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Phytoremediation of Arsenic by Macroalga: Implication in Natural Contaminated Water, Northeast Iran

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Abstract: This research has been accomplished in two separated phases. The aim of the first phase was to evaluate arsenic concentration in the surface water and plants in Chelpo area in different seasons. In addition, pH and DO in water samples were measured. The aim of this study at second phase was to investigate the efficiency of arsenic removal from surface water using macro-alga; *Chara vulgaris*. Four reactors were used in this study with the initial arsenic concentrations of 50, 100, 200 and 300 $\mu\text{g L}^{-1}$. Arsenic concentration in water and algae, pH of the media, initial and final weight of *Chara vulgaris* were measured during the experiment. High concentrations of arsenic were found in surface water of Chelpo area. The maximum contamination of arsenic (150 $\mu\text{g L}^{-1}$) was observed in winter. It was found that the arsenic concentration in algae was high due to high affinity of the algae for arsenic uptake. The results of the second phase showed that *Chara vulgaris* could significantly remove arsenic from polluted water. The arsenic content of alga increased approximately to about 62.7 mg kg^{-1} dry weight in 19 days of exposure in the reactor with initial concentration of 300 $\mu\text{g L}^{-1}$. The high arsenic accumulation ability of *Chara vulgaris* could reduce arsenic by averagely 66.25% in the contaminated water of the reactors.

Key words: Arsenic removal, macroalgae, northeast Iran, phytoremediation

INTRODUCTION

Arsenic is causing a global epidemic of poisoning and has been associated with skin lesions, cancers and other symptoms. Natural contamination of freshwater by arsenic has become a crucial water quality problem in many parts of the world such as China, India, Vietnam, Bangladesh, Italy, Iran, etc (Gabriella and Cini, 2004; Ghassemzadeh *et al.*, 2003; Chakraborti *et al.*, 2003; Rahman *et al.*, 2001; Chowdhury *et al.*, 1999). One of the main causes of this widespread arsenic poisoning is the growing current trend around the world of drinking water from polluted underground and surface water, naturally and anthropogenic sources. The range of arsenic concentrations found in natural waters is large, ranging from less than 0.5 to more than 5000 $\mu\text{g L}^{-1}$. Typical concentration in freshwater are less than 10 $\mu\text{g L}^{-1}$ and much higher concentrations are found in groundwater (Smedley and Kinniburgh, 2002).

Contaminated soils and waters with heavy metals pose a major environmental and human health problem, which may be partially solved by the phytoremediation technology. Phytoremediation uses plants to concentrate elements and compounds from the environment and

metabolize them in their tissues. Knowledge is scarce regarding arsenic phytoremediation in the environment (McCutcheon and Schnoor, 2003; Lasat, 2002; Raskin and Ensley, 2000).

This research has been accomplished in two separated phases. In the first phase the investigation involves the seasonal and spatial changes of As within algae and the naturally contaminated surface water of Chellpo river. The Chellpo area is part of the Koh-e-Sorkh mountain range, located north of Kasmar in Khorasan Province, northeastern Iran (Fig. 1). This area is composed of the variety of rock types that are aged in Cenozoic. Geomorphologically, the drainage systems are dendritic because of shale and marls. In this study the samples have been collected from small tributaries that flow toward the major Chellpo River and from the river itself (58°30' - 58°33' E and 35°35' - 35°36' N). The minimum and maximum temperature for the study area, in 20 years period, is -6-36°C with average annual precipitation of 225 mm, from February to May (Ghassemzadeh and Karimpour, 2002). Tributaries and Chelpo River waters are the only sources for drinking water, domestic and agricultural uses in this semiarid region, however some rural people uses these waters directly for domestic and drinking and not through

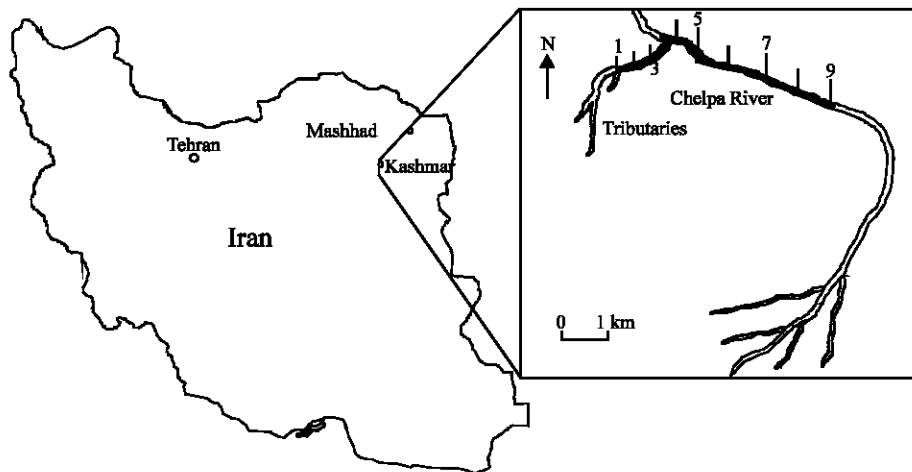


Fig. 1: Location of the study area (Chelpo, Northeast Iran)

the water supplies system. We have previously shown that soil and drinking water sources in this region have severe environmental problems regarding arsenic contamination (Ghassemzadeh *et al.*, 2006). The absence of any type of industrial activity and intensive agriculture on the area means that it can be considered as a pilot area for the investigation of natural concentrations of arsenic. It was hypothesized that arsenic pose contamination risks in the drinking water from natural sources in this region and the possible of health problems in the area (Ghassemzadeh *et al.*, 2003). Thus, in this study we looked at As levels in the water and algae. The aim of the second phase was to determine the capacity of arsenic accumulation by *Chara vulgaris* under the influence of arsenic in the water. The ultimate goal was to assess the possibility to use *C. vulgaris* for bioindication and phytoremediation of arsenic contamination in Chelpo water and similar polluted area.

MATERIALS AND METHODS

Water collections and analyses: Field investigations were carried out from March 2005 to June 2006. The sampling was usually carried out between 10:00 am - 14:00 pm at approximately three month intervals. A total nine sampling stations were selected from small tributaries and Chelpo River (Fig. 1). Air and water temperatures, pH and dissolved oxygen were measured at the field according to standard methods (APHA, 1995). Water samples from tributaries and river were obtained by immersing the mouth of an acid-washed polyethylene container approximately 20 cm below the surface. The samples were kept immediately on the ice and then transported to the laboratory, Ferdowsi University for the

determination of total arsenic using a Shimadzu model AA-670G with a GFA-4B graphite furnace atomizer (Arbab-Zavvar, 1982).

Algae collections and analyses: The algae were collected in four seasons manually and were sampled as near as to the water sampling location as possible. The algae samples were washed thoroughly at the field to remove as much debris and associated biota as possible and placed immediately in plastic bags and kept them on the ice in darkness, transported to the Ecology laboratory, University of Mashhad. In the laboratory, the samples washed thoroughly in deionized water and kept frozen in zip-lock bags for arsenic measurements. Before arsenic analyses, the samples were dried to a constant weight at 110°C and then 10 cc nitric acid was added to 1 g of dried samples, left them for 24 h. After that, the samples were heated until to evaporate the acid. Then, 10 cc nitric and 10cc sulfuric acids were added to the samples and kept them for 24 h. The samples again heated for 1 h (60-70°C) and were then filtered. The samples store until time of analysis by using the method of Arbab-Zavvar (1982).

Experimental section: In this experiment, arsenic phytoremediation by macroalga, *Chara vulgaris*, were tested. Because of, this macroalga absorbed high arsenic (according to the arsenic analysis on the field samples) and also they adapted to the environment of study area. To perform this experiment, 5 glass reactors were used. For complete simulation of the environment in the experimental reactors, *Chara vulgaris*, water and sediment were transferred from an unpolluted area in the upstream (similar region with no arsenic contamination) to the laboratory. At the beginning of the experiment, 60 g of the

plant (the wet weight) and 14 L of water were added to each reactor under the natural light conditions. After seven days (for algae adaptation to new environment and any changing due to transportation), arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) was added in different concentrations, 50, 100, 200, 300 $\mu\text{g L}^{-1}$ to the reactors 1, 2, 3 and 4 respectively, (R 1, R 2, R 3, R 4). Arsenate selected because it is the dominant arsenic species in the water phase under oxidation condition as it was reported before (Ghassemzadeh and Karimpour, 2002). The fifth reactor was chosen as a control and no arsenic was added. The reactors were aerated continuously, stimulating field condition. Furthermore, to compensate for water evaporation, some distilled water was added to the reactors daily and also the pH was measured. The experiments went on for 19 days and the concentration of arsenic in the water and plant were measured on the twelfth and nineteenth days (three replicates).

RESULTS AND DISCUSSION

Arsenic in water and algae from the Chelpo river:

Table 1 summarizes the Arsenic concentrations in water from the Chelpo river sampling sites. All sites were well oxygenated at the time of sampling as revealed by the high dissolved oxygen concentration and high percent saturation of dissolved oxygen. The As (V) is most abundant form in the study area (Ghassemzadeh and Karimpour, 2002) and which is derived from fault zone where the Paleogene deposits have been cut by faults and the ore deposits formed along these zones. Therefore as has formed in this area and is visible in alteration zones along the outcrop belt. The original

rocks are mainly fine grained siliciclastics that have been deposited in a relatively closed basin in central Iran within an intracontinental basin (Mazlomi, 1992).

Arsenic levels ranged 0 to 150 $\mu\text{g L}^{-1}$ in the water. The total arsenic in the water was highest in the winter due to high rainfall in the area and washing out arsenic from natural sources. Andreae (1978) reported levels 0.001 to 0.043 $\mu\text{g L}^{-1}$ in lakes and rivers. Murdoch and Capobianco (1979) reported arsenic levels of 0.006-0.250 $\mu\text{g mL}^{-1}$ in lakes located in an area that had been a center for mining activity. The arsenic level in Coeur d'Alene River, Idaho (a mining industry area) ranged from 0.11-1.64 $\mu\text{g L}^{-1}$ (Mok and Wai, 1990). Most studies regarding to arsenic contamination have focused on anthropogenic sources and industrial contamination in particular. Less study has been done on the contaminated water only with the natural sources. Present study area is an example of long-term arsenic contamination occurring naturally in a non-industrial area.

Arsenic levels ranged 13.3-660.9 mg kg^{-1} as dry biomass Table 2. Absorption of arsenic by algae showed the possibility of arsenic accumulation by macroalgae, *Chara vulgaris*. Previous studies have implicated that aquatic plants can play a key role in remobilizing of arsenic (Reay, 1972; Lee *et al.*, 1991; Knauer and Hemond, 2000; Robinson *et al.*, 2003; Meharg and Macnair, 1991; Pickering *et al.*, 2000). Wagemann *et al.* (1978) reported As levels in water from Keg Lake, 700-1500 $\mu\text{g mL}^{-1}$ and in aquatic macrophyte in excess of 3000 mg kg^{-1} . Lee *et al.* (1991) studied the concentration of arsenic in ten varieties of aquatic plants. They showed that the concentrations of arsenic in these plants partially reflected the concentration of arsenic in pool water, which ranged from 0.002-0.251 $\mu\text{g mL}^{-1}$. The levels of As in

Table 1: Arsenic concentration (μgR^{-1}), pH and dissolved oxygen (mgL^{-1}) in the samples (Chelpo river)

Station	Fall			Winter			Spring			Summer		
	As (μgR^{-1})	pH	DO (mgL^{-1})	As (μgR^{-1})	pH	DO (mgL^{-1})	As (μgR^{-1})	pH	DO (mgL^{-1})	As ($\mu\text{g L}^{-1}$)	pH	Do (mgL^{-1})
1	28.8±0.4	8.43	6.78	69.6±0.4	7.76	11.00	37.9±0.4	8.35	6.91	-	-	-
2	21.9±0.4	8.75	8.70	141.2±0.4	8.25	10.90	15.8±0.4	8.11	7.95	-	-	-
3	21.3±0.4	8.50	7.90	150.0±0.4	8.28	10.90	44.1±0.4	8.73	7.44	1.2±0.4	8.10	9.18
4	9.4±0.4	8.66	7.43	9.8±0.4	7.67	10.90	0.0	8.70	7.43	5.6±0.4	7.92	9.33
5	11.9±0.4	8.80	7.70	22.5±0.4	7.57	10.20	0.6±0.4	8.74	7.48	4.8±0.4	8.20	8.31
6	16.9±0.4	7.88	4.50	13.7±0.4	8.17	10.40	2.8±0.4	8.64	7.47	10.0±0.4	8.47	9.44
7	6.3±0.4	7.96	5.70	35.3±0.4	8.12	10.90	6.2±0.4	8.72	7.14	9.6±0.4	8.20	8.99
8	10.0±0.4	8.71	8.12	26.5±0.4	7.96	11.25	6.2±0.4	8.91	7.42	13.7±0.4	8.02	8.91
9	14.4±0.4	8.11	7.50	7.8±0.4	8.21	11.90	9.0±0.4	8.52	6.64	10.0±0.4	7.94	8.24

- : No data

Table 2: Arsenic concentration in the algae samples from the Chelpo river (mg kg^{-1} dry biomass)

Station	1	2	3	4	5	6	7	8	9
Winter	-	660.9±0.4	212.5±0.4	28.9±0.4	19.5±0.4	109.4±0.4	35.2±0.4	74.2±0.4	57.8±0.4
Spring	112.6±0.4	95.9±0.4	39.9±0.4	59.2±0.4	41.4±0.4	36.7±0.4	41.4±0.4	25.3±0.4	41.5±0.4
Summer	-	-	52.2±0.4	41.8±0.4	30.5±0.4	13.3±0.4	70.3±0.4	15.7±0.4	90.8±0.4

- : No data

Chara vulgaris in this study was, $440.8 \pm 2.2 \mu\text{g g}^{-1}$ dry weight where the concentration of water was $0.251 \mu\text{g mL}^{-1}$. They also studied the uptake of arsenic by *Hydrilla verticillata* Casp. in the laboratory and indicated that the uptake of arsenic was a function of initial arsenic concentration in the water. They showed that the aquatic plants are more tolerant to arsenic than terrestrial plants. Knauer and Hemond (2000) demonstrated the importance of green alga, *Chlorella* sp. in arsenic reduction in aquatic freshwater environment where polluted with large quantities of heavy metals. They showed arsenic (arsenate) to enhance growth conditions and highest cellular content of arsenic with the highest reduction on arsenic in the culture, arsenic detoxification. However, growth rate, final cell yield and cellular arsenic content were all enhanced by higher arsenate concentrations in the cultures grown at a low concentration of phosphate. They also found the similar results from the field data. In present study area, the ponds also were oligotrophic with phosphate concentrations less than 0.01 mg L^{-1} (Babae, 2006). Freshwater plants have evolved mechanisms specially, when growing in water naturally high in arsenic, for rapid detoxication of arsenic species (Lee *et al.*, 1991; Knauer and Hemond, 2000). The bioaccumulation of arsenic was investigated in aquatic plant, *Lemna gibba* L. by Mkandavire and Dude (2005) under deficient and sufficient phosphate. They stated that bioaccumulation of arsenic was significantly higher for p-deficient (0.0136 mg L^{-1}) than sufficient cultures where the uptake may increase as a result of ion competition with phosphate. Under the field condition in our study the environment was oxygen rich that most of arsenic is probably arsenate which compete with phosphate under the low phosphate condition.

Arsenic accumulation by *Chara vulgaris*: The arsenate uptake by the plants (Fig. 2) was significantly influenced by the initial concentration of arsenate in the media and the time of harvest ($p < 0.05$). The total arsenic accumulation of all plants exposed to different concentrations of arsenic at 12 days was significantly lower than at the 19 days exposure ($p < 0.05$). This finding corresponds also to that of Meharg and Macnair (1991) who reported that the rate of arsenic uptake correlated with the time of arsenic exposure.

The ability of macroalgae *Chara vulgaris* to take up large amounts of arsenic from substrates containing relatively low concentrations of this element indicate the plant may have potential for phytoremediation by extracting arsenic from contaminated water. In present experiment, the arsenic taken up by the *Chara vulgaris* reduce the arsenic concentration in the solution of reactor 1 by 75% at the end of experiment. The percent of arsenic removal in all reactors are shown in Fig 3. Before any conclusion can be drawn in this area, however, an

investigation is needed of the plants biomass production and growth in aquatic environment. Plant growth rate is a crucial factor for showing tolerance of the test plant to exposure to arsenic. The results in Table 3 shows that all

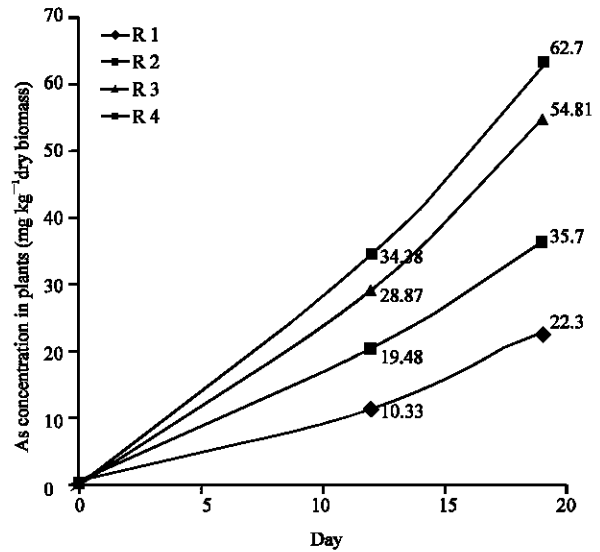


Fig. 2: The concentration of arsenic in the plants in the experiments.

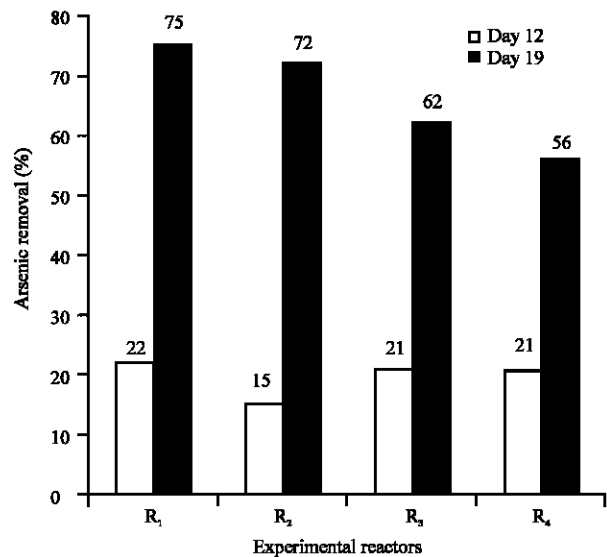


Fig. 3: The percentage of the arsenic concentration decreases in different reactors during the experiment

Table 3: The changes of *Chara vulgaris* weight during the experiment

Reactor	Plant weight (at first)	Plant weight (at the end)	Percent of weight increase
Control	60	73.20±0.68	22.0
R ₁	60	71.38±0.47	19.0
R ₂	60	66.84±0.63	11.4
R ₃	60	63.43±0.33	5.7
R ₄	60	62.81±0.64	4.7

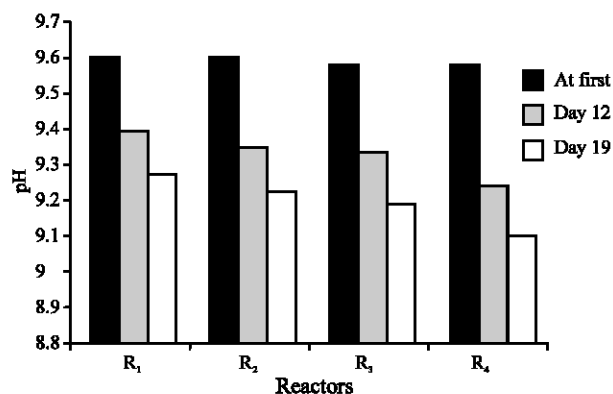


Fig. 4: pH changes in reactors

of the plants could survive well in the solutions and the fresh weight of the plants increased as the exposure period increased. Of course the increase is much higher in reactors with lower initial concentrations of arsenic. As the element concentration increases in the water, a sudden increase in the plant element concentration occurs. When this phenomenon occurs, the plants show toxicity and biomass production is reduced.

A change in pH of the medium was observed in this experiment Fig. 4. The pH of each reactor showed some fluctuation with a trend for slight decreases. A pH change may affect the bioavailability of arsenic. Mok and Wai (1990) reported that an acid pH (pH<4) would enhance the mobility of arsenic in the water phase. Decreasing pH levels are thus indirectly correlated with arsenic availability for plant uptake. Other investigators have reported that there was a better correlation between plant growth and total arsenic (Woolson, 1971; Walsh, 1975). Meharg and Macnair (1990) observed a decrease in arsenate uptake with an increase of pH value. Arsenate uptake was optimal at pH 5 where $H_2AsO_4^-$ was the dominant anion in solution. As the pH increased to pH 8 in which $HAsO_4^{2-}$ was the dominant form, arsenate uptake decreased.

CONCLUSIONS

The data presented in this study represent the first evaluation of natural levels of As in natural waters from Koh-Sorkh (Chelpo area), Kashmar, northeast Iran. Mean concentrations of As from 34 samples exceeded WHO 2003 limits ($10 \mu g L^{-1}$) for drinking water. The most affected areas correspond to the ore contains As. The results also reveal that arsenic accumulates considerably in algae samples from the Chelpo river. This indirectly proves that arsenic contamination exists in this site. In the experiment, the high arsenic accumulation ability of *Chara*

vulgaris to reduce arsenic on average by 66.25% in the solution shows that the *Chara vulgaris* has potential for arsenic phytoextraction from contaminated waters. Arsenic phytoremediation by *Chara vulgaris* can be used in the study area and similar polluted area.

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REFERENCES

- Andreae, M.O., 1978. Distribution and speciation of arsenic in natural waters and some marine algae. *Deep-Sea Res.*, 25: 391-402.
- APHA, American Public Health association, American Water Works Association and Water Environment Federation, 1995. *Standard Methods for the Examination of water and wastewater*. 19th Edn., American Public Health Association, Washington, DC., pp: 1260.
- Arbab Zavar, M.H., 1982. Arsenic in Environment. Ph.D Thesis, University of Southampton, England.
- Babae, Y., 2006. Determination of Arsenic in natural waters from Kashmar-Koohsorkh province and investigation the probability of its removal by regional plants. M.Sc Thesis. Amirkabir University of Technology, Tehran, Iran, pp: 116.
- Chakraborti, D., A. Hussam and M. Alauddin, 2003. Arsenic: Environmental and health aspects with special reference to groundwater in South Asia. *J. Environ. Sci. Health Part A, Toxic Hazad Substance Environ. Eng.*, 38: xi-v.
- Chowdhury, T.R., G.K. Basu, B.K. Manda, G. Samanta and U.K. Chowdhury *et al.*, 1999. Arsenic poisoning in Ganges Delta. *Nature*, 401: 545-546.
- Gabriella, T. and R. Cini, 2004. Heavy metals in drinking waters from Mount Amiata (Tuscany, Italy). Possible risks from arsenic for public health in the province of Siena. *Sci. Total Environ.*, 327: 41-51.
- Ghassemzadeh, F. and M.H. Karimpour, 2002. *Environ. Studies of Arsenic in Chelpo, Kashmar, Khorasan, Province*. Research Report for Ferdowsi University of Mashhad, Mashhad, Iran, pp: 120.
- Ghassemzadeh, F., M.H. Arbab Zavar and M.H. Karimpour, 2003. Environmental studies of arsenic in the Chelpo area, northeast, Iran. The Ecological Society of America 88th Annual Meeting, 3-8 August, Savannah, Georgia, USA., pp: 121.

- Ghassemzadeh, F., M.H. Arbab-Zavar and M. Geoffrey, 2006. Arsenic and antimony in drinking water in khohsorkh area, Northeast Iran possible risks for the public health. *J. Applied Sci.*, 6: 2705-2714.
- Knauer, K. and H. Hemond, 2000. Accumulation and reduction of arsenate by the freshwater green alga *Chlorella* sp. (Chlorophyta). *J. Phycol.*, 36: 506-509.
- Lasat, M.M., 2002. Phytoextraction of toxic metals: A Review of biological Mechanisms. *Environ. Qual.*, 31: 109-120.
- Lee, C.K., K.S. Low and N.S. Hew, 1991. Accumulation of arsenic by aquatic plants. *Sci. Total Environ.*, 103: 215-227.
- Mazlomi, A.R., 1992. Study of geology and geochemistry of gold deposits of Ali-Abad area and alteration zones in Koh-Sork, north of Kashmar. M.Sc Thesis, Faculty of Geoscience, Shahid Beheshti University., Tehran, Iran, pp: 123.
- McCutcheon, S.C. and J.L. Schnoor, 2003. Phytoremediation Transformation and Control of Contaminants. Wiley Intersci and New York, pp: 987.
- Meharg, A.A. and M.R. Macnair, 1991. Uptake accumulation and tranlocation of arsenate in arsenate-tolerant *Holcus lanatus* L. *New Phytol.*, 117: 225-231.
- Meharg, A.A. and M.R. Macnair, 1990. An altered phosphate uptake system in arsenate-tolerant *Holcus lanatus* L. *New Phytol.*, 116: 29-35.
- Mkandavire, M. and E.G. Dude, 2005. Accumulation of arsenic in *Lemna gibba* L. (duckweed) in tailing waters of two abandoned uranium mining sites in Saxony, Germany. *Sci. Total Environ.*, 336: 81-89.
- Mok, W.M. and C.M. Wai, 1990. Distribution and mobilization of arsenic and antimony species in the Coeur d'Alene River, Idaho. *Environ. Sci. Technol.*, 24, 102-108.
- Murdoch, A. and J.A. Capobianco, 1979. Effects of mine effluents on uptake of Co, Ni, Cu, As, Zn, Cd, Cr and Pb by aquatic macrophytes. *Hydrobiologia*, 64: 223-231.
- Pickering, I.J., R.C. Prince, M.J. George, R.D. Smith, G.N. George and D.E. Salt, 2000. Reduction and coordination of arsenic in Indian Mustard. *Plant Physiol.*, 122: 1171-1177.
- Rahman, M.M., U.K. Chowdhury, S.C. Mukherjee, B.K. Mondal, K. Paul and D. Lodh *et al.*, 2001. Chronic arsenic toxicity in Bangladesh and west Bengal, India. A review commentary. *J. Toxicol. Clin. Toxicol.*, 39: 683-700.
- Raskin, I. and B.D. Ensley, 2000. Phytoremediation of Toxic Metals, Using Plants to Clean up the Environ. John and Wiley and Sons, Inc., New York, pp: 304.
- Reay, P.F., 1972. The accumulation of arsenic from arsenic rich natural waters by aquatic plants. *J. Applied Ecol.*, 9: 329-332.
- Robinson, B., C. Duwig, N. Bolan, M. Kannathasa and A. Saravanan, 2003. Uptake of arsenic by New Zealand watercress (*Lepidium sativum*). *Sci. Total Environ.*, 301: 67-73.
- Smedley, P.L. and D.G. Kinniburgh, 2002. A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochem.*, 17: 517-568.
- Wagemann, R., N.B. Snow, D.M. Rosenberg and A. Lutz, 1978. Arsenic in sediments, water and aquatic biota from lakes in the vicinity of Yellowknife, Northwest Territories, Canada. *Arch. Environ. Contam. Toxicol.*, 7: 169-191.
- Walsh, L.M. and D.R. Keeney, 1975. Behaviour and Phytotoxicity of Inorganic Arsenicals in Soils. In: *Arsenical Pesticides*. Woolson, E.A. (Ed.), Am. Chem. Soc. Symp., Ser. 7, pp: 35-52.
- Woolson, E.A., J.H. Axley and P.C. Kearney, 1971. The chemistry and phytotoxicity of arsenic in soils. I. Contaminated field soils. *Soil. Sci. Soc. Am. Proc.*, 35: 938-943.
- WHO, 2003. Environmental Health Organization Guidelines for Drinking-Water Quality. Health Crssiter.