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Cadmium uptake and Phytoremediation potential of three Aquatic Macrophytes of Meghalaya, India

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Abstract

Laboratory experiments were performed to evaluate the Cd 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 Cd 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 Cd uptake by these macrophytes showed a linear relationship for S. mucronatus and for R. rotundifolia with the exposure time period (2–10 d). Cd 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 8^{th} day in all the three aquatic macrophytes and translocation factor (TF) was at the 2^{nd} day for S. mucronatus and R. rotundifolia and at the 10^{th} day for M. intermedium respectively. The experimental results demonstrated that these three aquatic macrophytes have a phytoremediation potential for removing Cd from Cd-contaminated water.

Keywords: *Scripus mucronatus*, *rotala rotundifolia*, *Myriophyllum intermedium*, cadmium uptake, bioconcentration (BCF), translocation factor (TF).

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. Cadmium is one of the potent heavy metals found in the earth's crust. There are several sources of Cd in the environment which includes both from the natural and man-made sources. Notably, natural emissions and man-made industrial effluents emitted by ore processing industries and plants have immensely polluted water.

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[2] 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[3]. 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[4].

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

Material 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 2011 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 [5] 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 Cd 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 Cd concentrations at a time interval of 2, 4, 6, 8 and 10 days. Cd of analytical grade, were supplied as $CdCl_2$ (Himedia) were used as the source of Cd. 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 was also set up where no Cd was added into the medium were not added.

For digestion, the plant samples were carried out according to Kara and Zeytunluoglu[6]. Atomic Absorption Spectrophotometer (AAS 3110, Perkin-Elmer) was used to determine the Cd 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[7].

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

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[8].

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

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 11.5. 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 Cd concentrations.

Results and Discussion

Accumulation of cadmium: Cadmium 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 cadmium concentration of 1, 2, 4, 8 and 16mg/L, the cadmium content (figure-2) in *S. mucronatus* roots increased to the maximum 1584, 2334, 4561, 4979 and 2749 µg/g dry weight and in case of shoots it was 560, 1197, 1353, 2069 and 574 µg/g dry weight at 2^{th} , 4^{th}, 6^{th}, 8^{th} and 10^{th} day of harvesting and accumulation ranges from 136-4979 µg/g dry weight in roots and 60-2069 µg/g dry weight in shoots. The maximum accumulation was on the 8^{th} day at 8mg/L in both the roots and shoots and minimum was on the 2^{nd} day (1mg/L) in roots and 4^{th} day (1mg/L) in shoots. In *S. mucronatus*, the accumulation of cadmium in the root and shoot increased significantly ($p<0.05$) from 2 to 8 days however, there is no significant increase ($p<0.05$) of metal accumulation in the root and shoot from 8^{th} day to 10^{th} days and this may be suggest that *S. mucronatus* approached its maximum accumulation on the 8^{th} day.

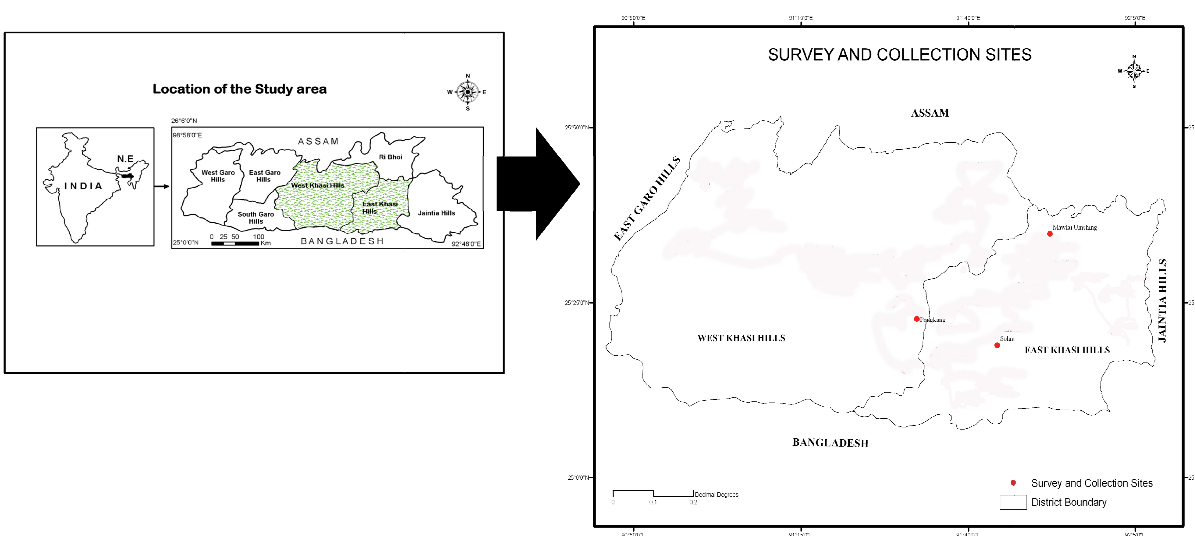

Figure- 1
Map showing location and collection sites of aquatic macrophytes

Cadmium content in the roots and shoots of *R. rotundifolia* (figure-3) was 1597, 2343, 2437, 2448 and 2540 µg/g dry weight and 2567, 2713, 2884, 3309 and 2881 µg/g dry weight respectively at 2th, 4th, 6th, 8th and 10th day of harvesting. Cadmium accumulation ranges from 240-2540 and 340-3309 µg/g dry weight in root and shoot. The maximum accumulation was on the 10th day (16mg/L) in roots and on the 8th day (16mg/L) in shoots while minimum accumulation was found on the 2nd day (1mg/L) for both roots and shoots. In *R. rotundifolia*, cadmium accumulation is significantly different ($p<0.05$) at different concentrations of Cd and time.

Cadmium content in *M. intermedium* roots and shoots (figure-4) was 4646, 5360, 7344, 4097 and 1982 µg/g dry weight and 3012, 5619, 8300, 6845 and 6321 µg/g dry weight at 2th, 4th, 6th, 8th and 10th day of harvesting. Cadmium accumulation ranges from 262-7344 and 249-8300 µg/g dry weight in root and shoot. The maximum accumulation was on the 6th day (16mg/L) in both the roots and shoots, whereas, minimum accumulation in the roots was on the 10th day (1mg/L) and at 2nd day (1mg/L) in shoots. Cadmium accumulation in the roots and shoots treated with 8 and 16mg/L is significantly different ($p<0.05$) in Cd accumulation with time. In control plants, Cd accumulation were below detection limit in all the three experimental plants.

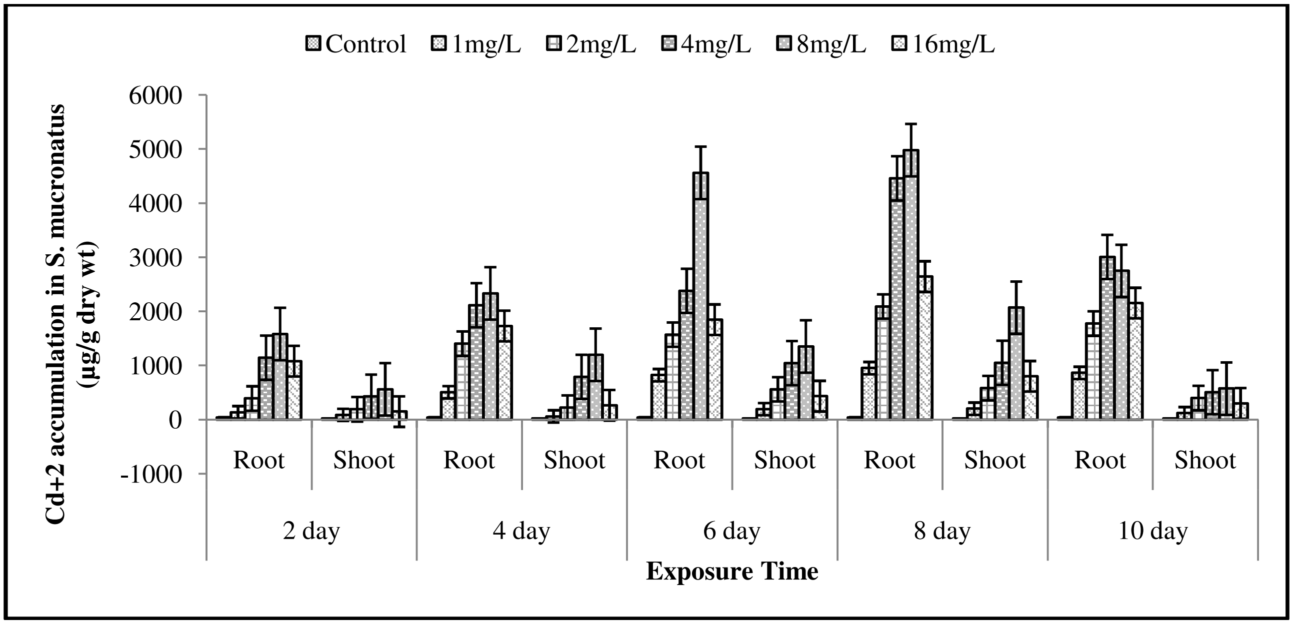

Figure-2
Cadmium accumulation in roots and shoots of *S. mucronatus*

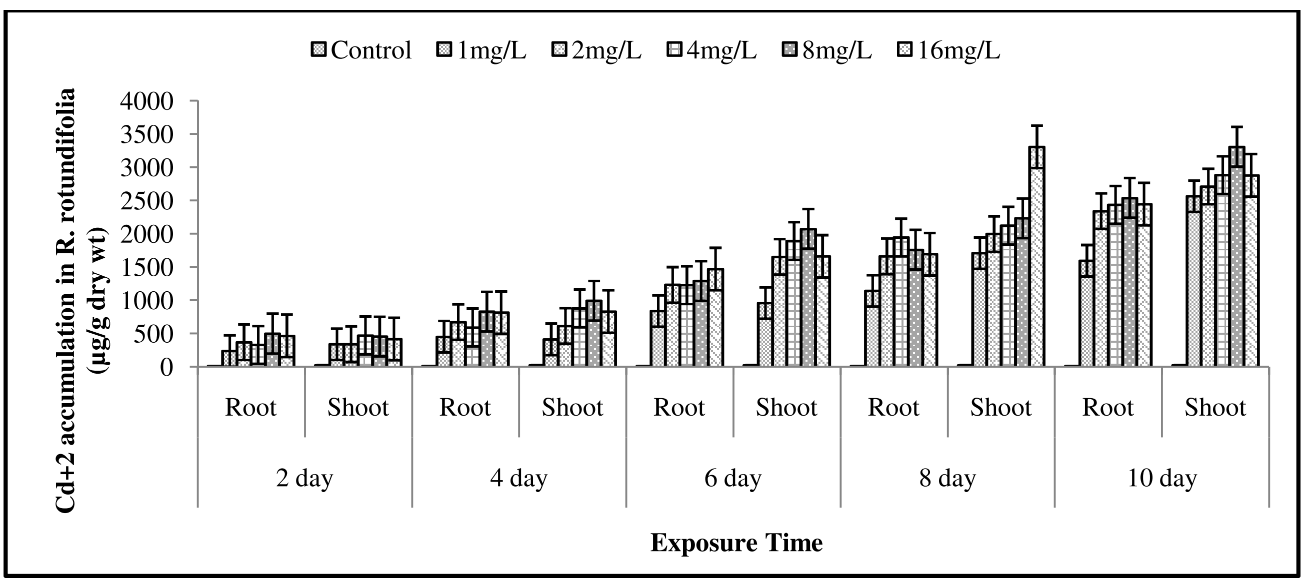

Figure- 3
Cadmium accumulation in roots and shoots of *R. rotundifolia*

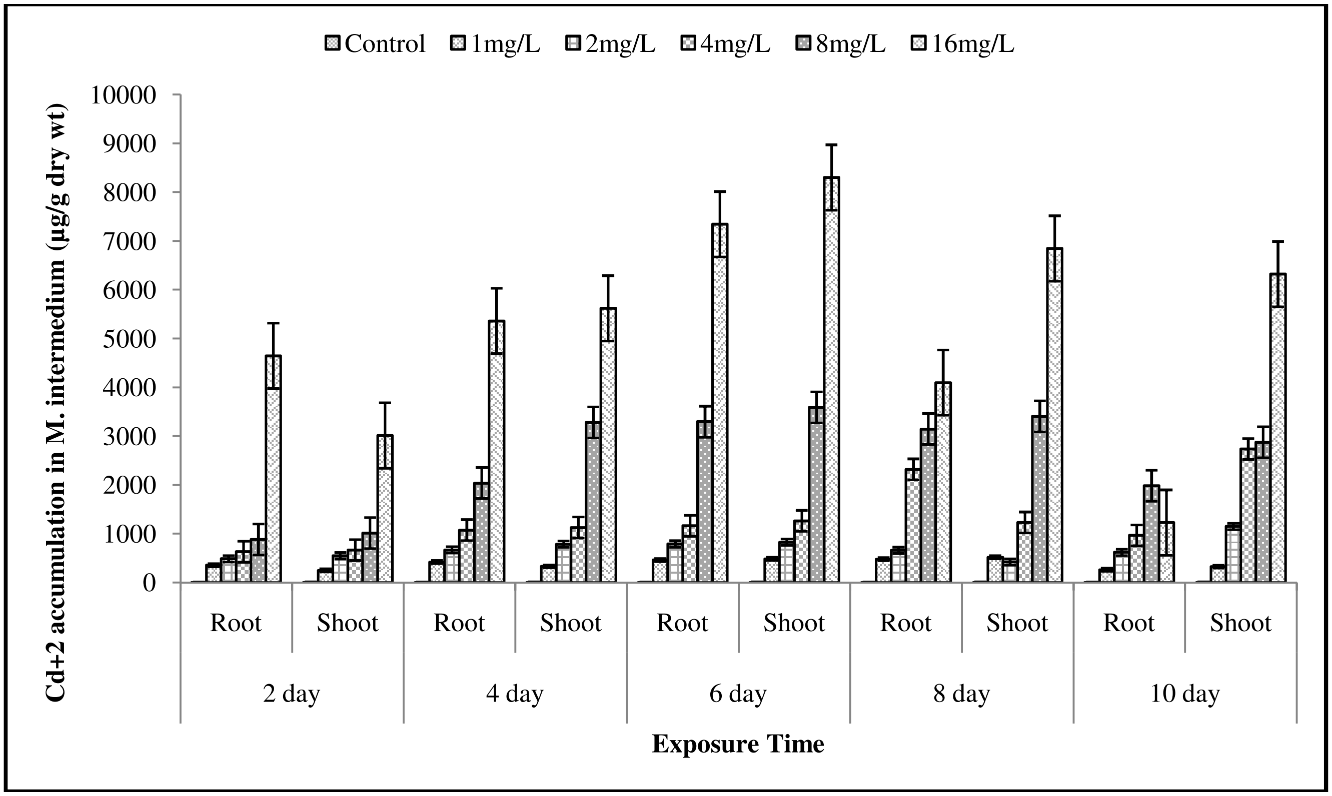

Figure- 4
Cadmium accumulation in roots and shoots of *M. intermedium*

Generally it is suggested that the important uptake route in plants are the roots, and it is expected that roots will have a higher uptake as compared to the shoot [9]. Cd ion is fast mobile and can be easily taken up by the roots and translocate to the upper parts of the plants [10]. In the roots of *S. mucronatus* the accumulation of Cd is directly proportional with the increase of Cd in the medium which corroborates with the findings by Deng *et al.*[8]; Sersen *et al*[11]; Sasmaz *et al.*[12] and Hussain *et al*[13]. It was found out that the roots of *S. mucronatus* accumulate higher Cd concentrations as compared to the shoots part. The accumulation of Cd in the shoots of an emergent plant generally dependent on the roots as its primary source[14]. 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[15] which is observed also in *S. mucronatus* with a long fine roots system and have a higher Cd concentration in the roots by increasing rootwater contact. Thus, higher concentrations of Cd in the roots of *S. mucronatus* which corroborates with earlier studies of Stoltz and Greger[16]; Ali *et al.*[17]; Phetsombat *et al.*[18].

Hadad *et al.*[19] reported that submerged plants have a greater surface area as compared to non-submerged plants and their shoots are in constant contact with the water medium which may help them to bioconcentrate more nutrients and metals. However, in the present study it was observed that the considerable amount of cadmium accumulated by *M. intermedium* and *R. rotundifolia* was in the shoots but not in roots. The shoots of the two submerged species also receives Cd form the roots but however due to their constant contact with the medium they may have also accumulate Cd directly from the medium to a greater extent as compared to *S. mucronatus* an emergent species which corroborates with the findings of Dunbabin and Bowmer[20].

Correlation and multiple regression analyses were conducted to examine the relationship between cadmium uptake by *S.mucronatus*, *R. rotundifolia*, *M. intermedium* and potential predictors (concentrations of cadmium 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 cadmium concentration in the medium for *R. rotundifolia*, and *M. intermedium*, indicating that with the increase in cadmium concentration in the medium tend to have a significant uptake of cadmium into the plant tissues but uptake is not significantly correlated with the cadmium concentration in the medium in the case for *S.mucronatus*. However, in all the three macrophytes the cadmium uptake is not significantly correlated with time i.e., the number of days does not have any significant outcome on the uptake of cadmium.

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 (µg/g dry wt)	2114.53	748.903			
Time (in days)	6.00	104.779	.298	188.334	.298
Concentrations (mg/L)	5.17	53.983	.409	133.219	.409

* 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 (µg/g dry wt)	2313.61	306.996			
Time (in days)	6.00	42.952	.147	94.081	.147
Concentrations (mg/L)	5.17	22.129	.925***	304.005***	.925

* 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 (µg/g dry wt)	3409.82	691.564			
Time (in days)	6.00	96.757	.087	119.705	.087
Concentrations (mg/L)	5.17	49.850	.926***	653.045***	.926

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

The multiple regression model with all two predictors produced $R^2 = .202$, $F(2, 27) = 4.660$, $p < .001$, $R^2 = .868$, $F(2, 27) = 96.762$, $p < .001$ and $R^2 = .855$, $F(2, 27) = 86.573$, $p < .001$ for *S. mucronatus*, *R. rotundifolia*, and *M. intermedium* respectively. As can be seen in table 2 and 3, the concentration of cadmium in the medium had significant positive regression weights, indicating with higher cadmium concentration in the medium were expected to have higher cadmium uptake in *R. rotundifolia*, and *M. intermedium* but not in the case for *S.mucronatus* (table-1). Time i.e., number of days did not contribute to the multiple regression model and it is does not have a significant regression weights, indicating that uptake of cadmium in all the three macrophytes does not fully depend on time period.

Bioconcentration factor (BCF) of cadmium: 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 values of cadmium for all the three experimental plants increased with exposure periods till the 8th day of exposure time and decreased with futher increase of exposure period. The maximum (1377) BCF value was obtained in *S. mucronatus* (table-4) treated with 4mg/L of cadmium after 8th day of harvesting while it was 956 in *R. rotundifolia* (table-5) and 995 in *M. intermedium* (table-6) at 1mg/L of cadmium after 8th day exposure. 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 [21]. In the present study, the BCF values of *S. mucronatus* was 1377, which suggest that it maybe be considered as a good accumulator of cadmium (BCF: 1377) as compared to *R. rotundifolia* (BCF: 956) and *M. intermedium* (BCF: 995) and they may be considered as a moderate accumulator. The response of aquatic macrophytes to cadmium concentration vary from species to species in terms of metal accumulation and BCF, some have shown to have a very high BCF value of cadmium[18,22], while some have a low BCF values[18,].

Translocation Factor (TF) of cadmium: 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 are known as hyperaccumulator plants. The TF values for *R. rotundifolia* and *M. intermedium* under different treatments are shown in table-8 and table-9. From the tables it appeared that in most of the treatments the TF value was greater than one indicating that cadmium is translocate from the roots to shoots parts whereas in *S. mucronatus* (table-7) the TF values were found to be less than one which indicates a lesser amount of cadmium is translocate from roots to shoots. In *R. rotundifolia* and *M. intermedium*, there is a significant increase in cadmium translocation, but with the increase of cadmium concentration in the the medium there is a reverse effect on the cadmium translocation to the shoot part, but not in the case of *S. mucronatus*. It may be suggested that the cadmium translocation to the aerial parts of *R. rotundifolia* and *M. intermedium* reached saturation or reduced or more or less static at a higher cadmium concentration in the medium (8 and 16 mg/L). The roots of the emergent aquatic plant (*S. mucronatus*) accumulate higher concentrations of cadmium than shoots, which indicate that limited cadmium translocation occur from the roots to the shoots[12]. The decreased cadmium in the *S. mucronatus* leaves of the present study may be to casparian bands acting as effective barrier for the movement of cadmium into the stele[28].

Cadmium ions are non-essential nutrients for many plant species but they are readily taken up by roots and translocate to the shoots[29]. The high accumulation of cadmium in roots and shoots of submerged species of *R. rotundifolia* and *M. intermedium* was also observed by Lu *et al.*[30]. Translocation of ions is possible apoplastic in the phloem and acropetally in the xylem[9], therefore, *R. rotundifolia* and *M. intermedium* being a submerged plant the roots and shoots are in direct contact with the cadmium concentrations in the medium and it may has the chance of direct cadmium accumulation via the shoots (apoplastic translocation) thereby, high cadmium accumulation was observed in the shoots of *R. rotundifolia* and *M. intermedium* but not in the case of *S. mucronatus* an emergent plant.

Table-4
Bioconcentration Factor for cadmium in *S. mucronatus*

Cd concentration (mg/L)	Bioconcentration Factor				
	2d	4d	6d	8d	10d
1	225	566	1018	1156	984
2	292	814	1067	1338	1088
4	393	727	857	1377	878
8	268	441	739	881	415
16	77	125	143	215	153

Table-5
Bioconcentration Factor for cadmium in *R. rotundifolia*

Cd concentration (mg/L)	Bioconcentration Factor				
	2d	4d	6d	8d	10d
1	580	715	803	956	890
2	434	645	737	912	825
4	450	722	781	841	783
8	357	457	509	499	465
16	260	316	332	359	338

Table-6
Bioconcentration Factor for cadmium in *M. intermedium*

Cd concentration (mg/L)	Bioconcentration Factor				
	2d	4d	6d	8d	10d
1	605	750	946	995	587
2	517	728	808	540	883
4	324	550	607	888	926
8	237	665	861	819	607
16	479	686	978	684	472

Table-7
Translocation Factor for cadmium in *S. mucronatus*

Cd concentration (mg/L)	TF values				
	2 day	4 day	6 day	8 day	10 day
1	0.65	0.12	0.24	0.21	0.14
2	0.49	0.16	0.36	0.28	0.23
4	0.37	0.37	0.44	0.24	0.17
8	0.35	0.51	0.30	0.42	0.21
16	0.14	0.15	0.23	0.30	0.14

Table-8
Translocation Factor for cadmium in *R. rotundifolia*

Cd concentration (mg/L)	TF values				
	2 day	4 day	6 day	8 day	10 day
1	1.42	0.92	1.43	0.91	0.90
2	0.91	0.91	1.49	1.20	1.02
4	1.14	1.34	1.54	1.60	1.13
8	1.50	1.20	1.09	1.27	1.19
16	1.61	1.16	1.18	1.35	1.13

Table-9
Translocation Factor for cadmium in *M. intermedium*

Cd concentration (mg/L)	TF values				
	2 day	4 day	6 day	8 day	10 day
1	0.70	0.79	1.05	1.08	1.24
2	1.13	1.18	1.04	0.64	1.86
4	1.05	1.05	1.09	0.53	2.83
8	1.15	1.61	1.09	1.08	1.45
16	0.65	1.05	1.13	1.67	5.15

Conclusion

In the present study, a laboratory experiment was carried out where all the external factors are controlled against Cd contamination in water. The present study indicates that all the three experimental plants were suitable for the phytoremediation of Cd contamination from water. Therefore, *S.mucronatus*, *R. rotundifolia* and *M. intermedium* could be useful for phytoremediation of Cd from contaminated water. Furthermore, field experiments are needed to carry out their Phytoremediation potentials of these plants for phytoremediation technique.

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