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A Comparative Study on the Bioaccumulation of Cr^{+3} and Cr^{+6} by an Emergent Macrophyte *Scirpus mucronatus*

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Abstract: A laboratory experiment was conducted to examine Cr^{+3} and Cr^{+6} uptake capacity of *Scirpus mucronatus*. The selected macrophytes were transferred to the laboratory containing nutrient solution enriched separately with 1.0, 2.0, 4.0, 8.0 and 16 mg/L of $K_2Cr_2O_7$ and $Cr(NO_3)_3$ and were separately harvested after 2, 4, 6, 8 and 10 days. The bioaccumulation study showed a linear relationship between chromium uptake with the exposure time (2-10 days). The calculated linear regression coefficients (R^2) between uptake and exposure time were found to be in the range of 0.577-0.800 and 0.972-0.994 for Cr^{+6} and Cr^{+3} respectively. In both the experiments, the concentration of chromium was found to be higher in the roots than the shoots of *S. mucronatus*. The maximum accumulation, bioconcentration factor (BCF) and translocation factor (TF) value were calculated at 2343.6 µg/g dry weight, 489 and 0.33 for Cr^{+3} and 1044 µg/g dry weight, 1034 and 0.68 for Cr^{+6}, respectively. *S. mucronatus* has the ability to accumulate Cr^{+6} from the surrounding water with a BCF value $>10^2$ but poorly translocate Cr^{+3} and Cr^{+6} (TF < 1) to the aerial parts.

Key words: *Scirpus mucronatus*, Cr^{+6}, Cr^{+3}, bioconcentration factor (BCF), translocation factor (TF).

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+} (Mortvelt and Giordano, 1975; Bartlett and James, 1979). Chromium is introduced into the ecosystem as a result of different 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) and other anthropogenic sources (Barros et al., 2004; Sune et al., 2007) and at higher concentrations causes serious environmental contamination in soil, sediments and groundwater (Su et al., 2005). Heavy metal contamination can be remediated using a variety of technologies, viz chemical, physical or biological. Methods such as precipitation (Rebhun and Galil, 1990), reduction (Brewster and Passmore, 1994), artificial membranes (Geckeler and Volchek, 1996) and ion exchange (Markus and Kertes, 1969) are used to remove toxic metals from industrial effluents but they are expensive, relatively inefficient and in most cases they generate a great amount of waste which is difficult to dispose off.

In recent years, interest has been focussed on the study of aquatic macrophytes as promising candidates for pollutant uptake and biological indicators of heavy metal in aquatic systems (Wolverton and McDonald, 1979; Martin and Coughtrey, 1982; Gersberg et al., 1986; Bishop and Taylor, 1989; Delgado et al., 1993; Jenssen et al., 1993; Ozimek et al., 1993; Sen and

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Bhattacharyya, 1994; Aoi and Hayashi, 1996; Maine et al., 1998, 1999, 2001). The ability of some plant species to accumulate high concentrations of heavy metals in roots or leaves can make a significant difference in the heavy metal removal. This methodology is known as phytoremediation which is considered to be safe and relatively inexpensive alternative since it is performed in situ and solar driven. Many plants have been known to accumulate heavy metals and other nutrients from contaminated waters and can be exploited for remediation of wastewaters (Jain et al., 1989; Boonyapookana et al., 2002). However, selection of any plant species for removal of metal ions from polluted water will also depend on ease of plant growth, nature and amount of biomass produced, its stage of development and level of metal in the environment (Black, 1995). According to Zayed et al. (1998), some plants were reported to be accumulators of specific metals (*Salvinia natans* for Hg or *Lemna polyrrizha* for Zn), and others have the ability to reduce the levels of heavy metals in polluted water such as *Ceratophyllum demersum*, *Spirodella polyrrhiza*, *Bacopa monnieri*, *Hygrorrhiza aristata*, *Vallisneria spiralis* and *Alternathera sessilis, Typha* sp., *Phragmites* sp., *Scirpus* sp., *Leersia* sp., *Juncus* sp. and *Spartina* sp. (Rai et al*.,* 1995; Vajpayee et al*.,* 1995; Shanker et al*.,* 2005).

It is against this background that the present study was undertaken to carry out a comparative study of bioaccumulation of Cr^{+6} and Cr^{+3} by *S. mucronatus* under laboratory conditions and the experiments were carried out under carefully controlled environmental conditions to eliminate the effects of all environmental factors and these findings may be relevant for phytoremediation of chromium from contaminated water.

Materials and Methods

S. mucronatus (L.) Palla ex Kerner is a species of the family Cyperaceae (Sedge Family). There are about 104 genera and more than 5000 species world-wide. The chief importance of sedges lies in their forming a major natural constituent of wetlands and riverside vegetation, where their densely tangled rhizomes contribute to erosion control and water purification. They are perennial and occur in a variety of aquatic habitats.

S. mucronatus was collected from unpolluted water body in Mawlai Umshing (Lat 25″36.76′36°N, Long 91″05.11′54°E) Meghalaya, India, were transferred to the laboratory in polyethylene bags and washed several times with tap and deionised water to remove adhering soil. Plants of similar size and height were selected and acclimatized for 15 days in hydroponic containers comprising deionised water and half strength Hoagland's solution (Hoagland and Arnon, 1950) under laboratory conditions (light:dark cycle 16:8 h, temperature 24±1°C, illumination 3500 Lux provided through normal bulb). Various concentrations (1.0, 2.0, 4.0, 8.0 and 16.0 mg/L) of each element, Cr^{+6} (K_2CrO_4) and Cr^{+3} ($Cr(NO_3)_3$), were prepared by diluting the stock solution (1000 mg/L) separately in 1 L deionised water with 5% Hoagland's solution. The acclimatized plants were then transferred to 10 L containers containing metal supplemented medium. AR, Himedia was used as the source of the metals. Three sets (comprising three containers for each concentration) were placed separately under above mentioned conditions.

Plants placed in 5% Hoagland's solution without metals served as control. One set of each concentration was harvested after 2, 4, 6, 8 and 10 days of the treatment and washed three times with deionised water. The washed samples were separated into roots and shoots (including leaves and floral parts) and adhering water was carefully removed using absorbent paper. Samples were then dried in an oven at 70±5°C for 48 h. The oven-dried samples were chopped and finally ground to ensure homogeneity for facilitating organic matter digestion. For digestion, the plant samples were carried out according to Kara and Zeytunluoglu (2007). Metal contents in plant samples were determined by using Atomic Absorption Spectrophotometer (AAS 3110, Perkin-Elmer).

The bioconcentration factor (BCF) is a useful parameter to evaluate plant's potentiality to accumulate metal; it provides the ability index of a plant to accumulate metals with respect to metal concentration in the substrate and it was calculated on a dry weight basis (Zayed et al., 1998).

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

Translocation of heavy metal from roots to aerial part is generally expressed as translocation factor (TF) and it indicates the internal metal transportation of the plant. The translocation factor was determined as a ratio of metal accumulated in the shoot to metal accumulated in the root (Stoltz and Greger, 2002; Deng et al*.*, 2004). This evaluates the extent of metal translocation from roots to shoots.

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

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

Statistics Analyses

One-way analysis of variance (ANOVA) and multiple linear regression 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. The significant difference between treatment means for different parameters was tested at $p < 0.05$ using a Fisher least significant difference (LSD) test.

Results and Discussion

The data on accumulation are presented in Figures 1 and 2, BCF (Figures 3 and 4) and TF (Table 1) for Cr^{+3} and Cr^{+6} in *S. mucronatus* at different concentrations and exposure time. Although chromium accumulation by plants was found to be concentration and duration-dependent, the Cr^{+3} and Cr^{+6} content in the roots and shoots of *S. mucronatus* were analyzed at regular intervals. The metal content were found to be 285.67, 289, 576, 781 and 393.67 µg/g dry weight and 1226.33, 1937.33, 2153.67, 2343.67 and 1841.33 µg/g dry weight, in the shoots and roots respectively for Cr^{+3}, and for Cr^{+6} are 481, 1982, 2428, 3010 and 3923 µg/g dry weight in the roots and 127, 86, 194, 683, 461 µg/g dry weight in the shoots after 10 d harvesting. The amount of chromium accumulated by plant tissues (roots > shoots) varied significantly (ANOVA, $p < 0.05$); maximum accumulation for Cr^{+3} in shoot was 781 µg/g dry weight and in root was 2343.67 µg/g dry weight after 8 d exposure at 16 mg/L, whereas for Cr^{+6} it is 4053 µg/g dry weight at 16 mg/L on the 8th day in the root and 1044 µg/g dry weight at 4 mg/L on the 8th day in the shoot. Highest BCF value for Cr^{+3} was found to be highest at concentration 1 mg/L (489) on the 8th day and for Cr^{+6} at 2 mg/L (1034) on the 10th day of exposure time. The BCF values were found to

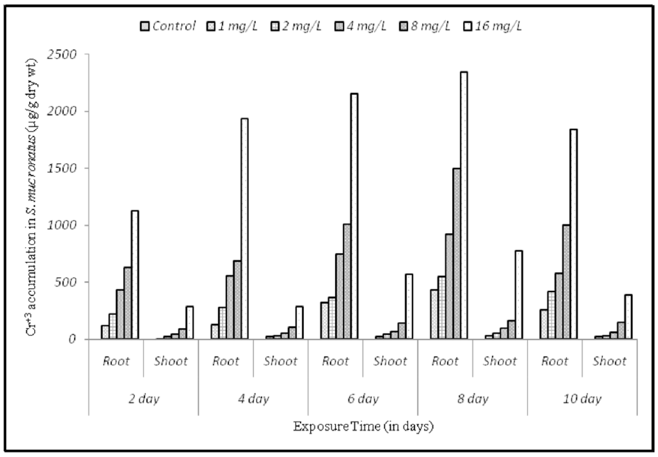

Figure 1: Cr^{+3} accumulation in the roots and shoots of *S. mucronatus*.

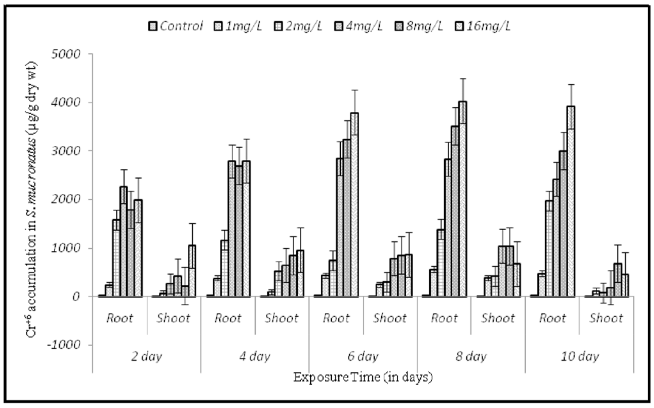

Figure 2: Cr^{+6} accumulation in the roots and shoots of *S. mucronatus*.

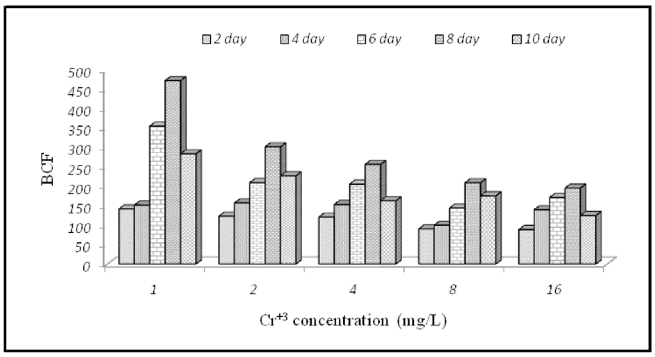

Figure 3: The bioconcentration factor (BCF) values of Cr^{+3} in *S. mucronatus* at different metal concentrations and exposure times.

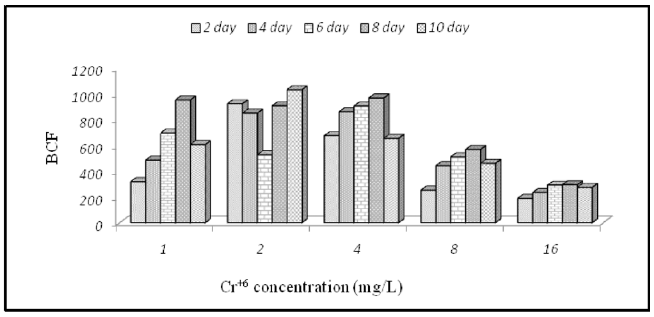

Figure 4: The bioconcentration factor (BCF) values of Cr^{+6} in *S. mucronatus* at different metal concentrations and exposure times.

Table 1: Translocation factor in *S. mucronatus*

Cr concentration (mg/L)↓	*TF values*									
	2d		4d		6d		8d		10d	
	Cr^{+6}	Cr^{+3}	Cr^{+6}	Cr^{+3}	Cr^{+6}	Cr^{+3}	Cr^{+6}	Cr^{+3}	Cr^{+6}	Cr^{+3}
1	0.28	0.15	0.26	0.18	0.58	0.09	0.68	0.68	0.26	0.26
2	0.17	0.11	0.46	0.11	0.41	0.13	0.31	0.31	0.04	0.04
4	0.19	0.11	0.23	0.10	0.28	0.10	0.37	0.37	0.08	0.08
8	0.13	0.14	0.32	0.16	0.26	0.14	0.30	0.30	0.23	0.23
16	0.53	0.25	0.34	0.15	0.23	0.27	0.17	0.17	0.12	0.12

decrease with increasing Cr concentration in growth medium. Highest Translocation Factor was 0.33 for Cr^{+3} and 0.68 for Cr^{+6}.

Results indicated that plants have the ability to uptake chromium from the surrounding solution in agreement with the reports of earlier studies that aquatic plants tend to adapt themselves to cope-up with chromium toxicity (Mangi et al., 1978; Staves and Knaus, 1985; Gupta et al., 1994; Garg and Chandra, 1994). The metal uptake ability of different plant tissues of aquatic macrophytes varied with species. In the present study also, the chromium uptake was higher in the roots in comparison to shoots which corroborate with the findings of Srivastav et al. (1994), Wolverton and McDonald (1975), Gupta et al. (1994) and Vajpayee et al. (2001). The absorption pattern in the present study corroborated with the findings of Sinha and Chandra (1990), Smith et al. (1989) and Qian et al. (1999) where emergent species have high accumulations in roots and lowest accumulations in shoots. The difference in the ability of plants to accumulate heavy metals has been related to differences in their root morphology (Maine et al., 2001). A plant with numerous roots would accumulate more metal than one tap roots. *S. mucronatus* also possess numerous fine roots and they accumulate more chromium at the high concentration in the root as compared to the aerial parts. Cr^{+3} and Cr^{+6} are being poorly translocated from the roots to the aerial parts. It was reported that, in general, plants have a low capacity to translocate Cr (Kabata Pendias and Pendias, 1984) and Cr^{+6} is more water-soluble than Cr^{+3} and both chemical species are easily taken up by plants (Vajpayee et al., 2000). One conclusive point is that Cr^{+6} is much more mobile and soluble than Cr^{+3} and the internal concentrations of Cr^{+6} in plants were many fold higher than those of plants exposed to Cr^{+3} (Mei et al., 2002; Shahandeh and Hossner, 2000). Siegel (1973) reported that Cr^{+3} forms complexes with ±COOH groups which inhibit the translocation of metal from root to shoot. Thus the present study is in accordance with the above findings where translocation of Cr^{+6} is more from the roots to the shoots as compared to that of Cr^{+3}.

Uptake of Cr^{+3} and Cr^{+6} versus Chromium Concentration Exposure Levels

However, in the present study, two important parameters are taken that is exposure time and concentration of metals to which the *S. mucronatus* are exposed. In case of Cr^{+3}, the uptake and accumulation increased linearly with an increase in treated Cr^{+3} concentrations (Figure 5). However, it was curvilinear in case of Cr^{+6} (Figure 6). The calculated linear regression coefficients (R^2) between uptake and exposure time were found to be in the range of 0.577–0.800 and 0.972-0.994 for Cr^{+6} and Cr^{+3} respectively. In *S. mucronatus*, the regression coefficients of Cr^{+3} at 2nd, 4th, 6th, 8th and 10th days, the linear relationship was much better at each exposure level as compared to Cr^{+6}. The outline of Cr^{+3} accumulation in *S. mucronatus* within the contact levels established with the reported pattern of Hasan et al. (2007) and Abhilash et al. (2009); but in case of Cr^{+6} the curvilinear pattern observed corroborates with the findings by Tateuyama et al. (1979).

Uptake of Metals versus Time of Exposure

The pattern of Cr^{+6} uptake in the root and shoot of *S. mucronatus* with respect to time of exposure at higher concentrations showed that the uptake occurred in two stages. The first stage uptake rate extended up to four days followed by a second stage with the enhanced uptake occurring between 4 and 8 days as shown in Figure 7.

The overall Cr^{+6} uptake rate seemed to be also dependent on the exposure level of chromium present in the solution. The biphasic nature was clearly evident at each stage of exposure while at the lower concentrations of 1.0 mg only the single stage of biphasic nature can be identified.

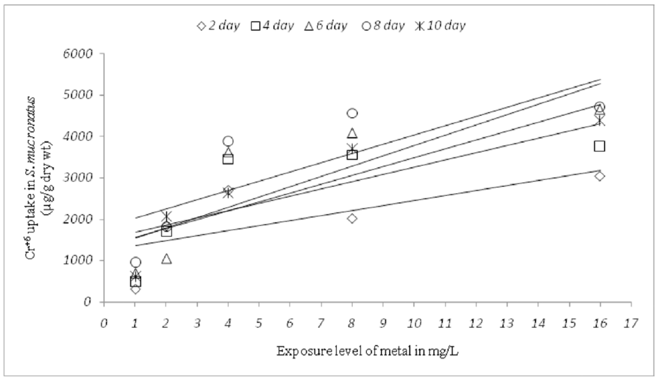

Figure 5: Linear relationship of Cr^{+3} concentration in water versus plant parts (root and shoot) during various exposure days.

Cr^{+3} uptake in the root and shoot of *S. mucronatus* (Figure 8) was also observed to be biphasic but it exhibits only one stage in contrast to the two stages in Cr^{+6} uptake rate. Thus, the present observations showed that the extent of chromium uptake by the experimental plants was dependent on the concentration of the metal in the solution as well as the length of exposure to the metal which is in accordance with the study by Hasan et al. (2007).

Correlation and multiple regression analyses were conducted to examine the relationship between chromium uptake by *S. mucronatus* and potential predictors (concentrations of chromium in the medium and time). Tables 2 and 3 summarize the descriptive statistics and analysis results for Cr^{+6} and Cr^{+3}. As can be seen, each of the uptake is positively and significantly correlated with the concentration in the medium for both Cr^{+6} and Cr^{+3}, indicating that with the increase in concentration

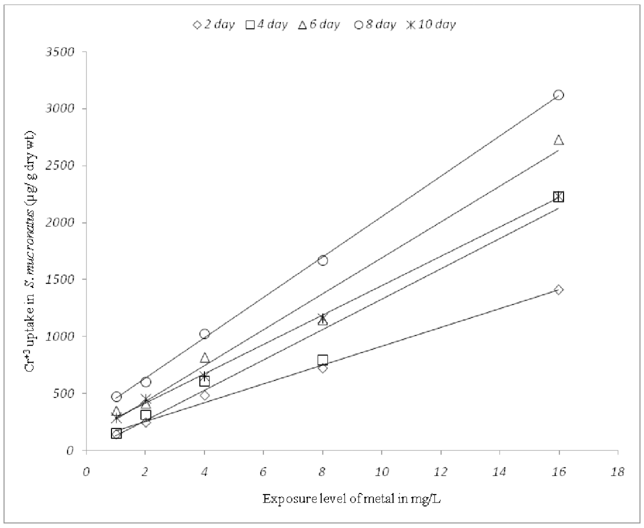

Figure 6: Linear relationship of Cr^{+6} concentration in water versus plant parts (root and shoot) during various exposure days.

in the medium it tends to have higher uptake of Cr^{+6} and Cr^{+3} into the plant tissues. However, in the case of Cr^{+6}, the uptake is not significantly correlated with time i.e., the number of days whereas it is vice versa in case of Cr^{+3} indicating that with the increase of time it will have higher uptake of Cr^{+3}.

The multiple regression model with all two predictors produced $R^2 = 0.642$, $F(2, 27) = 26.97$, $p < 0.001$ for Cr^{+6} and $R^2 = 0.893$, $F(2, 27) = 122.102$, $p < 0.001$. As can be seen in Tables 2 and 3, the concentration of chromium in the medium had significant positive regression weights, indicating that higher chromium concentration in the medium were expected to have higher Cr^{+6} and Cr^{+3} uptake by the plants. Time i.e.,

Table 2: Summary statistics, correlations and results from the regression analysis for Cr^{+6}

Variable	*Mean*	*Std*	*Correlation with uptake*	*Multiple regression weights*	
				B	β
Uptake	2221.9000	1640.37349			
Time (in days)	6.0000	2.87678	0.144	81.875	0.144
Concentrations (mg/L)	5.1667	5.58374	0.804***	236.099***	0.804

**p* < 0.05 ***p* < 0.01 ****p* < 0.001

Table 3: Summary statistics, correlations and results from the regression analysis for Cr^{+3}

Variable	*Mean*	*Std*	*Correlation with uptake*	*Multiple regression weights*	
				B	β
Uptake	809.1780	835.5758			
Time (in days)	6.0000	2.8768	0.182*	52.825*	0.182
Concentrations (mg/L)	5.1667	5.5837	0.931***	139.362***	0.931

**p* < 0.05 ***p* < 0.01 ****p* < 0.001

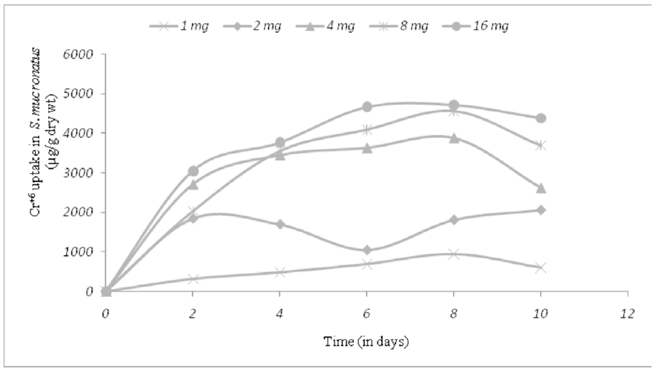

Figure 7: Cr^{+6} uptake in *S. mucronatus* as a junction of time from various concentrations of Cd solutions.

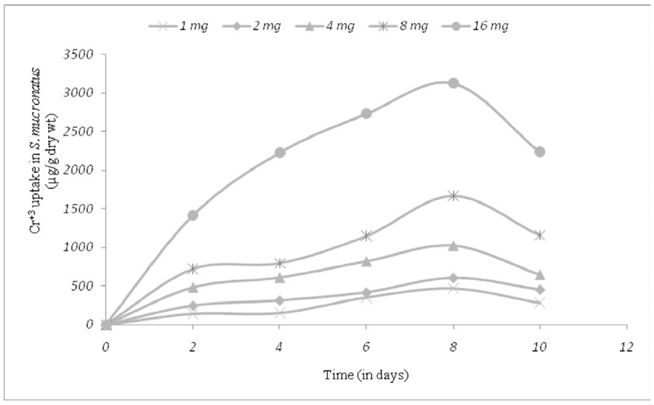

Figure 8: Cr^{+3} uptake in *R. rotundifolia* as a junction of time from various concentrations of Cd solutions.

number of days did not contribute to the multiple regression model in the case for Cr^{+6} while for Cr^{+3} time had significant positive regression weights indicating that with the increase of time it was expected to have higher Cr^{+3} uptake by the plants.

From the view of phytoremediation, a good accumulator should have the ability to concentrate the heavy metal in the tissue; for example, a BCF more than 1000 (Zayed et al., 1998) are generally considered evidence of a useful plant for phytoremdiation. Chandra (2004) reported that high bioconcentration factor values were found in *C. demersum* (15,330-31,400) and *H. reticulatum* (11,394) and Arora et al. (2006) also reported in *Azolla microphylla* (4617) and *A. filiculoides* (2977). However, in the present study, the BCF values of *S. mucronatus* for Cr^{+3} is 471 and Cr^{+6} is 1034. Since Cr^{+6} BCF was above 1000, this plant can be considered as a good accumulator of Cr^{+6} as compared to Cr^{3+} which can be considered as a moderate accumulator.

Conclusions

The study shows that *S. mucronatus* could efficiently reduce the Cr^{+6} content in wastewater and the maximum accumulation was found in the roots than in shoots, Cr being poorly translocated from the roots to the aerial parts. Based on this study, *S. mucronatus* could be a candidate for phytoremediation of Cr contaminated water. Further, more studies are needed to evaluate the on-site application of these plants for phytoremediation.

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