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Cadmium and chromium induced physiological changes in sesban seedlings

Lipsa, M. Mohanty, H. K. Patra and C. Pradhan[Ψ]

Post Graduate Department of Botany, Utkal University, Bhubaneswar - 751 004

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ABSTRACT

The present hydroponic study depicts the adverse impacts of heavy metals like cadmium (Cd) and chromium (Cr) on some physiological parameters of sesban (*Sesbania sesban* L. Merill.) seedlings. The seedlings were exposed to varying concentrations of $K_2Cr_2O_7$ and $CdCl_2$ (10-100 μM) for 14 days and different parameters like root and shoot length, biomass of root and shoot, chlorophyll and proline contents of the seedlings were measured. Root and shoot length was reduced to nearly 35% in presence of 100 μM of $CdCl_2$ and $K_2Cr_2O_7$ for 14 days and it was found that Cr has more inhibitory effect on root and shoot length than Cd. The fresh biomass content of the seedlings was reduced by 80% with increased heavy metal concentrations. Cadmium has more inhibitory effect on shoot biomass in comparison to Cr. Dry biomass of 14 days treated sesban seedlings was reduced by 90% with 100 μM of $CdCl_2$ and $K_2Cr_2O_7$. Chlorophyll content was reduced by 30%-50% with the metal treatments and proline was accumulated in seedlings, even up to eight times more in comparison to control. Seedlings with chromium treatment showed more proline accumulation than cadmium treated seedlings. These changes in morphological and biochemical parameters of sesban seedlings at different growth periods can be used as important bio-indicators of Cd and Cr stress.

1. Introduction

Heavy metal induced environmental degradation and subsequent loss of soil fertility and crop productivity is an emerging issue now-a-days. Some heavy metals play an important role in plants cells as micronutrients, while others in trace concentration have stimulating and toxic effects on plants (Mohanty and Patra, 2011). Major sources of heavy metals in environment are from anthropogenic sources including traffic emission, industrial emission, domestic emission and weathering of building, mining, smelting, waste disposal, urban effluent, vehicle exhausts, sewage sludge, pesticides, fertilizers (Sezgin *et al*., 2003; Montagne *et al*., 2007). Heavy metals in toxic concentrations can cause damage to plants by altering major physiological and metabolic processes (Hossain *et al*., 2010; Villiers *et al*., 2011). Although heavy metals are natural constituents of soils and occur naturally in the environment, now-a-days, contamination of soils by toxic metals and metalloids is of major concern worldwide (Villiers *et al*., 2011).

Various deleterious effects of heavy metals like cadmium (Cd) and chromium (Cr) have been reported by researchers worldwide (Zhuang *et al*., 2009; Mohanty and Patra, 2011). Chromium is known to have some beneficial role in animals, but it is toxic to plants. Cadmium is non-essential for plant and animal metabolism and it has both phyto- as well as zootoxic effects. Both the heavy metals enter the environment through different anthropogenic activities, including the mining and smelting of ores, industrial activities, municipal waste disposal and fertilizer application. Out of many oxidation states of chromium, Cr

[Ψ] *Corresponding author; Email:*chinmay_pr@yahoo.com

(III) and Cr (VI) are most stable forms in environment and among these two, hexavalent Cr is more toxic than the trivalent one. Several studies have reported that chromium interferes with metabolic processes and its phytotoxic effect is exhibited in the forms of reduced growth and biomass, foliar chlorosis, stunting and finally plant death (Zou *et al*., 2006; Mohanty and Patra, 2012). This heavy metal causes oxidative damage to biomolecules such as lipids and proteins (Shanker *et al*., 2004).

Cadmium (Cd) has been shown to cause delay in germination, induce membrane damage, impair food reserve mobilization by increased cotyledon/embryo ratios of total soluble sugars, glucose, fructose and amino acids (Rahoui *et al*., 2010), mineral leakage leading to nutrient loss (Sfaxi-Bousbih *et al*., 2010), accumulation in seeds and over-accumulation of lipid peroxidation products (Ahsan *et al*., 2007; Smiri *et al*., 2011) in seeds. It has been reported to reduce the germination percentage, embryo growth and distribution of biomass, and inhibit the activities of alpha-amylase and invertase, impair membrane integrity by high MDA content and lipoxygenase (LOX) activity (Sfaxi-Bousbih *et al*., 2010), reduce water content, shoot elongation and biomass (Ahsan *et al*., 2007). Cd stress can also cause stunted growth, chlorosis, leaf epinasty, alters the chloroplast ultrastructure, inhibits photosynthesis, inactivates enzymes in CO_2 fixation, induces lipid peroxidation, inhibits pollen germination and tube growth, and also disturbs the nitrogen (N) and sulfur (S) metabolism. The present investigation has been undertaken to assess the phytotoxicity of Cr and Cd on seed germination, growth and physiological changes in *Sesbania sesban* (L.) Merril. The study will be beneficial for assessing the heavy metal phytotolerane potential of the plants under metal stress.

2. Materials and methods:

Dry seeds of *Sesbania sesban* (L.) Merril, commonly known as sesban were procured from Central Rice research Institute, Cuttack (India). In order to study the effect of Cr and Cd on germination percentage of the sesban seeds, the seeds were germinated in petriplates taking different concentrations of $K_2Cr_2O_7$ and $CdCl_2$ (i.e. 10 µM, 25 µM, 50 µM, 75 µM and 100 µM) and distilled water was taken in another petriplate as control (Mohanty and Patra, 2012).

Two-days-old surface sterilized germinated seeds were transferred onto well aerated Hoagland's nutrient solution (half strength) as control and Hoagland's solution supplemented with varying concentrations of $K_2Cr_2O_7$ and $CdCl_2$ placed in hydroponic culture vessels under controlled growth condition (Mohanty and Patra, 2013) for 14 days growth period and at 7-day interval the seedlings were collected to study different growth as well as physiological parameters.

The extraction and estimation of chlorophyll content of leaf tissue was performed by using 80% cold acetone following the method of Porra (2002). Chlorophyll and carotenoid contents of seedlings were estimated as done by Mohanty and Patra (2012) by taking absorbance at 663.6, 645.6 and 470 nm.

Proline content was estimated following the method of Bates *et al*., (1973) by measuring the absorbance value at 520 nm.

3. Results and discussion:

3.1 Impact on seed germination

Maximum inhibition of seed germination was observed at 100 µM in both the Cr^{6+} and Cd^{2+} stress, which resulted in 17.8% inhibition in Cr^{6+} and 20% in Cd^{2+} treated seed whereas minimum was at 10 µM treatment resulting in 2.23% inhibition of seed germination (Fig. 1). The inhibitory effect of heavy metals on seed germination may be attributed to various toxic impacts on amylase, protease and ribonuclease enzyme activites thus retarding seed germination and growth of many crops (Ahmad and Ashraf, 2011; Mohanty and Patra, 2012). Cd and Cr induced inhibition in sesban seed germination could be also due to lower water uptake and transport in plants which ultimately leads to embryonic damage (Becerril *et al*., 1989).

3.2 Changes in growth parameters

Treatment of different concentrations Cr^{6+} and Cd^{2+} showed considerable changes in different growth parameters of sesban seedlings during 14 days of study period (Fig. 2, Fig. 3 and Fig. 4). Shoot length decreased remarkably with increase in Cr and Cd concentrations. The shoot length of the seedlings treated with lower concentration of both the

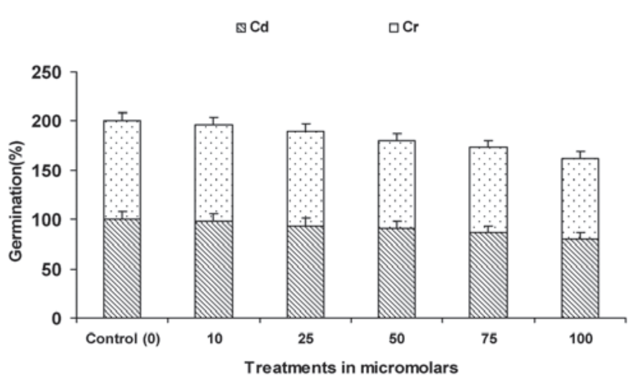

Fig. 1 Comparative study on inhibitory effect of Cd and Cr on germination of sesban seeds

metals (i.e., 10 µM) was recorded highest in comparison to all other concentrations.

Chromium exhibited more inhibition in shoot height in comparison to Cd, whereas Cd has more adverse effect on root length of the seedlings exposed for 14 days. The seedlings treated with 100 µM of both the metals had minimum root and shoot elongation (Fig. 2). The trend shows similarity with the findings of others in different plants (Zou *et al*., 2006; Mohanty and Patra, 2012; Mohanty and Patra, 2013). The seedlings treated with Cr^{6+} and Cd^{2+} metal ions showed relative decrease in the root growth (measured in terms of root length) in the similar order as observed in shoot growth study. The root and shoot fresh biomass gradually decreased at high concentrations of Cr^{6+} and Cd^{2+} treatments. Similar growth trend was found for dry biomass also (Fig. 3). The fresh biomass of both shoot and root tissues also decreased with increase in the concentrations of Cd and Cr in the medium. Root fresh biomass was more affected by Cr as compared to Cd, whereas the shoot fresh biomass was more affected by Cd. These heavy metals affect the enzymatic activities by which the food did not reach to the radicle and plumule (Srinivas *et al*., 2013) and probably this might be the reason behind inhibition in seed germination and reduction in seedling growth.

The dry biomass of root was found stimulated at 10 µM $CdCl_2$. It was noticed that both the root and shoot dry mass was significantly reduced at 100 µM treatments of both the metals. 50% reduction in root dry mass and 75% reduction in shoot dry mass was observed with 100 µM $CdCl_2$ treatment. Similar to the fresh biomass, Cr affected more the root dry biomass than Cd .

The reason behind decrease in seedling growth under Cd and Cr exposure could be due to the reduction in

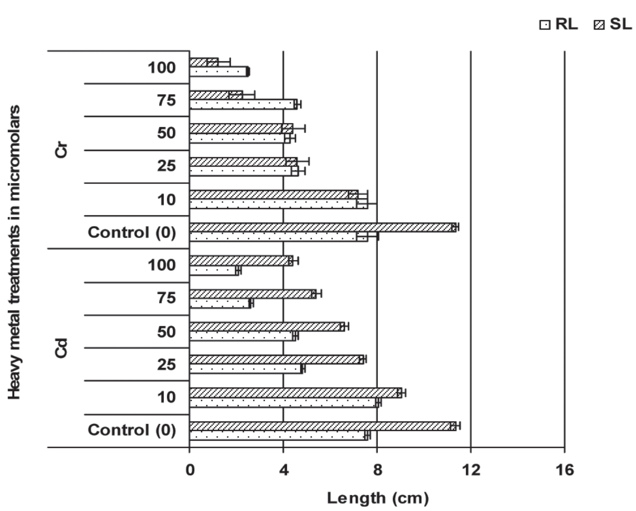

Fig. 2 Variation in root and shoot length of 14 days treated sesban seedlings in response to Cd and Cr stress.

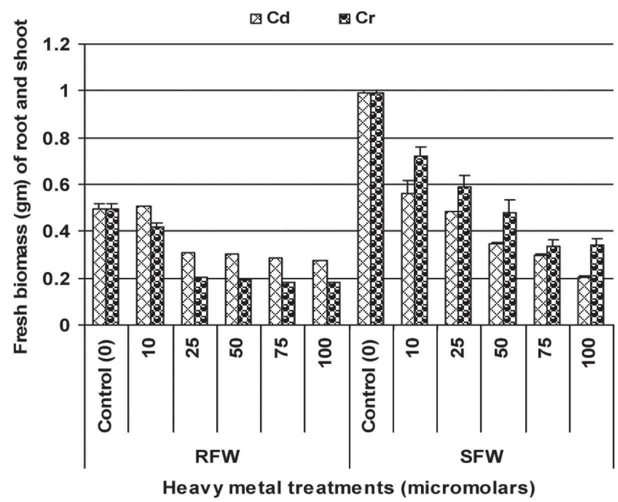

Fig. 3 Comparative effect of Cd and Cr on root and shoot fresh biomass of 14 days treated sesban seedlings. (NB: RFW and SFW: Root and Shoot Fresh Weight)

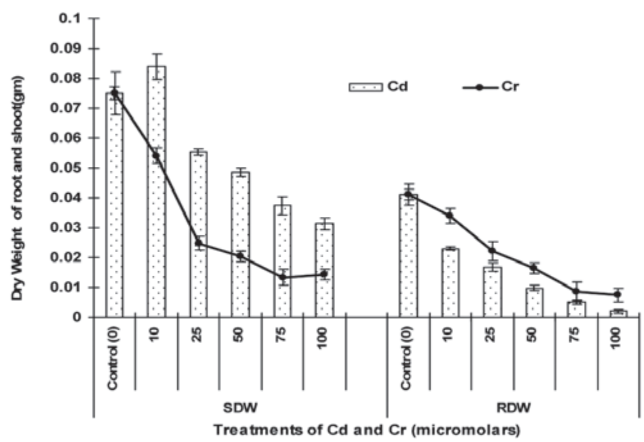

Fig. 4 Comparative effect of Cd and Cr on root and shoot dry biomass of 14 days treated sesban seedlings. (NB: RDW and SDW: Root and Shoot Dry Weight)

meristematic cells present in the growing tips of the seedling. Further, some enzymes contained in the cotyledon and endosperm cells become active and begin to digest and store food which is converted into soluble form and transported to the radicle and plumule tips ex: enzyme amylase convert starch into sugar and protease act on protein. The activities of these hydrolyzing enzymes might have inhibited by Cd and Cr as a result there was reduction in the growth of the seedlings.

3.3 Analysis of chlorophyll and carotenoid content

There was decrease in chlorophyll and carotenoid content with increase in the metal concentrations except for 10 µM of $CdCl_2$ treatments (Fig. 5). Beyond 50 µM of both $CdCl_2$ and $K_2Cr_2O_7$, reduction in chlorophyll and carotenoid contents was more than 40% in comparison to the control seedlings. The decrease was about 50% at 100 µM concentrations of both the metals. Generally, reduction of chlorophyll and carotenoids content may be due to Cd induced inhibition of δ-aminolaevulinic acid hydratase (δ-ALA). This enzyme converts δ-ALA into porphobilinogen in the synthesis of chlorophyll (Wouter *et al*., 2002). Also Cd inhibits the protochlorophyllide reductase by SH-interaction between heavy metals and SH group on the enzyme (Van Assche and Clijsters, 1990). In addition, heavy metals can cause substitution of Mg atom in chlorophyll molecules and led to breakdown in photosynthesis process as stated by Kupper *et al*. (1998) and Helene *et al*. (1998). Wouter *et al*. (2002) have also reported the decrease in chlorophyll and carotenoid contents with increases of Cd concentrations in growth media. The formation of chlorophyll pigment depends on the adequate supply of iron as it is the main component of the protoporphyrin, a precursor of chlorophyll synthesis. An excessive supply of chromium seems to prevent the incorporation of iron into the protoporphyrin molecule, resulting in the reduction of chlorophyll pigment (Datta *et al.*, 2011).

3.4 Analysis of proline

Proline content in the leaves of sesban seedlings was enhanced with increased concentrations of Cr and Cd in the medium. The proline content was found maximum at 100 µM concentrations and minimum at 10 µM concentrations of both the metals treated (Fig. 6). Proline accumulation was significantly high due to Cr in comparison to Cd. There was about seven times increase in proline accumulation due to Cd stress at 100 µM as compared to control whereas Cr treated seedlings showed nearly 40 times more proline accumulation (Fig. 6). Enhanced proline levels at higher concentrations of heavy metals have also been reported by other researchers which are considered as an important parameter to recognize the stress impact on plants (Levitt 1980; Khan *et al*., 2002; Mohanty and Patra, 2012). Proline accumulation in plants under Cd stress is induced by a Cd-imposed decrease of the plant water potential, and the functional significance of this accumulation would lie in its contribution to water balance maintenance. Accumulation of proline has been reported from increased proline biosynthesis which may be attributed to increased activity of δ-aminotransferase and reduced proline dehydrogenase activity for scavenging free radical formed by heavy metal stress (Bhamburdekar and Chavan, 2011).

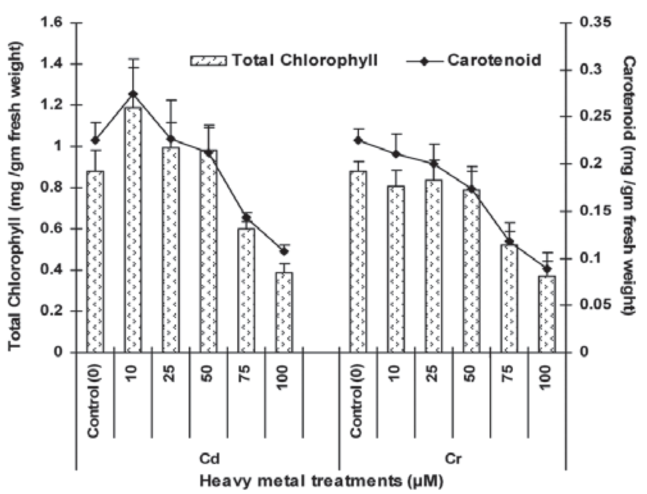

Fig. 5 Changes in pigment content of leaves of sesban seedlings after 14 days exposure to heavy metal stress.

4. Conclusion

In present investigation, it is concluded that Chromium and Cadmium treatments produced significant toxic effects on germination, seedling growth and physiological parameters of sesban seedlings. Increased concentration of both metals in the medium resulted in noticeable changes plant metabolism. The present preliminary study is an attempt to assess the morpho-physiological behavior of sesban seedlings under Cd and Cr stress which may become helpful in formulating strategies for phytoremediation programme in the soil polluted with these metals using *Sesbania sesban* plant.

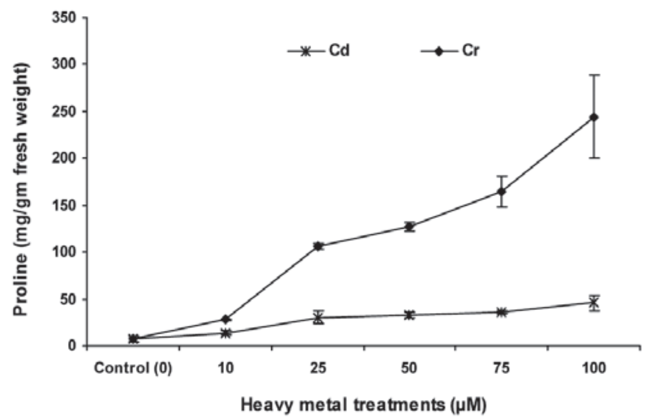

Fig. 6 Proline accumulation in 14 days treated sesban seedlings in response to Cd and Cr stress.

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