EFFECTS OF PHOSPHORUS FERTILIZATION ON ARSENIC UPTAKE BY WHEAT GROWN IN POLLUTED SOILS

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ABSTRACT

In this study we have examinated the results of two experiments on the uptake and distribution of arsenic (As) in roots, shoots, and grain of wheat grown in As-polluted soils and in an unpolluted soil irrigated with As-contaminated water in absence or presence of phosphorus (P) fertilization. Arsenic concentrations in wheat samples of the two experiments are higher than those in the plants grown on uncontaminated soil. In the experiments showed in this work, it is highlighted the role of P fertilization in preventing As uptake and translocation in wheat plants. These findings could have important implications to reduce the potential risk posed to human health by As entering the food-chain.

Keywords: phosphorus fertilization, arsenic, wheat, polluted soils.

INTRODUCTION

There is increasing concern worldwide regarding the contamination of soil with arsenic (As), and the potential risk to human and environmental health arising from such contamination (Smith *et al*., 1998). Arsenic contamination of soil can occur as a result of both natural sources and anthropic activities, including the use of arsenical pesticides and herbicides, atmospheric deposition, mining activity, waste disposal, and other sources (Mandal and Suzuky, 2002). Furthermore, in spite of the low geochemical abundance of As (average earth crust 1.8 mg kg^{-1}) some degree of arsenic dissolution might cause critical value is only 10 µg L^{-1}. High concentrations of As in groundwater has been reported from several countries including Argentina, Bangladesh, Chile, China, Hungary, Mexico, West Bengal (India), and Vietnam (Smith *et al*., 1998). New cases of high concentrations in drinking water have been reported when As analyses was included in routine water analysis. These cases included some areas of Italy where elevated as concentrations in groundwater and in soils were recently found (Costagliola *et al*., 2004).

Typical uncontaminated agricultural soils contain 1-20 mg kg^{-1} (Wauchope, 1983), but contaminated soils in mining or industrial areas contain levels as high as 45-2600 mg kg^{-1} (Brandsetter *et al*., 2002). In many cases, extensive use of As-rich groundwater has led to elevated As concentrations in agricultural soils, which may reduce soil productivity, be toxic to plants, and enter into the crops (Heikens, 2006. Martin *et al*., 2007). In fact, As is not an essential element for plants, but interferes with plant metabolism, inhibiting plant growth and crop yield (Barrachina *et al*., 1995; Abedin *et al*., 2002; Rahman *et al*., 2004). Therefore, minimizing soil-plant transfer of As is essential for agriculture on As-affected farmland.

In contrast, phosphorus (P), that is a chemical analogue of As (Adriano, 2001) and competes with As in plant uptake (Meharg and Macnair, 1992), is one of 17 essential elements required for plant growth (Raghothama, 1999), and often the overriding nutritional limitation in modern cereal farming (Runge-Metzger, 1995). It is estimated that crop yields on around 30-40 % of the world's arable land are limited by P availability (Runge-Metzger, 1995). The effect of P on the sorption/desorption of As in soil environments has received great attention, especially when P is used as a crop fertilizer (Peryea, 1998). The bioavailable fraction of As in soils to crop plants depends on different physical and chemical properties of soils. In fact soils rich in variable charge minerals (Al or Fe oxides) do not release As easily. Only large additions of P to high anion-fixing soils or alkaline pH or Fe and Al oxide dissolution may affect As solubility (Smith *et al*., 1998; Violante and Pigna, 2002). However, As sensitivity is intimately linked to P nutrition in plants. In rice seedlings, even cultivars found to be susceptible to arsenate can become more resistant by raising level of intracellular P (Geng *et al*., 2006; Wang and Duan, 2009). Gultz *et al*. (2005) reported that P availability and P demand, which are plant specific, have to be taken in account to predict uptake of As by crop plants. Therefore, As toxicity in crops can be more prevalent in situation where As contamination is found coexisting with low available P.

Much of the research on As in grain crops have focused on rice (*Oryza sativa L.*) (Abedin *et al*., 2002; Williams *et al*., 2005; Rahman *et al*., 2007). The work that has been conducted on wheat is less extensive (Tao *et al*., 2006; Zhao *et al*., 2007), with limited information regarding soil-root, root-shoot and shoot-grain assimilation and translocation (Williams *et al*., 2007). In Tuscany, Italy there is extensive soil contamination with As due to base mining and processing activities (Costagliola *et al*., 2004). Elevated As concentrations have been reported for Scarlino, close to Grosseto, in southwest Tuscany (ARPAT-UNIFI, 2003). Wheat (*T. durum*) is the main crop cultivated in this area. Grain is largely used for human consumption and as poultry feed. Also, the straw is used as fodder for cattle. The aim of this work was to evaluate the role of P fertilization on growth, As uptake and partitioning between different plant part (grain, shoot, root) in wheat (*T. durum*). In one experiment wheat was grown in two contaminated soils (Vetricella and La Botte) and in an uncontaminated soil (Padula) collected from Scarlino. The second experiment examined the effect of P fertilization on wheat grown in an uncontaminated soil, collected from a natural grass in Presenzano, Caserta, Italy, and irrigated with solutions containing As at three different concentrations.

MATERIALS AND METHODS

Some physical and chemical properties of the soils utilized in these experiments are presented in Table 1. The analyses were carried out as reported in previous works (Cozzolino *et al*., 2010; Pigna *et al*., 2009). A five-step sequential extraction procedure for As (Wenzel *et al*., 2001) was performed on contaminated soils (Table 1). The first extraction step (Step 1), performed with 0.05 M $(NH_4)_2SO_4$, represents the most labile As of the procedure. The second extraction (Step 2), with 0.05 M $NH_4H_2PO_4$, was used to assess the As fraction that can be specifically replaced by phosphate. Even if not as easily released as the first fraction, this one can also be considered as labile As. The following steps included extractions with 0.2 M ammonium oxalate at pH 3, in the dark (Step 3); with ammonium oxalate and ascorbic acid, in the light (Step 4); and an acid digestion with HNO_3 65% and HF 50% (Step 5). These three final fractions, representing As bonded to amorphous (Step 3) and crystalline (Step 4) hydrous oxides of Fe and Al, and residual As (Step 5), can be considered as nonlabile As. The sequential extraction was carried out in triplicate on 1 g of soil in 50-ml polypropylene centrifuge tubes to facilitate washing of the soil after each extraction thus minimizing any loss of the solids. The supernatants were separated by centrifugation (1700*g*) and filtered through 0.45-μm filters. The total soil arsenic concentration was determined by digesting the soils with concentrated HNO_3 and HF at 5:1 ratio. Total As concentrations of soil extracts and digests were determined by hydride generation-inductively coupled plasma spectrometry (HG-ICP – AES, Varian, Liberty 150).

Experiment 1. Vegetative responses of wheat grown on contaminated soils and uncontaminated soil with and without additional P

Experiments were conducted for seven months in an unheated greenhouse. Series of twelve pots were filled with 7 kg of each of the three soils with different arsenic content. Wheat (*T. durum* cv Creso) plants were sown directly in pots at a density of 10 seeds per pot. Fourteen days after sowing the seedlings were thinned to 3 plants per pot. All the pots were fertilized every 2 weeks with 80 mL of nutrient solution containing 29.1 mM N as NH_4NO_3 and 25 mM K as K_2SO_4. One third of the pots did not receive any phosphorus in the nutrient solution (P0 treatment); another third of the pots received 2.8 mM P as K_2HPO_4, corresponding to a fertilizer distribution of 75 kg ha^{-1} of P (P1 treatment); the last third of the pots received 5.6 mM P (150 kg ha^{-1}) included in the nutrient solution (P2 treatment). Thus, each of the three thesis with soils at different As content received three P treatments, and each treatment was replicated 4 times to give a total of 36 pots. The design was completely randomized and re-arranged every day.

Experiment 2. Vegetative responses of wheat grown on an uncontaminated soil and irrigated with water containing As at three different concentrations, with and without additional P

The wheat plants were grown for seven months in an unheated greenhouse. Wheat (*Triticum durum* cv. Creso) plants were grown in pots filled with 7 kg of the soil planted at a density of 10 seeds per pot, sown directly in the pots, and irrigated during the first 2 weeks with water. After this period the seedlings were thinned to 3 per pot, and were irrigated with water containing arsenate (Na_2HAsO_4) at four different concentrations: 0 (control treatment), 0.5, 1, and 2 mg L^{-1} of As, until the wheat grain was ripe. The range of arsenate concentrations was chosen to encompass the concentrations occurring in underground waters of the As-affected areas of world. Contaminated water was

Table 1. Selected physical and chemical properties of the soils in the two experiments.

Soil	pH (H_2O)	Organic matter	Sand	Silt	Clay	P_2O_5	As
		------------------------g kg^{-1}---------------------				-------mg kg^{-1}-----	
Experiment 1							
Padula	8.0	32.0	321	341	339	44.8	14
Vetricella	6.7	16.0	482	270	248	20.3	192
La Botte	8.1	40.0	293	365	342	16.9	304
Experiment 2							
Presenzano	5.70	23.0	131	388	275	13.5	6.8

added as required to maintain moisture content at 60% of water holding capacity. All the pots were fertilized each 2 weeks with 80 mL of nutrient solution containing 29.1 mM N as NH_4NO_3 and 25 mM K as K_2SO_4. Furthermore, in half of the pots 5.6 mM P as K_2HPO_4 was included in the nutrient solution in order to evaluate the influence of Phosphorus on As uptake by plants. Thus, there were 2 treatments without supplemental P (P-) and with supplemental P (P+). The design was completely randomized and re-arranged every day, and each treatment was replicated 4 times to give a total of 32 pots. As irrigation was stopped 1 week before harvest.

Contemporaneously, a germination assay was carried out. Thirty surface-sterilized seeds of wheat were placed on a filter paper, collocated on a Petri plate, moistened with aqueous solution of arsenate at four concentrations (0, 0.5, 1.0, 2.0 mg As L^{-1}). Each treatment was replicated four times. The seeds were incubated in dark at 24°C. Germinated seeds were counted 5 days after initiation.

Plant harvest and analysis

Seven months after sowing, wheat plants were harvested by cutting the stem 3-4 cm above the soil. Wheat spikes were collected and dried at 70°C to constant weight. The spikes were then dehusked by hand and the weight of the grain was recorded for each pot. Shoots and roots were washed with tap water and then rinsed twice with deionized water. The dry weights of the roots and shoots were determined after oven drying at 70°C for 48 h. Roots, shoots, and grain were analyzed for total concentration of As. All samples were ground using a PM 200 ball mill (Retsch) and were digested in a microwave (Milestone, Digestor/Dring Ethos 900). A sample of about 0.5 g was accurately weighted into a PTFE pressure vessel and 7 mL of HNO_3 (65%), 0.5 mL of HF (50%), and 2 mL of H_2O_2 were added. All glassware and plasticware were previously acid-washed with 3M HCl, and rinsed with deionized water. Arsenic concentrations in roots, shoots and grain were determined by flow-injection hydride generation atomic absorption spectrometer using a Perkin-Elmer AAnalist 700 interfaced with a FIAS 100 hydride generator. Hydride generation was used for roots, shoots and grain samples due to the lower detection limits of this technique (0.5 μg L^{-1}). All analyses were carried out in triplicate. In each analytical batch at least, one reagent blank and one internationally certified reference material (CRM), oriental tobacco leaves CTA-OTL-1, was included to assess precision and accuracy of the chemical analysis.

Statistical analysis

Data analyses were performed with Kaleidagraph 3.6. Treatment effects were determined by analysis of variance. Differences were considered as statistically significant at $p < 0.05$ (Tukey's test).

RESULTS AND DISCUSSION

Experiment 1. Vegetative responses of wheat grown on two contaminated soils and on an uncontaminated soil with and without additional P

Plant growth and As toxicity

The soils investigated in this study originated from sites where As contamination was due to high As content in the geological materials and to mining and industrial activities (Donati *et al*., 2005). The total As concentrations in the two polluted soils were clearly higher than Italian regulatory limits of As contamination in agricultural soils (20 mg kg^{-1}) (DM 471/99). The highest As concentrations was observed in La Botte soil (304 mg kg^{-1}) (Table 1).

It was observed that plant biomass decreased markedly with increasing As concentration in soils (Table 3). For plants grown without P addition (P0 treatment) there was a decrease in biomass production of 11%, 58%, and 60% compared to the plants that received P1 fertilization and of 16%, 65%, and 69% with respect to the plants with P2 fertilization (Table 3), in Padula, Vetricella and La Botte soils, respectively. These findings are comparable to results reported for rice (Abedin *et al*., 2002) and wheat (Liu *et al*., 2005). Root biomass decreased significantly ($p < 0.001$) with increasing concentration of As in soils. In P0 plant series, it decreased by 72%, and 83.6%, respectively in Vetricella and La Botte, compared to plants grown in unpolluted soil. Phosphorus application significantly increased root biomass in all soils and treatments, ameliorating the toxic effects of As in these soils. The interaction between As and P on the growth response was significant in shoots. The shoot biomass (shoot plus leaf biomass) decreased with increasing concentration of As in soils, especially in the P0 plants (Table 2), but P addition, both in P1 and P2 treatments, ameliorated the effects of As.

The grain yield of wheat was greatly affected by the application of P, ranging from 24.5 to 12.4 g pot^{-1} for the P2 treatment; from 23.6 to 8.6 g pot^{-1}, P1 treatment; 21.20 to 2.10 g pot^{-1}, P0 treatment (Table 2). For the non contaminated soil (Padula), the difference between P2 and P0 treatment was lower (14%) than for the two polluted soil, especially La Botte soil, where the increases in grain yield with respect with P0 treatment were 76% for P1 treatment and 84% for P2 treatment. According to our results, root biomass production responded more strongly to increasing soil As concentration compared to the shoot, especially in P0 plants. In the experiments reported here, in the presence of P supply, high As concentrations in the soils determined a moderate inhibition in wheat growth, including grain yield, especially at the highest P supply, compared to wheat growth in P0 treatment. Phosphorus has a protective effect with respect to As toxicity in wheat plant, particularly in La Botte soil, characterized by low P availability (Table 1). Studies on As toxicity have shown that

Table 2. Fractionation of arsenic in polluted soils (Vetricella, La Botte)

Soil	Step 1 F1	Step 2 F2	Step 3 F3	Step 4 F4	Step 5 F5	Σ Steps	Total	% recovery
	As (mg kg^{-1})							
Padula	-	-	-	-	-	-	14.0	-
Vetricella	0.1	12.6	32.2	107.5	19.7	184.0	192.0	95.8
La Botte	0.4	30.7	93.5	140.7	10.6	275.9	304.0	90.7

Table 3. Root, shoot, grain, total biomass in a wheat (*Triticum durum* cv. Creso) grown in unpolluted (Padula) and polluted (Vetricella, La Botte) soils at three levels of P fertilization.

	Root	Shoot	Grain	Total
	g pot^{-1}			
P0 treatment				
Padula	2.20 ± 0.10b	12.80 ± 0.60bc	21.20 ± 1.20b	36.20
Vetricella	0.60 ± 0.05f	6.50 ± 0.40f	3.00 ± 0.20f	10.10
La Botte	0.36 ± 0.04f	4.20 ± 0.20g	2.10 ± 0.10f	6.66
P1 treatment				
Padula	2.60 ± 0.20a	14.40 ± 1.05ab	23.60 ± 1.00a	40.60
Vetricella	1.40 ± 0.10cd	9.60 ± 0.60de	13.20 ± 0.73cd	24.20
La Botte	1.00 ± 0.10e	6.90 ± 0.55f	8.60 ± 0.45e	16.50
P2 treatment				
Padula	2.80 ± 0.20a	15.80 ± 1.30a	24.50 ± 1.20a	43.10
Vetricella	1.70 ± 0.10c	11.00 ± 0.50cd	16.00 ± 0.82c	28.70
La Botte	1.20 ± 0.10de	8.20 ± 0.40ef	12.40 ± 0.50de	21.80
Site	$p<0.0001$	$p<0.0001$	$p<0.0001$	
P fertilization	$p<0.0001$	$p<0.0001$	$p<0.0001$	
Interaction	$p<0.05$	$p<0.001$	$p<0.0001$	

root biomass decreased significantly ($p < 0.001$) with increasing concentration of As in soils. In P0 plant series, it decreased by 72%, and 83.6%, respectively in Vetricella and La Botte, compared to plants grown in unpolluted soil. P_i application significantly increased root biomass in all soils and treatments, ameliorating the toxic effects of As in these soils. The interaction between As and P on the growth response was significant in shoots. The shoot biomass (shoot plusleaf biomass) decreased with plant species not resistant to As suffer considerable stress upon exposure, with symptoms ranging from inhibition of root growth to plant death (Meharg and Macnair, 1991; Paliouris and Hutchinson, 1991; Barrachina *et al*., 1995). Arsenate acts as a phosphate (Pi) analogue and is transported across the plasma membrane via a Pi cotransport systems (Ullrich-Erebius *et al*., 1989). Once inside the cytoplasm arsenate competes with Pi, for example replacing Pi in ATP to form unstable ADP-As, leading to the disruption of energy flows in cells (Meharg, 1994). The effects of P nutrition on the mitigation of arsenate toxicity symptoms seem to be: 1) high plant P status leads to a down-regulation of the arsenate/Pi plasma-lemma transporters; 2) high cellular Pi levels results in greater competition with arsenate for biochemical processes where arsenate substitutes for Pi (Meharg, 2005).

Arsenic concentration and contents in wheat roots, shoots and grain

As concentration in wheat roots, shoots and grain increased significantly with the increase of total As concentrations in polluted soils and As concentrations of F1 and F2 fractions (labile fractions) (Figure 1). In fact, the total As and the sum of the F1 and F2 fractions were highly correlated with As root concentrations ($r = 0.976$ and $r = 0.897$, respectively) as well as with shoot ($r = 0.958$ and $r = 0.745$) and grain concentration ($r = 0.870$ and $r = 0.674$) in P0 treatment. High correlation coefficients were found also for As bonded to amorphous (F3) and crystalline (F4) hydrous oxides of Fe and Al ($r = 0.855$ and $r = 0.998$, respectively), but not for residual fractions. The results were almost the same in P1 and P2 treatments, although the correlation coefficients were lower. Arsenic concentration in the roots increased particularly in the P0 treatment. By increasing As concentration in the soils As root concentrations ranged from 0.28 to 3.50 in P2 plants and from 0.30 to 6.2 mg As kg^{-1} in P0 plants (Figure 1). Similar results were found in other studies investigating the influence of P on As accumulation by wheat (Tao *et al*., 2006).

Shoot As concentrations also rose with increasing As concentration in soil, more so for the P0 treatment. The highest shoot As concentration (2.45 mg kg^{-1}) was measured in the plants grown in La Botte soil in the absence of P fertilization. Our results are in agreement with those reported in previous studies (Barrachina *et al*., 1995; Quaghebeur and Rengel, 2003). The ability of As to accumulate in wheat has already been shown. Greenhouse pot experiments on wheat grown in soil spiked with 50 mg As kg^{-1} resulted in shoot levels of ~ 3 mg kg^{-1} d.wt (Williams *et al*., 2007). In wheat grown in As-polluted soil (range 53.8-709 mg kg^{-1}), contaminated by the Aznalcollar mine spill, levels of ~ 20 mg As kg^{-1} were detected in the shoots (Soriano and Fereres, 2003).

As concentration in grain in unpolluted soil (Padula) was only ~ 0.03 mg kg^{-1} in all P treatments, increasing, in the most polluted soil (La Botte), to 0.62, 0.50, and 0.44 mg kg^{-1} in P0, P1, and P2 treatments respectively. These values are similar or slightly higher compared to those found by Williams *et al*., (2007) in wheat grain. They reported levels of ~ 0.21 mg kg^{-1} in Scottish (East Coast) wheat grain, where the maximum soil As levels were of 18 mg kg^{-1}, and of 0.50 mg kg^{-1} in English (Cornwall and Devon counties) wheat grain, where the maximum As levels in soil was 201 mg kg^{-1}. Greater As accumulation levels (0.74 mg kg^{-1}) were observed in south-eastern Asia wheat (Norra *et al*., 2005), which was comparable to grain levels of 0.75, 0.71 and 0.69 mg kg^{-1} found in wheat grown in

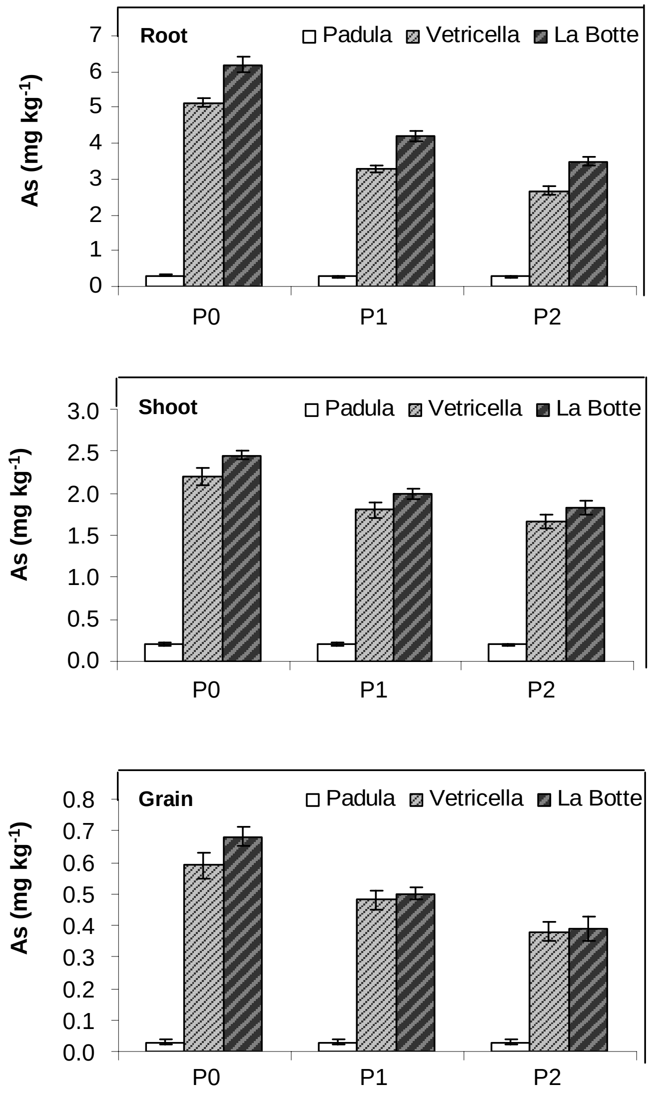

Figure 1. Total As concentration (mg kg^{-1}) in roots, shoots, grain of wheat (*Triticum durum* cv. Creso) grown in unpolluted (Padula) and polluted (Vetricella, La Botte) soils at three levels of P fertilization. Data are expressed as mean values ± SD (n=4) and have been analyzed by two-way analysis of variance.

a greenhouse experiment in 50 mg As kg^{-1} spiked soil (Williams *et al*., 2007).

In the present study, although As was primarily bound with Fe and Al oxides (Table 1), plant growth was stunted in the two contaminated soils in absence of P addition, especially in the soil with the highest As concentrations in more labile soil fractions and with low P availability (See Table 1; Cozzolino *et al*., 2010). Phosphorus fertilization seems to reduce the effects of As toxicity, promoting plant growth without increasing As concentrations in above-ground parts of plants and limiting shoot to grain transfer, particularly at highest P level.

These observations corroborate the reports by Meharg (1994) that P restricted the transfer of As from soil to the above-ground plant organs. Also, P nutrition could be involved in the decrease in reactive oxygen species and non-protein thiols production, formed during exposure to As in the shoot that cause tissue damage and lipid peroxidation, affecting shoot-grain transfer. Geng *et al*., (2006), in two different genotypes of rice seedlings, found higher stress resulting from As exposure in genotype with higher overproduction of enzymatic antioxidants and non-protein thiols.

Experiment 2. Vegetative responses of wheat grown on uncontaminated soil irrigated with water containing As at three different concentrations, with and without additional P

Plant growth and As toxicity

A downward trend was observed in plant growth with increasing As concentration in irrigation water (Table 4). For plants grown without P addition (P-) there was a decrease in biomass production of 15%, 52%, and 67% as As concentration in the irrigation water increased, with respect to control treatment. This reduction was less severe in the P+ conditions, 12%, 16%,

Table 4. Root, shoot, grain and total biomass in a wheat (*Triticum durum* cv. Creso) exposed to three arsenic concentration in irrigation water (0.5, 1.0, and 2.0 mg L^{-1}).

As conc. mg L^{-1}	Root	Shoot	Grain	Total
		(g)		
Control P-	1.80 ± 0.06	12.20 ± 0.80	8.40 ± 0.45	22.40
0.5	0.83 ± 0.05	11.90 ± 0.60	5.75 ± 0.22	18.48
1	0.57 ± 0.03	7.50 ± 0.42	3.00 ± 0.18	11.07
2	0.38 ± 0.03	4.60 ± 0.22	2.20 ± 0.12	7.18
Control P+	2.40 ± 0.16	13.00 ± 0.70	21.25 ± 1.05	36.35
0.5	2.30 ± 0.14	12.40 ± 0.74	17.60 ± 0.90	32.30
1	1.95 ± 0.10	11.80 ± 0.58	16.90 ± 0.88	30.65
2	1.60 ± 0.12	11.20 ± 0.65	14.30 ± 0.62	27.10

Values are expressed as mean ± standard deviation with n = 4 and 26% respectively (Table 3). These findings are similar to results reported for rice (Abedin *et al*., 2002) and wheat (Liu *et al*., 2005).

Root biomass decreased significantly ($p < 0.001$) with increasing concentration of As in irrigation water. In P- plants, by 54%, 68%, 79% at 0.5, 1, 2 mg As L^{-1} respectively, while by 4.2%, 19%, 33% in P+ plants. P_i application increased significantly root biomass in all treatment. Liu *et al*., (2005) reported a significant decline in root biomass production in wheat seedlings with the increase in arsenite and arsenate concentrations for all six varieties of *Triticum aestivum* studied. Quaghebeur and Rengel (2003), in the non tolerant species *Holcus lanatus*, found that at increasing As concentration in nutrient solution there was a decrease in the root and shoot dry weight, accentuated when the plants had no P supplied. Sneller *et al*. (1999) in hydroponic experiment did not observe any inhibition in root growth with up to concentrations of 0.58 mg of As L^{-1} in high-P (3.1 mg L^{-1}) treatment (on *Silene vulgaris*) but found 75% root growth inhibition in low P (0.31 mg L^{-1}) treatment. However, Abedin *et al*., (2002) observed no significant differences in rice root biomass as a result of P application.

The dry weight of shoots (shoot plus leaf biomass) was significantly influenced by the As X P interaction; the shoot biomass decreased with increasing concentration of As in irrigation water, especially in the P- plants. The grain yield of wheat (mass of grain pot^{-1}) was affected by the application of As in irrigation water. Grain yield was found to range significantly from 8.4 to 2.2 g pot^{-1} from the control to the highest As level, in P- plants (Table 3), a decrease in yield of 74%. While P+ plants exposed to 0.5 and 2 mg L^{-1} showed little/or moderate decrease in grain yield (17 to 33% respectively) with weights ranging from 21.25 to 14.30 g pot^{-1}. At lower As application rates there was no effect on root/shoot ratios in P+ plants, while at the lowest As treatment, a clear decrease of ratio was observed in P- plants (Table 3). It was demonstrated that root biomass responded more strongly to As application compared to the shoot, especially in P- plants.

In the experiments reported here, As concentrations of 0.5 – 2 mg L^{-1}, had a moderate inhibition in plant growth, including grain yield, in the presence of P_i. The differences in mean biomass between P- and P+ treatments at the higher As concentrations, also in these experiments, highlighted the role of P in preventing As toxicity and growth inhibition in wheat.

Arsenic concentration and contents in wheat roots, shoots and grain

Arsenic concentration in wheat root, shoot and grain, increased significantly with increasing As in irrigation water (Figure 2). Arsenic concentration in the roots increased particularly when P was added. By increasing As concentration in irrigation water from 0.5 to 2.0 mg As L^{-1} As root concentrations ranged from 0.63 to 2.06 and from 1.4 to 3.2 mg As kg^{-1} , respectively in P- and P+ plants. Similar results were found in other studies (Tao *et al*., 2006), investigating the influence of P_i on As accumulation by wheat.

Shoot As concentrations (mg kg^{-1}) increased in plants irrigated with As contaminated water, but no significant difference were found at 0.5-1.0 mg As L^{-1} treatments and no difference were observed due to P_i application. The highest shoot As concentration of 0.65 mg kg^{-1} was measured in the highest As treatment. As concentration in grain at the control treatment was only 0.05 mg kg^{-1} which increased to 0.24 mg kg^{-1} at concentration of 0.5 mg As L^{-1}, in P-

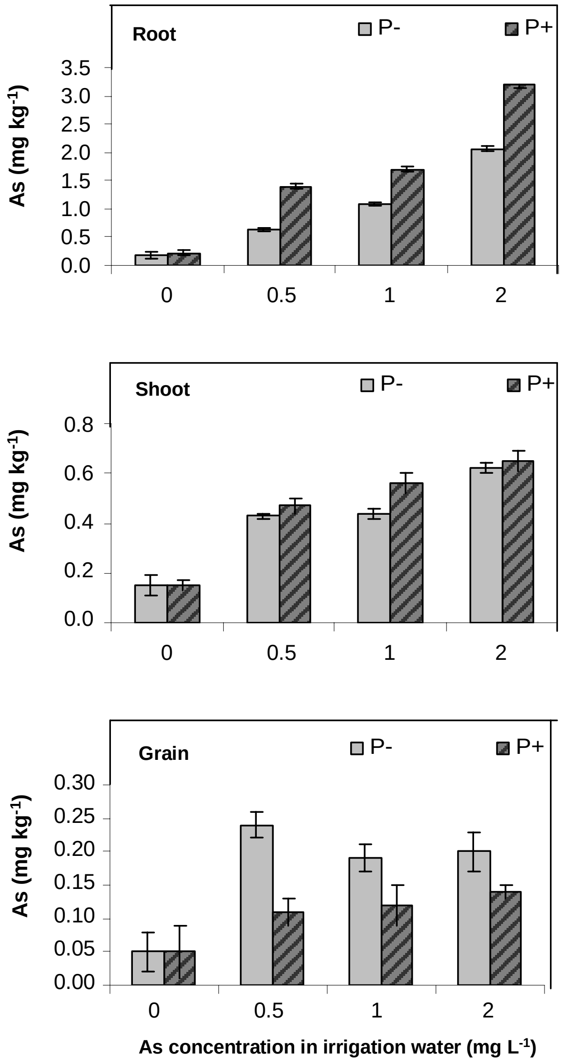

Figure 2. Total As concentration (mg kg^{-1}) in roots, shoots, and grain of wheat (*Triticum durum* cv. Creso) exposed to three As concentration in irrigation water. Data are expressed as mean values ± SD (n=4) and have been analysed by two-way analysis of variance.

plants, although remained constant for the next two arsenate treatments. In P+ plants, grain As concentrations was found to range slightly from 0.05 to 0.14 mg As kg^{-1} from the control to the highest As level (Figure 2). These results could indicate that at higher levels of As in irrigation water, the toxic element causes severe toxicity to wheat plant resulting in reduced growth rate and lowered translocation of As, as well as other nutrients from soil solution into the wheat grain. Similar results have been reported by Rahman *et al*. (2007) in rice plants and by Carbonell-Barrachina *et al*. (1997) in tomato and bean plants.

Figure 3 shows the As partitioning in wheat plant tissues. In P- plants for the highest As concentration in irrigation water As was found to be 72% in root, 21.5% in shoot and 6.5% in grain; whereas in P+ plants, at the same As concentration, 81% was in the root, 15.5% in shoot and 3.5% in grain. Results indicate that regardless P treatment, most of the As accumulated in wheat plant tissues, remains in root and the smallest amount in the grain, although this behaviour was more pronounced in P+ plants. In fact, P fertilization increased total As uptake (3.99 vs. 2.88 mg kg^{-1}, see Pigna *et al*., 2009), but the increase was restricted to the root. In rice plants, Abedin *et al*. (2002) also observed that a very large amount of As retained in root compared to its content in straw and grain. Other literature studies (Duxbury *et al*., 2002; Rahman *et al*., 2007) also reported similar results. Carbonell-Barrachina *et al*. (1997) in a similar experiment, found that in tomato plants, 83,2% of all the adsorbed As remained in the root system, 16,8% in the shoots and only 7,3% reached the leaves, while in bean plants only accumulated 13,2% of the total plant As in the roots.

As allocation to the above ground portion of cereal crops is undesirable, as it will cause contamination of the human food-chain, with wheat being a staple in many countries around the globe. The results presented here indicate P supply may result in lower As allocation to the above ground (Figure 3), which has practical application in soil-crop systems. Other authors (Lu *et al*., 2010) indicated also that in rice, maintaining higher P concentration and P/As molar ratio in rice shoot by variety breeding or soil management is one of the means for decreasing arsenic accumulation in rice grain.

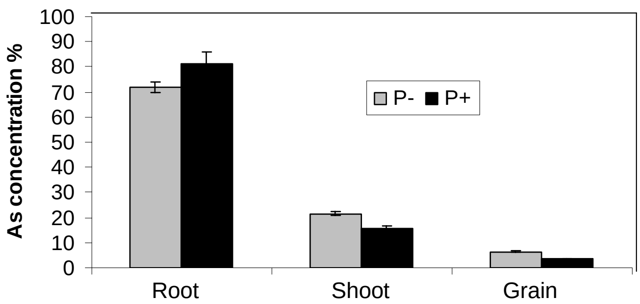

Figure 3. As partitioning (%) in root, shoot and grain of wheat plants (*Triticum durum* cv. Creso) exposed to highest As concentration in irrigation water (2 mg As L^{-1}) with (P+) and without (P-) P fertilization. Values are expressed as mean ± standard deviation with n = 4.

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CONCLUSIONS

Arsenic concentrations in wheat samples of the two experiments are higher than those in the plants grown on uncontaminated soil, and, although the daily intake of As calculated in these cases was lower than the daily permissible intake of As (0.15 mg, see Cozzolino *et al*., 2010 and reference therein), very low possible As intake from others foods (vegetables, meat, fish, eggs and milk) would be enough to increase the daily intake of arsenic above the limit value.

In the experiments showed in this work, it is highlighted the role of P fertilization in preventing As uptake in wheat plants. These findings could have important implications for human health and agricultural systems, since they suggest that it may limit the ingestion of As through the consumption of crops grown on contaminated soils and reduce yield losses.

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