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Chromium (CrVI) Toxicity and Tolerance in *Ipomoea aquatica* Forssk.: An Integrated Approach

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Abstract—*Plant based remediation techniques are showing increasing promise for removal of toxic metals from polluted water. This study investigates Ipomoea aquatica, a widespread macrophyte, for its toxicity and tolerance properties by exposing it to graded concentrations of Chromium (CrVI) ranging from 0.01 to 2 mg L^{-1} for 15 days. A dose and time dependent reduction of growth parameters of shoot height, new nodes and new leaves were observed on the 5th, 10th and 15th day of exposure when compared with those in control. Chlorophyll content and fresh weight estimated on the 15th day also showed dose and time dependent reduction. Based on the dry weight biomass, the Tolerance Index (Ti) of leaf, stem and roots of the plant can be defined as highly tolerant (Ti> 60) in all concentrations. The plant showed some visual symptoms like chlorosis of the older leaves, but complete withering or wilting was not encounter during this toxicity test. Being resistant to CrVI in terms of survival capacity above ambient concentration coupled with its capacity to proliferate by fragmentation through producing adventitious roots and lateral branches from nodes, Ipomoea aquatica has the prospect of being employed for phytoremediation of Chromium-contaminated wastewater.*

Keywords: *Toxic metals, Macrophyte, Growth, Tolerant, Phytoremediation, Wastewater*

"1. Introduction"

Chromium (Cr) is the seventh most abundant element in Earth's crust [17] and is a major metal contaminant in water, soil, and sediments due to its extensive industrial applications [30]. It can exist in oxidation states from -2 to 6 but mainly exist in the environment in the trivalent (CrIII) or hexavalent state (CrVI). CrIII is sparingly soluble and less toxic, while CrVI being more soluble in water, is highly toxic to biota [24]. CrVI has an extended residence time in ground and surface water [23]. Concerning plants, the essentiality of the role of Cr in plant metabolism is not precisely documented, and literature findings are contradictory in this regard; while some studies indicate that Cr is not essential in plants [14], others have shown that small additions of Cr have stimulating effects on plant growth and productivity [39, 11]. Chromium is also associated with decrease in plant growth and changes in plant morphology [23]. CrVI is carcinogenic to humans and classified as a Group A known human carcinogen [32].

Chromium concentration in rivers and lakes is usually limited to 0.5-100 nm [19]. An average of 2.89 µg L^{-1} of Cr was recorded in Indian rivers [7]. Mean wastewater concentrations of 410 µg L^{-1} CrIII and 296 µg L^{-1} CrVI were found at a dye plant [13]. Nowadays, majority of the leather industry preferred chrome tanning because of the speed of processing, color of leather, cost effectiveness and greater stability of the resulting leather. Cr-contaminated water and soil ultimately destroy crops and impart serious health hazards in human beings by entering through the food chain [34]. In developing countries like India, the effective management of chromium effluent has become a daunting task. Hence, contamination of water resources through chromium rich effluents imposed serious health hazards to biota [33].

Aquatic macrophytes inhabiting chromium polluted water bodies have been found to reduce the level of toxic metals [26]. The present study investigates the potential of an aquatic macrophyte, *Ipomoea aquatic* for biomonitoring, where hydroponic screening for chromium toxicity coupled with assessment of tolerance was performed. *I. aquatica* was selected on the basis of its geographical distribution, availability and adaptability. It covers not only large parts of India, but in the entire South, South-East and East Asia in terms of distribution and easy to propagate by fragmentation through production of roots and shoots from its nodes [8,5]. The distribution of this plant is also reported from other parts of the world like Africa, Australia and United States of America [3].

"2. Materials and methods"

Plant material and stock culture

Ipomoea aquatica Forssk. was collected from unpolluted water bodies at Irongmara, Silchar, Assam, India. The plants were grown in hydroponic tubs till new branches developed. The new branches were cut and planted in soil flooded with

50% Hoagland solution (HS). The pH of the nutrient media was adjusted in a range of 5.8-6.2. The soil and the nutrient medium were renewed every week and the reduced water level due to evaporation and transpiration was compensated by tap water. Healthy and fully grown shoots of similar shoot height were cut from the same mother plant, washed with tap water and acclimatized in 50 % Hoagland nutrient solution for one week at 25-27°C, 12 h light with an intensity of 100-120 $\mu mol^{-2}\ s^{-1}$ and 12 h dark periods [12, 18, 4].

Experimental Procedures

After the acclimatization period, plants of similar shoot height were exposed to graded concentrations of CrVI as $K_2Cr_2O_7$ (actual CrVI concentrations: 0.01, 0.1, 0.5, 1 and 2 mg L^{-1}). Control plants were also cultured in 50 % Hoagland nutrient media without $K_2Cr_2O_7$. Toxicological endpoints such as changes in shoot height, number of new nodes and number of new leaves with respect to those recorded at the beginning of the experiment (0 h) were observed on the 5th, 10th and 15th day. The fresh weight was taken on the 15th. The results were compared with those obtained from control plants.

The Tolerance index (T*i*) was to determine the ability of the plant to thrive in the given concentrations of CrVI. On the 15th day, T*i* of the plant was calculated as follows:

T*i*= Dry weight of the plant parts grown in chromium solution / Dry weight of the plant parts grown in control solution × 100

Dry weight of the plant parts referred to root, stem and leaf [37, 38]

Chlorophyll estimation was performed on the 15th day. 100g fresh leaf was homogenized with 80 % acetone, centrifuged, and the absorbance of the supernatant was taken at 662, 645 and 470 nm for chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*) and total carotene, respectively, in a spectrophotometer. The concentrations of these pigments were calculated following standard formula [20] with the extraction solution used as blank.

Statistical analysis comprised one-way analysis of variance (ANOVA) with least square difference (LSD) test at $p < 0.05$ using SPSS 20 for Windows.

"3. Results and Discussion"

Chromium toxicity and tolerance in *Ipomoea aquatica* was found to be concentration and duration dependent caused by enhanced chromium levels in the growth medium. The plant was able to survive the total exposure period of 15 days at all concentrations of CrVI, of which 2 mg L^{-1} was the highest. Toxicity symptom such as chlorosis was, however encountered. In like manner, severe chlorosis and necrosis of leaf in *Echinochloa colona* was reported at high concentrations of Cr in solution culture [28]. Chromium at 5 and 10 ppm in nutrient solutions produced chlorosis similar to iron chlorosis in oat plants [15]. Older leaves of *Salvia sclarea* treated with 5 ppm Cr showed interveinal chlorosis with the development of interveinal necrotic areas at the time of harvest in this plant [6]. Chromium stress also induced a significant decline in growth parameters of two barley genotypes [1]. Reduction in growth of plants under Cr stress was also observed in *Lolium perenne* [35], wheat, oat and sorghum [23], and *Datura innoxia* [36].

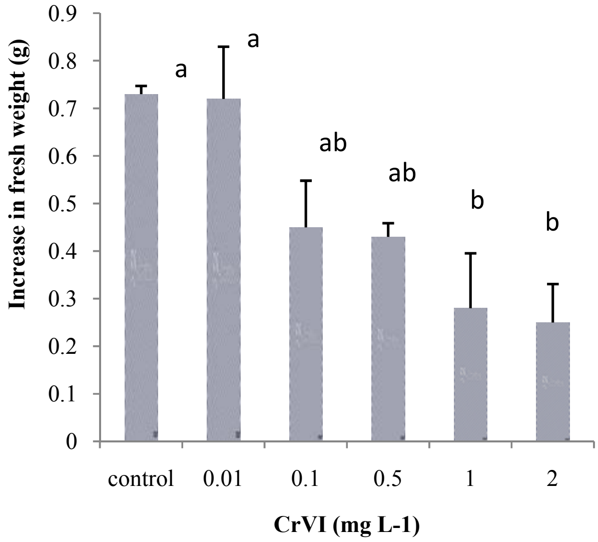

Fig. 1. Effect of CrVI on fresh weight of *I. aquatica* after 15 days. Values are given as Mean ± SE; Values with different superscript letters indicate significant difference at P < 0.05

Even low concentrations of CrVI inhibited the fresh weight of *I. aquatica* and the effect was more pronounced with high CrVI levels. The reductions were significant at 1 and 2 mg L^{-1} from that in control as presented by Fig.1. A significant decrease in fresh weights of shoot and roots was observed in sunflower (*Helianthus annuus L.*) exposed to Cr [2].

Table 1: Effects of CrVI on increase in (a) shoot height, (b) new node and (c) new leaf in *I. aquatica*

(a)

CrVI	Increase in shoot height		
(mg L^{-1})	5 D	10 D	15 D
Control	$13.2±2.3^a$	$23.2±5.4^a$	$30.2±3^a$
0.01	$14.5±0.3^a$	$19.1±0.6^a$	$29.5±2.3^a$
0.1	$5.9±2.2^b$	$8.3±1.7^b$	$13.2±3.2^b$
0.5	$3.9±1.2^b$	$8.8±3.6^b$	$11.4±4.2^b$
1	$2.9±0.6^b$	$5.9±0.8^b$	$8.1±1.8^b$
2	$2.6±0.5^b$	$4.5±0.5^b$	$7.4±1.3^b$

(b)

CrVI	Increase in new node		
(mg L^{-1})	5 D	10 D	15 D
Control	$3.3±0.3^a$	$4±0.5^a$	$4±1.1^a$
0.01	$2±0.9^a$	$4.3±0.3^a$	$6±0.9^{ab}$
0.1	$1.3±0.3^a$	$2.7±0.8^{ab}$	$3.7±0.6^{ac}$
0.5	$0.7±0.3^a$	$1.7±0.3^{ab}$	$2.3±0.3^{bc}$
1	$0.3±0.3^a$	$0.7±0.3^b$	$0.7±0.3^c$
2	$0.3±0.3^a$	$0.3±0.3^b$	$0.7±0.3^c$

(c)

CrVI ($mg\ L^{-1}$)	Increase in new leaf		
	5 D	10 D	15 D
Control	4 ± 0.6^{a}	4.7 ± 0.3^{a}	6 ± 1^{a}
0.01	2.7 ± 0.3^{ab}	5.3 ± 0.8^{a}	6 ± 0.6^{ab}
0.1	1 ± 0.5^{b}	3 ± 1.1^{a}	4.3 ± 1.2^{ac}
0.5	1 ± 0.5^{b}	2.6 ± 0.3^{a}	3 ± 0.5^{ac}
1	1.3 ± 0.3^{b}	1.7 ± 0.8^{a}	2 ± 0.9^{bc}
2	1.3 ± 0.3^{b}	1.3 ± 0.3^{a}	1.7 ± 0.3^{bc}

Values are given as Mean ± SE; Values with different superscript letters indicate significant difference at $P < 0.05$

The present study revealed that CrVI at 0.01 mg L^{-1} slightly increased the shoot height on the 5th day. The appearance of new node was also slightly increased at 0.01 mg L^{-1} CrVI on the 10th and 15th day. Chromium was never recognized as an essential element for plant growth, but some of its stimulative effects were reported [29]. It was also reported that lettuce grown in nutrient solutions was sometimes slightly benefited by 0.1 ppm of chromium. In some cases, chromium stimulated the growth of the plants at lower concentrations, whereas at higher concentrations it had definite growth retarding effects [37].

As shown in Table 1(a), there was decline in shoot length with significant effect at 0.1 mg L^{-1} CrVI as revealed by one way Anova at $p < 0.05$. Cr presence in excess amount within the plant can cause stunted growth of shoots, its phytotoxicity has been considered to be inhibitory for plant growth [9, 10]. Suppression of lateral shoots in *Salvia sclarea* with a diminishing trend with the increase in the dose of chromium was recorded [6]. The appearance of new nodes and new leaf were inhibited from 0.1 mg L^{-1} CrVI while it was enhanced at 0.01 mg L^{-1} on the 10th and 15th day as seen in Table 1(a, b). In tobacco, no specific toxic symptoms were marked; but shoot development was depressed and consequently no inflorescence developed [27]. Similar results of reduced shoot length and plant height with increased Cr content was reported in sunflower (*Helianthus annuus L.*) [2]. There were adverse effects of Cr recorded on plant height [27], such as in *Curcumas sativus, Lactuca sativa, and Panicum miliaceum* [16]. Because Cr transporting to the aerial shoot of the plant could have a immediate impact on cellular metabolism, and this may stimulate to the reduction.

Table 2: Effects of CrVI on content of chlorophyll in *I. aquatica* after 15 days

CrVI ($mg\ L^{-1}$)	chl a	chl b	Total Carotene
Control	1.43 ± 0.1^{a}	1.25 ± 0.1^{a}	0.88 ± 0.1^{a}
0.01	1.48 ± 0.2^{a}	1.18 ± 0.2^{a}	0.93 ± 0.05^{a}
0.1	1.17 ± 0.1^{a}	0.45 ± 0.2^{b}	0.87 ± 0.1^{a}
0.5	1.07 ± 0.1^{a}	0.59 ± 0.2^{b}	0.74 ± 0.1^{a}
1	0.34 ± 0.1^{b}	0.46 ± 0.2^{b}	0.38 ± 0.1^{b}
2	0.32 ± 0.1^{b}	0.4 ± 0.2^{b}	0.37 ± 0.1^{b}

Values are given as Mean ± SE; Values with different superscript letters indicate significant difference at $P < 0.05$

The results of the study revealed that low concentration of CrVI at 0.01 mg L^{-1} slightly enhanced the content of chl *a* and total carotene, but CrVI at 0.1 mg L^{-1} inhibited the content in the leaf of *I. aquatica*. On the other hand, a decline in chl *b* content was observed from low CrVI concentration (0.01 mg L^{-1}) which was found to increase with enhanced chromium concentrations (Table 2). Similar pattern of concentrations and duration-dependent chromium toxicity to chlorophyll content was also evident in *Vallisneria spiralis* [34]. Cr stress decreased chlorophyll content in two barley cultivars was documented [1]. Reduction in chlorophyll content with increase in chromium concentration may be due to accumulation and deposition of chromium by roots [28].

Table 3: Effects of CrVI on Tolerance index in *I. aquatica* after 15 days

CrVI ($mg\ L^{-1}$)	Tolerance index		
	leaf	Stem	root
0.01	84.6 ± 4.6^{a}	87.08 ± 2.8^{a}	73.13 ± 6^{a}
0.1	79.9 ± 3.2^{ab}	81.7 ± 6.8^{ab}	67.13 ± 5.8^{a}
0.5	70.5 ± 3.8^{ab}	72.7 ± 5.8^{ab}	63.8 ± 5.1^{a}
1	66.2 ± 8.7^{ab}	73.2 ± 7.6^{ab}	61 ± 3.1^{a}
2	63.9 ± 8.3^{b}	64.2 ± 5.7^{b}	61.7 ± 5.2^{a}

Values are given as Mean ± SE; Values with different superscript letters indicate significant difference at $P < 0.05$

As indicated by the T*i*, which was estimated on the basis of dry biomass, leaf, stem and root, *I. aquatica* tested in this work could be defined as highly tolerant (T*i*> 60) at all concentrations according to the scheme proposed by Lux et al. [22]. The results revealed highest tolerance in stem (T*i* = 87.08) followed by that in leaf (T*i* = 84.6) and the least in roots (T*i* = 73.13) as shown in Table3. The decline in tolerance was found to increase with enhanced in chromium concentrations. The results revealed that the tolerant nature of the plant might be because of internal detoxification mechanisms such as complexion complexation of metal with ligands [32].

"4. Conclusion"

The experimental results showed that Cr was toxic to different degrees at different stages of plant growth and development and also that the toxicity was concentration and medium dependent, although complete wilting was not encountered. This study gave information on chromium toxicity and tolerance in *Ipomoea aquatica,* which was likely to be useful for further studies like phytoremediation of CrVI polluted wastewater.

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