Assessment of Cr^{+6} Accumulation and Phytoremediation Potential of Three Aquatic Macrophytes of Meghalaya, India

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Abstract: ***A laboratory experiment was conducted to examine Cr^{+6} uptake capacities of three aquatic macrophytes (Scripus mucronatus, Rotala rotundifolia and Myriophyllum intermedium). The selected macrophytes were transferred to the laboratory containing nutrient solution enriched with 1.0, 2.0, 4.0, 8.0 and 16 mg L^{-1} of Cr^{+6} and were separately harvested after 2, 4, 6, 8 and 10 days. The bioaccumulation study showed a linear relationship for S. mucronatus, R. rotundifolia and M. intermedium plant parts with the exposure time (2–10 d). Cr^{+6} concentrations were found to be higher in the root than the shoot in S. mucronatus but reverse in the case of R. rotundifolia and M. intermedium. The maximum bioconcentration factor (BCF) and translocation factor (TF) value were calculated as 1034 and 0.68 for S. mucronatus, 444 and 2.36 for R. rotundifolia and 1048 and 3.37 in M. intermedium respectively. The experimental results demonstrated that the S. mucronatus and M. intermedium can be used for removal of Cr^{+6} from Cr^{+6} -contaminated water.***

Keywords: Scripus mucronatus, Rotala rotundifolia and Myriophyllum intermedium, Cr^{+6}, Bioconcentration factor (BCF)

1. Introduction

Chromium is an essential element for humans and animals (Mertz, 1967), but can be toxic to plants in its common oxidation states, $Cr^{+3}$and Cr^{6+} (Bartlett and James, 1979). Chromium is introduced into the ecosystem as a result of different anthropogenic and industrial activities such as in the production of steel and alloys, pigment manufacturing, plating, combustion of coal and oil, and leather tanning, chrome leather, chromium plating, wood preservation electroplating cleaning agents, catalytic manufacture and in the production of chromic acid and specialty chemicals (Shanker, *et al*., 2005, Sune, *et al.*, 2007). 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 Cr^{+6} and phytoremediation potential of *S. mucronatus R. rotundifolia* and *M. intermedium* for Cr^{+6} 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 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.

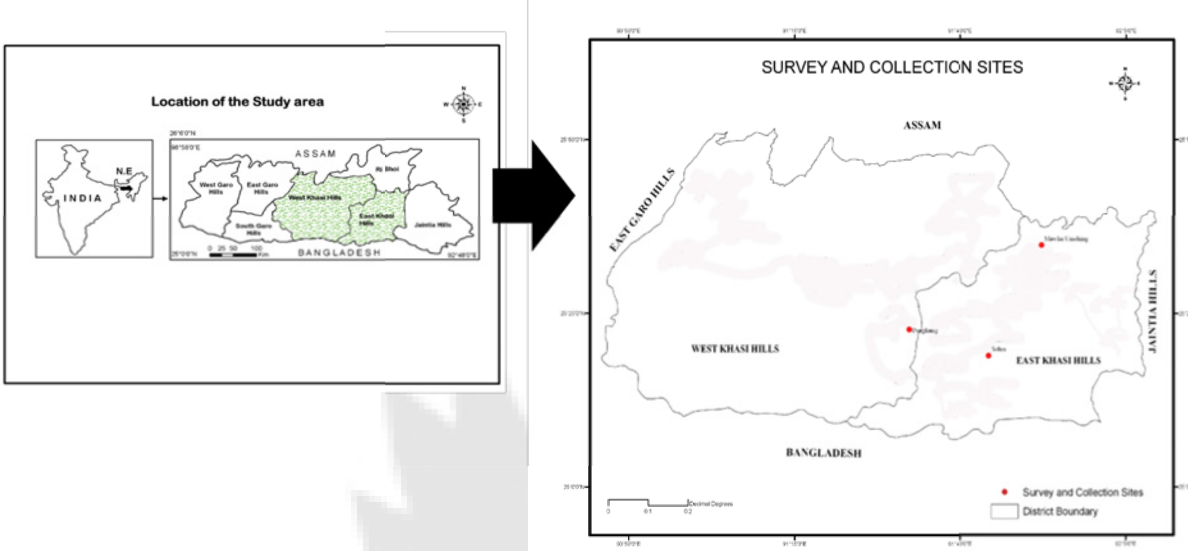

Figure 1: Map showing location and collection sites of aquatic macrophytes

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 Cr^{+6} 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 Cr^{+6} concentrations at a time interval of 2, 4, 6, 8 and 10 days. Cr^{+6} of analytical grade, were supplied as $K_2Cr_2O_7$ (Himedia) were used as the source of Cr^{+6}. Experiments were carried out separately for the three aquatic macrophytes under controlled temperature (24±1^{0}C) 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 Cr^{+6} were added into the medium was not added.

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 Cr^{+6} 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.

3. Statistics analyses

ANOVA and multiple linear regressions were performed for all the data to confirm their validity using SPSS 17. 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 Cr^{+6} concentrations.

4. Results and Discussion

4.1 Accumulation of Cr^{+6}

Cr^{+6} 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 Cr^{+6} concentration of 1, 2, 4, 8 and 16mg/L, the Cr^{+6} content (Fig-2) in *S. mucronatus* roots increased to the maximum 1994, 2802, 3793 4035 and 3923 µg/g dry weight in roots and in case of shoots it was 1052, 963, 871, 1044 and 683 µg/g dry weight at 2th , 4th, 6th, 8th and 10th day of harvesting and accumulation ranges from 250-4035 µg/g dry weight in roots and 70-1052 µg/g dry weight in shoots. The maximum accumulation was found on the 8th day (16mg/L) and minimum on 2nd day (16mg/L) of exposure time in both the roots and shoots. The accumulation of Cr^{+6} in the roots and shoots increased significantly ($p<0.05$) with lower metal concentration (1 to 2 mg/L) and passage of time (10th day) however, with the increased of concentration (4 to 16 mg/L) and exposure time (6th to 10th day) show no significant increase ($p<0.05$) of metals accumulation. This may be suggesting that *S. mucronatus* approached their maximum accumulation within 4th day of exposure time.

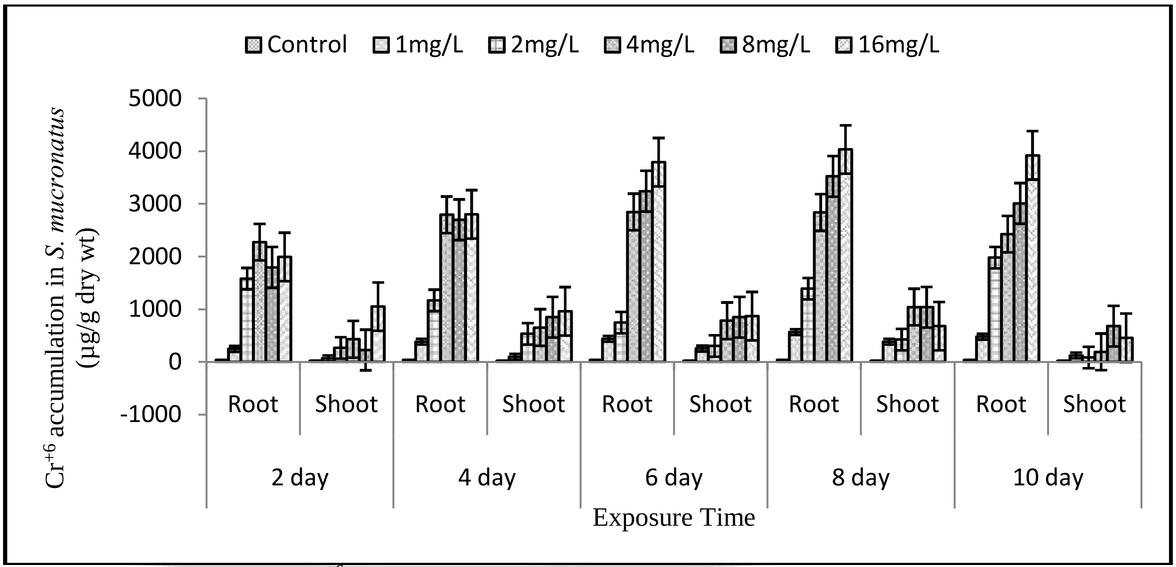

Figure 2: Cr^{+6} accumulation in roots and shoots of *S. mucronatus*

Cr^{+6} content in the roots and shoots of *R. rotundifolia* (Fig 3) was 398, 602, 807, 695 and 586 µg/g dry weight and 658, 819, 847, 724 and 661 µg/g dry weight respectively at 2th, 4th, 6th, 8th and 10th day of harvesting. Cr^{+6} accumulation ranges from 86-807 µg/g dry weight in roots and 125-847 µg/g dry weight in shoots. The maximum accumulation was on the 6th day (16mg/L) of exposure time in both roots and shoots, while minimum accumulation was on the 2nd day (1mg/L) in the roots and shoots. The accumulation of Cr^{+6} in the roots and shoots increased significantly ($p<0.05$) upto the 6th 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 reached a maximum at 6th day.

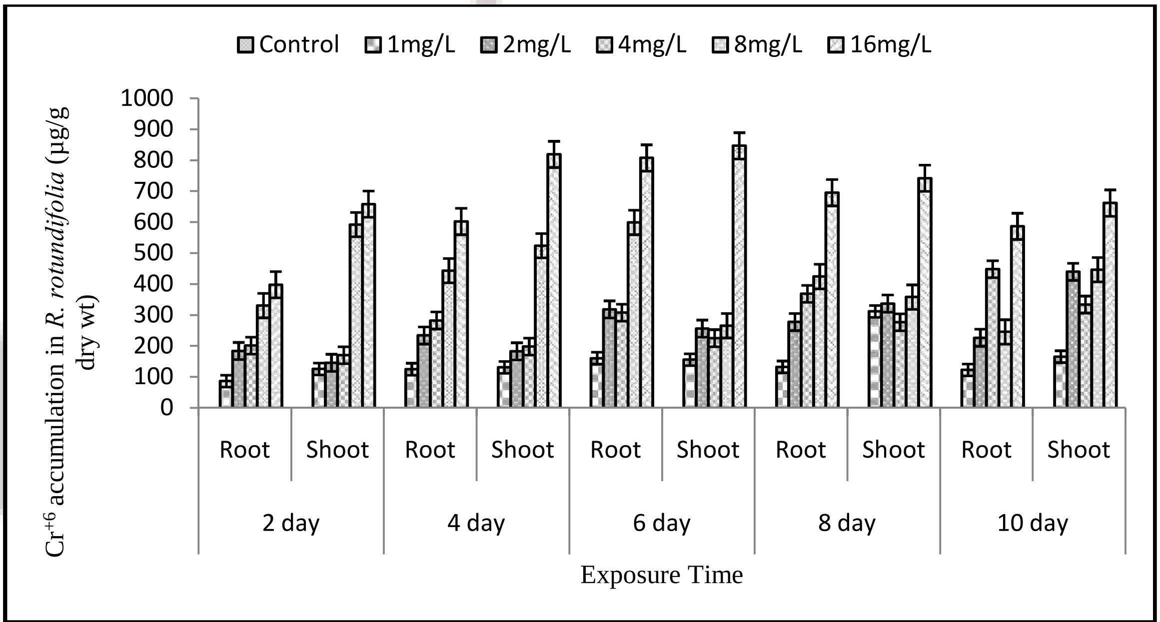

Figure 3: Cr^{+6} accumulation in roots and shoots of *R. rotundifolia*

Cr^{+6} content in *M. intermedium* roots and shoots (Fig 4) was 2738, 809, 2839, 2210 and 1966 µg/g dry weight and 854, 2639, 4048, 3789 and 2374 µg/g dry weight in the roots and shoots at 2th, 4th, 6th, 8th and 10th day of harvesting. Cr^{+6} accumulation ranges from 241-2839 µg/g dry weight in roots and 338-4048 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 Cr^{+6} in the roots and shoots increased significantly ($p<0.05$) with exposure time (2nd to 6th days) 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 Cr^{+6} in the tissue level may be approached its maximum accumulation on the 6th day. In control plants, Cr^{+6} accumulations was below detection limit in all the three experimental plants.

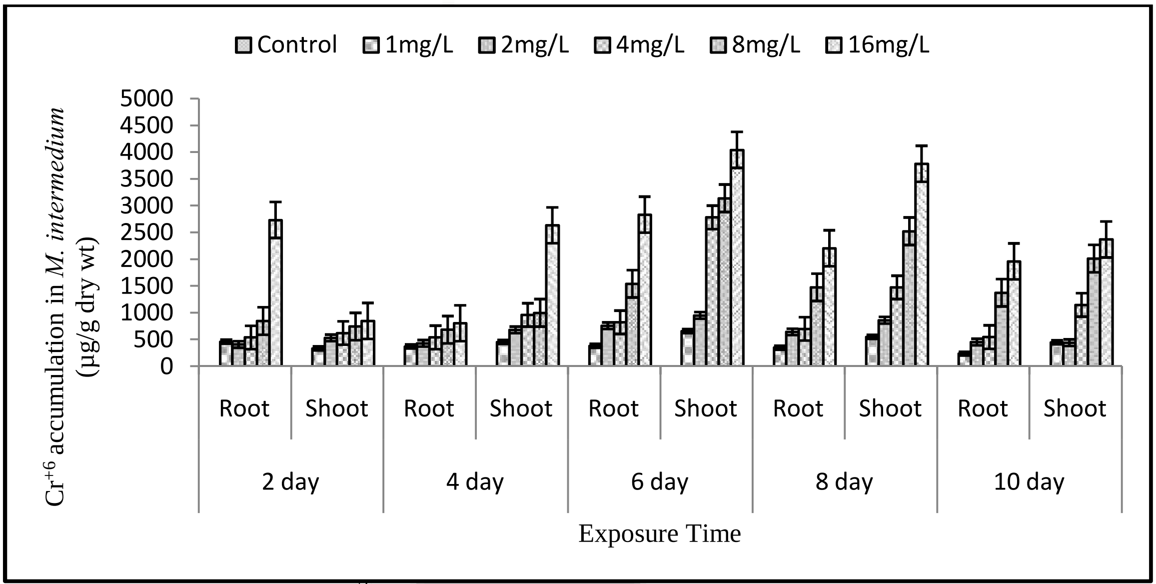

Figure 4: Cr^{+6} accumulation in roots and shoots of *M. intermedium*

Results indicated that all the three macrophytes have the ability to uptake Cr^{+6} from the surrounding solution as in agreement with the reports of earlier studies that aquatic plant tend to adapt themselves to cope-up with chromium toxicity (Gupta, *et al*., 1994; Garg and Chandra, 1994). The Cr^{+6} uptake ability to the roots and shoots parts of different aquatic macrophytes varied with species. In the present study, Cr^{+6} uptake was higher in the roots in comparison to shoots in *S. mucronatus* which corroborate with the findings of Vajpayee, *et al*., (2001). The absorption pattern in the present study corroborated with the findings of Qian, *et al*., (1999) where emergent species have high accumulates in roots and lowest accumulations in shoots. The accumulation of Cr^{+6} in the shoot of an emergent plant generally depend on the roots as its primary source (Maine, *et al*., 2001). 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 (Meharg and Macnair, 1991) which is observed also in *S. mucronatus* with a long fine roots system and have a higher Cr^{+6} concentration in the roots by increasing root water contact. In the present study, high concentration of Cr^{+6} in the roots of *S. mucronatus* was found which corroborates with earlier studies of Baldtantoni, *et al*., (2004).

Chromium concentrations in submerged macrophytes showed higher accumulations of Cr^{+6} in the shoots than emergent ones (Outridge and Noller, 1991). In the present study *R. rotundifolia* and *M. intermedium* accumulates more Cr^{+6} in the shoots than the roots which is in accordance to the findings by Chandra, *et al*., (1993); Rai, *et al*., (1996) in plants occurring in polluted waters. *R. rotundifolia* and *M. intermedium* showed higher levels of Cr^{+6} accumulation in their plant tissues which is similar with the findings of Garg and Chandra, (1990) and Rai, *et al*., (1995) in other submerged macrophyte, but disagree with the findings of Vajpayee, *et al*., (2001) where *Vallisneria spiralis* accumulate high concentration of Cr^{+6} in the roots when cultured in nutrient solution containing Cr^{+6}.

Correlation and multiple regression analyses were conducted to examine the relationship between Cr^{+6} uptake by *S.mucronatus*, *R. rotundifolia*, *M. intermedium* and potential predictors (concentrations of Cr^{+6} 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 Cr^{+6} concentration in the medium for *S.mucronatus*, *R. rotundifolia*, and *M. intermedium*, indicating that with the increase in concentration in the medium they tend to have higher uptake of Cr^{+6} into the plant tissues. However, in all the three macrophytes the Cr^{+6} uptake is not significantly correlated with time i.e., the number of days does not have any significant effect on the uptake of Cr^{+6} to the plant tissues.

The multiple regression model with all two predictors produced $R^2 = .666$, $F_{(2, 27)} = 26.97$, $p < .001$, $R^2 = .869$, $F_{(2, 27)} = 89.89$, $p < .001$ and $R^2 = .777$, $F_{(2, 27)} = 46.99$, $p < .001$ for *S. mucronatus*, *R. rotundifolia*, and *M. intermedium* respectively. As can be seen in Table 1, 2 and 3, the concentration of Cr^{+6} in the medium had significant positive regression weights, indicating with higher Cr^{+6} concentration in the medium were expected to have higher Cr^{+6} uptake in

S.mucronatus, *R. rotundifolia*, and *M. intermedium*. 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 Cr^{+6} in all the three macrophytes does not fully depend on time period.

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

Variable	*mean*	*std*	*correlation with uptake*	*multiple regression weights*	
				B	β
Uptake	2221.9000	1640.37349			
Time (in days	6.0000	2.87678	.144	81.875	.144
Concentrations (mg/L)	5.1667	5.58374	.804***	236.099***	.804

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

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

Variable	*mean*	*std*	*correlation with uptake*	*multiple regression weights*	
				B	β
Uptake	599.20	78.313			
Time (in days	6.00	10.957	.103	16.258	.103
Concentrations (mg/L)	5.17	5.645	.927***	75.227***	.927

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

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

Variable	*mean*	*std*	*correlation with uptake*	*multiple regression weights*	
				B	β
Uptake	2010.83	413.327			
Time (in days	6.00	57.829	.155	98.883	.155
Concentrations (mg/L)	5.17	29.794	.868***	284.322***	.868

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

4.2 Bioconcentration factor (BCF) of Cr^{+6}

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 608, 1034, 656, 462 and 274 in *S. mucronatus* (Table 4), 288, 333, 196, 86 and 78 in *R. rotundifolia* (Table 5) and 693, 452, 426, 425 and 271 in *M. intermedium* (Table 6) respectively after 10th day harvest. The maximum BCF was 1034 in *S. mucronatus* when treated with 2mg/L of Cr^{+6} at 10th day, 444 in *R. rotundifolia* in the 8th day at 1mg/L Cr^{+6} concentration and 1048 in *M. intermedium* in the 6th day at 1mg/L of Cr^{+6} concentration respectively.

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 (Zayed, *et al*., 1998). In the present study, the BCF values of *S. mucronatus* (1034) and *M. intermedium* (1048) was above 1000, which may be considered as a good accumulator of Cr^{+6} as compared to *R. rotundifolia* (444).

Table 4: Bioconcentration Factor for Cr^{+6} in *S. mucronatus*

Cr^{+6} concentration (mg/L)	*Bioconcentration Factor*				
	2d	4d	6d	8d	10d
1	320	486	697	956	608
2	926	852	528	909	1034
4	677	862	908	971	656
8	253	444	512	571	462
16	190	235	291	295	274

Table 5: Bioconcentration Factor for Cr^{+6} in *R. rotundifolia*

Cr^{+6} concentration (mg/L)	Bioconcentration Factor				
	2d	4d	6d	8d	10d
1	212	256	316	444	288
2	165	208	287	307	333
4	93	120	133	161	196
8	115	121	108	98	86
16	66	89	103	90	78

Table 6: Bioconcentration Factor for Cr^{+6} in *M. intermedium*

Cr^{+6} concentration (mg/L)	Bioconcentration Factor				
	2d	4d	6d	8d	10d
1	802	833	1048	908	693
2	475	560	860	757	452
4	293	378	904	546	426
8	200	212	586	501	425
16	224	215	430	375	271

4.3 Translocation factor (TF) of Cr^{+6}

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 and Greger, 2006). 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*., 2001), 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) was greater than one in most of the treatments indicating the translocation of Cr^{+6} 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. In *S. mucronatus* Cr^{+6} 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 Meharg and Macnair, (1991) and Aksorn and Visoottiviseth, (2004).

Table 7: Translocation Factor for Cr^{+6} in *S. mucronatus*

Cr^{+6} concentration (mg/L)↓	TF values				
	2d	4d	6d	8d	10d
1	0.28	0.26	0.58	0.68	0.26
2	0.17	0.46	0.41	0.31	0.04
4	0.19	0.23	0.28	0.37	0.08
8	0.13	0.32	0.26	0.30	0.23
16	0.53	0.34	0.23	0.17	0.12

Table 8: Translocation Factor for Cr^{+6} in *R. rotundifolia*

Cr^{+6} concentration (mg/L)↓	TF values				
	2d	4d	6d	8d	10d
1	1.46	1.05	0.97	2.36	1.35
2	0.79	0.78	0.80	1.22	1.94
4	0.85	0.70	0.73	0.75	0.74
8	1.79	1.18	0.44	0.84	1.82
16	1.65	1.36	1.05	1.07	1.13

Table 9: Translocation Factor for Cr^{+6} in *M. intermedium*

Cr^{+6} concentration (mg/L)↓	TF values				
	2d	4d	6d	8d	10d
1	0.73	1.22	1.71	1.56	1.87
2	1.29	1.58	1.25	1.34	0.97
4	1.15	1.77	3.37	2.10	2.09
8	0.88	1.46	2.03	1.71	1.46
16	0.31	3.26	1.43	1.71	1.21

Kahkonen, *et al*., (1997) and Augustynowicz, *et al*., (2010) reported that Cr^{+6} are mainly accumulated and sequestrated by the root system and are known for being poorly translocated inside plant tissues and thus there is usually no mobility of Cr^{+6} from the roots to the shoots and to the leaves, which is also correspond with the present study in *S. mucronatus* Cr^{+6}. The differential accumulation in roots and shoots suggests that interconversion between $Cr^{+6}$and Cr^{+3} may be occur in roots which lead to the translocation restriction of Cr^{+6} in plants to some degree. One hypothesis shown that Cr^{+6} is taken up actively by the sulfate carrier and immediately converted to Cr^{+3} in roots, possibly by the Fe(III) reductase enzyme which explain the similarities and dissimilarities between the two chromium ions inside the plants. Sen, *et al*., (1987) reported that Cr^{+3} reduces the mobility of Cr from roots to shoots as it is the predominant species of Cr in the roots and Siegel, (1973) also reported that the inhibition of translocation of Cr from root to shoot is that a ±COOH groups forms complexes with Cr^{+3}. Skeffington et al. (1976) also illustrated that Cr^{+3} and Cr^{+6} enter the vascular tissue with difficulty; however, once in the xylem, Cr moves more readily. Our results are in agreement with the earlier report by McGrath (1982) where high concentration of Cr^{+6} in roots and low concentrations in leaves indicate that only a fraction is transferred from roots to the aboveground parts of *S. mucronatus*. In this way, in *S. mucronatus* Cr^{+6} may be taken up from nutrient medium through the roots and the concentration in the roots is almost one order of magnitude higher than leaves which shows that Cr^{+6} does not significantly moved to above-ground parts which corroborates with the findings of Baldantoni, *et al*., (2004). The significant amount of Cr^{+6} concentrations in the shoots of *R. rotundifolia* and *M. intermedium* may possibly due to the uptake of Cr^{+6} by the shoots as they are in constant contact of shoots with the medium and in addition to translocation from roots thus this study corresponds to the findings by Sinha, *et al*., (2002); Shukla, *et al*., (2009) and Gupta, *et al*., (2011).

5. Conclusion

The study shows that *S. mucronatus* and *M. intermedium* could efficiently reduce the Cr^{+6} content in wastewater and can readily uptake in their plant parts. Based on this study, *S.mucronatus* and *M. intermedium* could be a candidate for phytoremediation of Cr^{+6} contaminated water. Furthermore, studies are needed to evaluate the on-site application of these plants for phytoremediation.

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