

Single and Sequential Extraction of Cadmium in Some Highly Calcareous Soils of Southwestern Iran

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Abstract:

Thirty-two surface soil samples with different physical and chemical properties were collected from calcareous soils of arid regions of Khuzestan Province, Southwestern Iran. Actual and potential cadmium (Cd) bioavailability was assessed by means of a greenhouse study, two different single extractions: 0.005 DTPA and 0.05 EDTA and two sequential extraction procedures: modified five-step Tessier method and the three-step BCR method. Extraction results were correlated with grain Cd uptake by Durum wheat (*Triticum durum* L.) grown under greenhouse conditions. Cadmium in the durum grain ranged from 0.064 to 0.273 mg kg⁻¹. The results showed better suitability of DTPA extraction in these highly carbonated soils than EDTA extraction for predicting Cd phytoavailability. DTPA –extractable Cd was positively related to soil electrical conductivity ($p < 0.01$, $r = 0.65$). Cadmium was predominantly associated with carbonate fraction in these soils (Tessier procedure 40% and BCR 44%). 1 M NH₄OAc which extracted the soluble + exchangeable fraction in the Tessier scheme may also provide useful information on Cd availability in studied soils.

Keyword: contamination, cadmium, sequential fractionation, wheat

1. Introduction

The chemical form of the metal determines whether it is bioavailable which are bound or associated with different soil constituents (Shuman, 1991). The degree of metal association with soil constituents depends on soil physical and chemical properties primarily soil pH, soil solution ionic composition, chloride concentration in soil solution, CEC, clay mineral content and types, organic matter content, calcium carbonate equivalent,

oxides of Fe, Mn, and Al, and redox conditions (Naidu *et al.*, 1994). Single and Sequential extraction techniques are widely used to characterize the metal fractions in soils and sediments and predict their mobility and bioavailability (Ahnstrom and Parker, 1999; Tessier, 1979). A single extraction method is designed to dissolve a phase whose elemental content is easily mobilizable and ideally is correlated with the

plant bioavailability of the element. In sequential extraction techniques, a sample is treated with a series of progressively harsher reagents to dissolve increasingly recalcitrant forms. In the case of heavy metals, these methods operationally estimate the amounts of metals, in various solid pools, which commonly termed exchangeable, carbonate bound, Fe–Mn oxide bound (Reducible), organic (Oxidisable) and residual. Ideally, the reagents are chosen to selectively attack a specific soil compartment with minimal dissolution of non-targeted phases (Ahnstrom and Parker, 1999). Although these techniques have been in use for four past decades (Gibbs, 1973; Tessier *et al.*, 1979), there are no universally accepted procedures in terms of reagents and their order in the sequence (Sutherland *et al.*, 2000). This variability limits both method validation as well as comparability of results obtained under multiple extraction regimes.

In order to establish a uniform extraction procedure the European Standards, Measurements and Testing Programme (formerly BCR) introduced the standardized sequential extraction procedure (Ure *et al.*, 1993). This procedure has been standardized and applied to a variety of matrices including sediments, soils; sewage sludge, mining wastes etc., and some modifications have been suggested during the evaluation of the scheme (Sahuquillo *et al.*, 1999; Sulkowski *et al.*, 2006). Of particular concern here is the fact that the BCR scheme considered the soluble, exchangeable and carbonates fractions as a single fraction with uniform potential bioavailability. However, in highly calcareous soils the low-solubility carbonate fraction is predominant (Rajaei *et al.*, 2006). This suggests that the BCR scheme may be inadequate for predicting potential Cd solubility and plant availability. As such, the potentially bioavailable fraction which correlates with plant uptake should be considered and separately distinguished in the sequential extraction schemes.

The soils of Khuzestan province, a primary crop production area in Southwestern Iran, are extremely carbonated, with more than 40 percent carbonate

content in most parts. Agricultural activities are extensive in the area and application of inorganic fertilizers, especially phosphorous, has caused elevated Cd levels in soils and grain crops produced in the area (Jafarnejadi *et al.*, 2011). Nevertheless, there has been very limited information on cadmium Cd status and, chemical forms, which is needed to evaluate the actual and potential Cd mobility and bioavailability in the region's soils.

To evaluate the adoption of the BCR scheme modified for carbonate rich soils (Sulkowski *et al.*, 2006) in calcareous soils of the region, we compared the results of this scheme with those of the extraction protocol of Tessier *et al.* (1979) which is, typically used for analysis of the calcareous soils of Iran (Abbaspour *et al.*, 2007; Jalali and Khanlari, 2008). These were also compared with standard single step extraction protocols (DTPA and EDTA). The objectives of this study, therefore, were (i) to evaluate the Cd phytoavailability by growing a wheat crop in a greenhouse condition, (ii) to determine the major soil fractions that contain bioavailable Cd in a wide range of soils of Khuzestan province using two single and two sequential extraction schemes.

2. Methods and Materials

2.1. The Study area and soil sampling

This investigation was conducted using 32 surface soil (0–20 cm) samples collected at different randomly selected farms in an area of approximately 4000 km², representing different soil types of calcareous soils in arid region of Khuzestan Province, Southwestern Iran, (47° 40' to 50° 33' N and 29° 57' to 57° 33' E). The climate vary from semiarid to arid with a mean annual precipitation and class A pan evaporation of 240 and 3000 mm, respectively (Farshi *et al.*, 1997). The physiography of the region ranged from piedmont plains to lowlands in the south. The soils are highly calcareous and are mostly classified as Entisols,

Aridisols and Inceptisols 17. The region's primary crops are wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), canola (*Brassica napus* L.), rice (*Oryza sativa* L.), and vegetables.

2.2. Soil analysis

Soil samples were air dried, ground, and passed through a 2-mm sieve. Analyses and methods (Table 1) included: particle size distribution (hydrometer method, Gee and Bauder, 1986); Soil Organic Matter (SOM) content (Walkley-Black procedure; Nelson and Sommers, 1996); Cation exchange capacity (CEC) by replacing cations with NaOAc (Chapman, 1965); Soil pH (glass electrode in saturated paste); and Electrical conductivity of saturation extracts by a conductivity meter. Calcium carbonate equivalent (CCE) was determined by neutralization with hydrochloric acid (Leoppert and Suarez, 1996), active calcium carbonate (ACCE) by the NH_4 -oxalate method (Drouineau, 1942), and manganese oxides (Mnox) were determined using the Loeppert and Inskeep method (1996). Total Cd concentration was determined by aqua regia procedure (ISO 11466), and all measurements of Cd concentration were performed by graphite furnace atomic absorption spectrometry (GFAAS) (PG 990).

2.3. Single and Sequential extraction

Single extractions were performed using DTPA and EDTA. For DTPA extraction 20 mL of DTPA 0.005 M and CaCl_2 0.01 M and TEA 0.1 M (pH 7.3) solution (Lindsay and Norvel, 1978) was added to 10 g soil in a 125 mL Erlenmeyer flask, shaken for 2 h in a reciprocal shaker, and filtered through a Whatman No. 597 filter paper. Similar procedure was used for EDTA (0.05 M, pH 4.65), except that the soil weight was 2 g and the shaking time was 1 h.

The sequential extraction methods of Tessier *et al.* (1979) as modified by Tsai *et al.* (2003) and BCR modified for carbonate rich soils (Sulkowski and Hirner, 2006) were used for partitioning Cd in the sampled soils. The outlines of these procedures are

presented in Tables 2 and 3. Cadmium contents of the final residues (following step 5 for Tessier and step 4 for BCR) was determined by digestion with aqua regia. All extractions were carried out on triplicate 1 g samples, for each soil, which were placed in a 50 mL centrifuge tubes of known weight.

Following each step, samples were centrifuged at 6000 rpm for 15 min, and the supernatant liquid was filtered through No. 597 Whatman filter paper.

2.4. Greenhouse experiment

Polyethylene pots containing 7 kg homogenized soil, which were passed through a 5-mm sieve, were prepared in triplicate for each soil. Each pot was treated sufficient N and P fertilizer before planting as $\text{Co}(\text{NH}_2)_2$ and H_3PO_4 . Four seeds of Durum wheat (*Triticum durum* L.) were sown in each pot and grown under greenhouse conditions. The pots moisture was kept near field capacity by frequent watering with de-ionized water. Plants were grown until maturation of the grain, and harvested. Grain were separated from shells, washed thoroughly with deionized water, oven-dried at 70°C for 72 h, ground and mixed thoroughly. Digestion of grain was carried out using $\text{HNO}_3\text{HClO}_4\text{H}_2\text{SO}_4$ hot block procedure and concentration of Cd was determined by graphite furnace atomic absorption spectrometry (GFAAS).

3. Results and discussion

3.1. General soil properties

General soil properties as well as the results obtained from single extraction of DTPA and EDTA are presented in Table 1. The soils covered a large range of soil properties in terms of electrical conductivity, organic carbon, clay, cation exchange capacity and calcium carbonate equivalent. The pH values of these severely carbonatic soils were alkaline ranged from 7.05 to 8.37. Calcium carbonate equivalent contents varied from 26.7 to 63.2 %.

Table1. Selected physical and chemical properties of soils (n = 32) as well as cadmium concentration in wheat grain for the soils used in the greenhouse experiment

Characteristics	Range	Median	Mean	SD
pH (water)	7.05 - 8.37	7.51	7.51	0.3
EC (dSm ⁻¹)	0.8 - 193.1	5.28	16.63	34.8
OC (%)	0.1 - 1.2	0.55	0.58	0.3
CEC (Cmol _c kg ⁻¹)	5.2 - 25.6	15.0	14.88	5.3
Sand (%)	8 - 60	24	27.7	14.0
Silt (%)	24 - 62	44	45.1	7.3
Clay (%)	12 - 46	28	27.2	11.1
CCE (%)	26.7 - 63.2	44.1	43.6	9.5
ACCE (%)	10.9 - 27.6	22.0	21.0	3.7
Mn Oxides (mg kg ⁻¹)	37.5 - 495	207.7	221.4	102.5
Total Cd (mg kg ⁻¹)	1- 3.3	2.2	2.1	0.65
EDTA Cd (mg kg ⁻¹)	0.45-1.65	0.9	0.95	0.4
DTPA Cd (mg kg ⁻¹)	0.04 - 0.15	0.06	0.065	0.02
Grain Cd (mg kg ⁻¹)	0.064 - 0.273	0.133	0.132	0.047

EDTACd : EDTA-extractable Cd; DTPACd: DTPA-extractable Cd; SD: Standard deviation

A wide range of EC was found, with a minimum of 0.8 dS m⁻¹ and a maximum of 193.1 dS m⁻¹. Total Cd concentrations in the soils varied from 1 to 3.3 mg kg⁻¹ with mean and median of 2.1 and 2.2, respectively. This is higher than the threshold recommended for calcareous soils (2 mg kg⁻¹; Wang, 1999) indicating Cd contamination of the studied soils mainly due to application of high-Cd phosphorous fertilizers (Jafarnejadi *et al.*, 2011). Total Cd was correlated (Table 4) only with soil organic carbon ($r = 0.48^*$) this

may be as a sequence of higher biomass production in areas with higher phosphorous fertilizers application.

3.2. Single extraction

Single extractions can be considered as tools indicating potential release of elements from particular soil constituents with which they are associated. EDTA-extractable Cd concentration varied from 0.45 to 1.65 mg kg⁻¹ with a mean of 0.95 mg kg⁻¹, whereas, DTPA-

Table 2. Scheme of metals fractionation proposed by Tessier *et al.* (1979) modified by Tsai *et al.* (2003)

Step	Fraction	Extractant	pH	Procedure
1	Soluble+Exchangeable	20 ml 1 M NH ₄ OAc	7	0.5 h shaking at 25°C
2	Associated with carbonates	20 ml 1 M NaOAc	5	5 h shaking at 25°C
3	Associated with Mn oxides	20 ml 0.1 M NH ₂ OH.HCl in 0.1 M HNO ₃		0.5 h shaking at 25°C
4	Associated with Fe oxides	20 ml 0.04 M NH ₂ OH.HCl in 25% w/v HOAc	2	6 h shaking at 96°C
5	Associated with organic matter	5 ml 0.1 M HNO ₃ / 10 ml H ₂ O ₂ 30% m/v + 15 ml NH ₄ OAc 3.2 M	-	5 h shaking at 85°C 30 min shaking at 25°C
6	Residual	aqua regia		

-extractable Cd concentration varied from 0.45 to 1.65 mg kg⁻¹ with a mean of 0.95 mg kg⁻¹, whereas, DTPA-extractable Cd ranged from 0.04 to 0.15 (mean = 0.065) mg kg⁻¹ (Table 1). EDTA extraction showed a higher potential mobilization of Cd than DTPA, releasing 45.7% of total soil Cd, whereas the percentage of total Cd extracted by DTPA was 3.1%. This difference may be attributed to the carbonate-dissolving nature of 0.05 EDTA (pH = 4.65) which thus releases part of the element fraction bound in carbonates (McGrath and Cegarra, 1992). The other possible factor is that EDTA dissolves iron oxyhydroxides (Borggard, 1988). As the proportion of metals fractions is controlled by the soil properties (Naidu *et al.*, 1994), correlation analysis was made to assess the soil properties controlling Cd fractionation in these soils. DTPA-extractable Cd Concentrations were positively correlated with soil electrical conductivity ($r = 0.65$, $p < 0.01$), it was reported before by Amini *et al.* (2005).

3.3. Sequential extraction

The two sequential extraction protocols tested have a different number of fractions, but their component

can be categorized into four roughly comparable equivalent fractions, acid soluble, reducible, oxidable and residual, which are associated with different forms and availability mechanisms. We use this general framework here to compare the results of the two protocols.

Acid soluble fraction:

This fraction is subdivided into two separate fractions, water-soluble + exchangeable and carbonate bound, in Tessier's scheme, which had mean concentration of 90.4 and 705 µg kg⁻¹, respectively (Table 5). As shown in Table 4, the water-soluble + exchangeable fraction was highly correlated with both DTPA-extractable Cd and soil electrical conductivity. Displacing Cd from exchange sites and forming complexes between anions, mostly chloride and Cd, may describe this positive correlation in saline soil conditions (Norvell *et al.*, 2000). The carbonate bound fraction, accounted for about 40 percent of total Cd, and was positively correlated with DTPA extractable Cd ($r = 0.39$; $p < 0.05$), clay content ($r = 0.42$; $p < 0.05$) and active calcium carbonate ($r = 0.44$; $p < 0.05$). Adsorption isotherms

of Cd in 20 highly calcareous soils of Southern Iran carried out by Maftoun *et al.* (2004) showed that CCE was the main factor controlling the adsorption capacity of Cd in these soils. Although the Cd extracted in the first equivalent fraction of the two sequential schemes was similar, only the water soluble + exchangeable fraction of Tessier, extracted by a neutral salt, provides useful information relevant to the plant-available fraction. Menzies *et al.* (2007) concluded that neutral salt solutions such as 1 M $\text{NH}_4\text{CH}_3\text{COO}$ and 0.01 M CaCl_2 tended to provide the best relationship between the soil extractable Cd and plant tissue accumulation. On the other hand, carbonates act as strong adsorbents for Cd, and carbonate bound fraction may not be released easily in these highly calcareous soils by weak reagents. As noted by McBride (1994), Cd can complex with carbonates via “solid solution” wherein Cd can substitute for Ca in the surface layers of CaCO_3 minerals. This fraction is only slowly plant-available but would be rapidly released when the carbonates are dissolved. Thus water soluble+exchangeable and carbonate-bound should be considered (and analyzed) as two different fractions. The highest percentage of total Cd (43.9%) was observed in the acid-soluble fraction of the modified BCR scheme ($912 \mu\text{g kg}^{-1}$), which was correlated with EDTA-extractable Cd ($r = 0.45$; $p < 0.01$), clay content ($r = 0.85$; $p < 0.01$), cation exchange capacity ($r = 0.55$; $p < 0.01$) and active calcium carbonate ($r = 0.43$; $p < 0.05$).

Reducible fraction: This fraction represents the Cd associated with Fe and Mn oxides that would susceptible to mobilization under altered redox conditions. This fraction is subdivided into separate Fe-bound and Mn-bound fractions in Tessier's scheme, with mean values of 399.4 and $219.7 \mu\text{g kg}^{-1}$ respectively. The total Cd extracted in the Fe and Mn oxides fractions of Tessier's scheme ($619.1 \mu\text{g kg}^{-1}$), was greater than the Cd extracted in the BCR Fe-Mn oxide fraction ($320 \mu\text{g kg}^{-1}$). These differences may be attributed to incomplete dissolution during the soluble + exchangeable phase of Tessier's procedure, allowing additional carbonate dissolution to continue during subsequent extraction steps (Gleyzes *et al.*, 2002).

Organic bound fraction: The average Cd extracted in Tessier scheme was $210 \mu\text{g kg}^{-1}$ soils whereas the BCR scheme extracted $169 \mu\text{g kg}^{-1}$. The two methods studied used H_2O_2 reagents for extracting the organic-bound fraction, but the oxidation time of Tessier's with H_2O_2 is longer which might be expected to released more metal than BCR. Nevertheless, this fraction accounted for 9.6 and 10.1 % of total extracted Cd, respectively. The presence of acetic acid also promotes the release of metals specifically sorbed on inorganic and organic substrates (Tessier *et al.*, 1979; Ahnstrom and Parker, 1999). The soil organic matter content was correlated with the metal amounts bound to the organic (0.38 ; $p < 0.05$) and residual (0.45 ; $p < 0.05$) soil phases when using the BCR procedure.

The relative amounts of Cd associated with the generally categorized (as mentioned previously) fractions in the BCR and Tessier schemes had similar rankings: Acid soluble > Reducible > Residual > Organic matter. (Figures 1 and 2) The ranking in the Tessier scheme followed the order: carbonate > residual > Fe oxides > Mn oxides > organic matter > soluble+exchangeable, which is likely due to the high carbonate and low organic matter content of the soil tested. Although the carbonate fraction contributes to the total labile fraction under more acidic conditions (McBride 1994), only the potentially bioavailable portion of carbonate-bound Cd should be considered in these highly calcareous soils. Hirsch and Banin (1990) reported that formation of the CdCO_3 may control the Cd solubility at high Cd concentrations in calcareous soils.

3.4 Grain uptake and soil extraction

Cadmium concentration in durum wheat grain varied from 0.064 to 0.273 mg kg^{-1} with a mean and median concentration of 0.133 and 0.132 mg kg^{-1} , respectively (Table 1). The maximum permissible limit for Cd concentration in wheat grain reported by the Commission Regulation of the European Communities (EC, 2006) is 0.2 mg kg^{-1} wet weight which indicates mean grain Cd concentration is not exceeded their threshold value.

Table 3. The BCR (Community Bureau of Reference) optimized sequential extraction procedure for high carbonate soils by (Sulkowski and Hirner, 2006)

Fraction	Extractant	Procedure
Soluble+Exchangeable+ Carbonate	40 ml 0.11 M CH ₃ COOH	16 h shaking at 25°C
Soluble+Exchangeable+ Carbonate	40 ml 0.11 M CH ₃ COOH	16 h shaking at 25°C
Soluble+Exchangeable+ Carbonate	40 ml 0.11 M CH ₃ COOH	16 h shaking at 25°C
Iron and manganese oxyhydroxides	40 ml 0.5 M NH ₂ OH.HCl pH 1.5 with HNO ₃	16 h shaking at 25°C
Organic matter + sulfides	10 ml H ₂ O ₂ 8.8 M	2 ×1 h shaking at 85°C
	50 ml CH ₃ COONH ₄ 1 M	16 h shaking at 25°C
Residual	aqua regia	

Grain Cd was closely and positively associated with EC ($r = 0.60$; $p < 0.01$), DTPA-extractable Cd ($r = 0.50$; $p < 0.01$) and the Tessier soluble+exchangeable fraction. Previous studies demonstrated that elevated concentrations of chloride thorough the formation of chloro-Cd complexes could be a explain for the increasing concentrations of Cd in plants, either through faster Cd diffusion to roots or transported chloro-complexes across the root membrane (Norvell *et al.*, 2000). The authors also believed that these complexes lead to Cd release from the solid to the solution phase, thereby enhancing solubility and mobility. The results showed that grain Cd as a phytoavailability index was positively correlated with soil DTPA-extractable

Cd ($r = 0.52$, $p < 0.01$), whereas, this correlation was not found in the case of EDTA-extractable Cd ($r = -0.12$, $p > 0.05$). It means that DTPA is more suitable for predicting Cd phytoavailability than EDTA in these highly calcareous soils. Among the sequential extraction fractions, grain Cd, positively correlated only with the water soluble + exchangeable fraction ($r = 0.43$; $p < 0.05$), which suggests that 1 M NH₄OAc (pH = 7) may also be a good index for phytoavailable Cd, as well as DTPA, in the studied soils.

Table 4. Correlation coefficients (r) for relationships between EDTA and DTPA-extractable, sequential extracted fractions, grain cadmium and selected soil properties (n =32)

Soil Properties	EDTA Cd	DTPA Cd	Grain Cd	Tessier						BCR				
				Ex.	Car.	MnOx.	FeOx.	OM.	Res.	Ex+Car	Mn+FeOx	OM.	Res.	Total
				Cd	Cd	Cd	Cd	Cd	Cd	Cd	Cd	Cd	Cd	Cd
EDTA _{Cd}	1	0.03	-0.12	-0.23	0.33	0.26	-0.08	-0.09	-0.03	0.45**	0.06	0.19	-0.23	-0.03
DTPA _{Cd}	0.03	1	0.50**	0.59**	0.39*	0.17	0.15	-0.23	-0.01	0.06	-0.18	-0.03	0.24	0.24
Grain Cd	-0.12	0.52**	1	0.43*	-0.10	-0.16	0.14	0.17	-0.16	-0.03	-0.20	-0.37*	-0.17	0.11
pH	0.18	0.09	-0.09	-0.27	-0.04	0.22	-0.08	-0.25	-0.04	-0.03	-0.15	-0.07	0.06	0.03
EC	-0.13	0.65**	0.60**	0.36*	-0.01	-0.12	0.01	-0.03	0.05	0.02	-0.39*	-0.15	0.35	0.27
OC	-0.16	0.23	-0.05	-0.17	0.26	0.01	0.31	0.10	0.28	0.14	0.11	0.38*	0.45*	0.48**
CEC	0.17	0.06	-0.22	-0.31	0.32	0.39*	-0.18	0.11	0.17	0.55**	0.04	0.30	0.01	0.22
Sand	-0.27	-0.07	-0.05	0.37*	-0.40*	-0.36*	-0.1	-0.13	-0.22	-0.75**	0.17	-0.13	-0.02	-0.28
Silt	0.01	0.15	0.37*	-0.22	0.12	0.10	0.26	-0.03	-0.04	0.15	-0.17	-0.27	-0.01	0.01
Clay	0.34	-0.01	-0.17	-0.32	0.42*	0.39*	-0.04	0.18	0.31	0.85**	-0.11	0.34	0.04	0.34
CCE	-0.10	0.12	0.22	0.10	0.25	-0.15	0.69**	-0.34	0.07	-0.13	-0.11	-0.08	0.30	0.20
ACCE	-0.5	0.18	0.11	-0.17	0.44*	0.25	0.40*	0.03	0.17	0.43*	-0.31	0.23	0.19	0.31
Mn Oxides	0.31	-0.12	-0.51**	-0.29	0.09	0.32	-0.45**	-0.01	-0.17	0.04	0.33	0.17	-0.30	-0.21

Table 5. Concentration of Cd released in each fraction of Tessier and BCR schemes ($\mu\text{g kg}^{-1}$).

Acid soluble			Reducible			
	Soluble +	Carbonate	Mn	Fe	Organic	Residual
	Exchangeable		Oxides	Oxides	matter	
Modified	90.4 ± 20	705 ± 83	219.7 ± 33	399.4 ± 106	210 ± 30	400 ± 120
Tessier						
Modified	912 ± 100		320 ± 70		168.8 ± 41	180.5 ± 158
BCR						

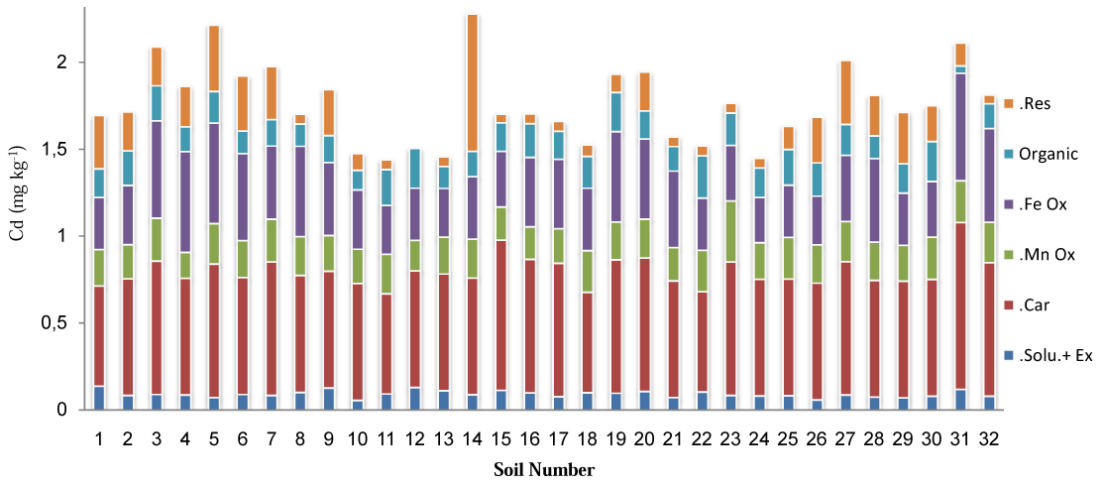


Figure 1. Distribution of solid-phase Cd fractions of the studied soils using the Tessier sequential extraction scheme

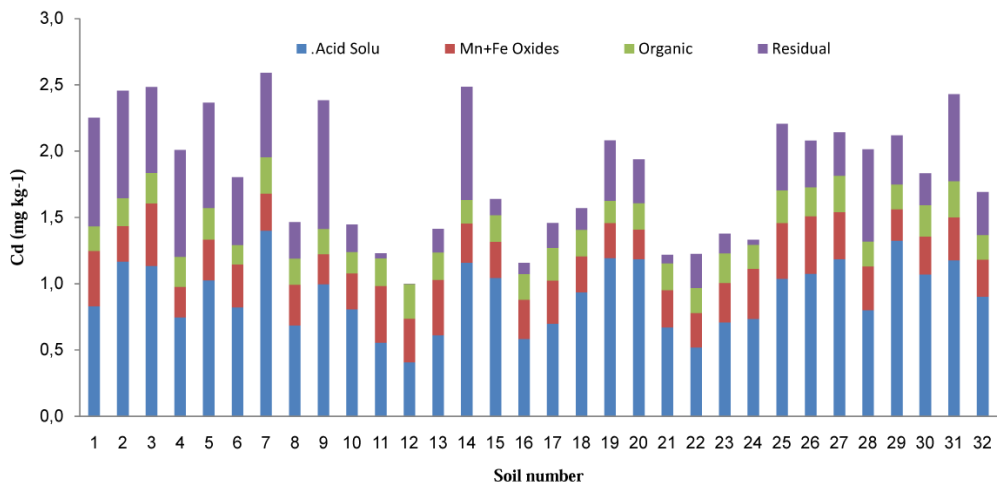


Figure 2. Distribution of solid-phase Cd fractions of the studied soils using the BCR sequential extraction scheme.

4. Conclusion

Among single-step extractions, DTPA correlated better with phytoavailable Cd in highly carbonated soils than did EDTA. The pH 7 NH_4AOc step of the Tessier protocol (water soluble + exchangeable) also correlated with phytoavailable Cd. While from a theoretical view point, the Cd released in the acid soluble fraction of the BCR scheme should equal the total of the Tessier soluble + exchangeable and carbonate fractions, the carbonate soils tested showed marked differences between the initial fractions of the Tessier and BCR schemes. Greater amounts of metal released in the acid soluble fraction of the BCR may suggests incomplete dissolution of carbonate phase in the Tessier scheme. Sulkowski and Hirner (2006) showed that 0.11 M acetic acid were not capable of complete dissolution of carbonate fraction in high carbonate soils and, thus proposed repetition of the acetic acid (step 1) extraction until the carbonates are completely destroyed to minimize metal readsorption. Although Tessier and BCR schemes use hydroxylamine hydrochloride for extracting the reducible fraction, differing experimental conditions (reagent concentration and temperature) resulted in the Tessier scheme step having a greater extraction capacity than that of the BCR. Among the sequential extractions tested, the Tessier scheme provided more information about Cd mobility and bioavailability than the BCR scheme for carbonate rich soils.

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