

Research Article

## Effect of citric acid on antioxidant activity of garden cress (*Lepidium sativum* L.) under chromium stress

Kobra Mahdavian \*

Department of Biology, Faculty of Science, Payame Noor University, Tehran 19395-3697, Iran

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### Abstract

Chromium imposed harmful morphological, physiological, and metabolic effects in plants. This study was aimed to evaluate the impact of citric acid (0, 2.5 and 5 mM), on different morphological and physiological characteristics of garden cress exposed to chromium-VI stress (0, 1, and 10 mM). Results showed that shoot and root length, fresh and dry weight, chlorophyll, carotenoids, and DPPH activity decreased under chromium, while anthocyanin increased. Also, at high chromium concentrations, the rate of accumulation of this metal in the shoot parts declined but increased in the roots. This study showed that the addition of citric acid (2.5, and 5 mM) with chromium (10 mM) significantly enhanced shoot and root length, fresh and dry weight, chlorophyll, carotenoids while a similar increase was observed in the combination of 1 mM chromium and 2.5 mM citric acid, 5 mM citric acid in combination with 1 mM chromium reduced shoot and root length, fresh and dry weight, chlorophyll and carotenoids. The addition of 2.5 and 5 mM citric acid along with chromium 1 and 10 mM significantly decreased Anthocyanin content. Besides, citric acid 2.5 and 5 mM alleviated the adverse effect of chromium 1 and 10 mM on DPPH activity. This means that the transfer from the root to the shoot will increase under the effect of citric acid at high chromium concentrations but in low concentrations of chromium, citric acid reduced the concentration of chromium in the shoot parts considering the chromium uptake and translocation factor results, garden cress is not suitable for phytoremediation. Hence, the citric acid played this role through the regulation of the antioxidant system to diminish the toxicity of chromium.

**Keywords:** Anthocyanin, Carotenoids, Phytoremediation, DPPH activity

### Introduction

*Lepidium sativum*, commonly known as garden cress is a fast growing annual herb that is native to Egypt and West Asia, although it is now cultivated in the entire world. Its seeds are rich source of proteins, dietary fiber, omega-3 fatty acids, iron, and other essential nutrients and phytochemicals. Garden cress is widely used in folk medicine for the treatment of hyperactive airways disorders, such as asthma, bronchitis and cough (Doke and Guha, 2014). The accumulation of heavy metals in the edible parts of plants is a serious threat for humans and animals (Anjum *et al.*, 2016; Wang *et al.*, 2017). Plants can easily absorb heavy metals along with necessary nutrients from the soil and transport them to shoots (Rizwan *et al.*, 2017; Jabeen *et al.*, 2016; Lopez-Luna *et al.*, 2016; Lukina *et al.*, 2016), cause them to enter the food chain (Wuana and Okieimen, 2011; Anjum *et al.*, 2015). Recent studies have shown the toxic effects of heavy metals on photosynthesis, growth, and plant biomass (Anjum *et al.*, 2017; Singh *et al.*, 2017).

Chromium is one of the foremost non-essential and hazardous metals for living organisms (Mantry and Patra, 2017; Atta *et al.*, 2013). Chromium is widely used in industrial processes. Chemical production is the

most important source of its release in soil and aquatic environments that can have harmful environmental effects (Riaz *et al.*, 2019; Tripathi *et al.*, 2016; He *et al.*, 2017). In general, the chromium in the earth's crust is in the range of 0.1–0.3  $\mu\text{g g}^{-1}$ . Nevertheless, distinct soils showed a varying concentration of chromium, a range of 15 to 100  $\mu\text{g g}^{-1}$  (Shahid *et al.*, 2017). Chromium mostly occurs in two redox forms i.e. trivalent ( $\text{Cr}^{3+}$ ) and hexavalent ( $\text{Cr}^{6+}$ ) (Afshan *et al.*, 2015; Ahemad, 2015; da Costa *et al.*, 2016; Aharchaou *et al.*, 2017). Chromium III is comparatively motionless, in contrast to highly bioavailable, solvable, and moving form: Chromium VI (Dhal *et al.*, 2013; Markiewicz *et al.*, 2015). Chromium-VI is extremely toxic, carcinogenic and causes necrosis, bronchitis, asthma, and dermatitis in humans (Farid *et al.*, 2017).

The high oxidation potential of chromium-VI makes it more permeable in biological systems (Sallah-Ud-Din *et al.*, 2017). Higher chromium accumulation can induce decrease in plant growth and delay in seed germination (Rout *et al.*, 2000; Tripathi *et al.*, 2015). Many studies have also shown the inhibitory effects of chromium on photosynthetic pigments that decreased gas exchange activities (Mathur *et al.*, 2016; Prasad, 2004; Rodriguez

\*Corresponding Author, Email: k.mahdavian@pnu.ac.ir

*et al.*, 2012). Oxidative stress caused by chromium disrupts the biochemical function and morphology of plants (Nguyen *et al.*, 2017; Ma *et al.*, 2017).

Revitalization of soil contaminated with metal via hyperaccumulator plants is an environmental strategy (Rascio and Navari-Izzo, 2011). For the bioremediation of chromium contaminated soil, the phytoextraction technique is extremely useful (Shakoor *et al.*, 2013). Nevertheless, the utilization of hyper accumulator plants is widely accepted to extract chromium from soil and water (da Conceicao Gomes *et al.*, 2017; Handa *et al.*, 2017). Many studies have investigated several hyperaccumulator species like Rapeseed (Gasco *et al.*, 2019), Mustard (Gill *et al.*, 2016), sunflower (Zehra *et al.*, 2020), for the phytoremediation of chromium contaminated-soil. Nevertheless, the upper concentration and accumulation of chromium may cause changes in biochemical and morpho-physiological properties which ultimately leads to low productivity and efficiency (De Maria *et al.*, 2013; Handa *et al.*, 2017).

The potential of the phytoextraction process is often optimized by adding some organic and inorganic chelators that increase the mobility and availability of chromium (Wiszniewska *et al.*, 2016; Kumar *et al.*, 2014). The utilization of organic acids may ameliorate metal absorption in non-hyperaccumulator plants like *Ricinus communis* L. (Zhang *et al.*, 2016). Numerous studies have reported the use of organic chelators so that citric acid playing an important role in increasing growth under the conditions of chromium (Afshan *et al.*, 2015; Habiba *et al.*, 2015).

Since there have not been any investigations on the citric acid- recovery within the chromium phytoremediation by the garden cress, present study was designed to (1) calculate the chromium absorption of garden cress plants; (2) distinguish how much use of citric acid increases the absorption of chromium by decreasing phytotoxic effects of the metal, (3) examine the chromium levels of toxic effects on different morphological and physiological characteristics of garden cress (4) determination of decreased phytotoxic effects of chromium by citric acid.

## Materials and methods

**Culture condition and treatments:** This experiment was designed and implemented in the July 2019 under greenhouse conditions in Payame Noor University of Kerman. The seeds of garden cress (*Lepidium sativum* L.) were sterilized with sodium hypochlorite (1%) for 5 min and then washed three times by distilled water. after 72 h at 25 °C. A pot experiment was conducted, and for this purpose, the plastic pots, with 1 kg capacity, were filled with sieved soil. Five garden cress seeds were sown in each pot and pots were replicated three times per concentration. The uniform germinated seeds were transferred to pots containing sand, clay, and humus (3:1:2) under a light density of approximately 100  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ , day/night temperatures of 25/20 °C under

16 h photoperiod. Chromium (0, 1, and 10 mM in the form of potassium dichromate:  $\text{K}_2\text{Cr}_2\text{O}_7$ ) and citric acids (0, 2.5, and 5 mM) were applied to the soil for 7 days during vegetative growth of plants. The leaves were harvested 21 days after treatment. The following 9 treatments were constituted for the present study: T<sub>1</sub>: Cr (0 Mm) + CA (0 Mm); T<sub>2</sub>: Cr (1 Mm); T<sub>3</sub>: Cr (10 Mm); T<sub>4</sub>: CA (2.5 Mm); T<sub>5</sub>: CA (5 Mm); T<sub>6</sub>: Cr (1 Mm) + CA (2.5 Mm); T<sub>7</sub>: Cr (1 Mm) + CA (5 Mm); T<sub>8</sub>: Cr (10 Mm) + CA (2.5 Mm); T<sub>9</sub>: Cr (10 Mm) + CA (5 Mm), treatments.

**Pigment analysis:** The pigments were extracted from leaf disks in 80% acetone. The chlorophyll and carotenoid concentrations were determined by spectrophotometry according to the method described by Lichtenthaler (1987).

To determine the concentration of anthocyanins, 0.1 g fresh leaves were mixed with 10 ml of acidified methanol (methanol: HCl, 99: 1, v:v) and kept overnight in the dark conditions. The absorbance was determined at 550 nm. Anthocyanin concentrations were calculated using an extinction coefficient of 33000  $\text{mol}^{-1} \text{ cm}^{-1}$  (Wanger, 1979)

**DPPH activity assay:** Methanolic extract of leaves was subjected to the free radical scavenging activity assay using the method described by Shimada *et al.* (1992). Each extract (0.2 mg  $\text{ml}^{-1}$ ) in methanol (2 ml) was mixed with 2 ml of a freshly prepared methanolic solution containing 100 ppm of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The mixture was shaken vigorously and remained for 30 minutes in the dark conditions. Then, the absorbance was measured at 517 nm.

**Biomass and chromium concentration measurement:** At the end of treatment, the length of the shoot and root was measured using a ruler. The plant samples were first washed with tap water and then rinsed with deionized water 3 times. Roots and shoots were weighed (FW) and dried in an oven at 70 °C for 48 h and weighed again to establish dry biomass (DW). Chromium (Cr) concentrations were determined in roots and shoots. Cr was determined by digesting 20–50 mg of oven-dried plant material in 2 ml of a 1–4 (v/v) mixture of 37% (v/v) HCl and 65% (v/v)  $\text{HNO}_3$  in Teflon cylinders for 7 h at 140 °C, after which the volume was adjusted to 10 ml with demineralized water. Cr was determined using a flame atomic absorption spectrophotometer (PG-990), as described in Mahdavian *et al.* (2016).

**Translocation factor (TF) measurement:** The translocation factor was calculated by dividing the metal concentrations in shoots and roots (Mattina *et al.*, 2003).

**Statistical design and analysis:** The data were presented as the mean of three replicates  $\pm$  standard error (SE). At 5% probability level, ANOVA was applied for data analysis by using SPSS software (Statistics Software, Version 17.0). Tukey's HSD post hoc test was applied for multiple comparisons of the means.

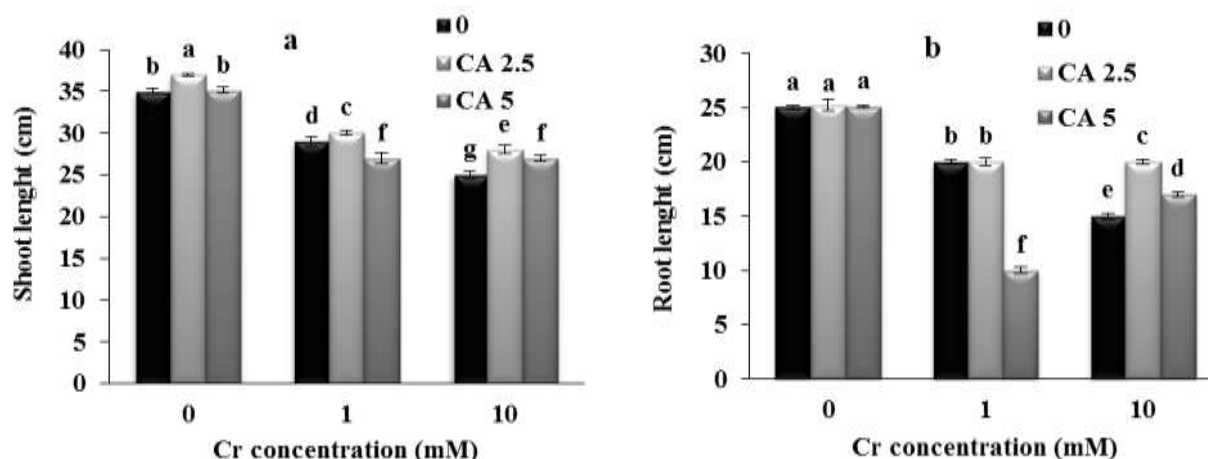


Fig. 1. Effect of citric acid and chromium stress on the shoot (a) and root length (b) of *Lepidium sativum* L. Values with similar letters are not significantly different at  $P < 0.05$ .

## Results

### Effect of citric acid and chromium on plant growth parameters:

The garden cress plant growth was significantly affected under chromium treatment (1 and 10 mM), compared to the control plants (Fig. 1). The chromium treatment at 10 mM reduced shoot lengths, with an average reduction of 29%, while this impact did not exceed 17% at 1 mM (Fig. 1a). Root length was found to be chromium dose-dependent, whereas similar decreases of shoot length were observed at both chromium doses to the controls (Fig. 1b). Similarly, data presented in (Fig. 2) indicated that chromium stress significantly ( $P < 0.05$ ) reduced the fresh and dry weight of shoots and roots (Fig. 2).

The citric acid 2.5 mM alone significantly enhanced growth parameters, compared to the control plants. The addition of citric acid (2.5 and 5 mM) with chromium (10 mM) significantly enhanced growth parameters while a similar increase was observed in the combination of 1 mM chromium and 2.5 mM citric acid, 5 mM citric acid in combination with 1 mM chromium reduced growth parameters. The maximum increase in shoot and root length was recorded (12% and 33%, respectively), under combined application of chromium (10 mM) and citric acid 2.5 mM, as compared to the plants treated with chromium (10 mM) alone. Similarly, the fresh weight of shoot and root were improved by 44% and 50%, respectively, under chromium 10 mM and citric acid 2.5 mM as compared to control. Also, the application of 2.5 and 5 mM of citric acid with 10 mM chromium significantly increased the dry weight of shoot and root but 1 mM chromium, only 2.5 mM citric acid increased the dry weight of roots and shoots (Fig. 1, 2).

**Effect of citric acid and chromium on photosynthetic pigments:** Chlorophyll (a, b, and total chlorophyll) and carotenoids contents significantly decreased in plants grown under chromium stress. The carotenoids and total chlorophyll content decreased by 11% and 17% respectively, in 10 mM, as compared to the control. However, foliar application of citric acid

significantly ( $P < 0.05$ ) improved the Chla, Chlb, total Chl, and carotenoid contents. The addition of citric acid (2.5 and 5 mM) alone significantly increased Chla, Chlb, total Chl, and carotenoid contents, compared to the control plants. Maximum total chlorophylls and carotenoids concentrations were obtained in the plants applied with citric acid (2.5 mM) only and minimum concentrations were obtained with 10 mM chromium treatment. The addition of 2.5 and 5 mM citric acid along with chromium 10 mM significantly enhanced total chlorophyll by 8% and 6% respectively while carotenoids by 5% and 12% respectively, as compared to the rest of the treatments (Fig. 3).

### Effects of citric acid and chromium on

**anthocyanin content:** Anthocyanin content in garden cress leaves significantly increased in chromium-stressed plants. The addition of 2.5 and 5 mM citric acid along with chromium 1 and 10 mM significantly decreased anthocyanin content. The addition of citric acid (2.5 and 5 mM) alone significantly decreased anthocyanin content, compared to the control plants. The addition of 2.5 and 5 mM citric acid along with chromium 1 mM significantly decreased anthocyanin content by 72% and 48% respectively while in chromium 10 mM by 82% and 71% respectively (Fig. 4).

### Effects of citric acid and chromium on antioxidant activity using DPPH activity assay:

Our result showed that significant changes in DPPH activity occurred in response to chromium. DPPH activity in garden cress leaves significantly reduced in chromium-stressed plants by 7.75% and 21.85% as compared to the control, respectively, in 1 and 10 mM of chromium. The addition of citric acid (2.5 and 5 mM) alone significantly enhanced DPPH activity, compared to control plants. The application of citric acid 2.5 and 5 mM alleviated this adverse effect of chromium 1 and 10 mM on DPPH activity. The citric acid 2.5 mM increased the DPPH activity of leaves by 132% and 147% respectively, under chromium 1 and 10 mM, compared with chromium treatment alone (Fig. 5).

### Effects of citric acid and chromium on chromium

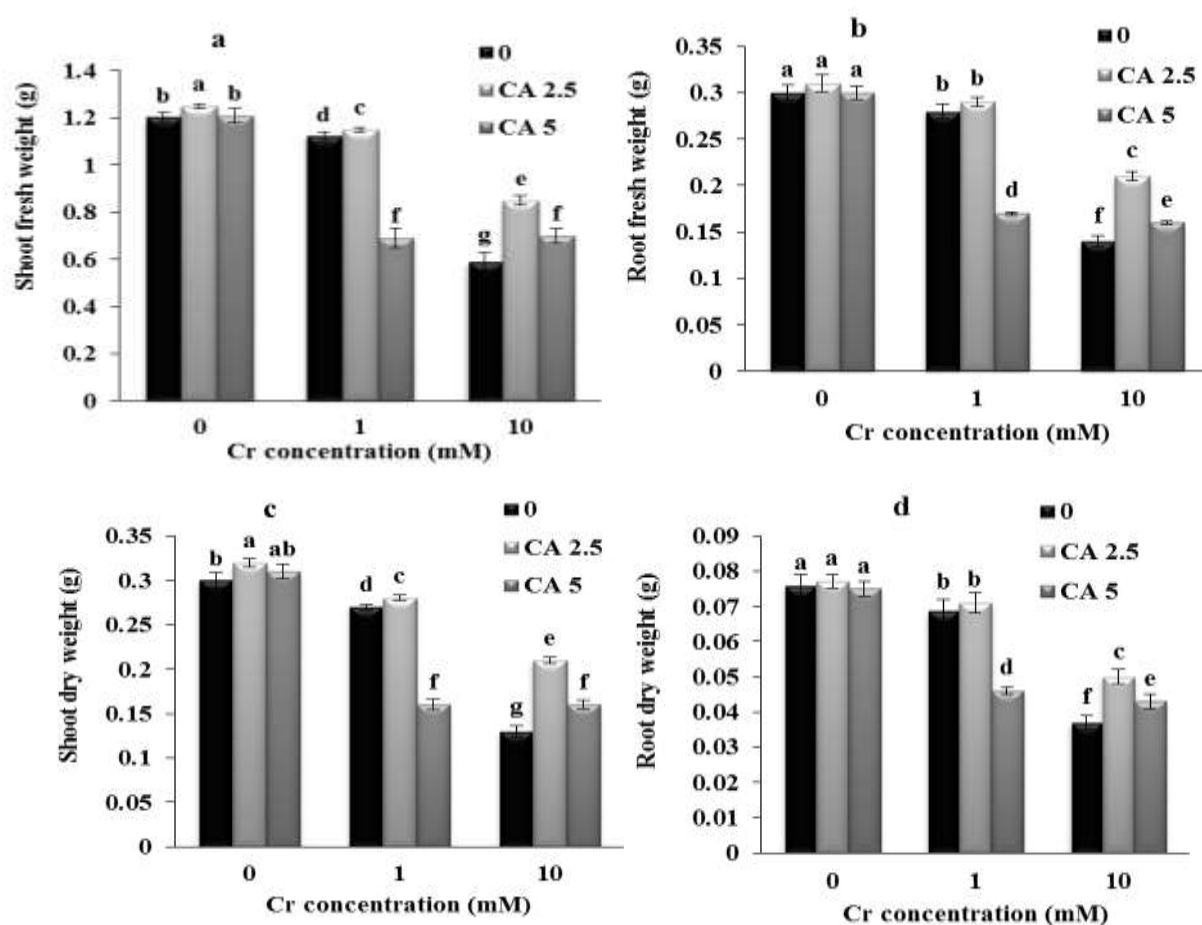


Fig. 2. Effect of citric acid and chromium stress on the shoot (a) and root fresh (b) and dry weight (c, d) of *Lepidium sativum* L. Values with similar letters are not significantly different at  $P < 0.05$ .

**uptake and translocation factor:** The results showed that with increasing chromium concentration in the soil, its concentration in the shoot of the garden cress plants decreased but in the root increased (Fig. 6 a, b). The highest chromium concentration ( $16.54 \text{ mg kg}^{-1}$  dry weight) was observed in 1 mM chromium, while in 10 mM chromium ( $3.60 \text{ mg kg}^{-1}$  dry weight), chromium adsorption was observed in shoot parts. Also, in the roots the highest chromium concentration ( $225.31 \text{ mg kg}^{-1}$  dry weight) was observed in 10 mM chromium. In the application of citric acid alone, no significant difference was observed in the concentration of chromium of shoots and roots in comparison with the control plants. In contrast, the soil amendment with citric acid produced an apparent reduction in chromium 1 mM accumulation in shoot parts while plants treated with 2.5 and 5 mM citric acid showed higher accumulation of chromium 10 mM in shoot parts compared with chromium contaminated soil (Fig. 6 a). The citric acid 2.5 and 5 mM application further reduced the concentration of chromium 1 mM by 8% and 66% in the shoots respectively, while in shoot, the concentration and accumulation were increased by 59% and 180% respectively under citric acid 2.5 and 5 mM with chromium 10 mM, compared with their respective

controls. Plants treated with citric acid showed higher accumulation of chromium in root parts compared with chromium contaminated soil (Fig. 6 b).

Analysis of variance showed that soil chromium concentration markedly impacted on translocation factor in garden cress plants. The translocation factor in the garden cress increased at a concentration of 1 mM chromium while decreasing at a concentration of 10 mM chromium (Fig. 6 c). In the application of citric acid alone, no significant difference was observed in the translocation factor in comparison with the control plants. The citric acid 2.5 and 5 mM application further reduced the translocation factor of chromium 1 mM by 6% and 67% respectively, while the translocation factor was increased by 31% and 81% respectively under citric acid 2.5 and 5 mM with chromium 10 mM, compared with their respective controls.

## Discussion

The toxicity of chromium greatly inhibited the growth and biomass of plants compared to the control (Antoniadis *et al.*, 2017). The results of this study indicated that chromium stress reduced the plant growth of garden cress plants. However, soil application of citric acid considerably improved the growth and

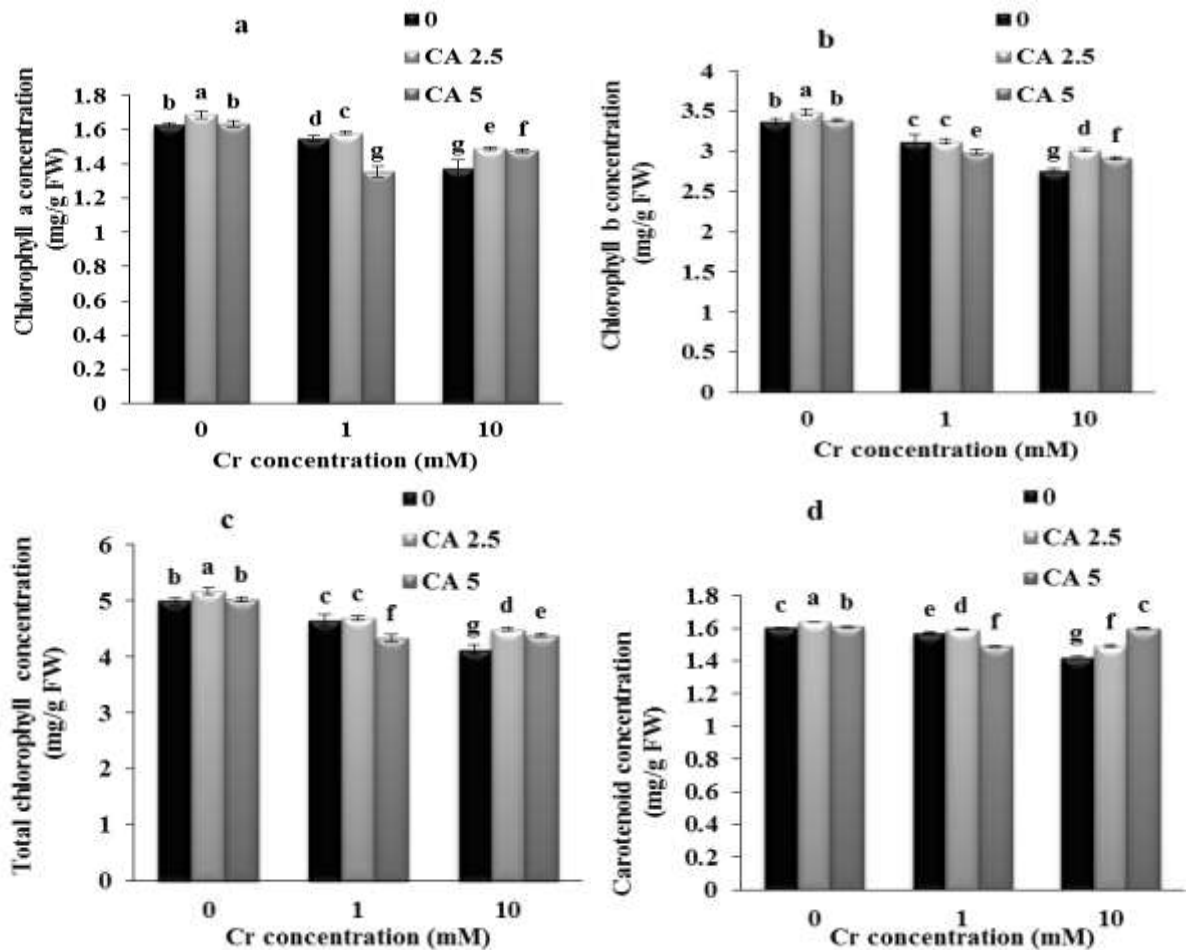


Fig. 3. Effect of citric acid and chromium stress on Chla (a), Chlb (b), total Chl (c), and carotenoid contents (d) of *Lepidium sativum* L. Values with similar letters are not significantly different at  $P < 0.05$ .

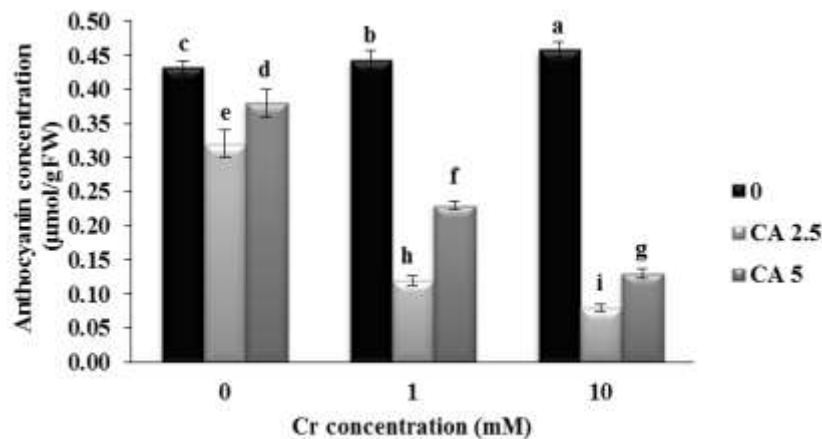


Fig. 4. Effect of citric acid and chromium stress on anthocyanin concentration of *Lepidium sativum* L. Values with similar letters are not significantly different at  $P < 0.05$ .

biomass production in garden cress plants that the role of CA as a growth promoting agent with a chelating potential against different heavy metals such as Cr (Afshan *et al.*, 2015). Increasing chromium concentration in soil considerably reduced the nutrient uptake and accordingly reduced growth and biomass of plants (Rivelli *et al.*, 2014; Tauqeer *et al.*, 2016). It has

been proven that chromium stress reduces growth and biomass production in sunflower (Saleem *et al.*, 2015), wheat (Dotaniya *et al.*, 2014), pea (Rodriguez *et al.*, 2012), rice (Hussain *et al.*, 2018), castor bean (Qureshi *et al.*, 2020), and cauliflower (Ahmad *et al.*, 2020).

The effect of citric acid on the growth parameters of garden cress plants considerably enhanced compared

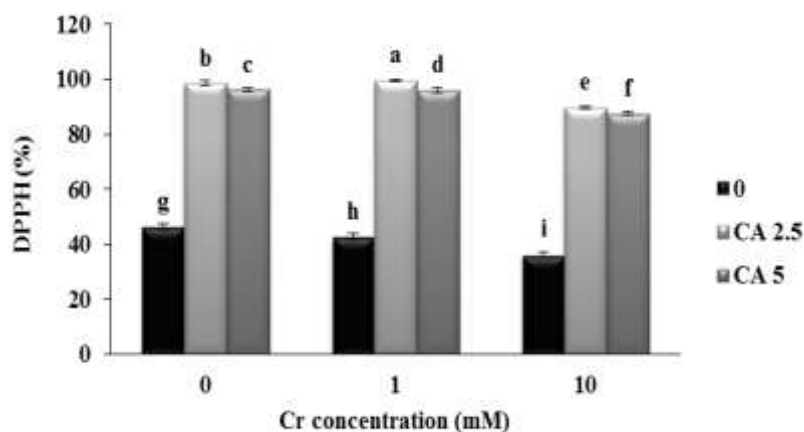


Fig. 5. Effect of citric acid and chromium stress on DPPH activity of *Lepidium sativum* L. Values with similar letters are not significantly different at  $P < 0.05$ .

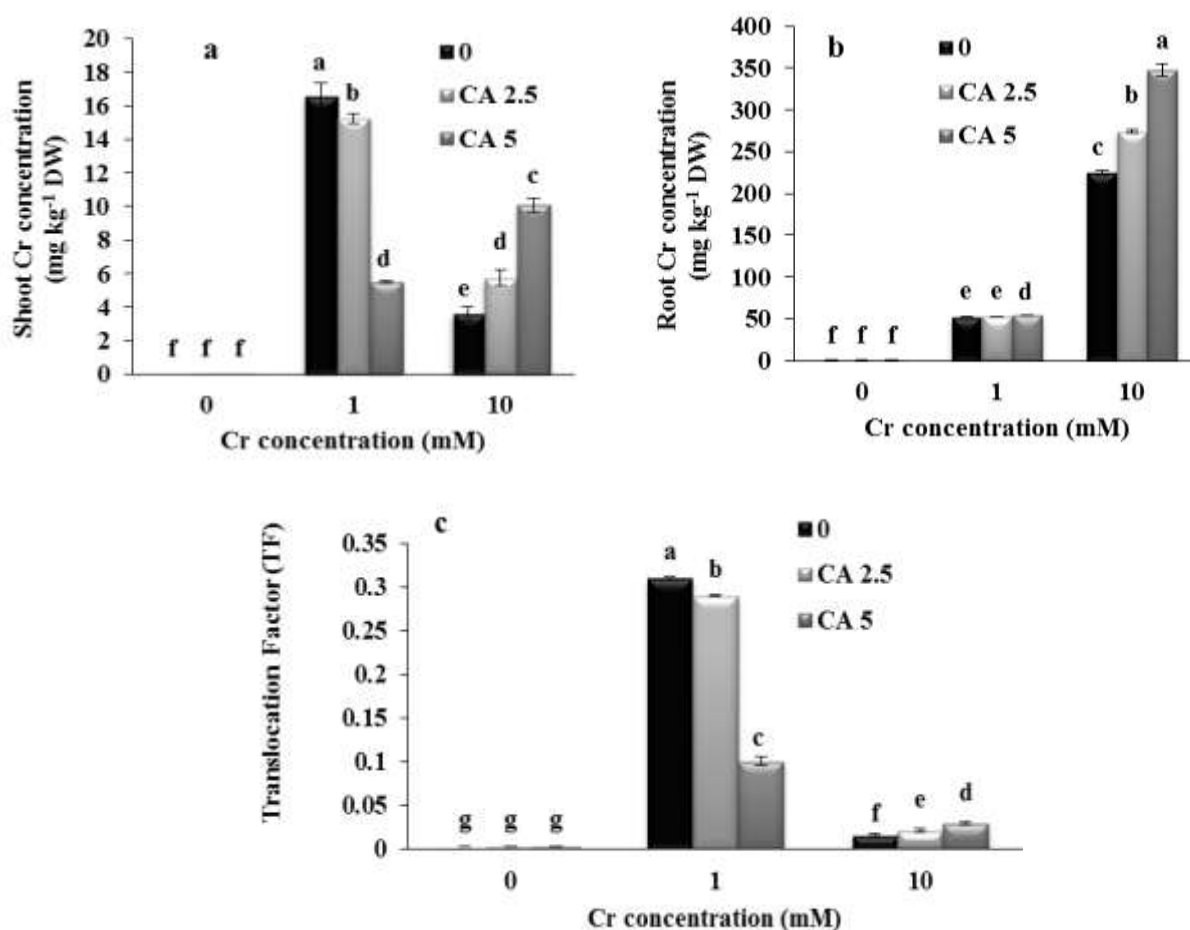


Fig. 6. Effect of citric acid and chromium stress on the shoot (a) and root (b) chromium concentration and TF (c) of *Lepidium sativum* L. Values with similar letters are not significantly different at  $P < 0.05$ .

with the control. The positive effect of citric acid is additionally attributed as a plant growth regulator because of its ability to increase nutrient uptake and protect against biotic and abiotic stresses (Freitas *et al.*, 2013). Studies have shown that citric acid also plays an important role in modulating the negative effects of cadmium and lead-heavy metals (Ehsan *et al.*, 2014;

Shakoor *et al.*, 2014). Afshan *et al.* (2015) declared that citric acid reduces the toxicity of chromium in *Brassica napus*. Similar findings have been observed in the cotton plant under copper stress (Zaheer *et al.*, 2015). Studies have shown that chromium reduces biomass in *Ricinus communis*, while the use of citric acid improves the reduction effect of chromium on plant biomass

(Qureshi *et al.*, 2020). Therefore, higher photosynthesis can lead to increase biomass (Rodriguez *et al.*, 2012).

Chlorophyll contents are as a criterion to evaluate heavy metal tolerance potential of plants (Huang *et al.*, 2011). In this study, chromium toxicity reduced photosynthetic pigments in the garden cress. These results are consistent with the findings of previous studies on in sunflower plants under the influence of chromium (Saleem *et al.*, 2015; Atta *et al.*, 2013). Reduction of photosynthetic pigments that were additionally determined in *Brassica napus*, *Brassica juncea*, mung bean, barley, and wheat under chromium stress (Afshan *et al.*, 2015; Adrees *et al.*, 2015; Gonzlez *et al.*, 2015; Jabeen *et al.*, 2016). Also, the accumulation of toxic chromium destroys the chloroplast structure of plants (Farid *et al.*, 2019; Najeeb *et al.*, 2011). Studies have shown that chlorophyll and carotenoid depletion is due to the breakdown of chloroplast structure, photosynthetic system, and electron transfer chain (Singh *et al.*, 2013). Similar to chromium, other heavy metals reduced photosynthetic pigments (Farooq *et al.*, 2016; Habiba *et al.*, 2015). In rice, chromium reduced the amount of chlorophyll so that chlorophyll *b* decreased more than chlorophyll *a* (Chen *et al.*, 2017).

The decrease in carotenoid content in our research might be due to increase accumulation of chromium that increased cellular levels of ROS (Jabeen *et al.*, 2016). Carotenoids have antioxidant activity that protects them to scavenge, hydrogen peroxide ( $H_2O_2$ ), superoxide radical, and singlet oxygen (Kang *et al.*, 2017).

The application of citric acid lessened the adverse effects of chromium on chlorophyll and carotenoid in garden cress plants. This might be the effect of CA application, which enhanced the uptake of essential nutrients as well as the formation of photosynthetic pigments (Farid *et al.*, 2017). It can be proposed that citric acid might have formed a chelate complex with Cr thereby reducing its toxicity. Similar findings of improvement in photosynthetic pigments were also observed in the plants under lead, cadmium, chromium, and nickel (Afshan *et al.*, 2015; Ehsan *et al.*, 2014; Shakoore *et al.*, 2014; Zaheer *et al.*, 2015). The addition of 5 mM citric acid also increased photosynthetic pigments in *Ricinus communis* under chromium stress (Qureshi *et al.*, 2020). In the present study, the decrease in carotenoid contents as a result of 5 mM CA in 1 mM Cr application could have been due to oxidative stress created by CA.

DPPH activity is used to determine the total antioxidant potential. The results of the present research showed a significant reduction in total antioxidant potential taken in the form of DPPH activity in plants under chromium stress. Our results are consistent with results of Tripathi *et al.* (2012), which showed a chromium-mediated decline in DPPH radical scavenging activity in rice plants grown in chromium-contaminated conditions. As so, Kundu *et al.* (2018) showed a considerable reduction in DPPH radical scavenging activity in Plantago under chromium stress.

However, in this study DPPH activity in garden cress leaves enhanced in 2.5 and 5 mM citric acid. Citric acid-mediated increase in antioxidant potential has been reported in *Ricinus communis* grown in chromium contaminated condition (Qureshi *et al.*, 2020). There are several reports on citric acid-mediated increase in antioxidant potential of plants under abiotic stress by inhibiting free radicals.

Anthocyanins have strong antioxidant potential, which plays an important role in chromium stress tolerance (Kovinich *et al.*, 2015; Habiba *et al.*, 2019). An increase in anthocyanins under metal stress has been reported in Arabidopsis (Baek *et al.*, 2012). The results of the present research showed a significant increase of anthocyanins content in plants under chromium stress. The addition of citric acid also mediated a significant decrease in this parameter under chromium stress.

The chromium-VI is considered to be much more toxic than chromium III (Gomes *et al.*, 2017). In this study, the results showed that with increasing chromium concentration in the soil, its concentration in the shoot of the garden cress plants decreased. However, our results do not match the old studies on chromium uptake in soil (Saleem *et al.*, 2015; Atta *et al.*, 2013). Studies show that chromium can be easily absorbed by some plants (Jabeen *et al.*, 2016; Adrees *et al.*, 2015). Our results showed that citric acid 2.5 and 5 mM treatment further decreased the absorption of chromium 1 mM in the garden cress. Therefore, our results are consistent with Qureshi *et al.* (2020), who found lower chromium-VI concentration in *Ricinus communis* in citric acid treatment. Also, in this study, citric acid 2.5 and 5 mM increased the concentration of chromium in the shoots of plants under 10 mM chromium stress, which represents chelating the role of citric acid in the absorption of heavy metals (Farid *et al.*, 2017; Najeeb *et al.*, 2009, 2011; Shakoore *et al.*, 2014). Citric acid showed the potential to vary the oxidation state of chromium-VI to chromium III (Lesniewska *et al.*, 2017). Citric acid efficaciously preserved plants from chromium-VI phytotoxic effects. Our results are consistent with previous studies that reported a decrease in the growth of wheat plants grown in chromium-media was regulated by chromium (Riaz *et al.*, 2019). Our results are consistent with previous studies that showed higher chromium accumulation in roots than shoots, this is due to the storage of chromium in the vacuole (Shanker *et al.*, 2005; Gupta and Sinha, 2006). The translocation factor calculates the potency of plants to transfer metals from the root to the shoot. It is given by the ratio of metal concentration in shoot and root. In this research, the citric acid 2.5 and 5 mM application further reduced the translocation factor of chromium 1 mM, while the translocation factor was increased under citric acid 2.5 and 5 mM with chromium 10 mM, compared with their respective controls. Plants with translocation factor higher than one will transfer heavy metals to shoot (Chen *et al.*, 2015; Adesodun *et al.*, 2010; Prasad *et al.*, 2001). While, plants with

translocation factor lower than one show lower capability transfer of heavy metal from root to shoot (Aran *et al.*, 2017). Therefore, our results indicate that the garden cress plant is not one of the hyperaccumulator plants in terms of chromium accumulation in the shoot parts. The results also showed that at high chromium concentrations in the soil, the rate of accumulation of this metal in the shoot parts declined. This means that the transfer from the root to the shoot will increase under the effect of citric acid at high chromium concentrations but in low concentrations of chromium, citric acid reduced the concentration of chromium in the shoot parts. Thus our results also proposed that citric acid treatment resulting in a reduction in oxidative damages imported by chromium-VI.

### Conclusion

The current research concludes that chromium considerably has toxic effects on the morphological and physiological characteristics of garden cress. The results showed that with increasing chromium concentration in

the soil, its concentration in the shoot of the garden cress plants decreased. Also, our results proposed that the transfer from the root to the shoot will increase under the effect of citric acid at high chromium concentrations but in low concentrations of chromium, citric acid reduced the concentration of chromium in the shoot parts. Therefore, the improvement in morphological and physiological characteristics of garden cress showed a reduction in the role of citric acid under chromium stress. The addition of citric acid protects the defense system and helps the plant resistance against high levels of chromium in the tissues by regulating the physiological and biochemical processes. Considering the above results, garden cress is not suitable for phytoremediation.

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### References

- Adesodun, J. K., Atayese, M. O., Agbaje, T. A., Osadiaye, B. A., Mafe, O. F. and Soretire, A. (2010) Phytoremediation potentials of sunflowers (*Tithonia diversifolia* and *Helianthus annuus*) for metals in soils contaminated with zinc and lead nitrates. *Water, Air, and Soil Pollution* 207: 195-201.
- Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F., Farid, M., Zia-ur-Rehman, M., Irshad, M. K. and Bharwana, S. A. (2015) The effect of excess copper on growth and physiology of important food crops: A review. *Environmental Science and Pollution Research* 22: 8148-8162.
- Afshan, S., Ali, S., Bharwana, S. A., Rizwan, M., Farid, M., Abbas, F., Ibrahim, M., Mehmood, M. A. and Abbasi, G. H. (2015) Citric acid enhances the phytoextraction of chromium, plant growth, and photosynthesis by alleviating the oxidative damages in *Brassica napus* L. *Environmental Science and Pollution Research* 22: 11679-11689.
- Aharchaou, I., Rosabal, M., Liu, F., Battaglia, E., Vignati, D. A. and Fortin, C. (2017) Bioaccumulation and subcellular partitioning of Cr (III) and Cr (VI) in the freshwater green alga *Chlamydomonas reinhardtii*. *Aquatic Toxicology* 182: 49-57.
- Ahemad, M. (2015) Enhancing phytoremediation of chromium-stressed soils through plant-growth-promoting bacteria. *Journal of Genetic Engineering and Biotechnology* 13: 51-58.
- Ahmad, R., Ali, S., Rizwan, M., Dawood, M., Farid, M., Hussain, A., Wijaya, L., Alyemeni, M. N. and Ahmad, P. (2020) Hydrogen sulfide alleviates chromium stress on cauliflower by restricting its uptake and enhancing antioxidative system. *Physiologia Plantarum* 168: 289-300.
- Anjum, S. A., Ashraf, U., Khan, I., Saleem, M. F. and Wang, L. C. (2016) Chromium toxicity induced alterations in growth, photosynthesis, gas exchange attributes and yield formation in maize. *Pakistan Journal of Agricultural Sciences* 53: 751-757.
- Anjum, S. A., Ashraf, U., Tanveer, M., Khan, I., Hussain, S., Zohaib, A., Abbas, F., Saleem, M. F. and Wang, L. (2017) Drought tolerance in three maize cultivars is related to differential osmolyte accumulation, antioxidant defense system, and oxidative damage. *Frontiers in Plant Science* 8: 1-12.
- Anjum, S. A., Tanveer, M., Hussain, S., Bao, M., Wang, L. C., Khan, I., Ullah, E., Tung, S. A., Samad, R. A., Shahzad, B. (2015) Cadmium toxicity in maize (*Zea mays* L.): Consequences on antioxidative systems, reactive oxygenspecies and cadmium accumulation. *Environmental Science and Pollution Research* 22: 17022-17030.
- Antoniadis, V., Shaheen, S. M., Boersch, J., Frohne, T., Du Laing, G. and Rinklebe, J. (2017) Bioavailability and risk assessment of potentially toxic elements in garden edible vegetables and soils around a highly contaminated former mining area in Germany. *Journal of Environmental Economics and Management* 186: 192-200.
- Aran, D. S., Harguinteguy, C. A., Fernandez-Cirelli, A. and Pignata, M. L. (2017) Phytoextraction of Pb, Cr, Ni, and Zn using the aquatic plant *Limnobium laevigatum* and its potential use in the treatment of wastewater. *Environmental Science and Pollution Research* 24: 18295-18308.

- Atta, M. I., Bokhari, T. Z., Malik, S. A., Wahid, A., Saeed, S and Gulshan, A. B. (2013) Assessing some emerging effects of hexavalent chromium on leaf physiological performance in sunflower (*Helianthus annuus* L.). International Journal of Scientific & Engineering Research. 4: 945-949.
- Baek, S. A., Han, T., Ahn, S. K., Kang, H., Cho, M. R., Lee, S. C. and Im, K. H. (2012) Effects of heavy metals on plant growths and pigment contents in *Arabidopsis thaliana*. Plant Pathology J. 28: 446-452.
- Chen, Q., Zhang, X., Liu, Y., Wei, J., Shen, W., Shen, Z. and Cui, J. (2017) Hemin-mediated alleviation of zinc, lead and chromium toxicity is associated with elevated photosynthesis, antioxidative capacity; suppressed metal uptake and oxidative stress in rice seedlings. Plant Growth Regulation 81: 253-264.
- Chen, Z., Zhao, Y., Fan, L., Xing, L. and Yang, Y. (2015) Cadmium (Cd) localization in tissues of cotton (*Gossypium hirsutum* L.), and Its phytoremediation potential for Cd-contaminated soils. Bulletin of Environmental Contamination and Toxicology 95: 784-789.
- da Conceicao Gomes, M. A., Hauser-Davis, R. A., de Souza, A. N. and Vitoria, A. P. (2016) Metal phytoremediation: General strategies, genetically modified plants and applications in metal nanoparticle contamination. Ecotoxicology and Environmental Safety 134: 133-147.
- da Costa, C. H., Perreault, F., Oukarroum, A., Melegari, S. P., Popovic, R. and Matias, W. G. (2016) Effect of chromium oxide (III) nanoparticles on the production of reactive oxygen species and photosystem II activity in the green alga *Chlamydomonas reinhardtii*. Science of the Total Environment. 565: 951-960.
- De Maria, S., Puschenreiter, M. and Rivelli, A. R. (2013) Cadmium accumulation and physiological response of sunflower plants to Cd during the vegetative growing cycle. Plant, Soil and Environment. 59: 254-261.
- Dhal, B., Thatoi, H. N., Das, N. N. and Pandey, B. D. (2013) Chemical and microbial remediation of hexavalent chromium from contaminated soil and mining/metallurgical solid waste: A review. Journal of Hazardous Materials 250: 272-291.
- Doke, S. and Guha, M. (2014) Garden cress (*Lepidium sativum* L.) seed - an important medicinal source: A review. Journal of Natural Product and Plant Resources 4: 69-80.
- Dotaniya, M. L., Das, H. and Meena, V. D. (2014) Assessment of chromium efficacy on germination, root elongation, and coleoptile growth of wheat (*Triticum aestivum* L.) at different growth periods. Environmental Monitoring and Assessment 186: 2957-2963.
- Ehsan, S., Ali, S., Noureen, S., Mahmood, K., Farid, M., Ishaque, W., Shakoor, M. B. and Rizwan, M. (2014) Citric acid assisted phytoremediation of cadmium by *Brassica napus* L. Ecotoxicology and Environmental Safety 106: 164-172.
- Farid, M., Ali, S., Rizwan, M., Ali, Q., Abbas, F., Bukhari, S. A. H., Saeed, R. and Wu, L. (2017) Citric acid assisted phytoextraction of chromium by sunflower; morpho-physiological and biochemical alterations in plants. Ecotoxicology and Environmental Safety 145: 90-102.
- Farid, M., Ali, S., Saeed, R., Rizwan, M., Bukhari, S. A. H., Abbasi, G. H., *et al.*, (2019) Combined application of citric acid and 5-aminolevulinic acid improved biomass, photosynthesis and gas exchange attributes of sunflower (*Helianthus annuus* L.) grown on chromium contaminated soil. International Journal of Phytoremediation 21: 760-767.
- Farooq, M. A., Detterbeck, A., Clemens, S. and Dietz, K. J. (2016) Silicon-induced reversibility of cadmium toxicity in rice. Journal of Experimental Botany 67: 3573-3585.
- Freitas, E. V. S., Nascimento, C. W. A., Silva Sousa, A. and Silva, F. B. (2013) Citric acid-assisted phytoextraction of lead: A field experiment. Chemosphere 92: 213-217.
- Gasco, G., Alvarez, M. L., Paz-Ferreiro, J. and Mendez, A. (2019) Combining phytoextraction by *Brassica napus* and biochar amendment for the remediation of a mining soil in Riotinto (Spain). Chemosphere 231: 562-570.
- Gill, R. A., Zhang, N., Ali, B., Farooq, M. A., Xu, J., Gill, M. B., Mao, B. and Zhou, W. (2016) Role of exogenous salicylic acid in regulating physio-morphic and molecular changes under chromium toxicity in black-and yellow-seeded *Brassica napus* L. Environmental Science and Pollution Research 23: 20483-20496.
- Gomes, M. A. D. C., Hauser-Davis, R. A., Suzuki, M. S. and Vitoria, A. P. (2017) Plant chromium uptake and transport, physiological effects and recent advances in molecular investigations. Ecotoxicology and Environmental Safety 140: 55-64.
- Gonzalez del, M., Gil-Diaz, M. and del Carmen Lobo, M. (2015) Response of two barley cultivars to increasing concentrations of cadmium or chromium in soil during the growing period. Biological Trace Element Research 163: 235-243.
- Gupta, A. K. and Sinha, S. (2006) Chemical fractionation and heavy metal accumulation in the plant of *Sesamum indicum* (L.) var. T55 grown on soil amended with tannery sludge: selection of single extractants. Chemosphere 64: 161-173.
- Habiba, U., Ali, S., Farid, M., Shakoor, M. B., Rizwan, M., Ibrahim, M., Abbasi, G. H., Hayat, T. and Ali, B. (2015) EDTA enhanced plant growth, antioxidant defense system, and phytoextraction of copper by *Brassica napus* L. Environmental Science and Pollution Research 22: 1534-1544.

- Habiba, U., Ali, S., Rizwan, M., Ibrahim, M., Hussain, A., Shahid, M. R., Alamri, S. A., Alyemeni, M. N. and Ahmad, P. (2019) Alleviative role of exogenously applied mannitol in maize cultivars differing in chromium stress tolerance. *Environmental Science and Pollution Research* 26: 5111-5121.
- Handa, N., Kohli, S. K., Thukral, A. K., Arora, S. and Bhardwaj, R. (2017) Role of Se (VI) in counteracting oxidative damage in *Brassica juncea* L. under Cr (VI) stress. *Acta Physiologiae Plantarum* 39: 1-15.
- He, X. L., Fan, S. K., Zhu, J., Guan, M. Y., Liu, X. X., Zhang, Y. S. and Jin, C. W. (2017) Iron supply prevents Cd uptake in *Arabidopsis* by inhibiting IRT1 expression and favoring competition between Fe and Cd uptake. *Plant and Soil* 416: 453-462.
- Huang, S., Han, Y., Yu, S., Gu, J. and Zhang, L. (2011) The effect of lead and copper on the growth and physiological response of water flower *Iris pseudacorus* L. *Fresenius Environmental Bulletin* 20: 2246-2250.
- Hussain, A., Ali, S., Rizwan, M., Ziaur Rehman, M., Hameed, A., Hafeez, F., Hafeez, F., Alamri, S., Alyemeni, M. N. and Wijaya, L. (2018) Role of zinc-lysine on growth and chromium uptake in rice plants under Cr stress. *Juornal of Plant Growth Regulation* 37: 1413-1422.
- Jabeen, N., Abbas, Z., Iqbal, M., Rizwan, M., Jabbar, A., Farid, M., Ali, S., Ibrahim, M. and Abbas, F. (2016) Glycinebetaine mediates chromium tolerance in mung bean through lowering of Cr uptake and improved antioxidant system. *Archives of Agronomy and Soil Science* 62: 648-662.
- Kang, L., Ji, C. Y., Kim, S. H., Ke, Q., Park, S. C., Kim, H. S. *et al.*, (2017) Suppression of the  $\beta$ -carotene hydroxylase gene increases  $\beta$ -carotene content and tolerance to abiotic stress in transgenic sweetpotato plants. *Plant Physiology And Biochemistry* 117: 24-33.
- Kovinich, N., Kanyan, G., Chanoca, A., Otegui, M. S. and Grotewold, E. (2015) Abiotic stresses induce different localizations of anthocyanins in *Arabidopsis*. *Plant Signaling and Behavior* 10: 1-4.
- Kumar, D., Tripathi, D. K. and Chauhan, D. K. (2014) Phytoremediation potential and nutrient status of *barringtonia acutangula* gaerth. tree seedlings grown under different chromium (Cr VI) treatments. *Biological Trace Element Research* 157: 164-174.
- Kundu, D., Dey, S. and Raychaudhuri, S. S. (2018) Chromium (VI) – induced stress response in the plant *Plantago ovata* Forsk in vitro. *Gene and Environment* 40: 21.
- Lesniewska, B., Gontarska, M. and Godlewska-Żylkiewicz, B. (2017) Selective separation of chromium species from soils by single-step extraction methods: A critical appraisal. *Water, Air, and Soil Pollution* 228: 1-14.
- Lichtenthaler, H. K. (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymology* 148: 350-382.
- Lopez-Luna, J., Silva-Silva, M. J., Martinez-Vargas, S., Mijangos-Ricardez, O. F., Gonzalez-Chavez, M. C., Solis-Dominguez, F. A., Cuevas-Diaz, M. C. (2016) Magnetite nanoparticle (NP) uptake by wheat plants and its effect on cadmium and chromium toxicological behavior. *Science of the Total Environment* 565: 941-950.
- Lukina, A. O., Boutin, C., Rowland, O. and Carpenter, D. J. (2016) Evaluating trivalent chromium toxicity on wild terrestrial and wetland plants. *Chemosphere* 162: 355-364.
- Ma, Q., Cao, X., Ma, J., Tan, X., Xie, Y., Xiao, H. and Wu, L. (2017) Hexavalent chromium stress enhances the uptake of nitrate but reduces the uptake of ammonium and glycine in pak choi (*Brassica chinensis* L.). *Ecotoxicology and Environmental Safety* 139: 384-393.
- Mahdavian, K., Ghaderian, S. M. and Schat, H. (2016) Pb accumulation, Pb tolerance, antioxidants, thiols, and organic acids in metallicolous and non-metallicolous *Peganum harmala* L. under Pb exposure. *Environmental and Experimental Botany* 126: 21-31.
- Mantry, P. and Patra, H. K. (2017) Combined effect of  $\text{Cr}^{+6}$  and chelating agents on growth and Cr bioaccumulation in flood susceptible variety of rice *Oryza sativa* (L.) cv. Swarna. *Annals of Plant Sciences* 6: 1573-1578.
- Markiewicz, B., Komorowicz, I., Sajnog, A., Belter, M. and Baralkiewicz, D. (2015) Chromium and its speciation in water samples by HPLC/ICP-MS – technique establishing metrological traceability: A review since 2000. *Talanta* 132: 814-828.
- Mathur, S., Kalaji, H. M. and Jajoo, A. (2016) Investigation of deleterious effects of chromium phytotoxicity and photosynthesis in wheat plant. *Photosynthetica* 54: 185-192.
- Mattina, M. I., Lannucci-Berger, W., Musante, C., White, J. C. (2003) Concurrent plant uptake of heavy metals and persistent organic pollutants from soil. *Environmental Pollution* 124: 375-378.
- Najeeb, U., Jilani, G., Ali, S., Sarwar, M., Xu, L. and Zhou, W. J. (2011) Insight into cadmium induced physiological and ultra-structural disorders in *Juncus effusus* L. and its remediation through exogenous citric acid. *Journal of Hazardous Materials* 186: 565-574.
- Najeeb, U., Xu, L., Ali, S., Jilani, G., Gong, H. J., Shen, W. Q. and Zhou, W. J. (2009) Citric acid enhances the phytoextraction of manganese and plant growth by alleviating the ultrastructural damages in *Juncus effusus* L. *Journal of Hazardous Materials* 170: 1156-1163.
- Nguyen, K. L., Nguyen, H. A., Richter, O., Pham, M. T. and Nguyen, V. P. (2017) Ecophysiological responses of young mangrove species *Rhizophora apiculata* (Blume) to different chromium contaminated environments. *Science of the Total Environment* 574: 369-380.

- Prasad, M. N. V (2004) Phytoremediation of metals and radionuclides in the environment: the case for natural hyperaccumulators, metal transporters, soil-amending chelators and transgenic plants. In: Heavy Metal Stress in Plants: From Biomolecules to Ecosystems (ed. Prasad, M. N. V.) Pp. 345-391. Springer, Berlin, Germany.
- Prasad, M. N. V., Greger, M. and Landberg, T. (2001) *Acacia nilotica* L. bark removes toxic metals from solution: corroboration from toxicity bioassay using *Salix viminalis* L. in hydroponic system. International Journal of Phytoremediation 3: 289-300.
- Qureshi, F. F., Ashraf, M. A., Rasheed, R., Ali, S., Hussain, I., Ahmed, A. and Iqbal, M. (2020) Organic chelates decrease phytotoxic effects and enhance chromium uptake by regulating chromium-speciation in castor bean (*Ricinus communis* L.). Science of the Total Environment In Press.
- Rascio, N. and Navari-Izzo, F. (2011) Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? Plant Science 180: 169-181.
- Riaz, M., Yasmeen, T., Arif, M. S., Ashraf, M. A., Hussain, Q., Shahzad, S. M., Rizwan, M., Mehmood, M. W., Zia, A., Mian, I. and Fahad, S. (2019) Variations in morphological and physiological traits of wheat regulated by chromium species in long-term tannery effluent irrigated soils. Chemosphere 222: 891-903.
- Rivelli, A. R., Puschenreiter, M. and De Maria, S. (2014) Assessment of cadmium uptake and nutrient content in sunflower plants grown under Cd stress. Plant, Soil and Environment 60: 80-86.
- Rizwan, M., Ali, S., Abbas, F., Adrees, M., Zia-ur-Rehman, M., Farid, M., Gill, R. A. and Ali, B. (2017) Role of organic and inorganic amendments in alleviating heavy metal stress in oil seed crops. 1<sup>st</sup> Ed. Oil Seed Crops: Yield and Adaptations under Environmental Stress 12. John Wiley and Sons, Ltd.
- Rodriguez, E., Santos, C., Azevedo, R., Moutinho-Pereira, J., Correia, C. and Dias, M. C. (2012) Chromium (VI) induces toxicity at different photosynthetic levels in pea. Plant Physiology And Biochemistry 53: 94-100.
- Rout, G. R., Samantaray, S. and Das, P. (2000) Effects of chromium and nickel on germination and growth intolerant and non-tolerant populations of *Echinochloa colona* (L.) Link. Chemosphere 40: 855-859.
- Saleem, M., Asghar, H. N., Khan, M. Y. and Zahir, Z. A. (2015) Gibberellic acid in combination with pressmud enhances the growth of sunflower and stabilizes chromium (VI) contaminated soil. Environmental Science and Pollution Research 22: 10610-10617.
- Sallah-Ud-Din, R., Farid, M., Saeed, R., Ali, S., Rizwan, M., Tauqeer, H. M., Bukhari, S. A. H. (2017) Citric acid enhanced the antioxidant defense system and chromium uptake by *Lemna minor* L. grown in hydroponics under Cr stress. Environmental Science and Pollution Research 24: 17669-17678.
- Shahid, M., Shamshad, S., Rafiq, M., Khalid, S., Bibi, I., Niazi, N. K., Dumat, C., Rashid, M. I. (2017) Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: a review. Chemosphere 178: 513-533.
- Shakoor, M. B., Ali, S., Farid, M., Farooq, M. A., Tauqeer, H. M., Iftikhar, U., Hannan, F., Bharwana, S. A. (2013) Heavy metal pollution, a global problem and its remediation by chemically enhanced phytoremediation: A review. Journal of Biodiversity and Environmental Sciences 3: 12-20.
- Shakoor, M. B., Ali, S., Hameed, A., Farid, M., Hussain, S., Yasmeen, T., Najeeb, U., Bharwana, S. A. and Abbasi, G. H. (2014) Citric acid improves lead (Pb) phytoextraction in *Brassica napus* L. by mitigating Pb-induced morphological and biochemical damages. Ecotoxicology and Environmental Safety 109: 38-47.
- Shanker, A. K., Cervantes, C., Loza-Tavera, H. and Avudainayagam, S. (2005) Chromium toxicity in plants. Environment International 31: 739-753.
- Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992) Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 40: 945-948.
- Singh, H. P., Mahajan, P., Kaur, S., Batish, D. R. and Kohli, R. K. (2013) Chromium toxicity and tolerance in plants. Environmental Chemistry Letters 11: 229-254.
- Singh, S., Tripathi, D. K., Singh, S., Sharma, S., Dubey, N. K., Chauhan, D. K. and Vaculik, M. (2017) Toxicity of aluminium on various levels of plant cells and organism: a review. Environmental and Experimental Botany 137: 177-193.
- Tauqeer, H. M., Ali, S., Rizwan, M., Ali, Q., Saeed, R., Iftikhar, U., Ahmad, R., Farid, M., Abbasi, G. H. (2016) Phytoremediation of heavy metals by *Alternanthera bettzickiana*: growth and physiological response. Ecotoxicology and Environmental Safety 126: 138-146.
- Tripathi, A., Tripathi, D. K., Chauhan, D. K. and Kumar, N. (2016) Chromium (VI)-induced phytotoxicity in river catchment agriculture: evidence from physiological, biochemical and anatomical alterations in *Cucumis sativus* (L.) used as model species. Journal of Chemical Ecology 32: 12-33.
- Tripathi, D. K., Singh, V. P., Kumar, D. and Chauhan, D. K. (2012) Impact of exogenous silicon addition on chromium uptake, growth, mineral elements, oxidative stress, antioxidant capacity, and leaf and root structures in rice seedlings exposed to hexavalent chromium. Acta Physiologiae Plantarum 34: 279-289.
- Tripathi, D. K., Singh, V. P., Prasad, S. M., Chauhan, D. K. and Dubey, N. K. (2015) Silicon nanoparticles (SiNp) alleviate chromium (VI) phytotoxicity in *Pisum sativum* (L.) seedlings. Plant Physiology And Biochemistry 96: 189-198.

- Wang, D., Guo, W., Zhang, G., Zhou, L., Wang, M., Lu, Y., Cai, D. and Wu, Z. (2017) Remediation of Cr (VI)-contaminated acid soil using a nano composite. *ACS Sustainable Chemistry and Engineering* 5: 2246-2254.
- Wanger, G. J. (1979) Content and vacuole/extra vacuole distribution of neutral sugars, free amino acids, and anthocyanins in protoplasts. *Plant Physiology* 64: 88-93.
- Wiszniewska, A., Hanus-Fajerska, E., Muszynska, E. and Ciarkowska, K. (2016) Natural organic amendments for improved phytoremediation of polluted soils: A review of recent progress. *Pedosphere* 26: 1-12.
- Wuana, R. A. and Okieimen, F. E. (2011) Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. *ISRN Ecology* 2011: 1-20.
- Zaheer, I. E., Ali, S., Rizwan, M., Farid, M., Shakoor, M. B., Gill, R. A., Najeeb, U., Iqbal, N. and Ahmad, R. (2015) Citric acid assisted phytoremediation of copper by *Brassica napus* L. *Ecotoxicology and Environmental Safety* 120: 310-317.
- Zehra, A., Sahito, Z. A., Tong, W., Tang, L., Hamid, Y., Wang, Q., Cao, X., Khan, M. B., Hussain, B., Jatoti, S. A., He, Z. and Yang, X. (2020) Identification of high cadmium accumulating oilseed sunflower (*Helianthus annuus*) cultivars for phytoremediation of an Oxisol and an Inceptisol. *Ecotoxicology and Environmental Safety* 187: 1-11.
- Zhang, H., Guo, Q., Yang, J., Ma, J., Chen, G., Chen, T., Zhu, G., Wang, J., Zhang, G., Wang, X. and Shao, C. (2016) Comparison of chelates for enhancing *Ricinus communis* L. phytoremediation of Cd and Pb contaminated soil. *Ecotoxicology and Environmental Safety* 133: 57-62.