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ORIGINAL RESEARCH ARTICLE

EFFECT OF HEXAVALENT CHROMIUM (Cr^{+6}) AND CHELATING AGENTS ON GROWTH, UPTAKE AND BIOCHEMICAL LESIONS IN FLOOD RESISTANT RICE GROWN UNDER WATER LOGGED AND DROUGHT CONDITIONS

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Abstract: The effect of hexavalent chromium (Cr^{+6}) were studied in rice plants by applying two concentrations of Cr, i.e. 10 mg and 50 mg/Kg of soil supplementing with various chelators. The effect of (Cr^{+6}) ions on the physical parameters such as root length, shoot length, fresh weight, dry weight, metal tolerance index and biochemical parameters such as chlorophyll, carotenoid, total sugar and protein content, catalase and peroxidase activity and the uptake of Cr in root and shoot were studied. A decrease in chlorophyll, carotenoid, sugar, protein content and enzyme activity was noticed at higher concentrations of Cr. Supplementation of ethylene diammine tetra acetic acid(EDTA), salicylic acid(SA) and citric acid (CA) as chelating agents also decreases the enzyme activity & other parameters as compared to plant treated with chromium only. Present investigation reports injurious effects of (Cr^{+6}) on different aspects of rice plants. Cr accumulation in plant parts is a matter of serious concern to human health as it causes cardiovascular diseases, kidney failure & cancer.

Key Words: hexavalent chromium, rice plants, soil

INTRODUCTION

Heavy metals are integral components of ecosystem with both essential and non-essential types. Cr is a non-essential toxic element to plants. The common anthropogenic sources of heavy metals in environment are wastewater irrigation, sludge applications, solid waste disposal, automobiles exhaust& industrial activities (Shi *et al., 2005*). Out of the two stable states, Cr^{+6} is considered to be more toxic than Cr^{+3} (Panda & Patra, 2000). Cr^{+6} is potent, toxic and carcinogenic to plants (Shankar *et al., 2005*, Liu *et al., 2008*, Mohanty & Patra, 2009). Crops grown in or close to the contaminated sites can uptake & accumulate these metals in their organs (Jarup, 2003). The effect of heavy metals on crops & humans causing functional disorder in their body organs due to exposure of low dose over a long time (Jianjie *et al.,* 2008). Heavy metals enter waste water from varieties of sources such as domestic, industrial & mining operations. Many of these dissolved metal ions such as copper, cobalt, nickel, zinc, chromium etc. are toxic to living organisms. Plants have a remarkable ability to absorb, translocate & accumulate heavy metals & organic compounds from the environment. When Cr entered into plant body, it can disturb many biochemical & physiological processes and caused oxidative stress to plants that ultimately reduced the growth and yield (Arun *et al., 2005*, Mohanty & Patra; 2011).

Heavy metals taken up by plants from contaminated soil and water are toxic to growth performance of plants and posses a hidden threat to consumers (Stobrawa *et al., 2008*). Cr toxicity results in the inhibition of plant growth, induced chlorosis, biochemical lesions, reduced crop yields, loss of enzyme activities. (Panda & Patra., 2000; Arun *et al.,* 2005; Mohanty *et al.,* 2010). Recent reports have demonstrated that through the proper application of chelating agents to the soil, relatively insoluble elements can be solubilised & made available for plant uptake.

MATERIALS AND METHODS

Rice (*Oryza sativa* L.) cultivar 'Swarna sub-1' was chosen for the present research work and potassium dichromate as the source of "hexavalent chromium" (Cr^{6+}). Graded dry uniform seeds of rice were surface sterilized by soaking in 0.1 %$HgCl_2$ solution for 5 minutes and then thoroughly washed with tap water and distilled water. Then the seeds were germinated in various pots containing Cr 10 mg and 50 mg/Kg of soil both in the presence and absence of chelating agents such as EDTA, SA and CA. Separate sets of plants were grown both under water logged condition and drought condition. The water logged condition was maintained by dipping the lower level of the stem of the plant. The water level was increased with growth of the plant. Water was supplied to the plant under drought condition only once at the time of germination.

After germination, various physical, biochemical parameters and chromium uptake were observed in 15 days rice seedlings. Growth of 15days rice seedlings were studied by measuring the root and shoot length and taking the fresh matter and dry matter content of the seedlings. The metal tolerance index (TI) was determined by following the formula as

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given by Iqbal and Rahmati (1992). The chlorophyll and carotenoid content was estimated by following the method of (Arnon, 1949; Porra, 2002). Catalase and Peroxidase were extracted and assayed as per the method of Chance and Maehly, 1955. Total Sugar & protein content were estimated by the method of Yoshida *et al., (1972)* and Nelson *et al., (1944)* respectively. The chromium uptake was observed by Atomic Absorption spectrophotometer.

Results

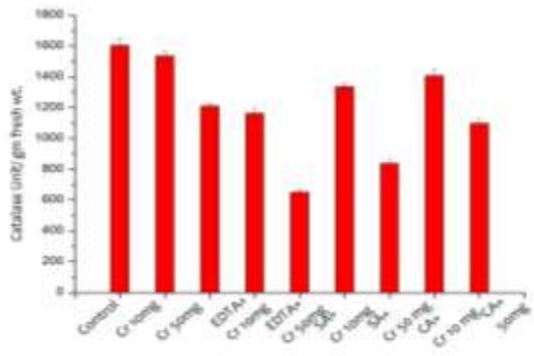
Physical parameters

Results of physical parameters of rice plants are presented in Table 1 &2 that depicted decrease in root length, shoot length, fresh weight, dry weight and metal tolerance index in Chromium treated plants as compared to control plants in both water logged and drought condition. Higher chromium content proved more toxic than that of lower chromium levels. Decrease in root length and shoot length, fresh weight, dry weight and is observed in plants grown in drought condition than in water logged condition. Chromium treated with chelating agent proved more toxic than Cr treated without chelating agent. Cr treatment at 10mg with EDTA and Cr treatment at 50mg with EDTA showed highest and lowest percentage of Tolerance Index in both water logged and drought condition respectively.

Physiological attributes

Results of physiological attributes are presented in Table 3 & 4 and figure 1 & 2 that depicted decrease in chlorophyll, carotenoid content, catalase and peroxidase activity, sugar and protein content in Cr treated plants than control plants grown both in water logged and drought condition. Cr showed negative effect on the formation and persistence of these chemical compounds in the leaves. Data shown in Table 3 & 4 and figure 1 & 2 for total chlorophyll, carotenoid, total sugar, protein, catalase and peroxidase activity gradually decreased with increase concentration of Cr in the rice plants. Decrease in all these parameters is observed in the plants grown under drought condition than water logged condition. Cr treated with chelating agent proved more toxic than that of only Cr.

1A



1B

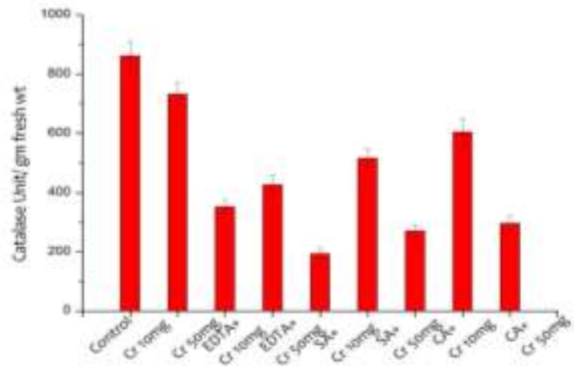
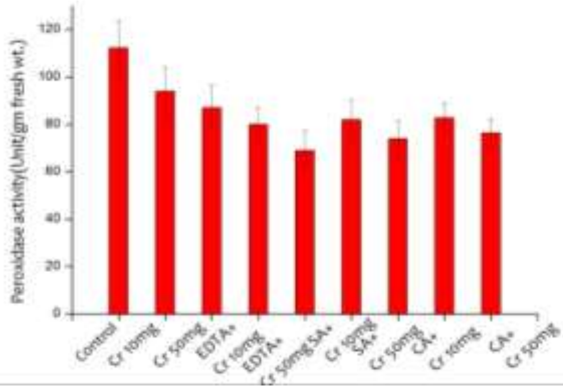


Figure 1A, B: Effect of Cr^{+6} and chelating agents on catalase activity of 15 days old rice seedlings grown under water logged and drought condition respectively.

2A



2B

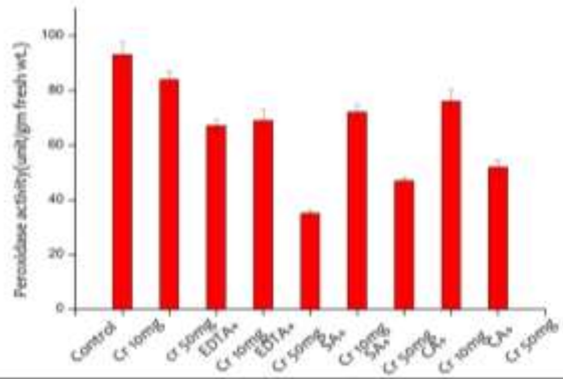
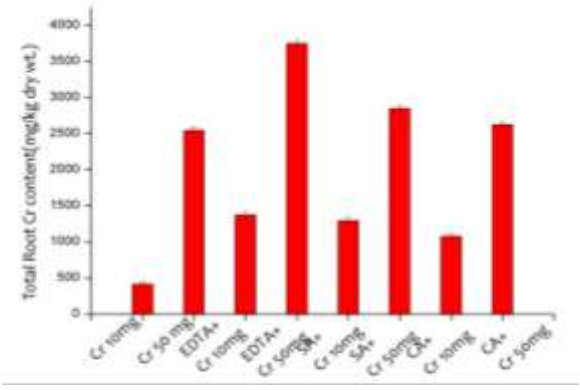


Figure 2A, B: Effect of Cr^{+6} and chelating agents on peroxidase activity of 15 days old rice seedlings grown under water logged and drought condition respectively.

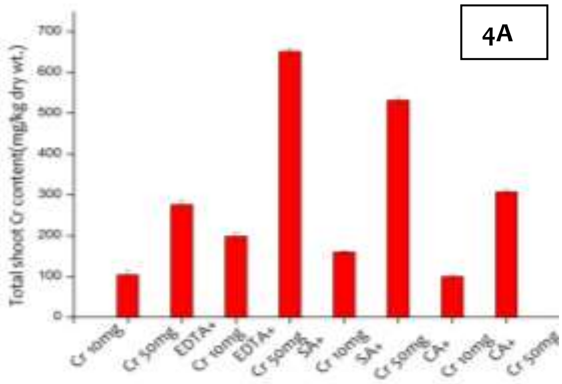
Accumulation of Cr in shoots and roots

Fig 3 & 4 depicted that more chromium was accumulated in the roots than shoots. Cr uptake increased with increase in its concentration. Plants treated with chelators absorbed more Cr than plants without chelators. Plants treated with EDTA and Cr showed more Cr uptake than SA with Cr than CA with Cr. The amount of Cr uptake is more in rice plants grown under water logged condition than drought condition.

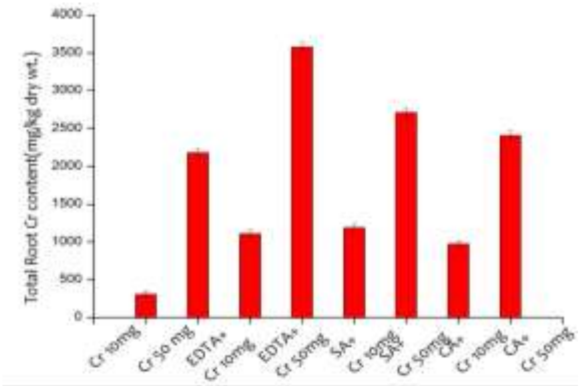
3 A



4A



3B



4B

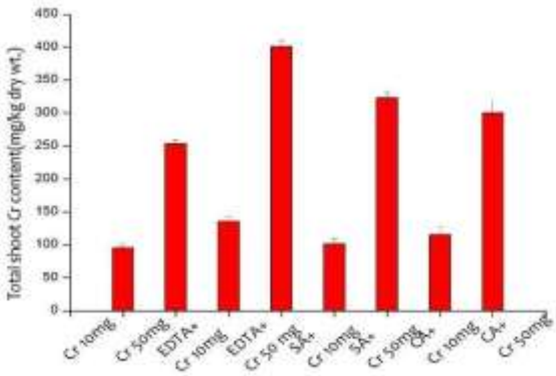


Figure 4 A, B: Effect of Cr^{+6} and chelating agents on total shoot Cr content of 15 days old rice seedlings grown under water logged and drought condition respectively.

Table 1: Effect of Cr^{+6} ions and chelating agents on growth Parameters of 15 days old rice seedlings grown under water logged condition. (Values are mean of three replicates±SEM)

Treatment	Root length in cm	Shoot length in cm	Root fresh wt. in gm	Shoot fresh wt. in gm	Root dry wt. in gm	Shoot dry wt. in gm	Metal tolerance index (%)
Control	22.2±0.531	27.5±0.481	0.925±0.021	5.892±0.431	0.093±0.031	1.357±0.035	--
Cr 10 mg	20.9±0.312	26.3±0.053	0.732±0.015	4.357±0.393	0.071±0.044	0.953±0.231	94.144
Cr 50 mg	18.7±0.430	24.2±0.312	0.598±0.007	2.839±0.275	0.042±0.005	0.398±0.592	84.234
EDTA+Cr 10mg	17.7±0.214	25.5±0.513	0.613±0.127	3.052±0.138	0.056±0.067	0.519±0.341	79.729
EDTA+Cr50 mg	16.1±0.391	23.1±0.235	0.329±0.032	1.452±0.247	0.031±0.092	0.276±0.435	72.522
SA+Cr10 mg	19.2±0.253	25.1±0.143	0.693±0.173	3.327±0.317	0.061±0.057	0.601±0.249	86.486
SA+Cr50 mg	16.8±0.124	23.7±0.374	0.415±0.191	2.539±0.152	0.035±0.019	0.315±0.531	75.675
CA+Cr10mg	19.5±0.173	25.9±0.095	0.701±0.231	3.117±0.178	0.069±0.007	0.693±0.219	87.837
CA+Cr50mg	17±0.483	24.3±0.215	0.467±0.313	2.612±0.197	0.039±0.025	0.359±0.232	76.576

Table 2: Effect of Cr^{+6} ions and chelating agents on growth Parameters of 15 days rice seedlings grown under drought condition. (Values are mean of three replicates±SEM)

Treatment	Root length in cm	Shoot length in cm	Root fresh wt. in gm	Shoot fresh wt. in gm	Root dry wt. in gm	Shoot dry wt. in gm	Metal tolerance index (%)
Control	19.3±0.432	24.2±0.521	0.773±0.051	4.771±0.231	0.081±0.009	1.103±0.132	--
Cr 10 mg	17.5±0.319	22.1±0.431	0.512±0.072	3.128±0.038	0.063±0.002	0.802±0.104	90.673
Cr 50 mg	13.9±0.515	19.7±0.372	0.373±0.061	2.339±0.097	0.039±0.009	0.309±0.173	72.020
EDTA+Cr10 mg	16.1±0.417	20.2±0.422	0.476±0.077	2.017±0.081	0.048±0.012	0.512±0.102	83.419
EDTA+Cr50 mg	12.2±0.214	17.3±0.215	0.238±0.093	1.007±0.042	0.029±0.003	0.201±0.019	63.212
SA+Cr10 mg	16.7±0.432	20.9±0.303	0.493±0.084	2.436±0.073	0.053±0.001	0.590±0.025	86.528
SA+Cr50 mg	12.9±0.191	18.1±0.205	0.305±0.075	1.578±0.088	0.034±0.023	0.276±0.017	66.839
CA+Cr10mg	17.1±0.352	21.7±0.373	0.501±0.065	2.973±0.065	0.059±0.032	0.671±0.031	88.601
CA+Cr50mg	13.2±0.296	18.7±0.512	0.332±0.052	1.993±0.059	0.037±0.004	0.301±0.007	68.393

Table 3: Effect of Cr^{+6} and chelating agents on Total Chlorophyll, Carotenoid, total sugar and protein content of 15 days old rice seedlings grown under flood condition.(Values are mean of three replicates±SEM)

Treatment	Total Chlorophyll content (mg/gm fresh wt.)	Carotenoid content (mg/gm fresh wt.)	Total sugar content (mg/gm fresh wt.)	Protein content (mg / gm fresh wt.)
Control	0.89 ± 0.59	5.8 ± 0.521	16.0 ± 0.781	24.2 ± 1.251
Cr -10 mg	0.63 ± 0.036	2.06 ± 0.414	10.5 ± 0.823	16.7 ± 1.313
Cr 50 mg	0.29 ± 0.022	0.52 ± 0.217	7.0 ± 0.625	11.3 ± 1.215
EDTA+Cr 10mg	0.37 ± 0.041	1.12 ± 0.312	6.2 ± 0.501	13.1 ± 1.109
EDTA+Cr50 mg	0.15 ± 0.028	0.25 ± 0.291	3.5 ± 0.431	8.3 ± 1.054
SA+Cr10 mg	0.49 ± 0.019	1.29 ± 0.345	8.3 ± 0.527	14.7 ± 1.092
SA+Cr50 mg	0.19 ± 0.051	0.37 ± 0.217	4.7 ± 0.479	9.2 ± 1.007
CA+Cr10mg	0.51 ± 0.062	1.35 ± 0.432	9.1 ± 0.875	15.2 ± 1.099
CA+Cr50mg	0.24 ± 0.031	0.45 ± 0.339	5.3 ± 0.673	9.9 ± 1.074

Table 4: Effect of Cr^{+6} and chelating agents on Total Chlorophyll, Carotenoid, total sugar and protein content of 15 days old rice seedlings grown under drought condition. (Values are mean of three replicates±SEM)

Treatment	Total Chlorophyll content (mg/gm fresh wt.)	Carotenoid content (mg/gm fresh wt.)	Total sugar content (mg/gm fresh wt.)	Protein content (mg/gm fresh wt.)
Control	0.65 ± 0.034	3.2 ± 0.175	14.1 ± 0.664	19.1 ± 1.337
Cr 10 mg	0.52 ± 0.025	1.63 ± 0.317	9.3 ± 0.731	14.3 ± 1.254
Cr 50 mg	0.39 ± 0.023	0.48 ± 0.332	6.7 ± 0.651	8.7 ± 1.207
EDTA+Cr10 mg	0.32 ± 0.037	0.83 ± 0.217	5.8 ± 0.532	10.4 ± 1.337
EDTA+Cr50 mg	0.17 ± 0.013	0.15 ± 0.131	2.5 ± 0.319	2.5 ± 1.025
SA+Cr10 mg	0.41 ± 0.033	0.95 ± 0.212	7.2 ± 0.529	11.8 ± 1.453
SA+Cr50 mg	0.25 ± 0.047	0.29 ± 0.103	3.9 ± 0.454	3.7 ± 1.098
CA+Cr10mg	0.47 ± 0.041	1.1 ± 0.205	8.3 ± 0.538	12.3 ± 1.535
CA+Cr50mg	0.29 ± 0.019	0.35 ± 0.191	4.3 ± 0.402	4.2 ± 1.024

Discussion

The results of the studies on the Cr and their interaction with chelators showed some interesting results.By adding chelators to the chromium, it was found that the seedlings of rice exhibited growth retardation (i.e. reduction in the root and shoot length, fresh weight and dry weight, metal tolerance index) as compared to the controlled seedlings. Cr treated plants depicted the slower rate of synthesis of chlorophyll, carotenoid, sugar and protein. The negative effect on dry matter was essentially an indirect effect of Cr on plants. Vernay *et al*., (2008) reported that D. Innoxia plants grown in presence of chromium hexavalent (Cr^{+6}) showed reduced growth leading to reduction in root and shoot biomass. Bahmanyar (2008) reported that Cr uptake in rice root was more than whole shoot and grain. The Present study shows some of the toxic effect of Cr and its uptake in the presence of different chelating agents such as EDTA, SA and CA. Cr interfere with several metabolic processes causing toxicity to plants as exhibited by reduced root growth, shoot growth, chlorosis, photosynthetic impairing and finally plant death (Scoccianti *et al.*, (2006). Samantray (2002) reported that (Cr^{+6}) stress induced a notable decrease in the total soluble protein content in the leaves of Cr sensitive mung bean cultivars. John *et al., (2009)* suggested that the reduction in total soluble protein content may be a natural toxicity response of seedlings exposed to (Cr^{+6}). Khanjani *et al.*, (2008) reported that watermelon irrigated by wastewater has high conc. of heavy metals like Ni, Cr, Pb, Zn and Mn in descending order from roots, stems and fruits. Mohanty & Patra (2012) reported that addition of chelating agents enhances the ability of plants to uptake Cr combatting against metal toxicity. The enhancement of heavy metal Cr uptake by different chelators differs from each other. In the present study, the Cr-uptake in root and shoot of rice was varied with different Cr-chelator combinations such as Cr-SA, Cr-CA, Cr-EDTA. By increasing the chromium concentration from Cr 10mg to Cr 50 mg, it was found that the seedlings of rice exhibited growth retardation and higher chromium uptake. The experiment marked a higher level of chromium accumulation at higher concentration of chromium treated plants. But the translocation of chromium to the aerial parts such as shoot was decreased as compared to the translocation of chromium to the roots. More Cruptake is observed in water logged plants than drought plants.

Tolerance to heavy metals in plants may be defined as the ability to survive in soil that is manifested by an interaction between a genotype and its environment (Macnair*et al., 2000*). Tolerance to Cr treatment was decreased as compared to control.

The Chelates enhanced the chromium uptake by forming mobile bound compounds. The Cr-uptake was increased with both external chromium concentration and the duration of the treatment. The decrease in catalase and peroxidase activity was possibly due to the inhibition of biosynthesis. The role of heavy metal chelators such as EDTA, SA &CA in enhancing the chromium uptake (heavy metal uptake) is very useful in the phytoremediation technology that exploits the natural ability of a green plant to accumulate variety of chemical elements and transport them to the above ground parts.

Conclusion

The present study demonstrated that hexavalent chromium is injurious to rice plant and it severely affects the growth and biomass. The study is proved in to the under laying causes of the harmful effect of chromium on plant growth and development by extensively investigating the physiological parameters. The main attributes of metabolic activity like chlorophyll, carotenoid, sugar, protein, catalase and peroxidase activity was seriously affected by high concentration of chromium in the soil. The plants containing even a very small amount of chromium can severely deteriorate the human health by entering the body through food chain. It is confirmed that the plants of flood resistant variety of rice "Swarna sub-1"grown under water logged condition mobilise more chromium from the soil than the plants, grown under drought condition.

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