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Chromium toxicity in *Moringa oleifera* Lam.

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This study investigated the toxic effects of different Cr concentrations (Control, 5, 25 and 50 mg/L) on hydroponically cultivated *M. oleifera* seedlings. At the end of the application, certain biochemical variations and changes in Cr and nutrient content (K, Zn, Ca, Cu, Fe, Mg and Mn) of the plant roots and shoots were determined. *M. oleifera* seedlings accumulated high Cr concentrations in roots and shoots. As a result, Cr led to nutrient deficiency by affecting the intake and transportation of the necessary macro and micro elements. Increase in phenolic compound content and non-protein SH groups may indicate that they played a role in Cr toxicity. Furthermore, the increases in H_2O_2 and MDA levels clearly demonstrated that Cr toxicity induced oxidative stress in *M. oleifera* cells.

Keywords: Chromium, *Moringa oleifera*, Physiological response, Toxicity

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Chromium is the 7th most abundant element globally and has a molecular weight of 51.1 g[1]. Although it could be found with a valence between +2 and +6, it is commonly found in terrestrial environments in trivalent (CrIII) and hexavalent (CrVI) forms[2]. Moreover, CrIII form is less active and less toxic in nature and is usually found bound to organic matter in the soil and aquatic habitats[3]. However, it is highly toxic for animals and plants at high concentrations[4,5]. CrVI has a long residence time in surface and ground waters[6]. CrVI, unlike CrIII, has a carcinogenic effect and is considered group I carcinogen[7]. Contamination of soil and groundwater has been a serious concern for scientists for a long time because of Cr utilization in various anthropogenic activities. However, Cr has been given little attention in botany, unlike other toxic heavy metals such as cadmium, lead, mercury etc. The effect of Cr toxicity on plants is observed at many levels, ranging from yield reduction, leaf and root undergrowth to enzyme activities and inhibition of mutagenesis[4]. Cr and Cr compounds are used in several industrial fields such as drilling muds, refractory steel, catalytic production, electroplating cleaners and chromic acid[8]. Industrial use of hexavalent (CrVI) chromium compounds includes cooling tower water treatment, metal plating, post tanning and wood preservation. It was determined that these anthropogenic activities increased the bioavailability and biostability of Cr and led to widespread environmental contamination.

Chromium compounds show high toxicity to plants by affecting growth and development in a negative way. Although there were not any adverse effect observed in some crops exposed to low levels of Cr (3.8 x 10-4 µM)[9,10], it is toxic to most of the higher plants at 100 µM/kg dry weight[11].

Moringa oleifera Lam. is in Moringaceae family of plants and is an active malnutrition medicine. *M. oleifera* is highly nutritive due to the presence of various basic phytochemicals in its leaves, pods and seeds. *M. oleifera* can be cultivated at a temperature of 25-35°C in several tropical and subtropical regions. It grows on sandy or loamy soils with slightly acidic and slightly alkaline pH and 250-3000 mm of net precipitation[12]. Seed cultivation method is preferred due to high germination rates. Seeds are expected to germinate within 5-12 days after planting and implanted in soil at a depth of 2 cm. It can also be grown from 1 m long and 4-5 cm diameter cuttings; however these plants may not develop a well-established root system. Such plants tend to be susceptible to the effects of drought and winds[13]. *M. oleifera* leaves are rich in minerals such as K, Fe and Ca, essential amino acids, vitamins and a number

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of glycosides[14,15]. Seeds have high (42%) fat content that could be used in medicine.

The present study aimed to determine the toxic effects of various Cr concentrations (0, 5, 25 and 50 mg/L) on *M. oleifera* seedlings cultivated under hydroponically controlled conditions.

Materials and Methods

Plant material and preparation procedure

M. oleifera seeds were germinated in perlite under 26±1°C and then seedlings were transferred to 2 L plastic vessels with aerated nutrient solution (4 seedlings per vessel). Elemental composition of the nutrient was 0.88 mM K_2SO_4, 2 mM KCl, 2.0 mM $Ca(NO_3)_2$, 100 µM Fe-EDTA, 0.25 mM KH_2PO_4, 1 mM $MgSO_4$, 1 µM H_3BO_3, 0.5 µM $MnSO_4$, 1 µM $ZnSO_4$, 0.2 µM $CuSO_4$ and 0.02 µM $(NH_4)_6Mo_7O_{24}$[16]. Pure water was used for preparing the nutrient solution. The seedlings were grown in a climate chamber (Snijders Scientific, Netherlands) (light/dark regimes of 16/8 h, light level ~120 $\mu E.m^2.s^{-2}$, temperature 26±1°C). After seedlings were acclimatized for 7 days in nutrient solution, they were supplied with 0, 5, 25 and 50 mg/L Cr as $K_2Cr_2O_7$ (CrVI). Solutions applied were changed every two days. The seedlings were harvested after 10 days then seedlings roots washed with deionized water three times. Root, stem and leaves were separated and frozen at -80°C in order to perform biochemical analyses.

Biochemical analyses

Fresh seedling leaves were weighted and then they were homogenized in 80% acetone. The supernatant was separated and absorbances were read at 470, 645 and 662 nm in a UV/VIS spectrophotometer. (CINTRA 202, Australia). The levels of chl-a and chl-b and total carotenoids were calculated using the formula by Lichtenthaler and Wellburn[17]. Phenolic compound content of the seedling parts was determined using Folin–Ciocalteu's reagent method by reading the absorbance at 765 nm wavelength according to the method by Ratkevicius *et al*.[18]. Gallic acid was used as a standard. Results obtained are expressed as mg of gallic acid equivalent (GAE)/g of fresh weight. H_2O_2 content was measured according to Sergiev *et al*.[19]. with minor modifications. After extraction with trichloro acetic acid (TCA) 0.1% and centrifugation, the supernatant was added to 1 M KI and 150 mM (pH 7.4) phosphate buffer. Then absorbance was read at 390 nm. Lipid peroxidation was analyzed by measuring the level of malondialdehyde (MDA), by a modification of the method by Zhou[20]. About 0.5 g fresh the seedling parts were homogenized in 5 mL 10% TCA, and the homogenate was centrifuged. The reaction mixture containing 2 mL extract and 2 mL TCA was heated at 95°C for 30 min and reaction was stopped by using an ice-bath. The absorbance of the mixture was determined at 532, 600 and 450 nm with a UV/VIS spectrophotometer. Non-protein thiols were determined after supernatant was mixed with Ellman's reaction mixture [5 mM EDTA and 6 mM DTNB 5, 5`-dithiobis (2-nitrobenzoic acid) in 150 mM phosphate buffer, pH 7.4]. The absorbance was taken after 20 min at 412 nm[21].

Data analysis

All analyses were repeated four times. SPSS 11.0 for Windows was used for the statistical analyses. The significance of differences was determined using the least significant difference (LSD) test.

Results

The present study aimed to determine the toxic effects of various Cr concentrations on *M. oleifera* seedlings cultivated under hydroponically controlled conditions. At the end of the application, it was found that the Cr content in seedling roots and shoots increased with the concentration of the application (Fig. 1A). Thus, it was found that the root Cr content at 5, 25 and 50 mg/L concentrations increased 37.2, 42.9 and 55.1 ($p<0.05$) times when compared to the control, respectively. Similarly, shoot Cr content at the same concentrations increased by 12.0, 25.2 and 48.1 ($p<0.05$), respectively.

It was found that chromium application affected macro and micro nutrient intake and transportation in *M. oleifera* seedlings. It was found that root potassium (K) content, a macro element, increased 8.7% with 5 mg/L Cr concentration ($p>0.05$), while it decreased 46.3% and 57.5% with 25 and 50 mg/L Cr concentrations, respectively ($p<0.05$). Similar findings were obtained with the shoots (Fig. 1B). The Ca content in *M. oleifera* seedling root and shoots increased with Cr application (Fig. 1C). The highest increases in roots and shoots were calculated as 86.4% and 32.8% ($p<0.05$) with 50 mg/L Cr concentration, respectively. The Mg content in *M. oleifera* seedling root and shoots increased with Cr application (Fig. 1.D). The highest increase in root Mg content was determined as 25.2% ($p<0.05$) with

5 mg/L Cr concentration and 32.0% ($p<0.05$) with 50 mg/L concentration in shoots. The variation in Cu concentration, a micro nutrient, decreased in all Cr concentrations in roots and shoots except 50 mg/L concentration in the shoots (Fig. 1E). The maximum decrease in root and shoots was determined as 33.3% ($p<0.05$) and 9.08% ($p>0.05$) in 25 mg/L Cr concentration when compared to the control, respectively. Although *M. oleifera* seedling root Fe content increased 17.9% and 8.1% ($p<0.05$) with 5 and 25 mg/L Cr concentrations, respectively, it decreased 25.3% ($p<0.05$) in 50 mg/L concentration. Seedling shoot Fe content decreased in all concentrations (Fig. 1F). The seedling root Mn content increased with 5, 25 and 50 mg/L Cr concentrations by 33.4%, 40.9% and 55.3% ($p<0.05$), respectively. Shoot Mn content increased with 25 mg/L Cr concentration and decreased with 50 mg/L Cr (Fig. 1G). Seedling root Zn content increased up to 35.7% ($p<0.05$) with Cr application. In contrast, shoot Zn content decreased up to 15.4% ($p> 0.05$) (Fig. 1H).

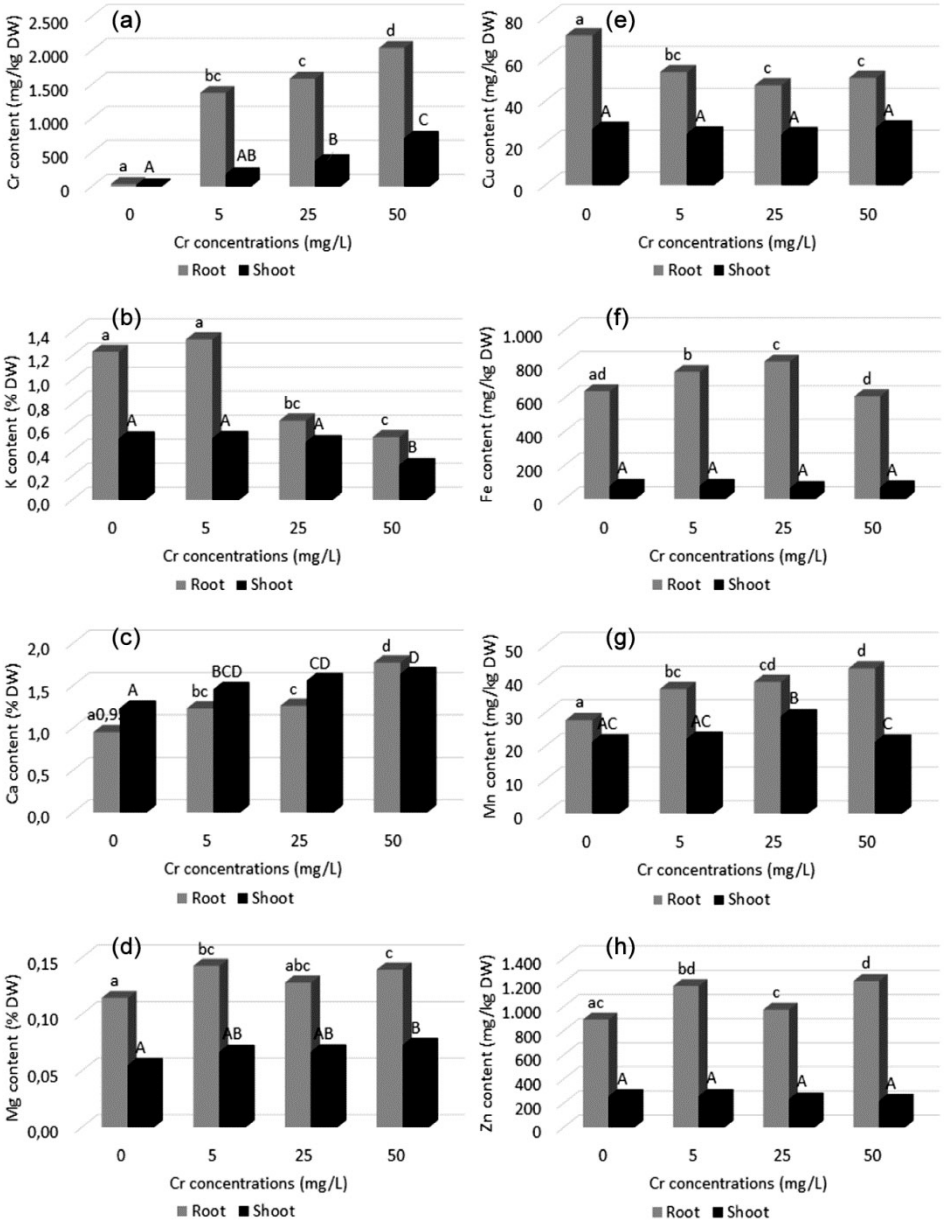

Fig. 1 — Effect of Cr application on element contents of *M. oleifera* seedling. Means with different letters are significantly different from one another (n=3) ($p<0.05$).

The *M. oleifera* seedling leaf photosynthetic pigment contents decreased with Cr application (Fig. 2). Chlorophyll-a (Chl-a) content decreased by 1.9%, 5.8 ($p>0.05$) and 15.7 ($p<0.05$) with 5, 25 and 50 mg/L Cr concentrations, respectively. Similarly, the highest decreases in Chl-b and carotenoid content in seedling leaves were calculated as 9.6% ($p>0.05$) and 20.8% ($p<0.05$) with 50 mg/L Cr concentration, respectively.

Certain biochemical changes were observed in *M. oleifera* root and shoot tissues as a result of Cr application. It was found that non-protein SH group content increased due to Cr stress. When compared to the control group, the highest increase in root and shoot non-protein SH group content were 162.3% and 75.1% ($p<0.05$) with 50 mg/L Cr concentration, respectively (Fig. 3A). Total seedling root phenolic compound content decreased with Cr application. On the contrary, the shoot phenolic compound content increased up to 7.3% ($p<0.05$) (Fig. 3B). The seedling tissue hydrogen peroxide concentration, a reactive oxygen species, increased with Cr application (Fig. 3C). Root tissue hydrogen peroxide content increased to 1.17 ($p> 0.05$), 1.57 and 2.36 ($p<0.05$) times in response to 5, 25 and 50 mg/L Cr concentrations, respectively when compared to the control. Similarly, the shoot hydrogen peroxide content increased to 1.9, 2.9 and 3.6 times with 5, 25 and 50 mg/L Cr application, respectively. Also, root and shoot MDA content increased with Cr toxicity (Fig. 3D). The seedling shoot and root MDA content increased by 4.1 and 6.9 times with 50 mg/L Cr application, respectively.

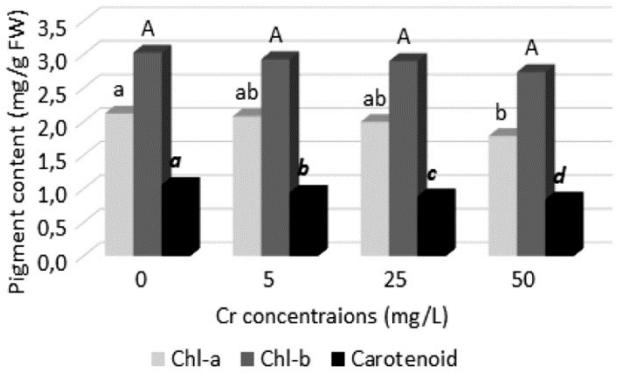

Fig. 2 — Effect of Cr application on photosynthetic pigment contents. Means with different letters are significantly different from one another (n=3) ($p<0.05$).

Discussion

Chromium is not an essential element for plants. The first contact between a plant and chromium occurs during the uptake of this element. The uptake of this heavy metal is through the carriers used for the uptake of metals necessary for the metabolism of plant. The toxic effects of chromium are mainly observed during the uptake, transport and accumulation of the metal. The metabolic pathway where Cr (VI) is transported is an active mechanism, which requires carriers that transport basic anions such as sulfate[22].

It was found that significant Cr amounts were accumulated in plant tissues after Cr application in *M. oleifera* roots and shoots. However, due to the structural similarities between Cr and certain requited

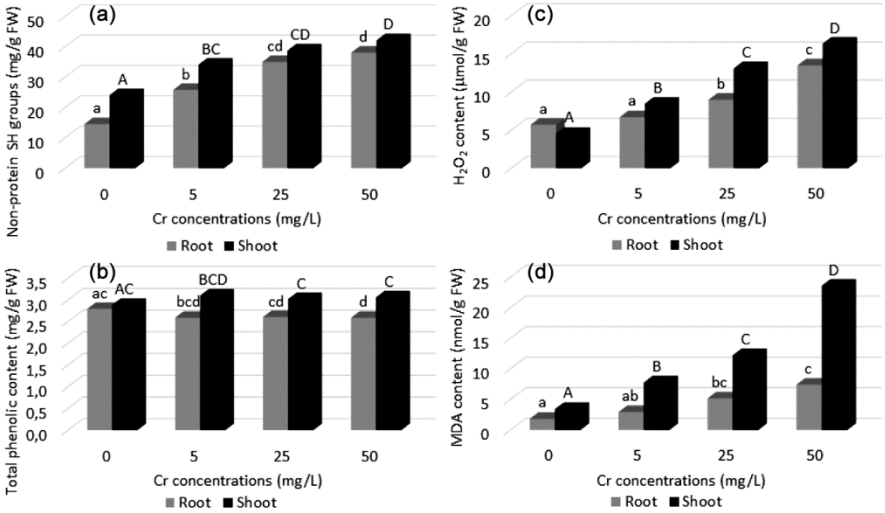

Fig. 3 — Effect of Cr application on non-protein SH groups (A), total phenolics (B), H_2O_2 (C) and MDA (D) contents of *M. oleifera* seedling parts. Means with different letters are significantly different from one another (n=3) ($p<0.05$).

nutrients, it could affect the mineral nutrition of plants through a complex mechanism. Based on present study findings, Cr application led to a change in macro and micro nutrient content in *M. oleifera* seedlings. Root and shoot macro element Ca and Mg content increased with Cr application. While K content increased with low concentration Cr application, it was determined that it decreased with the application of 25 and 50 mg/L Cr concentrations. *M. oleifera* seedling root Zn and Mn content increased with Cr application, while Cu and Fe content decreased in high Cr concentrations. In seedling shoots, it was found that Mn, Cu, and Fe content decreased with Cr application. Previous studies in the literature investigated the effect of Cr application on mineral nutrition. It was reported that K, Mg, P, Fe and Mn uptake decreased in soybean roots that included 9.6 µM Cr (VI) in nutrient solution[23]. Barcelo *et al*.[24] reported that P, K, Zn, Cu and Fe transport in plant sections were inhibited when the pea plant was exposed to Cr in nutrient solution. Sujatha and Gupta[25] stated that irrigation with tannery effluent led to micronutrient deficiency in several agricultural plants. Thus, it can be suggested that chromium induced nutrient imbalance by affecting the uptake and transport of macro and micro nutrients in *M. oleifera* seedlings consistent with the previous study findings,

Chromium application is an important factor that prevents photosynthesis in CO_2 fixation, electron transport, photophosphorylation and enzyme activities. Several studies reported that photosynthetic pigments decreased with Cr exposure[26,27]. In the present study, it was determined that leaf pigment content of *M. oleifera* seedling decreased with Cr application. Impaired δ-aminolaevulinic acid dehydratase activity that led to reduced photosynthetic pigment levels was observed in chromium-treated plants[28]. This might be attributed to the toxic effect of chromium on chlorophyll biosynthesis of *M. oleifera* seedling through direct inhibition of photosynthesis[29].

Phenolic compounds are agents that play a role in the response to biotic and abiotic stressors[30]. In *Jatropha curcas* L., Cr treatment enhanced the phenolic content in all parts of the plant[31]. Various studies reported other heavy metals led to increases in phenolic compound levels in plants[32,33]. *M. oleifera* root and shoot tissue phenolic compound content increased with Cr administration. The present study findings suggested that phenolic compound accumulation was an outcome of the robust seedling mechanism that controls and adapts to Cr toxicity.

Main SH groups in the structure of plants include cysteine, glutathione, phytochelatins and metallothionines. Most non-protein SH groups in plants include glutathione[34]. Glutathione is not only involved in the detoxifying of ROS but is also required for the synthesis of metal-binding properties such as phytochelatins[35,36]. *M. oleifera* seedling root and shoot non-SH group content increased with Cr administration. This increase was likely due to an increase in oxidative defenses or Cr-binding proteins of non-protein SH groups in Cr resistance in *M. oleifera* seedlings.

It is known that oxidative stress is an important pathophysiological event[37]. Previous studies demonstrated that Cr administration induces reactive oxygen species (ROS), leading to oxidative stress[38,39]. Among the ROSs, hydrogen peroxide (H_2O_2) is a product of O_2 with reduction of two electrons. H_2O_2 is a potentially reactive oxygen species, but not a free radical[40]. It is very dangerous since it could permeate cellular membrane and could reach cell compartments located far from the site of its formation[41]. Reactive oxygen species are known to damage cellular membranes by inducing lipid peroxidation[42]. The present study findings demonstrated that there was an increase in H_2O_2 and MDA levels in *M. oleifera* cells due to Cr application. The presence of these increases clearly demonstrated that Cr toxicity induced oxidative stress in the seedlings.

Conclusion

In conclusion, Cr application led to physiological and biochemical changes in *M. oleifera* seedling roots and shoots grown under controlled conditions. High Cr concentrations were observed in *M. oleifera* seedling roots and shoots. Thus, Cr induced nutrient irregularity by affecting the uptake and transport of the necessary macro and micro elements. Increases in phenolic content and non-protein SH groups may indicate that they might play a role in Cr toxicity. Furthermore, increases in H_2O_2 and MDA levels clearly demonstrated that Cr toxicity induced oxidative stress in *M. oleifera* cells.

Conflict of Interest

The Author declares that he has no conflict of interest.

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