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Full Length Article

Glutathione Alleviates Chromium Stress in *Helianthus annuus* Irrigated with Tannery Wastewater

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Abstract

Heavy metals pollution is a widespread problem in the present era and untreated wastewater being released from tanneries has huge amount of these toxins especially chromium (Cr). Untreated tannery wastewater is being fed to agriculture lands in Pakistan leading to huge safety concerns regarding Cr accumulation in soil, plants and its encounter with human. In this study, sunflower was grown as a test crop to evaluate the effect of glutathione (GSH) to cope with Cr stress. The GSH was applied as foliar application in 3 concentrations (0, 12.5 and 25 mg L^{-1}) along with 5 levels of untreated sewage water (containing 329 mg L^{-1} Cr VI) irrigation at 0, 25, 50, 75 and 100% concentration. Irrigation with untreated waste water severely affected growth (root and shoot dry weights, plant height and root length) and biochemical parameters [antioxidants, amino acid and ascorbic acid contents, malondialdehyde (MDA) and H_2O_2 contents] of sunflower. The foliar application of GSH significantly improved the growth and biochemical parameters of sunflower subjected to Cr stress. Higher application rate of GSH (25 mg L^{-1}) significantly increased the chlorophyll contents and plant growth parameters along with substantial cut in Cr uptake, amino acid contents, MDA and H_2O_2 contents at all levels of wastewater application. In conclusion, the foliar application of GSH counteracted the damaging effects of sewage water contaminated with Cr and improved sunflower growth.

Keywords: Antioxidant enzymes; Biomass; Chromium uptake; Malondialdehyde; H_2O_2

Introduction

Rapid industrialization possesses severe environmental concerns in developing countries. Most of the industries are discharging their untreated effluents into fresh water bodies and/or agriculture lands. Among these industries, tanneries had a mushroom growth and crossed the total number of 650 in Pakistan, generating more than 215036-gallon waste water per day (Qadir *et al.*, 2008). Kasur is one of the main hubs of these tanneries pouring their volumes into agriculture lands and fresh water canals (Hashmi *et al.*, 2017; Shafiq *et al.*, 2017). Acute shortage of fresh water enforces farmers to accept these contaminated effluents into their lands to grow different food stuff (Khalid *et al*., 2017; Rehman *et al*., 2017). These untreated tanneries waste waters contain considerable amounts of heavy metals like, cadmium (Cd) cobalt (Co), nickel (Ni), iron (Fe), lead (Pb), zinc (Zn) and Cr (Shafiq *et al.*, 2017). Chromium is the most relatable to tannery waste due to its use in leather tanning (Mottalib *et al.*, 2015) and 30–40% of Cr salt is drained into wastewater as it remains unused during process of tanning (Adeel *et al.*, 2012). Tannery waste water is loaded with high concentrations of Cr *i.e*., up to 362 mg L^{-1} which is far more than permissible limit *i.e*., 2.0 mg L^{-1} (Ashraf *et al.*, 2018), in our study it contains Cr concentration of 329 mg L^{-1} (Table 2). Chromium exists in two oxidation states in nature, Cr (III) and Cr (VI), but the later one remains more toxic to plants (Farid *et al.*, 2017a; Farid *et al.*, 2018). Chromium (VI) occurs in soil as chromate or dichromate anionic complexes and is class A carcinogen for human and animals hence its accumulation in soil is of major health concern (Kim and Dixon, 2002; Oliveira, 2012).

In Pakistan, sunflower (*Helianthus annuus* L.) is an important edible oil crop which is being cultivated on 82,151 ha and produced 40000 tons of oil every year (P.E.S., 2017–2018). Chromium being a highly toxic heavy metal can accumulate in sunflower in higher concentration and disturb morphological and physiological attributes (Rizwan *et al.*, 2016a, b). Sunflower being hyperaccumulator of Cr VI possess a potential of Cr phytoextraction from soil (Stoikou *et al.*, 2017; Farid *et al.*, 2018) but sunflower oil derived Cr influx into human food chain is of major concern. Plant growth regulators both enzymatic and non-enzymatic can be effectively used to

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mitigate the heavy metals stress. The glutathione (GSH) having molar mass 307.32 g/mol plays vital role in plants suffering from abiotic stresses including heavy metals. It is involved in the synthesis of phytochelatins (PCs) responsible for heavy metal tolerance mechanisms (Mohamed *et al.,* 2012). It is reported that GSH is involved in metal homeostasis and suppressing heavy metal driven oxidative stress (Seth *et al.,* 2012) as it serves very active part in scavenging of reactive oxygen species (ROS) (Asgher *et al.,* 2014; Khan *et al.,* 2016a). Exogenous application of GSH was reported to enhance photosynthetic activities, nutrient uptake and plant growth under Cd (Cao *et al.,* 2015) and Cr stresses (Zeng *et al.,* 2012; Qiu *et al.,* 2013) in rice (*Oryza sativa*) crop. Keeping in view the significant role of GSH as a growth stimulator under heavy metal stress, the present study was planned to evaluate the effect of foliar GSH application on physiological and biochemical attributes of sunflower, irrigated with untreated Cr contaminated tannery waste water.

Materials and Methods

This pot study was conducted in Government College University Faisalabad research area. Seeds of sunflower hybrid Faisalabad Hybrid (FH-614) were taken from Ayub Agriculture Research Institute (AARI), Faisalabad and used as experimental material. Prior to sowing, seeds were sanitized for 15 min in H_2O_2 (3%) and then washed carefully with distilled water 10 times. Experimental pots were filled with exactly 5 kg of sieved soil and 10 healthy seeds of sunflower were sown in each pot and irrigated with planned levels of tannery water. After germination of seeds, five morphologically homogenized seedlings were selected after 10 days of germination for the further experiment while other plants were pulled up and crushed into the same pot. By following Farid *et al.* (2017a), each pot was fertilized with a 500 mL solution containing 2.19 g L^{-1} N (as $(NH_2)_2CO$), 0.5 g L^{-1} P (as $(NH_4)_2HPO_4$) and 2.14 g L^{-1} K (as K_2SO_4) to fulfill standard amount of fertilizers. Pot experiment was planned with 5 levels of tannery waste water (WW) irrigation (0, 25, 50, 75 and 100%) collected from Kasur tannery industrial area and 3 levels of GSH (0, 12 mg L^{-1}, 25 mg L^{-1}) applied as foliar under Completely Randomized Design with 3 replicates. Glutathione (GSH, SIGMA, Germany) was dissolved in deionized water to prepare GSH solutions containing different concentrations for foliar application. Application of GSH was scheduled in 4 splits in 1st, 2nd, 3rd, and 4th week of vegetative growth of sunflower using hand held sprayer. The plants were irrigated with 500 mL waste water containing different concentration, prepared by diluting the raw tannery water and applied to each pot with 7-day interval.

Plant Growth, Agronomic Parameters and Sample Preparation

Soil and collected tannery water used for experiment were analyzed for basic properties and metal (Cr) contents described in Table 1 and 2. After 70 days of germination, crop was harvested and agronomic and growth parameters of plants were recorded. All plants per pot were washed with distilled water followed by air drying under cover and tissues were carefully separated. Then separated tissues were oven dried and grounded for digestion. For Cr determination, plant leaves, stem and roots were separately washed with distilled water and dried in air in lab environment followed by oven drying at 70ºC till constant dry weight was achieved.

Table 1: Soil physico-chemical properties used for the experiment

Texture	Sandy loam
Silt	15%
Sand	67.9%
Clay	17.10%
EC	1.96 dS m^{-1}
pH	7.61
SAR	1.89 (mmol $L^{-1})^{1/2}$
Available P	2.11 mg kg^{-1}
Organic matter	0.59 %
HCO_3^{-1}	2.51 mmol L^{-1}
SO_4^{-2}	11.44 mmol L^{-1}
Cl^-	5.45 mmol L^{-1}
$Ca^{2+} + Mg^{2+}$	13.98 mmol L^{-1}
K^+	0.04 mmol L^{-1}
Na^+	5.23 mmol L^{-1}
Available Zn^{2+}	0.77 mg kg^{-1}
Available Cu^{2+}	0.31 mg kg^{-1}
Available Cr^{6+}	0.16 mg kg^{-1}

Table 2: Characteristics of tannery wastewater used for irrigating plants

Parameters	Values
COD	2897 mg L^{-1}
BOD	876 mg L^{-1}
TOC	969 mg L^{-1}
Oil & grease	11 mg L^{-1}
pH	4.13
EC	91.8 dS m^{-1}
TDS	64968
Cr^{+6}	329 mg L^{-1}
K^+	41 mg L^{-1}
Carbonate	Nil
$Ca^{2+}+Mg^{2+}$	3.1 $mmol_c$ L^{-1}

Pigment Content Assay

For determination of biochemical parameters, plant parts were washed and preserved in liquid nitrogen. Fully extended and upper most leaves were selected for the determination of photosynthetic pigments at the end of 70 days of treatment. For the pigment extraction, a pre weighted fully matured fresh leaf was treated with 85% of acetone by holding the tubes in dark at 4ºC coupled with constant shaking to ensure homogeneity in extraction. The plant extract in tubes was centrifuged wisely at 4000 rpm for approximately 12 min, after that analyzed with the help of spectrophotometer (Halo DB-20/DB-20S) as

recommended by Metzner *et al.* (1965) and followed by Farid *et al.* (2017a) involving following equations (Lichtenthaler, 1987) for calculation of chlorophyll contents.

$$Chlorophyll\ a\ concentrtion\ (\mu L/\ mL) = 10.3\ \times E663 - E644$$

$$Chlorophyll\ b\ concentrtion\ (\mu L/\ mL) = 19.7\ \times E664 - 3.87 \times E643$$

$$Total\ Chlorophyll\ \ (\mu L/\ mL) = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Determination of Cr Concentration

A known mass (0.5 g) of dried plant tissue was carefully placed in the muffle furnace for 6 h at 650ºC till ash formation. After that, the ash of plants was completely dissolved in a solution having HNO_3 (3 mL) and $HClO_4$ (2 mL) and then diluted to 50 mL using distilled water. At last, atomic absorption spectrophotometer was used for the determination of Cr contents in given samples, following Ryan *et al.* (2001) as below:

$$Cr\ Concentration\ in\ tissue\ (mg/kg) = Solution\ Concentration\ (mg/L) \times \frac{Dilution\ soltion\ volum\ (mL)}{Tissue\ weight\ (g)}$$

Determination of Antioxidant Enzymes

Uppermost second, fully extended leaf was selected after 70 days of treatment, for the determination of peroxide dismutase (POD) and superoxide dismutase (SOD) activities. The activities of these antioxidant enzymes were efficaciously determined by using the method recommended by Zhang (1992) followed by Farid *et al.* (2017b). For SOD and POD determination, 1 g plant sample was frozen in liquid nitrogen and grounded with pre-cooled pestle and mortar followed by homogenization in 0.05 *M* phosphate buffer (pH 7.8). Mixture was filtered with four layered muslin cloth and filtrate was centrifuged for 10 min at 4ºC and 12,000×g. Resultant supernatant was used to determine SOD and POD contents following method of Zhang (1992) as followed by Farid *et al.* (2017b).

Evaluation of MDA and H_2O_2 Contents

The MDA (malondialdehyde) content in plant tissue was measured following method proposed by Heath and Packer (1968) with some essential changes mentioned by (Dhindsa *et al.*, 1981; Zhang and Kirham, 1994). For that, 5 mL 0.1% trichloro acetic acid (TCA) was added to 0.25 g of plant tissue to get it homogenized followed by centrifugation at 10,000×g. From this mixture 1 mL aliquot of supernatant was loaded with 4 mL TCA (20%) containing TA (0.5%). Mixture was then further heated at 95ºC for about 30 min followed by instant cooling in ice bath. Whole mixture was centrifuged once more at 10,000×g and known volume was used to measure specific absorption at 600 nm which was deducted from absorbance of supernatant noted at 532 nm. Extinction coefficient of 155 mM^{-1} cm^{-1} was used to determine the MDA contents.

Method proposed by Jana and Choudhuri (1981) was used to quantify hydrogen peroxide (H_2O_2) contents in plant tissue of test crop for which 50 mg of plant tissue was homogenized with 3 mL phosphate buffer (50 m*M*) having neutral pH of 6.5. Resultant suspension/ homogenate was attentively centrifuged for around 25 min at 6000×g and 3 mL of this derived solution was loaded with 1 mL of titanium sulphate (20% v/v, in H_2SO_4). Entire mixture was yet again centrifuged at 6000×g for around 15 min, then known volume of supernatant was loaded into spectrophotometer and absorbance was noted on 410 nm. Resultant absorbance was treated with 0.28 μmol^{-1} cm^{-1} of the extinction coefficient to obtain H_2O_2 contents.

Ascorbic Acid and Amino Acids in Plant

Contents of ascorbic acid in various parts of the plants were determined by following the method described by Mukherjee and Choudhuri (1983) after extraction in TCA. For amino acid content determination 0.5 g fresh leaf sample of plant was homogenized in given 10 mL of potassium phosphate buffer following the methods recommended by Hamilton and Van (1943).

Statistical Analysis

Analysis of variance (ANOVA) under CRD was implemented using Statistix 10.0 software. Tukey's post hoc test was used to determine pair wise comparison of treatments derived data means and level of significance.

Results

Plant Growth and Biomass

The data collected for plant growth and biomass revealed that plant height, root length, number of leaves and leaf area per plant were significantly decreased with the application of wastewater with varying concentrations (Table 3). Waste water application @ 100% has most detrimental impact on all parameters and foliar application of GSH has shown an incremental impact in all sets of plants. Foliar application of GSH in control (non-stressed) as well as in Cr stressed plants improved plant height, root length, number of leaves and leaf area per plant. In all waste water application levels, the foliar application of 25 mg L^{-1} concentration of GSH proved more beneficial compared to 12.5 mg L^{-1} (Table 3). Application of GSH had also shown incremental impact on plant biomass production in stressed and non-stressed plants which proved toxicity reversal and beneficial response of GSH under Cr stress.

Photosynthetic Pigments, Ascorbic Acid and Amino Acids

Data analysis showed that application of untreated tannery waste water significantly reduced chlorophyll (a and b)

Table 3: Impact of GSH application levels (0, 12.5 and 25 mg/L) on sunflower growth parameters under different application dilutions of Cr contaminated tannery waste water

Treatments	Tannery Waste Water level (%)					Tannery Waste Water level (%)				
GSH (mg/L)	0	25	50	75	100	0	25	50	75	100
	Plant Height (cm)					Root Length (cm)				
GSH 0	84.16±1.83c	79.82±2.0d	74.00±1.60e	65.12±1.61fg	58.47±1.53i	31.01±1.07c	28.07±1.15de	25.73±1.06fg	22.77±1.03hi	20.06±1.00j
GSH 12.5	89.63±1.99b	84.35±1.92c	78.73±1.69d	68.27±2.46f	61.25±2.38hi	33.36±1.11b	31.40±1.06c	26.95±1.15ef	25.04±1.05g	22.35±1.00i
GSG 25	94.71±1.47a	89.04±1.69b	83.26±1.72c	73.29±1.84e	62.70±2.74gh	35.68±1.14a	33.46±1.12b	29.00±1.15d	27.17±1.00def	24.28±1.08gh
	Number of Leaves (average per plant)					Leaf Area (cm^2)				
GSH 0	22.25±0.90de	22.06±0.95de	21.17±0.80e	19.72±0.70f	17.68±0.76g	159.82±3.43c	140.03±3.76ef	131.13±2.95ghi	120.10±3.64kl	117.43±3.36l
GSH 12.5	24.29±0.88bc	23.96±0.72bc	22.76±0.79cd	21.34±0.79e	19.59±0.67f	168.17±3.66b	148.23±4.51d	137.17±3.55fg	124.80±3.61jk	123.43±3.25jkl
GSG 25	26.27±0.83a	25.64±0.88ab	23.86±0.90bcd	22.72±0.91cde	21.28±0.85fef	179.87±5.39a	158.57±4.20c	144.23±3.01de	132.27±3.12gh	129.20±3.91hij
	Leaf Dry Weight (g)					Root Dry Weight (g)				
GSH 0	8.32±0.29d	7.99±0.32d	4.70±0.22ghi	4.43±0.30hi	3.26±0.31j	11.10±0.40c	9.61±0.36d	9.02±0.48d	8.32±0.38e	6.01±0.44f
GSH 12.5	8.97±0.28c	9.23±0.37c	5.21±0.27fg	5.30±0.30f	4.19±0.20i	12.36±0.46b	10.06±0.40d	11.31±0.54c	8.95±0.34d	6.66±0.31ef
GSG 25	9.84±0.38b	10.52±0.37a	5.29±0.32f	6.42±0.35e	4.91±0.33fgh	13.50±0.51a	11.04±0.52c	10.96±0.48cd	9.95±0.44cd	6.97±0.28ef
	Stem Dry Weight (g)									
GSH 0	0.78±0.02bc	0.80±0.03abc	0.70±0.03bcd	0.73±0.03bcd	0.53±0.02ef					
GSH 12.5	0.83±0.03abc	0.90±0.04b	0.80±0.04bc	0.77±0.02bcd	0.54±0.03ef					
GSG 25	0.90±0.04b	0.93±0.02a	0.90±0.02b	0.87±0.03bc	0.60±0.03g					

Values represented in this table are mean value of three replicates (± standard deviation) while small letters represent pair wise comparison and significant difference among different treatment combinations at $P < 0.05$

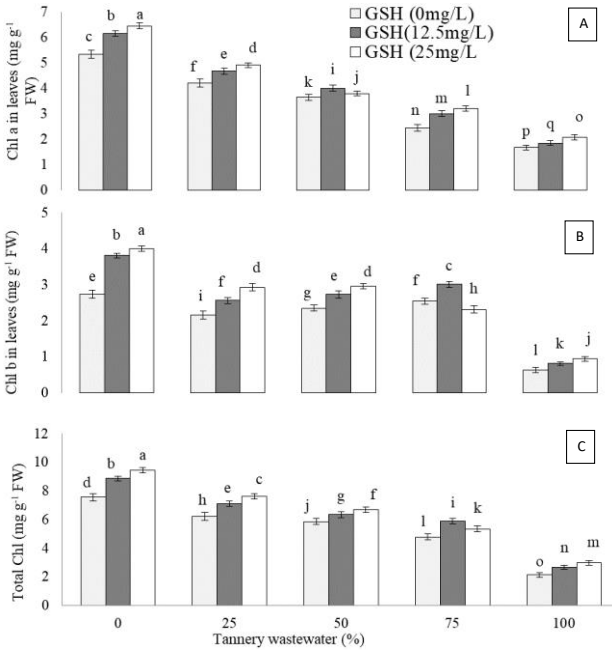

Fig. 1: Interactive effect of wastewater and GSH application on chlorophyll a (**A**), chlorophyll b (**B**) and total chlorophyll (**C**) contents of sunflower. Means with different letters differ significantly at 5% probability level

contents in sunflower. Waste water application at 100% proved most deleterious among all application levels for chlorophyll contents compared to control (Fig. 1). Upon interaction with waste water application, GSH foliar application at 25 mg L^{-1} proved most significant in increasing chlorophyll contents in sewage water treated (stressed) plants. Ascorbic acid and total amino acids contents in sunflower leaves and roots were also significantly varied with application of tannery waste water compared to control (non-stressed) plants. Ascorbic acid contents in Sunflower leaves and roots were minimum in 100% waste irrigated 0 mg L^{-1} GSH applied plants. While maximum ascorbic acid contents were observed in 12.5 mg L^{-1} GSH applied control (non-stressed) plants. In sunflower leaves, minimum ascorbic acid contents were observed in 100% waste water irrigated plants, while upon interaction, 12.5 mg L^{-1} application of GSH more significantly increased ascorbic acid contents than 25 mg L^{-1} GSH application in stressed and non-stressed (control) plants. Similar trend was observed in sunflower roots.

In plant leaves, Minimum amino acid contents were observed in sets where 100% waste water was applied, among which 25 mg L^{-1} GSH foliar sprayed plants showed minimum Amino acids contents followed by 12.5 mg L^{-1} and 0 mg L^{-1} GSH applied plants. Amino acid contents in roots showed varying trend with minimum concentration in control applied with 25 mg L^{-1} GSH. Maximum root amino acid contents were observed in 50 and 100% waste water irrigated 0 mg L^{-1} GSH sprayed plants (Fig. 2).

Antioxidant Enzymes

Analyzed data showed that SOD and POD activity was severely influenced by waste water stress (Fig. 3). In sunflower leaves, SOD contents were observed to increase in plants irrigated with 25% and 50% waste water compared to control (non-stressed) and application of 12.5 mg L^{-1} GSH has more incremental impact than 25 mg L^{-1}. Minimum SOD contents were observed in 0% waste water irrigated followed by 100% and 75% and GSH application at 25 mg L^{-1} has more incremental impact in these sets. The SOD activity in roots increased at 25% and 50% of wastewater stress followed by 100% and 75% waste water concentrations least being in 75%. The GSH application boosted the SOD activity in the roots of plants that were irrigated with wastewater. Minimum SOD contents were observed in 75% and 100% waste water irrigated plants and GSH application @ 12.5 mg L^{-1} showed more increment in SOD contents in both sets. Maximum root SOD contents

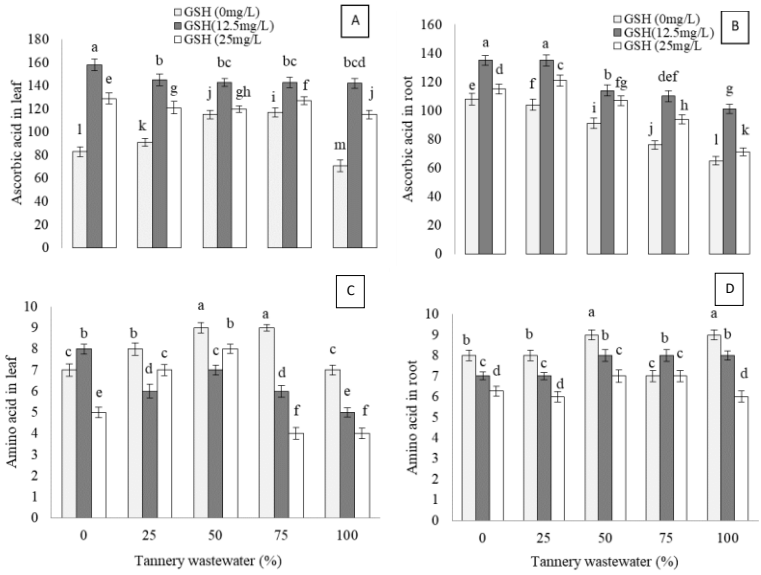

Fig. 2: Interactive effect of wastewater and GSH application on leave ascorbic acid (**A**), root ascorbic acid (**B**), leaves amino acid (**C**) and root amino acid (**D**) of sunflower. Means with different letters differ significantly at 5% probability level

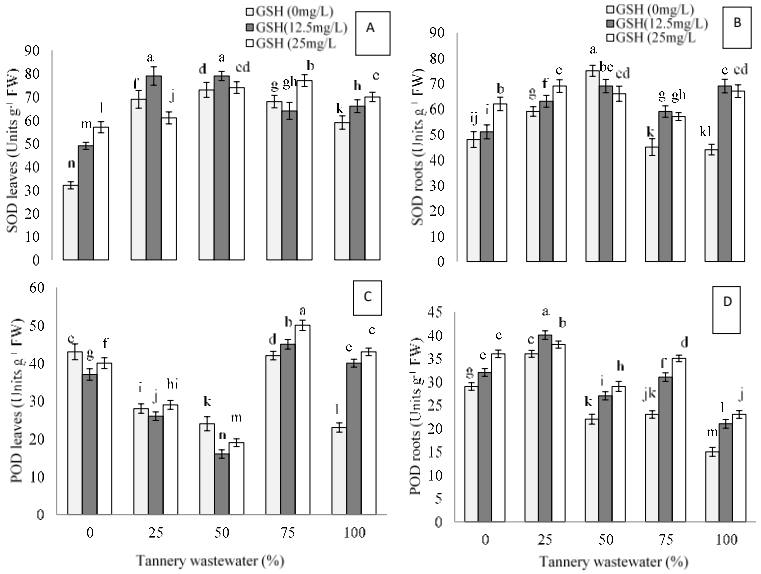

Fig. 3: Interactive effect of wastewater and GSH application on leave SOD (**A**), root SOD (**B**), leaves POD (**C**) and root POD (**D**) of sunflower. Means with different letters differ significantly at 5% probability level

were observed in sunflower plants irrigated with 50% waste water with no GSH application followed by 12.5 and 25 mg L^{-1}. In control and 25% waste water irrigated plants, application of GSH @ 25 mg L^{-1} significantly increased SOD contents compared to 12.5 and 0 mg L^{-1} GSH application.

The leaf POD contents in sunflower plants irrigated with wastewater were observed decreasing upon application of waste water in order of 75%>0%≃100%>25%>50%. The GSH application @ 25 mg L^{-1} proved beneficial for 25, 75 and 100% waste water irrigated sets while GSH 0 mg L^{-1} for control and 50% waste water irrigated plants. Analyzed data showed that waste water irrigation @ 25% increased root POD contents in sunflower compared to control with GSH application @ 12.5 mg L^{-1} being most influential. Higher concentrations of waste water irrigation imposed decline in root POD contents compared to control (in order of 75%>50%>100%) and GSH application @25 mg L^{-1} was more beneficial in these sets of plants (Fig. 3).

Sunflower plants experiencing Cr stress experienced a net increase in H_2O_2 and MDA contents compared to control (non-stressed). Sunflower plants fed with waste water irrigation showed increase in leaf and root H_2O_2 contents and GSH application showed

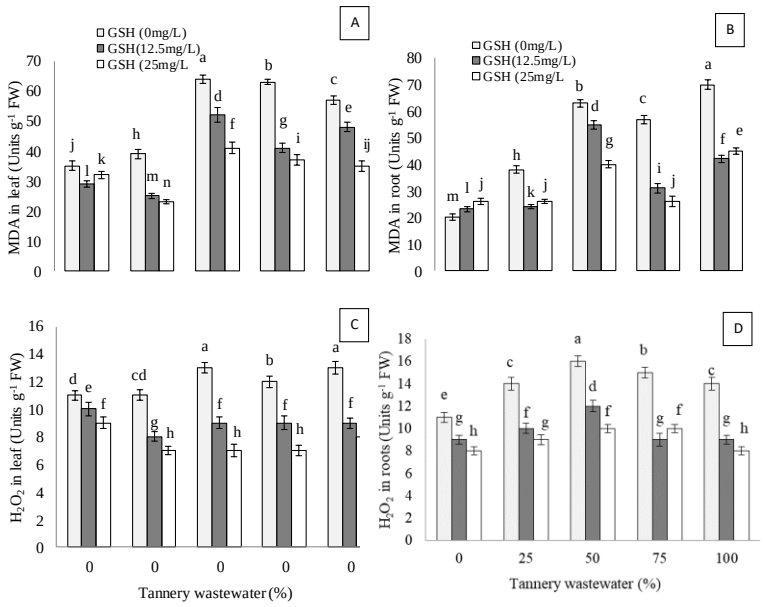

Fig. 4: Interactive effect of wastewater and GSH application on leave MDA (**A**), root MDA (**B**), leaves H_2O_2 (**C**) and root H_2O_2 (**D**) of sunflower. Means with different letters differ significantly at 5% probability level

decrease in leaf and root H_2O_2 contents (Fig. 4). Sunflower leaves and root MDA contents were also increased with waste water application and GSH application also demonstrated reversal impact on tissue MDA contents (Fig. 4).

Chromium Uptake

Data analysis showed that chromium uptake in sunflower tissues was in direct agreement with increasing waste water application with maximum Cr uptake in 100% waste water irrigated plants. Chromium accumulation was more in sunflower roots compared to its stem and leaves in all levels of waste water irrigation. Application of GSH decreased the concentration of Cr in leaves, stem and root of sunflower (Fig. 5). Foliar application of GSH at 12.5 and 25 mg L^{-1} significantly decreased Cr uptake compared to 0 mg L^{-1} and control (non-stressed) plants while 25 mg L^{-1} application being more prominent among two doses.

Discussion

The results of the current study revealed that the application of Cr containing waste water negatively affected the growth and biomass production of sunflower (Table 3). The untreated tannery waste water contains significant amount of Cr (Table 2) which cause toxicities in different plants (Ashraf *et al.*, 2018; Maqbool *et al.*, 2018;). Chromium causes toxicity by means of biochemical and physiological alterations in sunflower resulting in decreased growth and biomass production (Gopal and Khurana, 2011; Kolbas *et al.*, 2014; Farid *et al.*, 2017a; Maqbool *et al.*, 2018). Chromium, in both of its oxidation states distorts chloroplast contents, decreasing its auto fluorescence and volume

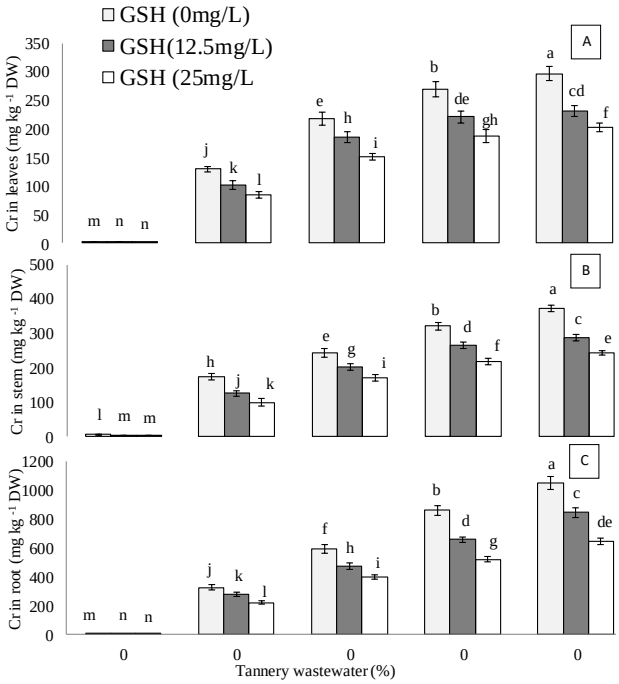

Fig. 5: Interactive effect of wastewater and GSH application on Cr in leaf (**A**), Cr in stem (**B**) and Cr in root (**C**) of sunflower. Means with different letters differ significantly at 5% probability levels

(Rodriguez *et al.*, 2012) leading to decreased photosynthesis and biomass production (Panda and Choudhury, 2005; Saleem *et al.*, 2018). In this study, Cr toxicity had significantly decreased biomass and growth of sunflower, 100% waste water application being the most detrimental (Table 3).

Foliar application of GSH significantly increased chlorophyll contents (Fig. 1) and biomass production of

plants (Table 3) in non-stressed as well as Cr stressed conditions. Photosynthetic pigments perform a very vital part in plants life by harvesting light and are responsible for growth and biomass production in plants. In this study, the Cr toxicity significantly decreased photosynthetic pigments (Fig. 1) which was due to Cr mediated disturbance in photosynthetic machinery (Saleem *et al.,* 2015; Gill *et al.,* 2016; Farid *et al.,* 2017a). Chromium induced ultrastructure distortion in chloroplast (Ali *et al*., 2013; Gill *et al.,* 2016) also causes destruction of photosystems. Glutathione is involved in Ascorbate-Glutathione pathway involved in scavenging ROS, thus play important role in cell organelle protection from ROS (Lou *et al.,* 2018). In our work, improvement in chlorophyll contents by application of GSH is due to minimization of ROS action on photosynthetic apparatus (Cai *et al.,* 2010; Cao *et al.,* 2015). Chromium mediated toxicity to photosystem functioning in plants (Ali *et al*., 2013) can be countered by exogenous application of GSH which protect photosynthetic and biochemical machinery of plants (Qiu *et al*., 2013; Asgher *et al*., 2017; Farid *et al*., 2017a). Foliar application of GSH can boost carotenoids contents and chlorophyll a, chlorophyll b as reported by many studies (Qiu *et al.,* 2013; Asgher *et al.,* 2014; Dixit *et al.,* 2016; Per *et al.,* 2016) and data of our study showed similar trends (Fig. 1).

Amino acids and their derivatives play significant role in decreasing heavy metal toxicity in plants (Sharma and Dietz, 2006) and heavy metal toxicity tend to vary these amino acid concentrations in sunflower. In our work waste water application has a variable impact on amino acid contents in sunflower and GSH application tend to decrease amino acid contents in sewage water irrigated sets with maximum impact of GSH at 25 mg L^{-1} foliar application. For tissue ascorbic acid, GSH application at 12.5 mg L^{-1} proved more effective than 25 mg L^{-1} application and showed an incremental impact on leave and root contents under waste water stress (Fig. 2).

Heavy metal toxicity in plants cause severe oxidative damage in plant tissues, to counter which, plant produces antioxidants (enzymatic like SOD, POD) and non-enzymatic GSH itself. Results of the present study revealed that wastewater stress showed dual impact on SOD and POD, mild stress enhanced the SOD and POD activities and severe stress reduced the activities of SOD and POD (Fig. 3). This is due to nutritional benefits of diluted waste water which become passive at concentrated levels of irrigation making Cr toxicity more prominent. Moreover, delicate Cr stress stimulates production and activity of antioxidants while higher Cr stress disturbs antioxidant enzymes system resulting in decline in enzymatic activity (Gill *et al.,* 2015). Our findings have similar trends in leaf, and root antioxidants (Fig. 3) explaining results of previous works (Meng *et al.,* 2009; Ali *et al.,* 2013; Maqbool *et al.,* 2018). Application of GSH enhanced the activities of both SOD and POD under different wastewater concentrations (Fig. 3) because it acts as a non-enzymatic antioxidant and support activity of enzymatic antioxidants (Anjum *et al.,* 2012; Khan *et al.,* 2016b). Previous investigations (Qiu *et al.,* 2013; Asgher *et al.,* 2014; Khan *et al.,* 2016a; Per *et al.,* 2016) also support our results proving foliar application of GSH a resourceful and effective approach.

Upon experiencing oxidative stress, plant tissue H_2O_2 and MDA contents remarkably increases as an indicator of oxidative stress (Ali *et al.,* 2013; Gill *et al.,* 2015; Maqbool *et al.,* 2018). Our results showed that increasing level of waste water concentration has led to higher MDA and H_2O_2 (Fig. 4) due to severe oxidative damage and membrane disruption (Gill *et al.,* 2015). Application of GSH has supportive role on membrane stability and oxidative damage suppression and mitigation leading to net decrease in MDA and H_2O_2 contents (Fig. 4). Our results are supported by previous findings (Khan *et al.,* 2016a; Per *et al.,* 2016; Asgher *et al.,* 2017). This decline in the MDA and H_2O_2 contents were attributed to the detoxification of Cr by GSH, this detoxification involves vacuolar sequestration and complexation of heavy metal with GSH (Sytar *et al.,* 2013).

The increasing wastewater concentration increased the Cr accumulation in all tissues of plants. The maximum concentration was founded in roots as compared to shoot (Fig. 5) (Ali *et al.,* 2013; Gill *et al.,* 2015; Maqbool *et al.,* 2018). The excessive Cr concentration in roots as compared to aerial parts of the plant is due to compartmentalization of Cr in vacuoles of roots, Cr precipitation in insoluble form of salts and immobilization of Cr by macro-molecule (Kanwal *et al.,* 2014; Ali *et al.,* 2015). Various studies have proved beneficial role of GSH application to decrease detrimental impact of heavy metals on growth of different plant by protecting cellular biochemical machinery (Qiu *et al.,* 2013; Khan *et al.,* 2016a; Asgher *et al.,* 2017; Ding *et al.,* 2017) and similar results were observed in sunflower plants under our investigation. Results indicated that the foliar application of GSH considerably reduced the uptake of Cr (Fig. 5). Decrease in metal accumulation in plant tissue is due to role of GSH in synthesis of phytochelatins (metal binding proteins) which play a vital role in metal stress tolerance (Mohamed *et al.,* 2012). That is might be due to the complexation of heavy metal with GSH (Sytar *et al.,* 2013).

Conclusion

Application of Cr-contaminated waste water decreased growth and biomass production in sunflower along with higher accumulation of Cr in different plant tissues, roots seemed more sensitive. Nonetheless, foliar application of GSH mitigated the adverse effects of Cr stress and improved sunflower growth and biomass due to enhanced activities of antioxidant enzymes and decrease in Cr uptake. Foliar application of GSH seemed a pragmatic option to grow various crops irrigated with tanneries waste water without any treatments.

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References

Adeel, S.S., A. Wajid, S. Hussain, F. Malik, Z. Sami, I.U. Haq, A. Hameed and R.A. Channa, 2012. Recovery of chromium from tannery wastewater by use of *Bacillus subtilis* in Gujranwala. *Pak. J. Pharm. Biol. Sci*., 2: 36–45

Ali, S., M.A. Farooq, T. Yasmeen, S. Hussain, M.S. Arif, F. Abbas, S.A. Bharwana and G. Zhang, 2013. The influence of silicon on barley growth, photosynthesis and ultra-structure under chromium stress. *Environ. Sci. Pollut. Res*., 22: 10601–10609

Ali, S., S.A. Bharwana, M. Rizwan, M. Farid, S. Kanwal, Q. Ali, M. Ibrahim, R.A. Gill and M.D. Khan, 2015. Fulvic acid mediates chromium (Cr) tolerance in wheat (*Triticum aestivum* L.) through lowering of Cr uptake and improved antioxidant defense system. *Ecotoxicol. Environ. Saf.*, 89: 66–72

Anjum, N.A., I. Ahmad and I. Mohmood, 2012. Modulation of glutathione and its related enzymes in plants' responses to toxic metals and metalloids – a review. *Environ. Exp. Bot*., 75: 307–324

Asgher, M., T.S. Per, S. Anjum, M.I.R. Khan, A. Masood, S. Verma and N.A. Khan, 2017. Contribution of Glutathione in Heavy Metal Stress Tolerance in Plants. *In: Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress*, pp: 297–313. Khan, M.I.R. and N.A. Khan (Eds.). Springer, Singapore

Asgher, M., N.A. Khan and M.I.R. Khan, 2014. Ethylene production is associated with alleviation of cadmium-induced oxidative stress by sulfur in mustard types differing in ethylene sensitivity. *Ecotoxicol. Environ. Saf.*, 106: 54–61

Ashraf, S., M. Afzal, K. Rehman, M. Naveed and Z.A. Zahir, 2018. Plant-endophyte synergism in constructed wetlands enhances the remediation of tannery effluent. *Water Sci. Technol.*, 77: 1262–1270

Cai, Y., L. Lin and W. Cheng, 2010. Genotypic dependent effect of exogenous glutathione on Cd-induced changes in cadmium and mineral uptake and accumulation in rice seedlings (*Oryza sativa*). *Plant Soil Environ*., 56: 516–525

Cao, F., Y. Cai and L. Liu, 2015. Differences in photosynthesis, yield and grain cadmium accumulation as affected by exogenous cadmium and glutathione in the two rice genotypes. *Plant Growth Regul*., 75: 715–723

Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Thorpe, 1981. Leaf senescence: correlated with increased level of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot*., 32: 93–101

Ding, S., C. Ma, W. Shi, W. Liu, Y. Lu, Q. Liu and Z.B. Luo, 2017. Exogenous glutathione enhances cadmium accumulation and alleviates its toxicity in *Populus* × *canescens*. *Tree Physiol*., 37: 1697–1712

Dixit, G., A.P. Singh and A. Kumar, 2016. Reduced arsenic accumulation in rice (*Oryza sativa* L.) shoot involves sulfur mediated improved thiol metabolism, antioxidant system and altered arsenic transporters. *Plant Physiol. Biochem*., 99: 86–96

Farid, M., S. Ali, M. Rizwan, Q. Ali, R. Saeed, T. Nasir, G.H. Abbasi, M.I.A. Rehmani, S.T. Ata-Ul-Karim, S.A.H. Bukhari and T. Ahmad, 2018. Phyto-management of chromium contaminated soils through sunflower under exogenously applied 5-aminolevulinic acid. *Ecotoxicol. Environ. Saf*., 151: 255–265

Farid, M., S. Ali, N.A. Akram, M. Rizwan, F. Abbas, S.A.H. Bukhari and R. Saeed, 2017a. Phyto-management of Cr-contaminated soils by sunflower hybrids: physiological and biochemical response and metal extractability under Cr stress. *Sci. Pollut. Res*., 24: 16845–16859

Farid, M., S. Ali, M. Rizwan, Q. Ali, F. Abbas, S.A.H. Bukhari, R. Saeed and L. Wu, 2017b. Citric acid assisted phytoextraction of chromium by sunflower; morpho-physiological and biochemical alterations in plants. *Ecotoxicol. Environ. Saf*., 145: 90–102

Gill, R.A., B. Ali, P. Cui, E. Shen, M.A. Farooq, F. Islam, S. Ali, B. Mao and W. Zhou, 2016. Comparative transcriptome profiling of two *Brassica napus* cultivars under chromium toxicity and its alleviation by reduced glutathione. *BMC Genom.*, 17: 885

Gill, R.A., L. Zang, B. Ali, M.A. Farooq, P. Cui, S. Yang, S. Ali and W. Zhou, 2015. Chromium-induced physio-chemical and ultrastructural changes in four cultivars of *Brassica napus* L. *Chemosphere*, 120: 154–164

Gopal, R. and N. Khurana, 2011. Effect of heavy metal pollutants on sunflower. *Afr. J. Plant Sci*., 5: 531–536

Hamilton, P. and D.D.V. Slyke, 1943. Amino acid determination and metal accumulation by *Brassica juncea* L. *Intl. J. Plant Prod*., 3: 1735–8043

Hashmi, G.J., G. Dastageer, M.S. Sajid, Z. Ali, M.F. Malik and I. Liaqat, 2017. Leather industry and environment: Pakistan scenario. *Intl. J. Appl. Biol. Forensics*, 1: 20–25

Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys*., 125: 189–198

Jana, S. and M.A. Choudhuri, 1981. Glycolate metabolism of three submerged aquatic angiosperms during aging. *Aquat. Bot*., 12: 345–354

Kanwal, U., S. Ali, M.B. Shakoor, M. Farid, S. Hussain, T. Yasmeen, M. Adrees, S.A. Bharwana and F. Abbas, 2014. EDTA ameliorates phytoextraction of lead and plant growth by reducing morphological and biochemical injuries in *Brassica napus* L. under lead stress. *Environ. Sci. Pollut. Res*., 21: 9899–9910

Khalid, S., M. Shahid, C. Dumat, N.K. Niazi, I. Bibi, H.F.S. Gul Bakhat, G. Abbas, B. Murtaza and H.M.R. Javeed, 2017. Influence of groundwater and wastewater irrigation on lead accumulation in soil and vegetables: Implications for health risk assessment and phytoremediation. *Intl. J. Phytorem*., 19: 1037–1046

Khan, M.I.R., N.A. Khan, A. Masood, T.S. Per and M. Asgher, 2016a. Hydrogen peroxide alleviates nickel-inhibited photosynthetic responses through increase in use-efficiency of nitrogen and sulfur, and glutathione production in mustard. *Plant Sci*., 7: 1–20

Khan, N.A., M. Asgher, T.S. Per, A. Masood, M. Fatma and M.I.R. Khan, 2016b. Ethylene potentiates sulfur-mediated reversal of cadmium inhibited photosynthetic responses in mustard. *Front. Plant Sci*., 7: 1–15

Kim, J.G. and J.B. Dixon, 2002. Oxidation and fate of chromium in soils. *Soil Sci. Plant Nutr*., 48: 483–490

Kolbas, A., L. Marchand, R. Herzig, E. Nehnevajova and M. Mench, 2014. Phenotypic seedling responses of a metal-tolerant mutant line of sunflower growing on a Cu-contaminated soil series: potential uses for biomonitoring of Cu exposure and phytoremediation. *Plant Soil*, 376: 377–397

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids pigments of photosynthetic biomembranes, *In: Methods Enzymology*, Vol. 148, pp: 350–382. Colowick, S.P. and N.O. Kaplan (Eds)

Lou, L., X., Li, J. Chen, Y. Li, Y. Tang and J. Lv, 2018. Photosynthetic and ascorbate-glutathione metabolism in the flag leaves as compared to spikes under drought stress of winter wheat (*Triticum aestivum* L.). *PloS One*, 13: 0194625

Maqbool, A., S. Ali, M. Rizwan, W. Ishaque, N. Rasool, M.Z. Rehman, A. Bashir, M. Abid and L. Wu, 2018. Management of tannery wastewater for improving growth attributes and reducing chromium uptake in spinach through citric acid application. *Environ. Sci. Pollut. Res.*, 25: 10848–10856

Meng, H., S. Hua, I.H. Shamsi, G. Jilani, Y. Li and L. Jiang, 2009. Cadmium-induced stress on the seed germination and seedling growth of *Brassica napus* L., and its alleviation through exogenous plant growth regulators. *Plant Growth Regul*., 58: 47–59

Metzner, H., H. Rau and H. Senger, 1965. Untersuchungen zur synchronisierbakeit einzelner pigmentmangel-Mutation von *Chlorella. Planta*, 65: 186–194

Mohamed, A.A., A. Castagna and A. Ranieri, 2012. Cadmium tolerance in *Brassica juncea* roots and shoots is affected by antioxidant status and phytochelatin biosynthesis. *Plant Physiol. Biochem.*, 57: 15–22

Mottalib, M.A., S.H. Somoal, M.S. Islam, M.N. Alam and M.N. Nurul Abser, 2015. Removal of chromium from tannery wastewater by tannery lime liquor; A very cost-effective method. *Intl. J. Curr. Res.*, 7: 16795–16798

Mukherjee, S.P. and M.A. Choudhuri, 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol. Plantarum*, 58: 166–170

Oliveira, H., 2012. Chromium as an environmental pollutant: insights on induced plant toxicity. *J. Bot.*, 2012: 1–8

Pakistan Economy Survey (P.E.S.), 2017–2018. *Finance Division Government of Pakistan*, Chapter 2, pp: 13–32. AgriculturePanda, S.K. and S. Choudhury, 2005. Chromium stress in plants. *Braz. J. Plant Physiol*., 17: 95–102

Per, T.S., S. Khan and M. Asgher, 2016. Photosynthetic and growth responses of two mustard cultivars differing in phytocystatin activity under cadmium stress. *Photosynthetica*, 54: 491–501

Qadir, A., R.N. Malik and S.Z. Husain, 2008. Spatio-temporal variations in water quality of Nullah Aik-tributary of the river Chenab, Pakistan. *Environ. Monit. Assess*., 140: 43–59

Qiu, B., F. Zeng and S. Cai, 2013. Alleviation of chromium toxicity in rice seedlings by applying exogenous glutathione. *J. Plant Physiol*., 170: 772–779

Rehman, M.Z., H. Khalid, F. Akmal, S. Ali, M. Rizwan, M.F. Qayyum, M. Iqbal, M.U. Khalid and M. Azhar, 2017. Effect of limestone, lignite and biochar applied alone and combined on cadmium uptake in wheat and rice under rotation in an effluent irrigated field. *Environ. Pollut*., 227: 560–568

Rizwan, M., S. Ali, M.F. Qayyum, Y.S. Ok, M.Z.S. Rehman, Z. Abbas and F. Hannan, 2016a. Use of maize (*Zea mays* L.) for phytomanagement of Cd-contaminated soils: a critical review. *Environ. Geochem. Health*, 9: 259–277

Rizwan, M., S. Ali, H. Rizvi, J. Rinklebe, D.C. Tsang, E. Meers, Y.S. Ok and W. Ishaque, 2016b. Phytomanagement of heavy metals in contaminated soils using sunflower: a review. *Crit. Rev. Environ. Sci. Technol*., 46: 1498–1528

Rodriguez, E., C. Santos, R. Azevedo, J. Moutinho-Pereira, C. Correia and M.C. Dias, 2012. Chromium (VI) induces toxicity at different photosynthetic levels in pea. *Plant Physiol. Biochem.*, 53: 94–100

Ryan, J., G. Estefan and A. Rashid, 2001. *Soil and Plant Analysis Laboratory Manual, International Centre for Agricultural Research in the Dry Areas (ICARDA)*. Aleppo and National Agricultural Research Centre (NARC), Islamabad, Pakistan

Saleem, M., H.N. Asghar, M.Y. Khan and Z.A. Zahir, 2015. Gibberellic acid in combination with pressmud enhances the growth of sunflower and stabilizes chromium (VI)-contaminated soil. *Environ. Sci. Pollut. Res.*, 22: 10610–10617

Saleem, M.U., H.N. Asghar, Z.A. Zahir and M. Shahid, 2018. Integrated effect of compost and Cr^{6+} reducing bacteria on antioxidant system and plant physiology of alfalfa. *Intl. J. Agric. Biol*., 20: 2745–2752

Seth, C.S., T. Remans, E. Keunen, M. Jozefczak, H. Gielen, K. Opdenakker, N. Weyens, J. Vangronsveld and A. Cuypers, 2012. Phytoextraction of toxic metals: a central role for glutathione. *Plant Cell Environ.*, 35: 334–346

Shafiq, M., T. Shaukat and A. Nazir, 2017. Modeling of Cr contamination in the agricultural lands of three villages near the leather industry in Kasur, Pakistan, using statistical and GIS techniques. *Environ. Monit. Assess*., 189: 423

Sharma, S.S. and K.J. Dietz, 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J. Exp. Bot.*, 57: 711–726

Stoikou, V., V. Andrianos, S. Stasinos, M. Kostakis, S. Attiti, N.S. Thomaidis and I. Zabetakis, 2017. Metal uptake by sunflower (*Helianthus annuus*) irrigated with water polluted with chromium and nickel. *Foods*, 6: 1–14

Sytar, O., A. Kumar and D. Latowski, 2013. Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. *Acta Physiol. Plantarum*, 35: 985–999

Zeng, F., B. Qiu, X. Wu, S. Niu, F. Wu and G. Zhang, 2012. Glutathione-mediated alleviation of chromium toxicity in rice plants. *Biol. Trace Elem. Res*., 148: 255–263

Zhang, J.X. and M.B. Kirham, 1994. Drought stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. *Plant Cell Physiol*., 35: 785–791

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*, pp: 208–211. Zhang, X.Z. (Ed.). Agriculture Press, Beijing, China

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