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Research Paper

Evaluation of Arsenic Accumulation in the Vegetables Okra *(Abelmoschus Esculentus)* and Bean *(Phaseolus Vulgaris)* Grown in Contaminated Soils

Chemistry

KEYWORDS : Arsenic contamination, Vegetable crops- okra and bean, Arsenic accumulation

Sukanya Chandra	Department of Chemistry, National Institute of Technology, Durgapur, Pin 713209, West Bengal, India
Rajnarayan Saha	Department of Chemistry, National Institute of Technology, Durgapur, Pin 713209, West Bengal, India
Parimal Pal	Department of Chemical Engineering, National Institute of Technology, Durgapur, 713209, West Bengal, India

ABSTRACT

Experimental investigations are carried out to determine the arsenic uptake by the vegetable crops like Abelmoschus esculentus (okra) and Phaseolus vulgaris (beans). Arsenic (As) was applied in the pots as an aqueous solution of sodium arsenate containing six different levels of arsenic as 0, 10, 20, 30, 40 and 50 mg/kg of soil. The highest concentration of total arsenic were found in the roots of all vegetables and decreased in the aerial portions of the plants. Arsenic accumulation followed the trend of roots>fruits>leaves>stems in okra whereas in case of beans the trend is roots>leaves>stems>fruits. The accumulation of arsenic in different parts of the plants increased with increasing concentration of the applied arsenic solution. The results showed that these crops accumulated arsenic from contaminated soil and as a consequence a significant quantity of arsenic may enter into the human body through food chain mechanism.

Introduction

Arsenic is the most toxic metalloid widely concerned and distributed in the environment (Tu and Ma 2002). It is present everywhere in nature and are usually discharged to soil, water and air during mining and refining activities (Andrea et al. 2004; Cheng 2003; Wei et al. 2007). The main sources of human exposure are through ingestion of arsenic contaminated groundwater and inhalation of arsenic dust particles. It can also be accumulated in humans via food chain. Crops and vegetables grown on the arsenic contaminated soil can also be a source of arsenic for human beings (Williams et al. 2005). As per the literature reviews some information regarding uptake of arsenic by plants and crops has been reported. Farid et al. (2003), reported that leafy vegetables concentrate higher contents of arsenic in aerial parts when compared to a variety of other vegetables grown in normal and arsenic contaminated soils. According to Vaughan (1993),

arsenic concentration in the edible parts of most plants is generally low. The lower concentration in aerial portions of plants may be due to the inability of plants to translocate arsenic beyond

roots (Smith et al. 2008). Wei et al. (2007), reported that arsenic is accumulated in plants at variable concentrations; 0.07-0.83 mg/kg in grains, 0.02-0.56 mg/kg in crops, 0.001-0.039mg/kg in pulse and 0.001-0.039 mg/kg in trees. Roy Chowdhury et al. (2002), reported that arsenic accumulation in different food products like leaves of vegetables, potato skin, rice, wheat, cumin, cereals etc generally varied within the range of <0.0004 and 0.693 mg/kg.

Arsenic commonly exhibit several different oxidation states of +5, +3, 0 and -3. Arsenic exists in natural water in a variety of chemical forms as As^{+3} (arsenite), As^{+5} (arsenate), MMA (Mono methyl arsenic acid) and DMA (Dimethyl arsenic acid). As^{+3} and As^{+5} are known to interconvert depending on the redox conditions of the surrounding environment (Masscheleyn et al. 1991). Chronic arsenic poisoning can cause serious health problems including cancers, hyperkeratosis, restrictive lung disease and ischaemic heart disease (Mandal and Suzuki 2002; Rossman 2003). Investigations were done by researchers regarding the phytotoxic effects of arsenic on the crops and the symptoms observed were inhibition of seed germination, reduction in plant height and root length, wilting and necrosis of leaf blades, decrease in shoot growth and lower fruit and grain yield (Liu et al. 2005; Abedin and Mehrag 2002; Carbonell-Barrachina et al. 1995; Burlo et al. 1999). In the Bengal-Delta basin, India widespread cultivation is done using arsenic contaminated groundwater. Vegetables and rice grow in plenty in this cultivation zone. Among the vegetables, okra (Abelmoschus esculentus) and beans (Phaseolus vulgaris) are very important crops that are commonly cultivated in this region and are selected for the study. Practically there has been no study on the absorption and accumulation of arsenic by okra but okra grown on arsenic contaminated water possesses a serious threat on human health in the Bengal-Delta basin. Similarly very limited works have been done for bean plants also. The present investigations therefore intend to study how these commonly consumed vegetables uptake and accumulate arsenic in its different parts, to identify the interactions between soil and crops' arsenic concentration and also to determine the effect of arsenic on the yield of these vegetable crops and its impact on the environment.

Materials and Methods

The soil used in the pot experiment was collected from a field in West Bengal which is known to be free of arsenic contamination. The soil samples were sundried, unwanted materials like grass, roots and stones were removed from it and the soil was crushed and sieved using a 2mm sieve and 5 kg soil was taken in 6 Litre plastic pots which were washed previously with tap water and dried and then mixed with urea, single super phosphate and muriate of potash for nitrogen, phosphorus and potassium content in the soil respectively. These fertilizers were mixed with the soil by hand. There were altogether 15 pots comprising 5 arsenic treatments with three replications for each and a control. The same treatments were done for okra as well as for beans. The plants were grown directly from seeds without any pretreatment and the seeds were purchased from a commercial seed breeder from Hooghly market of West Bengal, India. Seeds were soaked with water for 12 hours and were sown in a plastic tray and were then transplanted onto the plastic pots (one plant per pot) seven days after the germination of the seedlings. Arsenic was applied as an aqueous solution of sodium arsenate at 5 different arsenic levels of 10, 20, 30, 40 and 50 mg/kg of soil. No arsenic was added to the control soil.

After cultivation the plants were collected, the soil was brushed off, washed first with tap water, and then with distilled water and then oven dried at 70°C for 72 hours, after dividing into root, stem, leaves and fruit parts. Arsenic concentrations in different parts of the plants were analyzed by microwave digestion method. The dry mass of each part of plant were measured and then ground using a mortar. 3ml of concentrated HCl and 1ml of concentrated HNO_3 was added to 0.15-0.5 g of the ground sample in closed Teflon containers and then irradiated at 450 W for 4 minutes in closed Tephlon containers. Then 3ml of H_2O_2 was added to the mixture after cooling and once again irradiated at

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450 W for 4 minutes. Then the solution was diluted to 25 ml with distilled water after filtering and arsenic analysis was done using atomic absorption spectrophotometer (GBC, Avanta).

Transfer factor or Translocation of arsenic from soil to plant parts (Chamberlain 1983; Harrison et al. 1989) was calculated to determine the relative uptake of arsenic by the plants with respect to soil.

$$\text{Transfer factor with respect to soil } (TF_s) = \frac{\text{Concentration of arsenic in plant part}}{\text{Concentration of arsenic in soil}} = \frac{[As]_{\text{plant part}}}{[As]_{\text{soil}}}$$

For determination of relative movement of arsenic from roots to different plant parts, transfer factor with respect to roots (Barman et al. 2000) was calculated.

$$\text{Transfer factor with respect to root } (TF_r) = \frac{\text{Concentration of arsenic in plant part}}{\text{Concentration of arsenic in root}} = \frac{[As]_{\text{plant part}}}{[As]_{\text{root}}}$$

Statistical Analysis
Data were represented as the mean values ± Standard errors of three replicates. Results were analyzed by analysis of variance with SPSS 12 software and the means were separated by Tukey's multiple range test at $p < 0.05$.

Results and Discussion
Effect of arsenic on Physiological Parameters
Arsenic inhibited shoot length, leaf number, leaf size and fruit yield in bean and okra plants in comparison to the control which was negligible for small doses of arsenic treatments but became predominant in case of 40 mg/kg and 50 mg/kg arsenic treatments. Very slight yellow coloration and tiny brown spots appeared on leaves at highest arsenic concentrations. Visual symptoms of vegetative injury i.e. chlorosis or necrosis were not observed in either of the crops throughout this experiment and all crops were alive till the end of the study. This is in accordance with the observations of Rahman et al. (2009) in case of Beta vulgaris and Amaranthus gangeticus.

Arsenic Concentration and Accumulation in various Plant Parts and its Transfer from the Soil
Arsenic accumulation in okra plant followed the trend roots>fruits>leaves>stem. The highest arsenic concentration was found in roots followed by fruits regardless of applied arsenic concentration. In case of beans the trend is roots>leaves>stem>fruits which agrees with the findings of Cobb et al. (2000), where the accumulation of arsenic into shoots especially leaves is observed. In both the vegetable crops the lower concentration in other plant parts may be due to the inability of the plant to translocate arsenic beyond roots. The variations in the metal concentration in vegetables are due to the variations in their absorption and accumulation tendency. For both the crops it is seen that accumulated arsenic concentrations increased with increasing arsenic contamination, the arsenic concentrations in roots were far higher than those in the shoots. Similar observations were also noticed by Hong et al. (2010) in Cucumis sativus. The arsenic content in the stems of okra were considerably less than the other parts which may be due to the ability of the plant to translocate the element from stems to leaves and ultimately to fruits i.e. the edible part containing the toxic element enhances the risk of future generation. In case of bean plants the roots contain significant amount of arsenic and in the fruits it is very less. It means that translocation of arsenic in bean plants is very much restricted which may be due to some physical factors like pH, cation exchange capacity (CEC) and organic matter (OM) in the soil or may be due to some resistance offered by the plant body itself in absorbing and translocating the contaminant.

The accumulation pattern of arsenic in different parts of okra and bean plants grown in soils contaminated with different doses of arsenic are shown in Table 1.

Table 1 Concentration of arsenic(mg/kg) in different parts of okra and bean plants

Plant	Arsenic treatments in the soil in mg/kg	Roots	Stems	Leaves	Fruits
Okra (*Abelmoschus esculentus*)	10	4±0.44d	ND	1.66±0.17c	4.67±0.23a
	20	8±0.45c	ND	2.16± 0.20c	4.90± 0.12a
	30	12.17±0.33b	ND	2.4± 0.12bc	5.17± 0.14a
	40	13.2± 0.3ab	ND	3.16± 0.27ab	5.3± 0.40a
	50	14.2±0.56a	4.167±0.15*	3.5± 0.15a	5.5± 0.35a
Beans (*Phaseolus vulgaris*)	10	10.2± 0.42d	2.5±0.27c	2.95± 0.33d	ND
	20	11.08± 0.37d	3± 0.15c	5.21± 0.30c	ND
	30	14.04±0.39c	5.01± 0.12b	9.02± 0.51b	ND
	40	24.98±0.24b	5.5±0.44ab	10.10± 0.15b	ND
	50	32.02± 0.42a	7.01± 0.60a	12.04± 0.38a	ND

ND- not detected, * - As detected only at high concentrations, Values are means ± SE of three replicates (n=3); Means followed by same letters are not significantly different in one way ANOVA by Tukey's Test at $p<0.05$

It is seen from the above data that dry roots concentrated 0.86, 1.63, 2.35,2.49 & 2.58 times higher arsenic than dry fruits and 2.41, 3.70, 5.07, 4.18 & 4.06 times higher arsenic than dry leaves in case of okra at 10, 20, 30, 40 & 50 mg/kg arsenic treatments respectively where as in case of bean plants dry roots accumulated 3.46, 2.12, 1.56, 2.5 & 2.66 times higher arsenic than dry leaves and 4.08, 3.69, 2.8, 4.54 & 4.57 times higher arsenic than dry stems at 10, 20, 30, 40 & 50 mg/kg arsenic treatments respectively.

To determine the phytoextraction ability of the macrophytes transfer factor was calculated. The ratio of the metal in between the plant parts and soil measures the efficiency of the plant in removing metals from the soil and in translocating metals from the roots to the shoots. The ratio '> 1' means higher accumulation of metal in plant parts than soil (Barman et al. 2000).

The transfer factor with respect to root (TF_r) and transfer factor with respect to soil (TF_s) for okra plant is given in Figure 1a and 1b respectively and for bean plants TF_r and TF_s is given in Figure 2a and 2b respectively.

Fig 1a The transfer factor with respect to root (TF_r) of okra plant at different doses of arsenic

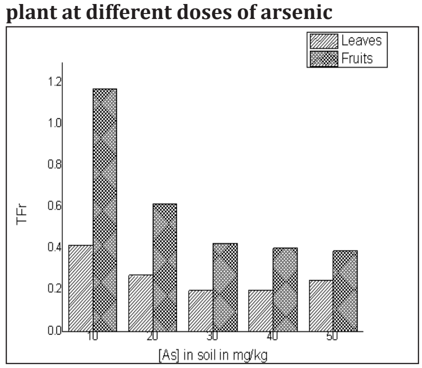


Fig 1b The transfer factor with respect to soil (TF_s) of okra plant at different doses of arsenic

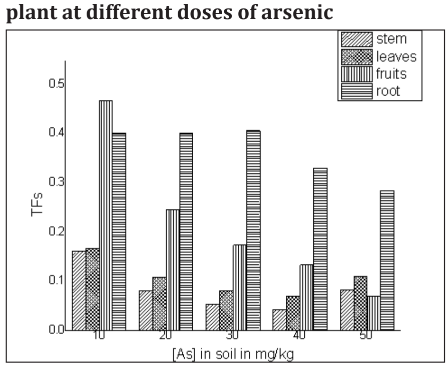


Fig 2a The transfer factor with respect to root (TF_r) of bean plant at different doses of arse nic

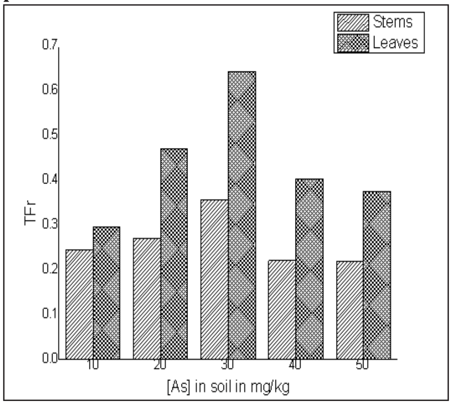
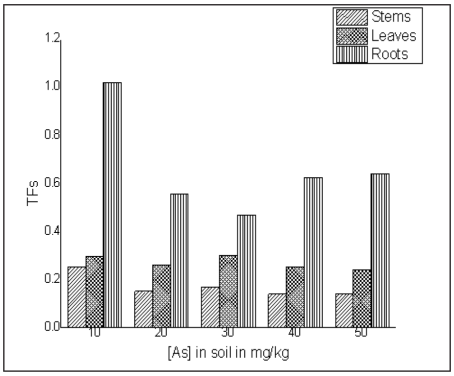


Fig 2b The transfer factor with respect to soil (TF_s) nic of bean plant at different doses of arsenic



From the above data it is clear that the transfer of metals from soil to different plant parts (TFs) and from root to different plant parts (TFr) is much lower than one which may be due to low adsorption capacity of the roots, restricted movement of the element from soil to shoots etc. It also probably explains the absence of toxicity symptoms in the plants. Similar observations were also noticed by Melo et al. (2012) in sunflower and castor beans.

Possibility of Human Exposure to Arsenic via Food Chain

From this experiment and also from previous reports it is clear that arsenic is accumulated in the plant tissues grown in soil contaminated with the element. Accumulation of arsenic in crops has been reported in maize (Sadiq 1986), barley and rye grass (Jiang and Singh 1994), potato, arum, amaranth, radish, cauliflower, brinjal (Mandal 1998). According to Mason et al. (2000) a decrease of arsenic levels with the increase of higher trophic level was observed. In this way considerable amount of arsenic enters the food chain via different pathways like 'soil-plant-man', 'plant-animal-man', thereby exposing human beings and other animals to the risk of arsenic poisoning.

Conclusions

The investigation shows that both the vegetables accumulated a significant amount of arsenic which increased with increasing arsenic contamination.

In general roots contain greater amount of arsenic than the edible parts. The uptake of arsenic by okra fruit is greater compared to bean fruit which may be considered as a resistive variety.

Extensive study and regular monitoring is necessary to find out which crop is maximum resistant to arsenic. The toxic effects of inorganic and organic compounds of arsenic on these crops need to be identified.

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