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A study on lead uptake and phytoremediation potential of three aquatic macrophytes of Meghalaya, India

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Abstract- Laboratory experiments were performed to evaluate the Pb 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 Pb 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 Pb uptake by these macrophytes showed a linear relationship for S. *mucronatus* and for *R. rotundifolia* with the exposure time period (2–10 d). Pb 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 2nd day for S. *mucronatus* and *R. rotundifolia* and at the 10th day for *M. intermedium* respectively. The experimental results demonstrated that these three aquatic macrophytes have a phytoremediation potential for removing Pb from Pb - contaminated water.

Index Terms- *Scripus mucronatus*, *Rotala rotundifolia*, *Myriophyllum intermedium*, Lead uptake, Bioconcentration (BCF), Translocation Factor (TF).

I. 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 (Kaplan, 1972), 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. Lead is a silvery-white highly malleable metal that occurs naturally in minute amounts within the Earth’s crust. Naturally lead is release to the environment from the Earth’s crust and mantle through volcanic activity and the weathering of rocks. However, these releases are very rare and the most significant sources of Pb discharge are those originated by anthropogenic activities such as mining, smelting, manufacture of pesticides and fertilizers, dumping of municipal sewage and the burning of fossil fuels that contain a lead additive and commercial products and materials like in paints, ceramic glazes, television glass, ammunition, batteries, medical equipment, electrical equipment (UNEP, 2010).

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 (Rebhun and Galil, 1990). 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 (Weiss *et al*., 2006). 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 (Maine *et al*., 2001).

The objective of the present study was to assess the uptake of Pb and phytoremediation potential of *S. mucronatus R. rotundifolia* and *M. intermedium* for Pb under laboratory conditions. The experiments were performed in a contained environmental set up inorder to eliminate all external environmental factors.

II. 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 figure 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 April 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 (Hoagland and Arnon, 1950) and kept for 15 days prior to experimentation for. After 15 days the acclimatized plants were transferred and maintained in 5% Hoagland’s solution containing working Pb 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 Pb concentrations at a time interval of 2, 4, 6, 8 and 10 days. Pb of analytical grade, were supplied as $Pb(NO_3)_2$ (Himedia) were used as the source of Pb. 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 ± 5^0C. 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 Pb was added into the medium.

For digestion, the plant samples were carried out according to Kara and Zeytunluoglu (2007). Atomic Absorption Spectrophotometer (AAS 3110, Perkin-Elmer) was used to determine the Pb 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 (Zayed *et al*., 1998)

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

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 (Deng *et al*., 2004)

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

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

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 Pb concentrations.

III. Result and Discussion

Accumulation of lead

Lead 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 lead concentration of 1, 2, 4, 8 and 16mg/L, the lead content (Figure-2) in *S. mucronatus* roots increased to the maximum of 2156, 2239, 2541, 3222 and 3765 µg/g dry weight in roots and in case of shoots it was 312, 753, 911, 1335 and 1781 µg/g dry weight at 2nd, 4th, 6th, 8th and 10th day of harvesting and accumulation ranges from 553-3765 µg/g dry weight in roots and 150-1781 µg/g dry weight in shoots. The maximum accumulation was found on the 10th day at 4mg/L in both the roots and shoots and minimum was on the 2nd day (1mg/L). In *S. mucronatus*, the accumulation of lead accumulation in the root increased significantly ($p<0.05$) upto the 8th day of exposure but when exposure time (8th to 10th days) from 2 to 8 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. The lead accumulation in shoots increased significantly ($p<0.05$) upto the 4th day and there after it showed no significant increased ($p<0.05$) in accumulation with further increased of exposure time from 6th to 10th day.

Lead content in the roots and shoots of *R. rotundifolia* (Figure-3) was 459, 517, 522, 637 and 546 µg/g dry weight and 488, 548, 568, 681 and 683 µg/g dry weight respectively at 2th, 4th, 6th, 8th and 10th day of harvesting. Lead accumulation ranges from 208-637 and 346-683 µg/g dry weight in root and shoot. The maximum accumulation was on the 8th day (16mg/L) in roots and on the 10th day (4mg/L) in shoots while minimum accumulation was found on the 10th day (1mg/L) for both roots and shoots. In *R. rotundifolia*, lead accumulation in roots and shoots increased significantly ($p<0.05$) upto 2nd day, but with further increase in exposure time and concentration it showed no significant increased ($p>0.05$) in accumulation.

Lead content in *M. intermedium* roots and shoots (Figure-4) was 462, 511, 495, 528 and 455 µg/g dry weight and 481, 496, 511, 469 and 457µg/g dry weight at 2th, 4th, 6th, 8th and 10th day of harvesting. Lead accumulation ranges from 244-528 and 358-511 µg/g dry weight in root and shoot. The maximum accumulation was on the 8th day (16mg/L) in the roots and on the 6th day (8mg/L) in shoots, whereas, minimum accumulation in the roots was on the 10th day (8mg/L) and on the 6th day (8mg/L) in shoots. Lead accumulation in roots increased significantly ($p<0.05$) in accumulation, however in shoots showed no significant increase ($p<0.05$) in accumulation. In control plants, lead accumulation was below detection limit in all the three experimental plants.

The major factor influencing in metal uptake efficiency of plants depends on the metal concentration in the medium (Rai and Chandra, 1992). The accumulation of lead observed in the roots of *S. mucronatus*, is much higher as compared than *R. rotundifolia* and *M. intermedium*. It was found out that the roots of *S. mucronatus* accumulate higher Pb concentrations as compared to the shoots part. The accumulation of Pb in the shoots of an emergent plant is generally dependent on the roots as its primary source (John *et al*., 2008,). 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 (Xie and Yu, 2003) which is observed also in *S. mucronatus* with a long fine roots system and have a higher Pb concentration in the roots by increasing root water contact. Thus, higher concentration of Pb in the roots of *S. mucronatus* corroborates with earlier studies of Stoltz and Greger, (2002); Deng *et al*., (2004); Phetsombat *et al*., (2006).

It has been reported that metal accumulate in a relatively higher amount in submerged species as compared to emergent species (Rai *et al*., 1995; Noller and Parker, 1996). Hadad *et al*., (2010) reported that submerged plants have a greater surface area as compared to non-submerged species and their shoots are in constant contact with the water medium which may help them to bioconcentrate more nutrients and metals. Welsh & Denny, (1979) accounted that high Pb accumulation in the shoots is by absorption of Pb from the medium, which may be the probably reasons for high Pb accumulation in the shoots of *R. rotundifolia* and *M. intermedium*. Adsorption may also contribute to high Pb accumulation in the shoots of submerged species from the surrounding medium. Pb accumulation in the shoots of *R. rotundifolia* and *M. intermedium* may also occur probably via root-mediated precipitation and intracellular uptake, apoplast and symplast transport which also contribute to the high Pb accumulation. Thus the present study corroborates with earlier findings of Welsh and Denny, (1979); Wolterbeek and van der Meer, (2002), Fritioff and Greger, (2006).

Correlation and multiple regression analyses were conducted to examine the relationship between lead uptake by *S. mucronatus*, *R. rotundifolia*, *M. intermedium* and potential predictors (concentrations of lead 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 lead concentration in the medium for *S. mucronatus* and *R. rotundifolia*, indicating that with the increase in cadmium concentration in the medium tend to have a significant uptake of lead into the plant tissues but uptake is not significantly correlated with the lead concentration in the medium in the case for *M. intermedium*. However, in case of *S.mucronatus* the lead uptake is significantly correlated with time i.e., the no of days have a significant outcome on the uptake of lead, but time is not significantly correlated with lead uptake in *R. rotundifolia*, and *M. intermedium*.

The multiple regression model with all two predictors produced $R^2 = .564$, $F(2, 27) = 17.49$, $p < .001$, $R^2 = .282$, $F(2, 27) = 5.294$, $p < .05$ and $R^2 = .093$, $F(2, 27) = 1.389$, $p > .05$ for *S. mucronatus*, *R. rotundifolia*, and *M. intermedium* respectively. As can be seen in Table 1 and 2, the concentration of lead in the medium had significant positive regression weights, indicating with higher lead concentration in the medium were expected to have higher lead 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 *S. mucronatus* and have a significant regression weights, indicating that the uptake of lead in *S. mucronatus* also depends on time period, whereas in *R. rotundifolia* and *M. intermedium* time did not contribute to the multiple regression model and it is does not have a significant regression weights, indicating that uptake of lead in these two aquatic macrophytes does not fully depend on time period.

Bioconcentration factor (BCF) of lead

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 1271, 1007, 1387, 599 and 270 (Table-4) in *S. mucronatus* 529, 446, 300, 108 and 61 in *R. rotundifolia* (Table-5) and 813, 373, 200, 83 and 52 in *M. intermedium* (Table-6) respectively after 10^{th} day harvesting. The maximum (1387) BCF value was obtained in *S. mucronatus* treated with 4mg/L of cadmium at 10^{th} day, while in *R. rotundifolia* the maximum BCF value of 879 was obtained on the 2^{nd} day (1mg/L). In *M. intermedium* also the maximum BCF value of 962 was obtain at 8^{th} day at 1mg/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 then 1000 (Zayed *et al*., 1998). In the present study, the highest BCF of Pb was 1387 in *S. mucronatus*, 879 in *R. rotundifolia* and 909 in *M. intermedium* which indicates that *S. mucronatus* was a good accumulator of Pb while *R. rotundifolia* and *M. intermedium* maybe considered as a moderate accumulator of Pb. High BCF values also reflects the high accumulation potential of the plants which is an essential factor for phytoremediation (Andra *et al*., 2010).

Translocation factor (TF) of lead

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 (Fritioff *et al*., 2005). Yanqun *et al*., (2005) 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 (Zhao *et al*., 2002), metals sequestration in the leaf vacuoles and apoplast (Lasat *et al*., 2000). According to Yoon e*t al*., (2006) 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) were greater than one indicating better translocation of lead from roots to shoots parts, whereas in *S. mucronatus* (Table-7) the TF values were less than one indicating less Pb translocation from roots to shoots parts.

Deng *et al*., (2004) reported that emergent species accumulated high concentrations of metals in their roots but much less in their shoots, and the accumulation increased further with increased external concentration which is in accordance with the present study for *S. mucronatus*. The lack of toxic effects of lead on plants may be due to that Pb is being bound to the cell wall, thus rendering its ineffective in acting as a strong metabolic inhibitor (Buddhari *et al*., 1983). The higher accumulation of lead in *S. mucronatus* maybe due to a relatively low mobility of Pb to the shoots which is indicated by the increased lead accumulation in roots with increased of lead concentration in the medium which is in accordance with the findings of Sharpe and Denny, (1976) and Buddhari *et al*., (1983).

Our findings showed that a significant amount of Pb 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 Pb from the medium in addition to translocation from roots. Nonetheless, accumulation of Pb in submerged plant shoots may be also due to transient releases or diffusion of metal in the water followed by rapid adsorption to shoot tissues.

IV. Conclusion

In the present study, a laboratory experiment was carried out where all the external factors are controlled against Pb contamination in water. The present study indicates that all the three experimental plants were suitable for the phytoremediation of Pb contamination from water. Therefore, *S.mucronatus*, *R. rotundifolia* and *M. intermedium* could be useful for phytoremediation of Pb from contaminated water. Furthermore, field experiments are needed to 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	1875.16	482.788			
Time (in days	6.00	67.547	.348*	185.058*	.348
Concentrations (mg/L)	5.17	34.801	.666***	182.413***	.666

* 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	793.33	157.394			
Time (in days	6.00	22.021	.080	10.776	.080
Concentrations (mg/L)	5.17	11.345	.525**	36.497**	.525

* 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	736.57	156.244			
Time (in days	6.00	21.860	.029	3.422	.029
Concentrations (mg/L)	5.17	11.263	.304	18.686	.304

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

Table-4: Bioconcentration Factor for lead in *S. mucronatus*

Pb concentration (mg/L)	Bioconcentration Factor				
	2d	4d	6d	8d	10d
1	703	961	1142	967	1271
2	422	505	611	630	1007
4	617	748	863	1140	1387
8	154	203	312	363	599
16	74	99	168	189	270

Table-5: Bioconcentration Factor for lead in *R. rotundifolia*

Pb concentration (mg/L)	Bioconcentration Factor				
	2d	4d	6d	8d	10d
1	879	836	794	782	529
2	424	467	501	508	446
4	218	234	262	289	300
8	100	119	120	152	108
16	55	66	62	82	61

Table-6: Bioconcentration Factor for lead in *M. intermedium*

Pb concentration (mg/L)	Bioconcentration Factor				
	2d	4d	6d	8d	10d
1	909	848	917	962	813
2	398	471	440	419	373
4	222	235	227	231	200
8	107	124	126	119	83
16	53	57	58	62	52

Table-7: Translocation Factor for lead in *S. mucronatus*

Pb concentration (mg/L)	TF values				
	2d	4d	6d	8d	10d
1	0.27	0.21	0.25	0.41	0.33
2	0.27	0.22	0.28	0.29	0.95
4	0.14	0.34	0.36	0.41	0.47
8	0.18	0.40	0.36	0.57	0.53
16	0.16	0.20	0.39	0.54	0.58

Table-8: Translocation Factor for lead in *R. rotundifolia*

Pb concentration (mg/L)	TF values				
	2d	4d	6d	8d	10d
1	0.98	1.07	1.03	1.41	1.84
2	0.84	0.96	1.00	0.95	0.3
4	1.02	1.03	1.00	1.03	1.37
8	1.57	1.36	1.04	1.02	1.21
16	1.07	1.05	1.34	1.07	1.01

Table-9: Translocation Factor for lead in *M. intermedium*

Pb concentration (mg/L)	TF values				
	2d	4d	6d	8d	10d
1	0.97	0.91	1.00	1.04	0.79
2	0.85	0.84	0.82	0.74	0.99
4	1.12	0.98	0.93	0.87	1.03
8	1.28	1.01	1.03	0.89	1.71
16	0.99	0.97	0.98	0.89	1.24

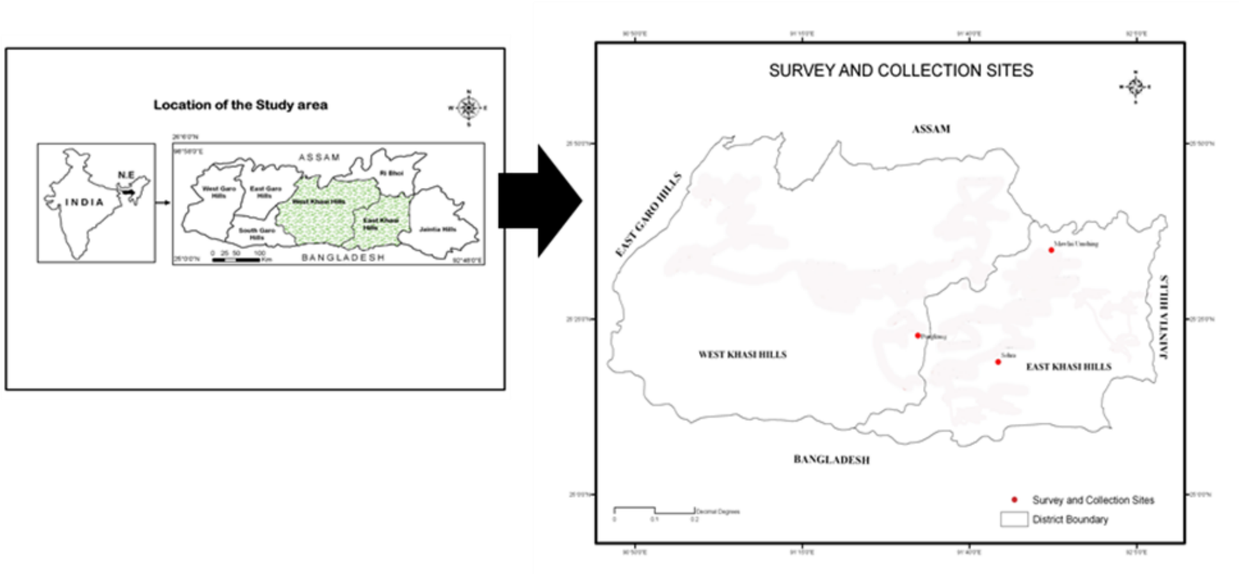

Figure- 1
Map showing location and collection sites of aquatic macrophytes

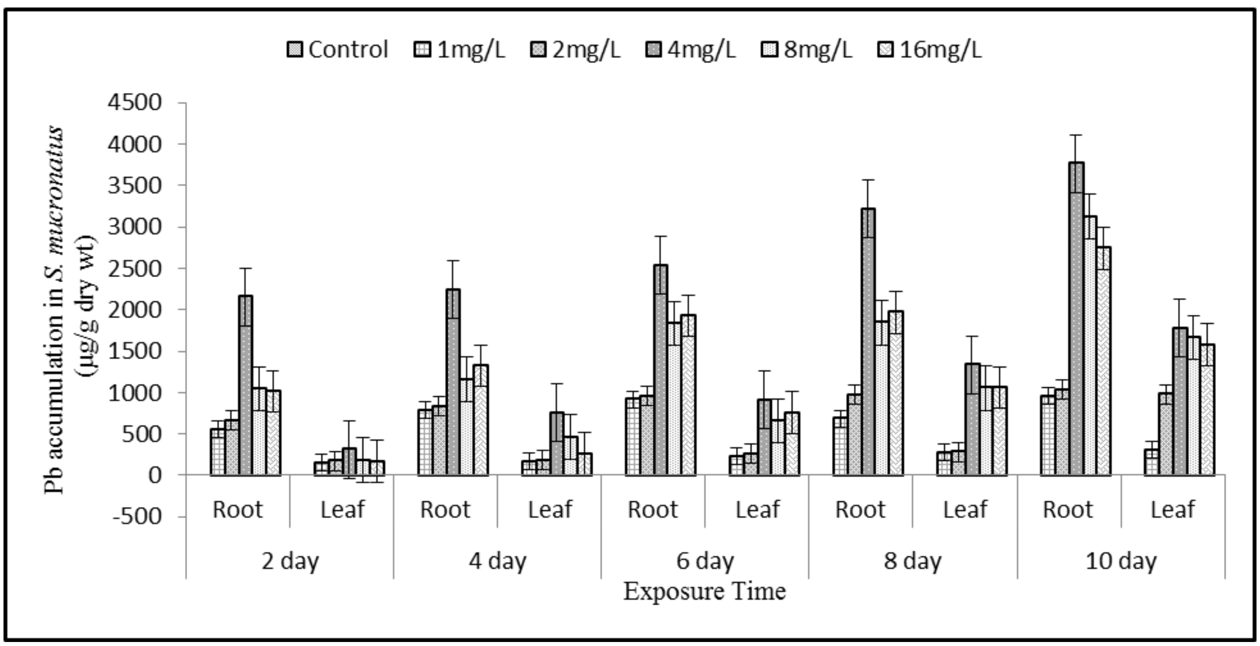

Figure-2
Lead accumulation in roots and shoots of *S. mucronatus*

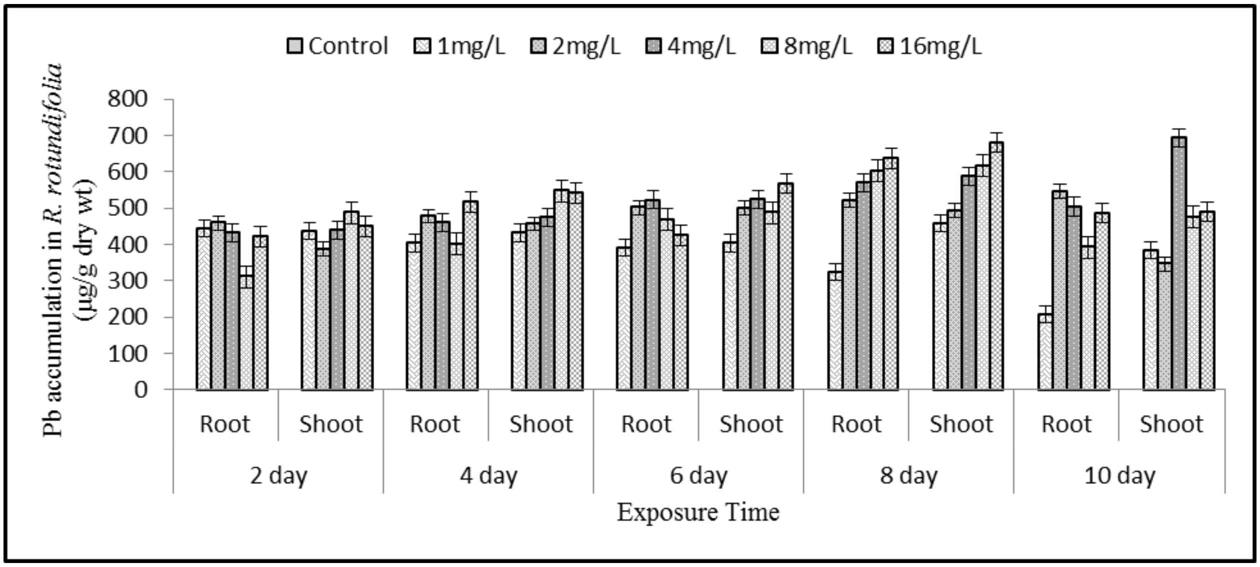

Figure- 3
Lead accumulation in roots and shoots of *R. rotundifolia*

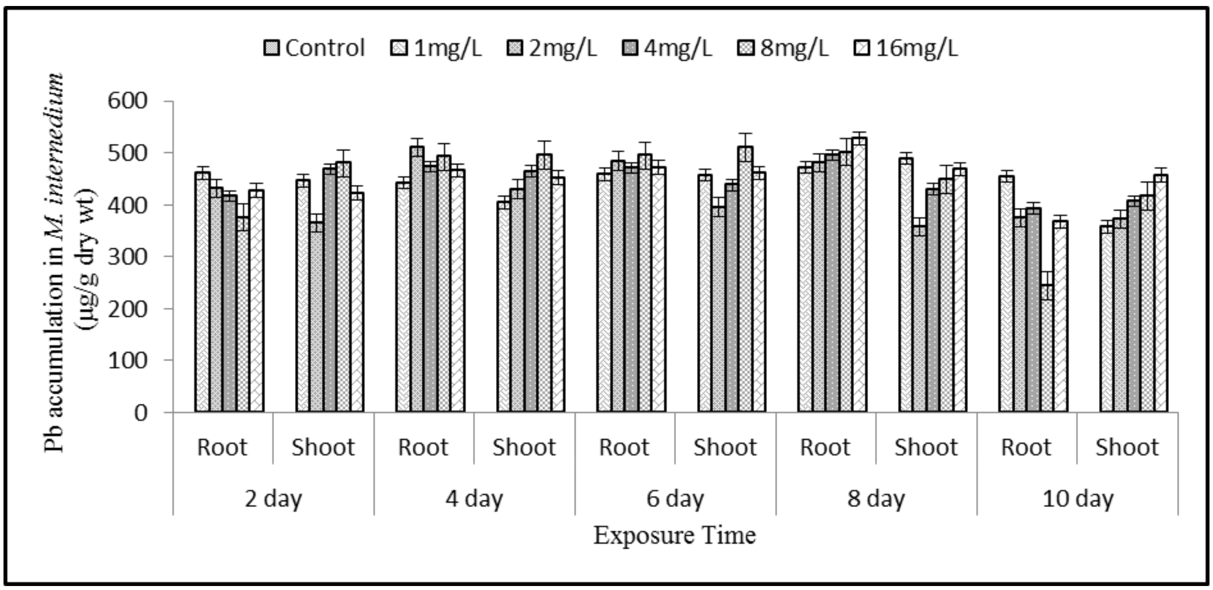

Figure- 4
Lead accumulation in roots and shoots of *M. intermedium*