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# PHYTOREMEDIATION POTENTIAL OF AQUATIC MACROPHYTE *AZOLLA PINNATA* R.BR. AND *SALVINIA MOLESTA* MITCHELL FOR REMOVAL OF CHROMIUM FROM WASTE WATER

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**Abstract:** In the present study the ability of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell to uptake Cr (VI) from aqueous environment as well as their potential application for phytoremediation were assessed. During 15 days of the experiment the ferns were grown on the nutrient solution containing hexavalent chromium ions, each in a concentration of control, 0.07, 0.09, 0.11 and 0.13 mg/l. The biomass productivity and relative growth of *Azolla pinnata* R.Br. was increased and in case of *Salvinia molesta* Mitchell it was decreased with increase in the metal concentration of the treatment medium. The protein content in different concentrations of nickel was found to be more as compared to the control but showed a decreasing trend with increased concentration. After 15 days of treatment, the concentration of Cr (VI) in the medium decreased between 72.86 – 97.69 % and 75.71 - 90 % by *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell respectively. Both the fern took a lesser quantity of metal from 0.07 mg/l treatments compared to 0.09 mg/l, 0.11 mg/l and 0.13 mg/l treatments. In the *Azolla pinnata* R.Br. tissue the concentration of heavy metal under investigation ranged from 0.106 - 0.216 mg/g dry weight and for *Salvinia molesta* Mitchell ranged from 0.094 -0.321 mg/g dry weight. Increasing concentration of Cr (VI) in the growth medium increased the bioconcentration factor of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell up to an optimum value of 1630.76 and 2469.23 respectively. The higher BCF of *Salvinia molesta* Mitchell indicated that it can be a better candidate for phytoremediation of Cr (VI) from the treatment solution than *Azolla pinnata* R.Br.
**Keywords:** *Azolla pinnata* R. Br., *Salvinia molesta* Mitchell, Phytoremediation, Bioconcentration factor.

## INTRODUCTION

Heavy metals containing industrial wastes were found to increase environmental stress. The common anthropogenic sources of heavy metals are waste water irrigation, sludge applications, solid waste disposal, automobile exhaust and industrial activities (Shi et al., 2005). Crops grown in or close to the contaminated sites can uptake and accumulate these metals in their organs (Jarup, 2003). The effect of Heavy metals on crop plants and human beings caused functional disorders in their body organs due to exposure of low dosage over a

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long period of time (Jianjie et al., 2008). Chromium is biologically inactive in a metallic state. There are mainly two stable oxidation states of chromium (viz. $Cr^{+6}$ and $Cr^{+3}$); $Cr^{+6}$ is considered to be more toxic than $Cr^{+3}$ (Panda and Patra, 2000). Hexavalent chromium ($Cr^{+6}$) rarely occurs naturally, but is usually produced from anthropogenic sources. It is used in various industries such as tanneries, chrome plating, cooling tower water treatment etc. It is classified as a group 'A' carcinogen and poses other health risks such as liver damage, dermatitis and gastrointestinal ulcers (Dokken et al., 1999). As the metal is non-biodegradable and toxic in nature, the toxicity of metals made threats on aquatic ecosystem. Hence, removal of hexavalent chromium by means of wastewater treatment is essential and important (Wittbrodt and Palmer, 1995) and become a major concern for protecting aquatic life. Reduction of hexavalent chromium to trivalent with subsequent immobilisation as the hydroxide is considered as one of the main strategies for chromium removal from waste water (Lazardis and Asouhidou, 2003).

In this study, attempts were made to investigate and compare the effects of various chromium concentrations on the growth and subsequent development of aquatic macrophytes (*Azolla pinnata* R.Br and *Salvinia molesta* Mitchell) through the study of consequent toxicological changes in their various morphological and biochemical parameters such as plant biomass, chlorophyll synthesis, protein level and heavy metal bioaccumulation in plant tissues. The present investigation emphasizes on the comparative chromium hyper-accumulation potential of macrophytes beneficial for future phytoremediation programmes in treatment of contaminated waste water.

## MATERIALS AND METHODS

### Experimental Design

The present study was undertaken with chromium ($K_2Cr_2O_7$) at 0.07 mg/l, 0.09 mg/l, 0.11 mg/l and 0.13 mg/l along with control (untreated). To assess the phytoremediation potential of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell for chromium, a hydroponic culture experiment was carried out in the controlled condition (Patnaik et al., 2012) in the growth chamber.

### Plant Growth in Hydroponics

Uniform sized approximately equal biomass of plants were grown in a plastic pot (Depth: 3" and Diameter: 20 cm) containing 750 ml of half strength Hoagland's nutrient solution with different concentrations of chromium for 15 days.

**Study of Plant Biomass**

After 15 days of plant growth under hydroponics they were weighed (Electronic balance: Sartorius). The fresh biomass as well as the dry biomass of each macrophyte under different chromium treatment conditions was studied initially and after 15 days of plant growth. The difference between initial weight and final weight gives the increase in biomass of the plants.

**Relative growth**

Relative growths (Lu et al., 2004) of the plants during the experiment were calculated as follows.

Relative growth = Final fresh weight / Initial fresh weight

**Chlorophyll Content**

Chlorophyll was determined according to the methodology devised by Porra et al. (1989). For measurement of chlorophyll content 0.5 g of each plant material were put into test tubes containing 10 ml of methanol and kept in dark for 24 hours. After this the supernatant solution from each test tube was taken for measurement of absorbance at 470 nm, 652 nm and 665 nm by the help of UV spectrophotometer.

Calculation (µg/ml)

$$\text{Chl a} = 16.29\ A_{665} - 8.54\ A_{652}$$

$$\text{Chl b} = 30.66\ A_{652} - 13.58\ A_{665}$$

$$\text{Chl (a+b)} = 22.12\ A_{652} + 2.71\ A_{665}$$

**Estimation of Leaf Protein Content**

For protein estimation 0.5 g of leaf sample was taken and homogenized in 10% ice cold TCA by a pre-chilled mortar and pestle and incubated overnight at 4 $^{0}$C. Then the sample was centrifuged at 10,000 rpm for 10 minutes and subsequently washed with 80% ethanol to remove phenolic compounds. Washed pellet was then suspended in a known volume of 0.1N NaOH. Then protein was estimated by Lowry’s method, 1951.

**Estimation of Total Chromium Content**

After 15 days of growth under different treatments of Cr, hydroponically grown aquatic macrophytes (control and treated) were oven dried. They were analyzed for total chromium bioavailability followed by digestion in a di-acid (perchloric acid and nitric acid) mixture in Microwave Digestion System-8 as per the methods prescribed by Bonet et al. (1991). The estimation of total chromium content was carried out using an Atomic Absorption Spectrophotometer (GBS Model 932AB plus)

**Bio concentration factor (BCF)**

$$\text{BCF} = \frac{\text{Concentration of metal in dried plant tissue } (\mu g/g)}{\text{Initial concentration of metal in external solution } (mg/l)}$$

Bio-concentration factor is a useful parameter to evaluate the potential of plants for accumulating metals (Lu et al., 2004; Mun et al., 2008).

**Percentage removal of heavy metals**

Removal of metals was calculated using the following equation (Khan et al., 2009):

$R\ (\%) = [(C_0 - C_t)/\ C_0] \times 100$

Where $C_0$ and $C_t$ represent the residual concentration of metal at the beginning of the experiment and at time of t, respectively.

**RESULTS AND DISCUSSION**

**Effect of chromium on biomass of aquatic macrophytes**

*Azolla pinnata* R.Br. with an initial fresh biomass of 2 g and *Salvinia molesta* Mitchell with an initial fresh biomass of 11.491 g, 11.311 g, 11.062 g, 11.424 g and 11.629 g were grown for 15 days in pots of control and different chromium concentrations viz. 0.07 mg/l, 0.09 mg/l, 0.11 mg/l and 0.13 mg/l respectively. After 15 days of culture the final fresh weight of both the plant species were recorded (Table 1, Fig. 1). The final fresh weight of *Azolla pinnata* R.Br. in control, 0.07 mg/l, 0.09 mg/l, 0 .11 mg/l and 0.13 mg/l of chromium treatment were recorded as 5.957 g, 10.322 g, 10.757 g, 10.993 g and 11.918 g respectively. The final fresh weight recorded for *Salvinia molesta* Mitchell with increased concentration of chromium were such as; control -10.564 g, 0.07 mg/l - 7.234 g, 0.09 mg/l - 6.981 g, 0.11 mg/l - 6.413 g and 0.13 mg/l - 6.130g.

**Table 1:** Changes in fresh biomass of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell after 15 days of plant growth in different concentrations of chromium

| Plant species | Chromium treatment (mg/l) | Biomass values (g) | |
|---|---|---|---|
| | | Initial fresh weight (g) | Final fresh weight (g) |
| *Azolla pinnata* R.Br. | Control (0.0) | 2±0 | 5.957±1.05 |
| | 0.07 | 2±0 | 10.322±0.465 |
| | 0.09 | 2±0 | 10.757±0.542 |
| | 0.11 | 2±0 | 10.993±1.387 |
| | 0.13 | 2±0 | 11.918±0.408 |
| | Control (0.0) | 11.491±0.248 | 10.564±1.278 |

| | | | |
|---|---|---|---|
| *Salvinia molesta* Mitchell | 0.07 | 11.311±0.197 | 7.234±2.148 |
| | 0.09 | 11.062±0.031 | 6.981±1.069 |
| | 0.11 | 11.424±0.363 | 6.413±0.36 |
| | 0.13 | 11.629±0.480 | 6.130±0.707 |

Values in the table are mean ± SD of 3 replicates

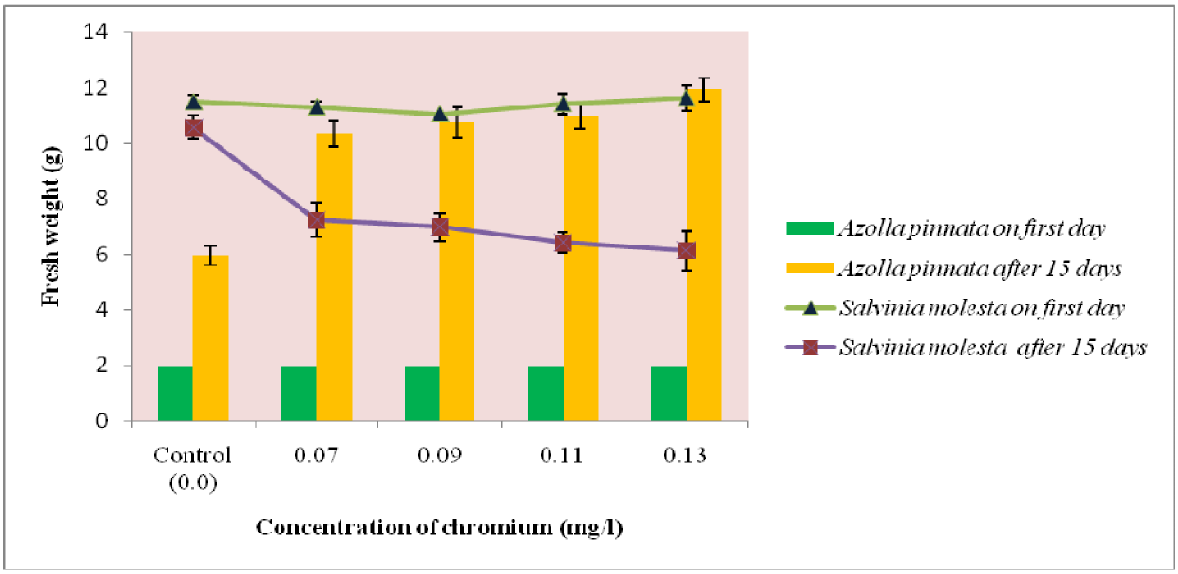


**Fig.1:** Biomass productivity (gram fresh weight) of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell after15 days of plant growth in different concentrations of chromium. Bars indicate mean± SD, where n=3

From the above observation it was concluded that *Azolla pinnata* R.Br. showed a positive response to elevated concentration of Cr in nutrient solution as evident from its increased biomass values, but caused a distinct limitation of *Salvinia molesta* Mitchell growth in the elevated concentrations relative to control after 15 days of treatment.

**Relative growth**

The relative growth of *Azolla pinnata* R.Br. was increased with an increase in the concentration of Cr in the liquid nutrient medium. The relative growth of the material in the control treatment was 2.978 and was increased up to 5.959 in 0.13 mg/l Cr concentration. The relative growth of *Salvinia molesta* Mitchell exposed to graded concentrations of chromium decreased significantly with respect to the control i.e. from 0.92 (control) to 0.527 in 0.13 mg/l Cr concentration. Average relative growth of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell for each treatment is presented in Table - 2 and Fig. 2.

**Table 2:** Average relative growth of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell in different chromium concentration after 15 days of culturing

| Plant species | Chromium treatment(mg/l) | Relative growth |
|---|---|---|
| *Azolla pinnata* R.Br. | Control (0.0) | 2.978±0.524 |
| | 0.07 | 5.161±0.232 |
| | 0.09 | 5.378±0.271 |
| | 0.11 | 5.496±0.693 |
| | 0.13 | 5.959±0.204 |
| *Salvinia molesta* Mitchell | Control (0.0) | 0.92±0.128 |
| | 0.07 | 0.64±0.192 |
| | 0.09 | 0.631±0.097 |
| | 0.11 | 0.561±0.049 |
| | 0.13 | 0.527±0.053 |

Values in the table are mean ± SD of 3 replicates

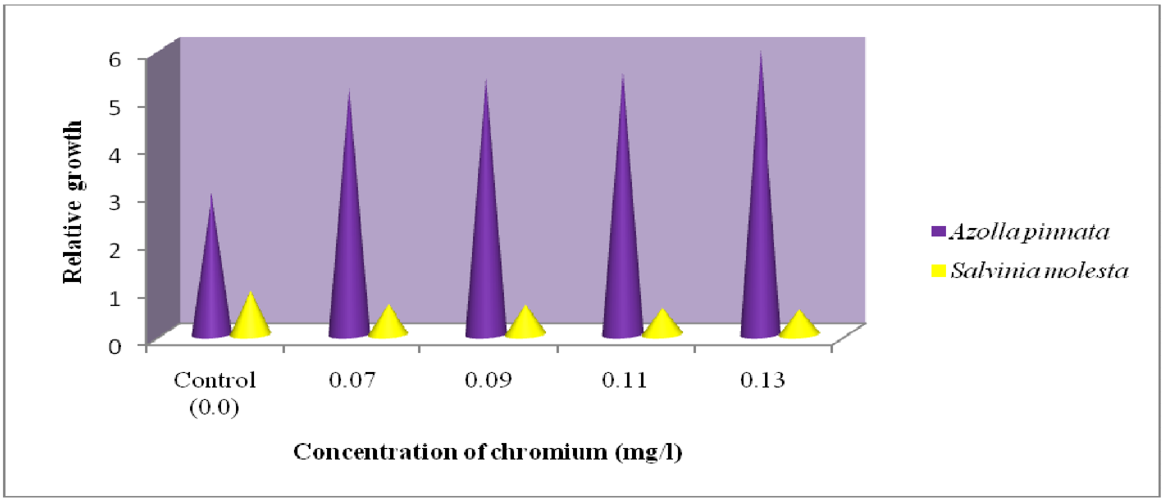


**Fig.2:** Average relative growth of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell in different chromium concentration after 15 days of culturing.

**Dry weight**

Maximum dry weight for *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell were observed in control solution with a value of 0.673 g and 0.481 g respectively while this value gradually decreased to 0.589 g and 0.336 g in 0.13 mg/l Cr concentration respectively (Table 3, Fig. 3).

**Table - 3:** Dry weight of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell after 15 days of plant growth in different concentrations of chromium.

| Plant species | Chromium treatment (mg/l) | Final dry weight (g) |
|---|---|---|
| *Azolla pinnata* R.Br. | Control (0.0) | 0.673±0.030 |
| | 0.07 | 0.474±0.014 |
| | 0.09 | 0.559±0.068 |
| | 0.11 | 0.571±0.025 |
| | 0.13 | 0.589±0.021 |
| *Salvinia molesta* Mitchell | Control (0.0) | 0.481±0.028 |
| | 0.07 | 0.458±0.016 |
| | 0.09 | 0.446±0.086 |
| | 0.11 | 0.368±0.044 |
| | 0.13 | 0.336±0.068 |

Values in the table are mean ± SD of 3 replicates

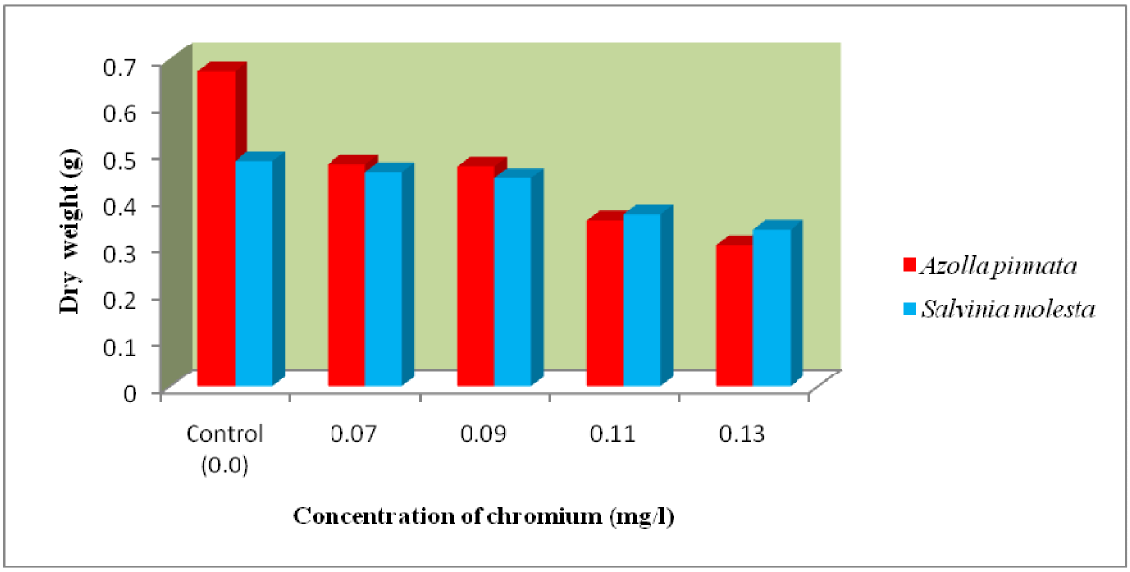


**Fig.3:** Dry weight of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell after 15 days of plant growth in different concentrations of chromium

**Effect of Chromium toxicity on chlorophyll content of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell**

The initial total chlorophyll content (mg/g fresh wt.) of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell was 0.055 mg/g fr.wt. and 0.103 mg/g fr.wt. respectively. After 15 days of inoculation the total chlorophyll content (mg/g fr. wt.) of *Azolla pinnata* R.Br. was decreased with increased concentration of chromium i.e. 0.07 mg/l - 0.138, 0.09 mg/l - 0.135, 0.11 mg/l- 0.122, 0.13 mg/l - 0.122, whereas the value for control was 0.143. Hence, the results revealed that the total chlorophyll content of *Azolla pinnata* R.Br. got increased as compared to the initial value, but got reduced with the increasing order of chromium concentrations in the nutrient solution. This might be due to the effect of Cr on chlorophyll content of the plants. The total chlorophyll content (mg/g fresh wt.) of *Salvinia molesta* Mitchell showed a decreasing trend in elevated chromium concentration treatment i.e. 0.07 mg/l - 0.2, 0.09 mg/l - 0.193, 0.11 mg/l - 0.147, 0.13 mg/l - 0.138 whereas the value in the control was recorded to be 0.212 (Fig. 4). Hence, this finding indicated that the total chlorophyll content of *Salvinia molesta* Mitchell got increased as compared to the initial value, but got reduced with the increasing order of concentrations. The chlorophyll pigments are present in thylakoid within chloroplast, and any damage brought to these structures could lead to denaturation of these pigments. It may be suggested that observed decrease in chlorophyll content at higher concentration of chromium might be due to breakdown of thylakoid and chloroplast envelope as was previously reported (Dodge and Law, 1974).

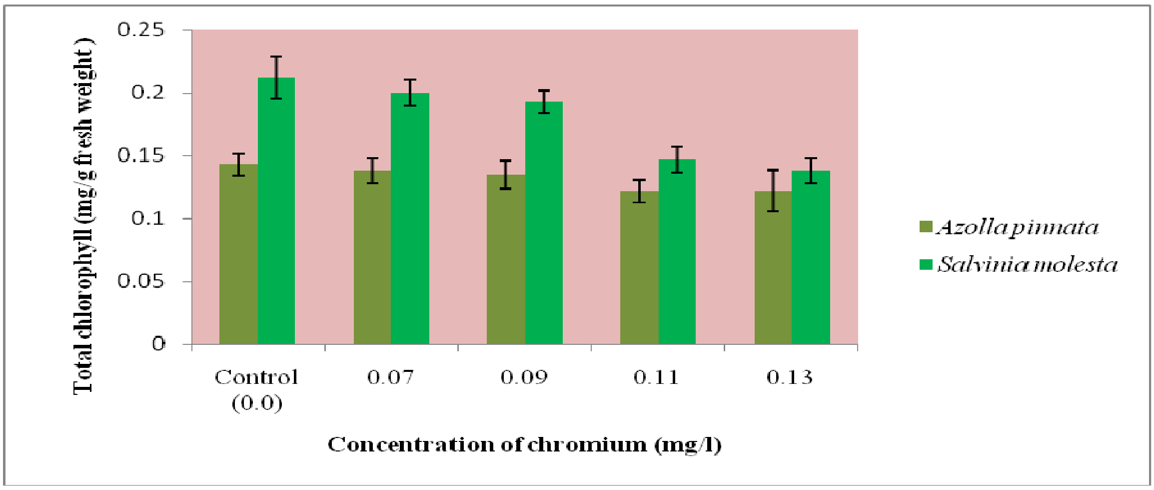


**Fig.4:** Total chlorophyll content (mg/g fresh wt.) of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell grown in different concentrations of chromium after 15 days of growth.

**Effect of chromium on protein content of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell**

In both the plant species the protein contents were observed to be decreased with the increase in concentration of chromium in the nutrient solution. The initial protein content of *Azolla pinnata* R.Br. was 1.198 mg/g fresh weight. However, the final protein content (mg/g fresh wt.) after 15 days of culture in control, 0.07 mg/l, 0.09 mg/l, 0.11 mg/l and 0.13 mg/l chromium concentration were recorded as 2.208, 1.727, 1.660, 1.632 and 1.580 mg/g fresh weight respectively. The initial protein content of *Salvinia molesta* Mitchell was 1.136 mg/g fresh weight and after 15 days the final protein content (mg/g fresh wt.) in control, 0.07 mg/l, 0.09 mg/l, 0.11 mg/l and 0.13 mg/l was observed as 2.624, 3.395, 3.222, 2.121 and 1.837 respectively (Fig. 5).

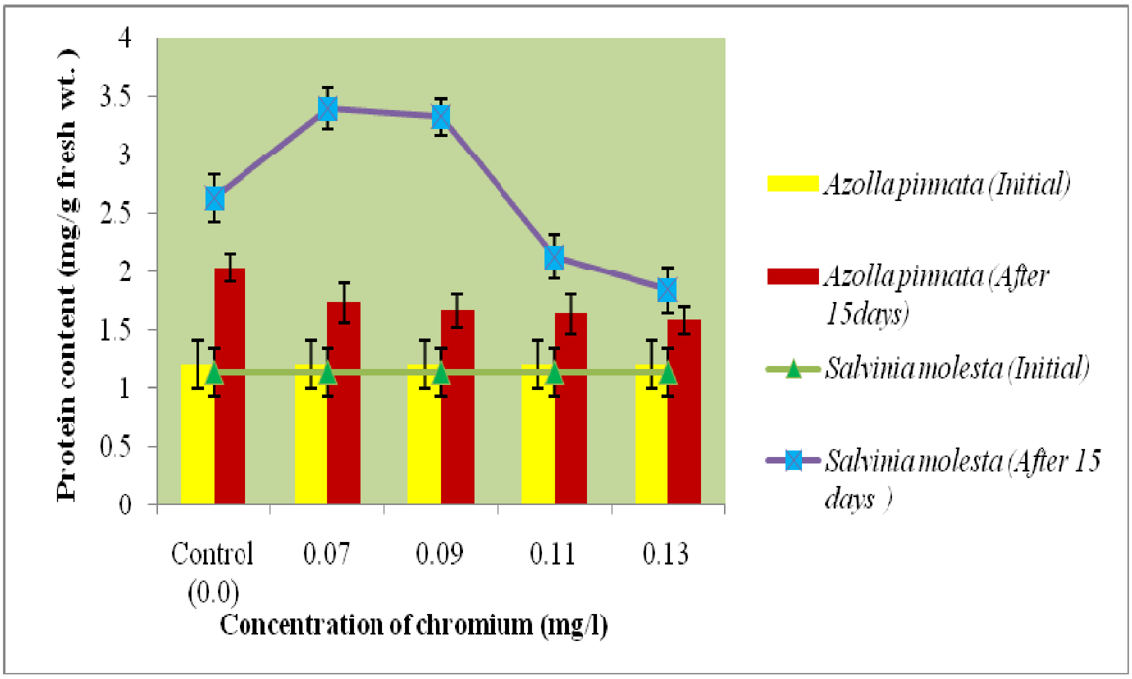


**Fig.5:** Protein content (mg/g fresh wt.) of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell grown in different concentrations of chromium before and after 15 days of treatment.

From the above observation it was concluded that after 15 days of treatment the protein content of *Azolla pinnata* R.Br. showed a decreasing trend from the initial value to the final value except control solution and also it was observed that the protein content in different concentration of Cr was less as compared to the control. This may be due to the Cr uptake by the fern which ultimately stressed on the protein level. After the end of the experiment the final protein contents of *Salvinia molesta* Mitchell in all the chromium concentrations were higher than its initial value before treatment. Again the final protein content (mg/g fresh wt.) first showed an increasing trend in comparison to control up to 0.09 mg/l chromium concentration and then declined with further increase in concentration. It may be due to the degradation of proteins in plants which could result in inhibition of nitrate reductase activity (Choudhury and Panda, 2004) and it could be correlated with the reduced photosynthetic activity, nitrogen metabolism and nucleic acid damage under Cr stress.

**Metal removal efficiency**

**A. Chromium removal from water**

Phytoremediation has been known as a promising, low cost and environmentally sustainable technology for the remediation of water polluted by toxic trace elements (Razinger et al., 2007). In the present investigation after 15 days of treatment period by *Azolla pinnata* R.Br. there remained 0.019, 0.008, 0.006 0.003 mg/l of Cr ions in the solution containing 0.07, 0.09, 0.11and 0.13 mg/l chromium respectively. In *Salvinia molesta* Mitchell the concentration of chromium ions reduced to 0.017, 0.018, 0.052 and 0.013 mg/l in the solution containing initial concentration of 0.07, 0.09, 0.11 and 0.13 mg/l chromium respectively (Table 4, Fig. 6).

**Table 4:** Concentration of chromium in *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell and in the nutrient medium after 15 days of treatment

| Plant species | Chromium treatment (mg/l) | Chromium content in plant (mg/g dry wt.) | Chromium content in water (mg/l) |
|---|---|---|---|
| *Azolla pinnata* R.Br. | Control (0.0) | 0 | 0 |
| | 0.07 | 0.106±0.001 | 0.019±0.003 |
| | 0.09 | 0.143±0.035 | 0.008±0.009 |
| | 0.11 | 0.176±0.016 | 0.006±0.002 |
| | 0.13 | 0.212±0.031 | 0.003±0.001 |
| *Salvinia molesta* Mitchell | Control (0.0) | 0 | 0 |
| | 0.07 | 0.094±0.006 | 0.017±0.006 |
| | 0.09 | 0.154±0.014 | 0.018±0.005 |
| | 0.11 | 0.254±0.083 | 0.15±0.003 |
| | 0.13 | 0.321±0.095 | 0.013±0.003 |

Values in the table are mean ± SD of 3 replicates

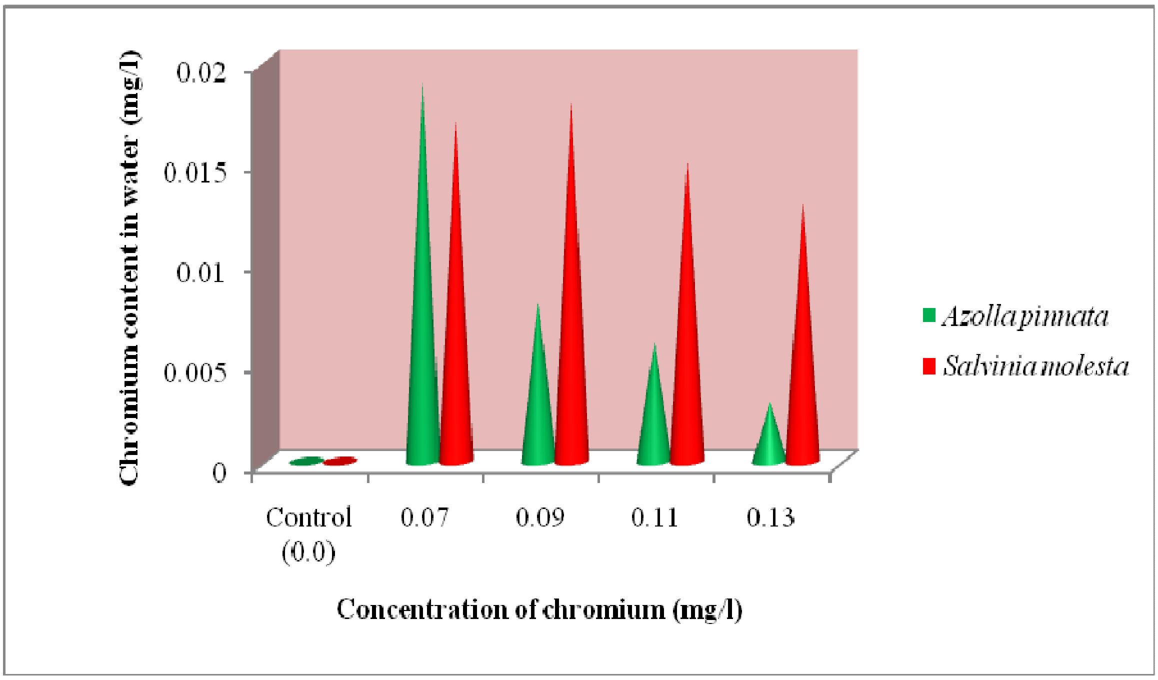


**Fig.6:** Concentration of chromium in residual treatment solution after 15 days of treatment by *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell

Fig. 7 and 8 shows the percentage of Cr removal for different initial concentrations by *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell. The result showed that after 15 days of treatment the maximum chromium reduction by both the plant species was observed in the treatment containing 0.13 mg/l chromium concentration and lowest in 0.07 mg/l chromium concentration. The percentage reduction of chromium by *Azolla pinnata* R.Br. was 72.86, 91.11, 94.54 and 97.69 in treatment solution containing 0.07 mg/l, 0.09 mg/l, 0.11 mg/l and 0.13 mg/l chromium respectively. Similarly for *Salvinia molesta* Mitchell the percentage reduction of chromium was 75.71, 80.00, 86.36 and 90.00 in treatment solution containing 0.07 mg/l, 0.09 mg/l, 0.11 mg/l and 0.13 mg/l chromium respectively.

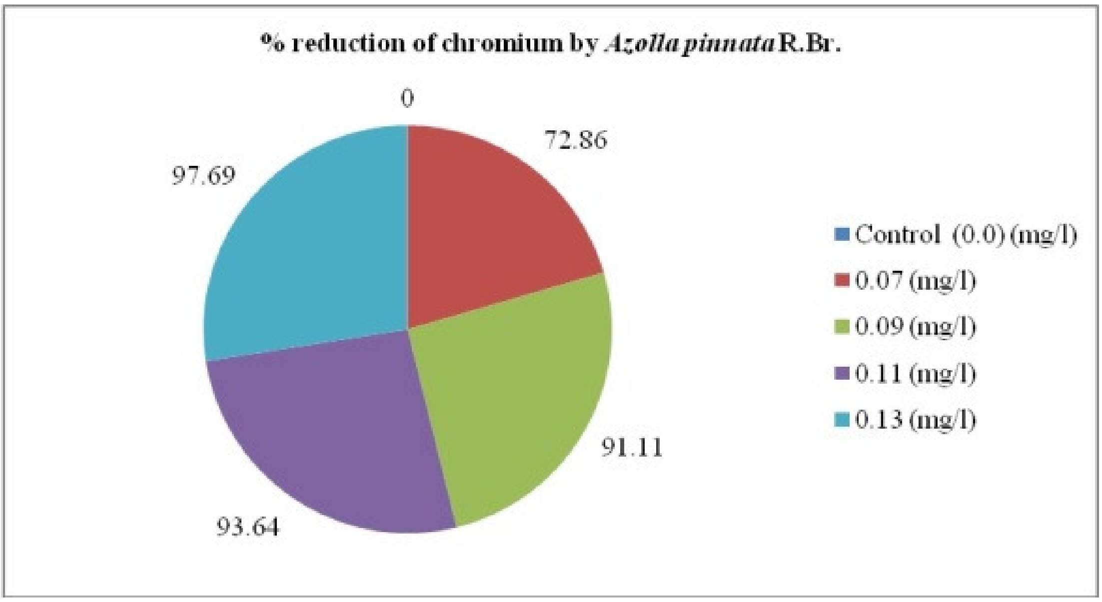


**Fig.7:** Percentage reduction of chromium from the different treatment solution by *Azolla pinnata* R.Br. after 15 days of plant growth

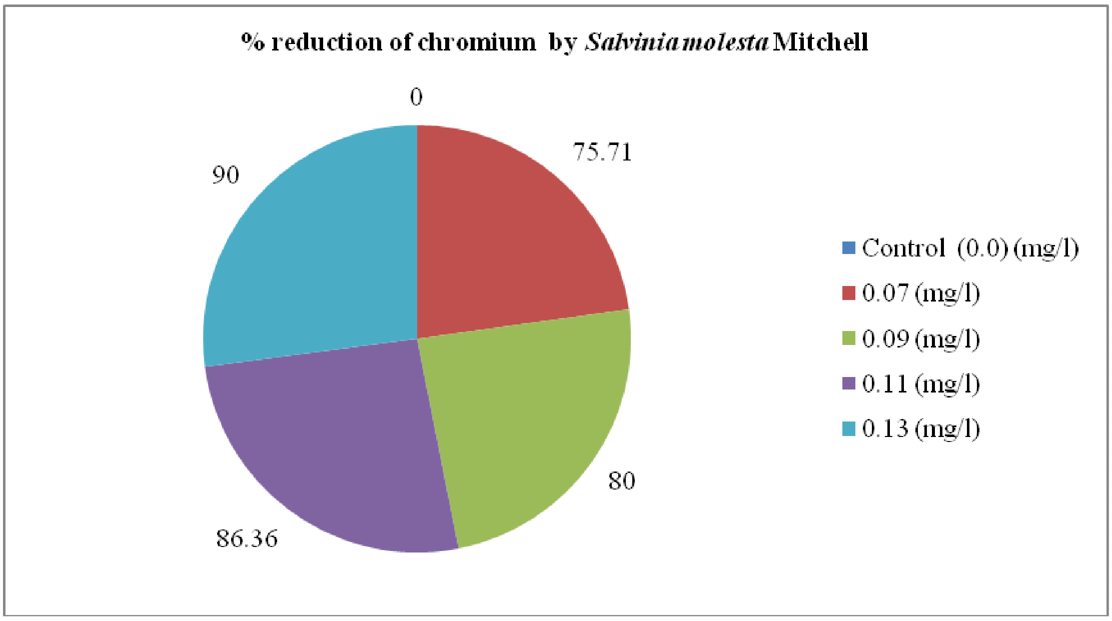


**Fig.8:** Percentage reduction of chromium from the different treatment solution by *Salvinia molesta* Mitchell after 15 days of plant growth

## B. Metal accumulation in plant

The chromium accumulation in both the plant species was increased with the increase in initial concentration of treated solution. The amount of chromium (mg/g dry wt.) accumulated in *Azolla pinnata* R.Br. was 0.106, 0.170, 0.162 and 0.164 when the medium was respectively supplied with 0.07, 0.09, 0.11 and 0.13mg/l chromium concentration. Similarly in *Salvinia molesta* Mitchell the chromium accumulation (mg/g dry wt.) in plant biomass was 0.094, 0.154, 0.373 and 0.321 in the treatment solution containing 0.07, 0.09, 0.11and 0.13 mg/l chromium concentration respectively. The chromium accumulation in plant sample was zero in control and showed an increasing trend with the increasing concentration of the heavy metal. Both the plant species showed increased accumulation of chromium, when the concentration of chromium in the medium was increased from 0.07 to 0.13 mg/l (Table 4).

To quantify metal accumulation in plant biomass, the bio-concentration factor (BCF) is more significant than the amount accumulated in plants since it provides an index of the ability of the plants to accumulate metal element with respect to the element concentration in water (Khellaf and Zerdaoui, 2009). Bioconcentration factors for *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell grown in different concentrations of chromium are given in Table - 5. In this study it was observed that the chromium BCF value for *Azolla pinnata* R.Br. were between 1514.29 and 1630.76 while for *Salvinia molesta* Mitchell between 1342.86 and 2469.23. In both the plant species lowest and highest BCF value was attained at 0.07 mg/l and 0.13 mg/l chromium concentration respectively. According to Razinger et al. (2007), plant which is considered as a good accumulator, must have a BCF over 1000, thus *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell could be considered and recommended as a good accumulator for chromium. Further, in between the two plant species BCF for *Azolla pinnata* R.Br. is less than that of *Salvinia molesta* Mitchell for a particular concentration. Therefore, *Salvinia molesta* Mitchell could be a better accumulator of chromium than *Azolla pinnata* R.Br.

**Table-5:** Variation of bio-concentration factor of *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell under different concentrations of chromium

| **Plant species** | **Chromium treatment (mg/l)** | **BCF** |
|---|---|---|
| *Azolla pinnata*R.Br. | Control (0.0) | 0 |
| | 0.07 | 1514.29 |
| | 0.09 | 1588.88 |
| | 0.11 | 1608.02 |
| | 0.13 | 1630.76 |
| *Salvinia molesta* Mitchell | Control (0.0) | 0 |
| | 0.07 | 1342.86 |
| | 0.09 | 1711.11 |
| | 0.11 | 2309.09 |
| | 0.13 | 2469.23 |

Values in the table are mean ± SD of 3 replicates

## CONCLUSION

It is evident from the study that, the floating hydrophyte *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell based treatment system proved to be a promising tool for the removal of chromium from the liquid medium. The favourable growth of *Azolla pinnata* R.Br and its growing biomass, has favoured rhizosphere activity in the liquid medium thereby enhancing metal uptake. The bio-concentration factor increased gradually with the increasing concentration of chromium and both the plant species growing in these concentrations showed higher accumulation capacities. The concentrations of chromium in the residual solution were below the permissible level of chromium for water. So it can be concluded that *Azolla pinnata* R.Br. and *Salvinia molesta* Mitchell are potential candidates for the removal of chromium from the polluted waterways.

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