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ORIGINAL ARTICLE

INFLUENCE OF ARSENIC TREATMENTS ON RESISTANCE AND ITS ACCUMULATION IN DIFFERENT WHEAT VARIETIES

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ABSTRACT: In this research we have investigated relationship between arsenate 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. As was applied with following concentrations of 0, 5, 25, 125 and 625 mgl^{-1}. With increasing As concentration in irrigation water, As levels of roots, shoots and seeds increased. Also, at 125 and 625 mg l^{-1} As concentration levels, the measured As concentrations of seed and shoot exceeded the tolerance limit. Among wheat varieties, Sardari variety (of contaminated area) had significantly less uptake of As compared with two other varieties. Besides, As concentrations in all wheat varieties followed the following order: root > shoot > seed.
KEYWORDS: Arsenate, Sardari, Pishtaz, Parsi, Food safety.

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, it's 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 (Kazia *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.,* 2005). 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

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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). 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 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 (Asher and Reay, 1979; Meharg and Macnair, 1994). 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 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.

MATERIALS 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.

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 Jardine, 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 Jardine, 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.

RESULTS

3.1. Concentration of As in root

Analysis of variance indicated that total As content was significantly influenced by wheat variety, arsenic treatment and interaction of variety * treatment (Table 2). The results showed that root As concentration in wheat varieties increased significantly with increasing As levels in irrigation water (Table 3). 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 mg kg^{-1} (Table 3). Also, 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 significant difference between cultivars were also observed (Table 4). So, the Parsi and Pishtaz from uncontaminated and Sardari from contaminated area had the highest and lowest As concentration in plant roots (Table 4).

Table 2: Analysis of variance for root, shoot and seed arsenic accumulation in wheat (*Triticum aestivum* L.).

S.O.V	df	Mean of square		
		Root arsenic	Shoot arsenic	Seed arsenic
Variety	3	992.22 *	655.09 *	8.44 *
Treatment	4	7893.02 *	4443.98 *	94.9 *
Variety*Treatment	12	96.94 *	123.77 *	1.4 *
Error	40	0.99	0.6	0.01
Total	60			

*= Significant at 5% level

Table 3: 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	2.8 e	1.3 e	0.08 d
	As_5	7.3 d	3.7 d	0.3 c
	As_{25}	14.6 c	7 c	0.5 c
	As_{125}	21.8 b	16.3 b	2.4 b
	As_{625}	51.1 a	24.6 a	3.4 a
Sardari (UC)	Control	3.1 e	1.5 e	0.08 e
	As_5	15.3 d	7.5 d	0.4 d
	As_{25}	34 c	15.4 c	0.6 c
	As_{125}	46.6 b	34.2 b	3.2 b
	As_{625}	73.2 a	54.9 a	4.3 a
Parsi	Control	2.7 e	1.3 e	0.06 e
	As_5	16.7 d	7.9 d	0.6 d
	As_{25}	35.8 c	17.3 c	0.9 c
	As_{125}	50.6 b	37 b	4.3 b
	As_{625}	75.9 a	57.6 a	5.4 a
Pishtaz	Control	3.2 e	1.4 e	0.07 e
	As_5	16.7 d	8 d	0.7 d
	As_{25}	36.3 c	17.8 c	1.1 c
	As_{125}	50.2 b	37.4 b	4.8 b
	As_{625}	75.9 a	56.3 a	5.7 a

C= Sardari of contaminated area and UC= Sardari of uncontaminated area. 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 4: mean comparison of root, shoot and seed As accumulation in wheat varieties.

Variety	Root arsenic	Shoot arsenic	Seed arsenic
		(mg kg^{-1})	
Sardari (C)	19.55 c	10.59 c	2.06 d
Sardari (UC)	34.46 b	22.74 b	3.4 c
Parsi	36.2 a	24.2 a	3.5 b
Pishtaz	35.51 a	24.26 a	3.6 a

C= Sardari of contaminated area and UC= Sardari of uncontaminated area. 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 As in shoot

Then, total arsenic uptake due to the different implemented levels of As treatments in shoot of wheat varieties were determined. Thus, Table 3 presents the results of arsenic uptake by plant shoots in pots. Shoot As concentration of wheat varieties showed a significant increase in all treatments. 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. Both root and shoot As concentration increased as As levels increased in treatments (Table 3). Our results demonstrate that root As concentrations increased more rapidly than shoot and that roots were more sensitive to As than shoots. Also, mean comparisons of As content in wheat varieties showed that Parsi and Pishtaz from uncontaminated lands and Sardari variety from contaminated lands with an average of 24.2 and 10.59 mg kg^{-1}, respectively were the highest and lowest As contents (Table 4)

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 treatments (Table 3).

3.3. Concentration of As in seed

Table 3 describes changes in accumulation levels As by wheat seeds under different As 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 $^{-1}$. 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 (Table 4).

DISCUSSION

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 4). 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; Marin *et al.*, 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) and Abedin *et al.*, (2002b) ranked plant parts according to the As concentrations as follows: root > straw > husk > grain (Bleeker *et al.*, 2003; Carbonell-Barrachina *et al.*, 1998; Carbonell-Barrachina *et al.*, 1997; 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 and As levels reduced gradually from 44% to 34%, 33.1% and 33.7% in plants grown from collected seeds of contaminated lands Sardari compared to Sardari, Parsi and Pishtaz varieties of uncontaminated area (Table 4). Also, there were a reduction of arsenic accumulation in wheat seeds and were up to 87% to 92%. 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 most of the As accumulated in root and the smallest amount in the seed. 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).

Overall, Sardari variety collected from contaminated area showed a better tolerance to As for which several explanations may be possible (Table 4). 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. While, avoidance mechanisms involve the exclusion of the toxic ion or limitation in absorption of a toxic ion. 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 et al.*, 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.

CONCLUSIONS

This study monitored the correlation between the arsenic content in different parts of tested wheat varieties. 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. 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.

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