Botanica Pacifica. A journal of plant science and conservation. 2022. 11(2): 89–93
DOI: 10.17581/bp.2022.11216

RESEARCH PAPER

Phytoextraction of Chromium VI by *Raphanus sativus* L. under exogenous application of citric acid

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Manuscript received: 26.04.2022
Review completed: 28.09.2022
Accepted for publication: 15.10.2022
Published online: 17.00.2022

ABSTRACT

The pollution of environment by toxic heavy metals has increased dramatically over the past decades due to various sources of contamination, mainly industrial and human activities. Soil is the main recipient of air and water contamination. In this study, we evaluated the ability of radish plants to accumulate chromium VI (Cr(VI)) (150 ppm) under the application of citric acid (CA) (15 mM). The results showed that Cr(VI) reduced shoot dry weight, root dry weight, hypocotyl dry weight, and total chlorophyll content. On the other hand, Cr(VI) increased H_2O_2, malondialdehyde, and antioxidant activities of the radish plant. However, the application of CA improved the growth and physiological parameters and antioxidant content (superoxide dismutase 5 % and 4 %, peroxidase content 24 % and 4.25 %, catalase 38 % and 28 %, and ascorbate peroxidase 4 % and 35 %) in root and shoot part respectively, and increases the accumulation of Cr(VI) in different parts of the radish plant.

Keywords: heavy metals, chromium VI, citric acid, antioxidants activities, radish

РЕЗЮМЕ

Бухади М., Элькуали М., Талби М., Амегрисси Ф., Фуграх Х. Фитоэкстракция хрома VI с помощью *Raphanus sativus* L. при экзогенном применении лимонной кислоты. За последние десятилетия загрязнение окружающей среды токсичными тяжелыми металлами из различных источников, главным образом, промышленной и человеческой деятельности, резко возросло. Почва является основным поглотителем загрязнений из воздуха и воды. В данном исследовании мы оценили способность растений редиса накапливать хром VI (Cr(VI)) (150 ppm) под действием лимонной кислоты (ЛК) (15 mM). Результаты показали, что Cr(VI) снижал сухой вес побегов, сухой вес корней, сухой вес гипокотиля и общее содержание хлорофилла. С другой стороны, Cr(VI) увеличил H_2O_2, малондиальдегид и антиоксидантную активность растений редиса. Однако применение ЛК улучшало рост и физиологические параметры и содержание антиоксидантов (супероксиддисмутазы 5 % и 4 %, пероксидазы 24 % и 4,25 %, каталазы 38 % и 28 %, аскорбатпероксидазы) 4 % и 35 %) в корнеплодах и побегах соответственно, и увеличивало накопление Cr(VI) в различных частях растения редиса.

Ключевые слова: тяжелые металлы, хром VI, лимонная кислота, антиоксидантная активность, редис

Chromium, which is found in many earth minerals, is an at least partially essential trace element. Its importance or toxicity depends primarily on the oxidation state. Chromium (III), for example, is an important trace element for humans and is important for many metabolic processes, such as glucose metabolism, so a deficiency can quickly lead to diabetes or growth disorders (Lin & Hung 2015). In contrast, Cr(VI), because its diffusibility through skin and cell membranes, is highly carcinogenic and allergenic (Filice et al. 2019). Chromium emissions come not only from anthropogenic sources but also from natural sources, such as volcanic eruptions, various biological processes and soil alteration. However, much of the environmental pollution comes from anthropogenic sources, such as the paper industry, the photographic industry, tape and battery production, and the tanning and electroplating (metal chrome plating) industry (Ismail et al. 2019).

The bioavailability, mobility and solubility of heavy metals in soil are very important. For this reason, organic acids are used and largely accepted because they decrease the risk of metal leaching and are ecologically durable due to their high degree of natural degradation (Wuana & Okieimen 2010). Several organic acids are presently being used to explore their potential to improve heavy metal bioavailability and plant growth, such as citric acid, ascorbic acid, salicylic acid, acetic acid and etc. (Osmolovskaya et al. 2018).

Recent studies show that Citric acid (CA) significantly increases the solubility of heavy metals and their acceptance by plants and may increase plant acceptance of other nutrients (Shakoor et al. 2014).The capacity of plants to absorb heavy metals can be significantly enhanced by the addition of CA, while a higher concentration of CA has intense phytotoxic consequences in certain plant species (Sinhal 2010).

The radish, *Raphanus sativus* L., is an annual or biennial vegetable plant, mainly cultivated for its fleshy hypocotyl, often eaten raw, as a vegetable. All parts of the plant are edible. The radish belongs to the Brassicaceae family, which is characterized by its high bioaccumulative capacity of heavy metals.

The aim of this study was to evaluate the effect of citric acid on the morphological and physiological parameters of *R. sativus* L. plants grown in contaminated soil, and the phyto-accumulation of Cr(VI).

MATERIAL AND METHODS

Treatments

In this study, we used soil artificially polluted with 150 ppm chromium (by the addition of a chromium VI solution), with or without the application of CA (15 mM), for the control we used garden soil. In general, we have four combinations (T1: Control plant, T2: CA (15 mM), T3: Cr(VI) (150 ppm) and T4: CA (15 mM) + Cr(VI) (150 ppm)). Five radish seeds were sown in each pot containing 2 kg of soil.

Plant sampling and analysis

Radish plants were harvested after five weeks of treatment to determine morphological parameters such as root, hypocotyl, leaf biomass, and physiological parameters such as Total chlorophyll content, H_2O_2, malondialdehyde (MDA), and antioxidants content (Superoxide dismutase (SOD), peroxidase content (POD), catalase (CAT) and ascorbate oxidase (APX)).

Chlorophyll content

Extraction was performed under cold conditions, with 4 ml of acetone, using 160 mg of fresh leaf material. After 10 min of centrifugation (5000 g / 4°C), the absorbance was measured at 645 and 662 nm using a double beam UV-6300PC spectrophotometer (VWR, Radnor PA). Pigment contents, expressed in µg.mg-1, were calculated using the following equations of (Lichtenthaler 1987):

chlorophyll a (mg/g) =11.24 × DO662 – 2.04× DO645;
chlorophyll b (mg/g) = 20.13 ×DO645 – 4.19×DO662;
total chlorophyll = chl a + chl b

The quantity of these pigments is expressed in mg per g of fresh matter.

Chromium accumulation

The standardized color method of Clesceri et al. (1998) was used to determine the concentration of Cr(VI) which forms a red-violet complex measured by spectrophotometry at 540 nm using a double beam UV-6300PC spectrophotometer (VWR, Radnor PA) with 1.5-diphnylcarbazide (DPC).

Malondialdehyde and Hydrogen peroxide content

The peroxidation of membrane lipids is estimated by determining the MDA content according to the method described by Šavicka & Skute (2010). Similarly, the content of hydrogen peroxide (H_2O_2) in leaves and roots was determined by the colorimetric method described by Jana & Choudhuri (1981).

Antioxidants enzymes

The Superoxide dismutase (SOD) and peroxidase content (POD) were measured according to the procedure described by Zhang (1992). The activity of catalase (CAT) was evaluated according to the protocol of Aebi (1984).The activity of ascorbate oxidase (APX) was calculated according to the method given by Nakano & Asada (1981).

Statistical analysis

The data presented in this study are the results of three replicates. The data were analyzed using SPSS version 24.0. To determine significant variations and pair wise comparison of data, we used ANOVA and Tukey post-hoc test (Khadraji et al. 2020).

RESULTS

Plant biomass

The plant biomass of radish (Fig. 1) was harmfully affected by the Cr(VI) metal. Unlikely the addition of CA (15 mM) enhanced the plant biomass under metal application and without metal application. The Chromium decreases root, hypocotyl and shoot dry weight of the radish plant by 43, 33, and 27 % respectively as compared to control. On other hand, the application of CA increases the root, hypocotyl, and shoots dry weight of radish plant under chromium metal.

Chlorophyll content

The chlorophyll content in leaves of radish was influenced by Cr(VI). The Metal caused a reduction about 34 % as compared to control plant. The application of CA improved the chlorophyll content, the maximum was observed at the control with CA applied plants (Fig. 2).

H_2O_2 and MDA content

In the presence of Cr(VI), CA application reduced MDA and H_2O_2 content in radish roots and leaves, the MDA content were 9 % lower in roots and 7 % lower in leaves, when CA was applied than the plant under Cr(VI) stress and without CA application .Similarly, significant reduction in H_2O_2 content in roots and leaves part was observed by the application of CA under Cr(VI) stress, H_2O_2 content was 4.4 % lower in roots and 4 % lower in leaves (Fig. 3).

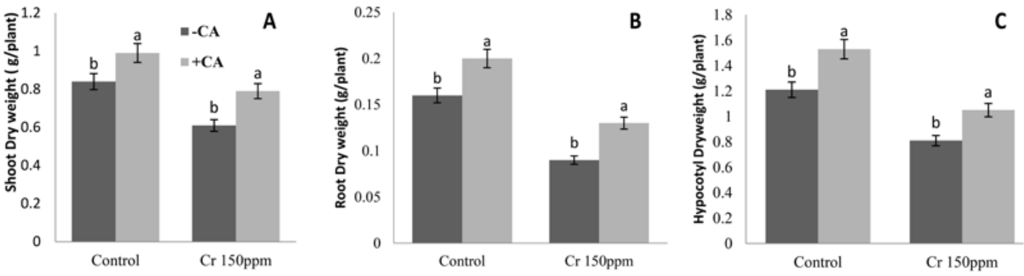

Figure 1 Effect of Cr(VI) on shoot (A), root (B) and hypocotyl (C) dry weight of radish plant grown in soil with or without CA

Antioxidant activities

The activities of antioxidants SOD, POD, CAT, and APX were significantly enhanced under the addition of CA (Fig. 4). The increase in SOD, POD, CAT and APX content under the ad-

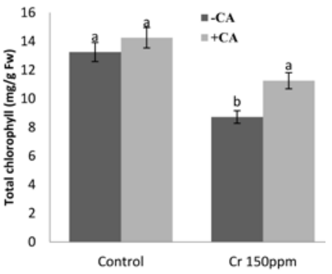

Figure 2 Effect of Cr(VI) on total chlorophyll in leaves of radish plant grown in soil with or without CA

dition of CA in chromium conditions was about 5, 9, 24 and 38 % in root and 4, 4.25, 28 and 35 % in leaves of radish plant, respectively. Generally, antioxidant activities was improved by CA application (under and without Cr(VI) stress).

Chromium accumulation

The CA application increase the accumulation of Cr(VI) in leaves, hypocotyl and root of radish by about 88, 27.57 and 33.91 % respectively, compared to the plant under Cr(VI) only (Table 1).

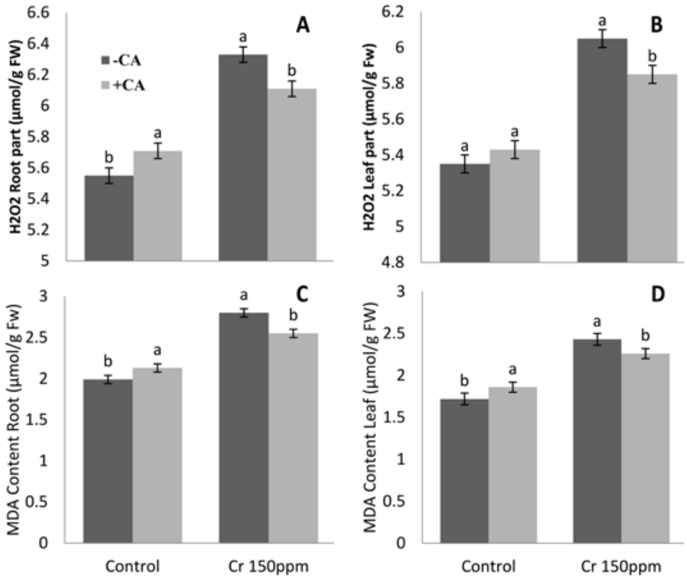

Figure 3 Effect of Cr(VI) and CA on H_2O_2 in root (A), H_2O_2 in leaves (B), MDA in root (C), MDA in leaves (D)of radish plant

Table 1 The effect of CA on chromium VI accumulation in radish plant.

Treatments	Chromium accumulation (mg/g)		
	Leaf	Hypocotyl	Root
Control	0	0	0
CA	0	0	0
Cr	1.51±0.75a	64.69±1.02a	35.74±0.85a
Cr + CA	2.85±0.03b	82.49±1.56b	47.86±0.25b

DISCUSSION

In the present study, the results showed that citric acid (15 mM) improves biomass and chlorophyll content in leaves of radish plant, we observed that plants treated with CA have more biomass and chlorophyll content compared to plants exposed to Cr(VI) without CA treatment. Similarly, Bouhadi et al. (2019) showed that the chromium affected the growth parameters and the chlorophyll content in the leaves of *Vicia faba* L. the results of Mohammed et al. (2021a, b) showed that the high concentration of Cr in soils negatively affects the physiological processes of plants. The same, results are found by Farid et al. (2017) who reported that CA application enhanced the chlorophyll content up to 19 % under Cr contamination. However, Hu et al. (2016) showed that chlorophyll content in leaves of *Lolium arundinaceum* was ameliorated in citric acid treated plants under heat stressed conditions. In addition, foliar application of CA increased photosynthetic parameters compared to treatments without CA. At 2.0 mM CA, total chlorophyll and carotenoid content increased by 50 and 62 %, respectively, compared to the same treatment without CA (Maqbool et al. 2018). Many studies (Afshan et al. 2015, Shahid et al. 2017, Shakoor et al. 2014) have reported that photosynthetic pigments of *Brassica napus* L under the toxicity of Cr and Pb was more significant with CA applied than with control without CA applied.

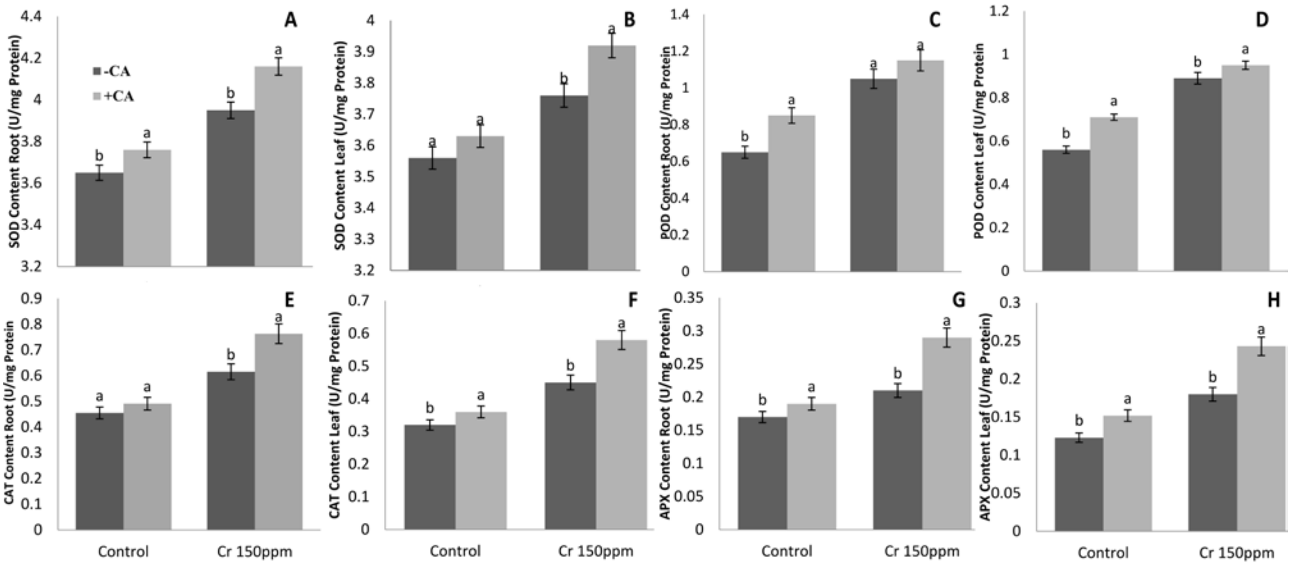

Figure 4 Effect of Cr(VI) on SOD in root (A), SOD in leaves (B), POD in root (C), POD in leaves (D), CAT in root (E), CAT in leaves (F), APX in root (G) and APX in leaves(H)of radish plant grown in soil with or without CA

The exposure of radish plants to chromium increased H_2O_2 and MDA content. However, the application of citric acid 15 mM reduced the H_2O_2 and MDA content in leaves and roots of radish plants under chromium treatment. The increase in MDA and H_2O_2 content in radish roots and leaves is a very strong indicator of lipid peroxidation and destruction of membrane structure. Similar, Farid et al. (2019) reported that exposure of maize plants to contaminated marble effluent increased the production of MDA and H_2O_2. Heavy metal-induced overproduction of ROS has also been previously reported (Anjum et al. 2016, Ashraf et al. 2017). However, the application of CA reduces oxidative stress. Several studies, reported that CA application reduced the excessive production of ROS in various plants under stress conditions (Ehsan et al. 2014, Najeeb et al. 2011).

Our results demonstrated that antioxidant activities increased under the application of chromium (without CA treatment), therefore, the application of CA significantly enhanced the antioxidant activities such as SOD, POD, CAT and APX in the roots and leaves of radish plants. Previously, many studies showed that antioxidant enzymes present as the main defense barrier against ROS generated under different types of biotic and abiotic stress, these enzymes work in synchrony to eliminate ROS (Choudhury et al. 2013, Shahid et al. 2017).Generally, plants with greater enzymatic activity are more tolerant to different types of stress. However, Farid et al. (2019) reported that antioxidant activities, i.e., APX, CAT, POD and SOD, were significantly improved in the roots and leaves of *Zea mays* L. by the application of CA compared to the non-citric acid-treated plant. In addition, the results of Maqbool et al. (2018) showed that foliar application of CA enhanced SOD, POD and CAT activities more than the respective treatments without CA, at 2.0 mM CA, CAT activity increased by 24 and 39 %, SOD activity increased by 20 and 12 %, POD activity increased by 79 and 24 %, in wastewater treatments at 50 and 100 % than the same wastewater treatments without CA.

Concerning chromium accumulation in different parts of radish plant, the results show that the application of CA enhanced the chromium accumulation in leaves, hypocotyl and roots by 88, 27.57 and 33.91 % respectively, compared to the plant exposed to chromium without treatment with CA. Farid et al. (2019) noted that CA application increase the accumulation of heavy metals in *Zea mays* L. plant. However, citric acid was the most effective chelating agent in increasing the concentration of Cu, Cr, and Pb in root and in the aerial part of chicory and castor bean (Bursztyn Fuentes et al. 2018). Unlike, Maqbool et al. (2018) reported that the foliar application of citric acid reduced Cr concentrations in plant (*Spinacia oleracea*) compared to the respective treatments without CA.

CONCLUSION

The results of this study show that the application of citric acid improved radish growth and stimulated the production of antioxidant enzymes, which can help the plant protect itself against ROS produced under the presence of chromium VI. Concerning the accumulation of chromium under the application of CA we have noted that CA increases the assimilation of CrVI, therefore CA has increased the solubilization of Cr(VI) in the soil and makes it more assimilable by the plant. Results confirm that CA can be successfully used in combination with the phytoremediation process for the depollution of soils contaminated by heavy metals.

LITERATURE CITED

Aebi, H., 1984. Catalase *in vitro* methods. *Enzymology* 105: 121–126.

Afshan, S., S. Ali, S.A. Bharwana, M. Rizwan, M. Farid, F. Abbas, M. Ibrahim, M.A. Mehmood & G.H. Abbasi 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(15):11679–11689.

Anjum, S.A., U. Ashraf, I. Khan, M.F. Saleem & L.C. Wang 2016. Chromium toxicity induced alterations in growth, photosynthesis, gas exchange attributes and yield formation in maize. *Pakistan Journal of Agricultural Sciences* 53: 751–757.

Ashraf, U., A.S. Kanu, Q. Deng, Z. Mo, S. Pan, H. Tian & X. Tang 2017. Lead (Pb) toxicity; physio-biochemical mechanisms, grain yield, quality, and Pb distribution proportions in scented rice. *Frontiers in Plant Science* 8:259.

Bouhadi, M., A. Ainane, M. Elkouali, M. Talbi, O. Cherifi, A. El Yaacoubi & T. Ainane 2019. Role of the macroalgae *Corallina officinalis* in alleviating the toxicity of hexavalent chromium on *Vicia faba* L. *Journal of Analytical Sciences and Applied Biotechnology* 1(2):60–64.

Bursztyn Fuentes, A.L., C. José, A. de los Ríos, L.I. do Carmo, A.F. de Iorio & A.E. Rendina 2018. Phytoextraction of heavy metals from a multiply contaminated dredged sediment by chicory (*Cichorium intybus* L.) and castor bean (*Ricinus communis* L.) enhanced with EDTA, NTA, and citric acid application. *International Journal of Phytoremediation* 20(13):1354–1361.

Choudhury, S., P. Panda, L. Sahoo & S.K. Panda 2013. Reactive oxygen species signaling in plants under abiotic stress. *Plant Signaling & Behavior* 8:23681.

Clesceri, L.S., A.E. Greenberg & A.D. Eaton 1998. *Standard methods for the examination of water and wastewater*, A.P.H. Association, Washington, 366 pp.

Ehsan, S., S. Ali, S. Noureen, K. Mahmood, M. Farid, W. Ishaque, M.B. Shakoor & M. Rizwan 2014. Citric acid assisted phytoremediation of cadmium by *Brassica napus* L. *Ecotoxicology and Environmental Safety* 106:164–172.

Farid, M., S. Ali, M. Rizwan, Q. Ali, F. Abbas, S.A.H. Bukhari & L. Wu 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., S. Farid, M. Zubair, M. Rizwan, H.K. Ishaq, S. Ali, U. Ashraf, H.A.S. Alhaithloul, S. Gowayed, F.P. Filice, J.D. Henderson, M.S. Li & Z. Ding 2019. Correlating live cell viability with membrane permeability disruption induced by trivalent chromium. *ACS Omega* 4(1):2142–2151.

Hu, L., Z. Zhang, Z. Xiang & Z. Yang 2016. Exogenous application of citric acid ameliorates the adverse effect of heat stress in tall fescue (*Lolium arundinaceum*). *Frontiers in Plant Science* 7:179.

Ismail, M., M.I. Khan, K. Akhtar, J. Seo, M.A. Khan, A.M. Asiri & S.B. Khan 2019. Phytosynthesis of silver nanoparticles; naked eye cellulose filter paper dual mechanism sensor for mercury ions and ammonia in aqueous solution. *Journal of Materials Science: Materials in Electronics* 30(8):7367–7383.

Jana, S. & M. Choudhuri 1981. Glycolate metabolism of three submerged aquaticangiosperms during aging. *Aquatic Botany* 12:345–354.

Khadraji, A, M. Bouhadi & C. Ghoulam 2020. Effect of soil available phosphorus levels on chickpea (*Cicer arietinum* L.) – rhizobia symbiotic association. *Legume Research* 43(6):878–883.

Lichtenthaler, H.K. 1987. Chlorophyll sand carotenoids: pigments of photosynthetic bio membranes. *Methods in Enzymology* 148:350–382.

Lin, C.-C. & Y.-L. Huang 2015. Chromium, zinc and magnesium status in type 1 diabetes. *Current Opinion in Clinical Nutrition and Metabolic Care* 18(6):588–592.

Maqbool, A., S. Ali, M. Rizwan, W. Ishaque, N. Rasool, M.Z. Rehman, A. Bashi, M. Abid & L. Wu 2018. Management of tannery wastewater for improving growth attributes and reducing chromium uptake in spinach through citric acid application. *Environmental Science and Pollution Research* 25(11):10848–10856.

Metzner, H., H. Rau & H. Senger 1965. Untersuchungenzur-synchronisierbaketieinzel-nerpigmentmangel-mutation von chlorella. *Planta* 65:186–194.

Mohammed, B., E. M'hammed, T. Mohammed & A. Tarik 2021a Effect of Chromium VI on edible plants and their health risks: case of Radish (*Raphanus sativus* L.). In: *E3S Web of Conferences, vol. 319*. doi:10.1051/e3sconf/202131901109.

Mohammed, B., T. Mohammed, E. M'hammed & A. Tarik 2021b. Physiological and physico-chemical study of the effect of chromium VI on the nutritional quality of maize (*Zea mays* L). *Procedia Computer Science* 191:463–468.

Najeeb, U., G. Jilani, S. Ali, M. Sarwar, L. Xu & W. Zhou 2011. Insights into cadmium induced physiological and ultra-structural disorders in *Juncuseffusus* L. and its remediation through exogenous citric acid. *Journal of Hazardous Materials* 186:565–574.

Nakano, Y. & K. Asada 1981. Hydrogen peroxide scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant & Cell Physiology* 22:867–880.

Osmolovskaya, N., V.V. Dung & L. Kuchaeva 2018. The role of organic acids in heavy metal tolerance in plants. *Biological Communications* 63:9–16.

Savicka, M. & N. Skute 2010. Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.). *Ekologija* 56:26–33.

Shahid, M, S. Shamshad, M. Rafiq, S. Khalid, I. Bibi, N.K. Niazi, C. Dumat & M.I. Rashid 2017. Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: a review. *Chemosphere* 178:513–533.

Shakoor, M.B., S. Ali, A. Hameed, M. Farid, S. Hussain, T. Yasmeen, U. Najeeb, S.A. Bharwana & G.H. Abbasi 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.

Sinhal, V.S. 2010. EDTA and citric acid mediated phytoextraction of Zn, Cu, Pb and Cd through marigold (*Tagetes erecta*). *Journal of Environmental Biology* 31:255–259.

Soliman, M.H. 2019. Efficacy of *Zea mays* L. for the management of marble effluent contaminated soil under citric acid amendment; morpho-physiological and biochemical response. *Chemosphere* 240:124930.

Wuana, R. & R.F. Okieimen 2010. Removal of heavy metals from a contaminated soilusing organic chelating acids. *International Journal of Environmental Science and Technology* 7:485–496.

Zhang, X.Z. 1992. The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. In: *Research Methodology of Crop Physiology* (X.Z. Zhang, ed.), pp. 208–211. Beijing Agric Press, Beijing.