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*Article*

# Interactive effects of arsenic and phosphorus on their uptake by wheat varieties with different arsenic and phosphorus soil treatments

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## Abstract

In this research we have investigated relationship between arsenate and phosphate uptake and its distribution in root, shoot, and seed of wheat varieties. Three wheat varieties were selected and grown in 7 Kg pots under controlled conditions among which, Sardari variety were collected from Iranian arsenic contaminated area and tested along with two other varieties Parsi and Pishtaz. The aim was to select a variety with low arsenate uptake ability with the aim of improving food safety and human health. Arsenic was applied with following concentrations of 0, 5, 25, 125 and 625 mg $l^{-1}$ in the presence or absence of P. With increasing As concentration in irrigation water, As levels of roots, shoots and seeds increased. Also, measurements indicated that As uptake rates decreased in the presence of P. Also, at 125 and 625 mg $l^{-1}$ As concentration levels, the measured As concentrations of seed and shoot exceeded the tolerance limit, regardless of P presence. Among wheat varieties, Sardari (of contaminated area) had significantly less uptake of As compared with two other varieties. Besides, P concentrations in all wheat varieties followed the following order: seed > root > shoot.

**Keywords** arsenate; contaminated area; food safety; phosphate; wheat.

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## 1 Introduction

Agricultural soils in many parts of the world are slightly to moderately contaminated by heavy toxic metal such as Cd, Cu, Zn, Ni, Co, Cr, Pb, and As (Yadav, 2009). 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 high (Smedley and Kinniburgh, 2002). Also, its environmental inputs can be through either natural (geogenic) or anthropogenic processes. Natural processes including volcanic eruption, weathering of rocks and minerals, fossil fuel, and forest fire can release huge amounts of As into the environment that may be transported over long distances as suspended particulates through both water and air. Anthropogenic activities are the main source of As in the environment, exceeding natural sources by 3:1 (Woolson, 1983). Among the anthropogenic sources, industrial effluents constitute the largest contribution. Industrial sources generally include coal-fired power plants, smelting, incinerations of wastes, wood preservation, and agriculture fertilizers (Mahimairaja et al., 2005). The present free style way of disposing agricultural, industrial and domestic effluents into natural water-bodies results in serious surface and groundwater contamination (Zandsalimi et al., 2011; karimi et al., 2010). During the last three decades, high concentrations of As in ground water have been reported in different regions of the world such as the USA, China, Chile, Bangladesh, Taiwan, Mexico, Argentina, Poland, Canada, Hungary, Japan, India, Vietnam, Nepal (Jack et al., 2003) and recently from Iran (Mosaferi et al., 2003; Karimi et al., 2009; Karimi et al., 2013). As 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 and Rahman, 2003).

As is typically considered a non-essential element for plants and its bioavailability depends on plant species and soil properties (Tao et al., 2006). The absorption of As by plants is influenced by the concentration of As in soil (National Research Council of Canada, 1977). In general, As availability to plants is highest in coarse-textured soils having little colloidal material and little ion exchange capacity, and lowest in fine-textured soils high in clay, organic material, iron, calcium and phosphate (National Research Council of Canada, 1978). 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 and van der Meer, 2002; Karimi et al., 2013). For example, Rice may take up As from the surrounding soil and the concentration of As in rice grains can reach elevated levels (Williams et al., 2007). The concentration of As in rice is usually below 0.5 mg $kg^{-1}$ (DW), but since it is common to eat approximately 200 g (DW) of rice per day in Asian diets (Zhu et al., 2008), the total amount of ingested As can reach levels 5-10 times higher than the daily limit set compared to drinking water (Sun et al., 2009). Beside, this conclusion is also true in the case of wheat. Rice and 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. To evaluate the possible health risk to humans consuming crops irrigated with As contaminated water, information is needed regarding the soil-to-plant transfer of As and to minimize the accumulation of As in plants consumed directly by humans, farm animals or wildlife (Meharg and Hartley-Whitaker, 2002). In addition, pesticides and fertilizers are the major sources of As in agricultural soils (Jiang and Singh, 1994). Numerous cases of As contamination of agricultural soils due to arsenic containing pesticides have been reported (Woolson et al., 1971; Peterson et al., 1981; Merry et al., 1986).

Arsenic can be found in both organic and inorganic compounds with variable oxidation states. Understanding the difference between inorganic and organic arsenic is important because some of the organic forms are less harmful than the inorganic forms. EPA has classified inorganic arsenic as a known human carcinogen (ATSDR, 2005). Arsenate, the dominant inorganic species of arsenic in aerobic/oxic environments, while arsenite 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 (Asher and Reay, 1979; Meharg and Macnair, 1994). Kinetic studies suggest that at least two phosphate uptake systems exist, a low and a high affinity system (Meharg and Macnair, 1990; Ullrich-Eberius et al., 1984). The understanding of the general patterns of 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 contaminated soils.

The aim of this study was to evaluate the accumulation rate of As in the presence and absence of P and also its effects on phyto toxicity, uptake and partitioning between different parts (seed, shoot, root) of three wheat varieties that grown in contaminated and uncontaminated soil. Also, to select a variety with a low arsenate uptake rate in order to improve food quality and safety.

## 2 Material and Methods

### 2.1 Growth conditions and treatments

The present experiments were conducted from September 2011 to June 2012 in a controlled condition greenhouse of Razi University. The greenhouse temperature was 14°C at night and 30 °C days, with an average photon flux of 825 mmolm$^{-2}$ s$^{-1}$. Three varieties of wheat (*Triticum aestivum* cv. Sardari, Parsi and Pishtaz) were selected for the study by the Sub-Center of Cereal Quality Control, Ministry of Agriculture of Iran. 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. These villages were selected on the basis of the high arsenic contamination and the inadequate supply of safe drinking water (Mosaferi et al., 2009). 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 l$^{-1}$ soil. The four As treatments used in this study represent either moderate or serious contamination dose levels in Iran. Each treatment was replicated 3 times. Furthermore, in half of the pots 5.6 mM P as $K_2HPO_4$ was added to the nutrient solution in order to evaluate the influence of P on As uptake by plants. Thus, there were two sets of treatments one supplemented with Pi (P−) and the other without Pi (P+).

### 2.2 Soil preparation and characteristics

Pots were filled with a coarse-silt loam Soil, collected from a local farm at 0-15 cm depth. It was crushed, mixed thoroughly and sieved through a 2 mm 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 potentiometer in a soil paste saturated with water and organic matter was determined by dichromate oxidation using the Turin method (Soon and 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 extract ant 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 was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Shimadzu, 6200) (Meharg and Jardin, 2003).

**Table 1** Physical and chemical properties of soil.

| Soil characteristics | Value |
|---|---|
| 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 |

### 2.3 Sampling and harvest procedure

When the wheat plants were harvested, they were thoroughly washed with tap water, and then with distilled de-ionized water, adhering water was then removed with filter paper. Root, shoot and seeds of each plant was separated and oven-dried at 70°C for 48 hrs, and dry weight was determined.

### 2.4 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 concentrations of As were measured by a hydride generation–atomic absorption spectrometer (Shimadzu, 6200). Hydride generation was used for samples due to their lower detection limits of detection (0.5 μg $l^{-1}$) (Meharg and Jardin, 2003).

### 2.5 Statistical analysis

All data were expressed as an average of three replicates. Treatment effects were determined by analysis of variance according to the General Linear Model procedure of the SPSS program. Duncan test at a 5% probability was used for post harvest comparisons in order to separate treatment differences.

## 3 Results

### 3.1 Concentration of As in root, shoot and seed

The results showed that root As concentration in wheat varieties increased significantly with increasing As levels in irrigation water, particularly in pots without P treatments (P-) (Table 2). So, by increasing As level in irrigation water from 5 to 625 mg $l^{-1}$, the measured As concentration levels of roots ranged from 7.31 to 75.9 and 3.4 to 74.9 mg $kg^{-1}$ in pots without P- and with P+ treatments (Table 2). Also, in pots without P- treatments, root As concentration was at the highest As level (625 mg $l^{-1}$) which were 7, 4.7, 4.5 and 4.5 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, Parsi and Pishtaz wheat varieties, respectively. The reduction of arsenic in different treatments followed the same pattern in three wheat varieties. So that, root As concentration in the presence of phosphate showed a significant reduction from 5 to 25 mg $l^{-1}$ treatments of As, But at 125 to 625 mg $l^{-1}$ treatments of As didn’t show a significant reduction (Table 2). Though, the Parsi and Pishtaz from uncontaminated area, and Sardari from contaminated area had the highest and lowest As concentration in plant roots (Table 3). Also, addition of phosphate to pots caused a significant reduction of As concentration in roots of Sardari variety (Table 3).

Then, total arsenic uptake due to the different implemented levels of As treatments in shoot of wheat varieties were determined. Thus, Table 2 presents the results of arsenic uptake by plant shoots in pots without $P^-$ and with $P^+$. Shoot As concentration of wheat varieties showed a significant increase in all treatments. Also, Pi application significantly decreased shoot As concentration in 5 to 25 mg $l^{-1}$ treatments of As. But, no significant differences were found at 125 to 625 mg $l^{-1}$ treatments of As (In all wheat varieties). In the absence of P, shoot As concentration was at the lowest As level (5 mg $l^{-1}$) and were in the ranged of 3.7, 7.5, 7.9 and 8 mg $kg^{-1}$, when irrigated with the highest As level (625 mg $l^{-1}$) increased to 24.6, 54.9, 57.6 and 56.3 mg $kg^{-1}$ in contaminated Sardari as well in uncontaminated Sardari, Parsi and Pishtaz varieties. Furthermore, in the presence of P, shoot As concentration increased slightly from 1.7, 3.5, 3.8 and 3.9 to 24.5, 54.5, 57.8 and 56.7 mg $kg^{-1}$ in all wheat varieties (Table 2). 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 (Table 2). 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), the wheat varieties investigated in this study accumulated relatively higher concentration of As in their edible parts; which this might represent a risk for animal and human health when this crop are grown on As contaminated soils and consumed. Except, Sardari variety of the one which was collected from contaminated area, that showed the lowest amounts of As in shoot at 5 mg l$^{-1}$ treatment of As, but the other wheat varieties showed higher As amounts in shoot than the standard limit in all pots without P-treatments. Although, in pots treated with phosphate at 5 mg As l$^{-1}$ treatment it did reduce the arsenic levels below the standard limit in all three wheat varieties (Table 2). The Parsi and Pishtaz varieties from uncontaminated area, and Sardari from contaminated area had the highest and lowest As concentration in plant shoots (Table 3). Also, addition of phosphate to pots caused a significant reduction of As concentration in shoots of Sardari variety (Table 3). But, shoot As concentration in the presence of phosphate did not show a significant reduction in Parsi and Pishtaz varieties (Table 3).

Table 2 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, 0.4. 0.6 and 0.7 mg kg$^{-1}$ Sardari seeds collected from contaminated lands verses Sardari, Parsi and Pishtaz from uncontaminated lands. But, at As level of 625 mg l$^{-1}$, As levels increased to 3.4, 4.3, 5.4 and 5.7 mg kg $^{-1}$. 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, 0.2, 0.3 and 0.4 increased to 3.1, 4.2, 5.4 and 5.7. 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 mg l$^{-1}$ treatment of As levels reduced below the tolerance limit. Furthermore, as mentioned, Sardari variety (seeds collected from contaminated and uncontaminated lands) reduced As levels below the tolerance limit in both 5 and 25 mg l$^{-1}$ of As (Table 2). The Parsi and Pishtaz varieties from uncontaminated area and Sardari from contaminated area had the highest and lowest As concentration in plant seeds (Table 3). Also, addition of phosphate to pots caused a significant reduction of As concentration in seeds of wheat varieties (Table 3).

**Table 2** Root, shoot and seed arsenic accumulation (mg/kg) in wheat varieties (*Triticum aestivum* cv. Sardari, Parsi and Pishtaz) exposed to four arsenic treatments (5, 25, 125 and 625 mg l$^{-1}$).

| Variety | Treatments (mg l$^{-1}$) | Root As content | Shoot As content (mg kg$^{-1}$) | Seed As content |
|---|---|---|---|---|
| Sardari (C) | Control(P-) | 2.8±0.21 ef | 1.3±0.1 e | 0.08±0.005 e |
| | As$_{5}$ | 7.3±0.48 d | 3.7±0.13 d | 0.3±0.05 d |
| | As$_{25}$ | 14.6±0.35 c | 7±0.16 c | 0.5±0.04 c |
| | As$_{125}$ | 21.8±0.35 b | 16.3±0.53 b | 2.4±0.08 b |
| | As$_{625}$ | 51.1±0.75 a | 24.6±0.31 a | 3.4±0.1 a |
| | Control(P+) | 1.4±0.15 f | 0.91±0.03 e | 0.008±0.003 e |
| | As$_{5}$ + P | 3.4±0.19 e | 1.7±0.14 e | 0.1±0.008 e |
| | As$_{25}$ + P | 7.2±0.31 d | 3.3±0.21 d | 0.3±0.01 d |
| | As$_{125}$ + P | 21.3±0.77 b | 16.1±0.93 b | 2.4±0.03 b |
| | As$_{625}$ + P | 49.9±0.35 a | 24.5±1.37 a | 3.1±0.04 a |
| Sardari (UC) | Control(P-) | 3.1±0.12 f | 1.5±0.11 ef | 0.08±0.003 f |
| | As$_{5}$ | 15.3±0.28 d | 7.5±0.11 d | 0.4±0.01 d |
| | As$_{25}$ | 34±1.52 c | 15.4±0.26 c | 0.6±0.03 c |
| | As$_{125}$ | 47.2±0.44 b | 34.2±0.51 b | 3.2±0.06 b |
| | As$_{625}$ | 73.2±1.3 a | 54.9±0.87 a | 4.3±0.1 a |

| | | | | |
|---|---|---|---|---|
| | Control(P+) | 1.8±0.13 f | 0.96±0.01 f | 0.03±0.01 f |
| | As $_{5}$ + P | 7.4±0.23 e | 3.5±0.12 e | 0.2±0.001 e |
| | As $_{25}$ + P | 16.6±0.25 d | 3.9±0.18 e | 0.5±0.01 d |
| | As $_{125}$ + P | 46.6±0.83 b | 34.2±1.2 b | 3.1±0.06 b |
| | As $_{625}$ + P | 72.8±1.73 a | 54.5±1.6 a | 4.2±0.09 a |
| Parsi | Control(P-) | 2.7±0.2 f | 1.3±0.1 f | 0.06±0.003 e |
| | As $_{5}$ | 16.7±0.1 d | 7.9±0.1 d | 0.6±0.005 d |
| | As $_{25}$ | 35.8±0.2 c | 17.3±0.2 c | 0.9±0.01 c |
| | As $_{125}$ | 50.6±0.3 b | 37±0.2 b | 4.3±0.06 b |
| | As $_{625}$ | 75.9±0.6 a | 57.6±0.4 a | 5.4±0.1 a |
| | Control(P+) | 1.6±0.1 f | 0.91±0.03 f | 0.03±0.003 e |
| | As $_{5}$ + P | 8.4±0.3 e | 3.8±0.1 e | 0.3±0.005 e |
| | As $_{25}$ + P | 17.2±0.1 d | 7.9±0.1 d | 0.6±0.01 d |
| | As $_{125}$ + P | 50.4±0.6 b | 37.3±1.05 b | 4.2±0.04 b |
| | As $_{625}$ + P | 74.9±0.4 a | 57.8±0.9 a | 5.4±0.008 a |
| Pishtaz | Control(P-) | 3.2±0.14 f | 1.4±0.15 e | 0.07±0.003 f |
| | As $_{5}$ | 16.7±0.48 d | 8±0.04 d | 0.7±0.04 d |
| | As $_{25}$ | 36.3±0.17 c | 17.8±0.14 c | 1.1±0.03 c |
| | As $_{125}$ | 50.2±0.19 b | 37.4±0.18 b | 4.8±0.04 b |
| | As $_{625}$ | 75.9±0.41 a | 56.3±1.4 a | 5.7±0.08 a |
| | Control(P+) | 1.6±0.09 f | 0.97±0.005 e | 0.04±0.002 f |
| | As $_{5}$ + P | 8.3±0.32 e | 3.9±0.05 e | 0.4±0.002 e |
| | As $_{25}$ + P | 17.9±0.06 d | 8.4±0.16 d | 0.7±0.004 d |
| | As $_{125}$ + P | 49.8±1.26 b | 37.4±1.3 b | 4.8±0.05 b |
| | As $_{625}$ + P | 74.3±1.71 a | 56.7±2.3 a | 5.7±0.06 a |

The data are expressed as mean ± S.E. C= Sardari of contaminated area and UC= Sardari of uncontaminated area. P+ and P- = with (P+) and without (P−) P fertilization. Data are expressed as mean values of n=3 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 3** mean comparison of root, shoot and seed As accumulation in wheat varieties.

| Variety | Root arsenic | Shoot arsenic | Seed arsenic |
|---|---|---|---|
| | | (mg kg$^{-1}$) | |
| Sardari P- (C) | 19.55±4.5 e | 10.59±2.3 e | 2.06±0.4 e |
| Sardari P- (UC) | 34.46±6.5 b | 22.74±5.2 b | 3.4±0.6 bc |
| Parsi P- | 36.20±6.7 a | 24.21±5.4 a | 3.5±0.7 ab |
| Pishtaz P- | 35.51±6.8 a | 24.26±5.3 a | 3.6±0.7 a |
| Sardari P+ (C) | 16.7±4.8 f | 9.34±2.5 f | 1.67±0.5 f |
| Sardari P+ (UC) | 29.2±7.1 d | 20.17±5.6 d | 3.03±0.7 d |
| Parsi P+ | 30.75±7.5 c | 21.58±5.9 c | 3.11±0.8 d |
| Pishtaz P+ | 30.74±7.4 c | 21.52±5.8 c | 3.25±0.8 cd |

The data are expressed as mean ± S.E. C= Sardari of contaminated area and UC= Sardari of uncontaminated area. P+ and P- = with (P+) and without (P−) P fertilization. Data are expressed as mean values of n=3 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.

### 3.2 Concentration of P in root, shoot and seed

The total phosphorus levels in root plants are shown in Table 4. There was not a broad variation of mean P concentrations among wheat varieties, ranging from 12.7 to 33.7 mg kg$^{-1}$ and 12.8 to 42.6 mg kg$^{-1}$ in pots without P- and with P+. Also, root phosphorus concentrations increased as the level of As increased. So, this increase was proportional to the increase of As concentrations in treatments. Furthermore, by adding Phosphate to the pots it did increase the concentration of P in the roots. Although, by escalating the As concentrations in treatments, the amount of phosphorus in roots increased from 48% to 68%. Moreover, most of the root P concentration was reported in Sardari variety collected from contaminated area (Table 5). On contrary, the results showed that P concentration in shoot significantly decreased as As levels increased (Table 4). Although, over all Pi application increased shoot P concentration in pots with P+ as compared with the one without P-. Also, at highest As level, Shoot P concentration levels decreased by 83.7% in Sardari variety of contaminated area, 66.9% in uncontaminated Sardari, 74.2% in Parsi and 64.2% in Pishtaz compared with the control. Although, this reduction was less severe in the treatments with P+, 66.6%, 58.4%, 61.6%, and 58% (Table 4). So, this study confirmed the higher ability of roots, to accumulate P, than shoot in As treatments (Similar behavior to arsenate resistant plants). Also, most of the shoot P accumulation was reported in Sardari variety collected from contaminated area (Table 5). Seed P concentrations of wheat varieties treated with different levels of As are summarized in Table 4. Grain P concentrations decreased with increasing level of As in all wheat varieties. However, it showed no significant differences at 125 and 625 mg l$^{-1}$ levels of As in pots treated with or without phosphorus (Table 4). Generally, the phosphorus concentration of grains in pots treated with P+ was higher than pots without P- treatments (Table 5). Thus, P concentrations in all wheat varieties followed the following order: seed > root > shoot (Table 5).

**Table 4** Root, shoot and seed phosphorus content (mg/kg) in wheat varieties (*Triticum aestivum* cv. Sardari, Parsi and Pishtaz) exposed to four arsenic treatments (5, 25, 125 and 625 mg l$^{-1}$).

| Variety | Treatments (mg l$^{-1}$) | Root P content | Shoot P content (mg kg$^{-1}$) | Seed P content |
|---|---|---|---|---|
| Sardari (C) | Control(P-) | 12.7±0.24 e | 25.3±0.51 b | 73.5±0.76 b |
| | As$_{5}$ | 14.7±0.65 de | 22.8±0.43 d | 66.3±0.42 c |
| | As$_{25}$ | 19.2±0.7 d | 15.2±0.41 f | 49.2±0.82 e |
| | As$_{125}$ | 27.3±0.48 c | 7.2±0.25 i | 42±0.24 f |
| | As$_{625}$ | 33.7±0.77 b | 4.1±0.20 j | 38.2±0.59 g |
| | Control(P+) | 13.1±1.1 e | 26.1±0.55 a | 82±1.31 a |
| | As$_{5}$ + P | 14.9±0.86 de | 23.5±0.48 c | 72.4±0.74 b |
| | As$_{25}$ + P | 25±1.2 c | 19.8±0.33 e | 63.8±0.73 d |
| | As$_{125}$ + P | 35.9±2.1 b | 11.2±0.4 g | 42.3±0.38 f |
| | As$_{625}$ + P | 42.6±3.1 a | 8.7±0.26 h | 38.7±0.71 g |
| Sardari (UC) | Control(P-) | 13.5±0.41 g | 25.1±0.58 a | 70.6±0.51 b |
| | As$_{5}$ | 15.1±0.73 f | 23.6±0.43 c | 64.8±0.34 c |
| | As$_{25}$ | 18.5±0.32 e | 16.4±0.35 f | 55.9±0.65 e |
| | As$_{125}$ | 22.2±0.64 d | 10.6±0.36 h | 31.5±0.31 f |
| | As$_{625}$ | 28.4±0.84 b | 8.3±0.25 j | 29.4±0.47 g |
| | Control(P+) | 13.5±0.33 g | 24.8±0.52 b | 77.7±0.62 a |
| | As$_{5}$ + P | 15.4±0.51 f | 23.1±0.31 d | 71.7±0.37 b |
| | As$_{25}$ + P | 20.2±0.42 d | 19.5±0.33 e | 61.7±0.59 d |
| | As$_{125}$ + P | 26.3±0.41 c | 12.1±0.35 g | 31.5±0.27 f |

| | | | | |
|---|---|---|---|---|
| | As$_{625}$ + P | 34.4±0.71 a | 10.3±0.23 i | 29.3 ±0.64 g |
| Parsi | Control(P-) | 12.9±0.47 f | 24.1±0.58 b | 57.2±0.65 b |
| | As$_{5}$ | 15.8±0.31 e | 22.2±0.44 d | 49.3±0.51 d |
| | As$_{25}$ | 18.3±0.62 d | 14.9±0.46 f | 40.7±1.3 e |
| | As$_{125}$ | 20.8±0.41 c | 9.3±0.35 i | 28.6±0.41 f |
| | As$_{625}$ | 26.2±0.45 b | 6.2±0.41 j | 20.4±1.07 g |
| | Control(P+) | 12.8±0.62 f | 24.8±0.29 a | 62.5±1.7 a |
| | As$_{5}$ + P | 15.5±1.03 e | 22.9±0.48 c | 53.05±0.45 c |
| | As$_{25}$ + P | 20.7±0.51 c | 18.3±0.37 e | 42.9±0.66 e |
| | As$_{125}$ + P | 25.3±0.83 b | 13±0.34 g | 28.7±0.55 f |
| | As$_{625}$ + P | 30.6±0.65 a | 9.5±0.31 h | 20.51±0.97 g |
| Pishtaz | Control(P-) | 12.7±0.25 g | 23.8±0.61 b | 51.6±0.41 b |
| | As$_{5}$ | 14.3±0.43 f | 22.5±0.48 d | 47.3±0.45 c |
| | As$_{25}$ | 19.2±0.35 e | 16.1±0.35 f | 37.7±0.53 e |
| | As$_{125}$ | 22.1±0.38 d | 11±0.41 h | 22.9±0.51 f |
| | As$_{625}$ | 24.6±0.41 c | 8.5±0.29 j | 17.5±1.11 g |
| | Control(P+) | 12.8±0.82 g | 24.1±0.55 a | 57.3±1.15 a |
| | As$_{5}$ + P | 15.7±0.54 f | 23.3±0.57 c | 50.2±0.46 b |
| | As$_{25}$ + P | 22±0.35 d | 18.7±0.34 e | 41.2±1.01 d |
| | As$_{125}$ + P | 26.5±0.71 b | 12.5±0.31 g | 23.2±0.72 f |
| | As$_{625}$ + P | 30.5±0.39 a | 10.1±0.33 i | 17.5±1.03 g |

The data are expressed as mean ± S.E. C= Sardari of contaminated area and UC= Sardari of uncontaminated area. P+ and P- = with (P+) and without (P−) P fertilization. Data are expressed as mean values of n=3 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** Mean comparison of root, shoot and seed phosphorus content in wheat varieties.

| Variety | Root phosphorus | Shoot phosphorus | Seed phosphorus |
|---|---|---|---|
| | | (mg kg$^{-1}$) | |
| Sardari P- (C) | 21.5±2.03 b | 16.80±1.38 d | 53.95±3.67 b |
| Sardari P- (UC) | 19.64±1.43 c | 14.84±1.18 g | 50.46±4.54 c |
| Parsi P- | 18.84±1.22 c | 15.40±1.26 f | 39.29±3.58 e |
| Pishtaz P- | 18.62±1.21 c | 16.38±1.32 e | 35.46±3.57 g |
| Sardari P+ (C) | 26.27±3.18 a | 18.02±1.77 a | 59.96±4.57 a |
| Sardari P+ (UC) | 22.09±2.04 b | 17.86±1.59 b | 54.46±5.41 b |
| Parsi P+ | 21.02±1.75 b | 17.70±1.72 c | 41.53±4.13 d |
| Pishtaz P+ | 21.53±1.76 b | 17.75±1.66 c | 37.92±4.15 f |

The data are expressed as mean ± S.E. C= Sardari of contaminated area and UC= Sardari of uncontaminated area. P+ and P- = with (P+) and without (P−) P fertilization. Data are expressed as mean values of n=3 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.

## 4 Discussion

### 4.1 As concentrations in plant tissues

The four As treatments used in this study represent either moderate or high contamination levels in Iran (Zandsalimi et al., 2011). Although varieties tested in this study differed in their response to As addition in soil, but, they all followed the same pattern. Further 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, seeds and As treatments by uncontaminated three wheat varieties. Arsenic concentration followed the order: root > shoot > seed in all wheat varieties (Table 3). The ranking of plant parts according to As accumulation is regularly used as "evidence" that aboveground edible parts are no risk to human health. It is, however, the absolute concentration of inorganic As in the edible parts that should be evaluated, regardless of As concentrations in other parts of the plant. The most As accumulation levels were in roots than any other plant parts which were also reported in rice (Marin et al., 1992; 1993), maize, English ryegrass, rape and sunflower (Gulz et al., 2005) their findings were similar to results reported in this experiments. For example, 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; Sneller et al., 1999).

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 46% to 34%, 33% and 34% in plants grown from collected seeds of contaminated lands Sardari compared to Sardari, Parsi and Pishtaz varieties of uncontaminated area. Also, there were a reduction of As accumulation in wheat seeds both in varieties irrigated with or without P. The roots to shoot and shoot to seed transfer factor of As (TF) were in the range of 0.5–0.6, and 0.07 to 0.1 in all varieties. Also, the results indicated that regardless of P treatment, most of the As accumulated in root and the smallest amount in the seed, 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 seed was under tight physiological control and the seed arsenic concentration level were much lower than the shoot. Also, findings were similar to results reported for wheat by both Pigna et al. (2009) and Zhang et al., (2009).

### 4.2 P concentrations in plant tissues

Also, Table 5 compares the changes of P accumulation levels in roots, shoots and seeds. as well, results showed significant different accumulation rates of phosphorus in roots, shoots and seeds among the pots treated with or without phosphorous. Moreover, the highest phosphorus concentration was observed in Sardari roots of contaminated area. Also, Parsi, Pishtaz and uncontaminated Sardari varieties didn't show a significant difference in root P accumulation. In contrast, phosphorus accumulation of shoots decreased. Besides, the lowest P concentration within the shoots found in Sardari variety collected from uncontaminated area. In addition, the highest P accumulation rates in seeds belonged to Sardari variety of contaminated lands.

Furthermore, Phosphorus has the most important role in bioaccumulation potential of As in different parts of plants. Considering the fact that P and As belong to the same chemical group, they stand for similar geochemical behavior in soils (Adriano, 2001). Also, P is frequently the most restrictive element for plant growth and the crop yield on 30%–40% of the world's arable land is limited by P availability (Runge-Metzger, 1995). 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 and 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.

Overall, Sardari variety collected from contaminated area showed a better tolerance to As for which several explanations may be possible. This tolerance to arsenic could be related to some physiological and biochemical adaptation strategies (Meharg, 1994). Also, some plants appear to have an exclusion mechanism which allows them to bioaccumulate heavy metals at different levels, thus avoiding the uptake of too much of a toxic element. Meharg and Macnair (1991) commented on the fact that internal tolerance mechanisms are considered to play a more significant role than avoidance mechanisms in the adaptation of plants to contaminated habitat. The definition of tolerance mechanisms which has been used by Meharg and Macnair (1991) refers to biochemical detoxification and limited movement of the metal ion once absorbed in the plant, or isolation of the ion within a cell.

In general, the tolerance to arsenic involves (1) complication of arsenic by such as peptides with SH-groups (Karimi et al., 2009), (2) reduction of As influx by suppressing phosphate/arsenate uptake systems (Meharg and Macnair, 1992; Meharg, 1994), and (3) enhanced production of antioxidants that detoxify free reactive oxygen species (ROS) produced in response to As (Hartley-Whitaker, 2001). In Meharg and Macnair's (1991) study on arsenate tolerance in certain grasses, they concluded that the evolved tolerance was due to an adaptation of the arsenate uptake system. Porter and Peterson (1975) found that grasses growing on mine wastes which contained high arsenic concentrations, tested plants developed a tolerance to the elevated levels compared to the grasses found on a site with low standard levels. In conclusion, the long contamination history of the surveyed areas in Iran, there was an evident exclusion mechanism of effective pressure toward As tolerance by the crops species. To summarize, based on the current available information, risks to food safety and yield are likely to increase with the buildup of As in the soil and irrigation water. Although the risks cannot be quantified for the time being, it is proposed to focus on preventing and minimizing input via irrigation and uptake by crops (Brammer, 2005; Duxbury et al., 2003; Karimi et al., 2009; Karimi et al., 2013). For example, P fertilization may reduce the effects of As toxicity without increase As concentrations in the above-ground parts of plants. This has practical importance in agriculture, since may reduce yield losses and improve yield quality. Furthermore, breeding crops tolerant to As with a low accumulation of As in seeds may reduce potential risks as well.

## 5 Conclusion

This study monitored the correlation between the arsenic content in different parts of tested wheat varieties and also the arsenic concentration of irrigation water in the presence or in the absence of P fertilization. Although, As concentrations which were used in this experiment could exist in areas with As contamination in groundwater and soil deposited by mining activities. Moreover, Arsenic concentrations in root shoot and seed increased with increasing As concentration in irrigation water. When Pi was added (P+), As concentration of root, shoot and seed decreased compared to plants without Pi (P-) treatments. So, the arsenic content in different parts of plants were found to be in the order of roots > shoots > seeds parts. Also, in 125 and 625 mg

$l^{-1}$ of As treatment levels, shoot and seed parts of all varieties showed arsenic concentrations higher than the Chinese food hygiene limit. Additionally, Sardari variety of contaminated area was found to be more resistant to arsenic contamination than the other studied wheat varieties. Also, our results have shown that there were considerable differences in arsenic transport rates into different plant organs. Besides, when P was applied it did reduce the arsenic uptake into the plant organs. Thus, based on the results of the present study, phosphorus application in rate according to the plant demands, leads to lower As accumulation in root, shoot and seed of wheat. This has practical importance in agricultural performance.

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