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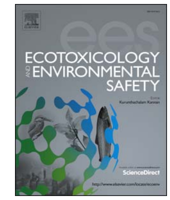

# Citric acid assisted phytoextraction of chromium by sunflower; morpho-physiological and biochemical alterations in plants

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ABSTRACT

Soil and water contamination from heavy metals and metalloids is one of the most discussed and burning global issues due to its potential to cause the scarcity of healthy food and safe water. The scientific community is proposing a range of lab and field based physical, chemical and biological solutions to remedy metals and metalloids contaminated soils and water. The present study finds out a possibility of Chromium (Cr) extraction by sunflower from spiked soil under chelating role of citric acid (CA). The sunflower plants were grown under different concentrations of Cr (0, 5, 10 & 20 mg $kg^{-1}$) and CA (0, 2.5 & 5 mM). Growth, biomass, gas exchange, photosynthesis, electrolyte leakage (EL), reactive oxygen species (ROS; malondialdehyde (MDA), hydrogen peroxide ($H_2O_2$) and the activities of antioxidant enzymes such as, superoxide dismutase (SOD), guaiacole values peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT) were measured. The results depicted a clear decline in plant height, root length, leaf area, number of leaves and flowers per plant along with fresh and dry biomass of all parts of plant with increasing concentration of Cr in soil. Similar reduction was observed in chlorophyll a and b, total chlorophyll, carotenoids, soluble protein, gas exchange attributes and SPAD. The increasing concentration of Cr also enhanced the Cr uptake and accumulation in plant roots, stem and leaves along with the production of ROS and EL. The activities of antioxidant enzymes increased with increasing Cr concentration from 0 to 10 mg, but decreased at 20 mg $kg^{-1}$ soil. The CA application significantly alleviated Cr-induced inhibition of plant growth, biomass, photosynthesis, gas exchange, soluble proteins and SPAD value. Presence of CA also enhanced the activities of all antioxidant enzymes and reduced the production of ROS and EL. The chelating potential of CA increased the concentration and accumulation of Cr in plant roots, stem and leaves. It is concluded that the sunflower can be a potential candidate for the remediation of Cr under CA treatment, while the possibility may vary with genotype, Cr level and CA concentration.

## 1. Introduction

Emerging issue of heavy metal accumulation in edible parts of plants is posing a severe threat to humans, animals as well as plants (Wang et al., 2017; Anjum et al., 2016). Plants readily uptake these metals from soil and water along with essential nutrients (Rizwan et al., 2017a; Jabeen et al., 2016) and translocate them to the above ground parts (López-Luna et al., 2016; Lukina et al., 2016; Anwar et al., 2016), giving a major route of entry to food chain (Anjum et al., 2015; Kamran et al., 2016). However, in trace amounts, few heavy metals like zinc (Zn), iron (Fe), copper (Cu) and cobalt (Co) are essential for proper growth and development of plants but, their higher levels can possess many toxic effects, leading to reduction in yield and quality of produce (Gill et al., 2016a, 2016b). Numerous recent studies describe the toxic effects of these heavy metals on plant growth, biomass, antioxidant defense systems and photosynthesis (See for example Anjum et al., 2017; Singh et al., 2017).

Chromium is one of the most toxic, non-essential carcinogenic heavy metals (Atta et al., 2013; Mantry and Patra, 2017). Extensive use of Cr in industrial processes such as, metallurgy, refractory materials,

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tanning and chemical manufacturing is the major cause of its release into the terrestrial and aquatic environment where it can exert deleterious ecological effects (Tripathi et al., 2016). Generally, Cr in Earth's crust is present in the range of 0.1–0.3 mg $kg^{-1}$. However, different soils showed variable background concentration of Cr, mostly ranging from 15 to 100 µg $g^{-1}$ (Shahid et al., 2017). Chromium is a transition element and can be found in six different oxidation states (Ahemad, 2015; da Costa et al., 2016). In surface waters, Cr mainly occurs in two redox forms i.e. Cr(VI) and Cr(III), With different bioavailability, mobility and toxicity level (Aharchaou et al., 2017; Afshan et al., 2015). Cr III is relatively immobile and sparingly soluble with limited ecotoxicological concerns, as compared to highly bioavailable, soluble and mobile Cr(VI) (Dhal et al., 2013; Farid et al., 2017; Markiewicz et al., 2015). Elevated oxidation potential of Cr(VI) makes it more toxic and capable of penetrating to biological system (Ramírez-Díaz et al., 2008; Sallah-Ud-Din et al., 2017). Higher Cr accumulation may cause delay in seed germination and reduce overall plant growth (Rout et al., 2000; Tripathi et al., 2015). Furthermore, the ions of Cr directly affect the biosynthesis of chlorophylls and photosynthetic pigments which result in reduced gas exchange activities and carbon assimilation (Mathur et al., 2016; Prasad, 2004). Ali et al. (2015a) and Islam et al. (2016) reported Cr as highly toxic metal that reduced the plants' photosynthesis, enzymatic processes and disturbed electron transport mechanism. Many recent studies also documented the inhibitory effects of Cr on plants' chlorophylls (Chl a, Chl b), carotenoids content (Rodriguez et al., 2012) and ultra-structural modifications (Bukhari et al., 2016). Chromium-induced oxidative stress has the ability to disturb biochemical functions of plants which may result in altered morphology and architecture (Nguyen et al., 2017; Ma et al., 2017).

For bioremediation of Cr contaminated soil, the phytoextraction technique is very useful (Shakoor et al., 2013). Moreover, the use of hyperaccumulator plants is widely accepted to extract Cr from soil and water for safe disposal (da Conceição Gomes et al., 2016; Handa et al., 2017). Recent studies evaluated many hyperaccumulator species like *Brassica napus* (Afshan et al., 2015), *Brassica juncea* (Gill et al., 2016a, 2016b), *Brassica campestris* L. (Dheri et al., 2007), *Helianthus annuus* (Fozia et al., 2008; Saleem et al., 2015), *Pennisetum purpureum, Panicum antidotale*, *Cucurbita pepo* (Lofty and Mostafa, 2014), *Spinacia oleracea* L. and *Polyalthia longifolia* (Anwar et al., 2011), for the phytoremediation of Cr contaminated soil. Sunflower (*Helianthus annuus* L.) is an important oilseed crop and a major source of edible oil all over the world (Rizwan et al., 2017a). Being a hyperaccumulator plant with ability to grow successfully under heavy metal toxicity and induced oxidative stress (Tassi et al., 2017). Sunflower has been reported to accumulate many heavy metals such as Cr (Fozia et al., 2008), Cd (Junior et al., 2014, 2015), Ni (Ahmad et al., 2011), As (Imran et al., 2013), Zn (Nehnevajova et al., 2012; Hao et al., 2012), Pb (Adesodun et al., 2010) and Cu (Lin et al., 2003). However, the higher concentration and accumulation of Cr may cause changes in biochemical and morpho-physiological attributes that ultimately lead to low productivity and yield (De Maria et al., 2013; Handa et al., 2017). Therefore, there is a need to protect plants from Cr stress to counteract the induced toxicity and oxidative damages caused by accumulation this metal in plants.

Although, phytoextraction is very easy, cost effective and efficient method, but is not recommended at large scale as a sole agent for remediation of Cr because of its low uptake and translocation to above ground biomass (Yıldız and Terzi, 2016). The capability of phytoextraction process can be optimized by the addition of some organic and inorganic chelators which enhance the mobility and availability of Cr by making chelator + Cr soluble complexes (Wiszniewska et al., 2016; Kumar et al., 2014). Recently, the use of organic chelators has been reported widely acceptable because of their low cost and high degradability as compared to expensive and highly leachable synthetic chelators (Habiba et al., 2015). Many studies documented the chelating potential and plant growth promoting role of citric acid (CA) under different heavy metals such as Cr (Afshan et al., 2015), cadmium (Cd) (Ehsan et al., 2014), lead (Pb) (Shakoor et al., 2014), Cu (Zaheer et al., 2015) and arsenic (As) (Farid et al., 2016).

**Table 1**
Properties of soil used for the pot experiment.

| Physicochemical properties | |
|---|---|
| Texture | Clay loam |
| Sand (%) | 24 |
| Silt (%) | 21 |
| Clay (%) | 55 |
| pH (1/2.5 soil to water ratio) | 6.6 |
| $EC_e$ (dS $m^{-1}$) | 2.94 |
| SAR $(mmol^{-1})^{1/2}$ | 6.54 |
| Organic matter (%) | 0.32 |
| Available P (mg $kg^{-1}$) | 2.18 |
| $HCO_3$ (mmol $L^{-1}$) | 3.54 |
| $Cl^-$ (mmol $L^{-1}$) | 2.31 |
| $SO_4^{2-}$ (mmol $L^{-1}$) | 6.60 |
| $Ca^{2+}$ + $Mg^{2+}$ (mmol $L^{-1}$) | 3.7 |
| $Na^{2+}$ (mmol $L^{-1}$) | 3.8 |
| $K^+$ (mmol $L^{-1}$) | 0.07 |
| Available $Cu^{2+}$ (mg $kg^{-1}$) | 0.30 |
| Available $Zn^{2+}$ (mg $kg^{-1}$) | 0.80 |
| Available $Cr^{+6}$ (mg $kg^{-1}$) | 0.18 |

The present study was designed to examine the Cr-induced toxic effects on various morpho-physiological and biochemical functions of sunflower along with ameliorative role of citric acid in stress alleviation and phytoextraction process.

## 2. Materials and methods

### 2.1. Soil treatments and Sunflower plant

Clay loam soil (55% clay, 21% silt, 24% sand) collected at Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan's botanical gardens from the depth of 0–15 cm, was used in the present study. To remove crop residues and debris, soil was dried and passed through a sieve of 2 mm diameter. The physicochemical properties of soil are presented in Table 1. Mature and healthy seeds of Sunflower genotype (Faisalabad Hybrid FH-614) were collected from Oilseeds Research Institute, AARI. After washing with 10% $H_2O_2$ and rinsing with deionized water, the seeds were sown in plastic pots lined with polythene bags each containing 5 kg soil spiked with increasing Cr concentration (0, 5, 10, 20 mg $kg^{-1}$ dry weight) in a wire house. Ten seeds were sown in each pot, followed by thinning after 15 days of germination to maintain 5 plants per pot. The removed plants were carefully crushed into the same pot. Each soil pot was fertilized with 500 mL solution after 15 and 30 days of germination. Fertilizer solution contained 2.14 g $L^{-1}$ K (as $K_2SO_4$), 2.19 g $L^{-1}$ N (as $(NH_2)_2CO$) and 0.5 g $L^{-1}$ P (as $(NH_4)_2HPO_4$).

### 2.2. Treatments

After 6 weeks of germination, juvenile plants were supplied with increasing concentration of CA (0, 2.5 and 5.0 mM). The following 12 treatments were constituted for the present study: $T_1$: Cr (0 mg $kg^{-1}$) + CA (0 mM); $T_2$: Cr (5 mg $kg^{-1}$); $T_3$: Cr (10 mg $kg^{-1}$); $T_4$: Cr (20 mg $kg^{-1}$); $T_5$: CA (2.5 mM); $T_6$: CA (5 mM); $T_7$: Cr (5 mg $kg^{-1}$) + CA (2.5 mM); $T_8$: Cr (10 mg $kg^{-1}$) + CA (2.5 mM); $T_9$: Cr (20 mg $kg^{-1}$) + CA (2.5 mM), $T_{10}$: Cr (5 mg $kg^{-1}$) + CA (5 mM), $T_{11}$: Cr (10 mg $kg^{-1}$) + CA (5 mM), $T_{12}$: Cr (20 mg $kg^{-1}$) + CA (5 mM), treatments. Each treatment was consisted of 3 replicates and the experimental pots were randomly rotated in the wire house. The pots were kept free from weeds throughout the experiment. Soil was spiked with different concentrations of Cr including 0, 5, 10, 20 mg $kg^{-1}$ dry weight. The available background level of $Cr^{+6}$ was found to be 0.18 mg $kg^{-1}$ soil. Citric acid solution, prepared in distilled eater, was