

TOXIC EFFECT OF ARSENATE ON GERMINATION, EARLY GROWTH AND BIOACCUMULATION IN WHEAT (*Triticum aestivum* L.)

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Damaging influence of arsenate(V) on germination and early growth of wheat (*Triticum aestivum* L.) was checked through pot bioassays. Four concentrations of As(V) i.e. 0.013, 0.025, 0.038 and 0.05 mg pot⁻¹ were used to spike sandy loam soil (sand: 76%, silt: 15%, clay: 9%). Different As(V) treatments in the soil resulted in significant reduction in germination rate (G%), germination index (GI) and relative germination rate (RGR), while increased in arsenic response index (ARI). Shoot growth and biomass were significantly declined by 40-90%, and that of roots were decreased by 50-99% in 2-week and 4-weeks old plants. Morphological symptoms like yellowing of leaf margins, stunt roots and stems were observed at higher dose (0.05 mg) of As(V) in 4-week seedling. The tolerance index (T_{index}) of seedling was decreased and metal accumulation was increased that revealed increase in the seedling sensitivity with increasing concentrations of As(V). Root accumulated more metal than the shoots. It was concluded that As(V) has potential to drastically reduce seed and seedling growth of wheat by accumulating in plant tissues, therefore it is required to assess uptake by plants growing in arsenic contaminated areas to address food security challenging in Pakistan.

Keywords: Heavy metal, arsenic, toxicity, cereal, wheat

INTRODUCTION

Arsenic (As) has been notorious as metaphor for poison, ranked amongst the group A of human carcinogens (WHO, 2006). Drinking water or food are essentially the key domains of As foyer to human population (Le *et al.*, 2000) and arsenic-loaded soil is focal source of As in both domains (Kim and Nriagu, 2000). Untreated industrial wastewater and unrestricted use of fertilizers, pesticides are some means of As in the soil (Eisler, 2004). Extensive utilization of naturally occurring As rich ground water in irrigation has resulted in accumulation of As in various domains of plant system through groundwater-plant-soil system ultimately transfer to food chain and has become serious matter of public health issue (Rahman *et al.*, 2008). Permissible limits of As in drinking and irrigation water are 0.01 mg L⁻¹, 0.1 mg L⁻¹, respectively and there is no safe level of As in food (FAO, 1985; FAO/WHO, 2002; WHO, 2008). Soil As is the major source uptake by the crops (Huang *et al.*, 2006). Previous literature showed a wide variation of As concentrations i.e., 0.007 to 7.50 mg kg⁻¹ in agricultural plants (Liao *et al.*, 2005; Dahal *et al.*, 2008; Bhattacharya *et al.*, 2009; Rahaman *et al.*, 2011). Studies on As(V) toxicity have shown that plant suffer considerable stress upon exposure, with symptoms ranging from inhibition of seed germination, root growth, shoot growth, plant height and sometimes leads to plant death (Fayiga *et al.*, 2007; Rahimi *et al.*, 2012; Bhattacharya *et al.*, 2012).

In Pakistan, As contaminated groundwater is extensively utilized both for irrigation and drinking nearly in all areas of

Punjab. Ground water of 32 districts of Punjab has been found to be contaminated with toxic level of As (PCSIR, 2000). It is most likely that As poisoning to the crop is related to soil-water concentration (Heikens, 2006). It was reported that areas located nearer to the river Indus system have relatively higher As concentration than those away from the river system. Three districts of Punjab i.e., Multan, Bahawalpur and Rahim Yar Khan, lying along the Indus River, were found to be highly contaminated with As (Ahmad *et al.*, 2004). Nevertheless, As contamination has also been recorded in cities lying near river Ravi i.e., Sheikhupura, Kasur, Sargodha and Jhang (PCSIR, 2000). So far, Javied *et al.* (2009) in their findings has declared the catastrophic situation in irrigation water, cultivated and uncultivated areas of the Multan due to As containing untreated waste of Pak-Arab factory. Abbas *et al.* (2010) detected the accumulation of some heavy metals including As in different vegetables grown in the Sindh province as well. Thus, widespread use of irrigation water contaminated with As, eventually leads to issues of food safety, food security and degradation of the environment (FAO, 2007).

The present study, therefore, was conducted in pots to assess the toxic influence of different concentrations of As(V) on seed germination, seedling growth and metal accumulation in *T. aestivum*.

MATERIALS AND METHODS

Healthy seeds of wheat variety (Sehar-2006) were surface sterilized with 3% H₂O₂ solution for 2-3 minutes and then

rinsed 3-4 times with sterilized distilled water. For pot trials, garden sub soil was collected from Botanical Garden of the University of the Punjab, Lahore, Pakistan and was analyzed by International Soil Reference and Information Centre (2002) for texture, organic matter (OM), pH, available phosphorus (P), and available potassium (K) before and after completion of the experiment.

Experiments were conducted in plastic pot of 6 cm x 10 cm filled with 350 g of pre-sterilized soil. Standard metal salt of *di*-Natriumhydrogenarsenate ($\text{Na}_3\text{AsO}_4 \cdot 7\text{H}_2\text{O}$) (MERCK, Germany) were used to make different concentrations of As(V). Metal-laden homogenized soil was prepared by mixing of 50 mL of metal solution @ 0.013, 0.025, 0.038 and 0.05 mg pot⁻¹ followed by soil aging (7 days), air drying and finally sieving (2 mm mesh size). Current selection of particular range of As(V) was based on reported level of that metal in soil and ground water of Pakistan (PCRWR, 2003). Metal spiking was followed by sowing of 10 surface sterilized seeds per pot. Each treatment was replicated thrice and a control set of treatment received distilled water only. Pots were arranged in a completely randomized design at an average temperature of 25±5°C and 12 hours photoperiod. Germinated seeds were studied after 7 days of sowing when both the plumule and radicle were appeared from the seeds. Germination rate (GR), germination index (GI), relative germination rate (RGR) and relative arsenic-injury rate (RAIR) were calculated for each treatment in pots by the following formulas:

$$\text{Germination rate} = \frac{\text{Total no. of germinated seeds}}{\text{Total no. of seed taken for germination}} \times 100$$

$$\text{Germination Index} = \frac{\text{Total no. of germinated seed}}{\text{Total no. of germinated days required}} \times 100$$

$$\text{Relative germination rate} = \frac{\text{Germination \% in treatment}}{\text{Germination \% in control}} \times 100$$

$$\text{Relative Injury Rate} = \frac{\text{Germination \% in control} - \text{Germination \% in treatment}}{\text{Germination \% in control}} \times 100$$

After germination, the seedlings were thinned out and six uniform seedlings were retained per pot. Two harvests (first after 15 and second after 30 days of sowing) were taken and data concerning length, fresh and dry biomass of shoot and root were recorded.

For determination of As concentration, dried powered of roots and shoots from each treatment was taken after 30 days of sowing and was digested with HNO_3 for 20 minutes. Each digested sample was analyzed on Atomic absorption spectrophotometer (Z-5000 Polarized Zemean) for metal accumulation (concentration x biomass) in plant tissues.

Tolerance of plant to As(V) was measured by assessing tolerance index (T_{index}). T_{index} was calculated by taking important parameters like length and dry weight of root and shoots against four different concentrations of metal at each of two growth stages by following formula (Mahmood *et al.*, 2007):

$$G_i = (G_x / G_{\text{max}}) \quad ; \quad T_{\text{index}} = \sum (G_x / G_{\text{max}}) n^{-1}$$

Where, G_i = normalized growth parameter; G_x = individual growth parameter; G_{max} = maximum value; T_{index} = Tolerance index.

Data regarding the assessment of toxic influence of As(V) on seed and seedling growth and metal accumulation in *T. aestivum* were analyzed through analysis of variance technique and means were compared by Duncan's Multiple Range Test (Steel and Torrie, 1997).

RESULTS AND DISCUSSION

The physico-chemical properties of soil before and after As(V) treatments are depicted in Table 1. Soil was sandy loam soil (sand: 76%, silt: 15%, clay: 9%). Untreated sandy loam soil (control) having organic matter 0.75%, pH 7.0, available phosphorus 6.0 mg kg⁻¹ and available potassium 87 mg kg⁻¹. Currently, estimation of soil pedological factors showed considerable difference in pH and organic matter of metal-laden and untreated soils. Increase in soil pH with decrease in organic matter due to rising As(V) doses could be owing to more solubility of As(V) and its sorption to organic matter (Moreno-Jiménez *et al.*, 2013). Many scientists have documented considerable influence of soil pH on As species, while presence of organic matter probably facilitate metal binding, distribution and retention to different soil fractions (Adriano, 2001; Wang and Mulligan, 2006; Fayiga *et al.*, 2007). Furthermore, it has been documented that As(V) find its way through roots via phosphate uptake system. Generally, common plasma membrane carriers are involved for the uptake of both arsenate and phosphate ions but phosphate ions hold greater affinity for carriers than the arsenate. However, the soil that was examined in present study showed no difference in phosphorus concentration at different doses of metal, it could seem that soil P did not significantly interfere with plant As uptake at any of these sites. Similar to results obtained in present study, Zandsalimi *et al.* (2011) also found no influence of phosphorus while exploring uptake of As by different plant species.

Table 1. Physico-chemical analysis of arsenate treated and untreated sandy loam soil (sand: 76%, silt: 15%, clay: 9%)

Treatments	Soil pedological factors			
	pH	OM (%)	K (mg kg ⁻¹)	P (mg kg ⁻¹)
Control	7.0	0.75	87	6.00
0.013 mg pot ⁻¹	7.3	0.74	86	6.00
0.025 mg pot ⁻¹	7.8	0.72	86	5.92
0.038 mg pot ⁻¹	8.0	0.72	85	5.95
0.05 mg pot ⁻¹	8.2	0.69	86	5.90

OM: organic matter; P: available phosphorus; K: available potassium

Values of GR, GI and RGR were significantly declined by 50-75%, while that of RAIR significantly increased up to 0.038 mg treatment dose, and while response to higher dose (0.05 mg) was not significantly differ from 0.038 mg (Table 2). Direct exposure of the radical to metal toxicity negatively influence on germination and early seedling growth and this negative effect became pronounced significantly with increasing concentrations of As(V); which could cause reduction of the plant growth and plant biomass (Bhattacharya *et al.*, 2012).

Root growth and biomass was more severely affected than that of shoot. At both harvests the root length, root fresh and dry biomass was significantly suppressed by 70-91%, 24-90% and 49-99%, respectively due to amendment of four different doses of As(V) in soil over control treatments. The highest concentration of 0.05 mg pot⁻¹ caused an abnormal germination (e.g. testa burst by radical but no growth of root), along with yellowing of leaf margins, stunt roots and stems. Increasing As(V) amount induced similar drastic effect on shoot growth parameters as was observed on root growth assays. There was 50-84%, 33-83% and 60-94% decline in shoot length, fresh weight and dry weight, respectively due to variously applied As(V) treatments in the soil (Table 3 and 4). Fall of both coleoptile and hypocotyl length is a distinctive response to toxic metals (Abedin and

Meharg, 2002). Reduced root length growth in response to As exposure has been reported by a number of investigators (Liu and Zhang, 2007; Zhang *et al.*, 2009; Talukdar, 2011). Reduction of root length growth with increase in As(V) concentration could be due to the fact that plant roots were the first point of contact for these toxic As species in the growth medium (Bhattacharya *et al.*, 2012). Shoot growth reduction could possibly be due to the decrease in meristematic cells present in this region and some enzymes in the cotyledon and endosperm cells. The enzymes in cotyledon and endosperm cells may become active and begin to digest store food while converts to soluble form. So when enzymatic activities were affected, the food could not reach to radical and plumule and in this way shoot growth and biomass were affected (Kabir *et al.*, 2008).

Metal concentration in both shoot and root increased significantly with rising As(V) level in soil, and root accumulated more metal than the shoots after 30 days of growth (Table 5). These results are in close conformity with those observed in previous literature that among the plant parts, As concentration was higher in roots, intermediate level in stem, leaves and lowest level in edible parts (Abedin *et al.*, 2002). In roots, As(V) likely to reduce to As(III) followed by complexation with thiol and possibly sequestration in the root vacuoles that could consequences in

Table 2. Effect of arsenate treatments on percentage germination, germination index relative germination rate and injury rate in wheat.

As(V) treatments (mg pot ⁻¹)	Germination rate (%)	Germination Index (%)	Relative germination rate (%)	Relative arsenic- injury rate (%)
0.013	50.0±1.41a	50.0±0.03a	50.1±0.03a	50.0±0.03c
0.025	37.5±1.41b	35.0±0.03b	37.5±0.03b	62.5±0.01b
0.038	25.0±2.12c	25.2±0.01c	25.0±0.01c	75.0±0.01a
0.050	25.0±0.71c	25.2±0.01c	25.0±0.01c	75.0±0.01a

Data are the mean values of n=3. In a column values with the different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

Table 3. Root and shoot growth of wheat as influenced by arsenate levels after 15 days of sowing.

Parameters	Treatments				
	Control	0.013 (mg pot ⁻¹)	0.025 (mg pot ⁻¹)	0.038 (mg pot ⁻¹)	0.05 (mg pot ⁻¹)
Shoot length (cm)	21.3±1a (0%)	10.5±0.71b (51%)	9.3±0.35c (56%)	5.2±0.35d (76%)	3.4±0.28e (84%)
Shoot fresh weight (g)	0.96±0.04a (0%)	0.62±0.02b (35%)	0.4±0.03c (58%)	0.23±0.03d (76%)	0.17±0.02e (82%)
Shoot dry weight (g)	0.14±0.04a (0%)	0.04±0.03b (71%)	0.036±0.02bc (74%)	0.02±0.02c (86%)	0.008±0.02d (92%)
Root length (cm)	44.2±1a (0%)	13.5±0.35b (69%)	8.8±0.28c (80%)	5.8±1.06d (87%)	3.8±0.28e (91%)
Root fresh weight (g)	0.21±0a (0%)	0.18±0.01b (14%)	0.15±0.01c (29%)	0.12±0.01d (43%)	0.05±0.04e (76%)
Root dry weight (g)	0.1±0a (0%)	0.027±0.02b (73%)	0.009±0.01c (91%)	0.003±0.02d (97%)	0.0001±0.01e (99.9%)

Table 4. Root and shoot growth of wheat as influenced by arsenate levels after 30 days of sowing.

Parameters	Treatments				
	Control	0.013 (mg pot ⁻¹)	0.025 (mg pot ⁻¹)	0.038 (mg pot ⁻¹)	0.05 (mg pot ⁻¹)
Shoot length (cm)	95±19a (0%)	78.6±2.25b (17%)	65±0.27c (32%)	50.7±1.8d (46%)	37.7±4.58e (60%)
Shoot fresh weight (g)	1.32±0a (0%)	0.97±0.05b (27%)	0.89±0.09bc (33%)	0.7±0.05c (47%)	0.46±0.04d (65%)
Shoot dry weight (g)	0.203±a (0%)	0.13±0.01b (35%)	0.1±0.01bc (50%)	0.07±0d (65%)	0.016±0.01e (92%)
Root length (cm)	187±2a (0%)	132±1.53b (29%)	89.5±2.65c (52%)	67±1.15d (64%)	30±2.52e (83%)
Root fresh weight (g)	0.87±0a (0%)	0.581±0.15b (33%)	0.54±0.05c (40%)	0.26±0.04d (70%)	0.1±0e (92%)
Root dry weight (g)	0.13±0a (0%)	0.096±0.01b (26%)	0.03±0.01c (77%)	0.0007±0d (92%)	0.0007±0e (92%)

Data are the mean values of n=3. In a row values with the different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test. Values in 'parentheses' indicate reduction rate in plant growth parameters with respect to control.

less uptake by the shoot (Zhao *et al.*, 2009). Comparatively fewer uptakes in shoot may be part of a defense strategy to avoid serious shoot damage (Hakmaoui *et al.*, 2007).

Table 5. Arsenic accumulation in plant parts (g g⁻¹ dry weight).

Samples	Treatments (mg pot ⁻¹)	Dry weight (g)	Arsenic accumulation (g g ⁻¹)
Shoot	0.000	0.140	0.0±0.00e
	0.013	0.040	0.2±0.01d
	0.025	0.036	0.3±0.02c
	0.038	0.020	0.4±0.02b
	0.050	0.008	0.4±0.04a
Root	0.000	0.100	0.0±0.00d
	0.013	0.027	1.0±0.05c
	0.025	0.009	1.4±0.06b
	0.038	0.003	4.0±0.08a
	0.050	0.0001	4.0±0.08a

In a column values with the different letters show significant difference ($P \leq 0.05$) on arsenic accumulation by shoot and root separately as determined by Duncan's Multiple Range Test.

Tolerance index is important tool to characterize tolerance of plant against stress (Orroño and Lavado, 2009). T_{index} calculated in current study was ranged from ≥ 0 to ≤ 1 showing extreme susceptibility of *T. aestivum* seedling to As(V). The tolerance index of *T. aestivum* seedling was inversely related to different As(V) treatment added to soil. The effect of different treatments (0.013, 0.025, 0.038 and 0.05 mg pot⁻¹) of As(V) on *T. aestivum* was more adverse on 15-days old plant (T_{index} : 0.37, 0.28, 0.13 and 0.11) as compared to 30-days old plant (T_{index} : 0.68, 0.45, 0.31 and 0.17), respectively (Table 6). The reduction in T_{index} was

well-linked with declined shoot and root growth and biomass that might have been consequences of irregularity in normal physiological mechanisms of wheat seedling with increasing metal doses (Khan *et al.*, 2006). However, 4-week seedling exhibited less sensitivity than 2-week ones probably avoidance and tolerance to metal ions in symplasm by complexation (Shi and Cai, 2009).

Table 6. Metal tolerance index of wheat
($T_{index} = \Sigma(G_x G_{max}^{-1}) n^{-1}$)

As(V) treatments (mg pot ⁻¹)	Metal tolerance index	
	1 st harvest	2 nd harvest
0.013	0.37	0.68
0.025	0.28	0.45
0.038	0.13	0.31
0.050	0.11	0.17

Conclusions: Increasing concentrations of As(V) imparted drastic influence on the germination and seedling growth of wheat, which indicated sensitivity of early development stages of wheat to arsenate. The findings specify an immediate danger by wheat crop growing in arsenic contaminated areas in Pakistan and direct attention to the wheat growers for periodically monitoring of crop for risk assessment and to avoid its entry in food chain.

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REFERENCES

- Abbas, M., Z. Parveen, M. Iqbal, Riazuddin, S. Iqbal, M. Ahmed and R. Bhutto. 2010. Monitoring of toxic metals

- (cadmium, lead, arsenic and mercury) in vegetables of Sindh. Kathmandu Univ. J. Sci. Engin. & Technol. 6: 60-65.
- Abedin, M.D., M.S. Cresser, A.A. Meharg, J. Feldmann and J. Cotter-Howells. 2002. Arsenic accumulation and metabolism in rice (*Oryza sativa* L.). Environ. Sci. Technol. 36: 962-968
- Adriano, D.C. 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability and risks of metals, 2nd Ed. Springer, New York.
- Ahmad, T., M.A. Kahlowan, A. Tahir and H. Rashid. 2004. Arsenic an emerging issue: Experiences from Pakistan. Proc. People-centered approaches to water and environmental sanitation. 30th WEDC International Conference, 25-29 Oct. 2004, Vientiane, Laos. pp.459-466.
- Bhattacharya, P., A.C. Sama, J. Majumdar and S.C. Santra. 2009. Transfer of arsenic from groundwater and paddy soil to rice plant (*Oryza sativa* L.): A micro level study in West Bengal, India. World J. Agric. Sci. 5: 425-431.
- Bhattacharya, S., N. De Sarkar, P. Banerjee, S. Banerjee, S. Mukherjee, D. Chattopadhyay and A. Mukhopadhyay. 2012. Effects of arsenic toxicity on germination, seedling growth and peroxidase activity in *Cicer arietinum*. Int. J. Agric. Food Sci. 2: 131-137
- Dahal, B.M., M. Fuerhacker, A. Mentler, K.B. Karki, R.R. Shrestha and W.E.H. Blum. 2008. Arsenic contamination of soils and agricultural plants through irrigation water in Nepal. Environ. Pollut. 155: 157-163.
- Eisler, R. 2004. Arsenic hazards to humans, plants, and animals from gold mining. Rev. Environ. Contam. Toxicol. 180: 133-165.
- FAO. 2007. Remediation of arsenic for agriculture sustainability, food security and health in Bangladesh.
- FAO/WHO. 2002. Joint expert committee on food additives (JECFA). Limit test for heavy metals in food additive specifications Explanatory note.
- Fayiga, A.O., L.Q. Ma and Q. Zhou. 2007. Effects of plant arsenic uptake and heavy metals on arsenic distribution in an arsenic-contaminated soil. Environ. Pollut. 147: 737-742.
- FAO. 1985. Water quality guidelines for maximum crop production. Food and Agricultural Organization/UN. Available online with updates at www.fao.org/docrep/T0551E.2006/9/1.
- Hakmaouia, A., M. Atera, K. Boka and M. Baronc. 2007. Copper and cadmium tolerance, uptake and effect on chloroplast ultrastructure. Studies on *Salix purpurea* and *Phragmites australis*. Z. Naturforsch. Sec. (C) J. Biosci. 62: 417-426.
- Heikens, A. 2006. Report on arsenic contamination of irrigation water, soil and crops in Bangladesh: Risk implications for sustainable agriculture and food safety in Asia. Food & Agric. Organization (FAO) 20: 1-46.
- Huang, R., S. Gao, W. Wang, S. Staunton and G. Wang. 2006. Soil arsenic availability and the transfer of soil arsenic to crops in suburban areas in Fujian Province, southeast China. Sci. Tot. Environ. 368: 531-51.
- International Soil Reference and Information Centre. 2002. Procedures for soil analysis. In: L.P. Van Fleeuwijk (ed.), International Soil Reference and Information Centre (ISRIC), 6th Ed. Food and Agricultural Organization of the United Nation, The Netherlands.
- Javied, S., S. Waheed, N. Siddique, M.M. Chaudhry, N. Irfan and M. Tufail. 2009. Arsenic pollution from phosphogypsum produced at Multan, Pakistan. The Nucleus 46: 219-224.
- Kabir, M., Z.M Iqbal, M. Shafiq and Z.R. Farooqi. 2008. Reduction in germination and seedling growth of *Thespesia populnea* caused by lead and cadmium treatments. Pak. J. Bot. 40: 2419-2426.
- Khan, N.A., I. Ahmad, S. Singh and R. Nazar. 2006. Variation in growth, photosynthesis and yield of five wheat cultivars exposed to cadmium stress. World J. Agric. Sci. 2: 223-226.
- Kim, M.J. and J. Nariagu. 2000. Oxidation of arsenite in ground water using ozone and oxygen. Sci. Tot. Environ. 247: 71-79.
- Le, X.C., S. Yalcin and M. Ma. 2000. Speciation of sub microgram per liter levels of arsenic in water: on-site species separation integrated with sample collection. Environ. Sci. Technol. 34: 2342-2347.
- Liao, X.Y., T.B. Chen, H. Xie and Y.R. Liu. 2005. Soil As contamination and its risk assessment in areas near the industrial districts of Chen zhou city, southern China. Environ. Int. 31: 791-798.
- Liu, X. and S. Zhang. 2007. Intraspecific differences in effects of contamination of cadmium and arsenate on early seedling growth and metal uptake by wheat. J. Environ. Sci. 19: 1221-1227.
- Mahmood, T., K.R. Islam and S. Muhammad. 2007. Toxic effects of heavy metals on early growth and tolerance of cereal crops. Pak. J. Bot. 39: 51-462.
- Moreno-Jiménez, E., R. Clemente, A. Mestrot and A.A. Meharg. 2013. Arsenic and selenium mobilization from organic matter treated mine spoil with and without inorganic fertilization. Environ. Pollut. 173: 238-244
- Orrono, D.I. and R.S. Lavado. 2009. Heavy metal accumulation in *Pelargonium hortorum*: Effects on growth and development. Int. J. Exp. Bot. 78: 75-82.
- Pakistan Council of Research in Water Resources (PCRWR). 2003. Arsenic contamination in ground water of Southern Punjab. PCRWR Ministry of Science and Technology, Government of Pakistan (Supported by UNICEF).
- Pakistan Council of Scientific and Industrial Research (PCSIR). 2000. A Report on ground water studies for

- arsenic contamination in Northern Punjab, Pakistan, Phases I & II.
- Rahaman, S., A.S. Chandra and D. Mukhopadhyay. 2011. Effect of water regimes and organic matters on transport of arsenic in summer rice (*Oryza sativa* L.). J. Environ. Sci. 23: 633-639.
- Rahimi, M., R. Farhadi, M.S. Balashahri, M.R. Abedi and K.A. Nasab. 2012. Effect of arsenic on medicinal plants. Int. J. Agron. Plant. Prod. 3: 755-758.
- Rahman, M.A., H. Hasegawa, M.M. Rahman, M.A.M. Miah and A. Tsmin. 2008. Arsenic accumulation in rice (*Oryza sativa* L.): human exposure to food chain. Ecotoxicol. Environ. Saf. 69: 317-324.
- Shi, G. and Q. Cai. 2009. Cadmium tolerance and accumulation in eight potential energy crops. Biotech. Adv. 27: 555-561.
- Steel, R.G.D. and J.H. Torrie and D. A. Dickey. 1997. Principles and procedures of statistics: a biometrical approach, 2nd Ed. McGraw-Hill Kogakusha, Tokyo.
- Talukdar, D. 2011. Effect of arsenic-induced toxicity on morphological traits of *Trigonella foenum-graecum* L. and *Lathyrus sativus* L. during germination and early seedling growth. J. Biol. Sci. 3: 116-123.
- Wang, S. and C.N. Mulligan. 2006. Effect of natural organic matter on arsenic release from soils and sediments into groundwater. 2006. Environ. Geochem. Hlth. 28:197–214
- World Health Organization (WHO). 2006. Guidelines for drinking-water quality, 3rd Ed. Vol. 1, Geneva.
- World Health Organization (WHO). 2008. Guidelines for drinking-water quality, 3rd Ed. incorporating 1st and 2nd addenda. Vol. 1. Recommendations. Geneva, World Health Organization. pp.306–308.
- Zandsalimi, S., N. Karimi and A. Kohandel. 2011. Arsenic in soil, vegetation and water of a contaminated region. Int. J. Environ. Sci. Technol. 8: 331-338.
- Zhao, F. J., J.F. Ma, A.A. Meharg and S.P. McGrath. 2009. Arsenic uptake and metabolism in plants. New Phytol. 181: 777–794.