Laboratory Study Of Arsenic Uptake And Phytoremediation Potential Of Three Aquatic Macrophytes Of Meghalaya, India

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Abstract: Laboratory experiments were performed to evaluate the As uptake capacity by three aquatic macrophytes (Scripus mucronatus, Rotala rotundifolia and Myriophyllum intermedium). The selected macrophytes were transferred to the laboratory containing nutrient solution and working As standard solutions of different concentrations (1.0, 2.0, 4.0, 8.0 and 16 mg L^{-1}) and harvested at regular time interval of 2, 4, 6, 8 and 10 days. The As uptake by these macrophytes showed a linear relationship for S. mucronatus, R. rotundifolia and M. intermedium with the exposure time period (2–10 d). As accumulation in the plant parts was higher in the roots for S. mucronatus but reverse in the case of R. rotundifolia and M. intermedium. The maximum bioconcentration factor (BCF) values were found at the 8th day in all the three aquatic macrophytes and translocation factor (TF) was at the 8th day for S. mucronatus and R. rotundifolia and at the 6th day for M. intermedium respectively. The experimental results demonstrated that these three aquatic macrophytes have a phytoremediation potential for removing As from As -contaminated water.

Keywords: Scripus mucronatus, Rotala rotundifolia, Myriophyllum intermedium, Arsenic uptake, Bioconcentration (BCF), Translocation Factor (TF).

1. Introduction

Water though an indispensable resource for human life is yet one of the most badly abused resources. For centuries, especially in urban areas, water has been polluted and used as dumping places for all sorts of domestic and industrial waste as well as sewage. Over 75 to 90 percent of people in developing countries are exposed to unsafe drinking water [1], hence proper water treatment is inevitable in order to ensure healthy life. Nowadays, apart from other common pollutants, heavy metals are considered as one of the most important water pollutants which may have a severe health problem. As contaminations from natural and anthropogenic sources has been reported in number of sites worldwide [2]. The extensive arsenic contamination in the groundwater have been reported in many countries, especially Taiwan, Argentina, India, Bangladesh, Mexico, Hungary, and Chile [3]. Arsenic is the 20th abundant element in earth crust [4], As is a semi-metallic element (atomic number 33, atomic mass 74.9) belonging to group V. The occurrence of As naturally in the environment is mainly from minerals and geogenic sources, but however extensive anthropogenic activities such as mining, burning of fossil fuels, use of arsenic containing chemicals in agriculture increases the As distribution in the environment [5]. As has been widely used in the fields of medicine, electronics, agriculture (pesticides, herbicide, insecticides, fertilizer, etc.), livestock (cattle and sheep dips), and as wood preservatives [6]. A variety of techniques which includes chemical, physical and biological technology have been used to remediate heavy metal contamination from soil or water. Toxic metals from industrial effluents have been remove by various other techniques such as precipitation, reduction, artificial membranes, and ion exchange, but however these techniques generate a huge amount of waste e.g., sludge, metal rich waste, etc which is difficult to dispose of and therefore, dangerous to the environment and they are also generally expensive, relatively inefficient [7]. Phytoaccumulation, one of the biological indicators which indicate the degree of absorption of heavy metals in plants has lately gained its applicability because its cost-effectiveness, long-term and ecological aspect [8]. Aquatic macrophytes have received great attention and have shown to be one of the candidates in the aquatic system for pollutant uptake and biological indicators of heavy metal [9]. The objective of the present study was to assess the uptake of As and phytoremediation potential of *S. mucronatus R. rotundifolia* and *M. intermedium* for As under laboratory conditions. The experiments were performed in a contained environmental set up inorder to eliminate all external environmental factors.

2. Materials and Methods

S. mucronatus an emergent and *R. rotundifolia* and *M. intermedium* are submerged macrophytes and they are one of the major natural constituent of wetland and riverside vegetation. They are sampled as shown in fig 1 from water body of Mawlai Umshing, (Lat $25^0$36'36.76N Long $91^0$54'05.11E), Cherrapunjee (Lat $25^0$19'01.38"N Long $91^0$48'36.51"E) and Pongkung (25°21'47.69" N 91°40'03.34" E), Meghalaya, India in the month of October 2012 and collected in polyethylene bags and transferred to the laboratory. Plants were washed several times with tap and distilled water in order to remove any adhering soils and plants of similar size, shape and height were selected and kept separately in a 40L capacity tank which contained half strength Hoagland's solution of pH = 7 [10] and kept for 15 days prior to experimentation for. The Hoagland solution was modified by omitting ferrous sulfate in order to prevent the As precipitation by iron. Nevertheless, high level of phosphorus in the nutrient medium may influence arsenic uptake by plants. After 15 days the acclimatized plants were transferred and maintained in 5% Hoagland's solution containing working As standard solutions of different concentrations 1.0, 2.0, 4.0, 8.0 and 16.0 mg $L^{-1}$and then they were exposed to As concentrations at a time interval of 2, 4, 6, 8 and 10 days. As of analytical grade, were supplied as As_2O_3 (Himedia) were used as the source of As. Experiments were carried out separately for the three aquatic macrophytes under controlled temperature (24±1^0C) and light (3500 Lux) conditions. After each time interval the plants were collected and washed with deionised water to remove any metal adhering to its surface. The washed plant samples were carefully dried the

adherent water using absorbent paper and then they are separated to roots and shoots. Samples were dried for 48h in an oven at 70±5^{0}C. The dried oven plant root and shoot was then chopped and finally powdered using a mortar and pestle to ensure homogeneity for facilitating organic matter digestion. One control plant groups were also set up where no As was added into the medium. For digestion, the plant samples were carried out according to [11]. Inductively Coupled Plasma Optical Emission Spectrometry (Ultima) was used to determine the As contents in plant root and shoot parts. The bioconcentration factor (BCF) is a useful parameter and it provides the ability index of a plant to accumulate metals with respect to metal concentration in the medium and it was calculated on a dry weight basis [12].

$$\text{BCF} = \frac{\text{Trace elememnts concentration in plant tissue } (\mu g g^{-1})}{\text{Initial concentration of the element in the external nutrient solution } (mgL^{-1})}$$

Translocation Factor (TF) is generally the translocation of heavy metal from roots to aerial part and indicates the internal metal transportation of the plant. The translocation factor is determined as a ratio of metal accumulated in the shoot to metal accumulated in the root [13]

$$\text{TF} = \frac{[\text{Metal}]\text{Shoot}}{[\text{Metal}]\text{root}}$$

Wherein, TF>1 indicates that the plant translocate metals effectively from the root to the shoot.

3. Statistics analyses

ANOVA and multiple linear regressions were performed for all the data to confirm their validity using SPSS 17.0. The data were all presented as mean ± standard error of three replicates. Fisher least significant difference (LSD) test was performed at $p < 0.05$ to check the significant difference between the means for different uptake at different As concentrations.

4. Result and Discussion

4.1. Accumulation of arsenic

As content in the roots and shoots of *S. mucronatus*, *R. rotundifolia* and *M. intermedium* showed increases in metal accumulation in the roots and shoots if metal concentrations and time period are enhanced. At As concentration of 1, 2, 4, 8 and 16mg/L, the As content (fig 2) in *S. mucronatus* roots increased to the maximum 1152, 2316, 5067, 9469 and 1716 µg/g dry weight in roots and in case of shoots it was 811, 1480, 1358, 4141 and 1236 µg/g dry weight at 2th , 4th, 6th, 8th and 10th day of harvesting and accumulation ranges from 59-9469 µg/g dry weight in roots and 29-4141 µg/g dry weight in shoots. The maximum accumulation was found on the 8th day (16mg/L) and minimum on 2nd day (1mg/L) of exposure time in both the roots and shoots. The accumulation of As in the roots and shoots increased significantly ($p<0.05$) upto the 8th day of exposure time in *S. mucronatus* but when exposure time 8th to 10th days however, there is no significant increase ($p<0.05$) of metal accumulation, which may suggest that accumulation in the roots reached a maximum at 8th day. As content in the roots and shoots of *R. rotundifolia* (fig 3) was 1419, 2890, 3050, 2830 and 2816 µg/g dry weight and 818, 1671, 3061, 7822 and 4878 µg/g dry weight respectively at 2th, 4th, 6th, 8th and 10th day of harvesting. As accumulation ranges from 242-3050 µg/g dry weight in roots and 263-7822 µg/g dry weight in shoots. The maximum accumulation was on the 8th day (16mg/L) of exposure time in both roots and shoots, while minimum accumulation was on the 10th day (1mg/L) in the roots and at 2nd day (2mg/L) in the shoots. The accumulation of As in the roots and shoots increased significantly ($p<0.05$) upto the 8th day of exposure time in *R. rotundifolia* but when exposure time 8th to 10th days however, there is no significant increase ($p<0.05$) of metal accumulation, which may suggest that accumulation in the roots reached a maximum at 8th day. As content in *M. intermedium* roots and shoots (fig 4) was 1393, 2420, 5984, 4362 and 3193 µg/g dry weight and 3010, 4248, 7164, 6512 and 4502 µg/g dry weight in the roots and shoots at 2th, 4th, 6th, 8th and 10th day of harvesting. As accumulation ranges from 265-5984 µg/g dry weight in roots and 156-7164 µg/g dry weight in shoots, while the maximum accumulation was found on the 6th day (16mg/L) of exposure time in both roots and shoots, whereas minimum accumulation in the roots was on the 10th day (1mg/L) and in the shoots was on the 2nd day (1mg/L) of exposure time. The accumulation of As in the roots and shoots increased significantly ($p<0.05$) with exposure time and concentration, however, when the exposure time was further increased from 6th to 10th day no significant increase ($p<0.05$) was observed. Thus it may be inferred that with increase in concentration and exposure time, the accumulation of As in the tissue level may be approached its maximum accumulation on the 6 day. In control plants, As accumulation was below detection limit in all the three experimental plants. Plant species and time of harvest is an important factor which influence the rate of As uptake by the plants [14]. The As accumulation in the roots and shoots of the three plant species exposed to As at 2 days was significantly lower than As accumulation at 6 and 8 days, this finding corresponds to that of [15]. The accumulation of As observed in the roots of *S. mucronatus* accumulate higher As concentrations as compared to the shoots part which corroborates with the finding of [14], [16]. The accumulation of As in the shoots of an emergent plant is generally dependent on the roots as its primary source [17]. Root morphology plays an important role in the ability of plants to accumulate heavy metals, generally plants with long, fine roots formed a larger root system which in turn helps in efficient acquisition of nutrients or metal than those plants which have a short and thick roots [18] which is observed also in *S. mucronatus* with a long fine roots system and have a higher As concentration in the roots by increasing root water contact. As concentration in most plant species are generally lower in the aerial part than in roots [19}, however, in the present study significant accumulation of As in the shoots of *R. rotundifolia* and *M. intermedium* has been observed where As concentration reached up to 7822 and 7164 µg/g dry weight, this may be due to the lack translocation barrier from roots to shoots [20], [21]. The shoots of submerged macrophytes are completely inundated in water and may have the ability to directly take up metals from the surrounding water due to their very thin cuticle (Jackson, 1998). In addition, As accumulation from water also depends on the plant's

capacity for fast growth and high biomass production even under moderate nutritional conditions and to overcome As phytotoxicity submerged macrophytes stimulates the activity of antioxidant enzyme and augmenting the synthesis of thiols [22], thus the experimental plants (*R. rotundifolia* and *M. intermedium*) may also be good accumulator for As-contaminated water owing to their higher tolerance to As toxicity. Correlation and multiple regression analyses were conducted to examine the relationship between As uptake by *S. mucronatus*, *R. rotundifolia*, *M. intermedium* and potential predictors (concentrations of As in the medium and time). Table 1, 2 and 3 summarizes the descriptive statistics and analysis results for *S. mucronatus*, *R. rotundifolia*, and *M. intermedium*. As can be seen each of the uptake is positively and significantly correlated with the As concentration in the medium for *S. mucronatus* and *R. rotundifolia*, indicating that with the increase in As concentration in the medium tend to have a significant uptake of As into the plant tissues but uptake is not significantly correlated with the As concentration in the case for *M. intermedium*. However, in case of *M. intermedium* the As uptake is significantly correlated with time i.e., the no of days have a significant outcome on the uptake of As, but time is not significantly correlated with As uptake in *S. mucronatus* and *R. rotundifolia.* The multiple regression model with all two predictors produced $R^2 = .542$, $F(2, 27) = 15.95$, $p < .001$, $R^2 = .661$, $F(2, 27) = 26.34$, $p < .001$ and $R^2 = .525$, $F(2, 27) = 14.89$, $p< .001$ for *S. mucronatus*, *R. rotundifolia*, and *M. intermedium* respectively. As can be seen in Table 1 and 2, the concentration of As in the medium had significant positive regression weights, indicating with higher As concentration in the medium were expected to have higher As uptake in *S. mucronatus* and *R. rotundifolia* but not in the case for *M. intermedium* (Table-3). Time i.e., number of days also contribute to the multiple regression model in case of *R. rotundifolia* and *M. intermedium* have a significant regression weights, indicating that the uptake of As in *R. rotundifolia* and *M. intermedium* also depends on time period, whereas in *S. mucronatus* time did not contribute to the multiple regression model and it is does not have a significant regression weights, indicating that uptake of As does not fully depend on time period.

4.2. Bioconcentration factor (BCF) of arsenic

Bioconcentration factor (BCF) value indicates the ability of the plant to accumulate metal in their tissue parts. The BCF values at different cadmium concentrations (1, 2, 4, 8 and 16mg/L) were evaluated at 2, 4, 6 8 and 10 day. The BCF value was 691, 649, 490, 288 and 184 in *S. mucronatus* (Table 4), 928, 702, 868, 803 and 481 (Table 5) in *R. rotundifolia* (Table 5) and 794, 601, 510, 943 and 476 in *M. intermedium* (Table 6) after 10^{th} day of harvesting. The maximum BCF of 937 and 962 was obtained in *S. mucronatus* and *R. rotundifolia* respectively when treated with 1mg/L of As at 8^{th} day. However, in *M. intermedium* the maximum BCF value of 1346 was obtained at 8^{th} day at 8mg/L of concentration. Plants which have the ability to accumulate heavy metal in the tissues are generally classified as a good accumulator. Generally it is considered that a plant useful for phytoremediation should have a BCF value greater than 1000 [12]. In the present study, the BCF value of *M. intermedium* was 1346 which suggest it as a good accumulator of As as compared to *S. mucronatus* (BCF-937) and *R. rotundifolia* (BCF-962). Based on BCF values *S. mucronatus* and *R. rotundifolia* may be considered as a moderate accumulator.

4.3. Translocation factor (TF) of arsenic

Translocation Factor (TF) in plants is the ratio of heavy metal accumulation in the shoots parts to the roots. Translocation of heavy metal in plants are generally dependent on plant species, type of heavy metals and various environmental factors like pH, redox potential (Eh), temperature, salinity [23]. Yanqun *et al*., [24] reported that a TF value greater than 1, the plants are considered as an accumulator species, whereas TF lesser than 1 is an excluder species. The TF>1 indicated that there is a transport of metal from root to leaf probably through an efficient metal transporter system [25], metals sequestration in the leaf vacuoles and apoplast [26]. According to Yoon e*t al*., [27] TF value more than 1 of plant species indicates their hyperacumulation potential and is known as hyperaccumulator plants. In the present study, the TF values in *R. rotundifolia* and *M. intermedium* (Table 8 and 9) was greater than one in most of the treatments indicating the translocation of As from roots to shoots parts as compared to *S. mucronatus* (Table 7) where the TF values was less than one, although As translocation in *R. rotundifolia* and *M. intermedium* occurred and continued to go on during the whole experiment, it was slightly decreased at higher arsenic concentration. Plants initially accumulate As into their roots through phosphate uptake pathway, i.e., active apoplastic or symplastic mechanisms and translocate to the shoots and leaves, the amount of As translocated from roots to shoots indicates the phytoremediation efficiency of that plant. In *S. mucronatus* As is accumulated primarily in the root system which is the strategy developed to tolerate As phytotoxicity by limiting upward transport of As which corresponds to the findings of [14], [15]. Low acropetal translocation efficiency of As was observed in *S. mucronatus*, which is in accordance with previous findings of [28]. Zhao *et al*., [28] reported that the formation of As-phytochelation (PC) complexes in roots and possible subsequent sequestration in root vacuoles limits the translocation of As from roots to shoots which maybe the probable reasons for low translocation of As in *S. mucronatus*. However, in submerged plants the translocation of As occurs probably via phloem, which mainly transports photosynthate [29]. Recent studies in submerged macrophytes confirmed two directions of transport (acropetal and basipetal) for cadmium and copper [30] but however the mechanism of arsenic transport in submerged macrophytes is still unknown. Our findings showed that a significant amount of As is accumulate in shoots of *R. rotundifolia* and *M. intermedium* possibly due to a constant contact of shoots with the medium and the shoots may directly uptake As from the medium in addition to translocation from roots.

5. Conclusion

In the present study, a laboratory experiment was carried out where all the external factors are controlled against As contamination in water. The present study indicates that all the three experimental plants were suitable for the

phytoremediation of As contamination from water. Therefore, *S.mucronatus*, *R. rotundifolia* and *M. intermedium* could be useful for phytoremediation of As from contaminated water. However, these results were obtained from laboratory experiments. Field experiments are now needed carry out their phytoremediation potentials of these plants for phytoremediation technique.

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Table 1. Summary statistics, correlations and results from the regression analysis in *S.mucronatus*

Variable	mean	std error	correlation with uptake	multiple regression weights	
				B	β
Uptake	2237.4	897.4			
Time (in days	6.0	125.5	.196	188.6	.196
Concentrations (mg/L)	5.1	64.6	.709***	352.2***	.709

* p < .05 ** p < .01 ***p<.001

Table 2. Summary statistics, correlations and results from the regression analysis in *R. rotundifolia*

Variable	mean	std error	correlation with uptake	multiple regression weights	
				B	β
Uptake	2738.57	752.6			
Time (in days	6.0	105.3	.329	308.8**	.329
Concentrations (mg/L)	5.16	54.2	.744***	360.2***	.744

* p < .05 ** p < .01 ***p<.001

Table 3. Summary statistics, correlations and results from the regression analysis in *M. intermedium*

Variable	mean	std error	correlation with uptake	multiple regression weights	
				B	β
Uptake	3312.6	1251.2			
Time (in days	6.0	175	.681***	898***	.681
Concentrations (mg/L)	5.1	90.1	.247	168	.247

* p < .05 ** p < .01 ***p<.001

Table 4. Bioconcentration Factor for arsenic in *S. mucronatus*

As concentration (mg/L)	Bioconcentration Factor				
	2d	4d	6d	8d	10d
1	347	688	878	937	691
2	286	630	554	910	649
4	233	431	509	831	490
8	212	434	625	803	288
16	123	232	402	851	184

Table 5. Bioconcentration Factor for arsenic in *R. rotundifolia*

As concentration (mg/L)	Bioconcentration Factor				
	2d	4d	6d	8d	10d
1	671	665	749	962	928
2	304	456	631	1725	702
4	401	567	819	975	868
8	444	570	764	912	803
16	140	205	264	666	481

Table 6. Bioconcentration Factor for arsenic in *M. intermedium*

As concentration (mg/L)	Bioconcentration Factor				
	2d	4d	6d	8d	10d
1	543	700	857	937	794
2	271	422	984	724	601
4	300	555	723	599	510
8	332	764	1346	1122	943
16	275	417	822	680	476

Table 7. Translocation Factor for arsenic in *S. mucronatus*

As concentration (mg/L)	TF values				
	2d	4d	6d	8d	10d
1	0.33	0.26	0.37	0.61	0.50
2	0.39	0.42	0.28	0.89	0.50
4	0.58	0.54	0.37	0.84	0.54
8	0.56	0.74	0.19	0.75	0.58
16	0.70	0.60	0.27	0.44	0.72

Table 8. Translocation Factor for arsenic in *R. rotundifolia*

As concentration (mg/L)	TF values				
	2d	4d	6d	8d	10d
1	1.44	1.67	1.74	2.44	2.83
2	0.76	1.24	1.38	3.47	2.22
4	0.70	0.63	0.58	1.92	2.02
8	0.28	0.58	1.00	1.84	2.48
16	0.58	0.52	0.54	2.76	1.73

Table 9. Translocation Factor for arsenic in *M. intermedium*

As concentration (mg/L)	TF values				
	2d	4d	6d	8d	10d
1	1.05	1.43	1.76	1.87	0.40
2	0.40	0.84	2.71	1.02	1.03
4	1.47	2.10	1.69	1.70	1.45
8	1.07	1.73	1.09	1.35	1.36
16	2.16	1.76	1.20	1.49	1.45

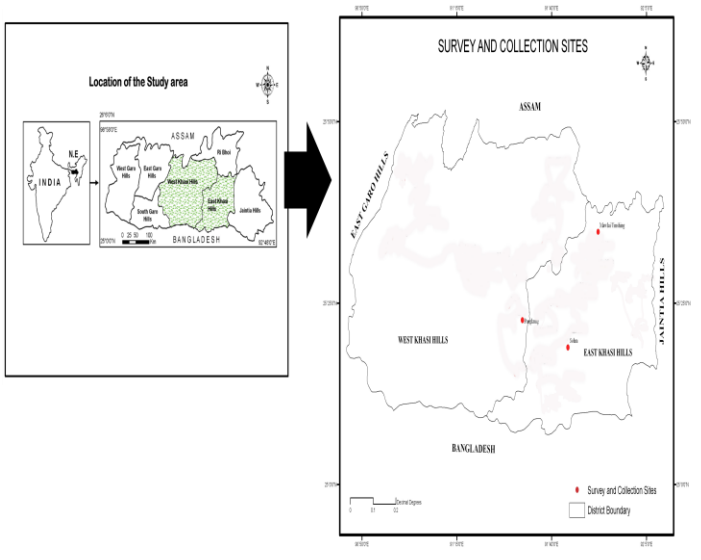

Fig 1. Map showing location and collection sites of aquatic macrophytes

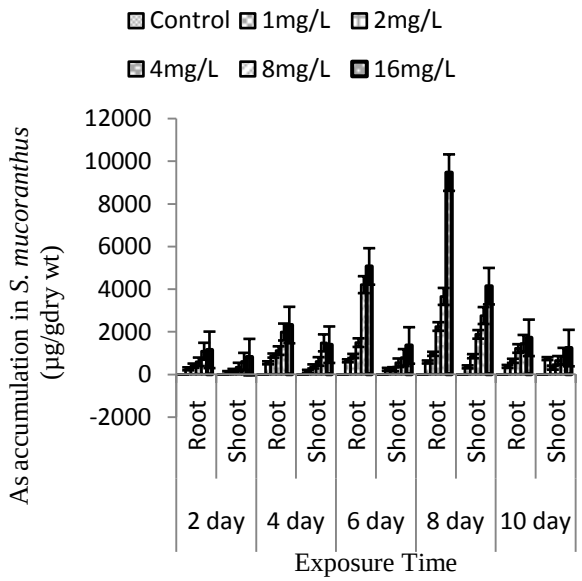

Fig 2. Arsenic accumulation in roots and shoots of *S. mucronatus*

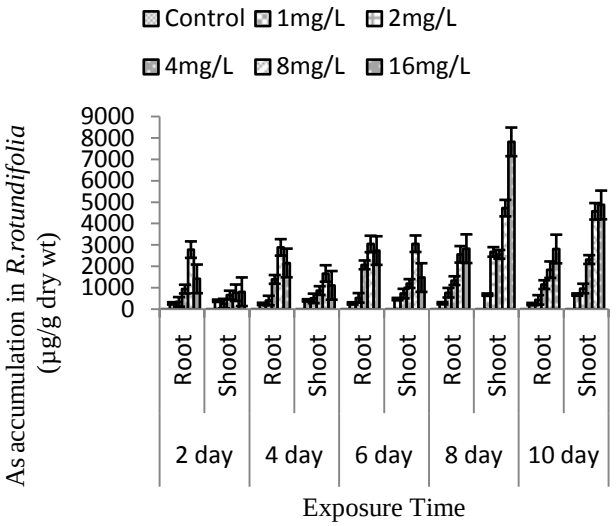

Fig 3. Arsenic accumulation in roots and shoots of *R. rotundifolia*

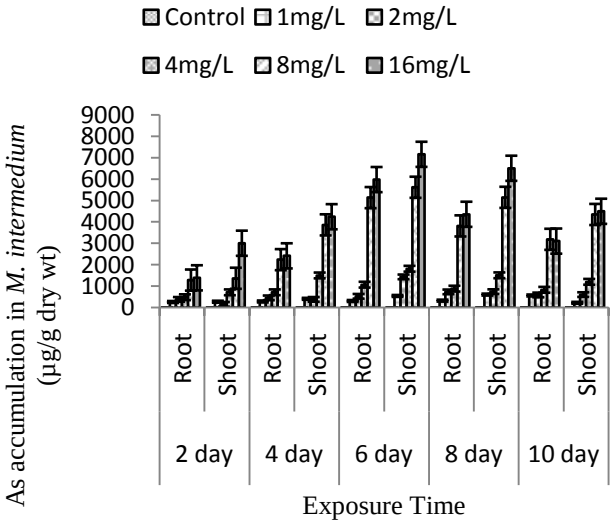

Fig 4. Arsenic accumulation in roots and shoots of *M. intermedium*