

**MARIGOLD (*TAGETES PATULA*) AND ORNAMENTAL ARUM
(*SYNGONIA SP.*) AS PHYTOREMEDIATORS FOR ARSENIC IN POT SOIL**

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Abstract

Marigold (*Tagetes patula*) and ornamental arum (*Syngonia sp.*) were grown on As spiked soils up to 10 mg As per pot or per kg soil to assess their properties as phytoremediators. Fifteen days old marigold and young plants of ornamental arum were transplanted to soils. At flowering stage, the maximum arsenic concentration in the root, shoot and leaf of marigold were 1987.12, 5.45 and 3.50 mg/kg dry mass, respectively. The maximum arsenic concentrations in root and shoot of ornamental arum after two months were 14.27 and 12.14 mg/kg dry mass, respectively. The arsenic accumulating property of the *Tagetes patula* and *Syngonia sp.* appeared to be good sources to be exploited to phytoremediate arsenic contaminated soil.

Introduction

The arsenic present in soils may pose a risk to the environment by providing a pathway for the transfer of arsenic from soil into the food chain via plant uptake (Huq and Naidu 2005). There is a great need for environmentally sound and cost effective technologies capable of reducing arsenic in soils to environmentally acceptable levels. In recent years, phytoremediation has generated increasing interest worldwide, because it serves both the purposes (Chaney 1983, Terry and Banuelos 2000). Plants used for phytoremediation should tolerate local conditions, having a high biomass production, be easily propagated, and have the ability to accumulate high concentrations of the contaminant, or facilitate its breakdown in the soil (Robinson *et al.* 1999, Raskin and Ensley 2000). Recently a few arsenic hyper accumulating plants *viz.* Chinese brake fern *Pteris vittata* L. (Ma *et al.* 2001) and another fern *Pityrogramma calomelanos* L. (Francesconi *et al.* 2002) have been reported. Considering soil contamination by arsenic through irrigation water (Huq and Naidu 2005) and a need for its remediation, the authors tried to find some plant species which can accumulate arsenic. They chose a very common and locally popular flowering plant, marigold (*Tagetes patula*), and a leafy ornamental arum plant (*Syngonia sp.*), commonly grown in courtyards, apartments and pots as an ornamental plant.

The objectives of this study were to (i) examine the accumulation and distribution characteristics of arsenic in the marigold (*T. patula*) and ornamental arum from an arsenic contaminated soil and (ii) assess the potential of these two plants for phytoremediation of arsenic contaminated soil.

Materials and Methods

The experiment was carried out in two phases. In the first phase, young plants of marigold and ornamental arum were grown in soils containing different levels of residual arsenic from a previous experiment. In that experiment, the pots (containing 3 kg soil in each) received As ranging from 10 to 100 mg/kg soil.

In the second phase, a pot experiment was carried out in a net house with two Aeric Haplaquept soils representing two series, the Dhamrai (used for marigold) and the Sonatola series

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um). Four and two kilogram of the composite bulk soil samples were thoroughly homogenized for both the soils respectively before transferring to pots. The potted Dhamrai soil was spiked with 0.0, 0.5, 2.5, 5.0 and 10.0 mg As/kg and the Sonatola soil was spiked with 0.0, 0.05, 0.1, 0.5, 1.0, 2.0, 3.0, 5.0, 8.0 and 10.0 mg As/kg soil. The treatments were added as solution of sodium meta-arsenite (NaAsO₂). The soils were fertilized as per requirement of the crops (BARC 1997). Soils in the pots were allowed to equilibrate for two weeks. Young plants of a hybrid variety ('Infa') of 15 days old marigold (*T. patula*) was procured from BRAC nursery. Young plants of ornamental arum were collected from the Dhaka University Botanical garden. Four seedlings of each plants were sown in the pots and allowed to grow. The pots (in triplicates) were randomly arranged. Watering of the plants was done with As-free tap water. Marigold plants were harvested at flowering stage and the ornamental arum was harvested two months after transplanting.

The harvested marigold plants were separated into root, shoot, leaf and flower whereas the ornamental arum plants were separated into root and shoot. The plant samples were first air-dried and then oven-dried at 70 ± 5°C for 48 hours. The dried plant samples were grounded and sifted through a 0.2 mm sieve. After harvest, soil samples in each pot were also sampled. The soil samples were air-dried, homogenized and was screened to pass through a 0.5 mm sieve. Plant samples were extracted with HNO₃ while the soils were extracted with aqua-regia (Portman and Riley 1964) for analysis and water soluble As was extracted at a soil : water ratio of 1 : 5 for 24 h.

The various physical, chemical and physiochemical properties of the soils were determined (Table 1) following procedures described in (Imamul Huq and Alam 2005). Both plant and soil were analyzed for total arsenic by hydride generated atomic absorption spectrometry (HG-AAS) while iron and manganese were determined by AAS. Certified reference materials were carried through the digestion and analyzed as part of the quality assurance/quality control protocol. Reagent blanks and internal standards were used where appropriate to ensure accuracy and precision in the analysis of arsenic. Each batch of 20 samples was accompanied with reference standard samples to ensure strict QA/QC procedures.

Table 1. Some basic properties of selected soils.

Soil	Sand (%)	Silt (%)	Clay (%)	Textural class	pH	OM (%)	N (%)	P (%)	K (%)	As (mg/kg)		Fe (%)	S (%)
										Total	WS*		
Dhamrai	7	49	44	Silty clay	6.3	1.40	0.12	0.07	0.32	1.66	0.23	7.52	0.17
Sonatola	10	69	21	Silt loam	5.4	1.22	0.09	0.06	0.28	0.55	0.08	5.26	0.13

* WS = Water soluble.

Results and Discussion

Marigold: The preliminary experiment setup with young plants of marigold on soils previously contaminated with various levels of As for a different experiment showed that the plants have extracted arsenic from soil and accumulated it to its different parts. The roots

contained higher amounts (0.129 - 5.400 mg/kg) of arsenic than the shoots (0.009 - 1.839 mg/kg) or leaves (0.015 - 2.959 mg/kg) on dry mass basis. There was however, no evidence of arsenic in the flowers. Considering the whole plant, the arsenic concentration ranged between 0.033 and 2.256 mg/kg dry weight with a bio-concentration factor (ratio of plant arsenic concentration to water-soluble arsenic in soil. Ma *et al.* 2001) of 0.68 - 28.2 with an average of 6.92; a transfer factor (ratio of arsenic concentration in plants to that in soil (Farrago and Mehra 1992) of 0.03 - 4.29 with an average of 0.91 and a translocation factor (ratio of arsenic concentration in above ground biomass to that in root. Ma *et al.* 2001) of 0.09-0.45 with an average of 0.24. Farrago and Mehra (1992) have considered that when the transfer factor for any particular element is 0.1, then the plant can be considered as excluding the element from its tissues. Alternatively, it could be postulated that if the value is greater than 0.1, the plant shows an affinity to the element. Except for a few samples, the transfer factor was above 0.1 with an average value of 0.91. This value is several times greater than Farrago and Mehra's (1992) recommended value. So, it may be said that the extraction of arsenic by marigold could be taken as an indication of the plant's affinity to bioaccumulate As from soil. The arsenic concentration of the plants and the corresponding soils after harvest are presented in the Fig. 1(a). The descriptive statistics are shown in the Table 2. Pearson correlation (r) of soil and plant is 0.137 at 0.564 per cent level of significance.

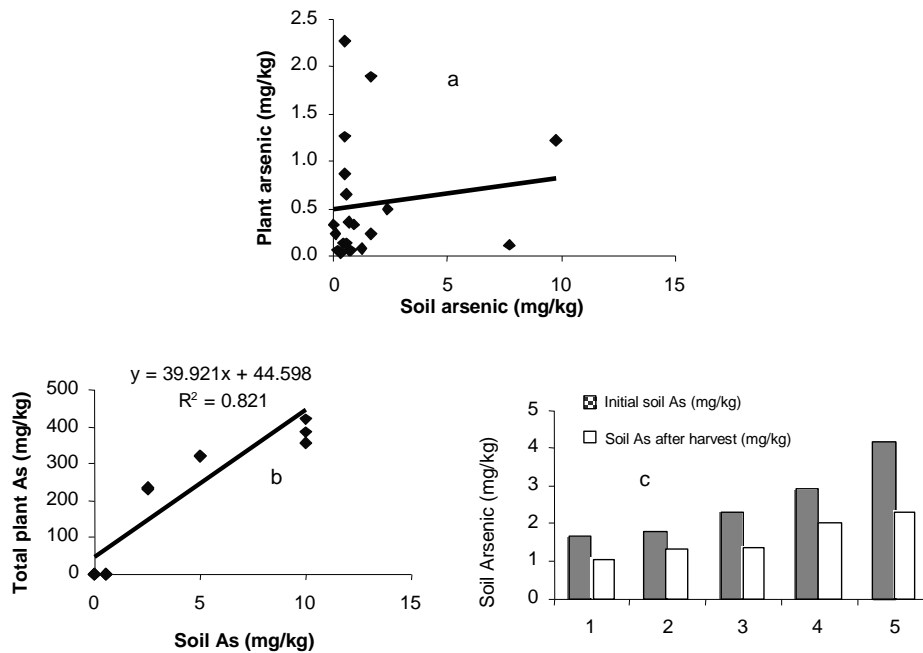


Fig. 1. (a) Arsenic concentration (mg/kg) of marigold (d.w.) grown on an As contaminated soil. (b) Arsenic conc. (mg/kg) of marigold (d.w.) grown on soil spiked with different levels of As (mg/kg). (c) Arsenic conc. (mg/kg) in soil before plantation and after harvest of marigold.

Table 2. Descriptive statistics of soil and plant arsenic for marigold.

Variable	N	Mean	Median	Tr mean	SD	Se mean	Min.	Max.	Q1	Q3
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Soil	20	1.54	0.61	1.17	2.54	0.57	0.23	9.72	0.41	1.55
Plant	20	0.54	0.28	0.47	0.64	0.14	0.03	2.26	0.08	0.81

Based on this observation a pot experiment with spiked arsenic at 0.5 - 10 mg/pot with the Dhamrai soil was initiated. The average values of arsenic concentration in different parts of the plant are shown in Table 3 and the changes in the whole plant arsenic with soil arsenic is presented in Fig. 1(b). Besides, arsenic concentration in soil before plantation and after plant harvest is shown in Fig. 1(c). Only the mean values are used to draw the figures. It is clear that the plants extracted a significant portion of soil As thereby reducing the soil arsenic. The paired t test value of mean difference between soil arsenic before plantation and soil arsenic after plant harvest = 0 (vs. not = 0); t value = 5.57; p value = < 0.001, 95 % CI for mean difference: (0.589, 1.326). The P-value indicates that there has been a significant mean difference between soil arsenic before plantation and soil arsenic after plant harvest. This indicates significant extraction of soil As by the marigold. Bio-concentration, transfer factor and translocation factor are also shown in the Table 3. The maximum amount of As was found in roots followed by shoots and leaves. Transfer factor for each treatment is very high which indicates marigold has a very high affinity and an extraordinary capability to extract arsenic from contaminated soil. This substantiated the findings from the preliminary experiment.

Table 3. Arsenic concentration in different parts of marigold in relation to spiked soil arsenic with bio-concentration and transfer factor.

Treatment	Arsenic concentration (mg/kg)				Bioconc.	T. F.	t. f.
	Root	Shoot	Leaf	Total			
Control	2.51	0.39	0.12	0.72	3.11	0	0.076
0.5	5.33	0.81	0.50	1.40	6.10	2.81	0.106
2.5	1429.18	1.48	0.91	233.05	1013.28	93.22	0.001
5	1587.74	3.82	2.00	318.99	1386.90	63.80	0.001
10	1987.12	5.45	3.50	387.41	1684.40	38.74	0.002

Bioconc. = Bio-concentration, T. F. = Transfer factor, t. f. = Translocation factor.

The arsenic concentration in plants increased significantly ($F = 59.64$, $p < 0.001$) with increasing As concentration in soil. The regression equation is, total plant As (mg/kg) = $44.6 + 39.9 \times$ treatment As (mg/kg); $R\text{-Sq} = 82.1\%$; $R\text{-Sq}(\text{adj}) = 80.7\%$. ANOVA test was done and the results also showed that the As treatment had a significant ($F = 515.70$, $p < 0.001$) effect on arsenic concentration in the whole plants.

Ornamental arum (Syngonia sp.): The ornamental arum grown on contaminated soil showed arsenic concentrations ranging from 0.547 - 11.601 mg/kg dry weight/plant, respectively. The concentration of As in roots of the plant ranged from 0.62 - 19.15 mg/kg dry mass and the shoot of the plants contained As ranging from 0.27 - 7.16 mg/kg dry mass. Considering the whole plant the bioconcentration factor was 6.75 - 143.22 with an average of 44.99; a transfer factor of 0.11 - 6.84 with an average of 1.58 and a translocation factor of 0.04 - 1.46 with an average of 0.56 were observed. The arsenic concentration of plants and corresponding soils are graphically presented in the Fig. 2(a). The descriptive statistics are shown in the Table 4. Pearson correlation (r) of soil and plant was 0.402 at 0.028 per cent level of significance. This is a better relationship compared to marigold.

Table 4. Descriptive statistics of soil and plant samples for ornamental arum.

Variable	N	Mean	Median	Tr mean	Sd	Se mean	Min.	Max.	Q1	Q3
Soil	30	3.72	2.43	3.47	3.19	0.58	0.08	11.88	1.02	6.37
Plant	30	3.64	2.76	3.35	2.88	0.53	0.55	11.60	1.17	6.13

In the pot experiment (Sonatola series) with *Syngonia sp.* the results obtained are shown in table 5 and Fig. 2(b). The arsenic concentration in plants increased significantly ($F = 301.46$, $p < 0.001$) with increasing As concentration in soil. The regression equation is, total plant As (mg/kg) = $- 0.045 + 1.34 \times$ initial total soil As (mg/kg); $R^2 = 91.5\%$; R^2 (adj) = 91.2% . ANOVA test also showed that the As treatment had a significant ($F = 59.01$, $p < 0.001$) effect on arsenic concentration of the whole plant. The arsenic concentration in soil before plantation and after plant harvest is shown in figure 2(c). Only mean values were used to draw the figures. From Fig. 2(c) it is clear that the soil arsenic concentration was reduced significantly after harvest. The paired t test value of mean difference between soil arsenic before plantation and soil arsenic after plant harvest = 0 (vs. not = 0): t value = 9.29; p value = < 0.001 , 95 % CI for mean difference: (1.263, 1.976). The low p value confirms the significant mean difference between soil arsenic before plantation

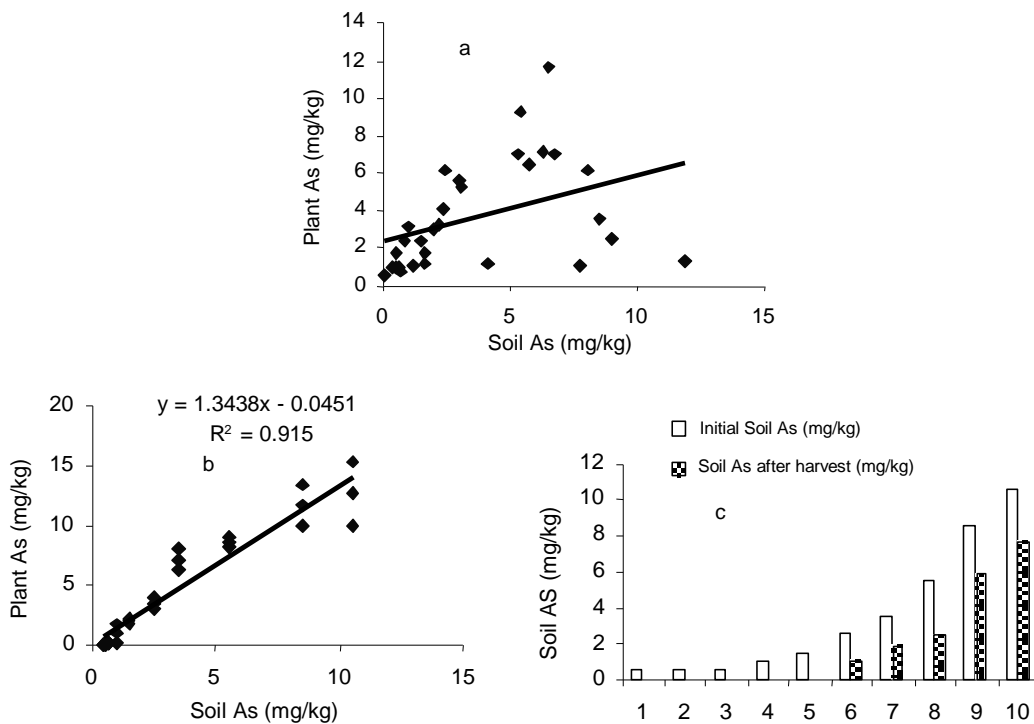


Fig. 2 (a) Arsenic conc. (mg/kg) of ornamental arum (d.w.) grown on As contaminated soil. (b) Arsenic conc. (mg/kg) of ornamental arum (d.w.) grown on soil spiked with different levels of As (mg/kg). (c) Arsenic conc. (mg/kg) in soil before plantation and after harvest of ornamental arum.

Table 5. Arsenic concentrations (mg/kg) in different parts of ornamental arum (d.w.).

Treatments (mg As per kg soil)	
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	Control	0.05	0.1	0.5	1	2	3	5	8	10
Root	0.04	0.09	0.19	1.43	2.86	5.07	11.51	9.99	12.22	14.27
Shoot	0.04	0.08	0.14	0.64	1.42	2.50	4.63	8.64	11.05	12.14

and soil arsenic after plant harvest. Moreover, the concentration of As in the soils spiked with 0.05, 0.1, 0.5, and 1 mg As/kg soil as well as control (0.547 mg/kg As) were below detectable limit (BDL) after plant harvest. Considering the whole plant, a bioconcentration factor of 0.04 - 189.10 with an average of 57.71; a transfer factor of 0.06 - 2.26 with an average of 1.02 and a translocation factor of 0.34 - 1.91 with an average of 0.75 were observed. It could thus be concluded that a significant amount of soil arsenic has been removed from the soil by the growing plants (ornamental arum).

From the observations with the two plants, it could be concluded that they have the characteristics to hyperaccumulate As from soil and could be used as a possible phytoremediators. The ornamental arum, however, is a better phytoremediator than marigold in extracting As from soil. Huq and Naidu (2005) have shown that edible arum is also a hyperaccumulator for As. Experiments under field conditions might further these observations.

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References

- BARC. 1997. Fertilizer Recommendation Guide-1997. M.M. Rashid, M. Idris Ali, N.I. Bhuiyan, A.K.M. Hossain, F. Huq, A.B. Talukder, G.M. Panaullah, M. Nuruzzamzn, and M.U. Miah (Eds.), BARC Soil Publication No. **41**. 1997. Published by the BARC. Dhaka. pp.1-196.
- Chaney, R.L. 1983. Plant uptake of inorganic waste constituents. *In: Land treatment of hazardous wastes.* J.F. Parr, P.B. Marsh, and J.M. Kla (Eds.), pp. 50 - 76. Noyes Data Corp, Park Ridge, NJ.
- Francesconi, K., P. Visoottiviseth, W. Sridokchan and W. Goessler. 2002. Arsenic species in an arsenic hyperaccumulating fern, *Pityrogramma calomelanos*: A potential phytoremediator of arsenic-contaminated soils. *Sci. Total Environ.* **284**: 27-35.
- Huq, S.M. Imamul and Ravi Naidu. 2005. Arsenic in Ground Water and Contamination of the Food Chain: Bangladesh Scenario, *In: Natural Arsenic in Ground Water: Occurrence, Remediation and Management.* Bundschuh, Bhattacharya and Chandrasekharam (Eds.). A.A. Balkema Publishers, New York. pp. 95-101.
- Imamul Huq, S.M. and M.D. Alam (Eds.). 2005. A Handbook on Analyses of Soil, Plant and Water. BACER-DU, University of Dhaka, Bangladesh, xxii+246pp.
- Ma, L.Q., K.M.M. Komar, C. Tu, W. Zhang, Y. Cai and E.D. Kennelley. 2001. A fern that hyperaccumulates arsenic. *Nature (London)* **409**: 579.
- Portman, J.E. and J.P. Riley. 1964. Determination of arsenic in seawater, marine plants and silicate and carbonate sediments. *Anal. Chem. Acta.* **31**: 509-519.
- Raskin, I. and B.D. Ensley. 2000. Phytoremediation of toxic metals: Using plants to clean up the environment. John Wiley & Sons, New York.
- Robinson, B.H., B.E. Clothier, T. Mills and R.R. Brooks. 1999. Phytoremediation of heavy metal contaminated soils. *In: Abstracts. Second International Conference on Contaminants in the Soil Environment in the Australia-Pacific Region.* 12-17 December 1999, New Delhi, India. pp. 466-467.

Terry, N. and G. Banuelos. 2000. Phytoremediation of contaminated soil and water. Lewis Publ., Boca Raton, FL.

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