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Phosphorus–arsenic interactions in soils in relation to arsenic mobility and uptake by wheat varieties

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Abstract. Arsenic (As) in soil, water, and food is a global health concern due to its toxicity, even at very low concentrations. Arsenic accumulation in cereal crops represents an important pathway for human exposure to arsenic from the environment. In this study we have investigated relationship between arsenate and phosphate (Pi) uptake and distribution in root, shoot, and grain of wheat (Sardari variety collected from Iranian arsenic contaminated area and control population). Arsenic was applied at concentrations of 0, 5, 25, 125 and 625 mg l^{-1} in the presence or in the absence of P fertilization. So, with increasing arsenic concentration in irrigation water, As levels of roots, shoots and grains increased. Also, measurements indicated that arsenic uptake rates decreased in the presence of P. Furthermore, arsenic accumulation was higher in the root as compared with aerial parts (shoot and grain). Also, at 125 and 625 mg l^{-1} arsenic treatments, the measured arsenic concentrations of grain and shoot exceeded the tolerance limit, regardless of P presence. Sardari variety (of contaminated area) had significantly less uptake of arsenic compared with control population. Our results showed that arsenic concentration in root, shoot and grain, decreased significantly with the application of P. These results will be beneficial to predict and reduce the risk of arsenic entrance into the food chain.

Key words: Arsenic, Uptake, Phosphorus fertilization, Wheat, food chain.

Introduction

Arsenic (As) is a ubiquitous trace element with mean lithosphere concentration of 5 mg kg^{-1}. In soils, As level is generally around 5-10 mg kg^{-1} and concentration above 20 mg kg^{-1} soil is considered toxic (Smedley & Kinniburgh 2000, Yadav 2009, Kahrizi et al. 2011). Arsenic toxicity has been known for centuries, and has recently received increased attention because of its chronic and epidemic effects on human health. It can enter the environment through weathering, biological and volcanic activity. Anthropogenic inputs from agricultural and industrial practices, such as the application of pesticides and chemical fertilizers, wastewater irrigation, precipitation from heavy coal combustion and smelter wastes and residues from metalliferous mining, increase the levels of As contamination in soil, ground water and surface water (Zhang et al. 2002, Zandsalimi et al. 2011, Karimi et al. 2013a). During the last three decades, high concentrations of As in groundwater have been reported in different regions of the world such as Iran (Mosaferi et al. 2003). Arsenic contaminated ground water is not only used as a source of drinking water, but also extensively used for irrigation in some regions (Kazia et al. 2009). Long term use of As contaminated water for irrigation has resulted in elevated As levels in agricultural soils (Meharg & Rahman 2003).

Arsenic is typically considered a non-essential element for plants and its bioavailability depends on plant species and soil properties (Tao et al. 2006, Azizi & Kahrizi 2008, Karimi et al. 2010, 2013b). Crop and vegetable production can benefit from knowledge of habitats and external conditions which might promote a higher accumulation of As in edible parts of the plants (Wolterbeek & van der Meer 2002). Wheat are the main cereal cultivated in world. Grain is largely used in human food and also as feed for poultry. Also, straw may be used as fodder for cattle (Maniee et al. 2009, Kahrizi et al. 2009, Mohammadi et al. 2010, Kahrizi et al. 2010, Geravandi et al. 2011, Ahmadi et al. 2012).

To evaluate the possible health risk to humans consuming crops irrigated with As contaminated water, information is needed regarding the soil-to-plant transportation of As and to minimize the accumulation of As in plants consumed directly by humans, farm animals or wildlife (Meharg & Hartley-Whitaker 2002).

Arsenic can be found in both organic and inorganic compounds with variable oxidation states. Arsenate is the predominant species of As under toxic conditions, while arsenate species dominates under anoxic conditions (Sadiq 1997). Arsenate, which is chemically very similar to orthophosphate, is thought to enter the root cell by the same uptake mechanism as phosphate in a variety of organisms (Meharg & Macnair 1994). The understanding of the general patterns accumulation and speciation of As in plants could help to elucidate the implications for dietary uptake of As from crops and vegetables cultivated in As containing soil. The hypothesis was that plants cultivated in As-containing soil will accumulate As into the edible plant parts. Therefore, the present study was conducted to examine the effect of arsenic on phyto toxicity, uptake and partitioning between different parts (grain, shoot, and root) of wheat that grown in uncontaminated soil and irrigated with solutions containing As at the presence or absence of Pi fertilization. These results will be beneficial to predict and reduce the risk of arsenic entrance into the food chain.

Materials and methods

Growth conditions and treatments

Experiments were conducted from September 2011 to June 2012 in greenhouse of Razi University. The greenhouse temperature ranged from 14 to 30 °C, with an average photon flux of 825 mmolm^{-2} s^{-1}. Sardari variety of wheat (*Triticum aestivum* cv. Sardari) was selected for the study. Seeds of contaminated Sardari were collected from populations growing in six contaminated villages of Bijar County, in the Northeast Kurdistan province, West of Iran, grid reference 34° 442 to 36° 302 North, and, 45° 312 to 48° 162 East. Control population of Sardari variety was sourced from fields of Kermanshah province, grid reference 34°18′15″N 47°03′54″E. Wheat 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 four per pot. A solution of As ($Na_3AsO_4.12H_2O$) was mixed thoroughly with the soil at a rate of 0 (control), 5, 25, 125 and 625 mg kg^{-1} soil. Each treatment was replicated 3 times. 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 Pi on As uptake by plants. Thus, there were two treatments without supplemental Pi (P−) and with supplemental Pi (P+).

Soil preparation and Soil characteristics

Soil per pot was a coarse-silty loam, collected from a local farm at 0-15 cm depth. It was crushed, mixed thoroughly and sieved through a 2mm mesh. A composed sample from this soil was collected for physico-chemical analysis. Some soil properties are presented in Table 1. Soil properties were determined as follows: pH was determined by potentiometry in a soil paste saturated with water and organic matter was determined by dichromate oxidation using the Tiurin method (Soon & Abboud 1991). For determination of CEC the soil was extracted with 1 M NH_4OAc at pH 7.0. Total phosphorus concentration was determined by colorimetric method using 0.5 M $NaHCO_3$ as the extractant Olsen method (Olsen et al. 1954). The particle size distribution (sand, silt and clay) was analyzed by the hydrometer method (Ashworth et al. 2001). The arsenic concentration in soil digest was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES Shimadzu 6200).

Table 1. Physical and chemical properties of soil.

Soil characteristics	Values
Clay (%)	50.60
Silt (%)	20.98
Sand (%)	26.74
pH (1:2.5 H_2O)	7.51
CEC (mequiv/100 g)	11.7
Organic matter (%)	1.38
Total phosphorus (mg/kg)	78.6
Total As (mg/kg)	5.53

Total As analysis

Oven-dried plant materials were digested in nitric acid on a heating block, and the temperature was at 100°C for 1 h, then at 120°C for 2 h. Reagent blank and standard reference were used to verify the precision of analytical procedures. The As concentrations were measured by a hydride generation–atomic absorption spectrometer (Shimadzu 6200) (Meharg & Jardin 2003).

Statistical analysis

Treatment effects were determined by analysis of variance according to the General Linear Model procedure of the SPSS statistical package version 16.0. Two-way ANOVA was performed to test the significant differences for all measurable variables in Pi x As interaction. Duncan's multiple range (DMRT) test was performed to compare among the groups for significant differences. Difference from control was considered significant as P< 0.05 or very significant as P< 0.001. All the values presented in this paper were expressed as the means of three replicates ± standard error (S.E).

Results

Concentration of As in root

Results of data analysis of root As concentrations are given in Table 2. Analysis of variance showed that there were significant differences between group, treatment, and group × treatment interactions (p<0.05) (Table 2). The results showed that root As concentration in wheat variety increased significantly with increasing As in irrigation water (Table 3), particularly in without P treatments (P-). So, by increasing As level in irrigation water from 5 to 625 mg l^{-1}, the measured As concentration levels of roots ranged from 2.8 to 73.2 and 1.4 to 72.8 mg kg^{-1} in pots without P and with P treatments, respectively (Table 3). In P- treatments, root As concentration at the highest As level (625 mg l^{-1}) were observed 7 and 4.7 times higher than the lowest As level (5 mg l^{-1}) in contaminated area of which Sardari seeds were collected and uncontaminated area of Sardari varieties, respectively. Sardari of contaminated area had the lowest As concentrations of roots. Also, addition of phosphate to pots caused a significant reduction of As concentration in roots (Table 3). The reduction of arsenic in different treatments followed the same pattern in wheat plants (contaminated and uncontaminated Sardari). So that, root As concentration in the presence of phosphate showed a significant reduction in 5 and 25 mg l^{-1} treatments of As, But at 125 and 625 mg l^{-1} treatments of As didn't show a significant reduction.

Table 2. Analysis of variance for root, shoot and seed arsenic in wheat plants.

S.O.V	df	Mean of square		
		Root arsenic	Shoot arsenic	Seed arsenic
Group	2	1418.9*	993.6*	1.25*
Treatment	8	3453.8*	1610.4*	17.15*
Group* Treatment	16	178.1*	234.4*	0.312*
Error	40	1.52	1.29	0.01
Total	60			

* = Significant at 5% level

Concentration of As in shoot

Statistical analysis showed significant effect of group, treatment, and group × treatment interactions in shoot As concentration (p<0.05) (Table 2). Total arsenic uptake was determined for the different treatments in wheat shoots. Table 4 presents the results of arsenic uptake by plant shoots in pots without P and with P treatments. Shoot As concentration of wheat plants showed a significant increase in all treatments. Also, Pi application significantly decreased shoot As concentration in 5 and 25 mg l^{-1} treatments of As. But, no significant differences were found at 125 and 625 mg l^{-1} treatments of As. In the absence of P, shoot As concentration at the lowest As level (5 mg l^{-1}) were in the ranged of 3.7 and 7.5 mg kg^{-1}, when irrigated with the highest As level (625 mg l^{-1}) increased to 24.6 and 54.9 mg kg $^{-1}$ in contaminated Sardari as well in uncontaminated Sardari, respectively. Also in the presence of P, shoot As concentration increased slightly range from 1.5 and 3.5 to 24.5 and 54.5 mg kg^{-1} in wheat varieties, respectively (Table 4). Both root and shoot As concentration increased as As levels increased in both with or without P treatments, even though in pots without P- these ratios were much higher. Our results demonstrate that root As concentrations increased more rapidly than shoot and that roots were more sensitive to As than shoots.

In spite of, the maximum As concentration allowed in fodder plants by the law is 4 mg kg^{-1} on a dry weight basis (Zhang et al. 2009). Wheat variety investigated in this study accumulated relatively high As concentrations in their edible parts; this indicates that wheat might represent a risk for animal and human health when this crop grows on As polluted

Table 3. Root arsenic accumulation (mg/kg) in wheat plants exposed to four arsenic treatments.

Varieties	As treatments (mg l^{-1})					As + P treatments (mg l^{-1})				
	0	5	25	125	625	0	5	25	125	625
Sardari (C)	2.8ef	7.3d	14.6c	21.8b	51.1a	1.4f	3.4e	7.2d	21.3b	49.9a
Sardari (UC)	3.1f	15.3d	34c	47.2b	73.2a	1.8f	7.4e	16.6d	46.6b	72.8a

C= Sardari of contaminated area and UC= Sardari of uncontaminated area. Data are expressed as mean values of n=4 and have been analyzed by two-way analysis of variance. Means followed by the same letter within columns are not significantly different by Duncan test at the 5% level.

Table 4. Shoot arsenic accumulation (mg/kg) in wheat plants exposed to four arsenic treatments.

Varieties	As treatments (mg l^{-1})					As + P treatments (mg l^{-1})				
	0	5	25	125	625	0	5	25	125	625
Sardari (C)	1.3e	3.7d	7c	16.3b	24.6a	0.91e	1.5e	3.3d	16.1b	24.5a
Sardari (UC)	1.5ef	7.5d	15.4c	34.4b	54.9a	0.96f	3.5e	3.9e	34.2b	54.5a

C= Sardari of contaminated area and UC= Sardari of uncontaminated area. Data are expressed as mean values of n=4 and have been analyzed by two-way analysis of variance. Means followed by the same letter within columns are not significantly different by Duncan test at the 5% level.

Table 5. Seed arsenic accumulation (mg/kg) in wheat plants exposed to four arsenic treatments.

Varieties	As treatments (mg l^{-1})					As + P treatments (mg l^{-1})				
	0	5	25	125	625	0	5	25	125	625
Sardari (C)	0.08e	0.3d	0.5c	2.4b	3.4a	0.008e	0.1e	0.3d	2.4b	3.1a
Sardari (UC)	0.08f	0.4d	0.6c	3.2b	4.3a	0.03f	0.2e	0.5d	3.1b	4.2a

C= Sardari of contaminated area and UC= Sardari of uncontaminated area. Data are expressed as mean values of n=4 and have been analyzed by two-way analysis of variance. Means followed by the same letter within columns are not significantly different by Duncan test at the 5% level.

Table 6. Compare means of root, shoot and grain As concentration in wheat groups.

Varieties (Groups)	Root As (mg kg^{-1})	Shoot As (mg kg^{-1})	Seed As (mg kg^{-1})
Contaminated Sardari P-	19.55 c	10.95 c	1.35 c
Un contaminated Sardari P-	34.46 a	22.74 a	1.7 a
Contaminated Sardari P+	16.7 d	9.34 d	1.23 d
Un contaminated Sardari P+	29.2 b	20.17 b	1.6 b

C= Sardari of contaminated area and UC= Sardari of uncontaminated area. Data are expressed as mean values of n=4 and have been analyzed by two-way analysis of variance. Means followed by the same letter within columns are not significantly different by Duncan test at the 5% level.

soils. Although, in pots treated with phosphate at 5 and 25 mg As l^{-1} treatment it did reduce the arsenic levels below the standard limit in all wheat plants (Table 4).

<u>Concentration of As in seed:</u>
The results of statistical analysis showed significant difference for seed As concentration in group, treatment and interaction of group × treatment ($p<0.05$) (Table 2). Table 5 describes changes in accumulation levels As by wheat seeds under different As and As × P As treatments. In pots without P treatments, As concentrations in pots treated with 5 mg l^{-1} of As the measured data were 0.3 and 0.4. mg kg^{-1} Sardari seeds collected from contaminated lands verses Sardari from uncontaminated lands. But, at As level of 625 mg l^{-1}, As levels increased to 3.4 and 4.3 mg kg $^{-1}$, respectively. Also, in pots with P treatments, as As levels in irrigation water augmented from 5 to 625 mg l^{-1}, As concentrations from 0.1 and 0.2 increased to 3.1 and 4.2. Our study showed that As concentrations of grains exceeded the tolerance limit described by Zhang et al. (2009) up to 0.5 mg kg^{-1}. Even though, the Sardari variety which seeds were collected from contaminated area significantly showed the lowest levels of As in grain. Also, among the pots which were fertilized with P only at As levels of 5 and 25 mg l^{-1} treatment of As levels reduced below the tolerance limit.

Discussion

The four As treatments used in this study represent either moderate or high contamination levels in Iran (Zandsalimi et al. 2011). The experiments showed that As uptake by seedlings, which followed Michaeli-Menten kinetics, increased with increasing As concentrations in the irrigation solution. So that, there is a relationship between As concentrations of wheat roots, shoots, grains and As treatments. Arsenic concentration followed the order: root > shoot > grain in wheat varieties (Table 6). The most As accumulation levels were in roots than any other plant parts which were also reported in rice (Marin et al. 1992), maize, English ryegrass, rape and sunflower (Gulz et al. 2005). In pot experiments with rice plants exposed to As added via As in irrigation water, Abedin et al. (2002a,b) ranked plant parts according to the As concentrations as follows: root > straw > husk > grain. (Bleeker et al. 2003, Carbonell et al. 1998, Carbonell-Barrachina et al. 1997, 1998, Hartley-Whitaker et al. 2001).

Also, there was a decrease in the shoot As concentration level than the root of wheat plants grown without P (P−) and As levels reduced gradually from 44% and 34% in plants grown from collected seeds of contaminated lands Sardari compared to Sardari variety of uncontaminated area. Even though, this reduction was less severe in pots irrigated with P+ 44% to 30.9% (Table 6). Also, there were reductions of As accumulation in wheat grains both in irrigated with P- or without P+ and were up to 87% to 92%. The roots to shoot and shoot to grain transfer factor of As (TF) were in the range of 0.5–0.6, and 0.07 to 0.1 (Mahdiah et al., 2012). Also, the results indicated that regardless of P treatment, most of the As accumulated in root and the smallest amount amount in the grain, although this behavior was more pronounced in pots with P. The results matches with the studies in rice reported by Williams et al. (2007) and their data indicated that export of arsenic from the shoot to the grain was under tight physiological control and the grain arsenic concentration level were much lower than the shoot. Also, findings were similar to our results were reported for wheat by both Pigna et al. (2009) and Zhang et al. (2009).

Phosphate in plants is important for energy transfer and protein metabolism (Marschner 1995). In view of the fact that, As is a chemical analogue to Phosphorus, As may exert toxicity to plants by interfering with many physiological functions performed by P. Therefore, P should play a critical role in a plant's protection against As phytotoxicity (Meharg & Hartley-Whitaker 2002). Meharg et al. (1994) postulated that Pi and arsenate are accumulated by plant roots via the same uptake system, and that the Pi–arsenate uptake system is much more efficient in accumulating Pi compared with arsenate. Geng et al. (2006) indicated that fertilization by P may reduce the effects of As toxicity by restricting As accumulation in the above-ground parts of plants. This has practical importance in agricultural systems, since may reduce yield losses and improve yield quality. Also, P could decrease the reactive oxygen species and non-protein thiols production, formed during exposure to As that cause tissue damage and lipid peroxidation (Hartley-Whitaker et al. 2001). Fitz and Wenzel (2002) have reported that the effects of P on the uptake and toxicity of As in plants depends on plant species, chemical speciation of As, growth medium and the experimental conditions. Our results were in agreement with these findings, which reported P restricted the transfer of As from the soil to the above-ground plant organs.

Conclusions

The present study describes the relationship between arsenate and phosphate uptake and distribution in root, shoot, and grain of wheat (Sardari variety collected from Iranian arsenic contaminated area and control population). Arsenic accumulation was higher in the root than aerial parts (shoot and grain). Sardari variety (of contaminated area) had significantly less uptake of arsenic compared with control population. Furthermore, our results showed that arsenic concentration in root, shoot and grain, decreased significantly with the application of phosphorus.

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