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EFFECT OF CHROMIUM ON MORPHOLOGICAL AND BIOCHEMICAL RESPONSES ON AQUATIC MACROPHYTE *PISTIA STRATIOTES* L. (WATER LETTUCE)

S. B. Kakkalameli [*1], Azharuddin Daphedar [2], M. Govindappa [1] and T. C. Taranath [2]

Davangere University [1], Shivagangotri, Davangere - 577002, Karnataka, India.
Environmental Biology Laboratory [2], P. G. Department of Studies in Botany, Karnatak University, Dharwad - 580003, Karnataka, India.

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Correspondence to Author:
S. B. Kakkalameli

Davangere University, Shivagangotri, Davangere - 577002, Karnataka, India.

E-mail: dubotsiddu@gmail.com

ABSTRACT: The current work reports an ecofriendly approach to study morphological and biochemical responses of *Pistia stratiotes* L. (Water lettuce) were investigated. The assessment of morphological, biochemical parameters, and accumulation of chromium in free-floating macrophyte exposed at its various concentrations *viz*. 0.25, 0.50, 1.25, and 2.5 ppm of chromium at the regular intervals of 4, 8, and 12 days, respectively. *Pistia* showed morphological toxicity like withering of roots, chlorosis, and necrosis at higher concentrations (2.5 ppm) of chromium. However, the plant showed normal growth at a lower concentration of 0.25 and 0.50 ppm. The evaluation of biochemical parameters of test plants showed Total Chlorophyll, Carbohydrate, and Protein content was increased at lower concentrations (0.25 ppm) and decreased at higher concentrations (2.5 ppm) of chromium. The toxicity of chromium was found to be directly proportional to its concentration and duration of exposure. It is evident from the results that the accumulation of chromium can produce adverse effects on the morphology of test plant species at a higher concentration of chromium. This may be attributed to the stress-induced by chromium.

INTRODUCTION: The rapid development of industrialization and urbanization, coupled with an alarming rate of population growth, has resulted in the large scale pollution of aquatic ecosystems by industrial and domestic wastewater discharge. Our environment, which consists of biotic and abiotic functions, is a part of the giant ecosystem called the biosphere or ecosphere. Biosphere complexly interfaces with other systems: hydrosphere, lithosphere, and atmosphere. Each of the systems is unique, with its own physicochemical factors and biotic components.

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Man-made pollutants affect the quality, causing abnormal functioning of these ecosystems and thus adversely affect plant species, animals, including humans.

Man-made pollutants can be broadly classified into biodegradable and non-biodegradable categories. Non-biodegradable or persistent organic pollutants (POPs) include Aluminum tins, Mercurial salts, Long-chain phenolic compounds, Dichlorodiphenyltrichloroethane (DDT), metals like Cu, Mn, Zn, Cr, and Ni, *etc*., which mostly do not degrade and further results in bio-magnification. Domestic wastewater is one of the quantifiable sources of biodegradable pollutants. Heavy metals are so heavy that even at lower concentrations can cause toxicity to humans and other forms of life. Microbial degradation is considered the best option to counter such toxic pollutants.

From a biological point of view, heavy metals can be divided into two categories: essential and non-essential. However, essential heavy metals have also been reported to be toxic at high concentrations. These heavy metal influences at the cellular level affecting the entire metabolic process, whereas the intracellular and extracellular accumulation of metals is usually energy-driven processes and thus can take place only in living cells [1, 2, 3, 4]. The presence of heavy metals in water and wastewater is mainly due to the industrial effluent sewage disposal or into the water bodies. The occurrence of toxic metals in pond, ditch, and river water affects the lives of local people that depend upon these water sources for their daily requirements [5]. Several of the submerged, emergent, and free-floating aquatic macrophytes are known to accumulate heavy metals [6, 7]. Macrophytes are aquatic plants growing in or near water that is emergent, submerged, or floating. Macrophytes are beneficial to the lake because they provide food, shelter, and breeding habitats for fish and aquatic invertebrates.

Not all macrophytes are effective for heavy metal remediation. Earlier reports reported that on certain aquatic macrophyts includes *Phragmites communis, Scirpusla custris, Eichhornia crassipes, Spirodela polyrrhiza, Chara corallina, Vallisneria spiralis, Alternanthera sessilis and Hygrorrhiza aristata Elodea canadensis, Egeriadensa, Ceratophyllum demersum, Bacopa monnieri, Limnanthemum cristatum, Potamogeton malaianus, Nymphoides peltata, Hydrilla verticillata* and the algal macrophytes, *Hydrodictyon reticulatum*, have been found suitable for the removal of different metals [8, 9, 10].

P. stratiotes is a monotypic genus, and free-floating herb mostly thrives best in still water or streams of minimal flow. Being warm loving, it is abundantly found in tropical and subtropical regions of *P.stratiotes* are a perennial and having light yellow, green leaves arranged in rosettes. Unbranched clusters of long fibrous roots extending from the centre of underwater rhizome (Rhizome bears root pockets). Seed production is limited, and propagation is mainly by offsets and buds. Leaves are densely clothed on both surfaces with short depressed hairs, which give the foliage a velvety appearance. Chromium (Cr) occurs naturally at trace levels in most soils and water, but disposal of industrial waste and sewage sludge containing Cr compounds has created a number of contaminated sites, which could pose a major environmental threat. Cr primarily exists as a soluble, highly toxic Cr_6 anion as against its reduced form Cr_3, which is less soluble and less toxic. Reduction /oxidation reactions between the two states are thermo-dynamically possible under physiological conditions [11], and hence reduction of Cr_6 to Cr_3 is potentially useful for remediation of Cr affected environments [12].

MATERIALS AND METHODS:

Collection of Test Plant Species: The plant Species of *P. stratiotes* belonging to the family Araceae were collected from Srinagar pond near Karnatak University, Dharwad, Karnataka, India.

Experimental Design: The collected young and healthy plants were acclimatized for two weeks in the experimental tubs of 10-liter 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 at the interval of 4, 8, and 12 days, tap water was used as control. After treatment, the plants were washed with double distilled water for morphometric and biochemical analysis.

Assessment of Morphometric and Biochemical Responses of Heavy Metals: The study of biochemical response reveals using standard protocols of chlorophyll, carbohydrate, protein and proline were estimated. The chlorophyll estimation was carried out as suggested by the standard method of Arnon and Hongland [13], the protein by the method of Lowry (1951) using Bovine Serum Albumin (BSA) as a standard, carbohydrate by phenol sulphuric acid method [14] using glucose as a standard and proline by Bates method (1973).

Statistical Analysis: The experiments were carried out using statistical analysis IBM (SPSS windows software) version 20. The standard error (Mean ± SE) was performed from three replicates for every set of data.

RESULTS AND DISCUSSION:

Plant Morphological Changes and Chromium Accumulation: The morphological changes of *P. stratiotes* in response to chromium are presented in

Table 1. *P. stratiotes* showed morphological toxicity at 0.25 ppm of chromium. However, at higher concentrations (2.5 ppm) the lower rosette of leaves becomes whitish-yellow, resulting in degradation and withering of leaves. The root caps get degenerated, and roots become dark brown in color. Our investigation revealed that the lower concentration of chromium (0.25 ppm) promotes the root length (12.93 cm), laminal length (2.93 cm), and breadth (3.53 cm) when compared with control at 12 days of exposure. The higher concentration (2.5 ppm) of chromium was found to inhibit the root length (7.96 cm), laminal length (1.83 cm) and breadth (2.37 cm) at 12 days of exposure (Table 1). The results were statistically not significant at $p<0.01$ and $p<0.05$ level at all tested concentrations when compared to control. From the data, it is evident that the chromium accumulation in *Pistia* was directly proportional to its concentration and duration of exposure. Our results are in substantiation with that of [15, 16].

In general, the aquatic macrophytes show normal growth and no visible symptoms at 0.5 ppm chromium treatment. The leaves are a greenish healthy and marginal increase in their size at 0.5 ppm. The exposure of Chromium to the plants to 2.5 and 5 ppm was found to inhibit the growth of the plant, resulting in etiolation and withering of leaves. In cellular activities of plants are affected by heavy metal pollution, including mineral nutrition, photosynthesis, respiration, membrane structure, gene expression, and other properties [17] Plant growth is a function of the complex interplay between sources and sinks limitations of the two main organs of a plant, the root, and shoots system, establishing functional equilibrium [18]. Heavy metals lead potential pollutants that readily accumulate at the cellular level, causing physiological, biochemical, and ultrastructural changes due to chromium toxicity resulting in dysfunction in plants. Heavy metal toxicity may cause multiple direct and indirect effects in certain tissues of plants such as the epidermis; cortex, and endodermis. There are several substantiations to show that metal toxicity has a direct effect on growth inhibition of many plants, cereals, pulses, vegetables, forages, and trees [19].

Recent studies suggest that the induction of metal toxicity caused by two reasons:

(a) Inhibition of cell division and (b) decrease of cell expansion, which can be disturb mitochondrial outer membrane permeabilization during oxidative stress, stopping oxidative phosphorylation, ATP production, and inhibition of cell division or cessation of mitosis due to heavy metal stress [20, 21].

The 2.5 ppm of chromium found to inhibit root and plant length with an exposure duration of 4, 8, and 12 days for *P. stratiotes*. The change in the pigment content in heavy metal exposed plants is one of the first visible symptoms. Additionally, these changes are used as an indicator of photosynthetic damage in plant tissues. Similar observations were observed with heavy metals (Ni and Cd) uptake, distribution, and accumulation in plants. Their effects on physiological and morphological traits have been summarized in several recent reviews [22, 23]. Though beneficial (Ni) or harmless (Cd) at lower concentrations, both elements induce phytotoxic effects at concentrations higher than the tolerance threshold of the different plant species since they are taken up through metal transporters with low specificity [24, 25, 26].

Many physiological processes are impaired by stress Cd and Ni, resulting not only in common but also in specific symptoms of metal toxicity [27, 28, 29]. The morphological changes observed in *Salvinia. adanata*, in response to chromium shows symptoms of morphological toxicity and normal growth. However, at higher concentrations, leaves turn yellowish-white, the margin of the lamina becomes dark brown, brownish discoloration, and shows degeneration of root. These results are in agreement with those reported for aquatic plants in general [30].

A decrease in the number of roots is a well-documented effect due to heavy metals in trees and crops. It was reported that the order of metal toxicity to new root primordial in *Salix viminalis* is Cd>Cr>Pb whereas root length was more affected by other heavy metals. The adverse effect of chromium on plant height and shoot growth was reported. Growth inhibition by Chromium can be attributed to chromosomal aberrations, which lead to inhibition of cell division. It is known that in many plant species, the mobility of Cr is low due to the fact that there are barriers or lack of transport mechanism suitable for Cr transport from roots to shoot lengths activities of enzymes catalase and peroxidase were found to be significantly decreased as compared to control. Several workers have reported that symptoms like reduced growth, Chlorosis necrosis, leaf epinasty, red-brownish discoloration due to Chromium metal toxicity [31].

TABLE 1: EFFECT OF CHROMIUM ON MORPHOLOGY OF *PISTIA STRATIOTES* L.

Conc. (ppm)	Exposure Duration (in Days)								
	4	8	12	4		8		12	
	Root length			Length	Breadth	Length	Breadth	Length	Breadth
Control	12.5±4.75	13.16±4.93	13.63±5.24	3.23±0.14	3.5±0.15	3.36±0.23	3.56±0.18	3.53±0.03	3.63±0.17
0.25	12.7±3.08	12.83±3.13	12.93±3.07	2.73±0.39	3.36±0.27	2.83±0.44	3.46±0.31	2.93±0.40	3.53±0.33
0.5	10.46±1.29	10.73±1.12	10.63±1.18	2.4±0.15	3±0.25	2.46±0.18	3.03±0.21	2.53±0.17	2.94±0.22
1.25	9.26±0.43	8.93±0.31	8.83±0.21	2.26±0.48	2.7±0.37	2.2±0.47	2.63±0.37	2.16±0.52	2.53±0.38
2.5	8.36±2.82	8.06±2.02	7.96±2.11	2.06±0.24	2.6±0.30	1.96±0.24	2.46±0.26	1.83±0.26	2.37±0.21

Values are expressed in cms, Mean values ± Standard Error

TABLE 2: EFFECT OF CHROMIUM ON BIOCHEMICAL PARAMETERS OF *PISTIA STRATIOTES* L.

Concentration (ppm)	Exposure duration (Days)	Biochemical parameters			
		Total Chlorophyll	Carbohydrate	Protein	Proline
Control	4	1.47±0.00	26.7±0.16	5.37±0.17	0.53±0.05
	8	1.48±0.00	26.58±0.34	5.49±0.25	0.59±0.00
	12	1.51±0.00	26.43±0.20	5.62±0.09	0.85±0.03
0.25	4	1.36±0.02	27.43±0.10	5.78±0.14	0.97±0.07
	8	1.64±0.02	27.04±0.30	5.61±0.34	2.52±0.16
	12	1.32±0.03	26.58±0.39	5.74±0.53	2.98±0.18
0.5	4	1.04±0.01	27.12±0.26	3.9±0.34	2.97±0.07
	8	1.23±0.00	26.31±0.13	3.49±0.24	1.58±0.15
	12	1.12±0.00	26.19±0.20	3.25±0.25	1.03±0.28
1.25	4	1.09±0.00	26±0.20	3.67±0.26	1.42±0.08
	8	0.9±0.00	25.46±0.23	3.28±0.13	1.92±0.20
	12	0.89±0.00	25.85±0.24	3.07±0.33	1.2±0.26
2.5	4	0.93±0.03	24.96±0.10	2.9±0.25	0.77±0.05
	8	0.87±0.00	24.5±0.30	2.84±0.38	0.69±0.25
	12	0.85±0.01	24.85±0.33	2.79±0.11	0.1±0.39

* Values are expressed in µg/gm tissue, Mean values ± Standard Error

Effect of Chromium on Biochemical Parameters of *P. stratiotes*: Most of the aquatic plants growing in a contaminated site have the ability to tolerate the high intensity of metal concentration. The accumulation of the high amount of heavy metals without showing any phenotypic damage/ expressions, but there are certain alterations in their internal physiology [32]. The reduction of photosynthetic pigments is mainly due to metal stress, which is very common in chloroplasts and plays a significant role in modifying many metabolic pathways through the biochemical and physiological process [33].

Hadif *et al.*, [34], suggested that the amount of chlorophyll such as chlorophyll a, chlorophyll b, total chlorophyll (a+b), and chlorophyll (a/b) ratio, were affected by the chromium concentration in the leaves of paddy. It is found that the higher concentrations of chlorophyll decrease with increase in the concentration of chromium. The results exhibit there has been an increased in the total chlorophyll content, which varies from (1.88 mg/g to 1.94 mg/g at 0.25ppm) Chromium during exposure duration of 4th and 12th days. The results are statistically significant when compared to control. Similar results were found in *Marselia sp*. (16. 94%). Total chlorophyll content showed strong negative correlations with Fe (0.974, *Eclipta sp*.), Cr (0.997 *Eclipta sp*.), Cd (0.998, *Eclipta sp*.), Pb (0.999, *Alternanthera sp*.), Zn (0.981, *Marselia sp.)* and Cu (0.998, *Eclipta sp*.). Reduction in chlorophyll content was due to the substitution of central Mg^{2+} with metal ions; besides, excess accumulation of metals can also lead to higher chlorophyllase activity and the destruction of the structure of chloroplasts.

Similarly, chlorophyll concentration in *S. polyrhiza* was negatively correlated with Pb exposures nutrient enrichment attenuated the observed reduction in chlorophyll concentration caused by

Pb exposures. When *Spirodela* fronds were exposed to Pb concentrations of 5 mg/L or higher, a dose-dependent decrease of chlorophyll pigments were also observed, with minimum chlorophyll "a" value of 0.414 mg/g fresh weight on day 7 at 50 mg/L compared to 1.601 mg/g in controls [35].

A decrease in soluble sugar in all the aquatic plants was also noticed due to high metal stress, which is consistent with the findings of Warrier and Saroja [36]. *Marselia sp.* Showed the highest reduction of soluble sugars (53.28%), which is highly sensitive to environmental stress. The reduction of sugar content in stressed plants probably corresponds with the photosynthetic inhibition or stimulation of respiration for higher energy requirements [37].

A significant negative correlation exists between soluble sugars Fe (0.983, *Eclipta sp*.) and soluble sugars Cr (0.983, *Ipomea sp.*). Both biotic and abiotic stress may inhibit the synthesis of some proteins and cause protein misfolding leads to inhibiting the expression of genes [38].

TABLE 3: TWO WAY ANOVA FOR EFFECT OF BIOCHEMICAL PARAMETERS OF *P. STRATIOTES* L.

	Total Chlorophyll	Carbohydrate	Protein	Proline
F-Value Between Concentration	32.944	5.706	0.918**	29.381**
F-Value Between Duration	815.7**	37.573**	64.494**	111.04**
Days* Concentration	41.795	0.924	0.473	1.193

*Significant at 0.01 level, **Significant at 0.05 level

There are several earlier investigations on the metal accumulation capacity of *Ipomea sp*. [39], *Eclipta sp*., *Marselia sp*. [40], *Alternanthera sp* and *Typha sp*. [41]. Aquatic macrophytes showed varied affinities for different metal uptake. Most of the aquatic macrophytes exhibited higher metal accumulations in the leafy vegetative parts except *Typha sp*., which indicated high metal concentrations in its underground part due to its well-developed roots and rhizome system. Among amino acids, Proline responds most sensitively to stress conditions [42]. Proline accumulation is accepted as an indicator of environmental stress, including metal contamination considered to have important protective roles [43]. The Proline content of the macrophytes species exhibited strong positive correlations with Fe (0.967, *Ipomea sp*.; 0.978, *Eclipta sp*.) and Cr (0.998, *Eclipta sp.)*, The proline accumulation changes in plant tissue may be explained by a decrease in proline degradation or an increase in proline biosynthesis and hydrolysis of proteins [44].

The biochemical effects of chromium on *P. stratiotes* are presented in **Table 2**. The 0.25 ppm of chromium promotes the Chlorophyll synthesis by 1.36 mg/g and 1.32 mg/g at 4 and 12 days of exposure when compared to control. At higher concentrations (2.5 ppm) inhibit the synthesis of chlorophyll by 0.93 mg/g and 0.85 mg/g at 4 and 12 days of exposure **Table 1**. The higher concentration (2.5 ppm) of Chromium promotes the synthesis of carbohydrates at 4 and 12 days of exposure by 24.96 mg/g and 24.85 mg/g when Compared to control, **Table 3**. The lower concentration (0.25 ppm) of Chromium promotes the synthesis of protein by 5.78 mg/g and 5.74 mg/g at 4 and 12 days of exposure. Whereas at higher concentrations (2.5 ppm) it promotes the synthesis of protein at 4 and 12 days of exposure by be 2.9 mg/g and 2.79 mg/g when compared to (Table 1).

The higher concentration (2.5 ppm) induces the accumulation of Proline with 2.77 mg/g and 4.1mg/g at 4 and 12 days of exposure when compared to control **Table 1**. The three-way ANOVA with two-factor interaction among the concentration, exposure duration, and parameters were applied to know the relation between concentration and exposure duration, concentration and parameters, and exposure duration and parameters. The factors are related but not at the significant level in **Table 2**.

CONCLUSION: In the present investigation, it has been observed that the test plant species *P. stratiotes* showed visible symptoms such as Chlorosis and withering of roots at higher concentrations of Chromium. However, at the lower concentration, it showed normal growth. The total chlorophyll decreases with an increase in the concentration of Chromium. The lower concentration of Chromium promotes the production of chlorophyll, carbohydrate, and protein, whereas it showed a negative effect at higher concentrations. The Proline content has been found to increase with increased concentrations and duration of exposure. Thus, from the present investigation, it can be concluded that the higher concentration of Chromium is toxic to *P. stratiotes*, and its toxicity is directly proportional to its concentration and duration of exposure.

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