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RESEARCH ARTICLE

# CHROMIUM INDUCED MORPHOLOGICAL AND BIOCHEMICAL RESPONSES OF *Salvinia molesta* MITCHELL

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**ABSTRACT**

The experiments were conducted to study the morphological and biochemical responses of *Salvinia molesta* Mitchell to different concentrations of Chromium exposed for 12 days. The data on Morphological and Biochemical parameters were recorded at the interval of 4 days. Test plant showed visible symptoms such as chlorosis and withering of roots at higher concentrations of Chromium however, the normal growth was observed at lower concentration of 0.25 and 0.50 ppm. Total chlorophyll, carbohydrate and protein content increased at lower concentration and decreased at higher concentration compared to control. Proline accumulation in test plants and directly proportional to the concentration of Chromium which was due to the stress induced by Chromium. The toxicity of Chromium was found to be directly proportional to its concentration and duration of exposure.



## INTRODUCTION

The concentration of the metal ions in the environment resulting due to the rapid development of industries inhibit a variety of metabolic activities and also proved to be toxic to most organisms. Chromium is commonly found in oxidized trivalent ionic state in the environment, whereas its hexavalent form Cr (VI) is present in smaller quantities which originates from widespread use of this metal in various industries, such as metallurgical (steel, Ferro and non ferrous alloys) and chemical (Pigments, electroplating, tanning, others). The primary source of Cr (VI) include chromate chemicals used as rust inhibitors and is emitted as the particulate matter delivered during manufacture and use of metal chromates, chromic acid mist from the plating industry, waste from the manufacture of the steel and other alloys, bricks in furnaces, dyes, pigments and effluents from leather tanning and wood preserving industry (ATSDR, 1993d). Chromium can be found in several oxidation states of which the most stable and common forms of Chromium include trivalent Cr (III) and hexavalent Cr (VI) with different chemical properties (Bagchi *et al.,* 2002). The hexavalent form is the most toxic; and it usually associates with oxygen to form chromate ($CrO_4^{2-}$), this molecule can easily enter the cell membrane as an alternative substrate in the sulphate transport system.

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More than 1, 70,000 tons of Cr wastes are discharged to the environment annually as a consequence of manufacturing activities involving Cr in the processing of the leather. Cr compounds discharged in liquid, solid and gaseous waste forms into the environment, results in significant adverse biological and ecological effects (Kabata and Pendias, 2001). The presence of heavy metals in the environment is a major concern because of their toxicity to flora and fauna (Priyadarshini *et al.,* 2012). Industrial waste, geo-chemical structures and mining of the metals creates a positional source of heavy metal pollution in the aquatic environment (Gagmum *et al.,* 1994). Through the anthropogenic activities the emissions of Chromium exceeded the natural concentration in soil and water environment (Eva and Jan, 2001). Several laboratory studies have been carried out to assess the toxic effects of Chromium in higher plants and algae (Bishnoi *et al.,* 1993). In view of these the present investigation was undertaken to study the effect of Chromium on morphological and biochemical responses of *Salvinia molesta* Mitchell at different concentrations.

## MATERIALS AND METHODS

The test plants were collected from Srinagar pond near Karnatak University, Dharwad, India. The young and healthy plants were selected and acclimatized for two weeks in the experimental tubs of 10 litre capacity containing Hoagland solution. About 50g of plant material was introduced simultaneously into experimental tubs containing 0.25, 0.50, 1.25 and 2.5 ppm of Chromium and control was maintained in tap water. The plants were harvested at the end of 4, 8, and 12 days of exposure, thoroughly washed with distilled water and used for morphometric and biochemical analysis. Chlorophyll, carbohydrate, protein and proline were estimated using the standard protocols as a biochemical response. The chlorophyll estimation was carried out by following standard method of Arnon and Hongland (1940), the protein by the method of Lowry (1951) using Bovine Serum Albumin (BSA) as a standard, carbohydrate by phenol sulphuric acid method (Dubois *et al.,* 1956) using glucose as a standard and proline by Bates method (1973). The plants were maintained under laboratory conditions and all the experiments were carried out in triplicates and values are expressed as Mean±SE.

**Statistical analysis**

Statistical analysis of the data was carried out by employing two way ANOVA to know the significance between concentrations and durations of exposure using Statistical software SPSS ver.16.

## RESULTS AND DISCUSSION

**Morphological responses**

Heavy metals are the major environmental pollutants which enter the aquatic environment through industrial, domestic and agricultural wastes (Sankar *et al.,* 2008). They create a serious threat to aquatic flora and fauna as they do not degrade easily. The inhibition of root growth and leaf has been reported to be directly proportional to the concentration of Chromium. Heavy metal accumulation in aquatic macrophytes is known to produce significant physiological and biochemical responses (Satykala, 1997; Shanker, 2005; Choo *et al.*, 2006). The morphological changes in *Salvinia* in response to Chromium are presented in Table 1.

**Table 1. Effect of Chromium on Morphology of *Salvinia molesta* Mitchell**

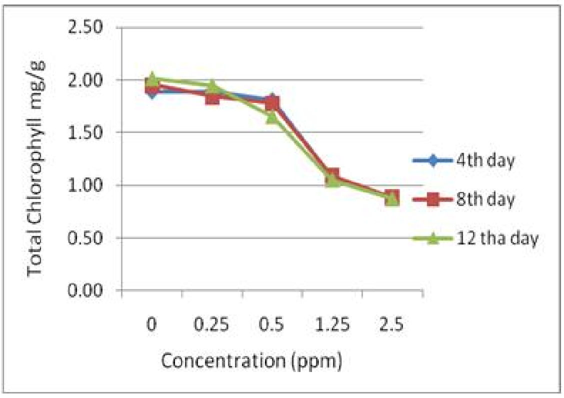
| Days | 4 | | | 8 | | | 12 | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration (ppm) | Root length (cm) | Leaf Length (cm) | Leaf Width (cm) | Root length (cm) | Leaf Length (cm) | Leaf Width (cm) | Root length (cm) | Leaf Length (cm) | Leaf Width (cm) |
| Control | 3.16±0.05 | 1.94±0.02 | 2.74±0.10 | 3.65±0.09 | 1.61±0.07 | 2.67±0.09 | 4.74±0.10 | 1.50±0.10 | 2.06±0.03 |
| 0.25 | 3.90±0.12 | 1.56±0.02 | 2.54±0.09 | 4.10±0.03 | 1.47±0.02 | 2.50±0.06 | 5.74±0.08 | 1.57±0.02 | 2.28±0.04 |
| 0.50 | 3.21±0.07 | 1.60±0.03 | 2.58±0.14 | 3.98±0.01 | 1.56±0.03 | 2.59±0.15 | 4.15±0.02 | 1.66±0.03 | 2.80±0.10 |
| 1.25 | 3.0±0.04 | 1.72±0.01 | 2.54±0.04 | 3.14±0.11 | 1.30±0.03 | 2.30±0.10 | 3.10±0.02 | 1.48±0.01 | 2.30±0.10 |
| 2.5 | 2.9±0.10 | 1.83±0.08 | 2.70±0.10 | 3.11±0.07 | 1.58±0.01 | 2.48±0.03 | 3.09±0.06 | 1.53±0.15 | 2.58±0.04 |

Each value represents mean ± SE.

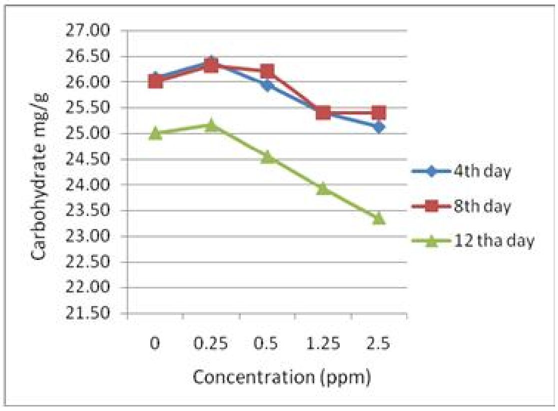
*Salvinia* shows no symptoms of morphological toxicity at 0.25 ppm of Chromium and shows normal growth. However, at higher concentration (2.5ppm) leaves turn yellowish white, margin of the lamina becomes dark brown and show degeneration of roots. Several workers have reported that the symptoms like reduced growth, chlorosis, necrosis, leaf epinasty and Reddish brown discoloration are due to metal toxicity. Reduced growth of tomato plants due to presence of Chromium in nutrient solution has been reported by (Nagoor, 1999). It has been concluded that the reduced growth in terms of shoot length and root length at increasing doses could be due to the effect of metal on auxin synthesis in plants (Rolli *et al.,* 2013). Excess doses of Chromium has been reported to cause adverse effect on the iron metabolism of paddy plants which resulted in reduced concentration of total carbohydrate and chlorophyll (Anil *et al.,* 2006) it may also cause reduced photosynthesis resulting in stomatal closure, reduced intracellular spaces and alteration in chloroplast. The lower concentration 0.25 and 0.5 ppm enhanced the root length throughout the period of exposure. The root length of 3.9 and 3.21 cm for the concentration 0.25 and 0.5 ppm on 4$^{th}$ day, 4.1 and 3.98 cm on 8$^{th}$ day and 5.74 and 4.15 cm were observed on the 12$^{th}$ day of exposure. However, the root length decreased at the higher concentrations of 1.25 and 2.5ppm throughout the period of exposure showing the toxic effect of higher concentration of Chromium on root growth. Reduction in the leaf length and width was observed at every concentration of Chromium for the whole period of exposure which indicates that the toxicity of Chromium is directly proportional to its increasing concentration.

**Biochemical responses**

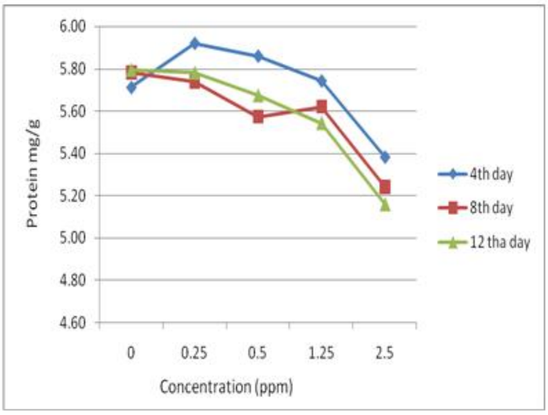
The biochemical responses of *Salvinia molesta* to Chromium are represented in the Figure.1. (A-D), it is evident that chlorophyll content of *Salvinia molesta* decreases with increasing concentration and duration of exposure. The chlorophyll content of water lettuce showed a similar trend of decline, corresponding to the increase in the concentration of Cr (VI) (Qin *et al.,* 2011). This decline in chlorophyll content under heavy metal stress could be attributed to the inhibition of important enzymes of biosynthesis such as α-aminolevulinic acid dehydrogenase (α-ALAD) and photochlorophllide reductase (Ouzounidou *et al.,* 1993; Satykala and Jamil, 1997). The carbohydrate production is promoted at the lower concentrations of Chromium throughout the period of exposure but it decreased at the higher concentration of Chromium indicating the negative effect of Chromium over the carbohydrate production at the higher dose. The similar kind of trend was noticed in case of protein production where the protein production promoted at the lower concentration but it decreased in higher concentration. The higher heavy metal concentration interferes the carbohydrate metabolism which can be attributed to its negative impact on carbohydrate production (Cook *et al.,* 1998). The tolerance mechanism in plants under stress has been associated with accumulation of amino acids. The production of amino acids and protein content were observed to gradually reduce with gradual



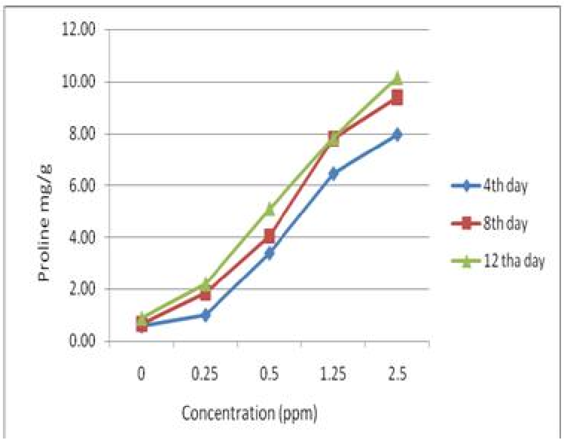

A




B




C




D

**A) Total Chlorophyll B) Carbohydrate C) Protein D) Proline**

increase of Chromium concentrations. The nitrogen content of plants has been reported to get reduced by metal stress; ultimately the amino acid content is also to get reduced (Grill *et al.,* 1986). In general Chromium adversely affects several physiological activities and produce severe stress (Assche, 1990; Samantary 2002). The proline content in the test plants increased at every concentration of Chromium showing direct proportionality to the increased concentration of Chromium. Increase in the proline content in plants can be attributed to different types of stress such as salinity, drought, low and high temperature, and heavy metals (Ibarra *et al.,* 1988; Kishor *et al.,* 1995). Substantial increase has been reported in the Proline content of leaves of wheat with increasing heavy metal concentrations (Panda *et al.,* 2003). The toxicological impact of Chromium Cr (VI) has been concluded to originate from its action as an oxidising agent, as well as from the formation of free radicals during the reduction of Cr (VI) to Cr (III) occurring inside the cells (Niebor and Jusys, 1988). Proline accumulates in variety of plant species in response to stress, under stress conditions proline may act as an osmotic adjustment mediator, a sub cellular structures stabilizer, a free radical scavenger and a redox potentional buffer (Molinari *et al.,* 2007).

### Conclusion

In the present investigation, it has been observed that the test plant species *Salvinia molesta* Mitchel showed visible symptoms such as chlorosis and withering of roots at higher concentrations of Chromium. However, at the lower concentration it showed the normal growth. The total chlorophyll decreases with increase in concentration of Chromium. The lower concentration of Chromium promotes the production of chlorophyll, carbohydrate and protein, where as it showed negative effect at higher concentration. The proline content has been found to increase with increased concentrations and durations. Thus from the present investigation it can be concluded that the higher concentration of Chromium is toxic to *Salvinia molesta* and its toxicity is directly proportional to its concentration and duration of exposure.

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