interpretation of the complex mixture of Hg-chemical forms in the soil samples was made simpler by performing, beside bulk XAS investigations, microanalyses on soil thin sections by combined µ-XRF/µ-XRD (micro X-ray Fluorescence/micro X-ray Diffraction) and µ-XANES, with a resolution of 20 µm. The information coming from the micro-scale was then used to understand the bulk data. µ-XRF maps were collected to localize microscopic Hg-containing particles in areas of several hundreds of µm². Simultaneous to µ-XRF spectra, microdiffraction patterns were collected in each point of the map, to identify possible crystalline Hg-mineral forms or mineral associations. Once points of interest were localized, µ-XANES spectra were collected and compared with the spectra of known Hg-standard compounds. In one of the samples, Hg-containing particles with a size above 50 µm were clearly identified as HgS in the form of cinnabar by combined µ-XRF/µ-XRD. For microscopic Hg-containing particles with no detectable diffraction, µ-XANES spectra were collected. In general, two main characteristic XANES spectra (S1 and S2) could be collected from Hg-rich spots at the microscopic level. Interestingly, all the bulk XANES spectra from all soil samples could be fitted by a linear combination of the microscopic S1 and S2 spectra. However, the S1 and S2 spectra could be only partly interpreted by simply combining the XANES spectra of Hg-standards such as: cinnabar (HgS), Hg (0), calomel (Hg₂Cl₂), and corderoite (Hg₃S₂Cl₂). The presence of these forms is also in agreement with the chemical behaviour of the soil samples as assessed by sequential extractions and thermal desorption analyses. Also, more complex chemical forms beside those hypothesized seem to be present as it can be inferred by the interpretation of the bulk EXAFS spectra. In conclusion, notwithstanding the diffuse Hg-pollution in the investigated area, it seems that Hg is speciated in scarcely soluble and hardly mobilisable forms.

Keywords: Mercury; polluted soils; X-ray.

Session 3. Soil-Root-Microbe Interaction & Their Effects on Biophysical Transformation, Fate, and Toxicity of Metal and Metalloids

S3-O10

Spectrophotometric Method for the Determination of Trace Amounts of Gold using Prochlorperazine Bismethanesulphonate

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Prochlorperazine bismethanesulphonate is proposed as a selective and sensitive reagent for the spectrophotometric determination of gold. The reagent forms a red species with gold (III) instantaneously in 4-7 M orthophosphoric acid medium. An 8-fold molar excess of the reagent is necessary for the full development of the color intensity. The red species exhibits an absorption maximum at 529 nm with a molar absorptivity of $4.14 \times 10^4 \text{ L mol}^{-1}\text{cm}^{-1}$. Beer's law is obeyed over the range 0.1-6.4 ppm of gold with an optimum concentration range of 0.3-5.8 ppm. The effects of acidity, time, and temperature, order of addition of reactants, reagent concentration and interferences from various ions are reported. The method has been used for the determination of gold in gold minerals and alloys containing silver, palladium, platinum, copper and zinc.

Keywords: Spectrophotometry; prochlorperazine bismethanesulphonate; gold.

S3-O11

Solid Speciation of Uranium at the Kurdai Uranium Ore Deposit Site (Kazakhstan)

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The Central Asian Republics were major producers of uranium for the nuclear weapon programme in the former Soviet Union. Extensive mining and milling activities resulted in large amounts of uranium tailing materials and waste rock deposits, which may have a potential radiological impact on the environment. To assess the long term impact on the environment, the knowledge of radionuclide partitioning is a significant factor in order to predict and explain soil-to-plant transfer factors. In the present work, new data are presented on concentrations of ^{226}Ra , ^{210}Po , ^{210}Pb , and natural uranium isotopes in soil samples from Kurdai uranium ore deposit site collected during field expeditions to Kurdai site, Kazakhstan in July, 2006. The mining activities in the Kurdai site generated approximately 6,280,000 cubic meters of radioactive waste in the form of rock spoil heaps. In addition, the association of uranium isotopes with different geochemical phases is investigated using a sequential extraction technique according to the standard procedures of Tessier *et al.* (1979). The uranium isotope disequilibrium was utilized as a key tool to identify sources of uranium in each geochemical phases. Soil samples were dried at 80°C, ground, homogenised and sieved through a 1 mm wire mesh. Cation exchange capacity, exchangeable calcium content, pH and organic matter were determined for the characterization of the soils. X-Ray diffraction analyses was performed to assess the selectivity of extraction for different geochemical

phases and to determine mineralogical composition. The radionuclides were determined using different techniques: instrumental gamma-spectrometry, alpha-beta counting and alpha-spectrometry analysis following radiochemical separation (Fe hydroxide co-precipitation, dissolution by concentrated HNO₃, separation of U by solvent extraction using tri-n-butylphosphate, and electroplating). The results show that ²²⁶Ra, ²¹⁰Po, ²¹⁰Pb, ²³⁸U concentrations in soils within the Kurdai site were in the range 410-2913 Bq kg⁻¹, 208-2181 Bq kg⁻¹, 304-2229 Bq kg⁻¹, 300-654 Bq kg⁻¹, respectively. According to gamma-spectrometry for majority of soil samples, the determined uranium-series radionuclides were near radioactive equilibrium, which indicate that it has not been processed (such as chemical leaching) and subjected to significant radionuclide removal. Sequential extraction analysis results revealed that uranium isotope was predominantly associated with geochemical phase sensitive reducible and strongly bound (60-70%) extractants. The radionuclides ratio (²³⁴U/²³⁸U>1) indicated that uranium originate as result of sorption from water by mentioned geochemical phases.

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Keywords: Solid speciation; uranium; X-ray.

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S3-O12

Use of Soil Trace Element Model (STEM) to Assess Long-Term Fate of Cadmium in Cropland Soils: Case Study of Intensive Corn (*Zea mays* L.) Production in Central Chile

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Potentially toxic trace elements, such as cadmium (Cd), may be introduced into cropland soils by the application of fertilizers and other amendments. Frequent use of phosphorus (P) fertilizers is the most relevant Cd inputs in agricultural soils. Different concentrations of this heavy metal have been found in P fertilizers commonly used in Chilean agriculture. The long-term repeated application of these fertilizers may lead to an accumulation of Cd in soils, therefore increasing the risk of its transfer to the food chain. The Soil Trace Element Model (STEM) is a recently developed mass balance-based model to assess the fate and transport of trace elements in cropland soils. The objective of this work was to evaluate, through the STEM, the impact of long-term use of P fertilizers on the accumulation of Cd in the soil. The model was applied to a typical Chilean corn production system characterized by intensive use of inputs such as herbicides and fertilizers. Field and laboratory experiments were conducted to investigate the dynamics of Cd in a clay loam Mollisol which is representative of central Chile region. Data from soil and plant analysis were used to determine model parameters. Typical management practices such as irrigation and biomass returned to the soil after harvest

were represented in our simulations. Climate information (precipitation and evapotranspiration) from the nearest meteorological station was used as well. Three possible scenarios were evaluated considering different Cd concentrations found in commercially used P fertilizers: 1) triple superphosphate (TSP) with the highest Cd concentration (regarded as an extreme case), 2) TSP with an average Cd concentration, and 3) monoammonium phosphate (MAP) with the lower Cd concentration. STEM simulation outcomes indicate that in scenario 1, after 46 years a total Cd concentration of 0.5 mg kg^{-1} may be reached, which indicate an anthropogenically contaminated soil. On a 100 years horizon, the total Cd concentration would be 0.72 mg kg^{-1} , 2.4 times the actual content. In scenario 2, after 85 years a total Cd concentration of 0.5 mg kg^{-1} may be reached, and after 100 years the total Cd concentration would be 1.8 times the actual content. In the scenario 3, the Cd content of the soil would be slightly increased after 100 years, indicating a low impact of MAP fertilization. Considering P dose recommendations for high-yield corn production, we estimated that fertilizers with a Cd concentration ranging from 3.0 to 4.0 mg kg^{-1} would not increase the actual Cd content of the soil because of a balance between the inputs of Cd and the outputs by plant extraction and the downward movement of the element below the plow layer.

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Keywords: Cadmium; cropland soils; *Zea mays*.

S4-O1**Pentachlorophenol Degradation in Two Biological Systems: Biobed and Fixed-Bed Column, Inoculated with the Fungus *Anthracophyllum discolor*****M.C. Diez^{1*} and G.R. Tortella²**¹Departamento de Ingeniería Química, ²Programa de Doctorado en Ciencias de Recursos Naturales. Universidad de La Frontera, Temuco, Chile. *E-mail: mcdiez@ufro.cl

Anthracophyllum discolor, a white-rot fungus of southern Chile, produces ligninolytic enzymes specially manganese peroxidase (MnP), and has been studied for its potential use on bioremediation of contaminated soils with chlorophenols. The main purpose of this study was to evaluate the pentachlorophenol (PCP) degradation in two biological systems (biobed and fixed-bed column). Both systems used an Andisol and were inoculated with *A. discolor* immobilized in lignocellulosic material. The biobed system consisted of a mixture of straw (50% v/v), peat (25% v/v) and topsoil (Andisol) (25% v/v). The biobeds were contaminated with three successive application of 100 mg kg⁻¹ of PCP each (0, 21 and 55 days). After the contamination, one biobed was inoculated with the fungus *A. discolor* immobilized on wheat straw, and the other was non-inoculated and was used as a control. During the incubation period (80 days at 25°C), CO₂ evolution, enzymatic activities (laccase and manganese peroxidase) and residual concentration of PCP were periodically evaluated. Fixed-bed columns (35 × 5 cm) packed with a mixture of 50% quartz sand and 50% of allophanic soil (Andisol) activated by acidification (pH 4.5) were used for PCP removal. Wheat grains colonized by *A. discolor* were put on the upper part of each column. The columns were operated in continuous and were fed with PCP (100 mg L⁻¹) under a flow rate of 1.5 mL min⁻¹, at room temperature during 29 days approximately. Samples were taken from the effluent of the columns at pre-determined time intervals and analyzed for the remaining concentration of PCP and for manganese peroxidase (MnP) and laccase enzymatic activity quantification. In biobed system, degradation of PCP occurred efficiently over the whole incubation period. However, it was significantly quicker in biobed amended with the fungi compared with non-inoculated biobed, mainly in the first 21 days. Over this time, no differences were found between inoculated and non-inoculated biobed on PCP degradation. A fraction of the initial PCP concentration was adsorbed (aprox. 40%) in each application by the biobed matrix. The MnP activity was the highest in inoculated biobeds, and the laccase activity was the highest in control biobeds. In fixed-bed column, PCP adsorption was high in the first 12 h and decreased suddenly up to 48 h. During the days 5 to 14, the PCP was efficiently degraded, and the concentration was stabilized in the range of 30 mg L⁻¹ in the effluent. After that, PCP concentration increased and the column was saturated on day 16. The lowest values of PCP coincide with the production of MnP, showing that *A. discolor* increased degradation of PCP. In conclusion, the results of this study demonstrate that PCP was efficiently removed by the two biological systems, both by adsorption and by biodegradation processes.

Acknowledgements: Investigation financed by FONDECYT 1050614 and DIUFRO GAP-2007 projects.

Keywords: Pentachlorophenol degradation; biobed; *Anthracophyllum discolor*.

S4-O2**Fate of Pentachlorophenol (PCP) and Dynamics of Chemical and Biological Properties in an Artificially PCP-contaminated Soil as Affected by Compost and Dissolved Organic Matter****R. Scelza, M.A. Rao* and L. Gianfreda***Dipartimento di Scienze del Suolo, della Pianta, dell'Ambiente e delle Produzioni Animali, Università di Napoli Federico II, Portici, Italy. *E-mail: mariarao@unina.it*

Pentachlorophenol is a highly chlorinated organic compound extensively used as a broad-spectrum biocide, particularly in the wood preservation industry. As with other xenobiotics, PCP can exist in soil in a strongly sorbed state and the size of this desorption-resistant fraction may increase with the length of time the chemical remains in the soil. Soil organic matter can enhance PCP adsorption and hinder its desorption whereas association with dissolved organic matter (DOM) in the soil solution can increase the water solubility and mobility of the chemical. Very limited information exists on the effect of pentachlorophenol on soil biological and biochemical properties which are highly sensitive to changes caused by management practices and environmental stress, and may provide an early warning of soil quality changes. The main goal of this study was to evaluate, in a long-term experiment (500 days) under laboratory conditions, the response of soil to PCP contamination and the relative effects and potential remediation capacities of compost and dissolved organic matter. Soil response was assessed by measuring the disappearance of PCP when added to a fresh, agricultural soil, with and without compost and dissolved organic matter, both added at two different rates and evaluating the dynamics of physical and chemical properties, microbial biomass, basal respiration and some enzymatic activities, related to carbon, nitrogen, phosphorus and sulfur cycling, in the contaminated soil amended or non-amended with the two compost or DOM doses. PCP concentrations declined progressively and significantly with time. This effect was most pronounced for the soils amended with the lower compost dose and with the two DOM amounts. The soil showed an endogenous microbial activity as indicated by basal respiration, microbial biomass and all the enzymatic activities tested (dehydrogenase, β -glucosidase, phosphatase, arylsulfatase, urease). Addition of the PCP severely depressed some of the tested biochemical properties suggesting an inhibitory effect on microbial activity. Conversely, higher basal respiration, and similar β -glucosidase and phosphatase activities were measured in comparison with the controls. No significant effects were observed following the addition of two doses of the compost or the DOM. Fungal colonies belonging to the taxonomic group of *Ascomycetes* and identified as *Byssoschlamys fulva* developed with time in all the PCP-contaminated samples. Growth of *B. fulva* *in vitro* in the presence of PCP showed that the isolate was tolerant to 12.5 and 25 mg L⁻¹ PCP and degraded 20% of its initial concentration in 8 d. Overall, the results indicate that many complex processes occurred in the contaminated soils and combinations of these determined the response to PCP contamination. The sorption of PCP to the soil matrix (which increased with time) and its degradation/transformation by indigenous soil microbial activity were likely involved. Both the processes appeared to be favored by the presence of dissolved organic matter.

Keywords: Pentachlorophenol; compost; dissolved organic matter.

S4-O3**Unraveling Plant-Soil-Microbe Interactions for Phytoremediation of Soil Organic Contamination: Limitations and Opportunities****J. Xu*** and Y. He*Institute of Soil and Water Resources and Environmental Science, Zhejiang University, Hangzhou 310029, China. *E-mail: jmxu@zju.edu.cn*

Success in phytoremediation can be facilitated through the deep unravelment of plant-soil-microbe interactions under stress from organic contaminants (OCs). Due to the innovative methods, such as rhizosphere *in situ* sampling, mass separated detection and biomolecular techniques, we eagerly anticipate significant insights into plant-microbe interactions in a contaminated rhizosphere, providing a solid mechanistic understanding for phytoremediation. Several key aspects regarding the limitations and opportunities for research in the academic field mentioned above will be presented as follows:

Innovation of rhizosphere in situ sampling techniques. The rhizosphere is a heterogeneous system, and is difficult to physically sample and precisely manipulate. Some sensitivity of measurement is inevitably lost in an attempt to acquire a fixed quantity of root-free, homogenized rhizosphere soil, since the minute volumes of rhizosphere would be diluted by the bulk soil during sampling. It is challenging, but necessary to develop in-situ sampling techniques and protocols, which take into account the spatial and temporal variability of chemical and biological changes in the rhizosphere in response to OCs stress.

Clarity for the adaptive regulation of plants under OCs stress. The lack of comprehensive knowledge of exudate chemistry is a major barrier to unraveling the plant-soil-microbe interactions since the techniques used to collect the root exudates are often destructive, causing disturbance to the delicate interactions between the root and soil community. Given the exciting advances that have been achieved in the recent years in quantitative analytical approaches for measuring dissolved organic matter (DOM), we advance a new and alternative way to address this important scientific problem. A new approach to the direct and *in situ* study of rhizosphere C flow in the form of rhizosphere DOM will be presented.

Application of novel techniques for rhizosphere microbial identification. Recent advances in molecular methods provide an exciting opportunity to link rhizosphere microbial structure and function with the role they play in rhizodegradation. One of the most powerful and innovative techniques is stable-isotope probing (SIP), which relies on the incorporation of isotope-labeled substrates, such as fatty acids, DNA and RNA, into the biomass of the active microorganisms. Following extraction of the labeled biomass, insight into the structure and identity of the active members of a microbial community can then be gained with unprecedented clarity and precision. Application of this technique is expected to disclose microbial phylogeny with function, providing detailed information on the main microbial taxa involved in rhizodegradation.

Despite the limitations in the scope of this paper, we hope our discussion will inspire exciting and promising avenues for further research in plant-soil-microbe interactions for phytoremediation of soil organic contamination.

Keywords: Plant-soil-microbe interactions; phytoremediation; organic contamination.

S4-O4**Chlordecone Contamination of Root Vegetables in Long-term Polluted Soils in the FWI: Analysis of Levels of Pollution Provides a Decision Tool for Safe Production****M. Lesueur Jannover^{1*}, R. Achard¹ and Y.M. Cabidoche²**¹PRAM CIRAD, Petit Morne, BP 214, 97285 LE LAMENTIN (FWI), Francia.²INRA, UR 135 Agropédoclimatique de la Zone Caraïbe, Domaine de Duclos, 97129 PETIT BOURG (FWI), Francia. *E-mail: jannoyer@cirad.fr

Chlordecone is an organochlorine pesticide whose use is now forbidden but which persists in soils. It was used as an insecticide against the banana weevil from the 1970s to the early 1990s in the French West Indies. Late contamination of root vegetables (RVs) was revealed in 2002. In our work, we studied the mode of transfer of chlordecone from different types of tropical soil (Andosol, Ferralsol and Nitisol) to different RVs (dasheen, yam, sweet potato, turnip and radish) to explore plant sensitivity to contamination. We conducted complementary experiments: surveys, field trials, and mesocosm trials to link soil chlordecone content with plant chlordecone contamination. In all those experiments, RVs contamination was heterogeneous as soil pollution was heterogeneous, but dispersion of the chlordecone molecule was the same for each crop. Due to this dispersion, the soil type did not significantly affect contamination. Average contamination values measured in harvested organs were positively correlated to chlordecone soil content. Belowground organs themselves were contaminated heterogeneously depending on where there was direct contact between the organ and contaminated soil. The cortex was more highly contaminated than the pulp (up to ten times more). These two results thus support the hypothesis that contamination by direct contact between the chlordecone molecule and the plant organ is the most important mode of transfer in RVs. This has already been demonstrated in the yam *Discorea cayenensis*, whose tubers were chlordecone free even when the root system explored contaminated soil. Chlordecone diffusion into the plant was very weak suggesting that it is a passive process in RVs. A common model, based on morphology and duration of the soil/organ contact, accounted for the RVs contamination. We calculated an envelope curve of the relationship between soil chlordecone content and RVs contamination and the maximum transfer ratio observed was 1/5. This envelope curve which represents zero risk of contamination was calculated for crops on both Andosol and Ferralsol. This relationship is thus a decision tool to determine whether or not there is a risk involved in cultivating roots and tubers based on the result of soil analysis. With this tool, the risk of contamination can be foreseen at planting which is preferable to analyzing the product at the harvest stage. Further studies are in progress to determine the sensitivity of other mainly horticultural crops and fruits, and to analyze the contamination of aboveground organs. Factors that govern the bioavailability of the chlordecone molecule in the soil for the plants, like water, organic matter content, type of clay, need to be analyzed in more detail to better understand chlordecone transfer to plants and thus improve pollution management.

Keywords: Organochlorine pesticide; soil pollution; food safety.

S4-O5**Impact of Crop Residue Management on Sequestration of Soil Carbon, Transformation of Nitrogen and Irrigation Water use in Rice-Wheat Cropping System****N.S. Pasricha****Potash Research Institute of India, Dundaheha, Gurgaon, Haryana, India. *E-mail: nspasricha@sify.com*

Rice-wheat is the most popular cropping system followed on around 13.5 million ha of land in the South Asia extending across the Indo-Gangetic alluvial plain. To vacate fields for the timely sowing of wheat, farmers often burn rice straw in the fields in India. To overcome this problem, Zero-Till Seed-cum-Fertilizer Drill (ZTP) has been tried for sowing of wheat without any pre-sowing irrigation in the standing straw of rice (after the combine harvesting of rice) in a field experiment conducted for quantification of saving in organic carbon and irrigation water, and transformation of N in rice-wheat rotation. Rice (*Oryza sativa* cv. Sugandha-2, fine quality fragrant *Basmati* rice) was grown in rotation with wheat (cv. WL-343) on sandy loam soil in Gurgaon, Haryana, India. Six, 15 by 10 m plots, were arranged in two blocks. Micro plots of 1.2 by 1.2 m were maintained in each plot for N-15 studies. At maturity, rice crop was simulated combine-harvested in one block of 3 plots, leaving the anchored rice-straw and stubbles in the field. In the other block of 3 plots, rice was harvested up to ground level and the straw was removed from the field. Wheat was sown with Zero Till (ZT) in the standing rice straw with residual moisture in one block of three plots (no pre-sowing irrigation for wheat). In the other block of 3 plots, pre-sowing irrigation was given and wheat sown when soil came to workable conditions by conventional tillage practice (CT) providing two hoeing followed by planking. Total crop residue of rice returned to the soil surface in rice-wheat rotation with ZT than with CT (to wheat) was 3.13 t ha^{-1} . Total amount of organic carbon added to the soil through the above ground straw of rice in the ZT plots was 1.25 t ha^{-1} . On an average, 40% more of OC and around 45% more of N, P and K were returned with ZT as compared to CT practice. This practice of zero-tillage will prevent, on an average, 4.6 t ha^{-1} of CO_2 from emanating to the atmosphere. Zero-till wheat had lower N concentration than corresponding CT wheat indicating lower availability with zero tillage. Total N uptakes of N in wheat at harvest were lower in ZT (144.6 kg ha^{-1}) as compared to CT plots ($184.37 \text{ kg ha}^{-1}$). However, percent N derived from fertilizer at 44.62 was almost 8% higher than CT treatment, which were only 36.05. Soil profile (0-1.2 m) N content after harvest of wheat at 2612 kg ha^{-1} in CT plots was higher by 200 kg ha^{-1} than ZT plots at 2420 kg ha^{-1} . This could be ascribed to immobilization of the N, as residue decomposition is very slow in ZT plots due to lack of mixing. A mulched, non-plowed tillage system can potentially affect mineralization perhaps due to altered temperature and altered soil moisture content. Total amount of water needed to irrigate the wheat crop to full maturity was less by $1016.5 \text{ kg L ha}^{-1}$ in ZT treatment and there was a net saving of 100 mm ha^{-1} water in wheat. Water use efficiency in terms of $\text{kg wheat grain ha}^{-1} \text{ mm}^{-1}$ was higher by 16% in ZT plots as compared to CT treatment. Post-harvest moisture content in the soil profile (0-1.2 m) in ZT treatment was 30 mm higher than in CT plots. Fewer evaporation losses due to mulching effect of rice-straw in wheat could be the reason for higher moisture content in 0-1.2 m-soil profile in ZT plots.

Keywords: Soil carbon sequestration; nitrogen; rice-wheat cropping system.

S4-O6**Enantioselective Degradation and Unidirectional Chiral Inversion of 2-Phenylbutyric Acid by *Xanthobacter flavus* PA1****J.D. Gu*** and Y. Liu*Division of Microbiology, School of Biological Sciences, The University of Hong Kong, Hong Kong.***E-mail: jdgu@hkucc.hku.hk*

Linear alkylbenzenes, associated with detergents and chemical synthesis, are commonly detected in the environments. Microbial degradation of chiral 2-phenylbutyric acid, a carboxylic acid metabolite of linear alkylbenzene degradation, was investigated by using enantioselective high performance liquid chromatography (HPLC). A pure culture of bacteria, identified as *Xanthobacter flavus* strain PA1 based on 16S rRNA gene, was isolated from mangrove sediments of Hong Kong Mai Po Nature Reserve with ability to utilize the racemic 2-phenylbutyric acid as well as the single enantiomer as the sole carbon and energy source. Aerobic growth in batch experiments with the racemic compound showed that strain PA1 could degrade both enantiomers to completion and utilize them sequentially: the [S] enantiomer disappeared much faster than the [R] enantiomer. When the single pure enantiomer was used as the growth substrate, a unidirectional chiral inversion from [S] enantiomer to [R] enantiomer was observed. No difference was observed in the metabolites during the catabolism of individual enantiomers. The major two degradation intermediates were identified by liquid chromatography-mass spectrometry (LC-MS) and ¹H & ¹³C nuclear magnetic resonance (NMR) spectra as 3-hydroxy-2-phenylbutanoic acid and 4-methyl-3-phenyloxetan-2-one. These results provide evidence that the biochemical degradation pathway proceeds with an initial oxidation of the side chain before aromatic ring cleavage. This study reveals new evidence for enantiomeric inversion by pure culture of bacteria and shows the importance of examining the fate of both enantiomers of chiral pollutants in the environment.

Keywords: Enantioselective biodegradation; chiral inversion; *Xanthobacter flavus*.

S4-O7**Interactions of Bt Cry1Aa Toxin with Soil and Soil Components: Adsorption, Extraction and Persistence****N. Helassa, G. Daudin¹, S. Noinville², P. Déjardin³, J.M. Janot³, H. Quiquampoix¹ and S. Staunton^{1*}**¹*INRA-Biogéochimie du Sol et de la Rhizosphère, place Viala, 34060 Montpellier, France.*²*CNRS-LADIR, 2 rue Henri Dunant, 94320 Thiais, France.* ³*CNRS-Institut Européen des Membranes, 47 place Eugène Bataillon, 34095 Montpellier, France.* **E-mail: staunton@montpellier.inra.fr*

Genetically modified (GM) crops, which produce insecticidal Cry proteins from *Bacillus thuringiensis* (Bt), release the toxins into soils through root exudates and upon decomposition of residues. In contrast to the protoxin produced by the *Bacillus*, the protein produced in GM crops does not require activation in insect midguts and thereby potentially loses some of its species specificity. Although gene transfer and resistance emergence phenomena are well

documented, the fate of these toxins in soil has not yet been clearly elucidated. Cry proteins, in common with other proteins, are adsorbed on soils and soil components. Adsorption on soil and the reversibility of this adsorption is an important aspect of the environmental behaviour of these toxins. The orientation of the molecule and conformational changes on surfaces may modify the toxicity and confer some protection against microbial degradation. Adsorption will have important consequences for both the risk of exposition of non target species and the acquisition of resistance by target species. We have adopted different approaches to investigate the fate of Cry1Aa in soils and model minerals. In each series of experiments we endeavored to maintain the protein in a monomeric form (pH above 6.5 and a high ionic strength imposed with 150 mM NaCl). The adsorption and the desorbability of the Cry1Aa Bt insecticidal protein were measured on two different homoionic clays: montmorillonite and kaolinite. Adsorption isotherms obtained followed a low affinity interaction for both clays and could be fitted using the Langmuir equation. Binding of the toxin decreased as the pH increased from 6.5 (close to the isoelectric point) to 9. Maximum adsorption was about 40 times greater on montmorillonite (1.71 g g^{-1}) than on kaolinite (0.04 g g^{-1}) in line with the contrasting respective specific surface areas of the minerals. Finally, some of the adsorbed toxin was desorbed by water and more, about 36%, by high pH buffers, indicating that it was not extremely tightly bound. Moreover, the toxin was easily and quasi-completely desorbed using zwitterionic and non-ionic detergents. The evolution of the Amide I band measured by Fourier Transform Infrared Spectroscopy indicate pH-dependent changes in conformation of the protein when adsorbed on montmorillonite and silica. Adsorption on montmorillonite prevents oligomerisation. Adsorption was greater and caused a larger degree of conformational change when the silica was made hydrophobic. We have compared the persistence of Cry1Aa on various soils over several weeks varying microbial activity (inhibition or activation). We can thus distinguish between degradation of the protein and chemical fixation that leads to decreasing efficiency of extraction.

Keywords: Bt Cry1Aa Toxin; soil interactions; montmorillonite and kaolinite.

S4-O8

Carbon-13 NMR Spectroscopic Characterization of the HF-insoluble Organic Matter in some Palaeosols from the Loess Plateau, China

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We have used solid-state ^{13}C -nuclear magnetic resonance (NMR) spectroscopy to characterize the organic matter in five palaeosols and the topsoil from the Loess Plateau in north-central China that remained in the residue after extraction with HF. The Palaeosol samples were taken at different depths (10-76 m) along the 130 m thick loess-palaeosol type sequence near Luochuan, and ranged in age from 120 to 1070 kyr BP. The overlying surface soil was 10 kyr old. More than 60% of the initial C and N were lost during HF treatment, indicating that most of the organic matter was stabilized by interaction with the mineral

constituents, notably CaCO_3 . The ^{13}C -NMR spectra of the organic residue (after HF-treatment) were divided into four chemical shift (δ) regions: (I) $\delta = 160\text{--}220$ ppm, due to carboxylic acids and amides, accounts for <18% of the total spectral intensity; (II) $\delta = 110\text{--}160$ ppm, identifiable with aromatic rings and olefinic ($\text{C}=\text{C}$) structures, makes up 28–36% of the total organic carbon; (III) $\delta = 60\text{--}110$ ppm, assigned to O-alkyl and anomeric carbon, accounts for 19–33% of the total carbon; and (IV) $\delta = 0\text{--}60$ ppm, due to aliphatic structures and amino acid side chains in proteins, makes up 26–31% of the total intensity. The abundance of δ -region (I) carbon tends to diminish with an increase in Palaeosol age. On the other hand, the contribution of δ -region (II) carbon increases with depth and time of soil burial. This would indicate a selective enrichment (with age) of aromatic constituents in humic material formed by biological processes. We also propose that these aromatic structures are partly associated with charred residues, formed during vegetation fires that seem likely to have occurred across the Loess Plateau. The long-term survival of δ -region (III) carbon, normally assigned to O-alkyl functional groups in carbohydrate structures, may be due to physical protection within micropores. Alternatively, these groups may be associated with polyhydroxy acids rather than carbohydrates. Carbon in δ -region (IV) may largely be assigned to polymethylene structures as indicated by the intense peak near $\delta = 30$ ppm. Since the ratio of region IV to region I carbon is < 4, the surviving polymethylene structures may partly represent polymers of short-chain aliphatic acids. Although their abundance tends to diminish with increasing palaeosol age, these recalcitrant structures still make up 26% of the total organic carbon in the oldest palaeosol investigated (1070 kyr BP). The similarity of the spectra of the older Palaeosol samples to those of charred residues and soils containing charred material, published in the literature, is indicative of the refractory character of charred residues and their important role in long-term carbon sequestration.

Keywords: NMR spectroscopy; insoluble organic matter; Palaeosol.

S4-O9

Molecular Interactions of Pesticides in Whole Soils using Modern NMR Spectroscopy

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Pesticide sorption to soil organic matter (SOM) plays an important role in reducing pesticide bioavailability, and complicates bioremediation efforts. Elucidation of the mechanistic interactions of anthropogenic chemicals in whole soils as well as understanding the physical and chemical factors which influence their sorption is critical to understanding and eventually predicting their behavior in the environment. This presentation will introduce and explain a range of novel Nuclear Magnetic Resonance (NMR) approaches that can directly applied to whole soils to assess the molecular interaction of anthropogenic chemicals at the molecular level.

Information garnered includes: 1) Identifying the binding mechanism of contaminants in whole soils, 2) Identifying the soil components responsible for binding, 3) Monitoring the real-time kinetic transfer of pesticides from the solution-phase, through the gel-phase and finally sequestration into solid phase. High Resolution Magic Angle Spinning NMR will be briefly introduced as well as the concepts of single (STD) and double difference (STDD) saturation transfer NMR.

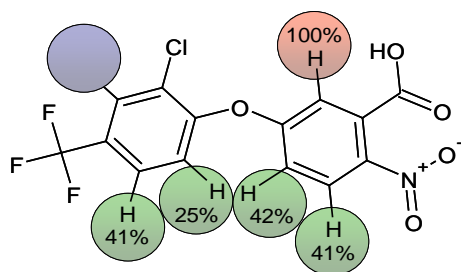


Figure 1. The binding epitope of Acifluorfen in a whole peat soil. Percentage (%) indicates the relative strength of interaction for each proton with the soil surface.

Results will be provided showing how binding mechanisms can be easily extracted from these experiments for a range of pesticides. For example, the binding epitope of acifluorfen in whole soil (Figure 1) shows how NMR can be used to gain specific information as to the molecular binding of pesticides in whole soil. Results indicate that dipolar interactions, H-bonding, hydrophobic associations and potentially π - π interactions are the predominant sorption mechanisms for pesticides at the soil-aqueous interface. It is evident that soil physical and chemical characteristics are highly influential in determining the mechanisms of pesticide sorption, as they significantly affect soil conformation. In particular, different binding mechanisms were observed for 1-naphtol in soil swollen using buffer versus D_2O , indicating that the K_{oc} alone may not be enough to accurately predict the behavior of a molecule in a real soil environment. Preliminary kinetic-based studies suggest that both the swelling solvent and soil moisture content significantly influence the sequestration of trifluralin. These studies demonstrate that HR-MAS and STDD NMR are powerful and versatile tools which can be applied to expand our knowledge of the mechanistic interactions of agrochemicals at the molecular level.

Keywords: Pesticide; NMR spectroscopy; anthropogenic chemicals.

S4-O10**Coupled LTA - PAS FTIR Approach for Studies of Organic Matter Dynamics and Function at the Interface of Soil Aggregates****L.P. D'Acqui^{1*}, A. Pucci^{1,2} and L. Calamai¹**

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LTA-PAS FTIR approach can be considered as an original tool useful to study the dynamics, function and protection of soil organic matter (SOM). Studies on SOM are generally carried out mainly using chemical extraction methods that modify both organic substances and mineral phases resulting in possible artifact. Low-Temperature Ashing (LTA) by oxygen plasma allows a controlled removal at low temperature of SOM from the mineral matrix of soil aggregates with minimal disturbance and damage to the inorganic constituents. Photoacoustic (PAS) -FTIR spectroscopy allows to obtain IR spectra from the surface of irregular tri-dimensional specimens. When LTA is coupled with PAS-FTIR spectroscopy, it is possible, at each step, to obtain spectroscopic evidences of the residual OM layers and, by difference, the spectra of the removed SOM. Therefore, the coupled LTA-PAS-FTIR approach can provide insights on the nature of SOM as well as the surface interaction of organic substances in undisturbed soil aggregates or in other solid materials.

Keywords: Organic matter; LTA - PAS FTIR spectroscopy; soil aggregates.

S5-O1**Geomicrobiology of Acidic Hot Spring from the Largest Geothermal Region, Kamojang, West Java, Indonesia****A. Akhmaloka*, H. Yohandini, P. Aditiawati and F. Madayanti***Biochemistry Research Division, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Jln Ganesha 10, Bandung, Indonesia. *E-mail: loka@chem.itb.ac.id*

Kamojang is one of the biggest geothermal regions in Indonesia. This area has a few hot springs divided into two types eg geyser and poll mad hot springs. Two hot springs, namely Kawah Hujan A (KHA) and Kawah Hujan B (KHB) represented both type of hot spring were chosen as model for assaying the biodiversity of thermophilic microorganism. The physico-chemical of the hot springs was differences. The KHA has pH at around 7.2 - 7.4, temperature at 80-86° C with concentration of Fe and sulfate ion at 0.08 and 156 mg mL⁻¹ respectively, while KHB has pH 1.8-1.9, temperature at 90-92° C and Fe and sulfate ion at 12 and 2,740 mg/mL respectively. Biodiversity of thermophilic microorganism from these hot springs were analysis using 16 SrRNA gene sequences combining with DGGE method. The biodiversity was measured by two approaches, culture dependent and independent strategies. The results showed that the KHA samples were dominated by gama-proteobacteria while the KHB samples were dominated by crenorchaeta (arkhae) and gama-proteobacteria. The organisms from KHA samples were less variation compared to that the KHB. From both hot springs we found a few uncultured organisms from culture dependent strategy and a few unidentified organisms based on phylogenetic analysis.

Keywords: Acidic hot spring; thermophilic microorganisms; DGGE.

S5-O2**Bioremediation Studies for the Clean-Up of Chlorinated Herbicide-Treated Agricultural Soils****V. Morgante, P. Miralles, M. Hernández, C. Flores, M. González and M. Seeger****Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Departamento de Química and Millennium Nucleus of Microbial Ecology and Environmental Microbiology and Biotechnology EMBA, Universidad Técnica Federico Santa María, Valparaíso, Chile.***E-mail: michael.seeger@usm.cl*

Herbicides are used world widely in agriculture and forestry. Chlorinated *s*-triazine herbicides (simazine, atrazine) are released in cultivation fields for weed control. Their persistence in the environment and potential toxicity for living organisms is of increasing concern. *s*-Triazines have been detected in surface and ground water in North America, Europe and Australia. Recently, *s*-triazines have been determined in watersheds close to agricultural fields in Chile. Bioremediation is an important biotechnology for the removal of persistent organic pollutants from soils. The low cost and *in situ* application are important advantages of this technology. The aim of this report was to analyze the effect of bioremediation on the fate of the herbicide simazine in agricultural soils. The native bacterium *Pseudomonas nitroreducens* MHP41 was characterized and selected as catalyst for the bioremediation studies. To describe simazine retention and availability in agricultural soils, batch experiments were conducted and the sorption coefficient was determined.

Herbicide depletion in soil microcosms was analyzed by HPLC. Microbial community changes during bioaugmentation were studied by cultivation-independent methods such as T-RFLP and FISH. Field studies were also performed. Distribution coefficient indicated moderate adsorption of simazine to these agricultural soils. Microcosm studies showed an increased degradation of the herbicide simazine in soil by bioaugmentation with *P. nitroreducens* MHP41. Microbial community analysis revealed that bioaugmentation with the bacterial strain MHP41 promotes changes in the structure of the soil microbiota. *Acidobacteria*, α -*Proteobacteria* and *Planctomycetes* are the phylogenetic groups most affected by bioaugmentation. In field studies, herbicide depletion was observed under natural environmental conditions using bioaugmentation. The fate of s-triazines in soil depends on physico-chemical as well as biological processes. Successful simazine biodegradation in agricultural soils was achieved by bioaugmentation with *P. nitroreducens* strain MHP41. This study showed that bioremediation could be useful for the clean-up of agricultural soils treated with pesticides.

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Keywords: Bioremediation; chlorinated herbicide; agricultural soils.

S5-O3

The Environmental Impact of Lignite Fly Ash Interacted with Quality of Soil and Rice Plant

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Lignite fly ash, a byproduct of coal combustion, was produced 75-85 % of total ash. 6,000-9,000 tones fly ash/day is a production from lignite electric power utility Mae Mao, Lumpang Province. Chemical composition of lignite fly ash consist of essential elements for plant and animal for both macroelements (P, Ca, K, S, Mg, etc.) and trace elements (Fe, Zn, Cu, Mn, Si, etc.) on one hand while another was contaminated with toxic heavy metals (Cd, Pb, Hg, Ni, As, etc.). Currently, fly ash is used in cement and concrete structures, brick manufacturing, golf ball and agricultural product as soil amendment because of its spherical-shape and hollow particle. Lignite fly ash, therefore possesses not only advantageous properties supported plant growth but also having the ability to inhibit plant growth such as heavy metals and particle size. Hence, the characteristics of lignite fly ash is important for effective the ash disposing and chemical composition is essential in assessing the environmental impact of lignite fly ash utilization. To clarify the possible positive and negative environmental impact of lignite fly ash interacted with quality of soil and rice plant, field experiment was conducted at paddy field of Amphore Mueng, Nakorn Nayok Province. The average particle size of lignite fly ash in this study was 155 μm , ranging from 0.49 to 830 μm , with spherical-shape and hollow. The experimental design was randomized complete block (RCB) with 3 replications of 4 application rate (0.5, 1, 2 and 4 t fly ash rai^{-1}) (1 ha = 6.25 rai). The result indicated that application of lignite fly ash 0.5-4 tones rai^{-1} to

clay soil (soil pH = 5.16) resulted in increasing soil pH around one pH unit. Moreover, Mn, Se, Si, Zn, As, and Cd also increased by increasing application rate of lignite fly ash when 0.005 M DTPA was used as soil extractant. The ash was sufficient potassium (K_2O) and magnesium (Mg) sources for plant growth with safe dose at the maximum rate 2 t fly ash rai^{-1} based on the heavy metal concentrations. In addition, root weight, root length and rice yield also were increased significantly. Lignite fly ash, therefore can be interacted as soil amendment to improve physical property of the soil and to avail as nutrients source for growth of rice with safe dose at the rate of 2 t fly ash rai^{-1} . The significant factors of lignite fly ash utilization lead to positive environmental impact included soil property, physical property and chemical composition of the ash, essential elements for plant and risk of heavy metals.

Keywords: Lignite fly ash; heavy metals; plant nutrients.

S5-O4

Oil-Contaminated Soil Microorganism: Isolation and Characterization for Biodiesel Production

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Lipase producing microorganisms (LPM) may catalyze hydrolysis or transesterification of triacylglycerols to fatty acids alkyl esters (biodiesel). In this sense, the main objective of this work was to isolate and identify LPM in oil and grease contaminated soil for future applications in biodiesel production from rapeseed oil. Samples from contaminated soil (with rapeseed oil), from an industrial facility and contaminated soil (with salmon grease) near to a fish wastewater treatment plant were collected. The samples were first inoculated at 150 rpm and 30°C in a liquid enriched selective medium (70g L⁻¹ polypeptone, 1 g L⁻¹ NaNO₃, 1 g L⁻¹ KH₂PO₄, 0.5 g L⁻¹ MgSO₄ 7H₂O, and 30 g L⁻¹ rapeseed oil as sole carbon source). Then, agar was added to the same medium for counting and isolating colonies at 20° C (solid medium). The colonies that showed growth were chosen. The characterization was carried out by 16S ribosomal RNA gene sequencing using the following primers: EUB933F, 358F and 907R. Lipase activity was measured spectrophotometrically using *p*-nitrophenyl-palmitate as substrate. Nine strains were isolated and characterized based on their ability to grow in rapeseed oil as the sole carbon source (7 from salmon grease contaminated soil and 2 from rapeseed oil contaminated soil). All strains were *Bacteria* domain. In salmon grease contaminated soil *Myroide*, *Arthrobacter*, *Bacillus* and *Serratia* genus were identified, whereas in rapeseed oil contaminated soils only *Bacillus* genus was identified. All strains showed lipase activity, being the highest 696 U (*Myroide*) and the lowest 118 U (*Serratia*). It is shown that soil contaminated with rapeseed oil or fish grease are possible sources of LPM, which could be useful in novel and innovative future applications for biodiesel production, such as whole-cell biocatalysts that unlike commercial lipase, requires no purification or complicate immobilization methods, minimizing biodiesel production process costs.

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Keywords: Oil-contaminated soil; microorganisms; biodiesel.

S5-O5**Ecological, Biochemical and Molecular Mechanisms Involved in Nutrient Acquisition and Heavy Metal Stress Alleviation by Mycorrhizal Plants****J.M. Barea****Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, Prof. Albareda 1, 18008, Granada, Spain. *E-mail: josemiguel.barea@eez.csic.es*

Mycorrhizas are mutualistic fungal-root symbiosis established by specialized soil-borne fungi and the majority of land plants. Arbuscular mycorrhiza (AM) is the most common mycorrhizal type which is formed by more than 80 % of plant species including almost all of the agronomical interest, and herbaceous and shrub species in natural ecosystems. The AM fungi are ubiquitous microbial fungi, whose origin and divergence have been dated back to more than 450 million years ago to critically contribute to plant evolution on Earth.

The AM fungi, after colonization of the root cortex of the host plant, improve plant capacity for nutrient acquisition. The AM role in nutrient supply to plants is exerted by the AM mycelium linking the geo-chemical and biotic portions of the soil ecosystem, thereby affecting rates and patterns of P and N cycling in both agricultural and natural ecosystems. In addition, the AM symbiosis improves plant health through increased protection against environmental stresses, whether they be biotic (e.g. pathogen attack) or abiotic (e.g. drought, salinity, heavy metals, organic pollutants), being able to enhance soil structure, through the formation of the aggregates necessary for good soil quality. The AM fungi can be therefore considered as an integral component to the function of root systems, usually acting in co-operation with other soil microorganisms in the so-called “mycorrhizosphere”.

The available information on the impact of AM fungi on plant functioning in both agricultural and natural systems, with special emphasis in stresses soils, is analyzed in this presentation. After a brief introduction of the AM fungal and symbiosis biology, the information is systematized as follows: (i) ecological, biochemical and molecular bases of the AM role on plant acquisition of P and N, in low-nutrient and degraded soils; (ii) ecological, biochemical and molecular bases of the AM role in increasing plant tolerance to heavy metals (HM); and (iii) evaluating the possibilities to use these micro-fungi as an environmental biotechnology tool for improving plant developments in low-nutrient and/or HM-contaminated environments.

Keywords: Nutrient acquisition; heavy metals stress; mycorrhizal plants.

S1-P1**Use of Natural Nanoclays as Support Material for Manganese Peroxidase Immobilization****F. Acevedo^{1*}, M. Cea², S. Sanhueza³, M. Calabi¹ and M.C. Diez⁴**¹*Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco.*²*Laboratorio de Suelos, Instituto de Agroindustria, Universidad de la Frontera, Temuco.* ³*Carrera de Ingeniería Ambiental, Facultad de Ingeniería, Ciencias y Administración, Universidad de La**Frontera, Temuco.* ⁴*Departamento de Ingeniería Química, Facultad de Ingeniería, Ciencias y Administración, Universidad de La Frontera, Temuco.* *E-mail: facevedo@ufro.cl

Various applications of extracellular enzymes from white-rot fungi as biocatalysts have been reported in pulp bleaching, wastewater treatment and soil remediation. However, their use in industrial applications has been limited by several factors, mainly the high enzyme cost, the low operational stability and the low or no recovery options. The enzyme immobilization has demonstrated to be a good technology that may allow their practical application. Fungal enzymes have been successfully immobilized on porous glass beads and on various clay minerals for soil pollutants degradation. Nanomaterials of natural origin, such as nanoclays, have not been proved yet. The aim of this study was to obtain nanoclays as support material for the immobilization of manganese peroxidase (MnP) from the white rot fungus *Anthracophyllum discolor*. Andisol of the South of Chile, from the Temuco Series, was used for nanoclays obtention. The clay fraction was obtained by a sedimentation method based on Stokes's law, without destruction of the organic matter. 5 g clay was suspended in 100 mL NaCl 1 M, with agitation for 48 h. The suspension was centrifuged at 3,000 rpm for 40 min and the supernatant was discarded. Clay was re-suspended in 50 mL of filtered and deionized water, under moderate agitation for approximately 1 h, and then centrifuged under the same previous conditions. The procedure was repeated 7 times. The recovered supernatants were dialyzed until a conductivity range between 0.5 to 0.8 μ S, and then concentrated. For its characterization, nanoclays were analyzed by transmission electronic microscopy and its isoelectric point was determined by microelectrophoresis. Electrophoretic mobilities were measured with a zeta meter (ZM-77) apparatus. Dilute dispersions (0.05 g/liter) were prepared in 10-3 M KCl. The pH was adjusted with 10-2 M HCl or NaOH. The mobilities were averaged and the zeta potential calculated using the Helmholtz-Smoluchowski equation. The results indicate that the obtained material corresponds to nanoclays with a particle average size less than 100 nm, without the presence of electronic diffraction and with an isoelectric point (pI = 3.2) closed to that of clay. Future studies will be focused on the evaluation of the immobilization efficiency of MnP from *A. discolor* in this new natural nanomaterial.

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Keywords: Nanoclays; enzyme immobilization; manganese peroxidase.

S1-P2**Adsorption of Added Cadmium in Relation to Properties of Some Alluvial Soils****I.A. Al-Hawas****Department of Environmental and Natural Resources, College of Agriculture and Food Science KFU, Saudi Arabia. *E-mail: ihawas@kfu.edu.sa*

In the present work an attempts have been done to study the adsorption capacity of Malaysian soils for cadmium, which consider as an environmental harmful metal. Six soils represent the major soil series used for agricultural production in Malaysia: Typic Sulfaquept (Sedu) Haplic Acrorthox (Segamat) Tropic Fluvaquent (Kundor), Orthoic Tropudult (Durian), Typic Paleudults (Melangit), and Typic Paleudults (Betu Anam) were used. The soils were very acid to slightly alkaline with the lowest pH value at 3.0 (Sedu) and the highest pH 7.3 at Melangit soil. The soils texture varied from silty to clayey. The highest clay content (87%) was found in Segamat series where the lowest was in Melangit which considered silty soil. The amount of Cd adsorbed by soil depends on their physico-chemical properties. The adsorption data were tested by three different models Langmuir, Freundlich and second order equation. For the Langmuir isotherm r^2 ranged between 0.943 to 0.998 with an average of 0.979, Freundlich isotherm r^2 ranged between 0.519 to 0.991 with an average value of 0.866 where the second degree (r^2) ranged between 0.366 to 0.997 with an average 0.900. This suggests that the Langmuir isotherm provides a good model of the sorption system. The adsorption capacity differs between soils. The highest adsorption capacity was found in Melangit and Kundure ranged between 1800 to 2500 $\mu\text{g soil}^{-1}$ Cd. Though Melangit is silty soil but it has high pH and high content of Calcite this suggesting the precipitation of Cd and the sorption of heavy metals may be on the edge due to the aluminol sites. The usage of added CdCl_2 as adsorbant metal, CdCl^+ tends to be desorbed from the clay to a larger extent than Cd and hence adsorbed as a monovalent than a divalent cation.

Keywords: Adsorption modeling; Cadmium; Clay.

S1-P3**Comparing Two Selective Chemical Treatments to Selectively Extract Iron from the Organic Matter of Volcanic Soil Samples****S. Aravena¹, C. Morales¹, C. Pizarro¹ and J.D. Fabris²**

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The chemical evaluation of the total amount and of the mineralogical distribution of iron in both soil-aluminosilicates and -organic matter (OM) fractions has been receiving special attention, in recent times. The main interests are centered on establishing differentiation criteria in soil grouping, regulating micro- and macro-nutrients dynamics in the soil-water interface and developing technological approaches to remediate contaminated areas. Chemical extraction treatments would be a more direct and practical laboratory alternative to selectively separate iron from minerals and iron-OM from soils. In this work, two selective chemical treatments for iron extraction from Fe-OM systems, namely 0.1 mol L⁻¹ sodium

pyrophosphate and 0.2 mol L⁻¹ citrate solutions, were assayed with the clay fraction (particle diameter, < 2 µm) and the bulk soil (sieved to 2 mm) samples from two volcanic soils from Chile: a Ultisol (sample labeled according to the locality's name, Collipulli, geographical coordinates of the sample sites are 36°58'S 72°09'W) and two Andisols (Digullín, 36°53'S 72°10'W; and Freire 38°57'S 72°36'W), in an attempt to compare the iron extractive ability of these methods. From chemical analyses, the clay and bulk Ultisol samples presented, as expected, higher iron content and lower OM amount than those from Andisols. The removal effectiveness of each of these two chemical treatments was found to behave differently. The iron extractive capacity was higher with citrate than with pyrophosphate for these clay fractions, whereas for bulk soils, the pyrophosphate treatment extracted iron more efficiently than citrate.

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Keywords: Chilean soils; pyrophosphate treatment; citrate treatment.

S1-P4

Use of Compost and *Azospirillum lipoferum* to Alleviate Saline Stress in *Capsicum annuum* Grown in Saline Soil

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One-third of all irrigated land is believed to be affected by the salt content, which is very common in arid and semi-arid regions. Salinity can cause a decrease in water uptake in plants, which reach toxic levels and reduce availability of nutrient and finally the growth of the plants is reduced. Soil amendments have been employed to ameliorate the adverse effects of salts, including plant-growth regulators, nitrogenous compounds, gypsum, and sugars. Recently, the use of microorganisms, compost, and humic acids have been shown to be more effective than gypsum, which is currently the standard amendment used to reclaim alkaline soils and reduce the effect of salt on seed germination and growth. Here we report on the degree of mitigation of salt stress in chili plants (*Capsicum annuum*), using compost and *A. lipoferum* as a soil amendment. We used chili seeds of the variety 'Ancho San Luis' germinated on peat moss. One month later, the seedlings were transplanted to pots containing saline soil (electrical conductivity 15 dS m⁻¹ EC) or the control (EC = 1 dS m⁻¹). Compost was mixed into the soil as an amendment agent at doses of 0%, 10%, and 20% (v/v). Some treatments involved plants inoculated with *A. lipoferum* (1×10⁸ UFC plant⁻¹) at the time of transplantation. Plants maintained under temperature and irrigation controls and harvested 3 months after the beginning of the treatments. Measurements of plants included height of plant, dry weight of the shoot and leaf, foliar area, and number of leaves. Chlorophyll content significantly increased a compost content of 10%, compared with controls (no compost). A synergic effect of compost with *A. lipoferum* increased root and leaf dry weight.

Keywords: Compost; *Azospirillum lipoferum*; alleviate saline stress.

S1-P5***Azospirillum lipoferum* and Humic Acids Induce Decrease of Salt Stress in Chili Plantlets (*Capsicum annuum*)****M. Bacilio-Jiménez*** and D. Covarrubias-Robledo*Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, B.C.S., México.***E-mail: mbacilio04@cibnor.mx*

To reclaim alkaline soils and reduce the effect of salt on seed germination and growth, several methods are currently used, including gypsum, compost and humic acids, and inoculation with *A. brasilense*. Simultaneous use of microorganisms and humic acid to alleviate saline stress represents another interesting possibility. Our objective was to evaluate the capacity of humic acids and *Azospirillum lipoferum* a plant growth-promoting bacteria together and separately for mitigating saline stress on plantlets of *Capsicum annuum* for 21 days after sprouting, under different levels of NaCl. Under in vitro conditions, seeds were germinated and cultivated under 0, 25, 50, and 75 mM NaCl for 21 days in an environmental chamber under axenic conditions. The treatments were: (1) Humic acid at a concentration of 1000 ppm; (2) *A. lipoferum* at a concentration of 10^6 UFC mL⁻¹; and (3) humic acid and *A. lipoferum* at the same concentrations. To evaluate mitigation, we measured length of root and shoot, number of secondary roots, root and shoot dry weight, root hair length, and production of root mucigel. The results indicate a synergic effect between HA and *A. lipoferum* on primary root, root hairs length, and secondary roots number, especially in high levels of salt where, for example, a 75 mM NaCl the root hair were from 35 to 150% longer than the other treatments. The humic acid treatment provided the best results by increasing dry weight of shoots and roots at practically all levels of NaCl, 10% better than the *A. lipoferum* treatment or the *A. lipoferum*-humic acid treatment. Higher production of mucigel, (two times more) was observed at 25 and 50 mM NaCl and was stimulated by *A. lipoferum*, but at 75 mM, mucigel production was stimulated by humic acid with amounts four times more than with the bacteria. These results suggest that a synergic effect between humic acid and *A. lipoferum* exists for mitigating saline stress in chili plants. However, in some cases, the mitigation is induced by humic acid or *A. lipoferum* independently, depending on the salt concentration and the target organ of the measured parameter.

Keywords: *Azospirillum lipoferum*; humid acids; salt stress.

S1-P6**Mulching for a Healthy Landscape****N. Bassirani****Department of soil science Zabol University, Zabol, I.R. Iran. *E-mail: N_Bassirani@yahoo.com*

A result of the geographical and ecological situation of Iran, as well as the blowing of unfavorable winds in the Central Desert, more than 80% of the 164 million hectare area of the country is subject to the conditions of arid and semi arid regions, characterized by low precipitation, ranged between 50 to 250 mm per year. At the present the area of the country's deserts and sandy soils is estimated to be 34 million hectares, and that of the poor and desertified rangelands which 12 million hectares correspond to the sandy, soils 5 million of

the same shifting sand dunes. For as long as trees have grown in forests, leaves and needles have fallen to the ground and formed a natural protective layer over the soil. This same protection can be given to the plants in our landscapes by mulching. Mulching can make a big difference in the success of your landscape. Mulches conserve soil moisture, allowing you to water less often; keep down weeds; reduce erosion; keep plant roots cool; provide winter protection; and make your yard more attractive. Mulches also simplify lawn mowing around trees and shrubs. A ring of mulch allows you to bring your lawn mower right up to the edge of the mulch, eliminating the need for string weeders. At the same time you are protecting tree trunks and surface roots from damage by mowing and clipping equipment.

Keywords: Mulching; healthy landscape; Iran.

S1-P7

Role of Planted Trees and Forest Plantations in Low Forest Cover Countries (LFCC) - Case Study of Iran

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The decrease of woodland and tree vegetation in and around rural and human settlements in Low Forest Cover Countries (LFCC) has raised concerns among the countries concerned and the international community. The loss of these resources impacts directly on the poor communities, which rely on trees and wooded formations to maintain their quality of life. To confront this situation, the planting of trees will become more and more frequent in order to create more wooded areas. To make these plantations sustainable, careful consideration must be given to their position in the overall land use patterns, and this paper draws attention to the role of trees planted both within and outside the areas formally classified as forest. Low forest cover raises various issues for which plantations, tree growing and the encouragement of regeneration have been identified as vital activities in order to: Replace the loss of natural forest and planted forest cover (reforestation); Introduce forest to sites that have never supported forest, or have not had non forest cover for a long period (afforestation); Improve degraded natural forest ecosystems; Expand tree cover on non-forest areas, rehabilitate degraded lands, restore soil fertility and control soil erosion; Provide services and goods that natural forests may no longer be able to meet, including the provision of fuelwood, fodder and non-wood forest products; Provide industrial wood and fuelwood; Ease human and animal pressure on limited natural forest areas; Improve the land and natural resource base, including water conservation, and Contribute to global ecological benefits such as the conservation of biological diversity and carbon sequestration.

Keywords: Planted trees; forest plantations; low forest cover.

S1-P8**Gypsum and Organic Matter Application in Salinity Field Trial Play a Major Role on Detoxification of Salt-Stressed Jasmine Rice (*Oryza sativa* L. spp. *indica*)****S. Cha-um^{1*}, Y. Pokasombat² and C. Kirdmanee¹**

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Jasmine rice, high cooking qualities as aromatic flavor, softening and long grain, has been reported as salt susceptible. The best quality of jasmine rice is generally cultivated in the northeastern region of Thailand, which is defined as inland salinity area. The salt contamination in the soil is one of the most barriers to reduce on growth and development of rice crop, especially in the seedling and reproductive stages, leading to low productivity. Soil physical and chemical improvements in the salinity area are alternative channels to enhance on detoxification of salt contaminant using exogenous calcium (gypsum) and organic matter (OM). In this study, rice grown in the exogenous application of gypsum and/or OM was 90% seed set, while rice grown in the soil without gypsum and/or OM treatment found seedless or lack of productivity. Sodium ion accumulation in both root and shoot organs of jasmine rice cultivated in the soil with exogenous gypsum and/or OM treatments was lower than those rice grown in the soil without gypsum and/or OM, while the potassium ion was enriched. In addition to, the low salt accumulation in exogenous gypsum and/or OM treated rice was positively related to pigment stabilization, photosystem II (PSII) function, and net-photosynthetic rate, resulting in high sugar content in the flag leaf with high yield. It should be concluded that the exogenous gypsum and/or OM in salinity field may be played as effective procedure to detoxify the damage from salts in salinity soil.

Keywords: Gypsum; organic matter; salt stress.

S1-P9**Contribution of Glomalin to Soil Organic Carbon in a Mediterranean Agroecosystem Managed under Two Tillage Systems****G. Curaqueo^{1*}, E. Acevedo², J. García³, G. Borie³, R. Rubio⁴, A. Morales⁴ and F. Borie⁴**

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The glomalin or GRSP (glomalin related soil protein) is a glycoprotein produced by arbuscular mycorrhizal fungi (AMF), presenting a high recalcitrance to microbial and enzymatic breakdown, and consequently is accumulated in the soil profile. GRSP might contribute to increase the soil organic carbon (SOC) and therefore the carbon sequestered into the soil due to its structural C content and the promotion of soil aggregation. Tillage systems affect all soil properties and obviously the microbiological characteristics, including glomalin

content and stability, influencing the C storage in the agroecosystems. We evaluated the influence of two tillage systems applied to a soil from Central Chile on its chemical, physical, and mycorrhizal properties including glomalin levels. Characteristics measured were: Total glomalin (GRSP), soil organic matter fractions (fulvic acid, humic acid and humin), C content, AMF spores number, NAGase activity (enzyme related with the biomass fungi), bulk density, and aggregate water stability at the upper horizon (0-5 cm). All these properties were evaluated in an experimental agroecosystem managed for 6 years by conventional tillage (CT), and no tillage (NT) in a wheat-corn rotation in a Mollisol (Entic Haploxerolls) in Central Chile. Additionally, we made a decomposition assay to measure glomalin recalcitrance, incubating soil samples during 250 days at 20°C and determining the level of GRSP every 50 days. Mean values in the 5 cm soil profile for GRSP were CT=3.96 and NT=8.16 mg g⁻¹. Soil organic matter fractions show 96.4% of humin, 0.5% of humic acid and 0.4% of fulvic acid in NT system, and 95.5% of humin, 0.6% of humic acid and 0.5% of fulvic acid in CT system. C content were CT=1.7% and NT=2.6%, where the glomalin-C represented 6.9% and 9.9% of total C for CT and NT, respectively. The AMF spore numbers were lower in soils under CT than NT system. NAGase enzymatic activity was high in NT in relation to CT. Soil bulk density was 0.884 g cm⁻³ in NT compared with 1.005 g cm⁻³ in CT. The aggregate water stability was higher in NT, with 59% in contrast to CT with 32% of aggregation. Glomalin decomposition assay shows that the glomalin levels decreased from T0 to T150 and increased from T150 to T250. Results showed that the NT systems exert a positive effect on all the characteristics measured in relation to CT. Another aspect to highlight is the important role of AMF and specifically the glomalin in the C sequestration in the soil of the Mediterranean Agroecosystem studied.

Keywords: Glomalin; soil organic carbon; tillage systems.

S1-P10

Kinetic Changes in Phosphorus Fractions during Aerobic Degradation of Dairy Dung

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Phosphorus (P) fractions in organic waste are modified during the aerobic degradation process. In this study, the changes in distribution of inorganic P (Pi) and organic P (Po) fractions were evaluated during the aerobic degradation of dairy cattle feces in laboratory-scale reactors for 105 d. Sequential fractionation procedure of Hedley was used. The kinetics data was described by power function. The fractionation procedure showed that P-residual fraction was strongly increased in time. Also, Pi fractions were gently increased. Thus, initial rate coefficients showed that Pi-H₂O and Pi-NaHCO₃ (around 6.6 mg P kg⁻¹ d⁻¹) were higher than Pi-HCl (5.3 mg P kg⁻¹ d⁻¹) and Pi-NaOH (4.2 mg P kg⁻¹ d⁻¹). Therefore available P was provided mainly by Pi weakly linked to aluminum and iron surfaces. In contrast, initial P release rate showed that Po fractions were quickly decreased in the following order: Po-

Session 1. Ecological Significance of Interactions Among Clay Minerals, Organic Matter and Biota

NaOH ($12.2 \text{ mg P kg}^{-1} \text{ d}^{-1}$) > $\text{Po-H}_2\text{O}$ ($9.9 \text{ mg P kg}^{-1} \text{ d}^{-1}$) > Po-HCl ($8.3 \text{ mg P kg}^{-1} \text{ d}^{-1}$) Po-HCO_3 ($6.9 \text{ mg P kg}^{-1} \text{ d}^{-1}$). P-residual fraction was increased through all the process of degradation, whereas Po fractions were decreased. This behavior was associated to strong interactions between organic macromolecules with aluminum, iron and calcium compounds. Therefore, useful tools focused to degradation of organic P compounds could contribute to increase P bioavailability in organic waste.

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Keywords: Phosphorus fractions; aerobic degradation; dairy dung.

S1-P11

Effect of Green Rust Formation on the Phosphorus Adsorption and Nitrogen Mineralization in Flooded Soils Collected from the Lower Orinoco River, Venezuela

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Water in soil restricts gas diffusion and limits the oxygen availability in soil with a consequent decrease in the soil redox potential. Under such circumstances, microbial dissolution of Fe (III) oxides can take place and the dynamics of the nutrients such as phosphorus (P) and nitrogen (N) can be affected. Under anaerobic soil condition biogenerated Fe(II) can experience secondary chemical reactions, which lead to the formation of aqueous complexes and/or the precipitation of ferrous solids as green rusts (GR) compounds $[\text{Fe}^{\text{II}}_{(1-x)}\text{Fe}^{\text{III}}_x(\text{OH})_2]^{x+} [(x/n)\text{A}^{n-}(m/n)\text{H}_2\text{O}]^{x-}$. In an earlier laboratory research with soil samples collected from a seasonal flooded forest in the lower Orinoco River, Chacón et al. (2005) hypothesized that anaerobic soil condition leads to the formation of the green rust compounds. In the field when the soils are exposed to the air, just at the end of the aquatic phase, we have detected dispersed spots of a blue-green color, which we have associated with precipitation of GR compounds. In this study we examined the N mineralization and adsorption P in samples collected from the GR spots and the adjacent soil as a control (C). Ten samples of both GR and C were taken at the end of the flood event in November 2006, following a completely random design. Nitrogen mineralization was carried out during eight weeks using an incubated method. Values of Nm (net nitrogen mineralization), No (potentially mineralizable nitrogen) and k were obtained following the Sanford and Smith (1972). Phosphorus adsorption was performed according to Waldbridge et al (1991). Theoretical adsorption maxima (b) were obtained fitting the data to the linear form of the Langmuir equation. The results showed that GR compounds were more active in the N mineralization, which leads to highest values of Nm and No. Ammonium-N predominates in both GR and C. The absence of nitrate forms was associated with the soil acidity. The P adsorption data were satisfactorily described by the Langmuir equation. Adsorption maxima value was lowest in the GR compounds. These minerals are active ion exchangers and sorbents of inorganic anions as CO_3^{2-} and SO_4^{2-} and organic acids. We hypothesized that the low b value obtained in the GR samples of this study could be obey to a strong competition among anions by the active adsorption sites of these compounds. Such low P adsorption

capacity could be indicative of a greater P bioavailability in the soil influenced by the presence of these surfaces. The above results allowed us to suggest a number of hypotheses concerning the mechanism that regulate the P and N availability in anaerobic soils influenced by the GR formation. In this study we proposed that the GR precipitation in the flooded soils of the lower Orinoco river are hot spots for P and N bioavailability. It is further suggested that this provides a mechanism for essential nutrient release which could be subsequently rapidly utilized by plants and microorganisms.

Keywords: Green rust formation; phosphorus adsorption; nitrogen mineralization.

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S1-P12

Organic Installment and Humidity Levels in Fodder Nopal

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When incorporating dung to the ground is increased the biological activity, the physical properties and chemical of the ground and the availability of nutrients for the plants is increased (Castilian 1980). The objective was to obtain the optimal interaction of dose of goat dung, fertilizing chemistry and levels of volume of water and their answer in the production of nopal for forage of the variety fodder Alpha. The investigation carried out in the Experimental Agricultural Field of the Faculty of Agriculture and Zootechnics of the Juárez University of the State of Durango located in the kilometer the 30 of the highway Gómez Tlahualilo Palace, Durango. Mexico. The treatments of the organic installment and the chemical fertilizer that were applied in the three blocks are according to the design of blocks at random with adjustment in strips.

Table 1. Factors and treatments studied in fodder nopal. DEP-FAZ-UJED. 2001.

FACTOR A		FACTOR B			
Dung and Chemical Fert. Levels		Water levels	application	Treatments	
		L/plant/ month			
A1	Control	B1= 10		A1B1	A3B3 A5B3
A2	40 ton ha ⁻¹ manure	B2= 20		A1B2	A4B2 A7B1
A3	80 ton ha ⁻¹ manure	B3= 30		A1B3	A5B1 A8B1
A4	120 ton ha ⁻¹ manure			A2B1	A5B2 A8B2
A5	40 - 80-00 Chemical Fert			A2B2	A5B3 A8B3
A6	80 - 80-00 Chemical Fert			A2B3	A6B1 A9B1
A7	120 - 80-00 Chemical Fert			A3B1	A6B2 A9B2
A8	40 -160-00 Chemical Fert			A3B2	A6B3 A10B1
A9	80 -160-00 Chemical Fert			A3B3	A7B1 A10B2
A10	120-160-00 Chemical Fert			A4B1	A7B2 A10B3

In analysis of the vegetative area and number of rackets the treatments of 40, 80 and 120 ton have⁻¹ of goat dung excel of the chemical fertilizer and the witness. The oppuntia responded suitably to the application of goat dung with 120 ton has⁻¹ and presents/displays the major yields and in number of rackets in comparison to the chemical fertilizer and the witness. The chemical properties of the ground (Ca, mg, Na, NO₃, pH and M.O.) presented/displayed major concentration in the goat dung treatments (80 and 120 ton has⁻¹). These values exceed the permissible limits, reason why it will be due to have well-taken care of with the application continues (for year) of these doses, ton is better to initiate with 80 120 has⁻¹ and later to diminish the dose according to a detailed ground analysis. The plant (rackets) in its quality analysis I present/display high levels of nutrients, where it excels the chemical fertilizer with 80-80-00, 40-160-00 and 120-160-00 kg ha⁻¹ in comparison with those of goat dung. In analyzed the physical properties of the ground they presented/displayed an improvement to the application of goat dung. The treatment of 120 ton has-1 of goat dung was the one that I present/display major humidity retention in the ground in comparison to the one of chemical fertilizer and to the witness.

Keywords: Goat dung; chemical fertilizer; nopal.

S1-P13

Management with Different Cover Crops and Phosphorus Fertilizers Affects Organic Matter Distribution in Savanna Soil Fractions

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The extension of the agriculture froontier in the savannas of Venezuelan Plains affects the functions of this ecosystem due to the perturbation of the physical and chemical protection mechanisms of soil organic matter (OM). Our objectives were to evaluate the effect of the cover crop-maize association managed with no tillage and different fertilizations on the organic matter fractions distribution in Savanna soils, as well as the stabilization mechanisms involved. The fertilization treatments applied to maize associated with *Brachiaria dictyoneura* (BDY) and *Centrosema macrocarpum* (CM), in a Savanna ecosystem localised in the Guárico State, Venezuela, were: IR: low level of inorganic fertilization using NPK compared with the average rates used by local farmers, RF: Phosphoric rock, IO: without fertilization. Total values of P, N, K applied were the same in all treatments, only the source of P was changed. Natural Savanna was used as a comparison control (NS). The experimental layout was big plots without repetition where a previous geoestatistic study of spatial variability was made. Six composed samples (0-5 cm) were taken in each plot (18 x 450 m). A soil fractionation was made using wet sieving (size) and density separation (sodium polytungstate $d = 1.85 \text{ g cm}^{-3}$). Free particulate OM no protected (OMf), free particulate OM between micro aggregates (OMfe), OM physically protected inside micro aggregates (OMi) and OM associated to clays and silts were obtained and total organic carbon was estimated in each fraction using a TOC auto analyzer. Grass cover (BDY) had more OMf and OMFe than NS and the legume cover CM ($p < 0.05$), while CM produced 10% more micro aggregates and OMi ($p < 0.05$), this cover crop promoted the microaggregation and the OM physical protection to similar levels than in the comparison

untreated system (NS). Cover-fertilization interaction (BDY-RF) increased the OMf and OMfe ($p < 0.05$). A minor OM quantity associated clay and silt was found in the BD-IR treatment, decreasing the OM chemical stabilization of savanna soil. The combination of soluble fertilizers with easily decomposable organic material showed by BDY (Padrino and Hernández-Hernández, 2004) decreased the OM chemical stabilization favouring the OMf production, which don't contribute to the soil stabilization, while the use of more recalcitrant organic material produced by the legume (CM) raised the OM physical stabilization.

Keywords: Cover crops; phosphorus fertilizers; soil organic matter.

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S1-P14

Soil Microbial Protein-Clay Complexes

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Bacterial enumeration in soil environments estimates that the population may reach approximately 10^{10} g^{-1} of soil and comprise up to 90% of the total soil microbial biomass. Bacteria are present as single cells or multicell colonies and often strongly adsorb onto mineral surfaces such as sand, clay, or clay-organic matter complexes. The interactions of microbes and microbial biomolecules with these minerals have profound impacts on the physical, chemical and biological properties of soils. In this work, microbes were propagated from a light clay-loam soil sampled from Teagasc, Carlow, Ireland, and the number of colony forming units (CFU) determined. Soluble microbial proteins (116 to 6.5 kDa) were isolated and the concentration of proteins evaluated using the Bradford Dye protein determination reagent. Quantified microbes and microbial proteins were sorbed to montmorillonite (M) and kaolinite (K) clay minerals and the equilibrium adsorption of microbes and microbial proteins determined as previously indicated. M showed a greater affinity for soil microbes and soil microbial proteins. To determine where soil microbes and soil microbial proteins are adsorbed on clay minerals and the nature of the interaction, complexes were characterized by X-ray diffraction (XRD), Scanning electron microscopy (SEM) and Fourier-transform infrared (FT-IR) spectroscopy. XRD showed that microbes and microbial proteins intercalated the interlamellar region of M resulting in M-microbial and M-protein intercalation complexes with a 001 d-spacing of 1.5 and 1.6 nm, respectively. XRD also confirmed that microbes and proteins did not penetrate K, indicating that adsorption occurred primarily on the external surfaces, as also suggested by SEM observation. SEM observations also indicated microbial and protein adsorption to the surfaces of the M. FT-IR spectra were indicative of M-microbes and -protein, and K-microbial and -protein complexes. The implications of these interactions on the total contribution of proteins and other microbial constituents to soils are currently being considered using novel NMR approaches. We also intend to look at degradation patterns and possible protection mechanisms afforded by clay complexes.

Keywords: soil microbes; soil microbial proteins; clay minerals.

S1-P15**Introduction about Microbial Biomass in Soil****H. Kheyroodin* and H. Antoun***Department of Soil Science and Agri-Food Engineering Laval University, Quebec, Canada, G1K 7P4.***E-mail: Hkhyroodin@yahoo.com*

Biomass, defined as all land and water based vegetation as well as all organic wastes, fulfilled almost all of humankind's energy needs prior to the industrial revolution. Soil microbes play an important role in how well the soil functions as a medium for plant growth and as an environmental buffer for clean water and air. Microbes increase soil quality by mineralization of nutrients for plants, processing residues into organic matter, increasing soil structure and degrading toxic compounds. The measurement of these microbes and their processes give us information as to how the soil in a particular ecosystem is functioning. The microbial biomass C, N, P, S are an important source for nutrient elements for the plants.

Keywords: microbial biomass; soil microbes; soil quality.

S1-P16**Management Soil Enzyme Activities in Long-term Tillage Application and Rotation in Agriculture-Ecosystems****H. Kheyroodin****Department of Soil Science in Semnan University, Semnan, Iran. *E-mail: Hkhyroodin@yahoo.com*

Agricultural practices that reduce soil degradation and improve agricultural sustainability are needed particularly for tropical/subtropical soils. No-tillage planting causes minimal soil disturbance and combined with crop rotation may hold potential to meet these goals. Soil enzyme activities can provide information on how soil management is affecting the potential to perform the processes in soils such as decomposition and nutrient cycling. Soil enzyme activities were investigated in a split-plot experiment (3 replications) where tillage (no till and conventional) was the main plot and crop rotation (soybean/wheat, S/W; maize/wheat, M/W or cotton/wheat, C/W) was the subplot. Soil samples were taken at 0-5, 5-10 and 10-20 cm depths in 1997 and 1998. The 0-5 cm layer under NT system showed increases up 68% for amylase, 90% for cellulase, 219% for arylsulfatase, 46% for acid phosphatase, and 61% for alkaline phosphatase. There were significant correlations of soil enzyme activities with total organic C, and C and N microbial biomass. These results showed that NT increased microbial activity and that soil enzyme activity is a sensitive indicator of alteration soil quality by management.

Acknowledgements: This work was supported by grants of the Minister of Education Iran.

Keywords: Enzyme activities; tillage application; soil quality.

S1-P17**Contribution of Humic Substances from Different Composts on the Content of Humin in a Tropical Soil****C.C. Lima^{1*}, E.S. Mendonça², I.R. Silva², L.H.M. Silva³, B.H. Peres² and A. Roig⁴**

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The mineralogical nature of the soil, as well as the quantitative and qualitative characteristics of humic and fulvic acids present in the composts may affect both the reactivity and the stability of humic substances (HS). Due to their recalcitrant structures and their interaction with the soil mineral matrix or occluded intra-aggregates, the hydrophobic HS become more resistant to microbial degradation, they persist longer time in the soil. The alkyl groups of hydrophobic character in this humic acid fraction of composts can role the incorporation of this humic fraction to the humin fraction of the soil. To evaluate this proposition it was added increasing doses (0, 13, 26, 52 e 104 Mg ha⁻¹) of five different composts in an Oxisol soil. The following wastes were used to obtain five composts: sweet cane bagasse (SCB), ashes of sweet cane bagasse (ASCB), poultry manure (PM), filter cake (FC) and castor oil plant residue (MR). The mixtures were as follows: a) SC: SCB+ASCB+PM (3:3:2); b) AS: SCB+ASCB+PM+ mineral fertilizers NPK (3:3:2); c) SM: SCB+ASCB+PM + serpentinite and micaxist powdered rocks (3:3:2 + 62.5 kg t⁻¹); d) FC: SCB+FC (2:1); and e) MR: SCB+MR (2:1). These composts were characterized chemically by ¹³C NMR, and estimated the quantity of functional alkyl groups of humic acids applied to the soil as composts. Thirty days after application of the treatments, soil samples were collected, and the organic matter fractionation in humic acids (HA), fulvic acids (FA) and humin (HU) were analyzed, and it was calculated the HA/FA and (HA + FA)/HU ratios. There was predominance of FA in relation to the HA after application of increasing doses of different composts to the soil, although they had higher levels of HA than FA; the application of the compost M-G contributed to obtain higher levels of HU in the soil; the incorporation of organic components to the fraction HU was governed by the content of alkyl groups of HA fraction of the composts.

Keywords: Humic substances; compost; tropical soil.

S1-P18**Modification Forms of P in Soils Affected by Ash from Forest Fires****P. De La Fuente, K. Manquian*, N. Arancibia, J.E. Förster and M. Escudey**

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In this work the impact in depth of ashes from native forest (NA), and pines (PA) and eucalyptus (EA) plantations on P forms in soils was studied. Ralún (Andisol) and Collipulli (Ultisol) soils were considered. Leaching columns to simulate the ash impact were used. Soils were packed into 10 cm of diameter columns to the depth of 25 cm according to their

respective field bulk densities. Sixty grams of ash were incorporated into the surface 5 cm of the packed columns. The columns were irrigated weekly with one pore volume of distilled water and gravity drained, for a period of 12 weeks. At the end of the leaching experiment, each soil column was cut open lengthwise and the profile was sectioned into five equal length segments. Each section was characterized for pH, organic carbon (OC), electrical conductivity (EC), and available P. Fractionation was used to estimate P chemical forms. Phosphate determination was carried out by the formation of phosphomolybdic acid, reduced to intensely colored molybdenum blue by ascorbic acid. In general, ash application results in higher values of pH, EC and available P in the first 0-5 cm section, and lower values of OC in the 0-5 and 5-10 cm sections, when compared with the corresponding control column section. No significant P leaching was observed; however, organic P increases in the 0-5 and 5-10 cm sections, showing that P forms are affected by the ash action. Ash addition results in a solubilization of previously soil fixed P, the impact depend on the origin of ash and follow the sequence NA>PA>EA.

Acknowledgments: This study was supported by FONDECYT N° 1070116 grant.

Keywords: Phosphorus; ash; forest fires.

S1-P19

Black Carbon Contribution to and Allophanic Soil as Affected by Agricultural and Natural Fire

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Wildfire and burning practices on agricultural land contribute to the formation of black carbon (BC) which is one of the chemically most stable forms of organic matter. BC is defined as a continuum of condensed aromatic structures, which are biologically refractory that remains as a residue from incomplete combustion processes. Literature on Soil Organic Matter (SOM) alteration by fire in natural systems is still scarce. Here, we examine the effect of wildfire severities in a pristine *Nothofagus* sp. rain forest and of several decades of stubbles burning of harvesting agricultural residues on BC contribution to young allophanic soils. Unburned control in adjacent area was also sampled. The amount and nature of BC was determined in each layer down to 0.4 m depth by solid-state ¹³C NMR spectroscopy and after chemical oxidation. With this method the elemental resistant carbon (OREC) was recorded. NMR spectra were integrated and four areas of ¹³C-NMR chemical shift assigned (carboxyl-C, O-substituted aryl-C, C-substituted aryl-C and O-alkyl-C). Alkyl C was excluded because of its aliphatic nature. Additionally we used radiocarbon dating of bulk forest soil to gain information on SOM age. Our results indicate that BC contents in agricultural land ranged between 5% to 11% of initial C stock and both, burned and unburned soils presented similar amount of BC. Unexpectedly, more BC (19%) was found in deep soil (0.2-0.4 m) of burned

site. In contrast, in the forest soils, BC content increased as fire severity increased from 7% in the control (unburned) to 16 % in the high severity treatment. On the other hand, BC was a distinct C pool from Al complexed organic matter as revealed by independent regression line between Al from pyrophosphate and Aryl C compounds (aromatics). This may indicate, that unlike observations made by other authors, BC in young allophanic soils is not stabilised by mineral interactions. Moreover, BC deposited on the soil surface across years can be transported vertically or lost horizontally by runoff, which could explain the low BC contents in both surface soils. In deeper soil layer, where radiocarbon age of 490 and 1.370 yrs were recorded, its preservation potential may be higher.

Keywords: Black carbon; allophanic soils; *Nothofagus* sp.

S1-P20

Interaction of Heavy Metals with Organic Matter of Chernozem in Model Experiments

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Compounds of metals with organic matter are obligatory soil components. Organic matter is active in bound of native and technogenic heavy metals (HMe). These interactions have the ecological significance because they suffer the alteration in the state as heavy metals, so organic matter. The object of investigation was the upper (0-20 cm) layer of an arable calcareous clay loamy ordinary chernozem with the properties: pH_{H2O} 7.2, CaCO₃ 11%, C_{org} 2.3%. Four kilograms of triturated (<5 mm) homogenized soil samples were placed in polyethylene vessels of 4 l, applied as dry acetates of Pb, Cu, and Zn separately at rates of 0-300 mg kg⁻¹ (7 variants in triplicate) and wetted to the total moisture capacity. The vessels were incubated under natural conditions for two years. Soil samples were analyzed before the beginning of the experiment and after 1 and 2 years of incubation. There was determined the total HMe content in soils (after the decomposition with HF + HClO₄). The HMe species were sequentially extracted according to the Tessier schema, combined with some parallel extractions. Compounds of HMe strongly bound to organic matter were extracted with a 3.2 M CH₃COONH₄ (AAB) solution in 20% HNO₃ after oxidation of the organic substances with 30% H₂O₂ under 85°C. Metal compounds extractable by an AAB + 1% EDTA solution are classified as the potentially mobile forms. Among with the exchangeable ions, these compounds include the organo-mineral complexes of metals. The content of HMe in all extracts was determined by AAS method. Some parameters of organic matter state were determined: C_{org} total, for humus: Cha, Cfa, elemental composition. The initial contents of the metals in the soil were 24, 44, and 65 mg kg⁻¹ for Pb, Cu, and Zn, respectively. The HMe content in the polluted soils exceeded their permissible critical level by 1.5-3 times. The 6- to 14-fold increase in the total content of metals in the contaminated soils was accompanied by an increase of HM compounds bound to organic matter by 17-30 times. A major part of the Pb and Cu applied to the soils entered into the organic matter. As a result, the share of these species increased to 23% of their total content in the soil. The enrichment of the organic matter with metals depended on the nature of the metal, they formed the following series: Pb > Cu > Zn. A major part of the metals retained by the organic substances in the chernozem (about half the total content of metals in the organic matter) was found in the mobile organomineral form. At the same

time some alteration were noticed in organic matter. After the incubation of a chernozem with metal salts for two years, the content of fulvic acids increased and that of humic acids decreased; the Cha:Cfa ratio decreased from 1.0 to 0.5. The contamination also affected the elemental composition of the organic matter; its H:C ratio increased, which was indirect evidence for an increase in the aliphatic segment of the humus acid molecules. It was supported that interaction between metals and organic substances in contaminated soils can result in their mutual mobilization, modification of humus acid molecules, the formation of more stable soluble complexes. The metal contamination can result not only direct toxicological effect. Complexation of metals with organic matter can activate the organic substances and disrupt the humus status of chernozem that is a relatively stable system points to its ecological hazard.

Keywords: Heavy metals; organic matter; chernozem.

S1-P21

Tillage-Induced Carbon Inputs in Soil in Rice-Wheat Cropping System: Farmer's Participatory Studies

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For sustainability of the rice-wheat production system, several resource conservation technologies are being promoted across the Indo-Gangetic plains of south Asia, where this cropping system covers an area of 13.5 million ha. The practice of burning the rice-straw for vacating the fields is common for timely seeding of following wheat crop. Burning of rice straw deprives soil its major source of organic matter (OM) and recycled plant nutrients. It has also become a source of atmospheric pollution. Zero tillage is one of such technologies which afford to recycle crop-residue in soil, thereby, helping not only to sequester OC but also prevents huge amounts of CO₂ emanating to the atmosphere due to burning of rice-straw in the fields. Farmer's participatory experiments were conducted at several sites across Punjab and Haryana states of India to compare the tillage effects on the carbon sequestration through efficient crop residue management. Results of 132 trials demonstrated a significant productivity increase in wheat by strategic tillage using zero-till-seed-cum-fertilizer-drill (ZT). The mean grain yield of wheat planted with ZT and conventional tillage (CT) were 4.25 and 3.52 ton ha⁻¹, respectively. This is mainly due to advancement of sowing with ZT, which ranged from 12 to 14 days. ZT technology reduced the turnaround time for sowing of wheat with residual moisture (no pre-sowing irrigation) having anchored rice-straw, by an average of 13.25 days. This practice helped, on an average incorporation of slightly more than 2.0 ton of C ha⁻¹. Changing to ZT saved approximately one million liters ha⁻¹ of irrigation water from sowing till harvest of wheat crop. Decrease in soil (5-10 cm) temperature by an average of 3°C as a result of mulching effect of anchored rice straw in ZT plots during end March and till the harvest of wheat, is helpful in decreasing the rate of depletion of SOM through oxidation.

Keywords: Tillage; induced carbon; rice-wheat cropping system.

S1-P22**Isothermal Titration Calorimetric Investigation on the Effects of Temperature, pH and Salt Concentrations on *Bacillus subtilis* Adsorption on Clay Minerals and Iron Oxide****P. Cai***

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The adsorption enthalpy changes (ΔH_{ads}) of *Bacillus subtilis* on montmorillonite, kaolinite and goethite were determined using isothermal titration calorimetry (ITC) at various temperatures, pH values and KNO_3 concentrations to clarify the bacteria-mineral adsorption mechanisms. The values of ΔH_{ads} were in the range of -3.3 to -14.7 kJ kg⁻¹ under experimental conditions, indicating the adsorption of *Bacillus subtilis* on minerals was exothermic. The ΔH_{ads} values of bacteria adsorption on minerals were not affected obviously by temperature and the absolute ΔH_{ads} values decreased with the increment of pH and the decrement of salt concentrations. These findings implied that hydrophobic effect and electrostatic interaction played an important role in *Bacillus subtilis* adsorption on clay minerals and iron oxides. Data obtained in this study provide valuable information for a better understanding of the interaction mechanisms between bacteria and minerals in soil and associated environments.

Keywords: *Bacillus subtilis*; Clay mineral; Isothermal titration calorimetry.

S1-P23**Use of Low Temperature Ashing Coupled with DRIFT-FTIR Spectroscopy to Characterize Soil Organic Matter Resistance to Degradation****A. Piedra Buena^{1*}, A. Pucci², P. Tinoco¹, G. Almendros¹ and L.P.D'Acqui²**

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Low Temperature Ashing (LTA) by oxygen plasma coupled with DRIFT-FTIR spectroscopy has been used to study the dynamics, function and protection of soil organic matter (SOM) in undisturbed aggregates. As the LTA technique can remove SOM from the mineral matrix of soil aggregates with minimal disturbance and damage to the inorganic constituents, the gradual oxidation of SOM can be monitored by both spectroscopic and chemical analysis. Undisturbed aggregates (0.5-1.0 mm size) from six different soils from the hilly area of Madrid (Spain), both under undisturbed forest and cultured/reforested plots of the same formerly forested areas, were subjected to LTA treatments of 1, 5 and 24 hours respectively, in order to obtain the dynamics of C removal. The samples before and after the removal of organic material at the different steps were analyzed by DRIFT-FTIR spectroscopy and the residual organic material in the aggregates was extracted by chemical methods. The findings showed that C removal dynamics by LTA can be related to the type of SOM and to its interactions with the different mineral constituents of the aggregates. The characterization of organic matter of undisturbed aggregates by DRIFT spectroscopy was compared with the one obtained by chemical methods of extraction.

Keywords: DRIFT-FTIR spectroscopy; soil organic matter; degradation.

S1-P24**Evidences of Organic Matter Affecting the Clay Fraction Mineralogy of Volcanic Soils****C. Pizarro¹, J.D. Fabris², R.C. Graham³, E. Murad⁴ and J.W. Stucki⁵**¹*Facultad de Química y Biología, USACH, Av. L. B. O'Higgins 3363, Santiago, 7254758, Chile.*²*Departamento de Química - ICEx, UFMG, Pampulha, 31270-901 Belo Horizonte, Minas Gerais,**Brazil.* ³*Soil & Water Sciences Program, Department of Environmental Sciences, University of**California, Riverside, CA 92521, USA.* ⁴*Bayerisches Landesamt für Umwelt, Dienststelle**Marktreidwitz, D-9560, Marktreidwitz, Germany.* ⁵*Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL 61801, USA. *E-mail: cpizarro@lauca.usach.cl*

Andisols and Ultisols forming on volcanic materials are abundant and widespread in Southern Chile. There, about 65% of all agricultural activities are usually carried out on these soils. Andisols are characterized by high organic matter (OM), relatively high amounts of iron oxides and poorly ordered aluminosilicates in the clay fraction; Ultisols have lower OM, higher iron oxide contents and a mineralogy mainly dominated by more crystalline compounds. The mineralogy of clay fractions from the B-horizon of two Ultisols (samples were collected in the localities of Collipulli and Metrengo, geographical coordinates of the sampling sites, 36°58'S 72°09'W and 38°50'S 72°37'W, respectively) and two Andisols (Diguillín and Freire, 36°53'S 72°10'W and 38°57'S 72°36'W, respectively) was determined in this study, in order to search for evidence of the role of organic matter in the crystallization mechanism of clay minerals in these volcanic Chilean soils. The mineralogical analyses were mainly based on powder X-ray diffraction (XRD), transmission electron microscopy (TEM), and Mössbauer spectroscopy (MS) data. The OM contents were evaluated by the Walkley-Black method. The clay samples were sub-divided into two sub-samples each and treated with dithionite - citrate - bicarbonate (DCB) and ammonium oxalate (OX) solutions, in an attempt to selectively remove iron oxides and aluminosilicates with different degrees of crystallinity. From chemical analytical data, the Ultisol samples were confirmed to contain lower organic matter content (2.4 mass%) than Andisols (6.4 and 8.0 mass% for Diguillín and Freire, respectively). Phyllosilicates (mainly kaolinite and halloysite), gibbsite and Fe-oxides coating aluminosilicate grains were identified in the clay fraction of the Ultisol samples. From 6 K-Mössbauer spectra, goethite, hematite, and maghemite were identified, along with some poorly crystalline iron oxyhydroxides, more easily removed by ammonium OX treatment. The XRD patterns for clay fractions of the Andisols resulted mostly in few broad reflections, which did not allow an unambiguous interpretation. After DCB or OX treatment, XRD patterns showed characteristic reflections of phyllosilicates. The 6 K-MS spectra of the clay fractions from the Andisols revealed hematite and some poorly crystalline iron oxyhydroxides that were not completely removed with the previous OX-treatment. In these volcanic Chilean soils, the organic matter accumulation tends to have an inhibitory effect on aluminosilicate stability and on the crystallization processes of iron oxides.

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Keywords: Organic matter; clay fraction mineralogy; volcanic soils.

S1-P25**Surface Thermodynamic Study on Adsorption of *Pseudomonas putida* and *Bacillus subtilis* on Kaolinite and Montmorillonite****X. Rong, Q. Huang^{*}, W. Chen, P. Cai and W. Liang***State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, China, 430070. *E-mail: qyhuang@mail.hzau.edu.cn*

Understanding the sorption process of microorganisms on soil components is fundamental in predicting their fate in soil environments. The sorption characteristics of gram-negative *Pseudomonas putida* and gram-positive *Bacillus subtilis* on kaolinite and montmorillonite were investigated by surface thermodynamic approach in the current study. The surface thermodynamics provides critical information on the relative magnitudes of the different forces involving in bacterial-mineral adsorption. The total free energy for the sorption of bacteria on clay minerals (ΔG) was assumed to be the summation of ΔG^H (hydrophobic interactions) and ΔG^{EL} (electrostatic interactions). Based on the state function, the magnitude of ΔG^H was determined from the different interfacial tensions (contact angles) present in the system. The ΔG^{EL} was calculated from Δ -potentials of the colloidal particles using three models, namely plate-plate, plate-sphere and sphere-sphere model by assuming the different shapes of the sorbate and sorbent, and the best plausible model was selected to obtain the ΔG . The calculated ΔG^H was -3.5 and -2.4 mJ m² for the sorption of *P. putida* on kaolinite and montmorillonite, respectively. The ΔG^H increased by 60.0% and 45.5% for the sorption of *B. subtilis* on the two clay minerals. The ΔG^{EL} was 0.882 and 0.827 mJ m² for the sorption of *P. putida* on kaolinite and montmorillonite, and the ΔG^{EL} decreased by 36.6% and 25.6% for the sorption of *B. subtilis*. The negative values of ΔG^H and the positive values of ΔG^{EL} suggested that the hydrophobic force favored the adsorption of bacteria on the two clay minerals, while the electrostatic force contributed negatively to bacterial adsorption. The magnitude of ΔG were -2.62 and -1.57 mJ m² for the sorption of *P. putida*, and -5.04 and -3.79 mJ m² for that of *B. subtilis* on kaolinite and montmorillonite, showing that the adsorption of bacteria on clay minerals was a thermodynamically spontaneous process. These results also imply that clay minerals possess higher affinity for the gram-positive bacteria in soil system. The quantification of bacterial adsorption to clay minerals in terms of free energy in this study would be helpful for a better understanding of the mobility of bacteria and the formation of bacteria-mineral complexes in soils and sediments.

Keywords: Bacteria; kaolinite; montmorillonite.

S1-P26**Is Bradford Reactive Soil Protein, Glomalin, Stabilised by Association with Allophane in Forest Soils of Martinique, French West Indies?****S. Staunton^{1*}, T. Woignier², P. Etcheverria³, F. Borie³, T. Chevallier⁴ and H. Quiquampoix¹**¹INRA, UMR 1222 (Biogéochimie du Sol et de la Rhizosphère), France. ²IRD, UR Seqbio, B.P. 213 Petit Morne, 97232, Le Lamentin, Martinique. ³Universidad de La Frontera, Casilla 54-D, Temuco, Chile.⁴IRD, UR Seqbio, Montpellier 34060, France. *E-mail: staunton@montpellier.inra.fr

Soil organic matter plays an essential role in mitigating the effects of rising levels of atmospheric CO₂ and the corresponding rise of temperature and increase in the frequency of extreme weather conditions. Glomalin, first reported in 1996 by Wright & Upadhyaya (1996) may be an important component of soil organic matter in terms of soil physical structure and resistance to breakdown. This glycoprotein is produced by the hyphae of arbuscular mycorrhizal fungi and secreted into the soil where it may accumulate. In some soils, glomalin may account for a large proportion of soil carbon, and on a global scale may represent an important carbon sink. Oxides of iron and aluminium are thought to protect glomalin and decrease its turnover time. In addition, it has been suggested that, in common with fungal polysaccharides, glomalin may play an important role in the stability of soil aggregates. We have studied the glomalin contents of soils sampled from A and B horizons of forest soils of Martinique, French West Indies. Both easily extracted (one short autoclave extraction in 20 mM sodium citrate solution at pH 7) and total (five 1-hour extractions in 50 mM sodium citrate solution at pH 8) glomalin contents were measured as Bradford reactive BSA-equivalent protein. The glomalin contents were compared to other soil properties. Glomalin contents ranged from about 1 to 36 g kg⁻¹ accounting for up to 10% soil organic carbon. Such levels of glomalin are high although even larger levels have been reported for Chilean Andosols. Glomalin contents were strongly correlated with organic carbon and allophane contents for surface soils. For sub-soils, glomalin content followed the same trend with organic C content as surface soils, but showed no significant relationship with allophane content. There was no correlation between the ratio of glomalin to total organic carbon content with the allophane content, suggesting that this mineral accorded similar protection to glomalin and other forms of stable organic matter. The glomalin content also followed a similar trend to that of soil respiration rate. However, since the latter was strongly correlated with organic carbon content, the effect is probably not direct. There was no correlation between the glomalin content and any other microbial parameter measured (microbial biomass, relative respiration rate or respiratory quotient). About half the soil glomalin was easily extractable in surface soils, whereas the fraction was smaller and more variable for B-horizon soils. This contrast between glomalin in surface and deeper horizons suggests that glomalin is transformed with time in contact with the solid phase.

Keywords: Glomalin; allophane; forest soils.

S1-P27**Radiocaesium Transfer in Mountainous Ecosystems in Central Balkan 20-Year after the Chernobyl Accident****M. Zhiyanski¹, J. Bech B.², M. Sokolovska¹, P. Tume^{3*} and J. Bech R.⁴**

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Following the Chernobyl accident a number of natural and semi-natural environments were contaminated by radiocaesium mainly through wet deposition. Radiocaesium deposited in these areas has a long term impact on the environment. This fact is attributable to the persistence of radiocaesium in all compartments of forests, pastures and natural meadows. During the last twenty years scientific efforts were directed towards the comprehension of the transfer phenomena of radionuclides in the ecosystems. Despite of this, few studies were conducted to compare the Cs-137 soil-to-plant transfer between native beech forest, pure spruce plantation and upland mountainous pasture in the Central Balkan Mountain in Bulgaria. We compared Cs-137 contents in forest floor, in upper mineral soil layers and in plant species in mountainous pasture, 45-years old natural *Fagus silvatica* L. forest and 45-years-old *Picea abies* Karst. forest. We indicated that 20-years after Chernobyl accident the main concentrations of Cs-137 are located in forest floor layers. The little thickness of forest floor was related with comparatively lower Cs-137 content. The higher organic C content in upper mineral soil layers was related with higher Cs-137 contamination and easier soil-to-plant transfer in the mountainous pasture. The relatively higher clay content in upper mineral soil under the plantation was related with lower transfer of Cs-137 towards spruces, despite of their surface root system. 20-years after the main pollution after the Chernobyl accident the transfer factors for caesium-137 for studied plant species were low and varied between 0.01 and 0.02. The grass vegetation had the highest Cs-137 soil-to-plant transfer compared with beech and spruce.

Keywords: Radiocaesium; mountainous ecosystems; forest soils.

S1-P28**Factors Influencing Competitive Sorption of Copper and Lead on Ferrihydrite and Goethite****J. Zhu^{1*}, L. Perelomov², V. Cozzolino³, M. Pigna³ and A. Violante³**

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Mobility of trace elements in soil environments is affected by sorption-desorption reactions, which are the predominant factors that control the bioavailability of heavy metals. Few studies have been carried out on the competition in sorption of heavy metals to soil

components and soils. Competition in sorption between two or more trace elements is of great importance for understanding their relative affinity for a given sorbents. Variable charge minerals selectively sorb polyvalent cations even when their surfaces are positively charged (solution pH lower than the PZC of the sorbents). Competitive sorption between two (or more) heavy metals seems to be affected by pH, surface properties of the minerals, nature and concentration of heavy metals and presence of inorganic and organic ligands. We have carried out experiments on the sorption of copper and lead added alone or together onto synthetic ferrihydrite and goethite as affected by pH, concentration of the heavy metals (initial Cu/Pb molar ratio of 0.5, 1 and 2), sequence of addition of the trace elements onto the sorbents and presence and concentration of oxalate and/or tartrate (among the most abundant low molecular-weight organic anions present in soil environments, mainly at the soil-root interface). At pH 4.5-5.0 the sorption isotherms of these two heavy metals fitted the Langmuir equation (L-type) and showed a higher affinity of copper for goethite, but a much greater affinity of lead for ferrihydrite. The sorption of both trace elements was strongly increased by increasing pH. In particular, the pH_{50} (the pH value at which 50% of the total sorption of the elements has occurred) was 4.90 and 5.90 for copper and 5.20 and 4.40 for lead on goethite and ferrihydrite respectively. In the presence of increasing concentration of oxalate (OX/Me molar ratio [R] ranging from 0 to 10) the pH_{50} of copper sorbed onto goethite decreased from 4.90 at R=0 to 3.85 at R=4 and then increased up to 4.74 at R = 10, whereas the pH_{50} of lead was less affected by the presence of oxalate. The two heavy metals strongly competed with each other for sorption sites on the surfaces of the oxides. At pH 4.5-5.0 the sorption of a given metal onto ferrihydrite and goethite was inhibited in the presence of increasing concentrations of the other element. By adding the two heavy metals together in equimolar amounts (25 mmol added per kg) on the oxides, copper sorption was reduced by lead of 35% onto ferrihydrite and of 11% onto goethite, whereas lead sorption was reduced of less than 5% onto ferrihydrite and 24% onto goethite. Competition in sorption between the two heavy metals was affected by the sequence of addition and the residence time of the first element added to a given sorbent. Finally, the presence of oxalate and/or tartrate previously added to ferrihydrite or goethite or added as a mixture with copper and lead strongly affected the competitive sorption of these heavy metals on the iron oxides.

Keywords: Heavy metals; ferrihydrite; goethite.

S1-P29

Comparison of Carbon Sequestration in Volcanic and Non-volcanic Soil of Chile

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The organic carbon (OC) stabilization and sequestration mechanisms differ due to the presence of aluminum (Al) in the soil solution, allophane and imogolite type materials in volcanic soils and the amount of silt + clay crystalline minerals in non volcanic soils. The objective of this work was to compare these factors of stabilization on OC immobilization in both, volcanic and non volcanic soils of Chile. We selected agricultural sites with similar

managements (cropping and grassland); all soil belongings to the order: Andisol, Ultisol, Molisol, Vertisol, Alfisol and Inceptisol (USDA classification). Soils samples were taken at 0-0.1, 0.1-0.2, and 0.2-0.4 m depth. The analysis were carried out for extractable Al with Na pyrophosphate (Al_p), acid ammonium acetate (Al_a), $CuCl_2$ (Al_{Cu}) and OC in: Whole soil, fractions 0-53 μm and the supernatant of pyrophosphate (C_p). We also determine water pH, bulk density and allophane content by Al/Si ratio. Our study confirmed previous results in non volcanic soils; Particles 0-53 μm (silt + clay) was positive and significantly related with whole soil OC ($R^2 = 0.5$) and the OC in 0-53 μm ($R^2 = 0.8$). The opposite being true in volcanic soils; instead, soluble C, C_p was highly related with soil OC and constituted ca. 43 % from this amount. Allophane content (about < 13 %) was positive and significantly related with the soil OC ($R^2 = 0.6$) and OC in the fraction 0-53 μm ($R^2 = 0.8$). The small amount of allophane may exert large influence on soil aggregation and it in turn on physical protection of soil organic matter. This was supported for the good relationship between allophane and total porosity of soils. We confirmed that the differences in soil organic C stabilization in volcanic with respect to non volcanic soils are due to the clay mineralogy and active Al, which complexed near half amount of whole soil organic C. The rest is assumed to be associated with Allophane *per se* and physically protected in soil aggregates. We also confirmed that Al_a (which is similar to $CuCl_2$ extraction) was highly and positively related with OC of volcanic soils.

Keywords: Carbon sequestration; allophane; volcanic soils.

S1-P30

Soil Organic Carbon Determination Associated to Active Al under Different Managements

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Decrease of soil organic carbon (SOC) proceeds from the conversion of natural to agricultural ecosystems and it has contributed to raise the level of atmospheric CO_2 . There is still little information about the mechanisms of stabilization of organic matter (OM) in allophanic soils. A key factor is the active Al complexed with organic compounds which has been previous reported by a significant and positive relationship with SOC. However, the quantity and quality of soil organic C bound to Al has not been quantified yet in our soils. The aim of this study was to determine the C associated with the Al (C_p) in the supernatant of Na-pyrophosphate at pH 10 extraction. C_p was measured by potassium dichromate in a range of 10 Andisols from Southern Chile, sampled between 0-40 cm. The results showed a good correlation ($R^2 = 0.72$, $P < 0.01$) between C_p and SOC. Approximately 45% of soil organic C corresponded to C_p , confirming that the active Al immobilized significant amounts of organic C in our allophanic soils.

Keywords: soil organic carbon; aluminum; allophanic soils.

S1-P31**Preparation of a Humic Acid/Polypyrrole Modified Pige Electrode and its Prospective Environmental Use****M. Antilén*** and J. Armijo*Departamento de Química Inorgánica, Facultad de Química, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, 6904411, Chile. *E-mail: mantilen@uc.cl*

Owing to their polyfunctionality, humic acids (HAs) are among existing natural organic compounds' most powerful chelating agents. Thus, many studies have demonstrated the important interaction between HAs and varied contaminants, which form complexes of different chemical-biochemical solubility and stability. This means that HAs might modify environment contaminants availability, transport, fixation, and toxicity. On the other hand, conductive polymers' properties offer an amply variety of applications, *e.g.*, doping-undoping process that can be carried out by electrochemical means and has been utilized as contaminants extracting method. Thus, the aim of the present work is the preparation of a humic substances modified PIGE electrode that will be evaluated regarding its potential environmental use for chemical species of interest, compared to those described in the literature. All electrochemical experiments were performed using a VoltaLab PGZ100 potentiostat system, in a glass three-compartment three electrodes cell under argon atmosphere. A paraffin-impregnated graphite electrode (PIGE) was used as working electrode. The counter electrode was a Pt wire and all potentials were measured with respect to Ag/AgCl reference electrode. Humic acid was transferred and immobilized onto the electrode surface by the abrasive transfer technique. Electrochemical growth of polypyrrole (Ppy) films was carried out on PIGE or PIGE/HA from 1mM pyrrole + 1 mM Na₂SO₄ + 3·10⁻² M H₂SO₄ by a potentiodynamic method applying 5 cycles between - 200 and 1000 mV. Next, two of the prepared electroodic surfaces (PIGE/HA/Ppy and PIGE/Ppy) were studied in the presence of 1mM AsO₄³⁻ + 1mM Na₂SO₄ + 3 x 10⁻² M H₂SO₄ (cell A) and in 1mM Na₂SO₄ + 3 x 10⁻² M H₂SO₄ (cell B) solutions respectively, in order to select the doping-undoping potential to perform the extractions (ExC). The total volume of electrolyte in each cell was always 10.0 mL. Each ExC was accomplished as follows: the PIGE/Ppy or PIGE/HA/Ppy modified electrode was immersed in A, potentiostatically perturbed (0.4 V) during 5 min, after which was immersed in B, where it was potentiostatized at -0.5 V during 5 min. Initially and after several ExC, arsenic concentration in A and in B was determined by ICP-OES spectroscopy using a Varian Liberty Serie II instrument. As(V) concentration found before and after the extraction using the PIGE/HA/Ppy showed an extracting efficiency of 0.34 mg L⁻¹ for 10 extractions, considering the electrode area as 28.25 mm². The observed efficiency corresponds to the incorporation of the HA into PIGE. As for the charge transfer, the process was not wholly reversible.

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Keywords: Humic acids; chelating agents; paraffin-impregnated graphite electrode.

S1-P32**Evaluation of The Oxidation Capacity of Humic Acids Extracted from Chilean South-Central Soils****M.A. González¹, M. Antilén^{1*}, J. Armijo¹, M. Briceño² and J. Canales³**¹*Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860,6904411, Santiago, Chile.*²*Universidad Arturo Prat, Casilla 121, Iquique, Chile.* ³*Universidad de La Frontera, Casilla 54-D Temuco, Chile.* *E-mail: mantilen@uc.cl

Humic acids (HAs) are soil constituents found as negatively charged colloids, biodegradable that can be stored for long periods of time. Also, these humic compounds present the properties of participating in redox reactions with elements such as Fe (II), Mn(IV), V(V), and Hg(II). The linkage of these materials to redox reactions and the scarce information in this respect, arouse interest concerning to their determination and characterization, as well as their likely applications to varied processes. The redox characterization will enable evaluation of, based upon cell potential and oxidation capacity of HAs, the actual possibility of utilizing these substances as base materials for feasible redox processes in analites of interest. Consequently, the aim of this work is to study and to establish the differences related to redox properties and acid-base characteristics of humic acids obtained from soils of the Chilean South-central region. Conductivity, pH, chemicals analysis, and organic matter for all soil samples [Collipulli (C), Colina (CL), Diguillín (D), and Ralún (R)] were determined. Extracted HAs, using the IHSS's recommended procedure, were characterized by elemental analysis, phenolic and carboxylic groups, UV-Vis, and FT-IR. Cell potential and oxidation capacity were determined by redox titration of a 50-mg sample at pH 7.0 using I₂ as titrant. End point was detected by means of a conjugated glass electrode and Pt under Ar atmosphere. Each titration took nearly 3-5 h because of the necessary stabilization required after each titrant addition. From the obtained cell potential, HA-D displayed the highest reduction values (438.3 ± 9.78 mV), becoming thus the humic fraction that possesses more energy to bring about oxidation processes. Analyses of the remaining samples enables establishing the following sequence: HA-C > HA-CL > HA-R (364.2 ± 39.7 mV). However, the cell potential must be evaluable concomitantly with the oxidation capacity since this allows establishing how much of the species of interest will be oxidized. As for HAs extracted from soils, a slight correlation of the oxidative capacity with the phenolic groups was found ($R^2=0.863$), which would enable associating the existence of these groups to a lesser oxidation capacity. According to the obtained results it may be inferred that the humic fraction of D-soil is the one having the most suitable redox conditions to oxidize chemical species of interest contained in soil-solution currently under survey using electrochemical techniques.

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Keywords: Humic acids; redox properties; acid-base characteristics.

S1-P33**Reduction of Ammonia Loss by Mixing Urea with Humic and Fulvic Acids Isolated from Peat Soil****S. Kasim¹*, O.H. Ahmed¹, N.M.A. Majid¹ and K. Yusop²**

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Surface application of urea is less effective due to high loss of N through volatilization. Rapid increase of pH (at soil microsite) and low soil exchangeable cations are some of the factors affecting urea-N efficiency. In order to improve urea-N use efficiency as well as contributing to reduction of environmental pollution, granular urea was mixed with liquid humic acid (LHA) only, liquid fulvic acid (LFA) only, and LHA + LFA isolated from a tropical peat soil. The presence of LHA only, LFA only, and LHA + LFA significantly reduced ammonia loss by 29.2, 11.4, and 8.1% respectively as compared to urea only (urea without additives). The high total acidity (417 to 583 $\mu\text{mol kg}^{-1}$) of these treatments may be one of the reasons for the significant reduction of ammonia volatilization. The other reason was that the treatments (LFA only and LHA + LFA) significantly retained ammonium ions compared to urea only. The high exchange properties of the treatments may have retarded urea hydrolysis and hence the reduction of ammonia volatilization. Urea amended with LHA only or LFA only or LHA + LFA could reduce ammonia loss, hence their potential of improving urea-N-use efficiency as well as contributing to the reduction of environmental pollution.

Keywords: Ammonia loss; humic and fulvic acids; peat soil.

S1-P34**Effect of Wildfire on Soil Organic Matter Dynamics and Quality in a South Chilean Andisol****Y. Rivas¹*, D. Huygens², O. Van Cleemput², R. Godoy¹ and P. Boeckx²**

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Old-growth forests of Southern Chile represent an important reserve of temperate forests in the World. The precipitation chemistry in Chile still reflects a close approximation of pre-industrial conditions. In this way, the productivity of the ecosystem depends on the internal cycling of soil organic matter (SOM). However, catastrophic events, such as wildfires can disturb this balance and produce important changes in the physical and biogeochemical properties of soils and as a consequence to the ecosystem sustainability. The isotopic composition of SOM is a useful tool for elucidating the different mechanisms of SOM decomposition processes. *Araucaria-Nothofagus* forests in the Andean mountains of Chile (38°S, 71°W) were affected by wildfire (February, 2002) providing an “experimental field” to study its effect on SOM. The objective of this investigation was to analyze the effect of the fire on the quality of SOM at different soil depths. We used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures coupled

to size and density fractionation to evaluate SOM dynamics. In the burned site, the different SOM fractions were depleted in $\delta^{13}\text{C}$ and enriched in $\delta^{15}\text{N}$ when compared to the unburned site. Our result respect to C dynamics suggest that: fire induces changes in C composition of the SOM, toward more depleted C compounds due to increase charcoal components. Respect to N dynamics, we found increased nitrification rate after fire and because nitrification discriminates against ^{15}N , this may in turn lead to a ^{15}N -enriched residual in soil. This conclusion indicates that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ can be used as proxy to assess the fire on C and N dynamics. This conclusion is noted, especially, in the upper soil layers (0-10 cm).

Keywords: Ammonia loss; humic and fulvic acids; peat soil.

S1-P35

Comparative Study of Structural and Physical-Chemical Characteristics of Nanoparticles Extracted from two Andisols of Southern Chile: Potential in Agricultural Application

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Chemical surface functionalization and surface structuring particularly on the nanometer scale play an increasing role in many fields of technological application. However, the production of nanoparticle with small diameters and a narrow size distribution is challenging and expensive. Therefore, natural nanoparticles are a cheap alternative if the size distribution is narrow and the extraction of the particles from soil material is economically efficient. Our goal is to provide nanostructured surfaces based on natural nanoparticles particularly for agricultural application (e.g. Biofertilizer or pesticides of controlled release). The first stage of our study contemplates the extraction and characterization of nanoparticles from two Andisols belonging from Piedras Negras and Temuco series of southern Chile. To obtain the nanometric fraction of soils (nanoclays covered with organic matter), first we separate the < 0.02 mm fraction by a sedimentation method based on Stokes's law. From this fraction, the nanoparticles were obtained according the methodology described by Li and Hu (2003). The nanoparticles were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), infrared spectroscopy (FTIR) and also, were determinated the isoelectric point (PIE) and specific surface area Brunauer-Emmett-Teller (SSA_{BET}) of both materials. Transmission electron microscopy micrographs revealed more or less condensed aggregates of nanoparticle and the obtaining material corresponds to nanoclays with a particle average size less than 50 nm for Andisols series Piedras Negras and less than 100 nm for Temuco series, which displaying homogeneous elemental compositions, with Al/Si molar ratios ranging ~ 2:1. SEM showed aggregates of nanoclays with variable pore size, which can adsorb high quantity of water generating microenvironments benefit for an adequate mass transfer. The FTIR spectra showed characteristic bands of the soil organic matter (fulvic and humic acids). Low values of PIE (3.2) are attributed to the organic matter covering of nanoclays. Variations in SSA_{BET} and in mesoporous and microporous between both materials were observed ($14 \text{ m}^2\text{g}^{-1}$ and $8 \text{ m}^2\text{g}^{-1}$ for Piedras Negras and Temuco series respectively). The shape of the nitrogen adsorption-desorption isotherms allows two types of curves BET, nanoparticles from Piedras Negras series presented a curve Type II that

suggest the presence of a relatively well-calibrated pore and a narrow pore-size distribution, and nanoparticles from Temuco series presented a curve Type III suggesting high microporosity and relatively low nitrogen-adsorbed volumes. From the results, we can conclude that both nanomaterials are promising to be used in biocatalysis or in other technological application, because of suitable confinement and enzymatic stabilization that those support nanomaterial can provide.

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Keywords: Nanoparticles; nanostructured surfaces; Andisol.

S1-P36

Natural Nanoclays: Physicochemical Characterization

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Nanotechnology is an emerging science with different applications in scientific and technological research. Studies with nanoclays have carried out to resolve environmental problems, due to its small size, high surface area, and charge characteristics. The nanoclays have a potential use in the pollution removal, mainly as adsorbent compounds and catalytic support. Allophane is the main component of the clays fraction of Andisols of Southern Chile. It is an aluminosilicate non-crystalline, amorphous to X-ray, which presents a short-range order in its structure with a predominance of Si-O-Al bonding (Wada and Wada, 1977). In these soils, the allophane is covered by amorphous iron oxide (hydrous ferric oxide and ferrihydrite), giving the Andisols typical features of iron oxides than allophane, even though it has been reported that Fe oxides normally not exceed 10% in these soils. The aim of this study was to characterize nanoclays from an Andisol of Southern Chile. Natural nanoclays were extracted using the methodology described by Liu et al. (2005) from a soil without organic matter. The nanoclays were characterized by the surface area and the micropores volume using the BET and Broekho and de Boer methods. The surface charge properties were evaluated by the isoelectric point (IEP), the Point of zero salt effect (PZSE) and the Zero point charge (ZPC), through electrophoretic mobility, potentiometric titration and Park's model (Parks, 1967), respectively. The natural nanoclays showed an Al/Si ratio of 0.24, the surface area of $124 \text{ m}^2 \text{ g}^{-1}$ and volume of micropore $0.018 \text{ cm}^3 \text{ g}^{-1}$. The shape of adsorption-desorption curves were similar to synthetic allophane with Al/Si ratio < 5 reported by Montarges-Pelletier et al. (2005). It suggests a wide pore size distribution and high content of mesopores. The zero point charges were 3.8 (ZPC), 7.0 (IEP) and 5.4 (PZSE). These values support that the superficial composition is different from their percentage composition. Therefore, the properties nanoclays evaluated in this study showed similar characteristics of aluminosilicate coated with iron oxides previously synthesized (Jara et al., 2006).

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Keywords: Natural nanoclays; allophane; aluminosilicate.

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Session 1. Ecological Significance of Interactions Among Clay Minerals, Organic Matter and Biota

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S1-P37

Allophane Nanoclays: The Potential Use to Increase the Catalytic Efficiency of Acid Phosphatase Enzyme

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Natural nanoparticles occur widely in soils derived from volcanic ash like Andisols. The more important constituent of Andisols is allophane, which is an aluminosilicate non-crystalline. These nanoclays have morphology spheroidal with an outer diameter of 3.5–5.0 nm, the thick wall (0.7–1.0 nm) is composed of an outer Al octahedral sheet and an inner Si sheet. Also, defects in the wall structure give rise to perforations of ~ 0.3 nm diameter. These nanoparticles form stable microaggregates, with pores within the nanoscale range with similar physical characteristics to silica nanomaterials which were very important in biocatalysis. Studies have reported that nanomaterials are more promising clays for enzyme immobilization compared to conventional materials. The aims of this study were: characterize natural clay and nanoclay with and without organic matter from an Andisol and ii) evaluate clay and nanoclays as support to immobilize acid phosphatase. An Andisol, Piedras Negras soil from Southern Chile was sampled within 0–20 cm of depth, sieved to 2 mm mesh and air-dried. One part of the soil was treated with 30% hydrogen peroxide to remove the soil organic matter. The separation of particle-size < 2 µm fractions was obtained by sedimentation procedures based on Stoke's law. Natural nanoclays were extracted using the methodology described by Liu et al. (2005). The clays and nanoclays were characterized by using energy dispersive X ray (EDX), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and electronic diffraction (ED). Synthetic complexes were formed by interaction between acid phosphatase and either allophanic clay or nanoclay, and used as model systems to simulate enzymatic reactions occurring in heterogeneous environment. The enzymatic activities of immobilized phosphatase on nanoclay, with or without organic matter, were measured with p-nitrophenylphosphate (pNPP) as substrate. The kinetics parameters (V_{\max} and K_m values) were calculated by a non-linear regression analysis according to the Michaelis–Menten equation. The TEM analysis showed the nanoclay presence (< 50) nm in the samples evaluated. The occurrence of amorphous and crystalline particles were observed by DE analysis in samples the clay and nanoclay. The SEM analysis the immobilized phosphatase showed cavities of different sizes. The enzymatic activity increased in 90 % compared with the free enzyme. Likewise, the kinetic parameters showed that the catalytic efficiency (between 60 and 70 %) and V_{\max} (between 110 and 130 %) were enhanced.

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Keywords: Natural nanoclays; allophane; aluminosilicate.

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S1-P38**Greenhouse Gases Emissions from Soils with Different Anthropogenic Influence in Southern-Central Chile****C. Muñoz^{1*}, L. Paulino¹, N. Stolpe¹, C. Ovalle² and E. Zagal¹**¹*Departamento de Suelos y Recursos Naturales, Universidad de Concepción, Campus Chillán, Chile.*²*Departamento de Producción Animal, Instituto Nacional de Investigaciones Agropecuarias (INIA) Quilamapu, Chillán, Chile. *E-mail: cristinamunoz@udec.cl*

The anthropogenic activities are potential sources of Greenhouse Gases (GHG) emissions where CO₂ and N₂O are of relevance in agricultural systems. In South America few information is available about *in situ* quantification of GHG from agricultural and agroforestry systems. In Chile, a national inventory was done based on factors proposed by the Intergovernmental Panel of Climate Change (IPCC) for the most representative systems, but revealed the lack of information for their particular characteristics of soil (mainly volcanic ones) and climate considering the most relevant productive activities at the country or region scale. A recently financed project of our research group has the main aims to assess biogeochemical processes related to GHG emissions (mainly CO₂ and N₂O) from soil under different anthropogenic influence, including agriculture uses and management of Southern-Central Chile. The methodology includes periodical *in situ* GHG sampling through non-fixed closed chambers (200 cm³), at least once a month and after events (meteorological or fertilization) during 3 consecutive years, simultaneously with records of meteorological data (e.g. precipitation, wind direction wind speed, relative humidity of air, temperature) and soil conditions (e.g. temperature, moisture and mineral N content as (–NO₃ and –NH₄). Gas sampling will be performed under different soil uses as native forests, exotic forest plantations (*Pinus radiata*), long-term prairie (*Medicago sativa*), agroforestry (with exotic species, intensive wheat (*Triticum aestivum*), oat (*Avena sativa*), prairie (*Trifolium* sp.) and maize crop (*Zea Maize*) rotation) including different soil and climate types. Also a no-till system under different nitrogen fertilization type and lime rates is considered as management field tray. The preliminary results indicate that the soil use intensity produce effect in CO₂ emission with major rate in agroforestry and pasture (with 52 t ha^{–1} year^{–1} each) compared with no-till (35 t ha^{–1} year^{–1}), but it is necessary to reinforce that information with measurements during 3 years at least. The N₂O emission rates were not well discriminated from different soil use due to very low amounts released. Sampling of CO₂ and N₂O at the other areas are to be started, and will add complementary information about the contribution of soil types on GHG emission from different scenarios of economic relevance.

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Keywords: Greenhouse Gases; climate change; anthropogenic activities.

S2-P1**Evaluation of *Rhizobium leguminosarum* Strains Effectiveness, Virulence and Ability of Competitiveness in Field Beans (*Vicia faba* L.)****A. Ansevica^{1*}, V. Steinberga², I. Alsina² and L. Dubova²**

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In agriculture systems, the bulk of the biological nitrogen fixation is derived from the cultivation of legumes. The inoculation of the legume seed material with active nitrogen fixing bacteria strains before sowing has a significant meaning for the increase of the legume yield. Inoculation can improve crop yields in cases where appropriate rhizobia are not present in the soil or the soil contains a significant proportion of nonnodulating or ineffective nitrogen-fixing strains. The successful use of rhizobial inoculants in the latter situation requires knowledge of the indigenous rhizobial population in terms of numbers, effectiveness, and competitive ability. The aim of the investigation was to detect the effectiveness, virulence and ability of competitiveness of *Rhizobium leguminosarum* strains in field beans (*Vicia faba* L.). The vegetation pot experiments were conducted at Institute of Soil and Plant Sciences of Latvia University of Agriculture and field experiment at agency Research institute of Agriculture of Latvia University of Agriculture. Before sowing field beans seeds 'Lielplatones vietējas' and 'Ada' were inoculated with *Rhizobium leguminosarum* strain: No. 110; No. 408; No. 501 and control (without inoculants) from the nodule bacteria collection of Institute of Soil and Plant Sciences of Latvia University of Agriculture. In the vegetation pot experiment was used three substratums. Plants were analyzed at six plant development stages: three leaves stage; butonisation; anthesis; pod formation; pod development; pod maturing. Analysed parameters: plant length; fresh weight; dry matter; protein amount and plant pigments. *R. leguminosarum* strains virulence and ability of concurrence were analysed by resistance on antibiotic and by molecular - genetics analyses. Taking into consideration the results of the experiment it has pointed out that all *Rhizobium leguminosarum* strains were active and inoculated plants formed nodules on the roots. It was found different *R. leguminosarum* strain effectiveness in different soils. Inoculation with *R. leguminosarum* strains doesn't significant impact plant pigments grown in different substratum. The field beans seed inoculation with *R. leguminosarum* strains significantly increased total protein amount.

Keywords: *Rhizobium leguminosarum*; Competitiveness; *Vicia faba*.

S2-P2**Determination of Essential Elements in nine Barley Cultivators (*Hordeum vulgare* L.) and its Soil, Grown at Wheat Research Station Tandojam, Sindh, Pakistan****S.A. Arain^{1*}, G.Q. Shar¹, T.G. Kazi² and L.A. Shar¹**¹Department of Chemistry, Shah Abdul Latif University Khairpur, Sindh, Pakistan. ²National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro.

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The mineral composition of nine different cultivars of barley (*Hordeum Vulgare* L.) seeds (Jau-83, Jau-87, Haider-93, Quina, Sadabahar, Bajawar-2000, Frontier-87, Sanober-96) and soil were analysed using flame atomic absorption spectroscopy. Bajawar-2000 was found to contain the higher sodium content. The essential bulk metals in the six cultivars were found to be dominant, where K was the highest and calcium was lowest. The decreasing order of essential trace metal concentration is Fe>Zn. All barley cultivars trial grown at Wheat Research Station Tandojam, Sindh, Pakistan for two years to compare the variation of mineral uptake. In this connection, wet ashing method used to destroy the organic matrix. The concentration in mg/Kg of the soil of that specific plot was observed 4424.1 ± 291.12 (Na), 1418.0 ± 556.5 (K), 3404.4 ± 327.0 (Ca) and 3975.2 ± 3847.8 (Mg), using reference material checked the validity of digestion method.

Keywords: *Hordeum vulgare*; Fe and Zn; *Vicia faba*.**S2-P3****An Innovative Biological Growth Promoter for Corn****M.R. Banerjee* and L. Yesmin**

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Application of plant growth promoting rhizobacteria (PGPR) for boosting plant growth and development is not particularly new in agriculture biotechnology. Yet attempt to successfully utilizing PGPR to develop commercial biological growth promoter to improve yield potential of agronomically important field crops are limited. In the present investigation, we tried to develop potential biological growth promoter for corn (*Zea mays*) to enhance performance and yield of the crop. Two sulfur-oxidizing PGPR, Corn01 and Corn02 were used as possible corn inoculants in the study. Corn seeds were treated with viable cells of selected strains of *Delftia acidovorans* (RAY 209) and *Achromobacter piechaudii* (RAY 12), designated as Corn01 and Corn02, respectively. Both rhizobacterial inoculant treatments were compared with the un-inoculated controls in several field trials conducted over three years in the mid-western corn belts in the United States. Corn inoculated with these bacteria showed increased plant vigor and yield. Increased root growth & development, and dark leaves were also quite evident in some of the trials. Upon comparison between Corn01 and Corn02 treatments, more positive and consistent results were observed due to the inoculation

of Corn02 in regard to plant vigor, root development and yield. Thus, the rhizobacterial inoculant Corn02 containing viable cells of *Achromobacter piechaudii* (RAY 12) could be utilized as a unique commercial biological growth promoter to improve corn performance and production.

Keywords: Biological growth promoter; sulfur-oxidizing PGPR; *Zea mays*.

S2-P4

Rapid Mass Multiplication of *Glomus mosseae* Inoculum

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Rhodes grasses (*Chloris gayana* Kunth) inoculated with *Glomus mosseae* were grown under the influence of a biotic factor- *Azospirillum*; and two abiotic factors - IAA, and Hoagland's solution. The effectiveness of each factor was evaluated by measuring mycorrhizal root colonization and spore numbers. The pot culture experiment was carried out under polyhouse condition and observations were recorded at 45 days, 90 days and 120 days of plant growth. The harvest date finely influenced the size of mycorrhizal inoculum. But, all biotic and abiotic factors had a greater influence on root colonization and spore numbers than harvest time. The factors (biotic, abiotic) on conjunction favorably enhanced root infection and sporulation as compared to their solo treatments, with *Azospirillum* + Hoagland's solution posing to be the best. This not only stimulated mycorrhizal development, but also accelerated the root growth. This investigation led to achieve large quantities of mycorrhizal root inocula within a relatively short time (45 days) and may help to large-scale utilization of arbuscular mycorrhizal fungi inoculant besides curtailing the production expenditure, which lacks mainly at present.

Keywords: *Glomus mosseae*; *Azospirillum*; IAA.

S2-P5

Mobilisation and Acquisition of Nutrients from Apatite and Biotite by Scots Pine Roots associated or not with a Mycorrhizosphere Bacterial Strain

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With the exception of N, soil minerals are the main source for all mineral nutrients required for tree growth in non-fertilized forest ecosystems. The major part of this nutrient reserve is, however, not directly available to trees as the nutrients are entrapped in the mineral phase. Therefore mineral weathering, a process which promotes soil nutrient bioavailability, is crucial to satisfy plant nutritional needs. A great variety of biological processes linked to plant root and micro-organism activities have been suspected to affect soil mineral weathering and to increase the nutrient supply to trees. The aims of this study are (i)

to quantify the respective effect of Scots pine roots inoculated or not with a mycorrhizosphere bacterial strain *Burkholderia glathei* PML1 (12) on the mobilization of essential nutrients such as Ca, P, K, and Mg issued from the weathering of apatite or biotite, two minerals frequently present in forest soils, and (ii) to assess the contribution of *B. glathei* PML1(12) to the nutrition of Scots pines. The strain *B. glathei* PML1 (12) was isolated in a forest soil mineral horizon from the ectomycorrhizosphere of *Scleroderma citrinum*-oak. This strain was selected within a collection of more than 500 soil bacterial isolates because of it showed highest abilities to produce weathering agents and to mobilize nutrients in different *in vitro* biotests. We carried out a column experiment in a growth chamber. Each column, containing a mixture quartz-biotite or quartz-apatite, was planted with one pine seedling except for the abiotic treatment. The half of the columns containing pine seedlings was inoculated with a calibrated suspension of *B. glathei* PML1 (12). Each column was supplied with a nutrient solution containing all the nutrients necessary to plant growth but absent from the mineral phase. The inputs and outputs of Ca, P, K, and Mg in solution as well as the immobilization of these elements by pine seedlings were measured. As regards to the biotite, the results revealed that Scots pine roots non-inoculated or inoculated with *B. glathei* PML1 (12) significantly increased K and Mg mobilization in comparison with the abiotic treatment. In addition, we observed that the inoculation of *B. glathei* PML1 (12) significantly increased K and Mg mobilization in comparison to the non-inoculated seedlings, resulting in an improvement of Scots pine nutrition and growth by a factor of 1.5. As regards to the apatite, the results also revealed that Scots pine roots non-inoculated or inoculated with *B. glathei* PML1 (12) significantly increased Ca and P mobilization in comparison with the abiotic treatment. Because the amounts of Ca and P immobilized by the pines during the experiment are not yet analyzed, we can not currently assess the effect of *B. glathei* PML1 (12) inoculation on nutrient mobilization and tree nutrition. We therefore observed the presence of oxalic acid, a strong mineral weathering agent, in the drained solutions only when Scots pine roots were inoculated with *B. glathei* PML1 (12). Our study demonstrates the capacity of a mycorrhizosphere bacterial strain to produce weathering agents and to increase the mobilization of nutrients from the soil mineral phases, thus improving tree nutrition.

Keywords: Apatite; Biotite; mycorrhizosphere bacterial strain.

S2-P6

Inoculation of Lettuce (*Lactuca sativa* L.) with Arbuscular Mycorrhizal Fungi (HMA) using Inorganic Matrix

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The soil ecosystem functioning largely depends on soil microbial activity. Arbuscular mycorrhizal fungi (AMF) are one of the most important beneficial microorganism associated with most of the vegetable species which improves nutrition and growth of host plant. In Chile, the area cultivated with vegetables is around 95,000 ha where lettuce occupies an area of 7,027 ha (VII Censo Nacional Agropecuario y Forestal, 2007). The objective of this work was to study the effect of inoculation with three AMF strains using an inorganic matrix and

lettuce type Roman cv. Blonde Maraîchère as host plant. Lettuce seeding was carried out in August, transplantation at the end of September and harvest in December 2007. Treatments included four inorganic matrixes: sand (S-1), perlite (S-2), vermiculite (S-3) and a mix of S-1, S-2 and S-3, (S-4); three AMF strains: *Glomus intraradices* (I-1), *Glomus claroideum* (I-2), *Glomus etunicatum* (I-3) and uninoculated control (I-4) with six replications, in a completely at random statistical design. The amount of inoculum accounted for the equivalent of 25 mL. The nursery stage covered was until 44 days after sowing (DDS) and harvest was at 90 DDS. Colonization AMF was quantified by staining the roots with tripan blue and observation under stereoscopic microscope. From 30 DDS, the number and breadth of leaves were weekly measured and chlorophyll measurements were carried out with a Minolta SPAD-502 in the first leaves avoiding aged leaves. After harvesting, dry weight of plants and leaves concentration of macronutrients Ca, Mg, K, P and Na were measured. During the cultivation, the visual appearance of inoculated plants was better than uninoculated being the former ones greater vigour, size and intensive green colour. The largest root colonization was reached with I-1 and I-2 with S-2, with a higher percentage (30%), introducing significant differences with control. However, when vermiculite (S-3) was used as matrix the highest colonization was observed in uninoculated plants due to native strains and coinciding with the highest dry weight obtained. The smallest dry weight was obtained when using S-4 as substrate and the inocula I-1, and I-3. The weight differences observed were mainly produced by the substrate rather than by inoculation, probably due to the short cycle of lettuce, lasting three months between planting and harvesting. According to the results obtained it is concluded that lettuce is a vegetable mycorrhization dependent and their performance depends on the AMF inoculum and the inorganic matrix used.

Keywords: Lettuce; arbuscular mycorrhizal fungi; inoculation.

S2-P7

Implications of the “Green Rust” on the Iron-Phosphorus Relationships in Flooded Soils of the Lower Orinoco River Venezuela

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Intermittent flooding and draining of soils in floodplain result in considerable temporal variability of soil oxygen. Under saturated soil conditions, water restricts gas diffusion and limits the soil oxygen availability. Under anaerobic soil condition biogenerated Fe(II) can experience secondary chemical reactions, which lead to the formation of aqueous complexes and/or the precipitation of ferrous solids as green rusts (GR) compounds $[\text{Fe}^{\text{II}}_{(1-x)} \text{Fe}^{\text{III}}_x (\text{OH})_2]^{x+} [(x/n)\text{A}^{n-}(m/n)\text{H}_2\text{O}]^x$. In an earlier laboratory research with soil samples collected from a seasonal flooded forest in the lower Orinoco River, we hypothesized that anaerobic soil condition leads to the formation of the green rust compounds. In the field when the soils are exposed to the air, just at the end of the aquatic phase, we have detected dispersed spots of a blue-green colour, which we have associated with precipitation of GR compounds. In this study we examined the soil P distribution and soluble Fe^{2+} content in ten samples collected from the GR spots and the adjacent soil as a control (C). Samples were taken at the end of the flood event in November 2006, following a completely random design. In both GR

and C samples, fine roots (< 2mm) were separated by manual extraction and the concentration of Fe^{2+} and total P was also determined. The results showed that available (resin-Pi), moderately available (NaOH-Pi) and occluded (HCl-, and residual-Pi) inorganic phosphorus (Pi) fractions were significantly higher ($P < 0.05$) in GR than in C samples. A similar trend to that of the Pi fractions was obtained in the different organic P (Po) pools (NaHCO_3 -, HCl- and NaOH- Po). Soluble Fe^{2+} in the C samples was well below to that obtained in GR. Fine roots extracted from the GR showed the higher soluble Fe^{2+} and total P. In this study we proposed that GR precipitation in the flooded soils of the lower Orinoco provide a small-scale spatial variability in the Fe cycle, which significantly increase the soil P bioavailability to plants growing in floodplains zones.

Keywords: Green rust; flooded soils; iron-phosphorus relationships.

S2-P8

Arbuscular Mycorrhizal Fungi in *Atriplex argentina* (Chenopodiaceae) from Saline Soils of Córdoba Province (Argentina)

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Plant root and arbuscular mycorrhizal fungi (AMF) symbiosis is one of many plant strategies in order to grow under a variety of stress conditions. The distribution of certain AMF has been related to different soil conditions (pH, P level, salinity, disturbance, etc). AMF in saline soil may improve plant salinity tolerance and growth. *Atriplex argentina* (Chenopodiaceae), a common native shrub of arid and semiarid zones of Argentina, is valued as a soil stabilizer and as palatable and nutritious forage for many herbivores. The aim of the present work was to study the relationship between seasonal changes in AMF root colonization and AMF in saline soils of Salinas Grandes (Córdoba, Argentina). Soil samples were collected from San José de Las Salinas and Árbol Blanco, Córdoba Province, in autumn and summer (1999). The AM colonization and AM fungal spores were quantified and identified respectively. Arbuscules, intercellular vesicles and inter- and intra-cellular hyphae were observed in *A. argentina* roots. The percentage of colonized root length differed between seasons, being higher in summer, but did not differ between study sites. The AMF most frequently found in the rhizosphere were species of the *Acaulospora* and *Glomus*. The data presented here provide useful information for possible reclamation of degraded areas with *A. argentina*.

Keywords: Arbuscular mycorrhizal, Salinity, *Atriplex*.

S2-P9**Growth of Peas *Pisum sativum* L. in the Presence of Diesel and Bacteria Consortia in Peat and Sandy Soils****L. Dubova^{1*}, O. Muter², I. Alsina¹, V. Steinberga¹, D. Zariņa², B. Līmane² and A. Mihailova²**¹Latvia University of Agriculture, 2 Liela Str., Jelgava LV-3001, Latvia. ²Institute of Microbiology and Biotechnology, University of Latvia, 4 Kronvalda Blvd., Riga LV-1586, Latvia.

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Remediation of contaminated soils, using plant-based systems, is known as a very perspective and rapidly developing area of biotechnology. Selective introduction of microorganisms with specific or broad-ranging biodegrading properties into a plant rhizosphere may accelerate remediation of soils polluted with organic chemicals. In this work, the results of the green-house experiments with soils artificially contaminated by diesel are presented. The seeds of peas were sown into peat and sandy soils amended with 1% and 3% of diesel, as well as inoculum of bacteria association, which was previously isolated from oil-contaminated sites. After 85 days, when peas were entering the bloom stage in the control samples, plant biomass was harvested. The presence of 1% diesel in sandy soil totally inhibited the growth of peas, although in peat soil an inhibition effect was about 50 %. Addition of bacteria inoculum did not influence this effect. Harvested biomass was also tested for moist and dry weight, height, content of chlorophyll a and b. Comparison of soil physico-chemical and biochemical properties after 85 days vegetation experiment with peas showed that the noticeable changes between soils exposed by diesel and without it were occurred. Thus, dry weight of peat soil without diesel was higher almost twice, as compared to the samples with diesel, i.e. 66 % and 34 %, correspondingly. The pH level of peat and sandy soil was increased in the diesel-containing samples and varied in the range of 4.6 - 5.8 for peat and 6.1-7.5 for sandy soil. Redox potential varied in the range of +49 to +117 mV for peat and +6 to -39 mV for sandy soil. The total microbial count in rhizosphere was 10-100 times higher than in average soil sample. The number of colony forming units on the selective medium with diesel was higher in the samples inoculated with bacteria association. This effect was more pronounced in the samples with sandy soil. Soil was tested also for its fermentative activity, i.e. urease, FDA, and dehydrogenase activity, as well respiration, which provided more complex soil characteristics on biochemical level. Toxicity study was performed using germination test. Summarizing, these results provided the additional information on plant response to diesel contamination in the context of toxicity study and perspectives for phytoremediation. Environmental protection by using nature's own resources would be both economically and environmentally beneficial.

Keywords: *Pisum sativum* L.; enzymatic activities; Sandy Soils.

S2-P10**Responsiveness To Arbuscular Mycorrhizal Fungi Of Seven Woody Species With Different Life Histories, Belonging To A Tropical Forests Cloudy In Miranda State, Venezuela****V.C. Flores^{1,2*} and G. Cuenca¹**¹Centro de Ecología, Instituto Venezolano de Investigaciones Científicas (IVIC), Venezuela.²Departamento de Ingeniería Ambiental, Universidad Nacional Experimental del Táchira (UNET), Venezuela.

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To assess the growth and responsiveness on arbuscular mycorrhizal fungi of pioneer species *Oyedaea verbesinoides*, *Heliocarpus americanus*, the intermediate *Palicourea fendleri*, *Vismia ferruginea* and *Hyeronima moritziana* and late *Richeria grandis* and *Guapira olfersiana*, germinated seeds, then seedlings were transplanted to plastic pots filled with sterile soil, apply four treatments: inoculated with the fungus *Glomus manihotis*, inoculation and fertilization triple superphosphate (TSP), plants with TSP as a reference and untreated controls. The mycorrhizal colonization was 84 and 63 and 34% for the pioneer species, intermediate and late respectively. Also, Responsiveness resulted in 0.9, 0.57 and 0.13 for pioneers, mid and late respectively, showing a response contrasting these species. In the pioneers the effect of mycorrhizal was evident in higher values for growth, the root to shoot dry matter ratio, root length and total absorption of specific nutrients, which will be enhanced in the presence of fertilizer, in the case of intermediate and late these values were low. In conclusion, it demonstrates the highly mycorrhizal status of pioneer species and the need to take into account these fungi to recover degraded areas.

Keywords: Arbuscular mycorrhizal fungi; woody species; tropical forests**S2-P11****Seasonal Changes in the Activity of Soil Enzymes of a Flooded Tropical Forest Gradient in the Lower Orinoco River, Venezuela****S. Flores*, N. Chacón and M. Rangel**

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Soil enzymes play an important role in the decomposition of the organic matter and nutrient cycling. The effects of the natural hydrological fluctuations on the activity of acid phosphatase, urease and deshydrogenase were studied along a flooded tropical forest gradient in Mapiro River, Venezuela. Soil samples were collected following a complete natural hydroperiod: end of the dry season (May 2004), end of the rainy season (November 2004) and end of the subsequent dry season (May 2005), and from three zones subjected to different flooding intensities: MAX, MED and MIN zones inundated for 8, 5 and 2 months per year respectively. Soil fresh of each zone and during the three collection periods, were incubated at 28°C and during 12 weeks. Throughout the collection dates, the results showed a decrease in the activity of the three enzymes from the MAX to the MIN zone, following the pattern of organic carbon of these soils. Regarding the seasonality, the three enzymes showed different patterns: Urease and deshydrogenase showed a peak in November 2004 while the acid phosphatase decreased. The increase in deshydrogenase and urease activity in November-2004 was expected considering that flooding brings with it a great contribution of labile organic matter, which with the fall of the water begins to be mineralized. At

the event of flooding are also associated processes of mineral dissolution which promotes the release of nutrients such as phosphorus (P). Therefore, a decline in the activity of acid phosphatase after the flood is logical if we consider that the P is being supplied from the geochemical pool.

Keywords: Soil enzymes; tropical forest; hydrological fluctuations.

S2-P12

Application of Bovine Dung, Yield and Extraction of Nutrients in Three Varieties of Alfalfa

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In the Lagoon Region approximately 39.259 are seeded has of alfalfa. This forage is an important component in the food rations of the milk bovine cattle, since it is a forage of protein high performance (19.5% PC). Reason why to look the suitable doses but of any organic product that is applied to the ground; he is determining in the agricultural production, protection and/or diminution of the contamination of the environment, etc. Logically to find the dose but suitable of dung in this case for the region it is extremely important, dice the different types from ground that exist, Reason why the objective of the work was to obtain the optimal dose of organic installment and the variety that more good interacts to increase the yield and quality of forage in alfalfa. The experiment was performed in the year of 2003-2004, in the locality of Ash of the North, Coahuila. They were evaluated, a treatment witness with chemical fertilizer (30-100-00) and five dung levels: 0, 40, 80, 120 and 160 ton ha⁻¹, using an experimental design of blocks at random with three repetitions. I am carried out the measurement of the nutrimental dynamics of materials CUF 101, Sundor and Altaverde, by means of samplings of plant to determine the extraction by the culture. Also, ground samplings and bromatológico were realized. Also forage yield was evaluated by cuts .y the answer of the applied dung. The green forage production not thus showed statistical differences for the dung treatments for varieties. Occurring the highest productions in cuts 7, 6 and 5, with productions of up to 18.7 ton ha⁻¹ for the Altaverde variety in cut seven, 18.26 and 18.27 ton ha⁻¹ for the Altaverde varieties and CUF 101 in 16.95 cut six and ton has-1 for the Altaverde variety in cut five. With respect to dung treatments, in cut seven it excelled the B5 treatment (160 ton Have of dung) with 20.9 ton of forage, followed of the B3 treatment (80 ton Have of dung) with 19.8 ton of forage; in the cut the six treatments B5 and B3 behaved equal with 20.3 ton ha⁻¹ of forage; in cut five it was the B4 treatment (120 ton of dung per hectare) but the excellent one with 20.5 ton ha⁻¹ of forage, with a slight increase in the production in the Altaverde variety. In regard to the dung the best treatments were those of 80, 120 and 160 ton ha⁻¹ with a production of 19.8, 19.1 and 20.7 ton ha⁻¹ respectively of fresh forage. The nitrogen extraction by the plant was around 180 kg ha⁻¹ in the dung treatments and of 130 kg ha⁻¹ in the chemical fertilizer treatment and the quality of forage was better in Altaverde genotype.

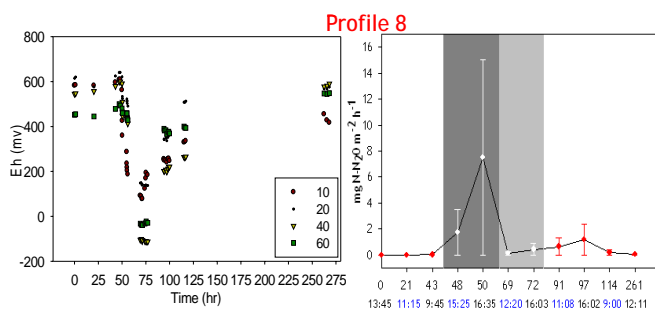
Keywords: Bovine dung; yield; alfalfa.

S2-P13

N₂O Emissions from Soil Irrigated with Untreated Wastewater in Central México**B. González-Méndez^{1*}, C. Siebe¹, S. Fiedler², J.M. Hernández³ and L.G. Ruiz-Suárez³**¹*Instituto de Geología, UNAM, México.* ²*Institut fuer Bodenkunde und Standortslehre, Univ. Hohenheim, Alemania.* ³*Centro de Ciencias de la Atmósfera, UNAM. México.*

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Nitrous oxide is a “greenhouse gas”, and it destroys the stratospheric ozone layer. Approximately 65% of the global N₂O emissions are emitted from agricultural fields (Huang, et al., 2004), and therefore this gas is considered within the Kyoto-protocol. Nitrous oxide is produced mainly during denitrification which occurs under anaerobic conditions and is the progressive reduction of nitrate (NO₃⁻) to nitrogen (N₂). In the field emissions occur in so called hot spots. The most important variables that regulate N₂O emissions from soils are the soil moisture, the soil temperature, the pH, and the availability of nitrogen and labile organic carbon (Huang *et al.*, 2004). Since reduction processes are more probable to occur in poorly drained and aerated soils, the redox potential is often determined to indicate the degree of aeration in the soil (Tan, 1998). This investigation was done in the Valle del Mezquital, State of Hidalgo, Central Mexico, where wastewater from the metropolitan area of Mexico-city is used to irrigate agricultural land since 1912. The objective of this study was to measure the N₂O emissions from a plot. This was achieved by simultaneous quantification of N₂O fluxes and redox potential



measurements before, during and after an irrigation event. The measurements were done in an alfalfa field of 5 ha. The field is irrigated by overland flow with untreated wastewater every 28 days. We installed 4 sampling sites within the field; two of them on vertic Phaeozems, and two on the lower flat part of the field with pellic Vertisols.

At each sampling point a soil profile was dug in which we installed in total 10 Pt electrodes at different depths. N₂O emissions were measured by the static chamber method (Teira, 1998). Statistical analyses were performed with the STATISTICA 6.0 software. Before irrigation (figure below), all soils had oxidic conditions with mean Eh values of 400 to 650 mV and very small N₂O emissions (0.01-0.04 mg N-N₂O m⁻² h⁻¹) occurred. During irrigation redox potentials (RP) decrease (-100 to 500 mV) and anoxic and suboxic conditions prevail (~60 to 400 mV) for the following two days; in this period N₂O is emitted reaching up to 3.42 mg N-N₂O m⁻² h⁻¹ in one of the Phaeozem subplots. As wastewater infiltrates and the top soil starts to dry out, a transition from suboxic to oxidic conditions (300 to 600 mV) is observed and the N₂O emissions decrease until the initial flux values are reached again. The N₂O emissions measured in this plot are comparable with the N₂O fluxes published for fertilized arable soils. Teira (1998), reported fluxes ranging between -0.166 to 1.620 mg N-N₂O m⁻² h⁻¹ for a clay rich agricultural soil.

Keywords: Nitrogen emissions; wastewater; soil irrigated.

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S2-P14**Carbon and Nitrogen Pools in Soil Physical Fractions Following Land Use Changes from Native Forest to Hoop Pine Plantation in Subtropical Australia****Y. He^{1,2*}, J. Xu¹ and Z. Xu²**

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Soil physical structure causes differential accessibility of soil organic matter (SOM), including carbon (C) and nitrogen (N) pools, to decomposer organisms and is an important determinant of SOM storage. Physical fractionation method of SOM in conjunction with elemental as well as isotopic analyses (C, N, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of those soil fractions are used in this study to determine the land use and management induced changes of SOM and associated processes in three adjacent sites of native forest (NF), first (1R) and second rotation (2R, including tree planting row (2R-T) and windrow of harvest residues (2R-W)) of hoop pine plantations in southeast Queensland, Australia. The results suggest that there is a greater accumulation of C and N in the light fraction (LF) of NF site than that of plantation sites (1R and 2R), and the C and N losses following conversion from mixed species NF to the single-species plantation are attributed to the reduction in C and N stocks for all physical fractions separated by wet sieving. In contrast, the C and N losses induced by the rotation practices (e.g., between 1R and 2R-T) are attributed to the C and N decreases in the LF and macroaggregates (250-2000 μm) only. The C and N increases upon the residue management (e.g., between 2R-W and 2R-T) are primarily attributed to the C and N increases in the LF and macroaggregates as well. Coupled with 30 soil chemical and biological parameters obtained in our previous studies, further principal component and multivariable regression analyses were conducted and the results showed that both the LF and macroaggregates were highly related to the status of C and N pools, the processes of N transformation and soil respiration, and the diversity of microbial communities, and thus could serve as diagnostic SOM fractions responsible for the changes in soil properties and processes induced by the land uses and management practices. Knowledge of the interactive relationships between aggregate classes within SOM and soil chemical and biological processes in this study represents a further step towards diagnostically measuring and understanding important soil C and N processes in response to the land use and management changes in the soil ecosystems such as forests in subtropical Australia.

Keywords: Carbon and nitrogen pool; native forest; pine plantation.

S2-P15**Current Information on Phytase-Producing Bacteria****M. Jorquera^{1*}, O. Martínez¹, P. Marschner², F. Maruyama³ and M.L. Mora¹**

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During last two decades there has been an increase in the biotechnological applications of microbial phytases in various fields, such as animal feed, processing and manufacturing of

food for human consumption, environmental protection, aquaculture and agriculture. Phytases are group of enzymes capable of releasing phosphate from phytate and they can be found in many organisms (plants, animals and microorganisms). Phytate is by far the most prevalent form of organic P in soils. Thus, phytase-producing bacteria (PPB) have attracted the attention of microbiologists and agriculturists as inoculants to improve the phosphorus-uptake by plants. The present study summarizes the current information on phytase-producing bacteria contained in public databases, such as National Center of Biotechnology Information (NCBI) and Swiss-Prot. The analyses of literature showed that phytases have been detected in a wide variety of bacteria, such as bacilli, enterobacteria, anaerobic rumen bacteria and pseudomonas. The analysis of protein sequences revealed that phytase-like proteins are found in a great diversity of bacterial groups, particularly in γ -proteobacteria. Based on amino acids sequences, phylogenetic analyses of representative phytase-like proteins into γ -proteobacteria shows clustering of the three classes of phytases (cysteine phytases, histidine acid phosphatases and β -propeller phytases) reported. The importance of bacterial phytases and PPB as potential biotechnological tools has been recognized in various fields. However, this study shows that only a limited number of bacterial phytases have been reported and studied, and our knowledge on mechanisms and factor regulating phytase activity is limited. The use of PPB for improving plant nutrition in the field also represents a promising potential for agriculture, although major efforts will be required to refine and implement this technology prior to its becoming economically viable.

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Keywords: Bacterial phytase, soil, bioinformatic.

S2-P16

Influence of the Temperature on Urease Activity in Andisols Fertilized with Urea

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Nitrogen (N) is an essential element for plant nutrition and is one of the most important factors that limits crop and pasture yields. In the soil-plant system, N availability mainly depends on the mineralization of organic N into ammonium (NH_4^+ -N) by urease enzymes from soil organic matter, organic residues and urea-based fertilizers. Thus, the changes in the urease activity can be used as an indicator indirect of the variation in the pool of potentially available N in a soil. The aim of this study was evaluate the effect of time, temperature and urea supply level on the urease activity in two Andisols. In order to evaluate the effect of soil temperature, duplicate 100 g soil samples of each soil [(Piedras Negras soil (PNS) and Freire soil (FS)] were treated with urea at rates equivalent to field applications of 0, 100, 200, 400 and 800 kg N ha⁻¹. The samples were incubated in plastic bags at 5, 10 and 21°C under aerobic conditions, and subsamples were taken at 24 and 48 h to determine the urease activity. To assay the urease activity a modified method of Nannipieri *et al.* (1980) was used. Soil sub-samples of 1 g were incubated with 4 mL of 0.1 M phosphate buffer (pH 7.1) and 1 mL of 1.067 M urea at 5, 10 and 21°C by 2 h, and after

incubation 5 mL of 2 M KCl were added to terminate the reaction. The N-NH_4^+ was determined by ion selective electrode. In both Andisols, the urease activity were fitted to a simple Michaelis-Menten kinetics and Michaelis-Menten constant (K_m) and the maximum enzyme reaction velocity (V_{\max}) were determined. Comparatively, PNS registered a higher urease activity than FS at 24 and 48 h of soil incubation. Although FS showed pH values slightly superior than those of PNS, the highest organic matter content of PNS (220 g OM kg^{-1}) may explain the greatest activation of the urease enzyme in this soil with respect to FS (170 g OM kg^{-1}) at all the temperatures. A differential activation of the urease was also observed by effect of the incubation time in each soil. Thus, the urease activity was raised in PNS as the incubation time increased from 24 to 48 h. In FS, an inhibition of urease occurred after 48h at 10 and 21°C, whereas at 5°C the enzyme activity increased. Likewise, urease enzyme was activated when the temperature increased from 5 to 21°C in both soils, but at 48 h of soil incubation the opposite behavior was observed in FS. In fact, the K_m decreased and V_{\max} increased with the increase of temperature in PNS and FS at 24 h and in PNS at 48 h of incubation. Furthermore, at 24 and 48 h of PNS incubation, the catalytic efficiency of the enzyme reaction (V_{\max}/K_m) increased 56.1 and 29.4%, respectively, with the increase of temperature from 5 to 21°C. In FS, the V_{\max}/K_m ratio increased 3.2 times at the highest temperature after 24 h of incubation, whereas it was reduced in 44.1% later. The decrease of both the urease activity and the V_{\max}/K_m ratio at 48 h of FS incubation suggest that most of applied urea was hydrolyzed before 24 h at 10 and 21°C. In contrast, the activation of urease and the slight changes in catalytic efficiency in PNS by effect of the temperature and incubation time indicates that the urea concentration was not a limiting factor for the enzyme activity, and denotes a slower urea hydrolysis in this soil compared with FS.

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Keywords: Nitrogen, urease activity, Andisols.

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S2-P17

The Possible Role of Bacteria Containing Bio-fertilizers in Sustainable Agriculture

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All operation and method, which try to reduce the environmental pollutions have and will have a main role in our life. Environmental protection is getting more important for the agrarian, because of the purpose of sustainable agriculture. Through the reduction of chemicals in agriculture can be provided the sustainability of surface waters. Bio-fertilizers containing less artificial compounds and plant growth promoting bacteria are good tools to reduce environmental damages and enhance the yield. The test plants were the sugar beet (*Beta vulgaris* L. sv. *sacharina* cv. *Bounty*) and the cucumber (*Cucumis sativus* L. cv. *Delicates*) We used the methods and materials written elsewhere (Lévai and Kovács, 2000). The bio-fertilizer was the bacteria containing Phylazonit MC®. The fresh weight of roots increased up to 20 % when bio-fertilizer was applied. The uptake of nutrients increased. The Phylazonit treatment modified the toxicity of Aluminium (Al), supposedly by the increasing uptake of elements responsible for the defense mechanisms in the plants. The mineralization

of wheat and sunflower stems was more intensive. The amounts of available P and K nutrients were higher, while the amount of N remained around at the same level in the soil, near the stems. The root fresh weight and the sugar contents were higher in the field experiment, measured in June. The PHYLAZONIT MC[®] treatment equalized the effect of 80 kg/ha nitrogen fertilization in the field experiments. The contents of „harmful” nitrogen, and other compounds in the sugar-beet were smaller than in the non treated-plants, which is advantageous for industrial processes. The extra yield, calculated in sugar, was: 2,6 t/ha. The application of PHYLAZONIT MC[®] increased the yields in the laboratory and in the field experiments. The physiological basis is: an increased chlorophyll synthesis, a more intensive uptake of nutrients causes a more intensive growth of leaves. Due to the more active bacterial soil life the level of several organic compounds, as malic acid, citric acid, and siderophores are higher, that makes the solubility and availability of nutrients easier in the rizosphere. We observed a moderate effect of Al-toxicity when PHYLAZONIT MC[®] was applied. Supposedly the complex formation of released organic acids by the bacteria prevents the uptake of Al, even under Al-stress. We came to the conclusion, that the PHYLAZONIT MC[®] is an alternative for replacing chemical fertilizers with a biologically active, environmentally protective agent.

Keywords: bio-fertilizer; ion-uptake; Al-tolerance.

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S2-P18

Impact of the Biodegradation of Bovine Manure in the Alfalfa Production

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This study had as general objective to determine the nutrimental necessities of alfalfa forage under several environmental conditions. The main agricultural problem in the Comarca Lagunera Region is That farmer do not have information about nutrient uptake for alfalfa forage and their effect on forage production. The alfalfa is the most important forage for cow milk production, and income for farmers. The experiments were established in three locations of Ejido Fresno del Norte Coahuila, Venecia and Horizonte Durango, respectively. The tow factors were studied alfalfa varieties as factor one in three levels, CUF 1001, Sundor and Alta Verde and the factor B had six levels: 0, 40, 80, 120, 160 ton ha⁻¹ of cow manure with one additional level of chemical fertilizer (30-100-00). The combination among factors gave 12 treatments which were established in a complete randomized block design with split block arrangement replicated three times. Plant and soil parameter were measured each harvest period to compare treatments such as dry and soil temperature and moist, respectively. Results indicated a low increment in a forage dry and wet production in Sundor variety in tree harvesting times. With respect to cow manure with a wet forage yields of 19.8, 19.1, and 20.7 ton ha⁻¹ at seven yield of forage respectively. The nitrogen uptake by plant was in average in chemical fertilizer and the control treatment. The soil temperature varies in average from 25 to 32°C in spring and from 15 to 19°C in winter this condition in part

explains the low yield in winter. As a main conclusion according to the results, a cow manure application at the beginning of alfalfa forage varies from 80-160 ton ha⁻¹ with only one application of cow manure.

Keywords: Manure; alfalfa; biodegradation.

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S2-P19

Management Strategies and Practices for Preventing Nutrient Deficiencies in Organic Crop Production

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In organic farming, synthetic fertilizers/chemicals are not applied to increase crop production. In the Canadian Prairie Provinces, most soils under organically farmed systems are deficient in available N for optimum yield. There are many organically farmed soils low in available P, and some soils contain insufficient amounts of S and K for high crop yields. The N deficiency in soil on organic farms can be corrected by growing N-fixing legume crops in the rotations, but if soils are deficient in available P, K, S or other essential nutrients, the only alternative is to use external sources to prevent their deficiencies. Field experiments are underway in Canada to determine the influence of management practices (crop diversification with deep taproot and shallow fibrous root crops, crop residue, green manure, legumes for seed/forage, cereal-legume intercropping) and amendments (*Penicillium bilaiae*, rock phosphate, elemental S, gypsum, compost manure, wood ash, alfalfa pellets) on crop yield. In the cropping systems study established in 1995, crop yields for organic system without any chemical input were 30-40% lower than the conventional system with high inputs. But, lower input costs plus price premiums for organic produce normally more than offset lower yields, resulting in favorable economic performance and energy efficiency. Legume, green manure, compost manure helped to replace nutrients lacking in the soil and improved crop yields. In the organic system, amount of P removed in crop exceeded that of P replaced and this can be a major yield limiting factor. In amendments experiments, there was small effect of granular rock phosphate fertilizer and/or *Penicillium bilaiae* in increasing soil P level and crop yield in the application year. Other findings suggested the use of elemental S fertilizer, gypsum, compost manure, wood ash or alfalfa pellets to improve nutrient availability, and yield and quality of produce. In conclusion, the findings suggest that integrated use of management practices and amendments has the potential to increase sustainability of crop production as well as improve soil quality plus minimize environmental damage.

Keywords: Organic crop; compost; *Penicillium bilaiae*.

S2-P20**The Enzymatic and Abiotic Hydrolysis of Glucose-1-Phosphate Adsorbed on Goethite: An ATR Infrared Spectroscopy *in situ* Study****R. Olsson^{1*}, R. Giesler² and P. Persson¹**¹Department of Chemistry, Umeå University, 901 87 Umeå, Sweden. ²Climate Impacts Research Centre, Department of Ecology and Environmental Science, Umeå University, Box 62, 981 07 Abisko, Sweden. *E-mail: Rickard.Olsson@chem.umu.se

In order to make phosphorus in organophosphates bioavailable, hydrolysis is usually a required step. This process may be catalyzed by exoenzymes present in soil. However, organophosphates have due to their phosphate moiety a strong affinity for environmental particles, which may affect their biodegradation. In this study the hydrolytic reaction is investigated with focus on a water-mineral interface at which adsorption of glucose-1-phosphate occurs. Glucose-1-phosphate has been used in bioassays as a model substrate and is found to be equally available as orthophosphate. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR FTIR) is a technique which allows *in situ* measurements of surface reactions occurring on e.g. metal oxide particles in aqueous solutions. In the present work ATR FTIR is used to monitor the enzymatic hydrolysis by a phosphatase from sweet potato of glucose-1-phosphate adsorbed at the water-goethite interface. Substrate desorption is investigated using the same molecular-level technique. The kinetics of hydrolysis and desorption, and the stability of the adsorbed glucose-1-phosphate in absence of enzyme will be discussed. The spectroscopic data will be presented together with results from quantitative measurements of levels of substrate and hydrolytic products, showing that abiotic hydrolysis due to surface sorption on goethite is not likely the reason to the bioavailability of glucose-1-phosphate.

Keywords: organophosphates; exoenzymes; ATR Infrared Spectroscopy.

S2-P21**Biological Fertility of an Intensive Farming Soil Affected by Various Management Practices****M.A. Rao^{1*}, R. D'Ascoli², R. Scelza¹, R. Marzaioli², F. Rutigliano² and L. Gianfreda¹**¹Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples Federico II, Portici, Italy. ²Department of Environmental Sciences, Second University of Naples, Caserta, Italy. *E-mail: mariarao@unina.it

In areas with intensive agricultural productions soil fertility can be depressed by practices addressed to optimize the crop and not attentive to store physical, chemical and biological fertility in long term. The preferential use of mineral fertilizers to the detriment of organic amendments leads to a decrease in soil productivity and plant growth. The depletion of the organic carbon content is a common response in these soils and a very strictly relationship with biological properties can be observed. Soil enzymes and microbial biomass are sensitive to the presence of organic matter and they are largely known as valid indicators of soil biological fertility. The aim of this work was to study the effect of organic fertilizations on some enzymatic activities (dehydrogenase, arylsulphatase, β -glucosidase, phosphatase, urease) and microbial biomass of an agricultural soil under crop rotation, sited in Campania

Region (Italy). At the beginning of the experimental plan, compost from vegetable residues, ricin seed exhaust and straw were supplied singly or in association on plots treated or not with a preventive sterilization by solarization and calcium cyanamide to control soilborne pathogens and weeds. The physical, chemical and biological analysis of the agricultural soil were carried out after the initial treatment (September 2005), after the first crop, lettuce (December 2005), and after pepper and lettuce rotation (January 2007). All plots amended with compost, and especially those treated with all three organic materials, showed higher contents of organic carbon and total nitrogen, correspondingly higher values of enzymatic activities and microbial biomass were detected. Conversely, solarization and calcium cyanamide depressed biochemical and biological parameters. At the last sampling amended plots showed a decrease of organic carbon content to the initial level registered in the control plot to indicate that the organic matter supplied by this compost or ricin seed exhaust was not stable and did not ensure a durable nutrient source.

Keywords: Biological fertility; soil enzymes; microbial biomass.

S2-P22

Microbial Mobilization of Mineral Phosphates Co-inoculated with Diazotrophic Bacteria on the Yield of the Pasture *Brachiaria decumbens* Stapf.

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The bioavailability of N and P in soils plays a fundamental role in the sustainable management of a pasture yield. The present research evaluates two microbiological processes: the mineral phosphate bioconversion by phosphate solubilizing microorganisms and the increase of nitrogen uptake by nitrogen fixing bacteria. A low P acidic soil was amended with the Venezuelan mineral phosphate (MP) of Monte Fresco; this mineral was used as an insoluble inorganic phosphate fertilizer. The phosphate solubilizer fungus *Penicillium rugulosum*, strain IR-94MF1, was evaluated in a randomized greenhouse experiment in conjunction with different diazotrophic rhizobacteria. These rhizobacteria include the following: *Enterobacter cloacae*, *Azospirillum amazonensis*, *Rhizobium* sp., and two unknown diazotrophic Gram-negative strains. The effect of these consortia was evaluated on the growth of the tropical pasture *Brachiaria decumbens* Stapf. The diazotrophic strain, which showed higher potential based on the dry matter yield of shoots and their P and N contents, was selected for the use of a field experiment. The microbial treatments were as follows: an uninoculated control, *P. rugulosum* strain IR-94MF1 alone and *P. rugulosum* strain IR-94MF1 co-inoculated with the rhizobacteria *E. cloacae* strain 17. The mineral phosphate was applied at a rate of 0 (control) and 750 kg ha⁻¹ using a bifactorial analysis of variance with repeated measurements over time. The study was performed during dry and rainy seasons in an established pasture of *B. decumbens*. The results for dry matter yield, %N and %P were evaluated for five cuts during seven months. If compared to the uninoculated controls, the inoculated treatments with and without the application of the MP, showed significant increases ($P < 0.05$) of the accumulated dry matter yield, and N and P contents based on a measurement of Kg/ha/7months. The application of the fungus with the nitrogen-fixing bacteria did not show significant statistical differences with respect to the productivity of *B. decumbens*. However, without the application of the MP, *E. cloacae* showed a positive tendency of 15% for the

accumulation of N in Kg ha^{-1} . It seems that both microorganisms, isolated from the Monte Fresco phosphate mine may function in a synergistic way when they are inoculated in a less rich nutrient rhizosphere. However, after the application of the MP, this positive tendency was reversed; the fungus MP solubilization capacity might activate native nitrogen fixing microflora allowing for better assimilation of the nutrients by the pasture. Moreover, the simple inoculation of the fungus *P. rugulosum* strain IR-94MF1 fulfilled the bioconversion of the MP resulting in an increase of 48% P in the shoots and 36% for the P accumulated relative agronomic efficiencies. Indeed, these bioinoculants could be considered as a sustainable biotechnological option, which could increase the nutritional value of *B. decumbens* pasture.

Keywords: *Penicillium rugulosum*; *Enterobacter cloacae*; mineral phosphate.

S2-P23

Use of Indigenous N₂-Fixing Soil Cyano-Bacteria for Low-Input, Sustainable Improvement of Overall Fertility of Degraded Lands in Semiarid Tropics

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For a sustainable improvement of both, biogeochemical performance and physical fertility of degraded soils in marginal lands of three Southern Africa Countries (Tanzania, Zimbabwe and South Africa), an holistic approach was adopted to assess the possibility of locally growing and applying to soil, of biomasses of selected strains of indigenous cyanobacteria. Some strains of these photosynthetic, autotrophic micro-organisms are able to fix atmospheric Nitrogen, and to produce exo-polysaccharides (EPS), capable to bind soil mineral particles, thus increasing aggregates stability. The main results of the investigations undertaken can be summarized as follows:

a) *Cyanobacteria identification and selection.* A large number of cyanobacteria strains (about 200) have been identified. Some of these strains were found able to fix considerable amounts of atmospheric Nitrogen and to produce abundant EPS. The most productive indigenous strains for each Country were successfully selected, purified and grown with suitable procedures and techniques specifically adapted to obtain large amounts of biomass, even under stress. These biomasses were used for laboratory and field studies and experiments aimed to increase permanently the overall fertility and productivity of degraded soils.

b) *Effects on chemical fertility.* Laboratory, greenhouse and field experiments demonstrated that cyanobacteria were capable to grow even under limiting conditions (low nutrients availability, low pH). In spite the metabolic cyanobacteria activity decreases the NO_3^- and NH_4^+ concentration, as they use these forms of N to grow, the overall soil C and N content increased. In soils with low nutrients content, cyanobacteria increased the amount of total and available phosphate, potassium, calcium and magnesium and also the concentration of micronutrients. In soils with higher inherent fertility in which cyanobacteria developed to larger extent, a decreased concentration of available macro- and micronutrients was found, due to sorption on the negative charged surface of the EPS produced by these micro-organisms. Moreover, cyanobacteria modify the SOM characteristics and increase the more stable humic fraction.

c) *Effects on physical fertility.* The results of laboratory, greenhouse, and preliminary field experiments showed an increase of aggregates stability due to cyanobacteria inoculation on poorly structured soils. This improvement appeared shortly after inoculation and increased with time and growth of cyanobacteria biomass. The increased stability is accompanied by changes of soils surface microstructure and formation of organo-mineral aggregates.

d) *Effects on crop growth.* Greenhouse experiments with rice and maize showed that inoculation with cyanobacteria had limited effect on the first crop, but the yield of the subsequent crop increased significantly. The effect of cyanobacteria on crop growth varied with soil types and this depended on the different extents of cyanobacteria establishment. Preliminary, short-term field experiments seem to confirm the beneficial effect of cyanobacteria on improving crops yield.

Keywords: Cyano-Bacteria; exo-polysaccharides; nitrogen.

S2-P24

Mineralization of Cow Manure and Their Impact in Production and Quality of Corn Forage under Subsurface Irrigation System

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At the present time the excessive chemical fertilizer use and the little use of organic amendments have brought with the deterioration of the physical, chemical and biological properties of the ground. On the other hand little technology has been generated to use this valuable remainder of the cattle activity. Combined to the previous thing the change of the system of traditional irrigation is urgency in the Lagunera Region. The work was made in the Agriculture research center of the Agriculture and Zootecnic Collage in the Ejido Venecia, County of Gómez Palacio, Durango, México. The variety of corn “San Lorenzo” (generated in this College) was planted in 1982 and the variety of Soya “Cajeme”. The exploration spaces were the following: Factor (cow manure): with the levels; B1 = 0 ton ha⁻¹ (control); B2 = 40 ton ha⁻¹; B3 = 80 ton ha⁻¹; B4 = 120 ton ha⁻¹; B5 = 160 ton ha⁻¹; B6 = 100-150-00 (chemical fertilization). Treatments distributions were made with a randomized block design using a split block arrangement replicated three times. The physical characteristics texture, capacity of field, witting point, usable humidity and soil temperature. Chemical variables also were measured such as: electrical Conductivity, organic Matter, pH, Ammonium and Nitrates. The main variables measured in the plant were: yield of green and dry forage. The physical-chemistries characteristics of the ground indicate that the soil is a clay until the depth of 90 cm and salty clay from 90-120 cm, which shows that this type of ground retains nutrients and water in significant form for the culture compared with any other type of mineral ground, since its water available (CC and PMP) is high in comparison with the other 10 textural classes that exist (Cadahia, 1998). pH in general is alkaline, the value of organic matter is poor (less than 1,1%) in the layer of 0-30 cm, and decreases up to 0,51% at 90-120 cm of depth. The value of EC (1.5 dSm⁻¹) is normal for the establishment of any agricultural crop. With respect to N-NO₃⁻ a poor ground is considered since the maxima concentration found was 17.5 mg kg⁻¹ to 0-15 cm and 3 mg kg⁻¹ in the layer 90-120 cm, indicating that the ground requires chemical or organic fertilization for a good development of the crop. The phosphorus content is considered low; the value of the potassium (k) 119.37 mg

kg-1 is high, which is a characteristic of the regions with calcareous soils. For the value of N-NH₄⁺, like for nitrate poor soil is considered with less than 14.52 mg kg⁻¹, in general terms these results are considered like normal for soil of the region. The forage production during years 2001 and 2002 had very similar values, not thus in 2003 that corn forage production decreases; nevertheless, the tendency was the same with respect to the manure treatments, where the 80 and 120 ton ha⁻¹ of cow manure had the highest production with 86 and 84 ton ha⁻¹ respectively of green forage for 2001, respectively; 84 and 86 t ha⁻¹ of green forage in 2002 and 73 and 72 ton ha⁻¹ of green forage for 2003.

Keywords: Cow manure; corn forage; irrigation.

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S2-P25

The Fate of Organic and Inorganic Phosphorus in Soils - Analysing a Single Root Model

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A mathematical single root model describing the effect of root exudates on the bioavailability of both organic and inorganic phosphorus is analysed using the method of nondimensionalisation and perturbation methods. Phosphorus (P) is mainly taken up by plants in the form of ortho-phosphate (Pi). The amount of total P bound to organic forms varies largely between soils and may contribute significantly to the P nutrition of plants. In order to be utilized by plants, organic P must be hydrolyzed to inorganic P by phosphatases which may be of plant or microbial origin. The proposed model includes solute transport in the rhizosphere as well as the effect of roots exuding both low molecular weight organic acids and phosphatases on the P uptake of a single root. Each of these processes has a characteristic time and spatial scale. When compared to each other, some processes may be dominant while other processes may appear negligible. Information about the importance of the processes compared to each other is useful for management decisions concerning the P nutrition of agricultural crops. The method of nondimensionalisation provides a mathematical tool to analyse this. It is a process of changing variables by scaling so that the new variables have no units. This procedure leads to a simpler form of equations with fewer parameters that are all dimensionless. These parameters describe the relative importance of different processes included in the model compared with each other and determine the behaviour of the solution. The proposed dimensionless model shall help to interpret the complex chemical, physical and biological interactions in the rhizosphere with regard to P and exudate control mechanisms, and lead to a better understanding of the relevant processes involved.

Keywords: Mathematical model; inorganic and organic phosphorus; nondimensionalisation.

S2-P26**Effects of “Helper” Microorganisms on Rhizobial and Actinorhizal Nitrogen-Fixing-Symbioses****M. Solans^{1*}, G. Vobis¹ and L.G. Wall²**

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The aim of the present study was to examine the effect of saprophytic actinomycetes as “helper” microorganisms on two different nitrogen-fixing-symbioses: rhizobial and actinorhizal. Experimental assays were carried out under laboratory conditions, employing the strains *Streptomyces* MM40, *Actinoplanes* ME3 and *Micromonospora* MM18, which were previously isolated from the rhizosphere of *Ochetophila* (*Discaria*) *trinervis* and characterized as rhizoactinomycetic microorganisms. Two symbiotic systems, *Medicago sativa*-*Sinorhizobium meliloti* and *Ochetophila trinervis*-*Frankia* were used. The corresponding plants were inoculated with the symbiotic strains *Sinorhizobium meliloti* 2011 and *Frankia* BCU110501, and coinoculated with the above mentioned rhizoactinomycetes strains. Double inoculations were performed either simultaneously or asynchronously, and the plants were fertilized with different N concentrations. In both nitrogen-fixing-systems, the nodulation and, consequently the plant growth were significantly stimulated by simultaneously or asynchronously coinoculations with rhizoactinomycetes strains, compared to those plants, which were inoculated only with the symbiotic strains. In asynchronous coinoculations, this effect was more evident when the plants were first inoculated with *Sinorhizobium* or *Frankia*, and later coinoculated with rhizoactinomycetes strains. However, the nitrogen-fixation rate, estimated as acetylene reduction activity (ARA/mg⁻¹) was not significantly modified by rhizoactinomycetes strains. The analysis of the nodulation kinetics with simultaneous or asynchronous coinoculations of rhizoactinomycetes with *S. meliloti* or *Frankia* suggests, that the effect of saprophytic strains operates in early stage of infection and nodule development, counterresting the autoregulation of nodulation by the plant. The inoculation of rhizoactinomycetes alone did not show any effect on plant growth. In spite of the different infection types -Alfalfa is infected via root hairs and *O. trinervis* is infected via intercellular invasion-, apparently the rhizoactinomycetes “helper” effect on nodulation may operate by a common step in both systems. As it was expected, a high N-concentration inhibited the nodulation by *S. meliloti* in single inoculations. On the contrary, in all coinoculations with rhizoactinomycetes strains, the development of nodules could be observed, even under high N-fertilization. These results on the effect of rhizoactinomycetes strains on the nitrogen fixing symbioses, open new perspectives on the applications of the nitrogen fixing plants as the agronomical-uses of legumes and as the restoration of degenerated forest soils (actinorhizal plants).

Keywords: *Ochetophila trinervis*; *Medicago sativa*-*Sinorhizobium meliloti*; nitrogen-fixing-symbioses.

S2-P27**Organic Fertilizer S in Hot-Water Extract from Calcareous Arable, Fallow and Forest Soils as Affected by Different Sources of Organic Carbon Additions**

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Hot-water extractable organic carbohydrates are reported to be mostly microbially-derived compounds that constitute a reservoir of readily available nutrients for plants. However, little is known about the impact of more or less labile carbon sources on the availability of organic sulphur (S) in hot-water extract. This study examined the effects of salicylate, glutamate and glucose on fertilizer S immobilization and the subsequent releases of fertilizer S in hot-water extract (HW-S) after incubation of three calcareous soils differing in organic C contents: an arable soil (2.3% organic C), a fallow soil (3.9% organic C) and a forest soil (7.3% organic C). The amounts of carbon of the three substrates were added at two rates (0 and 1000 mg kg⁻¹ soil) to the three soils after a 1-week soil pre-incubation at 25°C to regenerate microbial biomass and then incubated for 1, 2, 6 and 12 weeks more with a Na₂³⁵SO₄ solution (674 kBq kg⁻¹ dry soil and 20 mg S kg⁻¹ soil) prior to analysis. All soils received the same amount of 80 mg N kg⁻¹ soil as NaNO₃. HW-S was obtained after sequential extraction of SO₄²⁻-S with 0.009 M Ca(H₂PO₄)₂. The use of ³⁵S makes it possible to calculate the amount of fertilizer S immobilized and that of fertilizer S in hot-water extract. Fertilizer S immobilization gradually increased over time, indicating a progressive integration of fertilizer S in the structure of humified compounds via microbial biomass turnover. Across all soils, the average amounts of fertilizer S immobilized were in increasing order: control < salicylate < glutamate < glucose. For all substrates, the quantities of fertilizer S immobilized were on average in the order: arable soil < fallow soil < forest soil, indicating a largest fertilizer S immobilization in the forest soil mostly rich in organic C. In contrast, the average values of HW-S expressed as percentage of immobilized fertilizer S across all soils were in increasing order: glucose = glutamate < salicylate < control, suggesting a greater yield of recalcitrant microbial S products from labile organic carbon source additions such as glutamate and glucose, basically present in root exudates. The addition of salicylate, a breakdown product of lignin in the detritusphere, resulted in more percentage labile HW-S production compared with glucose and glutamate. Based on the largest S immobilization with glucose, the non difference in HW-S observed between glutamate and glucose suggests that glucose proportionally produced more labile HW-S than glutamate. This is reflected in the sulphate specific activity average values that were in the increasing order: glutamate < glucose < salicylate < control. This indicates a lowest turnover of fertilizer S in the presence of glutamate. Overall, our results showed that the efficient S immobilization induced by labile C sources such as glutamate and glucose was associated with a greater yield of recalcitrant S products as determined in hot-water extract. Freshly immobilized fertilizer S was more labile than the older one. Apparently, fertilizer S immobilization with glutamate engendered more recalcitrant S compounds than with glucose.

Keywords: Organic fertilizer S; organic carbon; forest soil.

S2-P28**Effects of Glucose on Sulphur Dynamics in Calcareous Arable and Fallow Soils****P.-C. Vong*, S. Piutti, S. Sle Zack-Deschaumes, E. Benizri and A. Guckert***UMR Nancy-Université-INRA Agronomie et Environnement Nancy-Colmar, 2 avenue de la Forêt de Haye, 54505 Vandoeuvre-lès-Nancy, France. * E-mail: Phuy-Chhoy.Vong@ensaia.inpl-nancy.fr*

The magnitude of microbial sulphur (S) immobilization as well as the synthesis of inducible enzymes such as arylsulphatase (ARS) are basically controlled by carbon (C) additions. In order to assess soil fertility after a period of fallow a better knowledge of S dynamics including S immobilization and ARS activities of a natural fallow soil (3.9% organic C) in comparison with an arable soil (2.3% organic C) is required. Thus, the objectives of this work were to (i) examine the effects of glucose-C additions, a representative labile organic C in the rhizosphere, on the intensities of S immobilization in relation with ARS activities and (ii) distinguish extra- and intra-cellular ARS activities induced by glucose-C applied to the two soils. The amounts of glucose-C were added at six rates (0, 125, 250, 500, 750 and 1000 mg kg⁻¹ soil) to the studied soils after a 1-week soil pre-incubation at 25°C and then incubated one week more (to promote microbial S immobilization over S remineralization) with a Na₂³⁵SO₄ solution (518 kBq kg⁻¹ dry soil and 20 mg S kg⁻¹ soil) prior to analysis. All soils received the same amount of 80 mg N kg⁻¹ soil as NaNO₃. Soil SO₄²⁻-S content was extracted with 0.009 M Ca (H₂PO₄)₂. The use of ³⁵S makes it possible to calculate the amount of fertilizer S immobilized. The ARS method used determines the total ARS which corresponds to the ARS located either outside the cells (extra-cellular ARS) or inside the cells (intra-cellular ARS). Recovery tests using 0.009 M Ca(H₂PO₄)₂ showed a 100% recovery of SO₄²⁻-³⁵S introduced in the sample and extracted immediately after addition. Therefore, physical immobilization (adsorption of SO₄²⁻-³⁵S onto soil particles) was excluded and the ³⁵S immobilized corresponded exclusively to microbial immobilization. Glucose-C additions increased both microbial S immobilization and ARS activities. Related to the rate 0 (control), largest increase in immobilized-S was observed in the arable soil (300.7%) compared with the fallow soil (153.1%). In contrast, the ARS activity increased by 16.4% in the arable soil versus 32.1% in the fallow soil. These results indicate that glucose proportionately affected more the intensities of immobilized-S than those of ARS. We found positive and significant correlation coefficients of ARS activities with immobilized-S in the arable soil ($r = 0.86$, $P < 0.05$) and in the fallow soil ($r = 0.83$, $P < 0.05$). Accordingly, by extrapolating to zero immobilized-S (or microbial biomass-S), the results showed a presence of extra-cellular ARS activity of 38.7 mg *p*-nitrophenol kg⁻¹ soil h⁻¹ in the arable soil and of 63.5 mg *p*-nitrophenol kg⁻¹ soil h⁻¹ in the fallow soil. Without glucose-C (rate 0), values of 3.7 and 20.0 mg *p*-nitrophenol kg⁻¹ soil h⁻¹ of intra-cellular ARS activity (difference between total ARS and extra-cellular ARS activity) were found in the arable and fallow soil, respectively. It was concluded that fallowing generated greater ARS activities. Fallow regime re-established soil fertility through increasing soil organic matter content and thereby enhancing the activities of extra- and intra-cellular ARS enzyme compared with the arable soil that is regularly disturbed by tillage, pesticide treatment and fertilization.

Keywords: Sulfur dynamics; calcareous soil; aryl sulphatase.

S2-P29***Eucalyptus* sp. Litter Deposition and its Colonization by Ectomycorrhizal Fungi****J. A. Zambrano^{1*}, M. Dutra², I. Ribeiro da Silva³, J.C. Lima³ and N. Félix de Barros³**¹Laboratorio de Biotecnología, CHEMTEC S.A.E. Capitán Felipe Gómez 1087, Ñemby, Paraguay.²Departamento de Microbiología, Universidade Federal de Viçosa-UFV, CEP 36571-000 Viçosa, Minas Gerais, Brasil. ³Departamento de Solos, Universidade Federal de Viçosa-UFV, CEP 36571-000 Viçosa, Minas Gerais, Brasil. *E-mail: ja.zambrano@yahoo.com

Eucalypt forests deposit high litter quantities on the soil surface, promoting an effective cycling of nutrients. This work aimed at evaluating the content and concentration of nutrients in the litter layer of a *Eucalyptus* sp. forest, grown for 2.5 years, in a mountainous area in the region of Viçosa, MG. Litter samplings were done in three topographical positions, namely Top, Slope, Lowland. The litter was separated in four fractions according to the degree of decomposition and analyzed for the contents and concentrations of P, Ca, Mg, and K. Litter colonization by ectomycorrhizal fungi and the activity of acid phosphatases of ectomycorrhizas present in the litter layer were also evaluated. Litter deposition, in kg ha⁻¹, decreased in the following order (p<0.05): Top, 2,460 > Lowland, 1,730 > Slope, 1,239. The larger fraction of the litter layer corresponded to that showing the most advanced level of decomposition, being composed of dark fragments of leaves and branches in direct contact with the soil. Generally, nutrient content in the forest litter, in Kg ha⁻¹, decreased as follows: Ca (33,4) > K, (4,9) > Mg (1,7) = P, (1,7). Ca content in the litter and its low concentration in the soil evidence the importance of the litter layer as a Ca reservoir for eucalypts. The highest concentrations and contents of P in the litter were verified in the Top position, however, the activity of acid phosphatases of ectomycorrhizas did not differ among the topographical positions evaluated. Fruit bodies of *Laccaria*, *Pisolithus*, *Scleroderma*, and of an unidentified fungus were observed in the area under study. The examination of the most decomposed litter layer and of the interface soil-litter showed an intense colonization by eucalypt roots, with the presence of eight distinct ectomycorrhizal morphotypes, hyphae, rhizomorphs, and basidiomes, suggesting an important role for ectomycorrhizal fungi in the cycling and storage of nutrients in the eucalypt forests.

Keywords: *Eucalyptus* sp.; litter deposition; ectomycorrhizal fungi.

S2-P30**Soil Microbial Biomass and Urease Activity in a Permanent Pasture under Different Grazing Systems****P. Núñez^{1*}, A.A. Jara², P. Cartes³, R. Demanet³ and M.L. Mora²**¹Instituto Dominicano de Investigaciones Agropecuarias y Forestales, La Vega, República Dominicana.²Departamento de Ciencias Químicas, Universidad de La Frontera, Temuco, Chile.³Instituto de Agroindustria, Universidad de La Frontera, Temuco, Chile. E-mail: pnunez@idiaf.org.do

In grassland systems, the pasture productivity can be influenced by the grazing management, due to its impact on soil microorganisms and, therefore, on biological processes that affects the nutrient cycling. The aim was to evaluate the soil microbial biomass carbon, microbial biomass nitrogen and the urease activity in a permanent pasture of Southern Chile under different grazing managements. During spring 2005 to winter 2006, four grazing systems were evaluated: frequent-heavy (FH), frequent-light (FL), infrequent-heavy (IH) and

infrequent-light (IL) and a no-grazing control (C), with three replicates, in a randomized block design. According with the results, the values of microbial biomass carbon fluctuated between 28 and 697 mg C kg⁻¹, whereas the microbial biomass nitrogen values ranged between 3 and 70 µg N g⁻¹ during the four seasons. Comparatively, the FH grazing system showed the highest average of microbial biomass carbon (297 mg C kg⁻¹) and microbial biomass nitrogen (21.6 µg N g⁻¹). On the other hand, the urease activity oscillated between 84 and 1137 µg N-NH₄⁺ g⁻¹ 2h⁻¹, and the highest enzyme activities occurred in the grazed pastures with annual averages between 319 and 339 µg N-NH₄⁺ g⁻¹ 2h⁻¹. Additionally, the pasture yield was improved by increasing the grazing intensity, and the greatest production was observed in FH (9.8 ton DM ha⁻¹) and IH (10.3 ton DM ha⁻¹). The results showed that, after grazing, a higher flux of organic residues in the soil stimulated the microbial biomass and, therefore, an increase of the urease activity and the microbial biomass -carbon and -nitrogen was observed. Thus, in grazing systems, the increase of the amount of residues in soil from both the plant and the cattle excretes (dung and urine) improves the biological fertility, the nutrient availability and the pasture production.

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Keywords: Microbial biomass; urease activity; grazing systems.

S2-P31

Dynamics of Nitrogen and Phosphorus Losses in a Volcanic Soil of Southern Chile

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The use of nitrogen (N) and phosphorus (P) in fertilizers in cattle production systems of Southern Chile has increased over the last ten years, so that it is expected that the environmental risk in the area will increase. Most of the production is based on grazing systems on volcanic soils, which are characterised by high organic matter content (>15%) and high P fixation capacity. The objective of this work was to establish the dynamic and main forms of N and P losses in runoff and leaching in a volcanic soil. The experiment was carried out on an Andisol of the Osorno soil series (Typic Hapludands). Nitrogen and P dynamics in surface runoff were determined using three surface lysimeters (5 x 5 m). Runoff samples were analysed for nitrate and ammonium. Total N was also determined and organic N (DON) was calculated as the difference between total N and the available N forms. Leaching losses were determined using the ceramic suction cups technique and samples were analysed as indicated previously for runoff samples. In runoff samples, reactive P (RP) and total P (TP) were determined and organic P (OP) was estimated as the difference between TP and RP for each sample. Of the total N lost in runoff, available forms represented on average c. 24% each, as the result of urine lost in runoff immediately after excretion and N lost after nitrate fertilizer application. Dissolved organic N represented c. 50% of total. This could be related to the high soil organic matter content in the topsoil and the expected high soil biomass activity registered at the site. A high proportion of the N leached (c. 70%) was lost as nitrate with ammonium averaging less than 10% of the total inorganic losses. Dissolved organic N in leachates samples represented

25% of total. Total P losses were mainly as RP (70% on average). Organic P losses represented only 30% of the total P lost in runoff. High soil organic matter played a major role on N and P dynamic losses. Thus, DON was the second most important N form in leachates and the main form in runoff losses. Fertilizer management played a fundamental role on P dynamic losses.

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Keywords: Nitrogen and phosphorus losses; volcanic soils; grazing systems.

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S2-P32

Effect of Acid Phosphatase Immobilized in Allophanic Clay on P Availability in Soil

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Organic-P represents 20–80% of the total P in most soils and consequently represents a significant reserve of potentially available P. Low phosphorus availability in soil, however, remains one of the largest constraints to agricultural production worldwide. Organic P contributes to the P nutrition of plants primarily after being mineralized into organic P. The rate of P mineralization depends on the activity of hydrolytic enzyme such as phosphatases. In soil, enzymes are naturally immobilized in inorganic, organic and organo-mineral soil constituents. The activity of immobilized enzyme and its catalytic behaviour strongly depends of the nature of the immobilizing support. In a previous work, we are demonstrated that the immobilization of acid phosphatase in allophanic clay increase their catalytic efficiency. Therefore, the immobilization of enzyme in this support could be increase the hydrolysis of soil organic substrates. However, the role of immobilized phosphatase as catalyst in hydrolysing soil organic P have not been well investigated. In order to estimate the role of immobilized phosphatase in P bioavailability in soils we are investigate the effect of addition of acid phosphatase immobilized on allophanic clay on P availability in soil under laboratory conditions. The Andisol soil used in this study has a organic matter of 9 % and 6 mg kg⁻¹ of Olsen P. For the immobilization of acid phosphatase, complexes were formed by the interaction between acid phosphatase and the clay fraction extracted from a Chilean Andisol. Two levels of enzyme (0.0029 and 0.0143 mg protein g⁻¹ of clay) were used for immobilization. Then, the soil was incubated with these complexes at 20°C by 8 days. For comparison, also was added a free enzyme to the soil and the control treatments was the soil without enzyme. At 0, 1, 4 and 8 days, samples of these soils were taken and the amount of available P, determined by the phosphomolybdenum blue method, was measured. By applying immobilized acid phosphatase to the soil, the amounts of soluble inorganic phosphate increased. The highest P content, 15 mg kg⁻¹, was obtained the fourth day for the treatments corresponding to complex with 0.0143 mg of enzyme. At this time, the available P increased by up to 150 % relative to soils without immobilized enzyme. Nevertheless, to the eighth day the available P concentration decreased to 12 mg kg⁻¹. It has been frequently reported that the activity of acid phosphatase can be controlled by the solution P concentration decreasing by a raise of inorganic P concentration. When complex with 0.0029 mg of enzyme was applied alone, the Olsen P increased from 6 to 10 mg kg⁻¹ at the fourth day. While, no significant differences in Olsen P content was observed by addition of free enzyme to the soil. This result indicate that the bioavailability of organic-P is likely to be largely dependent on the amount of

active phosphatases in soil solution. This result showed that acid phosphatase immobilized in allophanic clay can release available inorganic phosphates from soil.

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Keywords: Acid phosphatase; enzyme immobilization; allophanic clay.

S2-P33

Screening and Isolation of Phosphate Solubilizing Fungi from Soils of Southern Chile

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Chile has excellent conditions to become a major food producer and exporter, as it has done with fruit. In this way, free-living phosphate solubilizers fungi (phosphofungi) can play an important role in plant nutrition by allowing use part of accumulated P in the soil, which has low availability for plants, especially in volcanic soils. However, it is necessary to isolate and select native phosphofungi adapted to the climate-soil conditions in each region and well evaluated on their ability to solubilise phosphate. For this, a lot of liquid media have been suggested but all of them with different sensitivity. Hence, the aim of the study was: 1) To make a screening of native phosphofungi present in volcanic soils from southern Chile. 2) To asses the P solubilization capacity of a selected phosphofungus in four liquid media. The screening of phosphofungi was carried out from several soils of our region under different crops and managements using Martin medium agar-rose bengal-streptomycin, with $\text{Ca}_3(\text{PO}_4)_2$ or phytate-Ca as insoluble inorganic or organic phosphate source, respectively. A halo around the colony indicated solubilization. After 5 d incubation at 28°C an index of solubilization was calculated as the total ratio diameter / diameter of the colony. The colonies with high phytate-Ca solubilization capacity were analyzed for phosphatase (P-ase) activity secretion picking up on Martin medium supplemented with phenolphthalein phosphate; a red halo around these colonies revealed with ammonia vapours indicated secretion of P-ase. A phosphofungus strain with high index of solubilization (*Penicillium* sp.) was used as a model for starting the stage of election of a liquid media to assess phosphate solubilization capacity of the others strains. Erlenmeyer flasks with 50 mL of sterilized liquid medium were inoculated with three 0.5-cm-diameter of fungal mycelium grown on solid agar. The liquids media were as: Agnihotri, Asea-Wakelin, Pikovskaya and Nahas *et al.* $\text{Ca}_3(\text{PO}_4)_2$ was used as the P source which was previously autoclaved and then added to the sterile solutions. The growing conditions were: darkness, 20°C +/- 1, initial pH 6.5 +/- 0.2, intermittent orbital agitation 9 min. every 1 h. After 7 days the solid was separated from the liquid phase by Whatman N°1 filter paper. In the filterings soluble P and pH were determined. The solid phase was dried at 60°C for 24 h, and then calcined in a muffle furnace for 6 h at 500°C; the difference in weight between the two procedures accounted for the fungal biomass. At the controls it was applied the same treatment but without inoculum. All treatments were performed with four replicates. Phosphofungi were detected in the 90% of the soils analyzed, a few showed a promising solubilization capacity of $\text{Ca}_3(\text{PO}_4)_2$ or phytate-Ca on Martin medium. The highest solubilization indexes ranged 1.2-1.4 mainly found in medium with

phytate. There was P-ase secretion in 80% of phytate solubilizer colonies analyzed. The *Penicillium* sp. strain showed the highest solubilization capacity in the liquid medium of Asea-Wakelin. In conclusion, it is possible to isolate phosphofungi with high potential P solubilizing capacity to be used for mobilizing accumulated P in volcanic soils.

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Keywords: Screening; phosphate solubilizing fungi; Andisols.

S2-P34

Effect of the Co-inoculation with Two PGPRs Strains (C26 and C139) on Spring Wheat (cv. Pandora) in Volcanic Soils of Southern Chile

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Spring wheat (Pandora) was inoculated using two strains of PGPR (Plant Growth Promoting Rhizobacteria) C139 (*Pseudomonas* sp) and C26 (*Bacillus* sp.) both isolated from volcanic ash soils of Southern Chile. The taxonomy is being done. The effect was study on plants maintained at Santa Rosa Experimental field, Valdivia, South Chile (39°45' S and 73°14' O). Low levels of P Olsen (8 ppm) and mineral N (9.8 mg kg⁻¹) were determinated. Both strains showed at laboratory assays: Indols acids production and fixer Nitrogen (strain C26) and of solubilization P (C139). Seven treatments were established at field as follows: Control with full NP, C26 without N, C26 without N and P, C139 without P, C139 without N and P, C139 plus C26 without N and P and control without N and P. Plants were established at 400 plants m⁻² density in November 2007 and maintained until harvesting time. The Measured traits was total aerial biomass (g m⁻²), leaf area index (g m⁻²), spikes biomass (g m⁻²), radiation interception, leaf protein content, leaf pigments content (chlorophyll *a*, *b*, and carotenoids) and fluorescence of PSII, using Fv/Fm [Fv as variable fluorescence and Fm as maximal fluorescence]. These parameter was using of plant vitality indicator. Samples were taken at tillering initiation, flowering and harvest. Plants with C26 without N presented higher biomass (55%) and higher grain yield (57%) than control without NP and lower than Control NPK treatment (only 76.4% in biomass and 78.5% in grain yield). The treatment C139-P increased biomass and grain yield by 29% and 27% above the control -NP, respectively. At harvest co-inoculation treatments (C139 plus C26 without N and P) showed low yield and biomass than separated treatments (C26 without N). Non significant differences were observed in Fv/Fm parameter, chlorophyll and leaf between treatments at tillering initiation and flowering. All the inoculated treatments with C130 or C26 were significant different than control treatment (without NP) in grain yield and biomass. No positive interaction has been found between strains C26 and C139.

Acknowledgements: RHIBAC-Project European community and CONICYT contra part of RHIBAC-Project.

Keywords: PSII; PGPRs strains; volcanic soils.

S2-P35

Interactions between Fe and N Cycles: Role of Fougérite as a Competitor of Nitrate in Anaerobic Respiration and as a Direct Abiotic Reductor of Nitrate**F. Trolard*** and G. Bourrié[†]INRA UR1119, Géo chimie des sols et des eaux, Aix-en-Provence, France. *E-mail: trolard@aix.inra.fr

In moderately reducing conditions, anaerobic respirations rely on different electron acceptors, such as nitrate N(V) and ferric Fe(III) compounds, such as goethite or Fougérite. Fougérite is the natural green rust mineral responsible for the blue-green color of gley. It is a mixed Fe(III)-Fe(II) hydroxide with a brucitic-type layer and a well crystallized mineral with definite crystal structure: the excess positive charge of the layer is compensated in the interlayer by intercalated anions (Trolard *et al.*, 2007). As it contains Fe(III), Fougérite can act as an electron acceptor. Depending on the nature of Fe(III) compounds considered and on pH, nitrate can be reduced before or after Fe(III), so that nitrate and Fe²⁺ can coexist in solution. Thermodynamic calculations show that when Fougérite is not present, nitrate is reduced before goethite, while when Fougérite is present, it is reduced either before nitrate in acidic conditions (pH=6, Fig.1), but is reduced after nitrate in alkaline conditions (pH = 8.5). This implies that Fougérite can compete with nitrate as an electron acceptor in anaerobic respiration. Due to its labile character, Fougérite reduction proceeds easily and poises Eh so that nitrate does not undergo denitrification in anoxic conditions when Fougérite is present. Nitrate cannot be directly reduced by Fe²⁺, but Hansen *et al.* (1996) obtained the abiotic reduction of nitrate into ammonium by synthetic green rusts, *e.g.* GR-SO₄ in several hours. This is not denitrification, but abiotic reduction, and this is not a depolluting process, as ammonium is more toxic to fish than nitrate. The following mechanism can be proposed: the first step is an anion exchange between sulphate and nitrate in the interlayer. When the extent of the exchange is large enough, the GR structure changes from GR2 (2 layers of water molecule) to GR1 (1 layer), and the nitrate anion is located in between two Fe³⁺ cations, and surrounded by 6 divalent cations Fe²⁺ or Mg²⁺ in the upper layer and 6 others in the lower layer (Fig.2). In this situation, 8 Fe²⁺ can simultaneously donate an electron to N(V), while Fe – OH – Fe bonds transform into Fe – O – Fe bond (oxolation). The GR oxidizes into hematite. Thus N cycle cannot be fully understood without reference to biotic and abiotic interactions with Fe cycle.

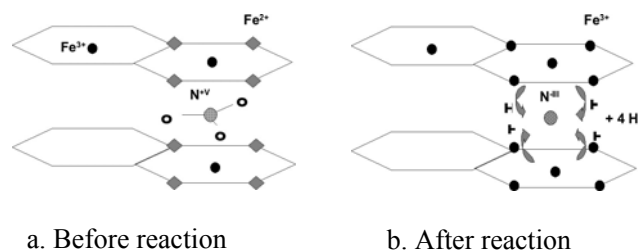
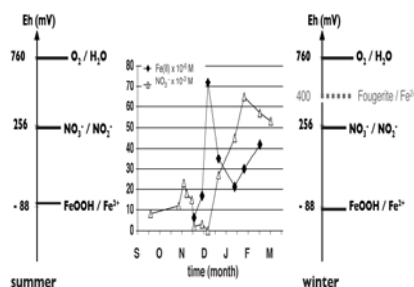


Figure 1: Scales of reduction with and without Fougérite. **Figure 2:** Mechanism for direct reduction of nitrate into ammonium by Fougérite.

Keywords: Fe and N cycles; Fougérite; anaerobic respiration.

S2-P36**Effect of Nitrogen Supply on Phosphate Dynamic in Perennial Ryegrass (*Lolium perenne* L.) Rhizosphere****C. Paredes^{1,2}, P. Yañez¹, A.A. Jara¹, R. Demanet³, F. Gallardo¹ and M.L. Mora^{1*}**¹Departamento de Ciencias Químicas, Universidad de La Frontera, Temuco, Chile. ²Programa de Doctorado y Magíster en Ciencias de Recursos Naturales, Universidad de La Frontera, ³Instituto de Agroindustria, Universidad de La Frontera. Avenida Francisco Salazar 01145, Casilla 54-D, Temuco, Chile.

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A rhizotron experiment was conducted to evaluate the ryegrass rhizosphere behavior in response to nitrogen fertilizers (N-NH_4^+ y el N-NO_3^-) supply. In the present work, the effect of N applications on pH values, phosphorous (P) availability, and oxalate concentration in both, bulk soil and ryegrass rhizosphere solution, were studied. The assay was carried out under environmental controlled conditions using soil samples (Chilean Andisol) previously incubated with P (300 mg P kg^{-1} of soil, using KH_2PO_4). Ryegrass plants (5 plants per rhizotron) were grown for a period of 12 days. Nitrogen doses of 0, 100 and 300 mg N kg^{-1} of soil were applied immediately after the rhizotron installation. The rhizospheric solution was collected with the micro-suction cup/rhizobox technique. The micro-suction cups were placed 0-2 mm, 3-5 mm and 6-8 in front of growing ryegrass fine roots. Results showed N-NH_4^+ supply diminished the rhizospheric pH. Thus, soil pH values of 5.55 and 5.21 were reached with N-NH_4^+ doses of 100 and 300 mg N kg^{-1} , respectively (average pH of 6.49 in control soil solution). P availability was increased when N-NH_4^+ was applied, nevertheless, for all N treatments rhizospheric solution P concentrations were lower than 1 mg P L^{-1} . A maximum P concentration of 0.7 mg L^{-1} was recorded with N treatment of 300 mg N kg^{-1} . On the other hand, oxalic acid concentration in the rhizosphere solution was increased in response to N-NH_4^+ applications. The highest oxalate concentrations were detected at the distances of 3-5 and 6-8 mm from the ryegrass root surfaces. The highest rhizospheric solution NO_3^- levels were obtained with N-NO_3^- doses of 300 mg N kg^{-1} , reaching a maximum of 562 mg N L^{-1} at 6-incubation day. For all treatments, results indicate that there is a difference of P concentrations in the bulk soil respect to the values detected in ryegrass rhizospheric solution. A decreasing gradient on P availability in the nearest distances to the roots was observed.

Acknowledgements: This work was supported by the FONDECYT project 1061262.**Keywords:** Ryegrass; rhizosphere; nitrogen.**S2-P37****The Role of Antioxidative Enzymes and Carboxylates Exudation on the Tolerance to Manganese Toxicity in Perennial Ryegrass (*Lolium perenne* L.)****M.L. Mora^{1*}, A. Ribera^{1*}, A. Rosas¹, E. Stohmann¹ and Z. Rengel²**¹Departamento de Ciencias Químicas, Universidad de La Frontera, Temuco, Chile. ²Soil Science and Plant Nutrition, School of Earth and Geographical Sciences, The University of Western Australia, Australia. *E-mail: aribera@ufro.cl

Manganese (Mn) is an essential micronutrient in plants, but may become toxic when present in excess in the rhizosphere. In Chile, most forage species are cultivated in soils derived from volcanic ashes, like Andisols. About 50% of Chilean Andisols have high soil acidity levels and hence, Mn amounts that may be highly toxic to plants. The biochemical mechanisms underlying differential tolerance to Mn toxicity in cultivars of perennial ryegrass, one of the dominant forage in southern Chile, are poorly understood. In this study, activity of antioxidative enzymes and root

exudation of organic acid anions were evaluated in four ryegrass cultivars grown in nutrient solutions with increasing supply of Mn. A growth reduction caused by Mn toxicity was smaller in Jumbo and Kingston than Nui and Aries cultivars. Shoot Mn accumulation varied among the ryegrass genotypes in the order Nui > Aries > Kingston > Jumbo. In general, ascorbate peroxidase (APX) and guaiacol (GPX) peroxidase activities increased proportionally to Mn excess. These enzymes were particularly strongly induced in shoot tissues of Mn-tolerant cultivars Jumbo and Kingston; correspondingly, lipid peroxidation was relatively low in these cultivars. Kingston was the cultivar best tolerating high tissue Mn concentrations; it also had the highest superoxide dismutase (SOD) activity. Thus, increased activity of antioxidative enzymes (such as peroxidases and especially SOD) in Mn-tolerant cultivars could protect their tissues against oxidative stress triggered by excess Mn. Mn toxicity induced root exudation of organic acid anions in all cultivars. In the case of Mn-tolerant cultivars, Kingston showed high exudation of oxalate and Jumbo high exudation of citrate. The findings obtained of root organic acid exudation measurements suggest that citrate was the most efficient organic acid anion in decreasing availability of external Mn. On the other hand, oxalate exudation may be linked to increased intracellular concentrations of this ligand that detoxify Mn by complexation.

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Keywords: Antioxidative enzymes; carboxylates exudation; manganese toxicity.

S2-P38

Effect of Urea Supply on Phosphate Fertilization and Nitrogen Forms in Perennial Ryegrass (*Lolium perenne* L.) Rhizosphere

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Nitrogen (N) fertilizers of acid reaction can affect phosphorus (P) availability in the rhizosphere and hence, reduce the growth and shoot P concentration in plants. A greenhouse experiment was conducted to evaluate the effect of nitrogen and phosphate fertilizers applications on nutrient distribution and forms in perennial ryegrass rhizosphere. The assay was carried out in pots using soil samples (Chilean Andisol) treated with 3 doses of N (0, 100 y 300 mg N kg⁻¹ of soil, using urea) and P (100, 200, 400 mg P kg⁻¹ of soil, using triple superphosphate). Ryegrass plants were grown for a period of 34 days. To study rhizospheric P depletion, 2 rhizobox were inserted in each pot. Results indicated that ryegrass dry weight (DW) and shoot P concentration significantly increased with increasing doses of P. In addition, shoot P concentration in ryegrass decreased in a doses dependent manner with N application. Shoot P concentrations ranged from 1540 to 2534 mg kg⁻¹. As expected, P availability in the rhizosphere increased in response to P supply. Moreover, the rhizospheric concentrations of N-NO₃⁻ and exchangeable aluminium were incremented at increasing N doses. Under all treatments, N- NH₄⁺ soil levels were low. The pH values diminished and varied from 4.1 to 5.4 in response to urea supply during the assay period. Thus, the highest pH variations were detected in samples treated with the higher urea doses. A decreasing gradient on P availability in the nearest distances to the roots was observed. P depletion conditions were provoked by N applications at the distance range of 0-2 mm from the root surfaces. These results allow concluding that the N fertilizers of acid reaction could reduce P uptake and availability in the ryegrass rhizosphere.

Acknowledgements: This work was supported by the FONDECYT project 1061262.

Keywords: Urea; rhizosphere; ryegrass.

S2-P39**Assessment of Interspecific Lines of *Oryza* spp. Inoculated with *Azotobacter chroococcum* and *Azospirillum amazonense* in Soil Typic haplustalf of Ibagué, Colombia****L.A. Castilla-Lozano^{1*} and M.M. Bolaños-Benavides²**¹Colombian National Rice, Federation, Fedearroz. ²Colombian Agricultural, Research Corporation, Corpoica, Palmira. *E-mail: arrozibague@hotmail.com

In rice crops, it is necessary to generate alternatives that reduce costs through the use of fewer nitrogen fertilizers, e.g. by using biological fixation of nitrogen (BFN) and breeding programs to obtain genotypes that are efficient in their nitrogen use. The genus *Oryza* has 20 wild species and two cultivated: *O. sativa* L. and *O. glaberrima* Steud. Wild species represent an additional source of genes for improving rice potential characteristics, then, the objective of this research was to evaluate the response of interspecific lines of rice (*Oryza* spp.) to inoculation with *Azotobacter chroococcum* and *Azospirillum amazonense* on the efficiency in the use of N, in a Typic haplustalf soil of the plateau of Ibagué, Colombia. Each material was evaluated with and without inoculation, with a biofertilizer with a concentration of 10^{10} CFU/mL of *A. chroococcum* (IBUN N° 142111) and *A. amazonense* (IBUN N° 121111). The seeds were inoculated with 1 cc of biofertilizer 250 g^{-1} per cultivar and were cultivated immediately after. Each cultivar, with or without inoculation, was studied with three doses of N: 0, 50 and 100%, 100% corresponded to 250 kg N ha^{-1} , the source used was granular urea (46% N). ANOVA was conducted in accordance with a subdivided plot design where the N rates corresponded to the main plot; with and without the inoculum in each subplot; and genotypes (10 in total) to each subplot with three repetitions. Tests were conducted by comparing the average DMRT (Duncan Multiple Range Test) and by a multivariate analysis. Inoculation with *A. chroococcum* and *A. amazonense* produced more vigorous rice plants, and more radical and shoot biomass, especially in the interspecific lines CT13941 and CT16049 when they received 50% of N. The use of N by interspecific rice lines produced a higher agronomic efficiency, when the plants received average doses of nitrogen fertilizer plus inoculation with *A. chroococcum* and *A. amazonense*, with the highest values in the lines CT13941, CT14524 and CT16049-1. The highest N recovery rate was obtained with the wild phenotype *O. rufipogon* Griff., with 50% N plus inoculation. In addition, there were differences between cultivars in this variable, where interspecific line CT16049-1 with 50% of N plus inoculation surpassed the commercial variety Fedearroz 50. The physiological efficiency was high on the interspecific lines CT16049-2 and CT1394-1 surpassing the other cultivars, with 100% N and without inoculation. The yield of interspecific lines CT13941 and CT13943 increased with the inoculation of the NFB and the implementation of 50% N, surpassing all other cultivars. It is considered a good alternative for reducing costs, by reducing the use of nitrogenous fertilizers and generating higher yields.

Keywords: biological nitrogen fixation, rice, interspecific lines.

S2-P40**Soluble Organic Matter and Humic Acids in Agricultural Soil Amended with Sewage Sludge and Sewage Sludge Compost****P.R. Vaca, J. Lugo de la Fuente* and J.P. Del Aguila***Laboratorio de Edafología y Ambiente, Facultad de Ciencias, Universidad Autónoma del Estado de México, Instituto Literario 100, Toluca 50 000, México. *E-mail: jlugo@uaemex.mx*

Biosolids are a valuable source of organic matter, N and P at low cost in agricultural soils. However, one of the main concerns regarding their application to the soil is the presence of potentially toxic elements (PTE). There are still some uncertainties about the final destination of PTE in the soil where sewage sludge is applied. For instance, metals introduced with sewage sludge can be released and become available to organism, but the organic matter can also immobilise them. The behaviour of DOM and HA in the soil and its effect on pollutant chemistry is of a particular interest to researchers, since de DOM and HA play a fundamental role in immobilisation and transport of pollutants. The effect of the sewage sludge or sewage sludge compost to the soil on the quality of the dissolved organic matter (DOM) and humic acids (HA) in the soil, was investigated under field conditions. The study site is located in an agricultural area of the municipality of Toluca, in Mexico's central region, where 9 land plots of 6 x 8 m each were defined and distributed in a Latin square. Three plots were used as controls (S) utilizing fertilizer (120-90-60 kg ha⁻¹ de N-P-K), another three were amended with 18 Mg ha⁻¹ of biosolid on a dry base (Sw-S) and the remaining three plots were treated with equal doses of sewage sludge compost (SwC-S). From the control and amended soil, were taken monthly samples throughout the agricultural cycle (June-November 2003), in order to carry out the pH analysis, organic matter content (OM), DOM and HA quality through the infrared spectroscopy (IR). The evaluation of the cationic exchange capacity (CEC), interchangeable cations (Ca, Mg, Na and K), organic carbon total, total nitrogen, as well as the total and available heavy metals content (Cd, Cu, Pb and Zn) were determined in an initial, intermediate and final period (June 2003, August 2003 and November 2003). The SwC-S showed the highest OM (4.2%), Nt (0.13%), CEC (16 Cmol kg⁻¹) and Cu (9.20 mg kg⁻¹). The Sw-S showed the highest C/N ratio (18.0), Ca²⁺ (7.2 Cmol kg⁻¹, Mg²⁺ (3.5Cmol kg⁻¹), K⁺ (0.28 Cmol kg⁻¹) and total Zn (31.7 mg kh⁻¹) content. The DOM of Sw-S indicated the presence of carboxyl groups, aliphatic compounds, lignin and protein content, while SwC-S showed an increased of carbohydrates. The behaviour of HA was different with respect to the DOM. The HA of SwC-S application showed an increased of proteins, lignins, carbohydrates and aliphatic compounds respect to S and Sw-S. No significant differences were found on total and available heavy metals content between S, Sw-S and SwC-S.

Keywords: Soluble organic matter; humic acids; sludge compost.

S2-P41**Molybdate and Phosphate Kinetic Sorption in Chilean Andisols****E.Vistoso^{1*} and M.L. Mora²**

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Sorption of molybdate and phosphate by soil constituents affect the mobility and bioavailability of Mo and P in soil-solution-plant systems (Barrow, 1999). Both anions are strongly and specifically sorbed on the soil (Goldberg and Forster, 1998), mainly on variable charge minerals (Al-oxides, Fe-oxides, allophane, imogolite). Several studies have described the kinetics of phosphate reaction in heterogeneous soil and clay minerals by various kinetic models. Nevertheless, reported information about the kinetic of molybdate sorption on soil constituents is very limited. The aim of this work was to compare the effect of time and soil properties on molybdate and phosphate sorption in Andisols with different properties. The sorption behavior was evaluated as a function of time (0-72 h) at ionic strength 0.1 mol L⁻¹ KCl (25°C) in four Chilean Andisols. Data from sorption experiments were fitted by the Elovich equation modified (Chien and Clayton, 1980). Results showed a fast sorption in the first 0.5 h, being 55% for molybdate and 62% for phosphate. For both anions the reaction continued slowly until 72 h reaching 90% for molybdate and 97% for phosphate. The release of OH⁻ ions at the solution was increased with the time, raising the pH in 0.85 units for molybdate sorption reactions and 0.65 units for phosphate reactions. The Elovich parameter α (sorption rate) value for both anions was relative to the organic matter content (O.M.) of soils, and also can be associated to humus-Fe and humus-Al complexes. The α values for molybdate were 2.24×10^{15} mmol kg⁻¹ h⁻¹ for Vilcún soil (15 % O.M), 2.49×10^{12} mmol kg⁻¹ h⁻¹ for Pemehue soil (16 % OM), 8.76×10^{10} mmol kg⁻¹ h⁻¹ for Osorno soil (20 % O.M.) and 3.11×10^7 mmol kg⁻¹ h⁻¹ for Piedras Negras soil (24 % O.M.), whereas for phosphate the α values were 3.89×10^7 mmol kg⁻¹ h⁻¹ for Vilcún soil, 5.21×10^{10} mmol kg⁻¹ h⁻¹ for Pemehue soil, 3.11×10^{12} mmol kg⁻¹ h⁻¹ for Osorno soil and 1.08×10^{16} mmol kg⁻¹ h⁻¹ for Piedras Negras soil. Desorption constant values (β) ranging from 0.47 to 0.28 for molybdate and 0.22 to 0.94 for phosphate from Vilcún to Piedras Negras soils. The Keq values determined by the relationship between α and β constants for Vilcún and Pemehue soils were higher for molybdate than phosphate, in contrast, Keq values for Piedras Negras and Osorno soils were higher phosphate than molybdate. Results showed that mineralogical composition and the O.M. content of the soils regulate the kinetic behavior of both anions. Thus, the sorption of molybdate was associated to higher affinity of the Fe-oxide and Al-oxide and the phosphate sorption was regulated by the humus-Fe and humus-Al complexes.

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Keywords: Molybdate; phosphate; kinetic sorption.

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S2-P42***Raoultella terrigena* and *Pseudomonas fluorescens* Effects on Wheat Biomass and Grain Yield in Volcanic Soils****P. Sandaña^{1,2*}, J. Oyarzún³, D. Pinochet² and L. Ciampi³**¹Escuela de Graduados, ²Instituto de Ingeniería Agraria y Suelos, ³Instituto de Producción y Sanidad Vegetal. Universidad Austral de Chile, Valdivia, Chile. *E-mail: patriciosandana@uach.cl

Plant growth promoting rhizobacterias (PGPR) are those exert beneficial effects on plant growth and development. Field and pot experiments were carried out, using volcanic soil, to evaluate wheat growth promotion produced by *Raoultella terrigena* and *Pseudomonas fluorescens* wheat. Both experiments had four treatments: (i) optimal phosphorus (P) fertilization; (ii) no P fertilization; (iii) no P fertilization plus *Raoultella terrigena* inoculation and (iv) no P fertilization plus *Pseudomonas fluorescens* inoculation. Treatments were arranged in a complete randomized blocks design with three replicates. Wheat seeds (cv. Pandora) in the field experiment were inoculated with 1.5×10^5 cfu seed⁻¹. In the field experiment, at grain harvest evaluation inoculated treatment with both PGPR increased significantly ($P < 0.05$) total aboveground biomass (34%), grain yield (33%), grain number (36%) and P uptake (25%) over the control without P fertilization. The pot experiment was planned growing ten inoculated plants (3.75×10^5 cfu plant⁻¹) in boxes of 375 cm² and 100 cm depth. Each box was put in 60° respect to ground and had a glass wall, in order to draw roots length at different times during growing period. At wheat heading stage evaluation *Raoultella terrigena* increased significantly ($p < 0.05$) aboveground biomass (248%) and root length (30%) over control without P fertilization. These results suggest a potential use of *Raoultella terrigena* and *Pseudomonas fluorescens* as wheat growth promoting rhizobacterias in volcanic soils.

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Keywords: *Raoultella terrigena*; *Pseudomonas fluorescens*; wheat.

S3-P1**Effect of Biosolid Incorporation on Cu, Cr, Ni, Pb and Zn Extractability in Mollisol and Inceptisol Soils of Central Chile****I. Ahumada^{1*}, M. Ávila¹, E. Contreras¹, L. Ascar¹, P. Richter¹ and M.A. Carrasco²**¹*Universidad de Chile, Facultad de Ciencias Químicas y Farmacéuticas, Departamento de Química Inorgánica y Analítica, Casilla 233, Santiago, Chile.* ²*Universidad de Chile, Facultad de Ciencias Agronómicas, Departamento de Ingeniería y Suelos. Casilla 1004 Santiago, Chile.***E-mail: iahumada@ciq.uchile.cl*

In Santiago city almost 100% of the urban wastewater will be treated by the end of 2010, which implies the generation of large volumes of biosolids that will need to be disposed to avoid an environmental problem. An interesting alternative to face the accumulation of these residues is their application in agriculture as soil amendment and as a complement of the soil fertilization. This could be an attractive option of disposal and recycling of these residues. Biosolid application to soil assumes a significant supply of nutrients and micronutrients essential to plants. Nevertheless, it has been found that biosolid application to soil incorporates trace metals and other organic toxic compounds that may interact with organic matter or other soil components. It is, thus, necessary to learn how biosolid incorporation affects agricultural soils in relation to availability of trace metals such as Cu, Cr, Ni, Pb, and Zn. The purpose of this study was to determine the effect of biosolid amendment on Cu, Cr, Ni, Pb and Zn extractability from the soil components through the BCR (Community Bureau of Reference) procedure, which considers three sequential extraction to obtain an acid-soluble fraction (metal soluble, exchangeable and bound carbonates), a reducible fraction (metal associate with iron/manganese oxides), an oxidizable fraction (metal bound to organic matter and as sulphides) and residual fraction. The study included agricultural soils of the Mollisol and Inceptisol order. Soil samples were collected from the surface soil layer (0- 20 cm) at six different sites close to the city of Santiago in the Metropolitan Region. These were amended with biosolids at rates of 0, 15, 30, 45 and 60 Mg ha⁻¹ and incubated for 60 days under controlled conditions of moisture and temperature. Among the metals determined in biosolids and soils, Zn, Cu and Cr showed the highest concentration. Total trace metal contents found were below the limits allowed by the Chilean regulation for both samples. Biosolid fractionation showed that Ni and Zn acid-soluble fraction was predominant and the incubation process increased this fraction for both metals, which confirms the greater availability of these metals in biosolids. In soils generally, without considering the residual fraction, the metals were found predominantly as reducible and oxidizable form and in the Zn case the first fraction was important too. Biosolid application to agricultural soils had effect on trace metal distribution in some of them, with an important increase in Zn availability in all the soils and Cu in some of them, while Cr, Ni and Pb showed no significant variations in most of them. A two-level factorial design was satisfactorily applied to assess the effect of biosolid application rate and the incubation time.

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Keywords: Biosolid; wastewater; mollisol and inceptisol soils.

S3-P2**Use of Sewage Sludge and Mussel Shells in Mine Soils Recovery****M.L. Andrade*, E.F. Covelo and F.A. Vega***Departamento de Biología Vegetal y Ciencia del Suelo. Universidad de Vigo, España***E-mail: mandrade@uvigo.es*

Opencast mines cause serious environmental impact like the destruction of natural soils, the extraction of important volumes of materials, and produce a large amount of waste because the ore is a small fraction of the total volume of the mined material. This causes the formation of new soils (Anthropic Regosols) on the accumulated wastes of the mine with low contents of nutrients and organic matter and unfavourable texture and structure. High levels of trace elements and the acidic drainage due to the oxidation of sulphide are also frequently common characteristics of the most mine tailings. The scarce vegetation leads to acidification of adjacent soils by leaching the mine spoils, giving rise to high environmental contamination. The extraction of metal ores causes generally a multielemental contamination of the environment. In addition, the natural metal content of the soils will be increased if materials with heavy metals are added. The restoration of soil function and mining soil quality is essential to long-term ecosystem stability. In Touro (Coruña, NW Spain, 42°52'34'' N, 8°20'40'' W) there were two opencast mines, Arinteiro and Bama, from which were extracted chalcopyrite and pyrrhotite between the years 1973 and 1988. The copper extraction stopped in 1988 and, since then, materials for road construction were extracted. The mine spoils cover a surface of 760 ha, and were formed of great piles of coarse rock fragments with strong slopes. In addition they did not begin to be recovered until two years ago. The only measures of the recovery made were the addition of sewage sludge, like source of organic matter, and mussel shells, like liming material. This study was conducted to evaluate, at four tailings from this opencast mine, the effects of sewage sludge and mussel shells on chemical characteristics and heavy metal content of minesoils. The superficial horizons of eight soils were sampled. Four come from the depleted copper mine of Touro treated with sewage sludge and mussel shells during two years and four soils without treatment. The main chemical limitations of untreated mine soils are their acidity (pH from 3.62 to 4.78), low organic matter content (from 1.12 to 12 mg kg⁻¹), low CEC (from 1.83 to 9.47 cmol₍₊₎ kg⁻¹), and Ca⁺² and Mg⁺² contents, and imbalance between exchange bases. The used amendments modified many of these characteristics. Thus, the treatment increased soil pH (varies from 7.8 to 8.1), organic matter content, that vary between 61 and 77 mg kg⁻¹, also it increased considerably the CEC (from 60 to 156 cmol₍₊₎ kg⁻¹), as a result of high increases of exchangeable Ca and Mn contents (from 51 to 137 and from 5.94 to 6.02 respectively). Nevertheless it continues the imbalances between exchange bases. All the soils contain high concentrations of Cu, Pb, Ni, and Zn. The Cu, Cr, and Ni levels surpass the maximum limits established in some reference guides. The used amendments increase the Cd, Cu, and Pb availability and diminish the total content. Nevertheless Cr, Ni, and Zn availability diminish and total content increase. It is probably due to the formation of Cu, Cd and Pb soluble organic complexes, and to Cr, Ni and Zn fixation when the pH increases until basic values. Therefore it is necessary to modify the dose of the amendments to reduce organic matter content and pH, and to balance the exchangeable cation content. Also it is advisable to add substances that adsorb heavy metals, like clay minerals, and fertilizers to ameliorate the mine soils. These used amendments, and the proposed ones, can improve the soil properties, and enhance soil functions and quality.

Key words: Minesoils; recovery; amendments.

S3-P3**Bacterial Community Structure Associated with the Rhizosphere of *Cirsium arvense* in an Italian Arsenic-Contaminated Soil**

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The rhizosphere bacterial community of a spontaneous plant (*Cirsium arvense*) inhabiting an Italian soil located in Tuscany Region contaminated with arsenic ($As_{tot} = 214 \text{ mg/Kg}$) was characterized by cultivation-independent and -dependent methods. FISH analysis showed that the dominant bacteria present in bulk and in rhizosphere fraction of soil belonged to *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Pseudomonas*. Both soil fractions contained high percentage of arsenate and arsenite resistant bacteria. Most of the culturable As-resistant isolates were affiliated to the *Alphaproteobacteria*. Some of the isolated strains possessed genetic determinants for As-resistance. Genes coding for arsenite efflux pump (*arsB* and *acr3p*) and arsenate reductase (*arsC*) were PCR amplified from both As (V) and As(III) resistant bacteria. Several aerobic microorganisms that are potentially involved in As-cycling were isolated from the enrichment cultures. When grown in a defined liquid medium containing an organic carbon source, a group of the As (III) resistant bacteria transformed efficiently arsenite to arsenate. Among them there were *Sinorhizobium* sp., and *Ancylobacter* sp. which rapidly grew chemolithoautotrophically. The microbial oxidation of As (III) to As (V) impact the mobility and speciation of arsenic in rhizosphere where plants could uptake arsenate via phosphate transporter. Moreover, As (III) oxidation is the basis for bioremediation of As(III) polluted systems, because As(V) can be immobilized onto strong adsorbents.

Keywords: *Alphaproteobacteria*; rhizosphere; arsenic-contaminated soil.

S3-P4**Speciation of Manganese in Oxisols of the Mining Area of Moanda (Gabon, Africa), and Its Possible Impact on Growth of Maize (*Zea mays*) and Cassia (*Chamaecrista rotundifolia*), and Heavy Metal Bioaccumulation, under Greenhouse Conditions**

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A set of soils with high manganese (Mn) content was collected in the manganese mining area of Moanda, in the South West of the Republic of Gabon. The prevailing mineral substratum is an ampelite from mid-Precambrian and the soils are developed on the 3-5 m

thick overlaying material, which is composed of ferruginous pisolites, the main weathering product of the ampelite. Assays with either locally cultivated species (maize, peanut, manioc...) or native legume grasses (*Pueraria phaseoloides*, *Crotalaria retusa*, *Chamaecrista rotundifolia*...) were run, both in the field and in the greenhouse, with the objective of optimizing the vegetation re-growth on the mine landscape. In the present work, a greenhouse experiment with maize (*Zea Mays*) and round-leaf cassia (*Chamaecrista rotundifolia*), grown on two soil materials, was carried out. The test material (S-Mn) was a composite of surface soils with high Mn content, developed on pisolitic deposits. The blank material (S-Bl) was a composite of surface soils with low Mn content, developed on weathered bedrock of the foothill, near Moanda. In soils composing the S-Mn material, the total Mn content was frequently higher than that of Al or Fe (100-300 % of soil weight, oxide basis; exchangeable Mn ranged from 0.2 to 1.5 cmol (+) kg⁻¹ and occupied from 100 to 650 % of the effective cation exchange capacity, concurrently with Al ions. When soil samples were submitted to ammonium oxalate (*Ox*) and citrate-bicarbonate-dithionite (*CBD*) reagents, Mn was highly soluble (700-900 %) in both reagents, Fe was moderately soluble in *Ox* (100-200 %) and *CBD* (400-600 %), and Al was much less extractable. The S-Mn material did not significantly modify the germination rate of both seeds, although the growth was frequently slower and the 60 day-old seedlings showed more yellowing and necroses on S-Mn than on S-Bl, especially for *C. retundifolia*. The accumulation of Mn in stems and roots of both plants was about 8 times higher on S-Mn than on S-Bl. Other metals (Cu, Co and Pb) followed the same tendency, but with much less differences and only in the roots of one or both plants. For most nutrients and other metals (Fe, Cr and Ni), bioaccumulation could be lower on S-Mn than on S-Bl. These results suggest that, in the peculiar type of Oxisol tested in our study, biological activity was still high and able to sustain the nutrition of the seedlings. The high dissolution of Mn by organic acids seemed to be responsible for the main disturbance in plant growth and metal mobility, especially in the absence of any fertilization.

Keywords: Manganese; oxisoil; maize.

S3-P5

Arbuscular Mycorrhizal Fungal Communities Associated to Four Metallophytes in a Mediterranean Ecosystem Affected by Copper Mining

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The arbuscular mycorrhizal fungi (AMF) form mutualistic symbiosis with most of terrestrial plants, which obtain a benefit from a greater nutrient uptake and an improved tolerance to abiotic stresses, including the heavy metal (HM) presence in the soil. The HMA diversity can be affected by high levels of HM, producing structural changes in the plant communities. In central Chile several ecosystems have been affected by the deposition of particulate matter and wastes originated in diverse mining activities, which have accelerated strong processes of loss of plant cover and diversity. Nowadays, these communities are only composed by some species able to tolerate high HM levels, specifically Cu, Zn and As. The

aim of this work was to study the AMF diversity in an ecosystem affected by mining activities, with the view to obtain native AMF ecotypes to use in bioremediation programs using endemic metallophytes from Central Chile. The communities of AMF associated to four plants (*Argemone subfusiformis*, *Baccharis linearis*, *Oenothera affinis* and *Polypogon viridis*) from a Mediterranean scrubland near the Ventanas smelter (Puchuncaví Valley, Valparaíso, Chile) presenting a strong Cu pollution and soil acidity, were analyzed. Spores collected from rhizospheric soil were morphologically characterized as well as by the use of PCR-TTGE analysis of RNA-SSU. The results showed a strong effect of the plant specie on the size and structures of the fungal communities, being the population size more than 600 spores in 100 g of soil in *O. affinis* rhizospheric soil, whereas in the other plant species the total spore community size was 3-6 times smaller. A high specificity was found between *O. affinis* and an AMF ecotype (*Acaulospora* sp1, probably a not previously described AMF specie), registering low specific richness ($S'=2$) and high dominance ($\lambda=0.97$). The other metallophytes presented AMF communities with high ecotypes richness (between 3.7 and 6 ecotypes) and homogeneity (H' between 0.86 and 1.51). *Acaulospora* sp1 was also found in the AMF communities associated to the other plant species analyzed, suggesting a greater adaptation to the stress conditions by high levels of Cu in the soil, in addition to a strong effect of the plant species on the fungal community structure. Moreover, there was a high specificity between *Acaulospora* sp1 and *O. affinis*, that could be related with a probable Cu bioaccumulator potential in this plant specie recently described, making possible its use in phytoremediation processes in ecosystems affected by mining activities in Central Chile.

Acknowledgments: This study was supported by Fondecyt 3070052 Grant.

Keywords: Arbuscular mycorrhizal; metallophytes; copper mining.

S3-P6

Mycorrhizal Fungal Propagules in a Mediterranean Ecosystem Polluted by a Copper Smelter in Central Chile

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Arbuscular mycorrhizal fungi (AMF) are present in the most of terrestrial ecosystems, contributing to the establishment and nutrition of plants through mutualistic symbiosis. In heavy metal (HM) polluted ecosystems, the arbuscular mycorrhizal (AM) symbiosis plays a fundamental role, increasing the presence and dissemination of metallophytes able to tolerate high levels of HM. In Chile the role that plays the AMF in ecosystems extremely affected by mining activities is still unknown, which is not related with the high number of mining deposits and ecosystems contaminated by mining practices. Based on the above mentioned, the objective of this study was to quantify the density of AMF propagules in HM polluted soils and to analyze the relation with the Cu pollution level. To carry out this study, rhizospheric soil of four metallophytes species present in a Mediterranean ecosystem influenced by the activities of the Ventanas smelter (ENAMI) at the Puchuncaví Valley (Valparaíso, Central Chile) was collected. The selected plants were *Argemone subfusiformis*, *Baccharis linearis*, *Oenothera affinis* and *Polypogon viridis*. The levels of available Cu fluctuated between 18.8 and 326 mg kg⁻¹ in the rhizospheric soil of *O. affinis* and *P. viridis*, respectively. On the other hand, the density of mycorrhizal mycelium were

Session 3. Soil-Root-Microbe Interaction & Their Effects on Biophysical Transformation, Fate, and Toxicity of Metal and Metalloids

found between 1.2 and 4.5 m g^{-1} in *B. linearis* and *P. viridis*, respectively, being this parameter highly correlated with the available Cu ($r=0,83$; $p<0,01$). The density of AMF spores was scarce in all the plant species (between 100 and 200 AMF spores in 100 g of soil), except in *O. affinis*, that showed densities near the 600 AMF spores in 100 g of soil. The mycorrhizal colonization fluctuated between 33.9% in *B. linearis* and 57.9% in *A. subfusiformis*. The rhizospheric soil of *P. viridis* presented the highest value for the acid P-ase activity ($2.6 \mu\text{g PNF g}^{-1} \text{h}^{-1}$); whereas *O. affinis* presented the higher value for dehydrogenase activity ($11 \mu\text{g INTF g}^{-1} \text{h}^{-1}$), 2 to 11 folds higher than in the other soils. These results are the first reported for AMF propagules densities in highly HM polluted environments in Central Chile, and suggest an important role of this kind of soil fungi in the establishment of some endemic metallophytes under this type of environmental stress. Particularly, *P. viridis* presents the highest mycelium density associated with high levels of P-ase activity (and available P), which could allow its growth in extreme conditions and that could support its inclusion in further phytoremediation programs. Moreover, this study gives new evidence on the role of AMF in soils polluted by mining activities, and suggests a strong influence of the plant in the development of the different AMF propagules in HM polluted soils.

Acknowledgments: This study was supported by Fondecyt 3070052 Grant.

Keywords: Mycorrhizal fungal; mediterranean ecosystem; copper smelter.

S3-P7

Bioavailability of As and P in the Rhizosphere of Plants Inoculated with Arbuscular Mycorrhizal Fungi

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Arsenic (As) is ubiquitous in the environment and it is highly toxic to most biological systems. Soil unproductiveness, due to build up of arsenic (As) concentrations in soils from human activities is an environmental concern. Elevated soil As concentrations generally decrease the ability of the soil to support an economically viable crop on contaminated sites, but phytotoxicity of As is often reached prior to accumulation levels that would be toxic to wildlife or to humans ingesting the plants. Understanding plant uptake metabolism of As is indispensable for the development of technologies that alleviate As toxicity in the crop plants. Arbuscular mycorrhizal fungi (AMF) can form symbiotic relationships with the vast majority of land plants, and are known to benefit the phosphorus (P) of the host plants by increasing P acquisition. Due to the physicochemical similarity between phosphate and arsenate, AMF are likely to have a strong influence on arsenate uptake and resistance. The fate of As in the rhizosphere and its interaction with P, in presence of AMF originating from different environment, has not yet been fully explored. In this study, we examined the role of AMF on As uptake in lettuce plants (*Lactuca sativa* cv. Trocadero) growing in a As-polluted soil collected from Tuscany, Italy. In particular, we tested the effectiveness of native AMF or inoculation with a commercial inoculum (colonized by *Glomus intraradices*) with and without P fertilizer on the growth and uptake of As by lettuce and their effects on As and P fractions in rhizosphere and bulk soil. The soil under investigations was a clay loam, alkaline (pH 8.2), containing 51 mg kg^{-1} total CaCO_3 , 7.5 mg kg^{-1} P (Olsen) and 250 mg kg^{-1} total As. Greenhouse pot experiments (six replicates each) were established in a randomized block

design. Plants were grown for three months, with and without a commercial inoculum (M+, M-) and two levels of P application (P- at 0 kg P ha⁻¹ and P+ at 90 kg P ha⁻¹). At harvest, soil (bulk and rhizo soil) and plant (root and leaf) samples were collected. Commercial inoculum and P application increased plant biomass enormously and the highest plant growth was in M+P+ treatment. Leaves and root As concentrations were slightly reduced by inoculation with commercial inoculum where P was added. On the contrary, M+P- treatments showed the highest As root concentrations. Root and leaves As and P concentrations in M-P- plants was lower compared with M+P- treatment. Root and leaves P concentrations in M+P+ was higher compared with M-P+ treatment. Analyses of rhizo and bulk soils at the harvest indicated that commercial inoculum plus P fertilization did not increase more available As fractions in the rhizosphere, but evidenced a P soil depletion respect to plants M-P+. Commercial inoculum protected its host plants from toxicity of excessive As through P nutrition by absorbing more P from the soil.

Keywords: *Glomus intraradices*; *Lactuca sativa*; arsenic.

S3-P8

Modelling of Heavy Metal Sorption and Retention by Antropic Regosols

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Many mine soils are chemically, physically, and biologically unstable and deficient. They are sometimes amended with sewage sludge and ashes but often contain heavy metals that increase the already high mine soils heavy metal contents. Cd, Cr, Cu, Ni, Pb, and Zn in mutual competition were added to five mine soils (Galicia, Spain). The studied mine soils (all Anthropic Regosols) are located at the former copper mine at Touro (Spain), from which road material is currently extracted, and at the lignite mine at Meirama. Ten solutions of mixtures of Cd, Cr, Cu, Ni, Pb, and Zn nitrates (between 10 and 400 mg L⁻¹) were prepared to obtain adsorption isotherms. Twelve grams soil samples were treated with 200 mL of solution, shaken for 24 hours at 25°C. After centrifugation, metal in solution was determined by ICP-OES. Desorption experiments were performed using the pellets resulting from adsorption experiments. Each sample was treated with 200 mL of an acetic acid (0.02M) and sodium acetate (0.02M) solution, buffered at pH 4.5 and shaken for 24 hours at 25°C. All of the experiments were performed in triplicate. Soil capacities for heavy metal sorption and retention were determined by means of distribution coefficients. In previous papers $K_{d\Sigma sp}$ proposed by Kaplan was adapted in order to evaluate the total sorption and retention capacity of all heavy metals that contain the added solution to soils. By means of $K_{d\Sigma sp}$ medium values and data of soil properties it was made a multiple linear regression analysis in order to predict the heavy metal sorption and retention capacities of mine soils. This analysis was made by backward stepwise. This predictive model could be applied to soils that present their characteristics within the interval established by the studied mines soils properties. The selected variables to elaborate these predictive equations were the contents of humified organic matter (HOM) and Fe, Mn and Al oxides (mg kg⁻¹), the cationic exchange capacity (cmol kg⁻¹) and the proportion in soils of sand, silt and clay. The obtained equations are:

Session 3. Soil-Root-Microbe Interaction & Their Effects on Biophysical Transformation, Fate, and Toxicity of Metal and Metalloids

Sorption: $K_{d\Sigma sp} = -0.32\text{ sand} - 1.09\text{ clay} - 1.92\text{ CEC}_e + 1.66\text{ FeOx} - 2.37\text{ MnOx} + 52.09$.

$R^2 = 0.99$.

Retention: $K_{d\Sigma sp} = -0.56\text{ sand} - 5.68\text{ clay} - 1.21\text{ HMO} - 15.50\text{ CEC}_e + 14.35\text{ FeOx} + 232.12$.

$R^2 = 0.98$.

The properties of studied soils that influence their sorption capacity and therefore they will influence the capacity of soils with similar characteristics are the sand and clay proportion, the cationic exchange capacity and the Fe and Mn oxides content (mg kg^{-1}). The variables that allows predict the heavy metals retention capacity of similar minesoils are the percentage of sand and clay, the cationic exchange capacity and the contents of humified organic matter and Fe oxides (mg kg^{-1}). Therefore the content of Mn oxides more influences the heavy metal sorption capacity of minesoils whereas after desorption experiments the minesoils humified organic matter influences their retention capacity. We represent the empirical data of heavy metals sorption and retention capacities as opposed to the predicted ones by means of multiple linear regression. They were obtained two correlation coefficients: Sorption = 0.99 and Retention = 0.975.

Keywords: Sorption; Desorption; Modelling.

S3-P9

Assessment of Heavy Metals in Selected Vegetables from Harichand Area in District Charsadda, Pakistan

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Consumption of vegetables and fish contaminated with the heavy metals Fe, Zn, Cu, Ni, Cr, Pb and Mn is the most likely route for human exposure in Harichand area in district Charsadda, Pakistan. The present study was undertaken to investigate the bioavailability of heavy metal in vegetables and to assess the extent of heavy metal contamination of vegetation due to irrigation with canal water on agricultural land of Harichand District Charsadda. - Digestion of samples (2 g each) was carried out using 10 ml nitric acid, according to the procedure used for soil samples (Lark, 2002) using Hitachi Zeeman Japan Z-8000, Atomic Absorption Spectrophotometer equipped with standard hollow cathode lamps as radiation source and air acetylene flame were used for absorption measurement of elements Cr, Cu, Ni, Zn, Cd, Pb and Fe. The present results demonstrate that the general order of decreasing metals concentration in the vegetables was $\text{Fe} > \text{Zn} > \text{Mn} > \text{Cr} > \text{Cu} > \text{Ni} > \text{Pb}$ and the decreasing order of metal contents in the soil sample was $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cr} > \text{Ni} > \text{Cu} > \text{Pb}$. -The present results demonstrated that the results show the presence of the heavy metals in vegetables, below the WHO standards.

Key words: Trace metals, Pollution, Bioavailability, Pakistan, WHO.

References

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S3-P10**Effect of the Aluminum (Al^{3+}) Toxicity on the Lipid Peroxidation, and Antioxidant Activity in Blueberry *Vaccinium corymbosum* L.****C. Inostroza-Blancheteau^{1*}, M. Reyes-Díaz², M. Alberdi³ and M.L. Mora³**

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In South-Central Chile where acid soils are predominant, blueberry (*Vaccinium corymbosum* L.) is an important agronomic activity. The acidity of these soils increases the aluminium phytotoxicity (Al^{3+}), with a concomitant decrease of the productivity and strongly yields reductions. Some alternative for the decrease of the concentration of exchangeable Al^{3+} is the application of calcium amendments. However, this application is only conditioned to the first centimeters of soil. Other alternative is the searching of genome of plants with aluminium (Al) resistance. Currently, did not exit studies involved with the identification of germoplasm of blueberry with Al resistance characteristics. In this work the Al response of three cultivars: cv. Brigitta, cv. Legacy and cv. Bluegold (the most important cultivars cultivated in Chile) was evaluated in a nutritive solution with different Al concentration (0, 25, 50, 100 y 200 μM Al^{3+}) at different times (0-20 days). The Al resistance level was estimated by the lipid peroxidation throughout the TBARS method in roots and by the antioxidant activity in roots and leaves using the DPPH method. The results showed that the three cultivars had a differential Al resistance ($P < 0.05$), being cv. Legacy the most resistance cultivar (5 to 10 mmol MDA g^{-1} FW). Cultivar Brigitta and cv. Bluegold showed higher lipid peroxidation (up to 83 mmol MDA g^{-1}) and therefore the greater Al sensitivity. These results are associated with a lower decrease of the antioxidant activity in roots with respect to the initial values in cv. Legacy (34%) following by cv. Brigitta (40%) and cv. Bluegold (58%). Interestingly, cv. Legacy showed initially a higher antioxidant activity (7.4 mg TE/gFW) in leaves than the other cultivars (2.4 mg TE/gFW), suggesting a constitutively larger antioxidant activity of cv. Legacy. This high antioxidant activity and its low lipid peroxidation suggest a higher Al resistance in cv. Legacy. We concluded that in the long term cv. Legacy showed the best behavior to Al stress, differently as showed previously in short term experiments performed by our group.

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Keywords: Blueberry; aluminium phytotoxicity; antioxidant activity.

S3-P11**Copper and Zinc Content in *Oenothera affinis* at Increasing Cu Levels as Affected by Different Ecotypes of Arbuscular Mycorrhizal Fungi****S. Meier, A. Seguel, F. Borie and P. Cornejo****Departamento de Ciencias Químicas, Universidad de La Frontera, Casilla 54-D, Temuco, Chile.***E-mail: pcornejo@ufro.cl*

Metallophytes can grow in soils with high heavy metals (HM) levels, as those that have received the deposit of wastes of mining activities. In Chile, copper (Cu) mining takes place in a vast geographic area, affecting several natural ecosystems. However, knowledge of metallophytes and its ability to stabilize or accumulate HM is scarce, and less known is the role of arbuscular mycorrhizal fungi (AMF) under these conditions. The AMF has several mechanisms allowing the establishment of plants in soil HM polluted, promoting phytostabilization and/or phytoextraction processes. The aim of this study was to analyze the effect of mycorrhizal symbiosis and the type of AMF on the Cu and Zn uptake and compartmentalization in *Oenothera affinis* at increasing soil Cu levels to establish its potential use in phytoremediation. For this, seedlings of *O. affinis* were grown in: i) rhizospheric soil of *O. affinis* with the native AMF inoculum (+M); ii) soil inoculated with *Glomus claroideum* (GC), and iii) sterile soil (-M). The growing substrate was sterile soil from a Mediterranean ecosystem near Ventanas smelter (Puchuncaví Valley, Valparaíso, Central Chile), which presented 178 mg kg⁻¹ of available DTPA-Cu. This soil, was used to form seven increasing Cu levels, which consisted of 0, without soil (a mixture of sand and sepiolite); 1, soil diluted with sand and sepiolite (1:1:1); 2, soil undiluted; 3, soil with 75 mg Cu kg⁻¹ (as CuSO₄ solution); 4, soil with 150 mg Cu kg⁻¹; 5, soil with 225 mg Cu kg⁻¹ and; 6, soil with 300 mg Cu kg⁻¹. Plants grew for 12 weeks and subsequently the contents of Cu and Zn in shoot and root, as well as the DTPA-available content of both HM in the soil, were quantified. The Cu concentration in shoot increased in all mycorrhizal treatments until level 3 (from 20 to 50-55 mg Cu kg⁻¹ in level 0 and level 3, respectively), and decreased at higher soil Cu levels (levels 4 to 6) up to 30-40 mg Cu kg⁻¹. In root was observed high Cu concentrations, especially in the +M treatments (up to 748 mg Cu kg⁻¹ in level 6), significantly different to the other treatments (up to 326 and 603 mg Cu kg⁻¹ in GC level 5 and -M level 6, respectively). The Zn was also preferentially concentrated in roots, being observed in this case that treatment -M presented a higher Zn concentrations (up to 313 mg Zn kg⁻¹ in level 6) compared to the other mycorrhizal treatments (up to 192 and 174 mg Zn kg⁻¹ in +M level 3 and GC level 3, respectively). The bioaccumulation of Cu was higher in the root compared to the shoot (up to 39 and 88 mg Cu kg⁻¹ in the treatment +M level 6, for the shoot and root, respectively); while the Zn was preferentially accumulated in shoot (up to 56 mg Zn kg⁻¹ in +M level 2 for the shoot and up to 30 mg Zn kg⁻¹ in -M level 1 for the root). Cu and Zn contents in both organs showed independence of the levels reached by both HM in the soil. These results suggest complexes mechanisms of HM homeostasis by *O. affinis*, in which the mycorrhizal association would favor the Cu transport and accumulation in the root, but could prevent the Zn accumulation in this organ. In addition, these results suggest a strong potential of the symbiosis between *O. affinis* and some ecotypes of HMA natives from soils polluted by high Cu levels to promote phytostabilization processes.

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Keywords: Copper; *Oenothera affinis*; arbuscular mycorrhizal.

S3-P12**Effect of High Biosolid Rates on Copper Mobility in Contaminated Soils****J. Mendoza*** and **T. Garrido***Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile, Casilla 233, Santiago, Chile.***E-mail: jmendoza@ciq.uchile.cl*

A strategy for heavy-metal stabilization in mining wastes and soils contaminated with mine wastes is the use of organic amendments, biosolids among them, in order to decrease concentration of metals in solution, leaching to deeper layers, and drifting by the wind. This practice, in turn, provides nutrients and organic matter favouring the growth of vegetation^{1,2}. In the present study, the effect is assessed of a biosolid applied at rates of 100 ton/ha to copper-contaminated soils. The study included two soils (0-20 cm depth) near the cities of Rancagua (Clay loam) and Santiago (Loam) in the central valley of Chile, with Cu total contents of 1046 and 475 ppm, respectively. The biosolid was obtained from a household wastewater treatment plant. Chemical analysis of both substrates was carried out with standardized methods for soils. For mobility studies, glass chromatographic columns were used, packed with 45 g of soil. The columns, previously moistened, were eluted with 10^{-4} M CaCl_2 at a flow rate of 0.1 mL/min, by means of a peristaltic pump. The eluate was collected in 300-drop fractions by means of a fraction collector. The biosolid was added at the top of the column. In the eluted fractions, determinations were done of pH, total Cu by FAAS, free Cu by ISE and organic carbon. Each column was prepared in duplicate and was eluted for about 48 h to obtain from 15 to 20 pore volumes (pv). In both soils, with no sludge application, an increase of eluted Cu was observed about the first 5 pv, decreasing then to undetectable values about 15 pv. With this volume, the accumulated total was 207 and 82 µg Cu for Loam and Clay loam soils, respectively. Rapid elution of dissolved organic matter (DOM) from the soil was observed, but its peak decreased faster than Cu peak and before 10 pv. Sludge application implied mobilization of a remarkable amount of copper and organic matter compared with control soils, even though the elution pattern of both analytes showed similar characteristics. The amount of eluted copper after 15 pv was 1137 and 148 µg, values which are 5.5 and 1.8 times higher than control for Loam and Clay loam soils, respectively. On the other hand, organic matter now mainly provided by sludge, showed increases of 4.3 and 2.6 times compared with control for the same soils, respectively. Application of mass balance determined that the Cu mobilized from soils by DOM corresponds to 881 and 16.4 µg, for Loam and Clay loam, respectively, which is about 9.4% and 3.8% of available Cu. These results would indicate that the mobile fraction of the biosolid organic matter has a significant effect on Cu mobility in soils contaminated by this metal.

Acknowledgments: This study was supported by FONDECYT, project N° 1080632

Keywords: Copper; DOM; contaminated soils.

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S3-P13**Effect of Aluminium (Al^{3+}) Toxicity on Total Soluble Sugars in Blueberry Cultivars (*Vaccinium corymbosum* L.)****R. Millaleo^{1*}, M. Reyes-Díaz², M. Alberdi³ and M.L. Mora³**¹Doctorado en Ciencias de Recursos Naturales, Facultad de Ingeniería, Ciencias y Administración, Universidad de La Frontera, Casilla 54-D, Temuco, Chile; ²Instituto de Agroindustria, Universidad de La Frontera; ³Departamento de Ciencias Químicas, Universidad de La Frontera.

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The aluminum toxicity (Al^{3+}) is the most important factor that inhibits the plants growth in acid soils, because it accumulates in the roots cells causing the inhibition of the cell elongation. The carbohydrates are direct products of the photosynthetic activity and they constitute an energy source and metabolites, which represent significant percentages of the dry weight of woody plants. The objective of this work was to determine the total soluble sugars (AST) content in three cultivars of blueberry (*Vaccinium corymbosum* L.) in Hoagland solution at different times (0, 7, 14 and 20 days) and aluminium (Al) content (0, 25, 50, 100 and 200 μM). The three cultivars showed different AST levels in leaves and roots under Al stress. Generally, cv. Legacy showed a decrease with Al treatments at different times in leaves, but a highest decrease (62%) was observed in cv. Brigitta at 200 μM of Al treatment compared with the control ones. In roots, cv. Legacy maintained the higher AST concentration with respect of the others cultivars under aluminium stress. Differently, cv. Bluegold did not change the AST concentration in leaves and roots. The role of AST in aluminium stress is unclear, since both high and low levels of AST can be involved in the improvement to the oxidative stress depending of species. We concluded that cv. Legacy showed a better acclimation under Al stress, existing an increase in the AST concentration in roots at different the times, maintaining the AST levels in leaves.

Acknowledgments: This study was supported by Bicentenario-Conicyt PSD-26 and FONDECYT N°1080372.

Keywords: Aluminum toxicity; total soluble sugar; *Vaccinium corymbosum*.

S3-P14**Arsenic and Cadmium Uptake by Field-Grown Corn (*Zea Maiz* L.): Modeling and Characterization****M. Molina^{1*}, M. Escudey², W. Chen³ and A.C. Chang³**¹Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile. ²Departamento de Química de los Materiales, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago, Chile. ³Department of Environmental Sciences, University of California at Riverside, CA 92521, USA.

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Potentially hazardous trace elements may be inadvertently introduced into cropland soils through applications of fertilizers and other soil amendments. In agricultural systems, much of the trace element removal from the soil will depend on the ability of crops to uptake these elements and translocate them from roots to above-ground biomass. Through plant uptake,

the elements accumulated in soils may be incorporated into the food chain to adversely affect consumers of the harvests. To characterize the risks, the trace element absorption process of plants must be quantified. We conducted a two seasons-long field experiment where grain corn was grown from seed to harvest. The data from soil and plant analyses was used to characterize the absorption of arsenic (As) and cadmium (Cd) in terms of plant uptake factors ($PUF = \text{element concentration in plant} / \text{element concentration in soil solution ratio}$), and of mean adsorption rates throughout the growing season. The cultivated soil was a clay loam Mollisol (Mixed, thermic, Ultic Haploxerolls), representing the soils used for intensive corn production in central Chile. This soil has been monocultivated with corn over a 15 year period, receiving high-dose application of P fertilizers. Soil samples were taken before sowing, and seven composite plant samples were taken in triplicate along each season. Soil and plant samples were analyzed to determinate the soil solution concentrations and total concentration of the elements, respectively. Total uptake of As and Cd by corn was $0.52 \pm 0.02 \text{ g ha}^{-1}$ and $0.43 \pm 0.02 \text{ g ha}^{-1}$, respectively. During the growing season the PUF decreased exponentially with time ($R^2 > 0.941$), indicating a mass dilution effect for both elements. The PUF decrease by approximately 3.5 times for As and 3.0 times for Cd from the early growth stages to the time close to harvest. This behavior has been attributed to a greater competition between roots and/or to the presence of less active roots as the plant develops to maturity. The concentration of As in plants was slightly higher than the Cd concentrations. On the other hand, the As concentration in soil solution was higher than Cd concentration. The Cd PUF was more than 6 times higher than the As PUF, indicating a higher bioavailability of soil Cd for corn plants. The uptake rate at a specific sampling date (mean uptake rate) followed a sigmoidal pattern ($R^2 > 0.917$). The maximum uptake rate were $3.9 \times 10^{-3} \text{ g ha day}^{-1}$ and $3.0 \times 10^{-3} \text{ g ha day}^{-1}$ for As and Cd, respectively. The maximum mean uptake rate was reached close to R1 stage (silking) for both trace elements and after that remained constant while the cumulative above-ground biomass was still increasing.

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Keywords: Arsenic; cadmium; corn.

S3-P15

Phytostabilization of Contaminated Soils in the Puchuncaví Valley, Central Chile

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Mediterranean ecosystems of the Puchuncaví valley (central Chile) have been exposed to massive contamination with sulfur dioxide and metal-rich particles due to emissions from the Ventanas copper smelter. The soils in the areas near the smelter are now sparsely-vegetated, severely-eroded, acid, and metal-contaminated. Phytostabilization is defined as use of plants to stabilize contaminated soil and reduce the movement of toxic elements from the soil to the environment. The objective of the study was to determine the effectiveness of lime and fertilizers for establishment and growth of *Oenothera affinis*, a local species that is tolerant to

metals. We carried out a two-year field experiment. The studied topsoils exhibited pH in the range of 4.0-4.5 (in salt extracts). Spatial distribution of total Cu concentrations was very heterogeneous in the studied topsoils, in the range of 310-750 mg/kg. The field plot was divided into 5 sub-plots of about 50 m² each. Two controls and three treatments were used: (1) calcium hydroxide [Ca(OH)₂], (2) nitrogen, phosphorus, and potassium fertilizers (NPK), and (3) Ca(OH)₂ + NPK. *Oenothera affinis* was propagated and transplanted into experimental plots. At the end of the first life cycle (after 6 months of growth), plants at each sub-plot were harvested, and shoot biomass was determined. During the second year of the experiment, natural growth of *Oenothera affinis* seedlings was monitored. Shoot biomass production and natural seedlings establishment were considerably higher in the case of the Ca(OH)₂ treatment in comparison to the control. In contrast, the NPK treatment did not produce any effect. The effect of Ca(OH)₂ + NPK was similar to that of Ca(OH)₂ alone. The data demonstrate a possibility of revegetation of contaminated soils of Puchuncaví valley by *Oenothera affinis* using lime, a low-cost agricultural amendment.

Acknowledgment: This study is funded by the FONDECYT projects 1050403 and 1085005.

Keywords: *Oenothera affinis*; phytostabilization; metal contaminated soil.

S3-P16

Effects of a Long-term Mercury Contamination on Soil Biological Properties as Assessed by Combined: Chemical, Biochemical, Ecotoxicological, and Molecular Methods

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Mercury (Hg) is a non-essential heavy metal that can pose serious risks to human beings and living organisms. However, its bioavailability and toxic effects are largely influenced by speciation. In the last years, combined chemical, biological and ecotoxicological studies on contaminated soils have increased importance to predict metal fate in the environment. The results obtained with these different approaches can be then used and compared to better categorize potential risk. In this research, different soil samples characterized by a long-term Hg-contamination were collected at two depths (0-10 and 40-50 cm) nearby an industrial area in the South of Italy ("Val Basento", Basilicata, Italy). These samples were studied for Hg total content, fractionation, phytotoxicity and influence on the bacterial community. Hg contamination ranged from 1 to 50 mg kg⁻¹ and most of it was speciated in scarcely soluble forms (eg., HgS, Hg₂Cl₂, Hg), as assessed by sequential extractions and thermal desorption experiments. Hg in soil is known to have a deleterious effect on the number of bacteria, microbial biomass and activities, and diversity within bacterial populations. Anyway, in these soils, the biochemical quality indexes investigated (biomass, different enzyme activities) apparently were not influenced by the different degrees of Hg contamination. Differently, the addition of Hg in soluble form (HgCl₂) showed a marked effect on these indexes. In addition, a quantitative approach based on enumeration of viable heterotrophic bacteria (VH) and mercury resistant bacteria (Hg^R) was used to analyze the

structure of the bacterial communities of the soils and to follow population's dynamics to the addition of HgCl_2 . Again, the number of VH in soils as well as the functional diversity investigated by colony development on solid TSA diluted medium, were not related to the Hg content of soils. The investigated soils exhibited low percentage of Hg^R resistant bacteria ranging from less than 0.001% to 0.25 % and mercury addition induced an increase in the number of Hg^R bacteria only in some studied soils, suggesting that in a soil, bacteria are not exposed to the same level of the toxicant. Gram-positive and Gram-negative Hg^R resistant bacteria capable to grow in a minimal medium containing HgCl_2 were also isolated and in some of them the presence of *MerA* and *MerB* genes was detected. Differently from bacteria, some influence of Hg concentration on seeds germination and root elongation was observed for *Lepidium sativum* L. and *Lycopersicon esculentum* L.. Probably, chelating agents exuded by plants can mobilize Hg thus increasing its bioavailability for root uptake. As a confirmation of this, spontaneous flora (*Glycyrhiza glabra* L.) collected in the investigated area showed non negligible amounts of Hg in the root system. In conclusion, the combination of the data obtained in this study showed that, despite a quite high Hg concentration, most of the Hg in these long-term contaminated soils was scarcely mobile and available and did not significantly influence the soil bacterial community. However, particular attention should be paid to Hg potential remobilisation that could be naturally favoured by the activity of plant roots.

Keywords: Mercury contamination; heterotrophic bacteria; *Glycyrhiza glabra*.

S3-P17

Effect of Native and Exogenous Mycorrhizal Fungi on the Growth of *Oenothera Affinis* and Glomalin at Increasing Copper Levels

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Pollution of natural ecosystems by mining activities is associated with the discharge of heavy metals (HM) enriched wastes. In these conditions of environmental stress, the arbuscular mycorrhizal fungi (AMF) may promote the establishment and growth of plants through the formation of arbuscular mycorrhizas. In addition, the glomalin, a glycoprotein produced by AMF and released to the soil in large amounts, can sequester and immobilize HM in the soil, improving the restoration of a plant cover through bioremediation technologies. The aim of this study was to analyze the effect of the mycorrhizal symbiosis and the type of AMF on the growth of *Oenothera affinis* and the accumulation of glomalin at increasing soil Cu levels. For this, seedlings of *O. affinis* were grown in: i) rhizospheric soil of *O. Affinis* with the native AMF inoculum (+M); ii) soil inoculated with *Glomus claroideum* (GC), and iii) sterile soil (-M). The growing substrate was sterile soil from a Mediterranean ecosystem near Ventanas smelter (Puchuncaví Valley, Valparaíso, Central Chile), which presented 178 mg kg^{-1} of available DTPA-Cu. This soil, was used to form seven increasing Cu levels, which consisted of 0, without soil (a mixture of sand and sepiolite); 1, soil diluted with sand and sepiolite (1:1:1); 2, soil undiluted; 3, soil with 75 mg Cu kg^{-1} (as CuSO_4 solution); 4, soil with $150 \text{ mg Cu kg}^{-1}$; 5, soil with $225 \text{ mg Cu kg}^{-1}$ and; 6, soil with $300 \text{ mg Cu kg}^{-1}$. The glomalin was determined as glomalin-related soil protein (GRSP), obtained as total (T-GRSP) and easily extractable (EE-GRSP) fractions and quantified by Bradford assay. The results showed a strong decrease in shoot biomass

production in all the AMF treatments used until level 3 (from 1.7-1.4 g pot⁻¹ at level 0 to 0.5-0.6 g pot⁻¹ in level 3). However, levels copper n°4 showed an increase in shoot biomass, which was higher in the treatments +M, reaching up to 1.1 g pot⁻¹ at level 6. Similar results were observed in root biomass production, but in -M was not observed an increase in root biomass at level 4 on higher (between 0.3-0.4 g pot⁻¹). At the other hand, the glomalin production was higher in all the Cu levels in the GC treatments (amounts of 25-30 mg T-GRSP g⁻¹ soil), compared to + M and -M (up to 25 mg g⁻¹ soil). At 0 levels of -M and +M both GRSP fractions represent 30-50% of the GRSP observed in the other levels, but all levels exhibit a similar GRSP amount in GC. These results suggest a greater adaptation of native AMF from the Cu polluted soil to high exogenous levels of this element, contributing to promote the *O. affinis* growth even at extreme Cu concentrations in the soil. In addition, higher levels of GRSP in GC suggest that their release to the soil could be one of the more important mechanisms by which this unadapted fungus can tolerate high Cu concentrations. However, further studies are needed to explain the divergent behavior observed at moderate and extreme Cu concentrations, and what are the fungi into the natural communities that provide the greatest benefits for its further use in bioremediation programs.

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Keywords: Mycorrhizal fungi; glomalin; copper.

S3-P18

Determination of Carbon Sequestration and Nitrate Leaching Under Different Irrigation and Dairy Manure Levels in Wheat (*Triticum aestivum* L.) on Typic Calciagris

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Nitrogen (N) losses from agriculture are negatively impacting groundwater, air, and surface water quality. New tools are needed to quickly assess these losses and provide nutrient managers and conservationists with effective tools to assess the effects of current and alternative management practices on N loss pathways. A field experiment was conducted to study the effect of different levels of irrigation and Farmyard manure on nitrate leaching in wheat (*Triticum aestivum* L.). Recommended dose of NPK Fertilizers was applied. One third dose of N and full doses of P and K was applied at the time of sowing and the remaining dose of N will be applied at second and third irrigation. Farmyard manure was applied (0, 150 and 300 kg ha⁻¹) in combination with different irrigation and nitrogen levels. A control of nitrogen and FYM was included for comparison. First treatment regarding Irrigation was applied after 30, 80 and 120 days. Second and third treatments of irrigation are applied after 30, 60, 90, 125 and 21, 42, 60, 80, 95, 115 days simultaneously. Samples for nitrate analysis at depth of 0-5, 5-10, 10-30, 30-60, 60-90 cm was taken out at initial and final stages of experiment. Carbon sequestration is analyzed at the end of experiment. The data obtained will be analyzed statistically by using Fisher's analysis of variance technique and difference among treatment means will be compared by using least significant difference test (LSD) at 5% probability level.

Keywords: Carbon sequestration; nitrate leaching; farmyard manure.

Session 3. Soil-Root-Microbe Interaction & Their Effects on Biophysical Transformation, Fate, and Toxicity of Metal and Metalloids

S3-P19

Propericiazine as a Reagent for the Spectrophotometric Determination of Osmium

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Propericiazine (PPC) is proposed as a new sensitive and selective reagent for the spectrophotometric determination of microgram amounts of osmium. PPC forms an orange red coloured species with osmium (VIII) or osmium (VI) instantaneously at room temperature in 6M hydrochloric acid medium. The orange red species exhibits maximum absorbance at 511 nm. Beer's law is valid over the concentration range 0.1-8.0 ppm for osmium (VIII) and 0.2-10 ppm for osmium (VI). Sandell's sensitivity of the reaction is 9.4 ng cm⁻² for osmium (VIII) and 13.5 ng cm⁻² for osmium (VI). The effects of time, temperature, acidity, order of addition of reagents, reagent concentration and diverse ions are investigated. The application of the proposed method in the determination of osmium content in synthetic ores and minerals has been explored.

Keywords: Propericiazine; osmium; synthetic ores.

S3-P20

Competitive Heavy Metal Sorption by Different Soils

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Heavy metals in the environment are posing significant threats to human health and the environment. In recent years, increased anthropogenic inputs of heavy metals in terrestrial environments have caused considerable concern relative to their impact on groundwater contamination. Soils are an important sink for these metals due to soils high metal retention capacities. Heavy metals are consistently, and simultaneously, added to soils in increasing quantities in the form of fertilizers, pesticides, livestock manures, sewage sludge, and industrial emissions. Many of these metals show moderate mobility under relatively acid soil conditions (pH 5-7) because of increased solubility and formation of soluble complexes with organic ligands. Two soils from Galicia (Spain) were selected. S-1 is developed in a serpentine-amphibolite area. Their pH is 6.9; the organic matter content is 52.59 mg kg⁻¹ and the CECe is 25.93 cmol kg⁻¹. The parent matter of S-2 is gneis, their pH is 5; the organic matter content is 67.59 mg kg⁻¹ and the CECe is 2.13 cmol kg⁻¹. Twelve solutions of mixtures of Cd, Cu and Pb nitrates (between 0.1 y 3 mmol L⁻¹) were prepared to obtain sorption isotherms. Six grams of soil samples were treated with 100 mL of solution, shaken for 24 hours at 25°C. After centrifugation, metal in solution was determined by ICP-OES. In this solution also they were analyzed the cations of the cationic exchange capacity (Al, Ca, K, Mg and Na). All of the experiments were performed in triplicate. The Cu and Pb sorption isotherms are curves tipe H in both soils. The obtained Cd isotherm in S-1 shows an initial

Session 3. Soil-Root-Microbe Interaction & Their Effects on Biophysical Transformation, Fate, and Toxicity of Metal and Metalloids

slope similar to the one of an H curve, but Cd sorbed amounts diminish in the most concentrated equilibrium solutions. The Cd sorption isotherm of S-2 shows an irregular curve and it is not similar to the ones established in the bibliography. In both soils Cd is the metal sorbed in least amounts, so that Cu and Pb compete favourably for sorption binding sites. S-1 sorbs highest heavy metals amounts than S-2. This fact is probably due to S-1 has a pH higher than the one of S-2. In addition the CEC_e of S-1 is higher than the one of S-2 and this factor also influences the heavy metal sorption capacity. Attending to the highest experimental sorbed amounts of each one of the studied metals, S-1 sorbs higher Cu amounts than Pb whereas S-2 sorbs higher Pb amounts than Cu. The amounts of each one exchangeable cations released in the sorption experiments were studied and compared with the amounts of sorbed heavy metals. Mg is the exchangeable cation that is in highest amounts in the sorption equilibrium solution of S-1 whereas Al is the exchangeable cation in highest proportions in the sorption equilibrium solution of S-2. Mg and Al are respectively the most abundant exchangeable cations in the CEC_e of the soils S-1 and S-2. So that, Pb and Cu are compete for sorption binding sites. The higher or lesser Cu and Pb sorbed amounts will depend mainly on soils pH and on their cationic exchange capacity.

Key words: Sorption, Metals, Soil.

S3-P21

Selection of Copper Resistant Bacteria in Copper-polluted Agricultural Soils from Central Chile

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Copper mining is the most important economic activity in Chile. Significant increases in copper concentrations in soils have been reported in agricultural areas located in the vicinity of mining activities. Copper is an essential micronutrient to all organisms but it can be toxic to plants and soil organisms at elevated concentrations. Tight control of intracellular copper concentration is thus necessary. To meet this challenge, bacteria have developed copper homeostasis and resistance mechanisms. It has been generally assumed that soil bacteria are more sensitive to metal stress than other soil organisms. For that reason, they may represent a relevant indicator for copper pollution in agricultural soils. The aim of the present study was to assess the effects of high copper concentrations on soil bacterial communities and to study the bacterial tolerance to metal pollution. To accomplish this goal, we used culture-dependent (isolation of copper resistant strains) and culture-independent approaches (ribosomal RNA intergenic spacer analysis [RISA]; PCR detection of copper resistance genes) for the examination of the bacterial community structure. Composite soil samples were collected from five sampling sites, located at variable distances from copper smelters. Soils presented variable total copper concentrations, ranging from 130 to 580 mg/kg. Soils with higher total copper concentrations (from 470 to 580 mg/kg) were located near copper smelters. Analyses of RISA fingerprint patterns suggested a higher bacterial diversity in control soils (< 165 mg/kg) compared to soils with high copper concentration. Predominant RISA bands were

observed in contaminated soils, probably related to bacterial groups selected by their metal resistance. Copper resistant bacteria were isolated from contaminated soils and presented a great metal tolerance (up to 275 mg/kg). Analysis by electron microscopy showed changes in the outer envelope and intracellular copper accumulation in some resistant strains. Detection of *copA* (copper transporting ATPase) and *pcoA* genes (multi-copper oxidase) in resistant strains suggests copper efflux and/or sequestration as resistance mechanisms. Our results suggest that soil bacterial communities respond to high copper concentration exposure by changes in microbial community structure and a selection of resistant strains. Future research will focus both on the evaluation of copper accumulation and the search of other genetic determinants involved in copper tolerance in resistant strains.

Acknowledgments: MILLENIUM P04/007-F and USM 130836 grants.

Keywords: Copper Resistant Bacteria; *copA* and *pcoA* genes; resistance mechanisms.

S3-P22

Competitive Adsorption of Copper and Lead on Volcanic Soil

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Chilean soils are characterized by their heterogeneous components due to the various interactions of their parental materials; which is dependent on the climatic conditions along the country. Volcanic soils represent a 70 % of the total agronomic land. The heavy metals retention depends on the nature and relative abundance of mineralized and organic phases which according to the geochemical soil solutions; control the adsorption processes and also regulates the transport of contaminants to another environmental compartments. The metal ion characteristics as size, charge and hydrated ion size are another factors to be considered in the competitive interactions between them and with others solution species for specific position or adsorption site. Ever more, the different reactive phases may offer adsorption positions of varied bonding energy to the solution species. The competitive adsorptions processes are relevant because heavy metals presence as nature or contaminant elements can be found simultaneously in the soil systems. Bioavailability of heavy metals is the potential fractions constituted by a soluble and absorbed metal content by plant species. The metal Bioavailability depends on its solubility and the adsorption capacity the soil colloidal fractions. Unfertilized Ralun Soil (41° 32' S 73° 05' W), classified as Andisol, was studied the more relevant chemical characteristic are: high content of organic matter, high cationic interchange capacity and high water retention content. The soil was characterized using the standard methods. Monocomponent and bicomponent adsorption isotherms was realized using bath experiment were studied by scanning electron microscopy (SEM) and x-ray diffractions were used to monitor Cu, Pb adsorptions on Ralun Soil and Microprobe analysis and microprobe profiles were made with a Link Isis energy dispersive spectrometry (EDS) micro analytical system on the SEM. From the studies realized can be appreciated that the adsorptions increment follows the sequence: Pb > Cu. The isotherm fits Freundlich and Langmuir models.

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Keywords: Competitive adsorption; volcanic soil; heavy metals.

S3-P23**Selenium-Induced Amelioration of the Oxidative Stress Triggered by Aluminium in Ryegrass Plants****P. Cartes^{1*}, A.A. Jara² and M.L. Mora²**¹*Instituto de Agroindustria,* ²*Departamento de Ciencias Químicas. Universidad de La Frontera, Temuco, Chile.*E-mail: pcartes@ufro.cl*

Aluminium (Al) toxicity is one of the major factors that limit the production and quality of pastures in Andisols of Southern Chile. Although Al is a non-transition metal, it has been suggested that Al induce oxidative stress in plants (i.e. Ezaki *et al.*, 2000; Yamamoto *et al.*, 2001). On the other hand, the protective role of selenium (Se) against oxidative damage in higher plants (i.e. Hartikainen *et al.*, 2005; Cartes *et al.*, 2005; Mora *et al.*, 2008), suggest an enhancement of the plant antioxidant properties due to Se additions under Al stress conditions. In order to evaluate the ability of Se to ameliorate the Al- induced oxidative stress in higher plants, ryegrass (*Lolium perenne* cv. Nui) was cultivated in a nutrient solution containing Al (0 or 200 µM) and Se (0 to 10 µM, as Na₂SeO₃) at pH 4.8. Al toxicity negatively affected the yield and the antioxidant system of ryegrass, and this effect was more apparent in the roots than in the shoots tissues. Aluminium addition of 0.2 mM decreased the dry matter (DM) production in about 17%, whereas Se additions up to 0.2 µM significantly increased the yield of plants treated with 0 and 0.2 mM Al. Aluminium- treated plants accumulated more Al in the roots than in the shoots, and a raise of Al concentration in the plant tissues was observed by effect of Se additions. Furthermore, Se concentration in roots and shoots was steadily increased at increasing Se supply levels, and the highest concentrations were registered in Al-stressed plants. In general, the addition of 0.2 mM Al activated the antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD) and ascorbate peroxidase (APX) in roots and shoots. When supplied at rates up to 2 µM, Se ameliorated the oxidative stress by decreasing the lipid peroxidation (TBARS), and the most noticeable reduction of damage of cellular membranes occurred plants supplied with Se and Al. The Se-dependent decrease in TBARS accumulation was accompanied by 50% decrease of the root SOD activity in Al- treated plants. This fact suggests that Se may counteract Al-induced oxidative stress by increasing the spontaneous dismutation of the superoxide anions produced under Al stress conditions. Furthermore, Al-treated plants showed thresholds for the reduction of lipid peroxidation at Se concentrations higher than those of plants supplied with only Se. This fact denotes the highest requirement of Se of Al-stressed plants to counteract the oxidative stress induced by Al toxicity.

Acknowledgements: PSD 26 CONICYT-UFRO and FONDECYT 1061262**Keywords:** Aluminium; Ryegrass; Selenium.**References**

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S3-P24**Short-term Aluminium Exposure Induced Rapid Changes in Proline, Carbohydrates and Aluminum Concentration in *Ugni molinae* and *Vaccinium corymbosum*****M. Reyes-Díaz^{1*}, Y. Rojas-Lillo², C. Inostroza-Blancheteau³, M. Alberdi⁴ and M.L. Mora⁴**¹Instituto de Agroindustria, Universidad de La Frontera, Casilla 54-D, Temuco. ²Programa de Magister en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile. ³Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Chile. ⁴Departamento de Ciencias Químicas, Universidad de La Frontera, Casilla 54-D, Temuco, Chile.*E-mail: reyesm@ufro.cl

Aluminum (Al) toxicity is a major agronomic problem in acid soils. In southern Chile, soils derived from volcanic ash (Andisols) with an acid pH are frequent. Currently, the most preponderant fruticultural activity in this region is blueberry cultivation (*Vaccinium corymbosum* L.). In this region also a native berry grows naturally (*Ugni molinae* L.). This species seems to be adapted to the Andisol conditions, being of great interest to know its Al response mechanisms and to compare with those of cultivated species such as blueberry. Thus, our aim was to investigate at the short term the uptake of Al and its effect on proline and carbohydrate concentrations in both species. Brigitta, Legacy and Bluegold cultivars of *V. corymbosum* and four ecotypes of *U. molinae* (Toltén, T. Schmidt, Carahue and Curacautín) were grown in a greenhouse in hydroponic solutions containing different Al concentrations for 0-48 h and allowed to recover. Previous own results indicated that Toltén ecotype of *U. molinae* was tolerant to Al toxicity, while Brigitta and Legacy cultivars were resistant and Bluegold cultivar was sensitive to this stress. The results showed that the Al accumulation was higher in roots than in leaves for both species, observing different patterns in Al uptake and transport to shoots. At the end of the Al treatment carbohydrate and proline concentrations in leaves decreased (19-48%) in Toltén and Carahue ecotypes of *U. molinae*, showing a posterior recovery. Contrarily, in leaves of cultivar Bluegold an increase of around 50% in carbohydrates and proline was found, maintaining similar values than the control by recovery. In *U. molinae* roots, carbohydrate concentrations practically did not varied during the treatments, whereas in Brigitta cultivar roots an increase (39%) was found at 24 h. No changes in proline concentrations of roots were found in cultivars and ecotypes, with exception of Bluegold cultivar and Carahue ecotype of *U. molinae* where an increase was observed. Statistically significant negative correlations between Al uptake and carbohydrates and proline were found in roots of Legacy cultivar and in leaves only with carbohydrates, whereas positive correlations between Al uptake and proline were found in roots of T. Schmidt and Curacautín ecotypes ($P < 0.01$). We concluded that the metabolites changes induced by Al did not correspond well with the degree of Al resistant of the investigated plants.

Acknowledgements: This study was supported by Bicentenario PSD-26 and Fondecyt 1080372.**Keywords:** *Vaccinium corymbosum*; *Ugni molinae*; aluminium.

S3-P25**Evaluation of Some Biological Activities in Four Landfill Municipal Soil from Southern Chile****M. Alvear^{1,2*}, M. Arriagada², P. Quezada² and Y. Sandoval²**¹*Departamento de Ciencias Químicas, Universidad de La Frontera, Temuco, Chile;* ²*Asociación de Municipalidades del Valle Central de la Región de La Araucanía (AMVACEN).***E-mail: malvear@ufro.cl*

In landfill soils, where the pollution is usually going on for many years is very important know about soil quality status. Microbial biomass and some hydrolytic enzyme activities are important soil quality indicators. A study was conducted to determine the effect of municipal solid wastes (MSW) on four landfill soils from southern Chile considering four situation: with MSW freshly near and far the methane columns sites, also with two and four years without received MSW sites as well as four exotic forest soil near the each landfill as "control". Both Lautaro's and Curacautín's landfills received about 12.6 tons; Victoria's landfill received 6.1 tons and Perquenco's landfill received 2.5 tons annually. Overall microbial activity of soil was determined by fluorescein diacetate hydrolyzing activity (FDHA). Active cells carry out such hydrolysis by a combination of enzymes like lipases, proteases and esterase. Active microbial cells transport FDA inside the cell where it is hydrolyzed to polar fluorescein. When the storage capacity of the cells with respect to fluorescein exceeds, it is released out. The FDHA, urease and manganese peroxidase activities were measured spectrophotometrically. All analyses were made in triplicate for each field replicate. All analytical results were calculated on the basis of oven-dried (105°C) weight. The results in all cases evaluated shown FDHA in landfill soils were significantly lower than four exotic forest soils due probably to lower organic matter and higher heavy metals content in landfill sites evaluated. Significant ($P < 0.05$) variations existed in the metal concentrations among the landfill soils and were due to the heterogeneity in the waste materials at the respective sites over the years. Microbial biomass activity was lower and correlated with bioavailable metals. This indicated that it is the bioavailable form, which renders more toxicity compared to the total metals. The metals affected microbial biomass and their activities in combination with each other found that when metals were present in a mixture, they behaved synergistically or additively. The landfill soils also contained significantly amounts of total N and the urease activity was major in sites with fresh MSW. However, manganese peroxidase activity was higher in exotic forest soils than landfill soils. Our results are also in agreement with those reported by other investigators. In conclusion, present study, carried out on landfill sites revealed that the soils of these sites are contaminated with some heavy metals; more particularly the bioavailable forms detrimentally influenced the microbial biomass and enzyme activities in such soils.

Acknowledgments: The authors extend their gratitude to Municipalidades of Lautaro, Perquenco, Curacautín and Victoria for their cooperation in soil sampling.

Keywords: Microbial biomass, Fluorescein diacetate activity, Urease activity, Landfill soil.

S3-P26**Plant Growth Promoting Effect of Selected Autochthonous Microorganisms (bacteria, yeast and/or arbuscular mycorrhizal fungi) in a Heavy Metals Multicontaminated Soil****R. Azcón^{1*}, M.C. Perálvarez¹, A. Roldán² and J.M. Barea¹**

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As plant (clover) development was limited in a heavy metal multicontaminated soil the effect of selected autochthonous microorganisms as bacteria (B), yeast (Y) and arbuscular mycorrhizal (AM) fungi used either as single or dual inoculants, were assayed to ascertain whether these microbial treatment can benefit plant growth, nutrient uptake, and metal translocation. The inoculated microorganisms increased shoot biomass by 148% (B), 162% (Y) and 204% (AM), and root growth, particularly in dual associations, and decreased metals translocation from soil to shoots. Dual inoculations also produced the highest symbiotic (AM colonization and nodulation) rates. The plant growth effect and the metal sequestration or bioaccumulation abilities demonstrated by these microorganisms seem to be involved in such effects. The activity of microbial antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APX) known to play important roles in cell protection against abiotic stress, changed slightly in the culture medium of the bacteria or yeast in presence of metals. Such activities alleviate cellular oxidative damage, which is an important protective mechanism against heavy metal stress. These results are an indication of adaptation to high metal concentration of these autochthonous microorganisms. Thus, they may be considered as an index of microbial metal tolerance. Microbial inoculations showed a bioremediation effect since reduced shoot metal concentration and increased nutrient in plants allowing growth developments and helping plant recovery of the multicontaminated soil. Therefore, the tested mycorrhizosphere interaction could be used as an environmental biotechnology tool for improving plant developments in heavy metal-contaminated environments.

Keywords: Heavy metals; autochthonous microorganisms; plant growth promoting.

S3-P27**Exudation of Organic Acids in Ryegrass and White Clover in Response to Different Phosphorous and Manganese Doses****A. Rosas¹, L. Pinilla^{2*}, D. Menezes-Blackburn² and M.L. Mora²**

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Soil chemical factors that limit root growth in acid soils reduce crop production, including Aluminium (Al) and manganese (Mn) toxicity, and also phosphorus (P) deficiency. Large areas of these grasslands are established in Southern Chile on acidic Andisols with both

ryegrass (*Lolium perenne* L.). The incidence of Mn toxicity depends of ambient factors, plant species, and accompanying fertilizer nutrients. Plants organic acid exudation is related to Al detoxification and P acquisition in the rhizosphere soil. This study investigated the effect of 5 levels of P (50 to 400 μ M) on perennial ryegrass tolerance to excess Mn in 4 levels (50 to 355 μ M). Ryegrass (cv. Nui) plants were cultivated for 30 days in nutrient solutions at pH 4.8, in a growth chamber with a light period of 16 h, temperature of 20°C. The plant response was evaluated through growth, and root exudation of 4 organic acid anions (citrate, malate, succinate and oxalate). Toxicity due to excess Mn supply in interaction with P deficiency resulted in significant reduction in plant dry weight. At highest rate of Mn supply (355 μ M), the ryegrass plants showed a reduction of approximately 50 % in DW compared with treatments 2.4 μ M of Mn. The decrease of the P supply from 96 to 44 μ M for ryegrass caused a significant reduction in DW due to P deficiency. Addition of P had a beneficial effect in reducing the severity of Mn toxicity. Ryegrass plants exuded the highest quantity of organic acids, especially oxalic and citric acid, in conditions of deficiency of P and Mn toxicity, thus enhancing tolerance to Mn toxicity in this forage specie. Oxalic and malic acid exudation rates were 7 fold higher in this condition than in P and Mn sufficient plants. It was observed an increase in exudation of oxalate (more than 80%), malate (89 %), citrate (27 %) and succinate (33%) in comparison with the plant supplied with adequate P and Mn.

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Keywords: Organic Acids; ryegrass and white clover; manganese.

S4-P1**Kinetics of Atrazine Degradation in an Andisol Amended with Liquid Cow Manure**

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The liquid cow manures (LCM) are used as amendments applied for soil, increasing organic matter, nutrient levels and also constitute a form disposal of animal wastes. This contributes to soil fertility, nutrient cycling and to prevent pollution of waters resources as rivers and streams near to the dairy farms. Incorporating LCM includes a high density of microorganisms that are directly involved in stabilization processes in amended soils. This soil amended condition determine, the bioavailability of several pesticides and the effectiveness in their action mechanism. Atrazine is an herbicide that belongs to the triazines chemical group, which experiment various degradation processes. The aim of this study was to establish the effect of the LCM dose and the incubation time of amended andisol on the kinetics of atrazine degradation. The Andisol belong to Los Lagos series, located in Futrono, Los Rios region. The LCM was obtained from the same place. The herbicide corresponded to an atrazine analytical standard (98% purity). Four mixtures of soil with LCM were incubated, applying LCM dose like field conditions: 0 (D0), 100 (D1), 200 (D2) and 300 thousand L ha⁻¹ (D3), during 1, 5, 10, 20 and 30 days. Then, atrazine was applied in field dose (3 Lha⁻¹). The kinetics of degradation was evaluated at 1, 5, 10, 20 and 30 days. Atrazine was quantified using high performance liquid chromatography (HPLC). This study showed that atrazine degradation is strongly influenced by the incubation time of mixtures soil and LCM and LCM dose. Amended soils with one day of incubation showed a high amount of extractable atrazine, suggesting that the microorganisms used initially organic material and N available in soil. At 10 and 20 days of incubation the amended soils presented a lower percentage of extractable atrazine than soil. It could indicate that alongside other process atrazine would be degraded. At 30 days of incubation there are not significant differences of extractable atrazine respect to the unamended soil. In general, D3 mixture showed a lower amount of extractable atrazine, probably due to adsorption processes that involve dissolving organic matter from LCM. These results suggest that the incubation time is a fundamental condition to atrazine effectiveness, and then atrazine could not be applied before 30 days from LCM disposal on soil. Probably, the LCM influence atrazine degradation due to, both nutrients and microorganisms present in it. Another important effect on bioavailability of atrazine is produce by a high amount of dissolved organic matter that is incorporate when excessively LCM disposal on soil was realized.

Acknowledgments: FONDECYT 1070568 and DI08-2014 projects.

Keywords: Atrazine kinetics degradation; Andisols; cow manure.

S4-P2**Effect of Incubation on the Distribution of Phosphorus Chemical Forms in two Andisols treated with Sewage Sludge****K. Manquían^{1*}, N. Arancibia¹, M. Molina², J.E. Förster¹ and M. Escudey¹**

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The objective of this work was to evaluate the effect of incubation on phosphorus (P) chemical forms in two Andisols (Nueva Braunau and Diguillin) treated with sewage sludge (SS). Incubation was carried out in pots of 10-cm diameter and 8-cm length filled with 625 cc of soil. Treatment involves a mixture of soil with SS (from El Trebal wastewater treatment plant). Sewage sludge was applied at a rate equivalent to 15 ton ha⁻¹ and 30 ton ha⁻¹ according to NCh2880 for agricultural soils and degraded soils respectively. A third treatment with a SS application of 60 ton ha⁻¹ was also included. All pots were incubated at 25°C for four months and watered weekly to keep the soils close to their water holding capacity. All pots were allowed to drain freely. Experiments were carried out in duplicate. After the incubation period, the P chemical forms were obtained by the sequential extraction procedure proposed by Steward and Oades and modified by Borie and Zunino for Chilean soils derived from volcanic materials. Before the experiment, the SS material underwent the same procedure. In addition, the available P was determined in all samples by the Olsen method. The results indicated that after the incubation the soils showed changes in P forms. The P mass balance showed that as result of SS application a higher amount the P was mineralized as compared to the control, in both soils. On the other hand, the residual P content in both soil decreased (69 to 88%), indicating that the incubation and the SS amendment contributed to the solubilization of recalcitrant P forms into more labile forms. The higher the SS rate, the higher the available P after the incubation. The theoretical sum of the available P of the soil and the SS before the incubation is lower than the experimental available P after the experiment. The difference between the theoretical and the experimental measurement increases with the SS rate. These trends were found in both soils. As a result of the changes in the P chemical forms, higher amount of this element is potentially available in the soil for plant growth.

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Keywords: Andisols; sewage sludge; phosphorus.

S4-P3**Dehydrogenase Activity during Bioremediation Process within a Venezuelan Savannah Soil Polluted by Mineral Oil-Based Drilling Cuttings****M. Arias***, P. Colombo, C. Rivas and M. Salcedo*PDVSA INTEVEP. Urb. Santa Rosa, Sector El Tambor, Los Teques, Miranda State, Venezuela.***E-mail: ariasdem@pdvsa.com*

Dehydrogenase activity is associated to microbes breathing processes, which is believed to be more dependent on both metabolic status and general biological activity than any other enzymes within the soil. This makes dehydrogenase enzyme a good microbial activity indicator in the bioremediation process of drilling cuttings polluted soils. The aim of this work is to assess dehydrogenase enzymatic activity as an indirect measurement of soil microbial communities capacity to transform biodegradable hydrocarbons (saturated & aromatics). Soil and mineral oil-based drilling cuttings were mixed up to obtain Oil and Greases (O&G) concentrations about 2, 3, 4 and 5% w/w . For cuttings treatment, worm humus (5% w/w), manure (1.5% w/w) and P:N 32:60 gm^{-2} fertilizers ratio were added. As a control, cuttingsless soil with the same organic conditioners proportions and P:N 8:15 ($g \times m^2$) fertilizers ratio was used. Mixtures were put within plastic cases during 124 days. Both dehydrogenase enzymatic activity and biodegradable hydrocarbons content changes within mineral oil-based drilling cuttings-soil mixtures were measured. Biodegradable hydrocarbons remotion of all mixtures reached about 50% for each different cuttings concentrations for 160 days. After assessment period, significant differences in dehydrogenase enzymatic activity for the evaluated O&G concentrations regarding to control soil ($p < 0.05$) were found for 124 days. Results suggest that dehydrogenase enzymatic activity during hydrocarbons biodegradation process decreases organic fractions degradation itself.

Keywords: enzymatic activity, drilling cuttings, worm humus.

S4-P4**Soil Degradation in South Asia with Special Reference to Iran- A Case Study of Natural Hazard****N. Bassirani****Department of soil science Zabol University, Zabol, I. R. Iran. *E-mail: N_Bassirani@yahoo.com*

As a result of the geographical and ecological situation of Iran, as well as the blowing of unfavorable winds in the Central Desert, more than 80% of the 164 million hectare area of the country is subject to the conditions of arid and semi arid regions, characterized by low precipitation, ranged between 50 to 250 mm per year. At the present the area of the country's deserts and sandy soils is estimated to be 34 million ha, and that of the poor and desertified rangelands which 12 million ha correspond to the sandy, soils 5 million of the same shifting sand dunes. Historical evidences and works remained from the ancient time testify the fact that these regions were covered by desert forests and plants, which were annihilated because of the wrong and excessive exploitation, the result of which has been the diminution of the vegetation resources and the conversion of the lands into deserts. It is estimated that the trends of desertification in the country is about 1% annually. Land degradation causes \$10 billion loss to South Asia annually. The environmental impact on the countries of the South by soil erosion and other forms of land

degradation is all too familiar. A recent United Nations study shows the high economic cost of such land degradations to the South Asian countries. Soil is one of the most vital of our natural resources. Together with water and air, it forms the very basis of life. Indeed, there is a thesis, quite difficult to refute, that the rise and fall of civilizations in history can be linked to the quality and management of their soil and land. For from the soil comes food and other crops and plants that provide medicine, clothing and so many other things we need for daily life. And the retention of soil in its natural state and habitat prevents erosion, river silting and flooding. When land is disturbed or degraded, the ecology is damaged. There can be rather serious effects in terms of soil erosion, loss of soil fertility and thus reduced plant growth or crop productivity, clogging up of rivers and drainage systems, extensive floods and water shortages. Environmentalists are familiar with the above cause-and-effect scenario. City planners and water and soil policy makers have to deal with the problem. And ordinary people experience and live in dread of the bad effects. But soil degradation and erosion is also an economic issue. The loss of soil and its effects cause tremendous losses to the economy. So much so that these costs may well outweigh the benefits of many development projects that give rise to the problem. If only the economic costs of soil erosion (and other environmental ills) were easily calculable. Then planners and politicians would think twice or thrice before allowing activities and projects that damage the land.

Keywords: Soil, Degradation, Natural Hazard, Iran.

S4-P5

Use of Free and Immobilized Laccase for the Decontamination of Soil Polluted with 2,4-Dichlorophenol

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Enzymatic treatment is currently considered an alternative method for the removal of toxic xenobiotics from the environment. Laboratory experiments demonstrated that the application of various phenoloxidases, such as peroxidase, laccase or tyrosinase, may lead to a complete decontamination of water polluted with phenols and anilines. The feasibility of soil treatment was demonstrated using a laccase from *Trametes villosa*. The soil was purposely polluted with ¹⁴C-labeled 2,4-dichlorophenol (2,4-DCP) and incubated with the free laccase or the enzyme immobilized on montmorillonite. After 14-day incubation, the binding amounted to 82% and 77% of the initial radioactivity for immobilized and free laccase, respectively. It is noteworthy to emphasize that free laccase proved to be very stable in soil. When aged in soil for 14 days before the addition of 2,4-DCP, the enzyme was able to immobilize 69% of 2,4-DCP. For comparison, immobilized laccase caused a 61% binding after the same aging period. In view of the results, laccase from *Trametes villosa* does not appear to require costly immobilization procedures before it is applied for soil remediation.

Keywords: Laccase; 2,4-dichlorophenol; *Trametes villosa*.

S4-P6**Degradation of Atrazine in an Agricultural Andisol Amended with Liquid Cow Manure****G. Briceño^{*1}, R. Demanet², M.L.Mora³ and G. Palma³**

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Application of animal manure amendments to agricultural soils is a common practice to improve soil fertility through the addition of essential plant nutrients. This practice may increase the potential for pesticides degradation by microorganisms with capacity to decompose these compounds. Andisols in Chile are generally cropped to maize (*Zea mays* L.) and their management includes the application of liquid cow manure (LCM) at rates higher than 100,000 L ha⁻¹, and the application of atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine) for broad leaf weed control. The application of LCM has produced an increase in weed populations, which has caused farmers to increase rates of atrazine application. In this work, we evaluated the influence of LCM application on atrazine degradation in an Andisol used for maize crop. The LCM was applied at rates equivalent to 0, 100,000, 200,000, and 300,000 L ha⁻¹, resulting in treatments S-0, S-100, S-200, and S-300, respectively. In the laboratory an aerobic incubation was conducted for 30 days. Then, was applied atrazine in concentrations of 0, 1, 2, and 3 mg kg⁻¹. The incubation was realized for 80 days and during this time were determined the residues of atrazine and the main degradation products by HPLC. Chemical analysis of atrazine residues revealed that the herbicide was rapidly degraded after 20 days in all studied soils. The residues of atrazine were from 7 to 13% and 1 to 5% for the days 20 and 40 respectively. Finally after 80 days were not detected residues of atrazine. Results showed that hydroxyatrazine (HA), deethylatrazine (DEA) and deisopropilatrazine (DIA) were detected in the extractable fraction of the amended and unamended soils. After 20 days of incubation, the highest levels were founded for DIA and HA, while that for day 40 of incubation were detected each one of the products but in lower concentration. According to the obtained results, the applications of LCM not modify the degradation of atrazine in the studied soil due to a stabilization of soil after 30 days of the LCM application. Atrazine in all the soils was rapidly degraded probably due to the existence of microorganisms adapted to the atrazine degradation.

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Keywords: Atrazine; andisol; liquid cow manure.

S4-P7**Effect of Atrazine in Enzyme Activity of an Andisol with Application of Liquid Cow Manure**

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Application of liquid cow manure on soil for agricultural use increase the organic matter content and constitute an important input of nutrients into the soil, increasing microbial activity through the direct addition of nutrients and microorganisms. Moreover these conditions cause the invasion of weeds that leads to increase the herbicide application dose, both pre-plant and postemergent herbicides, especially in cropped to maize, with the consequent environmental impact. The aims of this study are to assess the effect of dose of liquid cow manure (LCM) in the biological processes that modify the behavior of atrazine in the soil-slurry, determining time of stabilization of amended soil. The fluorescein diacetate activity (FDA) and the enzyme β -glucosidase activity are used to measure soil microbial activity. The soil used was an Andisol, belonging to Los Lagos series in Futrono. The liquid cow manure was obtained from the same place. The herbicide is an analytical standard of atrazine (98% purity). The LCM was added to soil at 0, 70, 140 and 200 mL kg⁻¹ and thoroughly mixed in polyethylene bags. The LCM dose used corresponded to 0 L ha⁻¹ (S0), 100.000 L ha⁻¹ (S100), 200.000 L ha⁻¹ (S200), and 300.000 L ha⁻¹ (S300). The soils were moistened to 70% of field capacity in a dark room at 20°C. The soils were incubated by 1, 10, 20, 30 days. By fulfilling the incubation time, the atrazine herbicide was applied in a dose field (3 L ha⁻¹). The biological activities are assessed in days 1, 10, 20, 30 for each incubation time. Three replicates for each treatment were used. The effect of liquid cow manure in the soil causes an increase in the hydrolytic activity FDA, due to pool of microorganisms that were added, and it is greater in substrates not stabilized. Besides, an increase in FDA activity can be explained by the toxic effect of some herbicides that cause the death of the microorganisms of soil. The breaking of the cells of the microorganisms killed in the soil, releases endonuclease enzymes; the hydrolase enzymes are the most abundant and therefore one would expect a temporary increase in FDA activity. For more than 20 days the values of FDA are similar to the soil. The herbicide application does not produce significant differences on FDA activity in soil. The β -glucosidase activity increased at the beginning of the experiment for all treatments, being more important to higher dose of liquid manure (S300), because the increase of organic matter that was added from liquid cow manure. But the activity decreased with the incubation time to become similar to soil activity. In treatments with herbicide application was not significant differences for all treatments.

Acknowledgments: Project FONDECYT N° 1070568 and DI08-2014.

Keywords: Atrazine; enzyme activity; andisol.

S4-P8**Microbiological Activity in an Andisol Amended with Dairy Slurry and Dimethenamid Application**

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Dimethenamid is a relatively new pre-sowing herbicide applied in Chilean forage maize crop. Some studies indicate that an important concentration of its degradation products in near groundwater to agricultural lands has been found. Also, livestock slurries are applied in forage maize during soil preparation, which increase both the population and aggressiveness of weeds, then decrease the efficacy and increase the requirements of herbicides. Considering this problematic, the aim of this study is evaluate the role of dairy slurry on microbiological processes that regulates the dimethenamid efficacy in slurry amended soils, in order to optimize its use. In this work both microbial respiration and hydrolysis of fluorescein diacetate activity (FDA) were evaluated on dimethenamid application to soil-slurry mixtures at different incubation times. For the mixtures preparation an Andisol (Los Lagos series) and dairy slurry were used. The slurry was applied in equivalent dose to 100, 200, and 300 thousand L ha⁻¹, also a control without slurry was considered. The mixtures were kept in conditions of incubation to 20°C with moisture control and darkness. The samples of amended soils were taken at different incubation times (1-60 days). At moment of sampling, dimethenamid was applied to every mixture to equivalent field dose of 2 Lha⁻¹. For the CO₂ measurement, the samples were located in closed glass flasks for evaluation by 60 days, whereas for the FDA measurement the samples were incubated again in the previous conditions. The results indicate that when dimethenamid is applied slight differences were observed, both in respiration rate and FDA of amended soil and soil, with regard to the soil without herbicide application. An important rising in CO₂ production and FDA were observed by increasing the slurry doses. The CO₂ rate production increased in the first 10 days and then declined reaching stable values from the 20 day. The FDA was low initially but increasing gradually up to reaching high and stable values from the 20 day. Thus, the effect of dimethenamid in the measured parameters suggest that dimethenamid would present a indicating that its degradation in soil could be principally a co-metabolic route and possibly occur a major quantity of degradation products that it must be evaluated, principally in later periods to 20 days where the hydrolytic activity of soil microorganisms was major, which would favor the production of degradation products.

Acknowledgments: Project FONDECYT 1070568 and DI08-2014.

Keywords: Dairy Slurry; dimethenamid; FDA

S4-P9**Effect of Soil and Biosolid Chemical Properties on Nitrogen Mineralization in Biosolid Treated Mollisol Soils**

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The disposal of biosolids generated by the municipal waste waters plant treatments constitutes an environmental problem. Their disposal on agricultural land every day acquire more relevance, however, in Chile there is not much experience and some research on soil-biosolids interactions are being carried out either *in vitro* conditions or in field experiments. The purpose of this work was to investigate the possible influence of soil and biosolid chemical properties on the nitrogen mineralization in soils from the Metropolitan Region (Chile) treated with biosolids taken from a landfill in a 3-year period. Three soils classified as mollisols were amended with 0 and 30 Mg dry-biosolid per hectare and incubated for 60 days at 28 °C. Biosolids were taken from the landfill twice a year for a period of three years. Soils and biosolids were characterized for pH, electrical conductivity, total N, organic matter, and total and DTPA-extractable heavy metals. Electrical conductivity (EC), pH, inorganic nitrogen (N_i), and DTPA-extractable heavy metals were analyzed after 0 and 60 days of incubation. A principal component analysis (PCA) of the data was performed using the Isfotat software. Before incubation it was possible to: 1) differentiate biosolids according to their chemical properties with more weight and find a negative relation between inorganic (N_i) and high contents of total Cr, Pb and Zn, and a positive relation between N_i and biosolid EC, 2) differentiate soils parting soil M with the lowest pH (6.4) and highest Cu-DTPA content (31.6 mg kg⁻¹). After the 60-day incubation, the soils amended with biosolids BET-1 and BET-2 presented the lowest rate of mineralization which was associated to the lowest biosolids EC (6.79 dS m⁻¹) and the highest total Cr content (335 mg kg⁻¹ of biosolids) in the case of biosolids BET-1, and in BET-2 to the highest EC (24.2 dS m⁻¹) and the highest Zn-DTPA content. (449 mg kg⁻¹ of biosolid). It is concluded that the rate of nitrogen mineralization in biosolid amended soils from the Metropolitan Region would be independent of soil type but it is affected by heavy metals and the electrical conductivity of biosolids. More research should be done related to the effect on nitrogen mineralization of total heavy metal contents in biosolids and bioavailable fractions of heavy metals in biosolid amended soils before considering biosolid application to land.

Acknowledgements: This research was supported by FONDECYT, Project 1080357.

Keywords: Biosolid; Mollisol soils; nitrogen mineralization.

S4-P10**White Rot Fungi: The Organisms Involved in the Carbon Recycling from Lignin as Ecosystem Bioremediators****E. Contreras, L. Sepúlveda and C. Palma****Departamento de Ingeniería Química, Universidad de Santiago de Chile, Santiago, Chile.***E-mail: cpalma@usach.cl*

The white rot fungi (WRF) are the main organisms involved in the carbon recycling of the lignin, which utilize an extracellular oxidative enzymes system. Nevertheless these basidiomycetes causers of the wood-decay, can not use directly the lignin as source of carbon and energy. The ligninolysis process which happens during the secondary metabolism provides bioavailable substrata. Therefore, the WRF produce various isoforms of the extracellular ligninolytic enzymes: laccases (Lac) and different peroxidases, including lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP). This last one brings together the catalytic properties of the LiP and MnP. The capacity of these fungi to mineralise an organic compounds variety offers an indisputable potential for use in bioremediation of ecosystems impacted by recalcitrant synthetic compounds. Additionally, its characteristic growth across the development of the hyphes, allows these to colonize different types of substrata, which also can have access to the soil's pollutants. Investigations in the last decade have shown the potential of the basidiomycetes of white rot and its enzymatic extracts for the xenobiotics pollutants elimination such as chlorinated organic compounds, simple and polycyclic aromatic hydrocarbons, nitro-substituted compounds, and modified polymers and dyes. The goal of this research is to study the enzymatic system capacity of the basidiomycete *Phanerochaete chrysosporium* for the recalcitrant pollutants degradation, especially textile dyes, and its potential use in bioremediation and reduction of environmental impact. The results obtained in *in vivo* culture indicate that the ligninolytic system of *P. chrysosporium* presents a partial or total ability to degrade/decolorize various azo dyes. The screening realized in static culture for the 50 mg l⁻¹ of dye showed decolorizing values between 99% and 60%, which correspond to Acid Black 1 and Reactive Orange 6 respectively. The evaluation of a continuous system with immobilized fungus which was fed with an increasing dye concentration as supplement of the culture medium indicated that the biological system was capable of responding satisfactorily up to 400 mg l⁻¹ for the Acid Black 1 and Reactive Blue 5, obtaining a decolorization greater than 70%. The bioreactor showed a stable operation for a period of the 35 d approximately. On the other hand with the purpose of establishing criteria for an effective operational strategy of dyes biodecolorization based on the application *in vitro* of the extracellular fluid of *P. chrysosporium*, was analyzed the effect of the Mn II concentration and the H₂O₂ dosage in the process kinetics. The decay of the dyes concentration in the time was fitted to an exponential model multistage. In all the cultures, only activity MnP was detected as being identified as the enzyme causer of the decolorizing process. Thermal stability essays of present MnP in the extracellular fluid indicated that the enzyme is stable up to 3 h to 40°C, nevertheless to 50°C was inactivated fastly. Finally it is possible to conclude that the white rot fungi constitute a versatile fungal consortium with high tolerance to toxic substances as the synthetic dyes, which have the potential for its application in the near future, in the bioremediation of wastewaters.

Keywords: White rot fungi; biosolid; carbon recycling; lignin.

S4-P11**Composite of Poly (ϵ -caprolactone)/ Starch/ Coconut Fiber Biodegraded by Fungi****L. Cordi^{1,2*}, A. Borin², P.D. Marcato², R.J. Poppi², L.H.I. Mei¹ and N. Durán²**¹Faculdade de Engenharia Química, UNICAMP, Campinas-SP, P.O. Box 6066, Brazil.²Instituto de Química, UNICAMP, P.O. Box 6154, Campinas-SP, CEP 13083-970, Brazil.

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Natural fiber, considered as disposal, when added into a polymer is possible to enhance the material resistance. The study of biodegradable formulation is important because among the polyhydroxyacids, poly (ϵ -caprolactone) (PCL) is more susceptible to the attack by microorganisms in controlled conditions. There are many organisms that are able to degrade biocomposites, but fungi may play a considerable role in degrading synthetic polyesters. *Fusarium* has many species that are plant and animal parasites. However, this genus is an important pigment producer and it can be applied as bioindicators to characterize contaminated soils by some herbicides as atrazine. *Fusarium* genus is known to produce enzymes as phenoloxidase, besides other enzyme that would be present in the biodegradation process as depolymerase. Then, the aim of this work was to verify the composite biodegradation containing different percentage of powder of coconut fiber in the presence of two fungi. PCL/starch composite containing 5, 10 and 15 % (w/w) of coconut fiber and the biodegradation were monitored by 60 days. After composite processing, an aerobic biodegradation assay of these materials was carried out. Samples of PCL/starch/fiber and fungal inoculum were added in sterile flasks. The experiment was performed at 28°C, 120 rpm for 60 days with *F. oxysporum* 07SD and *F. moniliforme* isolated from soil and kindly give by ESALQ-USP Collection (Superior School of Agronomy, Piracicaba, Brazil). After 60 days of biodegradation, the polymer was removed from the inoculum and gently washed with distilled water, followed by drying. The average molar mass lost and near infrared spectroscopy and morphological investigation of the composite before and after biodegradation was performed using Scanning Electron Microscopy Microscope (SEM) and Atomic Force Microscopy (AFM). The NIR and PCA shows a difference on the biodegradation by fungi between composite PCL/ Starch and Coconut Fiber (containing three different percentage of coconut fiber). The increase of fiber content in the composite was directly proportional to the biodegradation. The same result was observed by the average weight lost evaluation. These results were confirmed by SEM and AFM. Through these techniques was possible observed the fungal attack and the superficial morphology modification, especially the different fiber content. Both fungi present similar composites degradation behavior profile, for the same percentage of coconut fiber without any special culture conditions. However, the best biodegradation result was the composite exhibiting 15% coconut fiber content.

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Keywords: polyhydroxy acids, *Fusarium* genus, phenoloxidases.

S4-P12**Biodegradation of Hydrocarbons in Soils Enhanced by Addition of Hydrophobically Modified Vermiculite****S. Froehner***, E. Cardoso da Luz, J. Zeni and W. Furukawa*Universidade Federal do Paraná, Curso de Engenharia Ambiental, Centro Politécnico, Curitiba, PR, 81531900, Brazil. *E-mail: froehner@ufpr.br*

Due to the increasing of petroleum consumption, the numbers of oil spills also increased. In general, for removing oil and organic compounds from the soil, high cost technology are used. However, biodegradation, in situ or ex-situ, could be an alternative of less costly and more important, it is non aggressive to the environment. It is known that the biological treatment of an oil contaminated soil can be affected by the hydrocarbons (HC) structure and by the weathering processes, which decreases the bioavailability of pollutants to microorganisms. Weathering refers to the result of biological, chemical and physical processes that can affect the type of HC that remain in soil, enhance the sorption of hydrophobic organic contaminants as consequence decreases the rate and extent of biodegradation. As a general rule, only the fraction of HC dissolved in the aqueous phase is available for microbiological degradation while the sorbed fraction has a low bioavailability. The process can be enhanced by supplementing soils with modified clays. The chemical modified clay has hydrophobic environment to accommodate the organic contaminants, and mixed with soils can enhance the degradation process and the same time protect the microorganism community from toxic effects of contaminants. Pure clays do not offer the same hydrophobic properties and pure surfactant is expensive. Here, we investigated the biodegradation of naftalene and antracene in soils mixtured with modified vermiculite, common clay in Brazil. The vermiculite was modified replacing sodium and potassium cations by tetramethylammonium. With this modification on surface of the clay a hydrophobic environment was created to accommodate the naftalene and antracene and consequently increase the biodisponibility of both contaminants. Experiments were conducted mixing soil and VT-HDTMA (modified vermiculite) in proportions of 0, 2, 5, 10%, keeping aerobic conditions. The contaminants were spiked to have an initial concentration of 500 mg Kg⁻¹. Samples were taken every 10 days to analyze the presence of contaminants. The experiment was conducted for 90 days. We observed significant depletion of concentration of naftalene and antracene in those soils that VT-HDTMA was present and the time required for depletion of contaminantes decreased. Also the preference for degradation was for naftalene in all experiment with vermiculite was present in the mixture of soil. Finally, we can conclude that the presence of modified vermiculite can reduce the time required for degradation of organic contaminants. The most acceptable explanation for this observation is the increment of biodisponibility of compounds.

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Keywords: hydrocarbons; modified vermiculite; biodegradation.

S4-P13**Use of Hydrus 1-D to Describe the Transport of Nitrate in a Volcanic Soil Affected by Sewage Sludge, Sewage Sludge Ash, and *Pinus radiata* Ash Amendments****R. Fuentes^{1*}, L. Cáceres¹, M. Molina², S. Aravena¹, M. Cazanga¹, R. Calderón¹ and M. Escudey¹**¹Facultad de Química y Biología, Universidad de Santiago de Chile, Av. B. O'Higgins 3363, Santiago, Chile. ²Departamento de Ciencias Vegetales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Casilla 306 Correo 22, Santiago, Chile.

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Agriculture in Chile is an important activity which may lead to degradation of the soil, especially of volcanic ash derived soils. To be productive, volcanic soils require frequent adjustments of pH, replenishment of exchangeable Mg²⁺, and heavy phosphate applications. Municipal sewage sludge (SS), obtained from wastewater treatment processes, ash of the incinerated sewage sludge (SSA), and ash obtained from a *Pinus radiata* plantation fire (PA), appear to possess essential plant nutrients and dissolved minerals and good buffer capacity. When land applied, they may replenish the depleting nutrient reservoirs in these soils under cultivations. Sewage sludge has the capacity to improve P availability, porosity and hydraulic balance. However, they are also a potential source of pollutants to the soils and groundwater because of their heavy metals concentration and their content of a highly mobile anion such as nitrate. The objective of this work is to study the behavior of nitrate ion in columns of soil using the HYDRUS-1D software for a volcanic soil (Collipulli soil) having a set of experimental data to compare it with that obtained by simulation. Collipulli soil was packed to the depth of 25 cm into acrylic columns according to their field bulk density. On the surface of a column 30 g of SS, SSA and PA were incorporated, to test the capacity of Collipulli soil to retain chemical constituents when land applied. The columns were located in vertical position, flooded once a week with one pore volume of distilled water and drained by gravity for a period of three months. Sewage sludge ash was obtained by heating the SS at 500°C for two hours; PA was obtained at the field under forest fire conditions. Both were added on the surface of the soil column. Results shown that the Collipulli soil was able of retain or gradually release the nitrate. The amount leached depends on the treatment. The application of Hydrus 1-D considers experimental data of Collipulli soil as texture and nitrate affinity. Experimental nitrate leaching data are in good agreement with those simulated by the Hydrus 1D program. Therefore, the software should be considered to carry out nitrate transport and leaching studies on volcanic soils.

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Keywords: Hydrus 1-D; Volcanic Soil, *Pinus radiata*.

S4-P14**Characterization of Pet-Coke Fly Ashes and Potential Application in Heavy Metals Remediation****A. González^{1*}, J. Saez², S. Sanhueza² and R. Navia²**¹*Programa de Doctorado en Ciencias de Recursos Naturales,* ²*Departamento de Ingeniería Química. Universidad de La Frontera, Temuco, Chile. *E-mail: aixa@ufro.cl*

The generation of fly ashes is a global problem with severe implications for the environment. Their disposal imply the leaching of toxic substances through the soil to the groundwater, impacting negatively the terrestrial and aquatic ecosystems due to trace elements content in the solid residues, which can be considered harmful to the human health and to the environment. Therefore many investigations are aimed to find new applications for fly ashes as new raw materials and energy sources. The purpose of this study was the chemical, physical and mineralogical characterization of pet-coke fly ashes from fluidized bed combustion (PCFA) and of a low cost adsorbent (AnC) produced from PCFA and a recovered organic polymer from kraft pulp mill (lignimerina). It was too realized a test to determine the heavy metal removal efficiency from aqueous suspensions in batch test. The characterization was performed in order to obtain data about the chemical composition means of X-ray fluorescence (XRF), physical parameters (loss on ignition: LOI, grain size distribution and superficial area BET and crystalline forms of the produced adsorbent means of X-ray diffraction (XRD). The results indicated that the detected crystalline phase minerals in the low-cost adsorbent and PCFA were mainly portlandite, calcite and anhydrite, which corresponded with their chemical composition and data from petroleum coke fly ash, being the major element calcium with a value of a 32.5% (w/w) for the low cost adsorbent and 47% for PCFA. In relation with the physical properties, it was obtained that a 75% of the low-cost adsorbent sample presents a particle size of 3.55 mm, a specific surface area (BET) of 21.5 m² g⁻¹ and a medium pore radius of 20.77 Å, whereas PCFA present a lower BET specific surface area (4.66 m² g⁻¹) and small grain size (85µm). The copper (II) removal evaluation was performed in simple batch test with control pH between 4 and 5. The results indicated that PCFA and the low cost adsorbent present high affinity for copper, reaching the equilibrium time in 180 minutes with removal efficiency about 90% for the low cost adsorbent and in 240 minutes with removal efficiency near to 100% for PCFA. The adsorption isotherms showed a maximum adsorption capacity of 5.91 mg g⁻¹ for AnC and 4mg/g for PCFA. The obtained isotherm data were attempted to fit with Langmuir and Freundlich models. Accord to the results can state that Langmuir model describe better the adsorption results onto the produced adsorbent, with correlation coefficient between 0.6 and 0.96. Therefore, it can be proposed, that AnC could be used as reactive material in fixed bed technologies to reduce the concentration of heavy metals from wastewaters, due to their high particle size and copper removal efficiency. The low grain size of PCFA induces to think that it will present cementation problems in fixed bed technologies. Therefore, it is proposed that PCFA can be helpful as replacement material of clay layers in landfills to stop migration of heavy metals in soil and groundwater and by this way avoid the damage in the environment.

Keywords: Pet-coke ash; heavy metals removal; remediation.

S4-P15**Soil Nitrogen Transformations in a Dry Tropical Forest after Conversion to Pasture in the Llanos of Venezuela****A.F. González^{1,2*} and N. Dezzeo¹**¹*Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela.* ²*Universidad Nacional Experimental Sur del Lago “Jesús María Semprum”(UNESUR) Santa Bárbara, Zulia, Venezuela.***E-mail: afgonzal@ivic.ve*

This study was carried out in an area of tropical dry forest with dominant deciduous trees, located in the western Llanos of Venezuela. In this area, forest was converted to pasture by slash-and-burn. Estrella grass (*Cynodon nlemfluency* L) is grown for cattle use and is never fertilized. Soils were sampled in natural forest and in two pastures of different ages (5 and 18-year-old) on three periods along the year: at the beginning of the rainy season (May), at the end of the rainy season (November) and during the dry season (March). At each vegetation type, soil samples were systematically taken at twelve points using a 5 cm diameter soil core. At each point, undisturbed soil samples were collected at 0-5 and 5-10 cm depth. Total nitrogen (N) was determined in the soil samples collected at beginning of the rainy season, and was analyzed by digestion with concentrated H₂SO₄. Inorganic N (NH₄ and NO₃) was analyzed in all collected samples, and was extracted with 2M KCl. N mineralization was measured during 15 weeks incubation by Stanford and Smith (1972) method. Microbial N was extracted according to the CHCl₃ fumigation-extraction method in field-moist samples, and was determined by the alkaline persulfate oxidation method. The concentrations of total N, inorganic N, mineralized N and microbial N were measured using a Technicon Autoanalyser II. The values were corrected to dry soil. For each sampling period, the data sets from forest and pastures were subjected to one-way analysis of variance. Because clay content showed significant difference between forest and pastures, the clay percentage like co-variable to adjust the data was used. Means were separated with Tukey's test when statistical differences ($p < 0.05$) were observed among sites. A Mann-Whitney nonparametric test was used on data that did not meet assumptions for analysis of variance. Statistical analyses were performed using STATISTICA for Windows 6.0 (Statistica 2001). Soil clay content in the first 5 cm depth varied significantly ($p < 0.05$) between forest (31%) and 5-years-old pasture (43%). Soil moisture varied across all sites, sampling periods and soil depths, but the variations were not significantly. The highest soil moistures occurred in 5-year-old pasture, where the soil clay content was the highest. Total N concentrations in the first 5 cm were 2.86 in forest and 3.31 and 3.99 g kg⁻¹ in 18 and 5-year-old-pastures, respectively. These values were not significantly different. Microbial N concentration was strongly influenced by land use and season. In the first 5 cm soil, the forest showed the highest concentration ($p < 0.05$), while the 5-year-old pasture showed the lowest value. The highest NH₄-N and NO₃-N concentrations occurred in the 18-year-old pasture, both at 0-5 and 5-10 cm soil depth. The rate of N mineralization varied throughout the 15-week period between sites and depths, and was significantly ($p < 0.05$) higher in the 18 year-old pasture. Similarly occurred with the potentially N mineralization (No) and N mineralized cumulative (Nm). These results are comparable to others studies of a variety of fertile tropical soils, where the N concentrations and mineralization rates were lower in the young pasture than in the original forest, but they tended to rise with increasing pasture age.

Keywords: Soil nitrogen; tropical forest; pasture.

S4-P16**Tillage and Manure Effect on Soil Microbial Biomass and Respiration and on Enzyme Activities****H. Kheyroodin*** and **H. Antoun***Department of soil science and agri-Food Engineering Laval University, Quebec, Canada, G1K 7P4.***E-Mail: Hkheyroodin@yahoo.com*

The objective of this study was to determine the influence of both tillage and liquid manure application on soil microbial biomass, enzyme activities and microbial respiration in a meadow soil. The results obtained did not show any significant effect of tillage and manure on the microbial biomass C and N nor on soil acid phosphatase activity. However, these treatments significantly increased microbial biomass P, urease, alkaline phosphatase and ammonification rates. The maximum microbial activity was observed in surface soil layer both under conventional tillage and zero-tillage. In fact, microbial respirations (CO₂) of bacteria and actinomycetes were higher in the surface soil and increased with the level of manure. Tillage and manure application had no significant effect on fungal respiration but interaction between tillage and manure application significantly influenced soil urease and ammonification rates. We suggested that soil microbial biomass and enzyme activities were closely correlated to the N mineralization potential, N and C mineralization rates, total amounts of C or N, soil pH, ammonification rates and soil structural stability.

Keywords: Microbial biomass; enzyme activities; respiration.

S4-P17**Influence of Soil Types and Metals on Degradation and Bioavailability of Diverse Pesticides****D. Virág, A. Kiss*, T. Lehotay and L. Németh***EGERFOOD Knowledge Centre, Eszterházy Károly University, Eger H-3300, Eger, Hungary,**Leányka str. 6. *E-mail: attkiss@ektf.hu*

Metals play a key role in both biological systems as enzyme activators or protein structure stabilizers and in the environment as a major stress factors. Increasing contamination of soil with the residues of fertilizers, heavy metals, pesticides and several other compounds stimulated extensive studies on revealing their leaching properties, bioaccumulation and bioavailability. Influence of the mobility and transformation abilities of these contaminants on the potential environmental risk has lately thoroughly been examined. However in order to gain a complete picture and to be capable of proper interpretation of both environmental and biological implications, degradates of the contaminants should also be taken into account. As studies aiming at revealing interactions between transformation products of pesticides and metals in the soil have not been performed so far, our investigations fill in a real gap in understanding bioavailability and environmental behaviour of major spoil contaminant. It is well known that in some cases decomposition products of pesticides may exert more harmful and toxic effect than the parent compound. In addition to this fact metal ions may be bound to various extent to pesticides and their photolytic transformation products, thus the metal

content of the soil influences primarily the fate and environmental impact of the pesticides. The number and chemical feature of the major degradates of some pesticides has already been revealed, however no information is available on their complex forming abilities. The major objective of our research is the thorough examination of the interaction of photoproducts of diverse pesticides with the metal-components of soils. Three distinctive soil types (sandy, brownly and alluvial) and four different pesticides (acetochlor, carbendazim, chlorpyrifos, diuron) were involved in our studies. Investigations of the interaction of the degradates with metals extended to iron, copper, zinc, manganese and nickel. The photoproducts of the pesticides were formed by a special UV-source immersed into the suspension of diverse soil samples. Subsequently the degradation products and newly formed complex species were identified by GC-MS and HPLC-MS techniques. The metal-content of the soil samples were determined by AAS apparatus. Our experiment has been carried out via 3 stages: 1) as control samples soils of distinctive types were demetalized by EDTA solution in order the model circumstances in the total absence of metal components. The soil suspensions were treated with pesticides and then UV-irradiated; 2) Normal soil samples (without EDTA-treatment) were handled in the same manner as described above; 3) Solution of metals of different concentrations were added to the soil suspensions, followed with UV-treatment and isolation, then detection of newly formed metal complexes. The revealed interactions and detected new complex species contributes to a more extensive and appropriate interpretation of environmental fate, behaviour, bioavailability as well as possible harmful biological effects of pesticides.

Keywords: Metal degradation; pesticides; fertilizer residues.

S4-P18

Photodecomposition as a Key Factor of Influencing Fate of Pesticides in Nature

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Transformation of pesticides in the environment is a highly complex process affected by different factors. Both biological and physical-chemical factors play a role in the degradation, whose ratio depends on the actual environmental conditions. Major goal of our study was to investigate photolytic degradation of distinctive types of pesticides being frequently applied in practice. Relevance of the research is supported by the fact that few information is available on behaviour of pesticides used in agriculture, as well as reaction mechanism of photodegradation is not revealed yet. Our study aims at revealing specific details of photolytic degradation of pesticides as important soil contaminants, and to map biological effects of possibly toxic degradation products. The photolytic degradation of frequently applied pesticides of distinctive types (simazine, chlorpyrifos, acetochlor, carbendazim, EPTC) was effected by UV-light source. A special, immerseable UV-light source emitting light of 254 nm effected degradation. Subsequent to the irradiation of the sample the isolation of the degradation products was carried out by column-, and gas-chromatography. Identification of degradation species was implemented by mass spectrometry. Each of the studied 7 distinctive pesticides (metribusine, simazine, acetochlor, carbendazim, diuron, atrazine, chlorpyrifos) of definitely different chemical structure exhibited definite liability

to undergo photolytic decomposition. The degradation mechanisms of the mentioned pesticides were entirely revealed, and in each case 5 or 6 major degradation species have been detected. Loss of thiomethyl, alkyloxy, alkyl and chloro groups might be regarded as typical ways of transformation. The kinetic aspect of photodegradation was also revealed completely by GC technique displaying significant differences in each case. Significant differences were observed in the kinetic behaviour of the investigated pesticides by GC-monitoring. The kinetic curves of photolytic decomposition made it possible to compare photosensitivity and reaction mechanisms of the studied pesticides. 6 distinctive types of soil microbes have been applied as test-species to model and justify harmful biological effects of degradation products. We may claim the pesticide photodegradation may result in significant changes in soil microbiota, as well as formation of biologically harmful side-products. The research on revealing the exact reaction mechanisms of photolytic degradation of pesticides contributes not only to the proper understanding of environmental behaviour of pesticides, but also points out the possible environmental risk factors by identifying possibly toxic degradation products.

Keywords: photodescomposition; photodegradation; pesticides.

S4-P19

Temperature Effect on Interchange Selectivity of K-Ca and K-Mg on Chilean Volcanic Soils

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Four thousand forest fires had taken place annually in Chile from VIII to X Region. The soils damage increases with the soil reached temperatures. One of the main effects is the partial or total organic matter destruction which is related to the cation interchange capacity (CIC): K-Ca and K-Mg equilibrium. Interchange reactions consider competitive interaction between the cation and the soil adsorptive sites. The aim of this work is to establish, the temperature effect of heating conditions of the soils during a fire forest event, upon the interchange selectivity. The Ultisols: Collipulli (36° 58' S 72° 09' W) and Metrengo (38° 34' S 72° 22' W); Andisols: Diguillin (36°53'S 72°10'W), Nueva Braunau (41°19'S 73°06'W) and Ralun (41°32'S 73°05'W), belong to 0-20 cm profile. The cations interchange equilibrium were realized at constant ionic strength (0.05M) on calcined samples at 25°, 105° and 500°; the experimental data treatment was performed using Rothamund-Kornfeld semiempirical model; due to its advantage compared to other models on the parameter quality. The experimental data indicate that at the calcination temperature the dehydration of organic matter of the Andisol occurred at 25-105°C; from the data it can be observed that the CIC increased; but the pH value at equilibrium are kept constant. However between 105-500°C the cationic interchange capacity decreases as the temperature increases with the resulting increment of pH value at equilibrium due to the organic matter destruction and the carbonates formation. For variable charge soil a pH equilibrium increment implies an increment of CIC. Therefore at 500°C this effect is beyond the magnitude of organic matter destruction which can be proved with the experimental results obtained from the studied

Andisols. The changes of CIC and the increments of K selectivity with temperature increment are mainly related to the organic matter changes, rather than on a surface charge modification due to pH alterations at equilibrium. On the other hand Collipulli presents lower organic matter content compared to both Andisols; an increment of CIC is produced as the temperature increases. Ultisols present a greater selectivity for K compared to Ca. and Mg, especially at 25 and 105°C; but Metenco showed a decrease in the K selectivity compared to Ca; however for K-Mg there is no change with temperature increment which can be a result of the mineralogy. The selectivity for K in the studied equilibrium increases in the studied soils and the sequence selectivity found is: $K > Ca > Mg$ does not varied.

Acknowledgment: The authors thank a Project FONDECYT N° 1070116, DICYT-USACH 010741CS, Scholarships VRID and CONICYT.

Keywords: Interchange Selectivity; Volcanic Soil, cation interchange capacity, K-Mn.

S4-P20

Polycyclic Aromatic Hydrocarbons in Soils

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At the present time, soils are subject to continuously increasing anthropogenic impact. The prediction of processes occurring in soils requires diverse and systematic information on their status. Much attention should be given to monitoring different substances of anthropogenic origin, in particular, polycyclic aromatic hydrocarbons (PAHs), in different natural media, including soils. Most PAHs are toxic, mutagenic and carcinogenic compounds widely distributed in the environment and included in the list priority pollutants in EU and the USA. The aim of this work was to develop and optimize an algorithm for the determination and identification of PAHs in soils. Experiments were carried out on samples of peaty-podzolic-gleyic soil developed on coarse silty carbonate-free mantle loam at the Maksimovka station, Institute of Biology. These soils are prevalent on poorly drained broad ridges, gentle slopes, and flat depressions of the Komi Republic. The extraction of hydrocarbons was performed with n-hexane of high-purity grade distilled on a fractionating column 0.5 m in height. Ultrasonic treatment was performed in a UZU-0.25 ultrasonic bath (0.23 kW, 18 kHz). The fluorescence of hexane extracts and separated hydrocarbon fractions was measured on a Fluorat-02 liquid analyzer (a 270-nm filter in the excitation channel and a 315-nm filter in the registration channel). Chromato-mass-spectrometric studies were performed on a Shimadzu QP 5050A instrument (Japan). A relatively increased content of PAHs is observed in the upper peat horizons O1 and O2 with a higher microbiological activity and high carbon content compared to the mineral horizons, which can be explained by the formation of PAHs during the decomposition of litter. Peaty-podzolic-gleyic soil has a stagnant-percolative water regime, and peaty-litter PAHs are partly leached from organic horizons and almost uniformly accumulated in the eluvial layer. A zone with an increased content of PAHs is observed in illuvial horizons, which is related to their high density and poor filtrating properties. Deeper, the content of PAHs decreases abruptly down to horizon Cg. Using chromato-mass-spectrometry, PAHs with molecular weights of 166 (fluorene), 178

(anthracene), 202 (fluoranthene, pyrene), 228 (chrysene), 252 (benz[a]pyrene, benz[e]pyrene), and 278 Da (dibenzo[a,h]anthracene) were identified in organic horizons and PAHs structures with molecular weights of 166, 178, and 202 Da were found in the mineral A2hg and A2lg horizons. Thus, based on the experimental results, we developed an algorithm for the determination of polycyclic aromatic compounds in soils; optimal conditions were found for the extraction and separation of the PAH fraction. The distribution of PAHs in the profile of peaty-podzolic-gleyic soil was determined by fluorometry and chromato-mass-spectrometry. It was found that both low- and high-molecular PAHs occur in organic horizons and that lighter PAHs are mainly concentrated in mineral horizons.

Acknowledgements: This work was supported in part by the Russian Foundation for Basic Research, project № 07-04-00285 and the Russian Science Support Foundation.

Keywords: Cyclic hydrocarbons; Peaty-podzolic-gleyic soil; polycyclic aromatic hydrocarbons.

S4-P21

Microorganisms in Wastewater Sludge Contributed to Rapid Immobilization of Inorganic Nitrogen When Added to an Extreme Alkaline Saline Soil

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Addition of wastewater sludge to an alkaline saline soil of the former lake Texcoco induced a sharp decrease in concentrations of ammonium (NH_4^+) and nitrate (NO_3^-), important nutrients for a planned vegetation of the area. We investigated the factors that control this drop in inorganic N. Soil was amended with NH_4^+ , unsterilized or sterilized wastewater sludge while concentrations of NH_4^+ , NO_2^- and NO_3^- and emissions of CO_2 , N_2O and N_2 were monitored in an aerobic incubation experiment. An agricultural soil of Otumba was treated in the same way and served as control. Addition of unsterilized wastewater sludge increased the CO_2 emission rate > 2.0 times compared to soil amended with sterilized sludge. The mean concentration of NH_4^+ in soil was significantly lower when unsterilized sludge was added compared to soil amended with sterilized sludge. Addition of sludge decreased the concentration of NO_3^- with 50 mg N kg^{-1} soil in the Texcoco soil at day 7, but not in the soil amended with sterilized sludge. Emission of N_2O in the Otumba soil was mainly due to nitrification, while the denitrification process was the main contributor in the Texcoco soil. Microorganisms in the sludge reduced N_2O to N_2 when added to the Texcoco soil, but not the soil microorganisms. It was found that microorganisms added with the wastewater sludge accelerated organic material decomposition, increased NH_4^+ immobilization and induced immobilization of NO_3^- in the alkaline-saline Texcoco soil, while contributing to the reduction of N_2O to N_2 .

Keywords: Microorganisms; wastewater sludge; inorganic nitrogen immobilization.

S4-P22**Impact of 8-Yr Tillage, Crop Residue and N Fertilizer Management on Crop Yield, Nutrient Uptake, Soil Quality and Nitrous Oxide Gas Emissions in Northeastern Saskatchewan****S.S. Malhi^{1*} and R. Lemke²**¹*Agriculture and Agri-Food Canada, P.O. Box 1240, Melfort, Saskatchewan, Canada S0E 1A0.*²*Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada. *E-mail: malhis@agr.gc.ca*

A field experiment was conducted from 1998 to 2005 on a Gray Luvisol (Boralf) soil near Star City, Saskatchewan, Canada, to determine the influence of tillage (no tillage -NT and conventional tillage - CT), straw management (straw retained and straw removed) and N fertilizer (0, 40, 80 and 120 kg N ha⁻¹, except no N to pea phase) on seed and straw yield, mass of N and C in crop, organic C and N, inorganic N and aggregation in soil, and nitrous oxide (N₂O) emissions. The plots were seeded to 4-yr rotation of barley-pea-wheat-canola. Seed, straw and chaff yield, root and C mass, and N uptake increased with increasing N rate for barley, wheat and canola. Tillage and straw treatments generally had no effect on crop yield during the first three years. But, NT produced greater canola seed, straw and chaff than CT, whereas straw retention increased seed and straw yield compared to straw removal for canola in 2001. No-till also had greater seed, straw and chaff yield, N uptake in seed, and mass of C in seed, straw, chaff and root than CT for barley in 2002, but many of these parameters were greater under CT than NT for wheat in 2004 and canola in 2005. Straw retention had greater seed, straw and chaff yield, root mass or mass of N and C in seed, straw, chaff and root in most cases compared to straw removal for barley in 2002, pea in 2003 and wheat in 2004. Total organic C (TOC) and N (TON), and light fraction organic matter (LFOM), C (LFOC), and N (LFON) in soil were generally greater when straw was retained than when removed. Tillage did not affect TOC and TON in soil, but LFOM, LFOC, and LFON were greater or tended to be greater under NT than CT. The NT + straw retained treatment had the lowest proportion (34%) of wind-erodible (<0.83 mm) aggregates and greatest proportion (37%) of larger (>12.7 mm) dry aggregates, compared to highest (50%) and lowest (18%) proportion of corresponding aggregates in CT + straw removed treatment. This suggests a lower potential for soil erosion when crop residues are retained and tillage is eliminated. There was no effect of tillage, straw and N fertilization on NH₄-N in soil, but CT + straw retention tended to have higher NO₃-N concentration in surface soil than NT + straw removed. Concentration of NO₃-N in soil increased substantially with N rate \geq 80 kg ha⁻¹. Amount of N lost as N₂O was higher from N-fertilized than from zero-N plots, and it was substantially higher from CT than NT plots when N was applied. In conclusion, retaining crop residues along with no-tillage improved some soil properties and may also be better for environment and sustainability of high crop production. Although N fertilization improved crop production, it increased the potential for NO₃-N leaching and N₂O-N emissions, when applied in excess of crop requirements.

Keywords: Tillage; crop residues; fertilizer management.

S4-P23**Influence of Controlled-release Urea (Polymer-Coated ESN) on Seed Yield, Soil Nitrate-N and Nitrous Oxide Gas Emissions in Northeastern Saskatchewan****S.S. Malhi¹, C.A. Grant² and R. Lemke³**¹*Agriculture and Agri-Food Canada, P.O. Box 1240, Melfort, Saskatchewan, Canada S0E 1A0.*²*Agriculture and Agri-Food Canada, Brandon, Manitoba, Canada R7A 5Y3.* ³*Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada S7N 5A8. *E-mail: malhis@agr.gc.ca*

If the supply of N is synchronized with crop uptake, the risk of gaseous N emissions to the atmosphere and leaching of nitrate-N to the groundwater can be minimized. A field experiment was established in 2004 near Star City, Saskatchewan, Canada, on a Dark Gray Luvisol (Typic Cryoboralf) clay loam soil to determine the influence of controlled-release/coated urea (CRU) versus conventional urea on seed yield, nitrate-N accumulation in soil and gaseous N₂O-N loss to the atmosphere. The treatments included two sources of N (conventional urea and CRU), placement methods (side banded - SB, banded - B, broadcast, blend, split), N rates (0, 30, 60 and 90 kg N ha⁻¹) or timing of application (autumn and spring) in different combinations under conventional (CT) and zero (ZT) tillage systems. There was a substantial increase in seed yield with increasing rate of applied N under both ZT and CT, and seed yields and response trends were similar for both tillage systems. The N-use efficiency (kg seed kg⁻¹ of applied N) was highest at 30 kg N ha⁻¹ rate, but it did not produce the highest seed yield. Autumn-applied (banded) urea was less effective in increasing seed yield than spring-applied (side-banded) urea. The effectiveness of Autumn-applied urea was increased with CRU, where it produced higher seed yield than uncoated urea. This also suggests that CRU can be a good fit for winter wheat or other winter cereals or oilseed crops. For side-banded N in spring, CRU tended to produce higher seed yield than uncoated urea, suggesting CRU may have the potential to replace split applications under certain conditions. Split application of N (i.e., a half of N at seeding and the other half at tillering) tended to produce higher seed yields than uncoated urea. Spring 50:50 blend application of CRU and uncoated urea produced higher seed yield than uncoated urea. This suggests that blending urea with CRU may improve efficiency of applied N. Spring applied CRU, blended, and split application treatments all produced similar seed yields. Application of CRU resulted in lower accumulation of nitrate-N in soil in top 60 cm depth at harvest than uncoated urea, thus reducing the potential for N losses over the winter and in spring. CRU tended to reduce nitrous oxide emissions compared to uncoated urea. The findings suggest that under wetter soil moisture conditions in the Parkland region, CRU/coated urea can be an effective management tool in enhancing seed yield, while also minimizing potential for gaseous and/or leaching N losses over the winter and in early spring, particularly for Autumn-applied N. This may benefit both crop yield and environment.

Keywords: Nitrogen emissions; tillage systems; controlled-release urea.

S4-P24**Screening Method for Determination of Alkylphenol Ethoxylates and their metabolites in biosolids. Optimization of the Extraction Variables****A. Maricán*, J. Rivas, B. Sepúlveda, I. Ahumada and P. Richter***Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Olivos 1007, Independencia, P.O. Box 233, Santiago, Chile.***Email: adolfo.marican@gmail.com*

Biosolid beneficial utilization as potential fertilizers and their features as soil improvers turn them into a viable alternative, as opposed to a simple buildup of solid wastes. For a safe option in the use of biosolids in forest, agricultural or degraded soils it is necessary to control the presence of pathogenic organisms, heavy metals and organic pollutants. With regard to the last mentioned, biosolids contain high concentrations of alkylphenol ethoxylates which are widely used in commercial and household detergents. These compounds degrade during the wastewater treatment process to their more toxic, estrogenic, and lipophilic alkylphenols. The aim of this study is to develop a rapid and modern extraction methodology to determinate these xenobiotics from biosolids with potential use in agriculture. The analytes studied were 4-nonylphenol ethoxylate (4-NPE), 4-n-octylphenol (4-n-OP), and 4-nonylphenol (4-NP). The extraction methodology developed was based on pressurized solvent extraction (PSE), on an ASE 100 (Dionex). The extraction variables were optimized by a “central composite multivariate design”. The variables studied were: temperature (from 40 to 200°C), static time (from 5 to 60 min.) and solvent (different volume ratio of acetone/dichloromethane). This solvent mixture was previously selected among various single and mixed solvents, because it provided a more quantitative extraction. The other working conditions were: extraction cell, 10 mL; flush volume, 100%; purge time, 100 sec. and two static cycles. After the extraction process, the extracts were dried on a glass column, filled with Na₂SO₄, and then concentrated to 1 mL with N₂. The extracts were cleaned up on a glass column packed with Al₂O₃. A volume of 40 mL of ethyl acetate/toluene (3:1 v/v) mixture were passed through the column in order to elute matrix apolar components. After discard this fraction the analytes were eluted with 20 mL of a diethyl ether/methanol/formic acid (1:1:0.05 v/v) mixture. The solvent was exchanged to methanol, and concentrated to 2 mL previous to chromatographic determination. The final determination was carried out by HPLC-DAD (λ =280 nm, C-18 column, mobile phase: 60% Acetonitrile, 40% water, injection volume of 100 μ l). The optimal extraction conditions were: temperature, 153°C; static time, 60 min.; and solvent, acetone. Under these conditions the method was compared with Soxhlet extraction, using the same solvent (200 mL), 2 g of sample, and 24 h of extraction time. When real biosolid samples from Metropolitan Region were analyzed, the sum of the concentrations found for 4-NPE, 4-n-OP and 4-NP by Soxhlet extraction was 219 ± 45 mg kg⁻¹, and by the proposed method 460 ± 75 mg kg⁻¹. These results indicate that PSE method is much more quantitative than Soxhlet, the extraction time is decreased from 24 h to 2 h and the organic solvent used in the extraction is decreased to 10%.

Acknowledgements: The authors thank to FONDECYT (Projects 1070616 and 1080357) and CONICYT (D-21070345) for financial support.

Keywords: biosolids; alkylphenol ethoxylates; xenobiotics.

S4-P25**Chemical Fractionations and Adsorption of Cd in Chilean Soils under Intensive Corn (*Zea mays* L.) Monocultivation****M. Molina^{1*}, M. Escudey², W. Chen³ and A.C. Chang³**

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Cadmium (Cd) has been one the major trace elements of environmental concern because of its toxicity and mobility in the soil-plant-water system. In Chile, as much as 266 mg Cd kg⁻¹ of P-fertilizer can be incorporated to soils through fertilization. The mobility and transport of trace elements are dependent on the chemical form of the elements and on the ability of the soil to adsorb elements from the soil solution. The objective of this study was to investigate the distribution of forms and the adsorption of Cd in two soils representing the soils used for intensive corn production in central Chile. Soil samples were taken from the 0-25 cm of two Mollisols (Mixed, thermic, Ultic Haploxerolls) a clay loam (CL) and a clayed (C) soil. These soils have been monocultivated with corn over a 15 year period, receiving high-dose application of P fertilizers. Two laboratory experiments were carried out: 1) a sequential extraction method was employed to investigate the adsorbed, mineral and organic phase of Cd in the solid phase of soils; both, the Cd concentration in the soil solution and the total Cd concentration of soils were also determined; 2) adsorption isotherms were carried out applying 20 mL of different Cd solutions to one gram of dry soil (Cd concentrations ranging from 50 to 2000 µg L⁻¹ in 0.01 M NaNO₃ background solution) and equilibrated for 48 h. After that period, the suspension was centrifuged and filtered. The supernatant was separated for Cd determination and the solid underwent the chemical fractioning procedure to obtain the adsorbed and mineral forms of Cd. In all samples the Cd concentrations were determined by AAS-GF. Total Cd content of soils were 0.27 mg Kg⁻¹ and 0.33 mg Kg⁻¹ for the CL and C soil, respectively. These concentrations have been reported for soils under agricultural practices. The greater differences between soils were found in the concentration of Cd in soil solution and in the mineral phase. The Cd concentration in the soil solution of the CL soil was a 57% higher than that of the C soil. The recovery of the sequential extraction method was ~93% of the total Cd. The remaining Cd could be considered to be in the residual fraction, which is a very low reactivity phase. About 33%, 45% and 12% of the total Cd of the CL soil are in adsorbed phase, mineral phase and organic phase, respectively. For the C soil, most Cd is in the mineral phase (50%), and lesser amounts are in the adsorbed phase (36%) and the organic phase (9%). The linear partition coefficients ($K_d = \text{Cd in adsorbed phase} / \text{Cd in soil solution ratio}$) obtained from the adsorption isotherms indicate that the ability of the C soil to adsorb Cd was more than 2 times higher than that of the CL soil. The K_d were 455 L kg⁻¹ and 975 L kg⁻¹ for the CL soil and C soil, respectively. After the equilibrating time, most of applied Cd was found in the adsorbed phase in both soils. The amount of Cd found in the mineral phase accounted for the 8% and 12% of the total adsorption in the CL soil and C soil, respectively.

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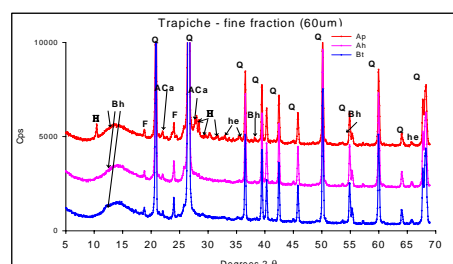
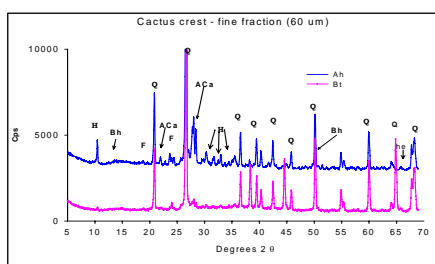
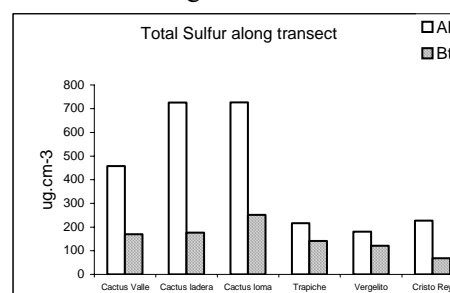
Keywords: Cadmium absorption; monocultivation; Chilean soils.

S4-P26

Properties and Acid Buffer Capacity of Soils Affected by Acid Deposition Near to the Gas Processing Facility Cactus in Southeast Mexico**L. Mora-Palomino and C. Siebe***Instituto de Geología, Universidad Nacional Autónoma de México, México.***E-mail: lmora@geol-sun.igeolcu.unam.mx*

Acid deposition produced by industrial facilities that emit sulfur dioxide and nitrous oxides affects vegetation, soil and aquatic systems in surrounding areas to the source. Its effects have been widely studied in temperate regions of industrialized countries, but little is known about its magnitude and behavior in tropical regions. Dominant soils in this region are Acrisols and Ferralsols, which are acid soils of variable charge and are considered vulnerable to acid deposition. In the Gulf of Mexico petroleum extraction activities are widely distributed, and large quantities of gases are emitted into the atmosphere. In this region the gas processing facility Cactus is located, which emits the largest amount of SO₂ to the atmosphere in the entire region. The climate is warming humid and the land surface surrounding Cactus is relatively old (Mid Tertiary to early Pleistocene) and deeply weathered soils have developed.

These soils are acid, have a small reserve of basic cations and are rich in quartz and kaolinite type clay minerals as well as iron and aluminum oxides, which have variable charge. The objective of this investigation was to study sulfate deposition, to characterize the soils and their changes due to acid deposition and to evaluate their buffer capacity. Dry acid deposition was quantified by exposing PbSO₄ capsules at different distances from the source; soil profiles were described and sampled along a transect and at different distances from the facility (0.7, 4, 8, 12 km). Also 3 profiles were sampled along a catena (crest, slope and valley) at the site of largest deposition. Suction cup lysimeters were installed at different depths along the transect to monitor the soil solution. The largest amounts of SO₂ are deposited within a 4 km radius around the facility (Figure 1). Soils in this area have more acid pH values and a smaller ratio of basic cations to exchangeable aluminum. Weatherable minerals introduced to these soils by the eruption of El Chichón volcano, are almost absent in the soils close to the facility (Fig. 2). Also the soil solution is more acid close to the facility and a larger Ca²⁺ and SO₄²⁻ concentration is observed. The soils near to the facility have the largest buffer capacity, since they are richer in clay and have the largest specific surface. The smallest buffer capacity was determined for the soils at 8 km distance; these soils are more sandy.



Keywords: Acid deposition; sulfur dioxide emissions; nitrous oxides emissions.

S4-P27**Behaviour of a Recombinant Ovine Prion Protein in Soil Humic and Humic-like Complexes****F. Russo, R. Scotti, M.A. Rao* and L. Gianfreda***Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples Federico II, Portici, Italy. *E-mail: mariarao@unina.it*

According to the “only-prion” hypothesis, prion proteins are the main agents of Transmissible Spongiform Encephalopathies (TSE). Contamination events, through buried contaminated animal carcasses, abattoir wastes or accidental dump, can lead soil to become a potential reservoir of TSE infectivity. As prion protein in infective form is strongly resistant to proteolytic attack, in soil PrP molecules could persist and interact with organic and inorganic colloids by electrostatic interactions, hydrogen bonds, van der Waals forces, etc. Biomacromolecules such as proteins can also be involved in the humification process by phenolic compound polymerisation, through coupling oxidative reactions, catalysed by abiotic catalysts such as Fe and Mn oxides or biotic catalysts such as peroxidases and polyphenoloxidases. The process is very fast and produces a population of polymeric products of different molecular structure and size. Further polymerization, self-assembly of different polymeric products, and/or association with mineral constituents as well as biomacromolecules quickly lead to the formation of more complex organic and organo-mineral complexes. The aim of this work was to study the entrapment of a recombinant, non pathogenic, ovine prion protein (recPrP) in polymeric aggregates of catechol, a humic-like precursor, obtained by birnessite ($\delta\text{-MnO}_2$) or laccase (from *Trametes versicolor*) catalysis. The adsorption of recPrP on humic acids extracted from agricultural soil was investigated, as well. Attention was also focused on the possible release of the protein from humic acids and from the formed organic and organo-mineral complexes, obtained by different catechol concentrations and sequences of addition of recPrP. Desorption and extraction were investigated by using weak and strong extracting agents. The high affinity of recPrP for phenol polymers and organo-mineral complexes promoted the complete entrapment of recPrP molecules in insoluble complexes whatever the catechol concentration or the catalyst. Complete protein adsorption on humic acids was also observed. Extraction tests of recPrP performed with hexametaphosphate and sarkosyl were successful only on humic acid complexes. Conversely solution of sodium dodecyl sulfate (SDS), the strongest agent, allowed extracting recPrP from all the systems. Overall, the results seem to indicate that entrapment and adsorption phenomena involving prion protein actually occur in soil. Furthermore, they suggest that the oxidative polymerisation of humic precursors can strongly immobilise recPrP. Therefore similar processes occurring with the infective protein could represent a mean to avoid the dissemination in the environment.

Keywords: Recombinant Ovine Prion Protein; humic acids, humic-like complexes.

S4-P28**Study of the Thermal Parameters of the Micellization of Surfactants as a Function of Hydrophobic Chain Length and Temperature****S.M. Salman*** and F. Mehreen*Department of chemistry, University of Federal Santa Maria (UFSM), RS, 97105-900, Brazil.***E-mail: salmanchemist80@yahoo.com*

The thermodynamics of various surfactants aggregation in aqueous solution was examined at 283.15 – 323.15 K by conductance technique. The degree of ionization (α) and temperature dependence of critical micelle concentration (CMC) were used to reveal the thermodynamics of micellization of surfactants by the application of mass action model. The standard heat of micellization ΔH_M° for most of the surfactants is positive i.e. $\Delta H_M^\circ > 0$, interpret that micellization process for all these surfactants is endothermic. From the large and highly positive values of standard entropy change of micellization (ΔS_M°) for all surfactants, except SDS, it can be deduced that the system becomes more and more random after micelle formation. The sum of ΔH_M° and $T\Delta S_M^\circ$ is free energy of micellization (ΔG_M°) which appeared to be negative for all surfactants enumerates that micellization is a spontaneous process.

Keywords: Micellization; surfactants; thermal parameters.

S4-P29**Effect of Dimethenamid on β -Glucosidase Activity Evaluated in Andisol with Liquid Cow Manure Application****K. Sanhueza^{1*}, P. Aguilera¹, G. Briceño¹, M. Candia¹, O. Candia¹, M.L. Mora^{1,2}, R. Demanet²**
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Corn is a resource used in dairies in southern Chile, is applied in the crop a lot of liquid cow manure (LCM). LCM have a significant impact on their development and reduce costs fertilization, generate an increased level of fertility as accordingly recycling of nutrients especially nitrogen and potassium, thus they increase the burden of weeds in the field. Therefore as increasing doses of normal herbicide application, including the group of chloroacetamides, costs increasing in production and there will be a greater impact on the environment. The soil fertility depends on biological quality and quantity of microorganisms and enzymes present. The aim of this study was to assessing the role exerted by slurry into the biochemical process that regulates the efficiency of herbicide dimethenamid, with different doses of LMC, determining the time of stabilization the soil-LCM-dimethenamid so as to maximize the effectiveness of herbicides through of enzyme activity β -glucosidase which is used to measure soil microbial activity. The Andisol belonging to Los Lagos series was used, located in Futrono, Los Rios, Chile; the LCM was obtained from the same place. The herbicide corresponded to dimethenamid (for analysis, 99.9% active ingredient). Was measured the weight of 4 bags containing 3kg of soil each one, the humidity was adjusted to

70% (field capacity), were applied 3 doses of liquid manure equivalent to normal doses in the field, of 100(S100), 200(S200) and 300(S300) thousand Lha⁻¹, and the control (S0) without application, the mixtures of soil with LCM were incubated at 20°C and with constant humidity. Samples were taken from each substrate on day 1, 10, 20 and 30 during incubation, to these samples; one doses of herbicide dimethenamid (dose field) were applicated plus a control without herbicide. And then, the activity of β -glucosidase at day 1, 10, 20, and 30 for each of the days of incubation, using 3 subsamples plus a control was measured. The enzyme β -glucosidase catalyzes the last step in the breakdown of cellulose to glucose and it is important in soils with a high percentage of organic matter, in this case provided by liquid manure. In all treatments of liquid manure there is initial increase in activity and then decreased, before to become stable, the difference can be seen in the reduction and stabilization of the activity, this takes importance in treatments with higher doses of liquid manure (S200 and S300), the stabilization of these samples is at 20 days of measurement, for other site, in the doses S100 and S0 after day 10 of measurement it becomes stable. There is no appreciable difference in the assessment of the β -glucosidase activity in treatments that apply herbicide, the reason is the dose field (2 Lha⁻¹), this is not high and does not interfere significantly in the enzyme activity. We suggest an experiment degradation of the herbicide to assess their efficiency at different doses of liquid manure.

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Keywords: Dimethenamid; β -glucosidase activity; liquid cow manure.

S4-P30

Expression of Aromatic Compounds Degradation Genes From *C. necator* JMP134 in Response to *Acacia caven* Exudates

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Cupriavidus necator JMP134 is a soil β -proteobacterium possessing a wide catabolic versatility towards aromatic compounds, being capable to use 2,4-dichlorophenoxyacetic acid (2,4-D), a powerful herbicide and soil pollutant, as a single carbon source. This bacterium was isolated from Australian soil impacted with 2,4-D used to eliminate weeds from agricultural soils, such as *Mimosaceae* family plants. The performance of soil bacteria is determined by both biological and physicochemical factors, like carbon source availability, pH and plant root exudates. The exudates comprise different molecular weight compounds such as proteins, amino acids, enzymes, aromatic compounds, and other secondary metabolites secreted by the plant into the rhizosphere. These can range from 10% of the net carbon assimilation by a plant, to as much as 44% of plant's total carbon. Soil microbes take advantage of this abundant carbon liberation, and modulate the expression of their catabolic genes to utilize these growth substrates. The aim of this study was to analyze the effects of the root exudates of *Acacia caven* (a *Mimosaceae* widely distributed in Chile), on *C. necator* JMP134 catabolic gene expression. In order to determine the ability of *C. necator* JM134 to utilize *A. caven* exudates as a carbon source, root exudates were collected from the plant after 24 days of growth in hydroponic cultures. *C. necator* was found to grow on exudates as sole

carbon source 4 h after inoculation. To assess the expression of catabolic genes in response to *A. caven* root exudates, transcriptional fusions of catabolic gene promoters to the *lacZ* reporter gene, were constructed. β -galactosidase activities were measured in *C. necator* derivatives harboring these fusions, when exposed to root exudates for 5 h. We found induction of *nagAa* promoter gene, involved in the salicylic acid degradation, mediated by these exudates; salicylic acid is a secondary metabolite of plants. Furthermore, we also found that these exudates induced, *pcaIJF* and *pcaHQ* genes, belonging to two central catabolic pathways for aromatic compounds degradation in bacteria. This work reports relevant information about the catabolic gene modulation in response to natural compound mixtures such as root exudates. Root exudates might improve bacterial adaptation and survival in soil, by stimulating both gene induction and growth, and thus may have positive effects on bacterial degradation of pollutant compounds.

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Keywords: Aromatic compounds degradation genes; *Cupriavidus necator*; *Acacia caven*.

S4-P31

Investigation on the Soil Infiltration Factors in Order to Increase Efficiency of Furrow Irrigation in Relation to Water Crisis

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Drought and water crisis (especially in recent years) has covered a vast part of Iran and caused a great concern among the farmers and officials. In order to solve this problem, few strategies can be suggested. Taking long-term decision for increasing the efficiency of irrigation is strongly suggested. At the same time ways in which the customized irrigation is optimized must be studied. An old way of irrigation in the country in large scale has been furrow irrigation that unfortunately no attempt has been made to optimize this method. Different designs for furrow irrigation are offered the most important of them are: 1) S.C.S method, 2) F.A.O method, and 3) walker and skogerbo method. The present study is offering a design method that is suited according to environmental conditions and the results obtained from this study. Attempt was made to identify the efficient length of the furrow. Keeping these in mind, a study field in Amirabad farm of college of Agriculture at Birjand University was chosen. Because in furrow irrigation the slope for land leveling slope distribution is very important, preparing the land and leveling was carefully done. Research on the soil infiltration (Double ring and input-output methods), advancing time and delaying time and measuring furrow cross section in different steps of the study.

Keywords: Furrow irrigation; drought; soil infiltration.

S4-P32**Analysis of Organic Matter at the Soil-Water Interface by NMR Spectroscopy: Implications for Contaminant Sorption Processes****M.J. Simpson*** and A.J. Simpson*Department of Physical and Environmental Sciences, University of Toronto, 1265 Military Trail, Toronto, Ontario, M1C1A4 Canada. *Email: myrna.simpson@utoronto.ca*

Contaminant sorption to soil organic matter (OM) is the main fate of nonionic, hydrophobic organic contaminants in terrestrial environments and a number of studies have suggested that both soil OM structure and physical conformation (as regulated by the clay mineral phase) govern contaminant sorption processes. A great deal of this evidence has come from macroscopic observations with contaminants and soil fractions as well as a recent mass balance approach where the sum of the parts exceeded the whole suggesting that the physical arrangement of OM in organo-mineral complexes may be more important than OM structure in sorption processes (Bonin and Simpson, 2007). In addition, recent studies with constructed organo-mineral complexes have suggested that aliphatic OM is preferred over aromatic moieties and suggests that clay minerals play an indirect role by governing the sorption of organic contaminants by controlling the surface accessibility of OM at the soil-water interface (Feng *et al.*, 2006; Simpson *et al.* 2006). To investigate this further, a number of soil samples were characterized by both solid-state ^{13}C Cross Polarization Magic Angle Spinning (CPMAS) NMR and ^1H High Resolution Magic Angle Spinning (HR-MAS) NMR. HR-MAS NMR is an innovative NMR method that allows one to examine samples that are semi-solid using liquid state NMR methods (ie: observe ^1H which is more sensitive than ^{13}C). With HR-MAS NMR, only those structures that are in contact with the solvent are NMR visible thus one can probe different components within a mixture using different solvents. The ^1H HR-MAS NMR spectrum of a grassland soil swollen in water (D_2O) is dominated by signals from alkyl and O-alkyl structures but signals from aromatic protons are negligible (the peak at $\sim 8.2\text{ppm}$ is attributed to formic acid). When the soil is swollen in DMSO-d_6 , a solvent which is more penetrating and capable of breaking hydrogen bonds, aromatic signals are visible suggesting that the aromatic structures are buried within the soil matrix and do not exist at the soil-water interface. The ^{13}C solid-state NMR data confirms that aromatic carbon is present in substantial amounts (estimated at $\sim 40\%$ of the total ^{13}C signal) therefore, the lack of ^1H aromatic signals in the HR-MAS NMR spectrum indicates that aromatic structures are buried and that the soil-water interface is dominated by aliphatic chains, carbohydrates, and peptides. The NMR data indicates that the mineral component of soils governs the physical conformation of OM at the soil-water interface. Consequently, one must consider that one-dimensional NMR methods provide an excellent overview of all the structures that are present but they do not provide direct information regarding which structures are available or accessible at the soil-water interface for contaminant interactions.

Keywords: Soil-water interface; contaminant sorption; NMR spectroscopy.

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A.J. Simpson et al. (2006). *Langmuir* 22:4498-4503.

S4-P33**Pulp Mill Sludge Addition on Volcanic Soil Characteristics and *Lolium perenne* Production****C. Pichihuencho¹, M.C. Diez² and F. Gallardo^{1*}**¹Departamento de Ciencias Químicas, ²Departamento de Ingeniería Química. Universidad de La Frontera, Temuco, Chile. *E-mail: fgallar@ufro.cl

The secondary sludge from pulp and paper industry contains high organic matter content, nitrogen and phosphorus and, low concentrations of heavy metals. This residual may presents an alternative as soil amendment improving physical, chemical and biological properties of the soil. The aim of this study was to evaluate the effects of the addition of different doses of the sludge (0, 10, 20 and 30 Mg ha⁻¹) on *Lolium perenne* production in volcanic soil (Freire Series) in field assays. The sludge was incorporated in the first 5 cm soil depth, and *Lolium perenne* seeds were sowed after 20 days of incubation. The assays were maintained for 135 days and at days 45, 75, 105 and 135 the plants were cut and soil and plants were analyzed, as well as the microbial diversity using molecular techniques (DGGE) was evaluated. The sludge addition improved physical and chemical properties of the soil increasing macro and micronutrients, as well as *Lolium perenne* biomass production. Soil pH rose from 5.50 to 5.72 with the highest dose of sludge, organic matter increased significantly from 10.59 to 17.18 with the addition of 30 Mg ha⁻¹. The biomass production in each cut increased with the increment of sludge addition. It is necessary to emphasize that with the minimal dose of 10 Mg ha⁻¹ of sludge, the biomass values were higher than values obtained with the control from the first stage (day 0) up to the final stage evaluated (day 135). It was also observed an increment in nitrogen and phosphorus content in aerial biomass, with significant difference between 30 Mg ha⁻¹ sludge dose compared with the control. The analysis of the microbial diversity showed that the application of the sludge does not modify the microbial community of fungi and bacteria even in the high doses applied. According to the results, secondary sludge from pulp and paper industry improved volcanic soil (Freire Series) increasing all micro as well as macronutrient content. The addition of sludge increased the availability of nutrients in the soil, therefore increasing its productivity. The highest production of *Lolium perenne* biomass was obtained with 30 Mg ha⁻¹ in field experiments.

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Keywords: Pulp mill sludge; volcanic soil; *Lolium perenne*.

S4-P34**Effect of Root Extracts of Both Lines and Cultivars of Red Clover (*Trifolium pratense* L.) on the Behavioural Responses of Red Clover Root Borer (*Hylastinus obscurus*) (Marsham) (Coleoptera: Scolytidae)****L. Manosalva^{1*}, F. Pardo², F. Perich², L. Parra¹ and A. Quiroz²**

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Red clover (*Trifolium pratense* L.) is one of the main forage legumes grown in North America and Europe. Soil-climate conditions in southern Chile are also favorable for the establishment of red clover stands. Although red clover is considered a perennial plant, the production decline to unsatisfactory levels within two years (Steiner & Alderman, 2003). The main factor associate to this reduction is the root borer, *Hylastinus obscurus* (Marsham) (Coleoptera: Scolytidae). At present there is no control for clover root borers other than crop rotation (Aguilera et al., 1996). Because this borer is an underground pest, chemical components that are associated to the root-ground matrix could have a role in the host acceptance by the root borer. This behaviour could be modulated by chemical compounds released to the rhizosphere. These compounds could vary quantitative and qualitatively through the time depending of the genotype. This study investigated the role of volatile compounds released from different aged of seven line and two cultivars of *T. pratense*. Seasonal variation of the olfactometric responses was also studied. Behavioural responses of the borer stimulate by different extracts was evaluated in an olfactometer and registered by means the software EthoVision 3.1. The results showed that root extracts of the seven lines and two cultivars were more attractants as the plant increased its physiological age. Plants of 7 months-old were attractants, however those of 3 months-old did not have a significant effect on the olfactometric response of *H. obscurus*. There were not significant differences among the olfactometric activities elicited from root extract of both lines and cultivars in different stages of growth.

Acknowledgements: Project FONDECYT N° 1070270

Keywords: Root extracts; *Trifolium pratense*; *Hylastinus obscurus*.

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S4-P35**Effect of Arsenate, pH and Aging on the Mineralogy, Physicochemical Properties and Reactivity of Aluminum, Iron and Mixed Iron-Aluminum Precipitates****A. Violante*, M. Pigna and V. Cozzolino***Dipartimento di Scienze del Suolo, della Pianta e dell'Ambiente, Università di Napoli, Italy.***E-mail: violante@unina.it*

Arsenic mobilization in soils is controlled by sorption/desorption processes, but arsenic also may be coprecipitated with aluminum and/or iron in natural environments. Coprecipitation involving arsenic with aluminum or iron has been studied because this technique is efficient for the removal of arsenic from polluted waters. We studied the mineralogy, surface properties, chemical composition and reactivity toward phosphate of aluminum, iron and mixed iron-aluminum (Fe/Al molar ratio of 1.0) oxides formed in the absence or presence of arsenate (initial As/Al, As/Fe or As/Fe+Al molar ratio (R) of 0, 0.01, or 0.1) and at pH 4.0, 7.0, or 10.0 and aged 30 or 210 d at 50°C. The removal of arsenate by phosphate from the samples formed at R = 0.1 was also studied. The samples were characterized using XRD, FT-IR, XPS and transmission electron microscopy (TEM). The chemical composition of the precipitates was determined by treating the samples with NH₄-oxalate/oxalic acid or with 6 M HCl. In aluminum systems and in the absence of arsenate, gibbsite (pH 4.0 or 7.0) and bayerite (pH 10.0) formed, whereas in the presence of arsenate the formation of short-range ordered materials was promoted. Poorly crystalline boehmite formed at pH 4.0 (R = 0.01 and 0.1), 7.0 and 10.0 (R = 0.1) and did not transform into Al(OH)₃ polymorphs even after prolonged aging. In iron systems and in the absence of arsenate (R = 0) goethite and/or hematite (with ferrihydrite at pH 4.0 and 7.0) crystallized, whereas at R = 0.01 formation of ferrihydrite increased and hematite crystallization was favoured over goethite. At R = 0.1, ferrihydrite formed in all the coprecipitates and remained unchanged even after 210 d of aging. Finally, in mixed iron-aluminum systems, in the samples formed at pH 4.0 (aluminous) ferrihydrite predominated, whereas in the precipitates obtained at pH 7.0 or 10.0 and R = 0 or 0.01 gibbsite and ferrihydrite or bayerite, gibbsite, hematite with small amounts of ferrihydrite formed, respectively. The coprecipitates obtained at pH 7.0 or 10 and R = 0.1 showed presence of ferrihydrite and poorly crystalline boehmite. The surface area and chemical composition of all the samples were affected by pH, R, and aging. Arsenate was found to be present mainly into short-range ordered materials. The sorption of phosphate onto the precipitates was affected by the mineralogy, specific surface area, amounts of arsenate in the oxides and chemical composition of the samples. The presence of short-range ordered materials, mainly those richer in aluminum, facilitated phosphate fixation, whereas arsenate present in the samples prevented phosphate sorption. Low percentages of the total arsenate coprecipitated in the samples formed at R = 0.1 were desorbed by phosphate, but usually more arsenic was removed from the samples synthesized at pH 10.0 than from those formed at pH ≤ 7.0. A comparison of the desorption of arsenate by phosphate from aluminum-arsenate, iron-arsenate and iron-aluminum-arsenate coprecipitates evidenced that phosphate has a greater capacity to desorb arsenate from aluminum than iron sites. Finally, we found that more arsenate was desorbed by phosphate from metal oxides on which it was added immediately after their formation than from oxides obtained coprecipitating the metalloid with aluminum and/or iron, attributed to partial occlusion of some arsenate anions into the framework of the coprecipitates.

Keywords: Arsenate; aluminum; coprecipitation

S4-P36**Effect of Pentachlorophenol on Ryegrass Biomass Production using Rhizotron Assays****C. Urrutia^{1*}, C. Paredes², M.L. Mora³ and M.C. Diez¹**¹Departamento de Ingeniería Química, ² Programa de Doctorado en Ciencias de Recursos Naturales,³Departamento de Ciencias Químicas., Universidad de La Frontera, Temuco, Chile.

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Common methods for remediation of pentachlorophenol (PCP) contaminated materials include land disposal, incineration, soil washing, and chemical extraction. Biodegradation by bacteria or fungi and phytoremediation by plants can destroy or transform this contaminant to less bioavailability forms. The degradation of PCP occurs under aerobic and anaerobic conditions in soils, and plant, bacterial and fungal extracellular phenol oxidases plays an important role in the degradation of this contaminant. The aim of this work was to evaluate the effect of PCP concentration on ryegrass rhizosphere and biomass production. The soil used in this study was an allophanic soil (Andisol) which was collected from 0-20 cm depth, and sieved (>2 mm). The assays were conducted using mini rhizotron system with 620g of allophanic soil, moistened to 65% of its water holding capacity, and contaminated with 50, 100 and 250 mg kg⁻¹ of PCP. Ryegrass (*Lolium multiflorum*) seeds were germinated, put on Taylor and Foy nutritive solution for 2 weeks. Six selected plants were placed on the top of each rhizotron, and samplers were installed to collect soil solution. The rhizotrons were maintained at 20°C in controlled temperature chamber, and periodically the soil solution was collected and analyzed for PCP and organic acids concentration. At the end of the experiment the soil of each rhizotron was analyzed for PCF and peroxidase activity, and plants for aerial and radical biomass. The allophanic soil used in this study (0-20 cm depth) presents pH of 5.9, organic matter content of 14.6 and carbon and nitrogen ratio of 11. Particle size distribution for the soil was 16.5 % sand, 58.2 % silt, and 25.7 % clay, and the relationship from Al₂O₃/Fe₂O₃/SiO₂ was 18.6/10.5/41.6 %, respectively. This soil possesses a highly pH-dependent variable surface charge, and presents a high adsorption capacity due to its physicochemical characteristics, mainly its high level of Al, Fe and Si oxides. The adsorption properties of chlorophenols are controlled largely by their degree of substitution and the resulting hydrophobicity. PCP presents a high affinity with soil organic matter, associated with their log *K*_{ow} value (5.01) and the p*K*_a value (4.75), being the adsorption strongly affected by soil pH in allophanic soil. The contamination of the soil with PCF affected Ryegrass biomass production. The total dry matter biomass (aerial and radical) obtained decreased with the increment of PCF concentration. The biomass of the samples with 50, 100 and 250 mg kg⁻¹ of PCP was 17.3, 18.5 and 29.6 % lower than the control sample without PCP.

Acknowledgements: Investigation financed by FONDECYT 1050614, CONICYT-CSIC 2007-144 and DIUFRO GAP-2007 projects.

Keywords: Pentachlorophenol; ryegrass; rhizotron assays

Session 5. Environmental Biotechnology: Biochemical and Molecular Mechanisms of Microbe-Plant-Root Interactions & Their Genomic & Proteomic Advances Pertaining to Restoration of Contaminated Soils

S5-P1

Isolation of Efficient Polyethylene Degrading Bacterial Species from Marine Ecosystem of Gulf of Mannar in India

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Polyethylene (LDPE) is advantageous as they are strong, light-weighted, and durable. However, they possess disadvantages such as they are resistant to biological degradation and harmful to the natural environment. These solid waste related problems pose threat to megacities. Biological degradation includes microbial degradation by microorganisms such as bacteria and fungi that consume the material. 15 (GM1- GM15) bacteria were isolated from the plastic wastes dumping places near sea shore in Gulf of Mannar, India. Among 15 bacterial isolates, GM5, GM7 were efficient to degrade the polyethylene. The efficiency test carried out by weight loss. GM5 and GM7 degraded the polyethylene by 13.73% and 12.15% of weight loss respectively and efficiency of degradation was confirmed by FTIR spectrometric analysis.

Keywords: Polyethylene; solid wastes; microorganisms; marine ecosystem.

S5-P2

Evaluation of Enzymatic Activity and Biodiversity in Soil with Methyl-Parathion Pesticide in Chinampas of Xochimilco

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Denitrification is one of the main steps of global Nitrogen cycle and consists in the reduction of Nitrate to molecular Nitrogen via Nitrous Oxide. It is the most important biological mechanism by which Nitrogen returns to the atmosphere from soil and water. Denitrification has also received considerable interest recently because yields N_2O emissions, it is an important greenhouse gas and a natural catalyst of stratospheric ozone degradation. Denitrifying bacteria are widely distributed in the environment and exhibit a high taxonomic diversity, but the increasing use of pesticides and fertilizers during the last decades has negatively affected denitrifying populations in agricultural soils. Recently, the development of molecular techniques based on direct DNA extraction from the environment, which enable identifying functional genes, has helped understand the relation between microbial community composition and nitrogen dynamics in ecosystems. Now, in order to further understand denitrification processes in ecosystems, it is necessary to integrate physicochemical and molecular analysis. We performed microcosms experiments aimed at examining the persistence of nitrate, nitrite and nitrous oxide reductase, as well as the effect on the dynamics of NO_3^- , NO_2^- , N_2O y N_2 in soil with methyl-parathion pesticide in the so-called “Chinampas” of Xochimilco, in Mexico from Náhuatl or Aztec, chinamitl, bulrush or cattail stalks lattice for hydroponics cultivation. Studying the dynamics of the enzymes contributes to understanding the controls over N_2O production when anaerobiosis is rapidly induced in soils. We analyzed these enzymes’ activity after different periods of aerobic incubation by gas emission and analyzed one functional genes by TGGE. Our results indicate that there is variation of abundances at genetic level and of gas emission in incubations times, pointing at a correlation between gas emissions and molecular analysis.

Keywords: Methyl-Parathion pesticide; denitrification; enzymatic activity.

S5-P3**Determination of Changes in the Microbial Community in a Soil Amended with Liquid Cow Manure and Application of Atrazine through Molecular Techniques****G. Briceño¹*, M. Jorquera², R. Demanet³, M.L. Mora¹ and G. Palma¹**¹*Departamento de Ciencias Químicas,* ²*Instituto de Agroindustria,* ³*Departamento de Producción Agropecuaria. Universidad de La Frontera, Av. Francisco Salazar 01145, Casilla 54-D, Temuco, Chile. *E-mail: gbriceno@ufro.cl*

Application of animal manure amendments to agricultural soils is a common practice to improve soil fertility through the addition of essential plant nutrients. Applications of organic amendments have also been shown to increase the soil microbial biomass and stimulate microbial activity. This effect of biostimulation has been associated to modify the behaviour of pesticides in soil. Andisols in Chile are generally cropped to maize (*Zea mays* L.) and their management includes the application of liquid cow manure (LCM) at rates higher than 100,000 L ha⁻¹. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1, 3, 5-triazine) is a herbicide widely used in corn production. However, the application of LCM has produced an increase in weed populations, which has caused farmers to increase rates of atrazine application through repeated application. The effects of herbicides on soil microbial communities are conventionally studied by techniques based on measuring their metabolic activities. However, in many cases detect significant effect. Recently, various molecular techniques, such as denaturing gradient gel electrophoresis (DGGE) have been developed to monitor temporal and spatial changes of the soil microbial community. In this study we investigated the response of microbial communities in unamended and LCM-amended soil treated with the herbicide atrazine. The LCM was applied at rates equivalent to 0, 100,000, 200,000, and 300,000 L ha⁻¹, resulting in treatments S-0, S-100, S-200, and S-300, respectively. In the laboratory an aerobic incubation was conducted for 30 days. Then, was applied atrazine in concentrations of 0, 1, 2, and 3 mg kg⁻¹. Bacterial community changes were assessed by 80 days after atrazine application using DGGE of polymerase chain reaction-amplified 16S rDNA fragments. The application of LCM at different doses modified the soil community, represented by appearance of new bands. The same tendency was observed with the application of atrazine at different concentrations in unamended and amended soils. Atrazine modified the soil community during the first 10 days after their application, and then returned at the previous condition. At day 1, 15 and 30 after LCM application, and at day 1 and 10 after atrazine application, major bands were excised from the gels and the DNA was cloned for sequence analysis. In amended soil the community was modified by the appearance of bacteria belonging of Phylum Bacteroidetes and Genus *Acinetobacter* mainly in amended soil S-300. While the dominant bacterium in unamended and amended soil with atrazine applications at different concentrations were belonging of Phylum Proteobacteria/Class Betaproteobacteria. The main changes were presented in unamended soil S-0. Our results indicate the existence of bacteria adapted to atrazine degradation in agricultural soil.

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Keywords: Microbial community; liquid cow manure; atrazine.

Session 5. Environmental Biotechnology: Biochemical and Molecular Mechanisms of Microbe-Plant-Root Interactions & Their Genomic & Proteomic Advances Pertaining to Restoration of Contaminated Soils

S5-P4

Perchlorate in Soils and Salary in the North of Chile, Iquique

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Perchlorate is regarded as a new emerging persistent inorganic contaminant because of its specific properties, such as highly soluble, mobile and stable in water, readily migrates to ground and surface waters that are used as drinking water and for irrigation. The Atacama Desert nitrate deposit in Chile used as fertilizer is also known to be a natural source of perchlorate. Recently it has also been reported that perchlorate is naturally formed in atmospheric processes. Perchlorate is known to interfere with iodine uptake by the thyroid gland. In 2005, the United States Environmental Protection Agency (US EPA) established an official reference dose (RfD) of 0.7 µg/kg/day of perchlorate and specified its drinking water equivalent level to be 24.5 ppb. Perchlorate can be uptake in plants, especially at environmentally relevant (low ppb) concentrations. In the present study we investigated the occurrence of perchlorate in the north of Chile, Iquique using IC-ESI-MS and illustrate the perchlorate pollution status in Chile. A total of 10 soils and 4 salary samples were collected from village around of Iquique. The soils in study is Canchones (6 samples), Tirana (4 samples) and Pintados Salary (4 samples). The quantification of perchlorate was performed by IC-ESI-MS, the methods detection limits (MDL) of perchlorate in soils were 0.03 ppb. The soils were characterized for pH, electric conductivity and bases of interchange. The result of the soils analyses, indicate the existence of perchlorate in 9 of the 14 soils in study: Canchones (496 µg/Kg, 430 µg/Kg, 140 µg/Kg and 280 µg/Kg), Tirana (257 µg/Kg, 600 µg/kg and 530 µg/Kg) and Pintados Salary (346 µg/kg and 1719 µg/Kg). The rest of soils is under the limit of detection of the method. The presence of perchlorate around of agricultural zones and habited of the north of Chile in close concentrations to the established for international organism, generate a situation of precaution because considering the mobility and persistence of perchlorate the transference toward water sources used for the human consumption and for the irrigation of traditional cultivations and of accumulative vegetables like lettuces and spinach, can represent a real risk for the health of the consuming potentials.

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Keywords: Perchlorate, inorganic contaminants; Atacama Desert.

S5-P5**Characterization of Phytate-Utilizing-Bacteria during Aerobic Degradation of Dairy Dung****B. Fuentes^{1,2*}, M. Jorquera³ and M.L. Mora⁴**¹Programa de Doctorado en Ciencias en Recursos Naturales, Universidad de La Frontera, Temuco, Chile. ²Departamento de Ingeniería Química, Universidad Católica del Norte, Antofagasta, Chile.³Instituto de Agroindustrias, Universidad de La Frontera, Temuco, Chile. ⁴Departamento de Ciencias Químicas, Universidad de La Frontera, Temuco, Chile. *E-mail: bfuentes@ucn.cl

Phytate-utilizing-bacteria (PUB) have potential biotechnological applications in agricultural fields, such as animal nutrition and soil fertility. Amendments of organic waste such as manure, compost or sludge contribute to increase phytate concentration in soil. Due to phytate is strongly linked to colloids of soil; it is stabilized in unavailable forms for plants nutrition. Improvement of P utilization could be achieved by using PUB to increase the availability of P in dung in order to produce an organic fertilizer rich in available P. In this study, culture-dependent methods, genetic and enzymatic analyses were used to characterize PUB populations during aerobic degradation of dairy dung. The culture-medium indicated the occurrence of PUB throughout degradation process. The phylogenetic analyses based on 16S rDNA gene sequences showed high diversity of PUB, which resulted similar to bacteria belonging to genus *Bacillus*, *Escherichia*, *Enterobacter*, *Ochrobactrum*, *Rahnella*, *Shigella* and *Streptomyces*. Two morphotypes with high phytate-utilizing capacity were genetically characterized as member of genus *Enterobacter* and *Rahnella*. Moreover, these morphotypes showed capacity to produce alkaline and acid phosphatases as revealed the enzymatic characterization by using Api Zym system. PUB could be useful tools to produce a fertilizer rich in available P to plants.

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Keywords: Phytate-utilizing-bacteria; aerobic degradation; dairy dung

S5-P6**Rhizospheric Bacteria Community Structure Perturbation of *Acacia caven* in the Presence of the Herbicide 2,4-Dichlorophenoxyacetic Acid and the Bioprotective Effect of *C. necator* JMP134 on the Plant****T. Kraiser*, M. Manzano and B. González**

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Cupriavidus necator JMP134, is a *b*-proteobacterium well studied for its great catabolic versatility and whose genome sequence is now available. This bacterium can degrade and use more than 50 aromatic compounds as carbon source. Among them, xenobiotic compounds like the 2,4-dichlorophenoxyacetic acid (2,4-D), used for many years as an herbicide. *Acacia caven* is a leguminous shrub belonging to the *Mimosaceae* family. This plant has a wide distribution on Chile, associated with dry places, with a great resistance to the drought. *A. caven* is sensitive to 2,4-D, when used at standard field concentration. The aim of this study

was to test the effect of the 2,4-D on *Acacia caven* and in its community structure of rhizospheric and non-rhizospheric bacteria, in the presence of *C. necator* JMP134. Using plant-soil microcosms with or without exposure to 2,4-D and inoculated or not with a green fluorescent protein tagged *C. necator*, we detected that this bacterium has a preferential distribution towards the rhizosphere of *A. caven*. The presence of 2,4-D clearly diminished the ratio of *C. necator* cells found in the rhizospheric soil compared with non-plant soil. The presence of the wild type strain protected the plant from toxicity of 2,4-D, because plants died in soil microcosms inoculated with a *tfdA* mutant, unable to degrade 2,4-D. The analysis of the bacteria community structure by the T-RFLP technique in the rhizospheric and non-rhizospheric soil of *A. caven* in the absence of 2,4-D, revealed that the community structure of these two kinds of soil were different. The presence of the herbicide in these plant-soil microcosms produces a remarkably decrease in the differences between the bacterial community structure of the rhizospheric soil and the non-rhizospheric soil. The non-rhizospheric bacterial community structure remained unchanged, but the rhizospheric bacterial community structure turned to be closer to that of the bacterial community that is not influenced by the plant. This effect of 2,4-D was observed even if the plant had been protected of the lethal effect of this compound by *C. necator* JMP 134. This, can be explained by a toxic effect of the 2,4-D on the rhizospheric bacteria or because a change of the composition of the exudates of the plant in a stress situation.

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Keywords: Rhizospheric bacteria community; *Acacia caven*; 2,4-dichlorophenoxyacetic acid.

S5-P7

Effect of Metal Speciation on the Functional and Morphological Responses of Soil Fungi: Case of Cu and Zn in *Trametes versicolor*

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Filamentous fungi are part of major biomass in soil and are key actors of soil functioning due to their ability to produce extracellular enzymes. It is known that metals can alter extracellular enzymatic activities as well as morphology in fungi. Thus fungal responses to metals were suggested to be used as biomarkers of metal exposure in soils. Such biomarkers are needed in the environmental risk assessment, to develop methods for measuring the impact of metal contamination on soil microorganisms. However it is not well known how the metal speciation, linked to the metal bioavailability, influences the soil fungi responses to the presence of metals thus limiting the predictive interest in such biomarkers. The present study aims at investigating both the morphological responses and the sensitivity and selectivity of extracellular oxidases produced by the filamentous fungus, *Trametes versicolor*, exposed to Cu or Zn, taking into account the speciation of these two metals. For that purpose, the activities of three peroxidases were used as biomarkers of exposure and of bioavailability: the laccase, manganese- and lignin-peroxidases. Activities were monitored from pure cultures performed in various liquid media exhibiting several levels of metal complexation in order to

vary metal bioavailability. Total metal contents from 0.1 μM to 1 mM and three liquid media, either rich or poor in organic ligands, were tested. Morphological responses were characterized in scanning electron microscopy. Theoretical speciation of Cu and Zn was calculated using the SOILCHEM thermodynamic program at the initial stage, and an Ion Selective Electrode was used to determine the free copper contents in the various media. Our results showed that Zn had no effect on the three oxidases tested. Cu however highly stimulated laccase and manganese-peroxidase activities. Lignin peroxidase was not measured in the control cultures without metals, but was found specifically produced after fungal exposure to Cu. Culture media exhibiting low complexing properties led to an increase in intensity and sensitivity of oxidase responses, suggesting a higher metal bioavailability. Moreover experimental speciation of Cu showed that Cu^{2+} contents were higher in the low complexing media. Theoretical speciation showed that only a few percentage of total Cu and Zn were in the free forms, the other forms were complexes of Zn and Cu with the organic or inorganic ligands. In our conditions, we were able to measure enzymatic responses to Cu at environmental levels of contamination (1 μM). Only Zn was found to impact the morphology of fungi during exposure. This result contrasted with the fact that no significant stimulation of oxidase activity could be detected in our experimental conditions. Our results show that the response of fungi is specific of a metal and depends on metal speciation: oxidases are stimulated in the presence of copper but no impact could be found on fungi morphology, while Zn had an impact on fungi morphology but had no effect on oxidase activities. This specificity and sensitivity confirm that fungal responses can be useful tools for metal ecotoxicity assessment that need to be tested in contaminated soils.

Keywords: Metal speciation, *Trametes versicolor*; Copper; Zinc.

S5-P8

The Metabolic Response of *Arabidopsis* to a Combination of High Light and Sulfate Deficiency

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One of the most crucial functions of plant cells is the ability to respond to environmental changes. Understanding the connections between a plant initial response and the downstream events is one of the open questions of plant biology. In the past effects of sulfate deficiency in plants has been intensively investigated to study the systems response to the applied stress. However, the majority of the performed experiments testing the response of plants to limiting sulfate levels were focused on single stress treatments applied to plants under controlled conditions. Responses of plants to sulfur nutrition and varied light intensities during growth have not been characterized so far. Chlorophyll fluorescence imaging and metabolite profiling were used to study the metabolic changes as read out for the plant response in leaf tissues of *A. thaliana* sulfate starved plants under different light intensities. Our study revealed a new pattern of response showing an initial event of inhibition in the photosynthetic

Session 5. Environmental Biotechnology: Biochemical and Molecular Mechanisms of Microbe-Plant-Root Interactions & Their Genomic & Proteomic Advances Pertaining to Restoration of Contaminated Soils

performance associated with alterations at metabolic levels of different pathways. Furthermore, sulfate deprivation causes the disruption of metabolic pathways involved in the acclimatization process of plants to high light, such as the antioxidant defence system.

Keywords: *Arabidopsis thaliana*; sulfate deficiency; high light

S5-P9

***Rhizopus oryzae* Immobilization for Biodiesel Production Using a Soil Extract Media**

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The use of chemical catalysts (acids and alkalines) in the transesterification reaction for biodiesel production have some negative aspects. Such as: difficult of glycerol recover, the need for wastewater treatment process and free fatty acids in the raw material can interfere with the reaction, generating saponified products. These problems can be minimized by using a catalytic enzyme (biocatalysts). The uses of extracellular enzymes are expensive (given its high purity level) and therefore are being used intracellular enzymes (whole cell) whose cost is closely related to the culture medium with which the organism grows. Soil is a known source of LPM (Lipase Producing Microorganism), such as *Rhizopus oryzae*. The main objective of this work was to immobilize *Rhizopus oryzae* using a growing low commercial value medium for produce biocatalyst particles for application in biodiesel production. *R. oryzae* was kept on potato dextrose agar plates (4%) and it was grown in a modified medium used for isolation of LPM from the soil and it consists of extract soil (10%), urea (0,02%) and rapeseed oil (3%) in 1 L of distilled water. The extract soil was prepared by stirring 5 min 100 g of sieved soil, 0.2 g CaCO₃ in 1 L of distilled water, filtered and sterilized. An airlift reactor with 550 mL of culture medium was inoculated with *R. oryzae* spores and zeolite was used as support material, the fungus was immobilized as result of natural growth. This process was carried out at 30°C and 1 lpm of airflow. After cultivation, the microorganism immobilized in zeolite was separated, washed with water for 1 minute and dried at room temperature for 48 h. To stabilize the lipase activity, the microorganism immobilized in zeolite was treated with a glutaraldehyde solution (0.1 - 1.0% v / v) at 25°C for 1 hour, washed with water and dried at room temperature for 48 h. For the transesterification reaction were used bottles of 30 ml with rapeseed oil and ethanol at 1:3 molar ratio and biocatalyst particles. The reaction was carried out in a shaker at 30°C and 150 rpm. The contents of alkyl esters were analyzed by gas chromatography. Conversion of rapeseed oil and alcohol into biodiesel evidenced the microorganism immobilization using soil extract medium and it increased with the glutaraldehyde concentration treatment. While this conversion was low it could be optimized and to compete with other culture mediums of higher commercial value that are normally used for growth of *R. oryzae*.

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Keywords: *Rhizopus oryzae*; microorganism immobilization; biodiesel.

S5-P10**Vegetable Meals as Substrate for Cellulase Production by *Asperguillus niger*****M. Riveros¹, Y. Esparza¹, M. Rubilar^{1,2} and C. Shene^{1,2*}**¹Departamento de Ingeniería Química, Universidad de La Frontera, Casilla 54-D, Temuco, Chile.²Agri aquaculture Nutritional Genomic Center, Technology and Process Unit, Universidad de La Frontera. Casilla 54-D, Temuco, Chile. *E-mail: cshene@ufro.cl

Cellulose is insoluble and crystalline; hence, it is largely resistant to enzymatic hydrolysis. In many biomass utilization schemes, the raw material is first treated with dilute acid at moderate temperatures to remove hemicellulose and to speed-up subsequent cellulose hydrolysis by enzymes. Worldwide, screening of whole cellulase preparations is done predominantly using the International Union of pure and Applied Chemists (IUPAC) standard filter paper assay (FPA). In this assay the dinitrosalicylic acid (DNS) method is used to determine reducing sugars (RS) released from filter paper hydrolysis. Because cellulase activity in whole broths is nonlinear in regarding enzyme concentration, dilution is required to a point where 2 mg of RS equivalents are released in 1 h at 50°C and pH 4.8. This amount of enzyme is defined as one filter paper unit (FPU). In this work cellulase production by two *Asperguillus niger* strains ATCC 20447 and ATCC 11414 was evaluated. Fermentations were carried out using rapeseed meal and a mixture of bran and wheat germ. For the submerged fermentations (SmF) the substrates (1% w/v) were inoculated with spores to a final concentration of 10^4 cell mL⁻¹. These fermentations were carried out at 25°C and enzyme production was followed during 7 days. In the solid state fermentation (SSF) the substrates were moisturized to 65% and inoculated with spores to a final concentration of 10^4 cell g⁻¹ dry matter. Incubation was carried out at 25°C in a water bath. Every 24 h two flask containing the fermented substrate were taken out for enzyme quantification. In the SmF, enzyme production presented a maximum (0.02 U) at 96 h with a significant reduction (0.007 U) after 120 h. Cellulase activity in the SSF of bran and wheat germ by *A. niger* ATCC 11414 presented a sustained increase and after 48 h, 0.56 U g⁻¹ dry matter was obtained. These results allowed us to conclude that best fermentation conditions for the production of cellulose by *A. niger* strains is SSF. The substrate also affected the enzyme production and best results were those obtained by using a mixture of bran and wheat germ. Probably the slow growth rate and water limiting conditions in SSF favor the enzyme production. Experiments for the optimization of fermentation conditions (moisture, fermentation time, size of the inoculum, substrate and substrate pre-treatment) are currently under study.

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Keywords: Vegetable meals; cellulase; *Asperguillus niger*.

S5-P11**Biomass Production by a Native *Thraustochytrium* Strain****A. Huaiquil¹, I. Hinzpeter², B. Quilodrán³, M. Rubilar^{1,4} and C. Shene^{1,4*}**¹Department of Chemical Engineering, Universidad de La Frontera, Casilla 54-D, Temuco, Chile.²Department of Government and Management, Universidad de Los Lagos, Casilla 557, Puerto Montt, Chile. ³Department of Natural Resources and Environment, Universidad de Los Lagos, Casilla 557, Puerto Montt, Chile. ⁴Agri aquaculture Nutritional Genomic Center, Technology and Process Unit,

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Biomass production by a native *Thraustochytrium* strain isolated from the coastal area of Budi Lake was study. Budi lake is the only salt lake of América, located at La Araucanía Region, Chile. The genus *Thraustochytrium* contains unicellular, zoospore-producing species of historically uncertain taxonomic affinity. These microorganisms have recently attracted the interest because of their capability to produce docosahexaenoic acid (C22:6, DHA), eicosapentaenoic acid (C20:5, EPA), and docosapentaenoic acid (C22:5, DPA). DHA is a major structural component of the gray matter of the brain and the eye retina and an important component of heart tissue. Regular DHA consumption has been shown to be important for proper development of the brain and eye in infants and supports good cardiovascular health. On the other hand, EPA acts as a precursor for eicosanoids, prostaglandin-3 (which inhibits platelet aggregation), thromboxane-3, and leukotriene-5 groups. In this work, the effect the carbon [glucose, rye, malt extract (ME) and malt syrup (MS)], and nitrogen (lupine and yeast extracts) sources on biomass production by our native strain was evaluated. The inoculum was grown for 48 h on media containing glucose 20 g/L, yeast extract 4 g L⁻¹, and monosodium glutamate (MSG) 2 g L⁻¹ in 50% artificial seawater. Flasks containing 100 mL of the following growth media: 1% (w/v) C-source, 0.4% (w/v) N-source and 0.2% (w/v) MSG, initial pH 7, were inoculated and incubated at 20°C and 250 rpm. The results indicated that the best C-source was glucose with a final biomass concentration of 2.2 g L⁻¹. Growth media based on ME and MS showed higher growth rates although final biomass concentrations were lower, 1.5 and 1.9 g L⁻¹, respectively, suggesting a limitation by an unknown nutrient. Best N-source for biomass production in flask experiments was YE. The fast pH increase observed in the lupine based media was responsible of the low biomass production and thus this media should be evaluated under controlled pH conditions. Lipid content in the biomass of Th4 strain was dependent on growth conditions (10-40%). Saturated fatty acids in the lipids produced when biomass was grown in the Glu-YE-MSG media were 35.6%; monounsaturated and polyunsaturated fatty acids were 7.8 and 56.6%, respectively, and DHA represented 43.8% of the polyunsaturated fatty acids. Addition of vitamins (thiamin, biotin, cyanocobalamin and Ca-pantothenate) and trace metals significantly increased biomass production under controlled pH conditions and highest biomass productivity was 0.033 g L⁻¹h⁻¹. Under this growth conditions specific growth rate was 0.04 h⁻¹.

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Keywords: *Thraustochytrium* strain; fatty acids; biomass production.

S5-P12**Consumption of *Ugni molinae* Turcz. Extracts and Its Effect on the Human Gut Microflora****N. Canquil¹, M. Riveros¹, M. Rubilar^{1,2} and C. Shene^{1,2*}**¹Department of Chemical Engineering. Universidad de La Frontera. Casilla 54-D. Temuco. Chile.²Agri aquaculture Nutritional Genomic Center. Technology and Process Unit. Universidad de La Frontera. Casilla 54-D, Temuco. Chile. *E-mail: cshene@ufro.cl

Murta (*Ugni molinae* Turcz) is a wild shrub growing in the South of Chile. Studies on the chemical composition of the leaves indicate the presence of phenolic acids, flavonoids and tannins. Dietary flavonoids have recently attracted the interest because *in vitro* and *in vivo* studies suggest they have a variety of beneficial biological effects, which may play an important role in the maintenance of human health. The flavonoids are potent antioxidants, free radical scavengers and metal chelators; they inhibit lipid peroxidation and exhibit various physiological activities including anti-inflammatory, antiallergic, anticarcinogenic, antihypertensive, antiarthritic and antimicrobial activities. Many plant phenols are known to possess antimicrobial properties, so their consumption might change the composition of the human gut microbial flora. The aim of this study was to investigate the antimicrobial properties of murta leaves against human gut microorganisms isolated from faecal human samples after the regular consumption of infusions. The fecal samples were collected from 6 healthy human subjects of different ages (0 to 50 years) and sex (three women and three men). Subjects maintained their usual lifestyles and dietary intakes throughout the two-weeks period of study. The volunteers consumed 200 mL of a murta leaf infusion (5 g/L), twice a day. Samples were collected in sterile specimen jars and immediately taken to the laboratory. A control sample was requested at the beginning of the study. Samples were also requested at 5th and 10th day, during the infusion consumption. A last sample was requested at the 15th day, time at which the infusion consumption was finished. The samples were homogenized in buffer sodium phosphate (0.1 M pH 6.5) to a final concentration of 10% w/v. A dilution series (10^{-1} to 10^{-4}) was made in the same buffer, and 100 μ L aliquot of each dilution was used to inoculate selective media by spread plating. Bifidobacteria, Bacteroides and Gram positive coccus were enumerated on blood agar; total anaerobic and aerobic bacteria on nutritive agar; clostridia on clostridial agar; coliforms on Wilkins Chalgren agar; *Lactobacillus* on MRS agar and *Staphylococcus* on Bair Parker agar. Bacterial stocks were isolated for the analysis of *in vitro* effect through the diffusion in agar method. The tested extracts were those obtained in water (100%) and water-ethanol mixture in volumetric ratio of 1: 1. The results showed that regular consumption of aqueous murta infusions do not affect the distribution of bacterial microflora population, at least in the concentration and frequency of consumption evaluated. The *in vitro* assays confirmed that aqueous murta extract do not have antimicrobial effects, even at higher concentrations. However, water-ethanol extracts displayed antimicrobial effect on 12 of the 70 isolate bacterial strains. It was also demonstrated that the *in vitro* antimicrobial effect was limited to aerobic strains.

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Keywords: *Ugni molinae*; flavonoids; human gut microflora.

S5-P13**Protease and Prolil-Endopeptidase Production by *Aspergillus niger* Under Different Culture Condition****Y. Esparza¹, A. Leyton¹ and C. Shene^{1,2,*}**¹Department of Chemical Engineering, ²Agri aquaculture Nutritional Genomic Center, Technology and Process Unit. Universidad de La Frontera, Casilla 54-D, Temuco, Chile. *E-mail: cshene@ufro.cl

The celiac disease (CD) is a permanent gluten intolerance. Gluten present in wheat, barley, rye and oat, is partially degraded in the gastrointestinal tract. The resulting proline rich fragments are sub-sequentially modified by tissue transglutaminase (tTG) and following reactions induce epithelial intestinal damage. Several studies have demonstrated that proline peptides are efficiently hydrolyzed by prolil-endopeptidase (PEP) produced by some *A. niger* strains, suggesting a new therapy for CD treatment. Very few references describe PEP production by *A. niger* and no one has evaluated the synthesis in solid state fermentation (SSF). In this work proteases and PEP production by two *A. niger* strains ATCC 20447 and ATCC 11414, was evaluated in submerged fermentation (SmF) and SSF. Culture media used in SmF contained for 1 L: KH₂PO₄ 1 g, K₂HPO₄ 2 g, KCl 0.5 g, MgSO₄·7H₂O 0.5 g, FeSO₄·7H₂O 0.01 g and variable levels of glucose, yeast extract and gelatin. Data for the optimization of culture media composition was obtained from flask experiments. The effects of pH, aeration and temperature were evaluated in batch fermentations. In the SSF a mixture of bran and wheat germ moisturized to 65% was used. Incubations were carried out at different temperatures. Protease activity was quantified by incubating the protein solution with azocasein solution (2,5 g/L) at 37°C for 20 min; the reaction was stopped through the addition of TCA 1 M. One unit of protease activity was defined as the amount of enzyme that increased the absorbance at 400 nm by 0.1 per min. PEP activity was assayed using a synthetic peptide Z-Gly-Pro-pNA (Sigma, 100 µM in DMSO 20%). The reaction was carried at 45°C for 1 h and the released pNA was measured spectrophotometrically at 410 nm. One unit of PEP activity was defined as the amount of enzyme that release 1mM of pNA per min. Highest protease activity in SmF was 884.6 U obtained after 14 days cultivated by using glucose 5 g/L, yeast extract 20 g/L, gelatine 10 g/L at pH 4.5. Growth temperature significantly (p<0.05) affected protease production suggesting that slow growth positively influence protease production. Maximum specific PEP activity (0.004 U/g) was obtained after 100 h at 25°C. In SSF by *A. niger* ATCC 11414 maximum protease activity was 130.4 U/g wet matter after 4 days incubation at 25°C; specific PEP activity was 0.038 U/g. These results confirm the hypothesis that SSF can be used for the production of PEP. Experiments for the optimization of conditions for the PEP production in SSF are currently in progress. The possibility of PEP therapy against CD is currently a very important biotechnological application, because the unique treatment against CD is a gluten free diet.

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Keywords: Protease; prolil-endopeptidase; *Aspergillus niger*.

S5-P14**Microbial Community Structure in Pentachlorophenol Contaminated Soil under Fungal Bioremediation Treatment Amended with Wheat Straw****M. Cea^{1*}, M. Jorquera¹, H. Langer², M.L. Mora¹ and M.C. Diez³**

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We analyzed, by denaturing gradient gel electrophoreses (DGGE), the structure of bacterial and fungal community in a pentachlorophenol (PCP) contaminated soil under fungal bioremediation treatment amended with wheat straw. The effect of the bioremediation treatment on autochthonous microorganisms was evaluated in simple microcosms. Briefly, 75 g of soil (35% of humidity) were put into glass recipient and different treatment were established: T1 (soil), T2 (soil contaminated with PCP), T3 (soil mixed with wheat straw), T4 (soil contaminated with PCP and mixed with wheat straw), T5 (soil mixed with *Anthracyllium discolor* grown in wheat), and T6 (soil contaminated with PCP mixed with *A. discolor* grown in wheat). The soil was artificially contaminated by spiking a stock solution of 10 g L⁻¹ of pentachlorophenol (PCP) diluted in KOH 0.1 mol L⁻¹ reaching a concentration of 250 mg of PCP per kg of soil. The samples were incubated at a controlled temperature (25±1°C) for 28 days. Water losses by evaporation were compensated weekly to maintain soil water content. Treatments were set up in triplicates. At the end of experiment, the analysis of 16S rDNA gene revealed that the addition of PCP stimulated the presence of γ -Proteobacteria (Xanthomonadaceae) and β -Proteobacteria (Burkholderiaceae). The addition of PCP plus straw also stimulated the presence of Enterobacteriaceae. In the case of fungus, the analysis of 18S rDNA gene revealed that PCP stimulated the presence of Saccharomycetes, Tremellomycetes and Agaricomycetes. The addition of straw showed an increment of other basal fungal lineales, such as Ascomycetes. However, the addition of PCP plus straw revealed a different pattern of stimulation. Under the presence of *A. discolor*, the addition of PCP plus straw showed predominance of member of Agaricomycetes and Ascomycetes. This work emphasizes the importance of autochthonous microorganisms involved in PCP degradation.

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Keywords: Microbial community; pentachlorophenol; fungal bioremediation.

S5-P15**Acquiring Tolerance to Solvents during a Vermicomposting Process**

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In this work, a cultivable, Gram-positive, solvent-resistant bacterium was isolated from vermicomposted olive wastes. The highest 16S rDNA sequence similarity (99%) was found with *Brevibacillus brevis*. The genome of the isolate, selected for TCE-tolerance, contained a nucleotide sequence encoding a conserved protein domain (ACR_tran) ascribable to the HAE1-RND family. Members of this family are hydrophobic/amphiphilic efflux pumps largely restricted to Gram-negative bacteria. Since no cultivable solvent-tolerant bacterium was detected in the unvermicomposted olive waste, a transfer of solvent-resistance genes from Gram-negative bacteria during the vermicomposting process could explain the presence of HAE1 transporters in *B. brevis* isolated from the vermicompost. The RND gene expression induced by different levels of pollution was determined with real time PCR in the bacterium cDNA. Under TCE stress conditions, the acquired nucleotide sequence was translated into proteins, and tolerance to solvents was conferred to the bacterium.

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Keywords: Tolerance; vermicompost; *Brevibacillus brevis*.



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