Effects of Cd and Cr Stress on Physiological and Morphological Traits of Two Cultivars of Wheat (*Triticum aestivum* L.) under Hydroponic System

Faiza Rafique[1], Abdul Nazir[1], Sabaz Ali Khan[2], Arshad Mehmood Abbasi[1], Ahmed Mahmoud Ismail[3,4*], Hossam M. Darrag[5], Ramy Yehia[6], Mohamed J. Hajjar[3]

[1]Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad, Pakistan

[2]Department of Biotechnology, COMSATS University Islamabad, Abbottabad, Pakistan

[3]Department of Arid Land Agriculture, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982

[4]Pests and Plant Diseases Unit, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia

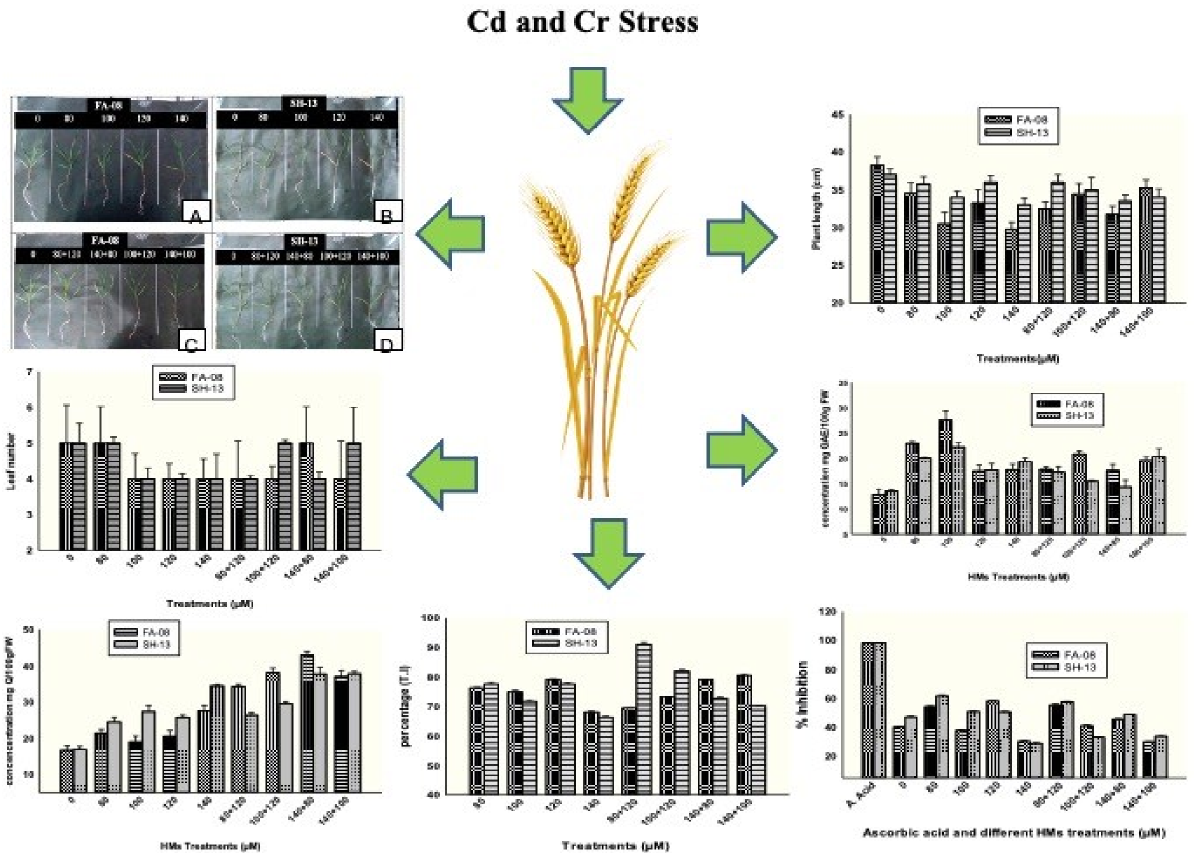
[5]Research and Training Station, King Faisal University King Faisal University, Al-Ahsa 31982, Saudi Arabia

[6]Department of Biological Sciences, College of Science, King Faisal University, Al-Ahsa 31982, Saudi Arabia

*Corresponding author: Ahmed Mahmoud Ismail (amismail@kfu.edu.sa).

Mobile phone: +966550364659.

Graphical Abstract



Abstract

Hydroponic experiment was conducted to evaluate the single and combine effect of different concentration of Cd (80 µM, 100 µM) and Cr (120 µM, 140 µM) on the seedlings of two wheat cultivars viz. FA-08 and SH-13 commonly grown in Hazara division of Khyber Pakhtunkhwa- Pakistan. High dose accumulation of Cd and Cr greatly affected the plant height and leaf number. The presence of Cd reduced the accumulation of Cr, whereas the effect of Cr on the accumulation of Cd depends on the concentrations of Cd used. Different treatments of these HMs (heavy metals) greatly fluctuate the total amount of phytochemicals in leaves of wheat seedlings. The application of Cd and Cr separately and in combination increased the total phenolics in all treatments compared to control groups which were tested on the 18th day of treatment. Highest levels of total phenolics were recorded in FA-08 when Cd is used in a concentration of 80µM. Flavonoid content was high in FA-08 at level Cd+Cr:100+140µM. SH-13 also depicted highest antioxidant activity at level Cd:80µM against all treatments as compared to FA-08. Cd and Cr behaved synergistically because the combined toxicity of Cd and Cr was less than Cd or Cr alone. The current study suggests that both wheat cultivars were tolerant to stress of Cd and Cr up to certain limit and high concentration reduced the contents of phytochemicals, this might cause decrease in the wheat yield.

Key words: Wheat, cadmium, chromium, reactive oxygen species, antioxidants, phenolics, flavonoids.

1. Introduction

The increasing concentration of organic and inorganic pollutants in our environment affects the soil properties and their products (Nagajyoti *et al*., 2010; Ali *et al*., 2013). Plants are more vulnerable to environmental stress because of their sedentary lifestyle than other organisms (Anjum *et al*., 2012). To avoid cellular damage plants, have a complex system of enzymatic and non-enzymatic antioxidants, to tolerate abiotic stress. Antioxidant concentration helps the plant to resist stress and maintain the balance between peroxidant and antioxidant reactions (Maleva *et al.,* 2012; Al Mahmud *et al.,* 2017). Phytochemicals are non-nutritional compounds having antioxidant properties due to the OH group (Koleva *et al*., 2002). Phenolic compounds are one of the stress responses and help the plant to maintain homeostasis, their adverse effect on plants is the generation of harmful active oxygen species, leading to oxidative stress. Phenolic contents in cereals are influenced by types, varieties and grain parts used. Phenolic acids and flavonoids are abundant phenolic contents found in cereals (Žilić *et al*., 2011; Žilić *et al*., 2012). Besides the well-studied antioxidant systems consisting of low-molecular antioxidants and specific enzymes, effective antioxidant flavonoids and phenolic acids play a potential role against stress. During heavy metal stress phenolic compounds can act as metal chelators and on the other hand phenolics can directly scavenge molecular species of active oxygen (Bartwal *et al*., 2013).

Heavy metals (HMs) unlike organic pollutants are non-biodegradable and persistent, enter humans through various routes and affect their health (Wuana & Okieimen, 2011; Adrees *et al*., 2015). Industrial effluents (electroplating, paints, batteries, mining, fertilizers, and pesticides) add cadmium (Cd) to the soil and plants uptake it through their roots and accumulate it in their shoot (Gill *et al*., 2012; Gill & Tuteja, 2011). A high concentration of Cd reduces photosynthetic efficiency in some plants by reducing the availability of Fe (II) and decreases the concentration of oxidative enzymes; superoxide dismutase, catalase, and peroxidase in plants (Mohamed *et al*., 2012). Chromium (Cr) is used in wood preservation, leather tanning, electroplating, and steel production and accumulates more in the plant's roots (Al Mahmud *et al*., 2017). Cr (VI) is known to be carcinogenic and causes several respiratory disorders (Kumar *et al*., 2013; Vajravel & Saravanan, 2013). Heavy metals, especially Cd, Pb, and Cr have no biological role in organisms (Plants, animals) and are toxic to them (Adrees *et al*., 2015). Cr toxicity causes a reduction in the quality of enzymes such as catalases, peroxidases, and reductases (Cervantes *et al*., 2001). The harmful or toxic substances in the environment affect various biochemical processes in living organisms (Hakeem, 2015; Shahid *et al*., 2014). The consumption of contaminated plants by humans causes many fatal diseases including cancer (Gill *et al*., 2012).

Wheat (*Triticum aestivum)* is an important cereal crop and a source of protein for the human population (Hithamani & Srinivasan, 2014). Wheat is nutritionally essential and rich in natural antioxidants and used in making food products to improve the health of consumers and the economy. It is an annual herb used as a staple food and comprises essential vitamins like B_6, B_{12}, A, and E that act as important antioxidants. Whole wheat grains prevent coronary heart diseases and certain cancers due to their antioxidant properties (Liangli, 2008; Shewry, 2009). Pakistan is 6^{th} in wheat production and 8^{th} in the number of the area under cultivation and 59^{th} in terms of yield and total calorie intake by the population of Pakistan is about 50% (Zulfiqar & Hussain, 2014). Being a staple food in Pakistan (5^{th} most populous country with a population exceeding 207.77 million) wheat, an area of 8.66 million hectares, 25.478 million tons of wheat can be produced (Chandio *et al*., 2016; Ali *et al*., 2018). In Pakistan, the high yield of wheat is dependent on irrigation water and the use of fertilizers (Chandio *et al*., 2016), so more use of fertilizers and polluted water may be sources of HMs pollution. The present study aimed to compare the concentration of phenolic acids and flavonoids in two commonly cultivated wheat varieties (FA-08 and SH-13) in the Hazara division and to evaluate the impact of Cd and Cr stress on the concentration of these bioactive compounds.

2. Materials and Methods

2.1. Plant cultivation

Pretreated seeds of two wheat cultivars, Faisalabad -2008 (FA-8) and Shahkar-2013 (SH-13) were purchased from agriculture extension department Abbottabad-Pakistan. Ten grams seeds of each cultivar were sown in loamy sand under controlled environmental temperature (18℃) in the greenhouse. After two weeks of germination, the seedlings were uprooted; their roots were washed with tap water to remove sand particles and transplanted to the hydroponic system placed in the greenhouse.

Hoagland solution was prepared by using the standard recipe mentioned in protocol (Hoagland and Arnon, 1950; Sharma *et al*., 1995; Ghani *et al*., 2015) for wheat crop with little modifications as shown in table1. All the salts were autoclaved, dissolved and pH of the solution was checked regularly which was between 6.7-7.02. Nutrient were supplied daily except on 3rd day, on first day 1/4th concentration of solution, which was increased to half and full concentration on 2nd and 4th day respectively.

Table1. Chemical formulae and concentrations of nutrients used

Chemical Formulae	Concentration (µM)	Chemical Formulae	Concentration (µM)
$Ca(NO_3)_2$	1000	$MnSO_4$	2.0
K_2SO_4	1000	$ZnCl_2$	0.5
$MgSO_4$	600	$CuSO_4$	0.3
KH_2PO_4	200	Na_2MoO_4	0.29
$CaCl_2$	5000	Fe-EDTA	200
H_3BO_3	1.0		

2.2. HMs treatment

In the current study Cadmium (Cd) and Chromium (Cr) concentrations used were 80 µM, 100 µM and 120 µM, 140 µM respectively. Nine different treatments, T_1 without heavy metals as control, Cd 80 µM, 100 µM, Cr 120 µM, Cr 140 µM, Cd + Cr: 80 + 120, Cd + Cr : 100 + 120, Cr + Cd : 140 - 80, Cr + Cd : 140 + 100 were used with complete randomized block design in triplicates. Heavy metals were applied after one month of seedling in an ascending order starting from lower to higher concentration.

2.3. Morphological observations

Plant samples (two seedlings) were randomly selected from each pot at the tillering stage after 15th day of HM treatment. The seedlings were washed with deionized water. A transparent ruler was used to measure the whole length of the plant (shoot + root) in cm and leaf numbers were counted to note the difference between the control and heavy metal treated seedlings. Tolerance index was calculated by using Wilkins, (1957) method.

$$Tolerance\,index\,\% = \frac{Observed\,value\,of\,\,root\,length\,in\,soution\,with\,metal}{Observed\,value\,of\,\,root\,length\,in\,solution\,without\,metal} \times 100$$

2.4. Extraction

For extraction the method described by Venkateswaran & Pari, 2003; Omoloye *et al*., 2007 was followed with little modifications. Three plants from each pot were selected for biochemical study; leaves were collected on 18th day of HM treatment and preserved at -20°C for two weeks. Briefly, 0.5 gram of preserved leaves was crushed in a mortar. The powdered material was shifted to falcon tube containing 10 ml methanol and placed in shaker (36℃) overnight. The next day mixture was centrifuged at 4000rpm for 10 minutes and supernatant was collected. Additional 10 ml of methanol was added to the pellet in falcon, vortexed and placed on a shaker for 1 hour. The solution is centrifuged; supernatant was collected and stored in in refrigerator at4°C until further analysis is done.

2.4.1 Total phenolics contents

Total phenolic contents (TPC) were determined by slightly modified Folin-Ciocalteu method as described earlier (Alves *et al*., 2010; Lin *et al*., 2011). 1.0 mL of leaf extract was added to labelled falcon tube, followed by 1 mL Folin-Ciocalteu reagent (1:15) solution and 2 mL of 6 % (W/V) sodium carbonate solution and left in dark for 90 minutes. Absorbance level was measured by using double beam UV spectrophotometer (Model No. $T80^+$) at 765 nm. The experiment was done in triplicate. The final concentrations of phenolic compounds in extract were expressed as gallic acid equivalents (GAEs).

2.4.2. Total flavonoids contents

Aluminum chloride ($AlCl_3$) method was used to determine the flavonoids content as described by Barroso *et al*., (2011). Briefly, in 1.0 mL of plant extract 0.5mL (5%W/V) of $NaNO_2$ solution was added and left for 5minutes. 0.5mL $AlCl_3$ (10 % W/V) was added followed by 2.0 mL NaOH solution (4 %W/V). The absorbance was measured at 510 nm using UV-Visible spectrophotometer (Model No. $T80^+$), was compared to the quercetin standard and was expressed as mg quercetin equivalents per g of sample.

2.4.3 Antioxidants

DPPH scavenging activity of wheat leaves was determined by method described previously by Aoshima *et al*., 2004 and Yu *et al*., 2002. 4.0 mL of 0.1mM DPPH (in methanol) was mixed with 1.0 mL leaf extract. The solution was then kept in dark at room temperature for 30 minutes. The antioxidant activity was determined by measuring absorbance of the solution at

517nm by spectrophotometer (Model No. T80$^+$). Blank DPPH solution was used as negative control and ascorbic acid was used as positive control and percent inhibition was measured with the following equation:

$$\% \, Inhibition = \frac{(A\,blank - A\,sample)}{A\,blank} \times 100$$

3. Result and Discussion

3.1 Effects of HMs on phenotypic parameters

After 15th day of HM treatment, comparison was made between the control and HMs treated seedlings. The uptake of HMs caused yellowing of the leaves and stunted growth of wheat seedlings. Cd was more toxic as compared to Cr stress and cause stunted growth in wheat plant (Ather and Ahmed, 2002). In the present study, it was found that growth of both cultivars of wheat as compared to control were more pronounced on the seedlings where the metals were applied singly (Cd: 100µM and Cr 140 µM) The length of the root was more affected with HMs as compared to aerial parts. Less number of leaves were counted at high dose of HM treatment (Fig.1). These symptoms on the leaves of wheat cultivars due to uptake of Cr. As both phosphate and sulphate transporters help in the uptake of chromium, hence lead to deficiency of macronutrients (N, K, Mg). The deficiency of these macronutrients showed the typical nutrient deficiency toxicity symptoms (Guarino *et al.,* 2020; da Conceicao Gomes *et al.,* 2017).

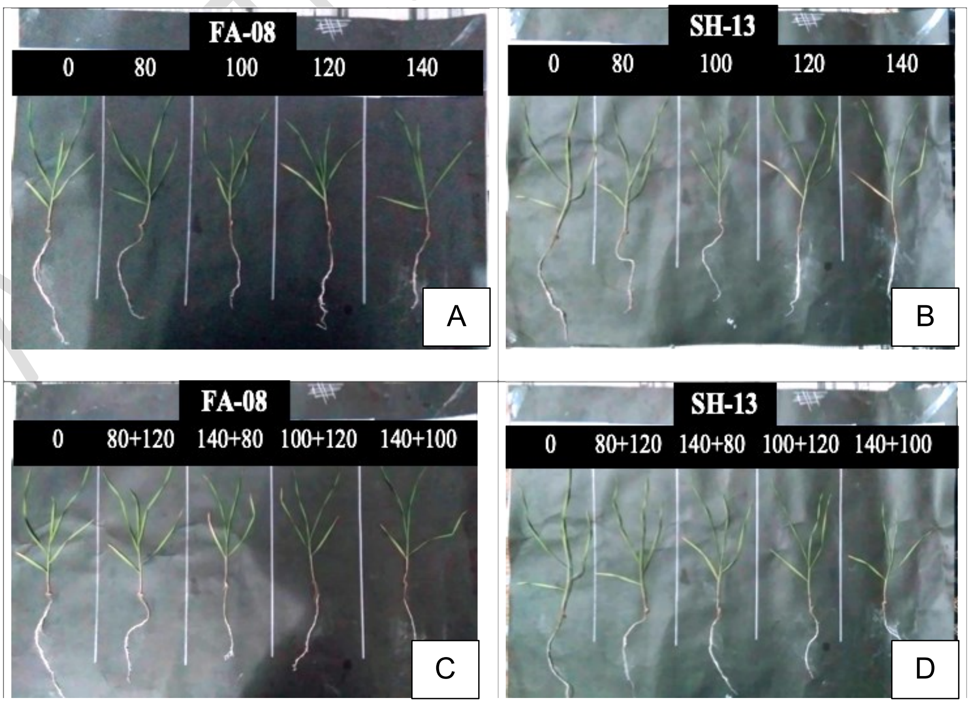


Figure 1. (A). Phenotypic comparisons between control and singly given stress of Cd and Cr of cultivar FA-08, (B). Control and singly given stress of Cd and Cr of SH-13, (C). Control and stress Cd-Cr in combination form of cultivar FA-08, (D). Control and stress Cd-Cr in combination form of cultivar SH-13.

3.1.1 Plant length

Both cultivars of wheat exhibited reduced growth when treated with HM. This reduced growth due to fewer numbers of leaves, stunted growth of root and stem. It was found that the whole length of the plant is significantly decreased with increased concentration of applied heavy metals Cd, and Cr. This effect was more pronounced in cultivar FA-08 as compared to cultivar SH-13. When Cd was applied in100µM concentration, FA-08 significantly decreased in plant length (30.5±1.50 cm) as compared to control (38.25±1.06 cm). This plant length of FA-08 was even decreased (29.75±0.95 cm) as compared with control (38.25±1.06 cm) with an increase level of Cr i.e., 140µM. Similarly, the seedling growth of SH-13 was also retorted (33±0.90) as compared to control (37.0±0.74) with higher concentration of Cr (Cr: 140 µM). At level Cd + Cr: 140 + 100 µM, where high dose of HMs were applied in combination, the cultivar SH-13 showed significant reduction in length as compared to cultivar FA-08 (Fig. 2). Similar growth pattern was reported in *Brassica napus* at high dose of Cd (100 and 500µM) Ali *et al*., 2013. It was found that Cr in combination Cd worked both synergistically and antagonistically depending on the type of plant species and affects the plant growth (Khan *et al*., 2018). In the present study it was observed that Cd reduced the accumulation of Cr. However, when Cr was applied individually, it effected the plant growth significantly
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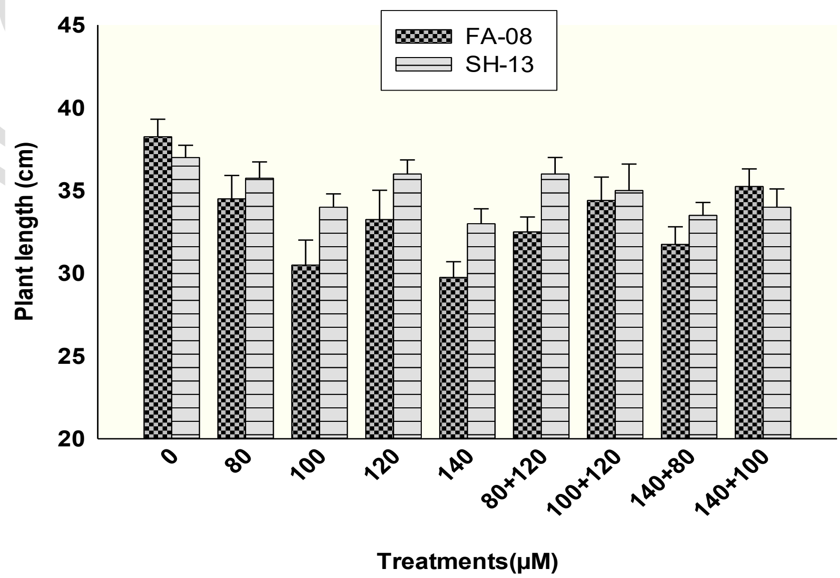

Figure 2. Comparison of plant length between control and heavy metals, Cd $(NO_3)_2$ μM/L (80, 100); $K_2Cr_2O_7$ μM/L (120, 140) stress seedlings and stress of Cd + Cr in combine form to both cultivars; FA-08, SH-13. Data are expressed as the mean ± SD of three replicates. Bars showed the significant difference between the control and treatments by *Tukey*-test at $P < 0.05$.

3.1.2 Leaf number

There was an inverse effect of higher concentrations of Cd and Cr on the number of leaves (from 5 to 4) in both cultivars (FA-08, SH-13). The cultivar SH-13 showed more reduction in leaf number as compared to cultivar FA-08. The number of leaves showed no significant difference between control and stress plants, but phenotypic conditions (phytotoxic symptoms) showed the difference between control and stress plants (Fig. 3). The number of leaves in wheat cultivar at (0.5, 1.0 mM) of Cr levels was less than half of control (Sharma *et al*., 1995). Cd at level 100, 500μM greatly reduced the number of leaves per plant as compared to their control group in *Brassica napus*, (Ali *et al*., 2013).

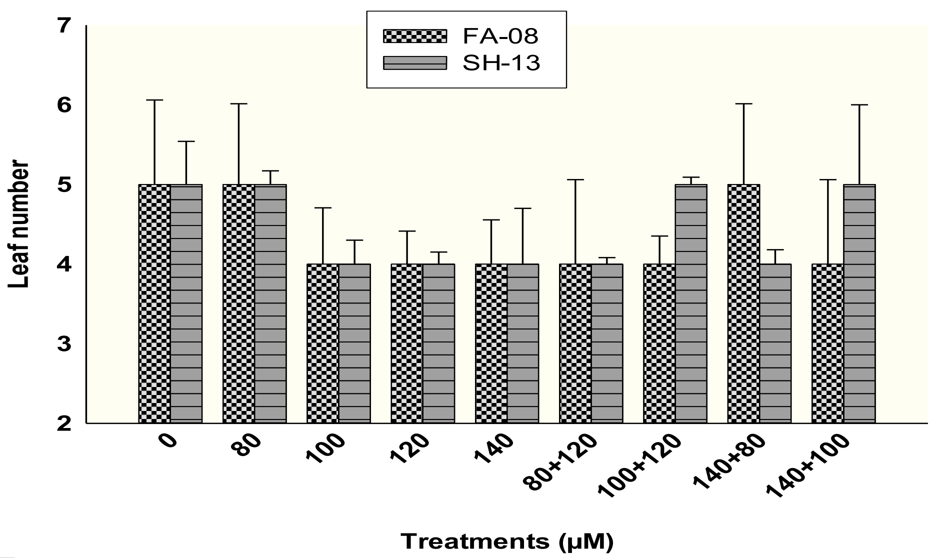

Figure 3. Comparison between control, HMs Cd, Cr stress seedlings and Cd + Cr in combination to both cultivars, FA-08, SH-13. Data are expressed as the mean ± SD of three replicates. Bars showed the significant difference between the control and treatments by *Tukey*-test at $P < 0.05$.

3.1.3 Tolerance index

Tolerance index of root showed that the cultivar FA-08 showed less reduction in length of root after15 days of treatment with toxic HMs as compared to cultivar SH-13. Root length after uptake of metals in cultivar FA-08 at levels Cd:100, Cr:140, where stress is given singly and Cr+Cd:140:80, Cr+Cd:140:100 showed less decreased in length as compared to SH-13. FA-08 at level, Cd+Cr:80+120, Cd+Cr:100+120 showed reduction in root length as compared to cultivar SH-13. At level Cr 140µM both cultivars showed significant difference from the control conditions. From the observations, both cultivars were tolerant to heavy metal because they are not fully dead but show toxicity symptoms, stunted growth and reduction in leaf number and leaf area (Fig. 4). Tolerance index help to know the tolerance of plant against high concentration of metal stress over a long time period (Ghosh & Singh, 2005). Tolerance index was calculated to know the length and biomass of stem, root and leaf, in *Brassica juncea* tolerance index was increased as the concentration of Zn was increased (Jamali *et al*., 2014; Chaudhry *et al*., 2020).

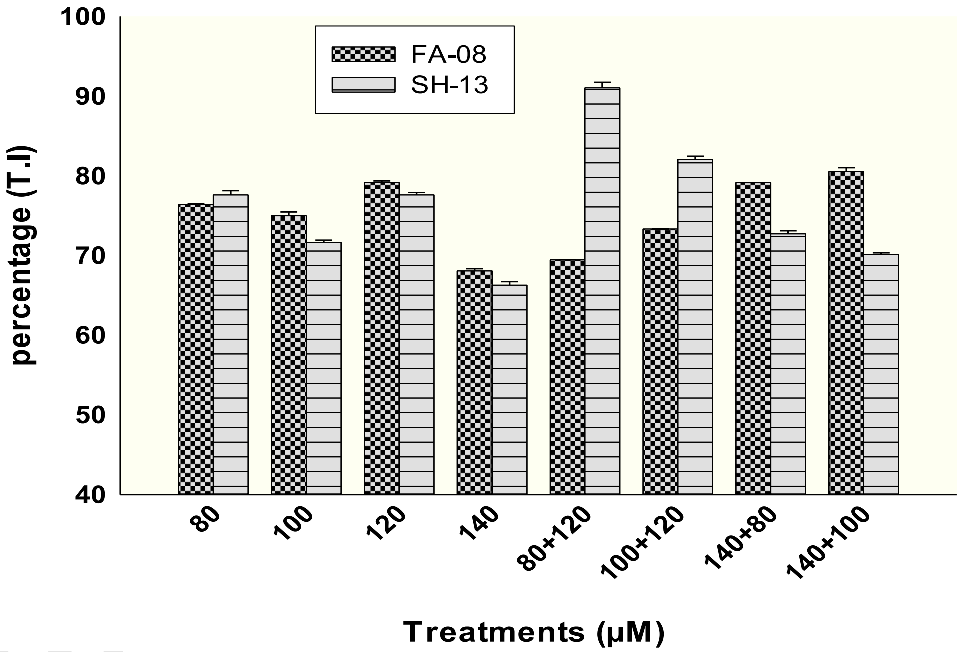

Figure 4. Tolerance index of two cultivars FA-08; SH-13, showed the effect of heavy metals Cd, Cr and tolerance of wheat cultivars by measuring root length at different concentration of metals and control was assumed as zero. Data are expressed as the mean ± SD of three replicates. Bars showed the significant difference between the control and treatments by *Tukey*-test at $P < 0.05$.

3.2. Effect of HMs on total phenolic contents

Wheat is rich source of bio-accessible polyphenols as compared to the other cereals (Hithamani & Srinivasan, 2014). In the present study it was found that the cultivar FA-08 possessed the highest phenolic concentration at Cd:100 (27.57±1.77) followed by Cd:100 (22.23±0.91) in cultivar SH-13. When Cd and Cr were applied in combination with each other, it was found that cultivar FA-08 exhibited the highest concentration of phenolics (20.83±0.62) when Cd and Cr used in a concentration of 100 µM and 120 µM respectively. However, in cultivar SH-13, highest value of phenolics was found (20.33±1.59) when Cr and Cd were used in a concentration of 140 µM and 100 µM respectively. Fig. 5 depicts the total phenolic contents in both cultivars treated with Cd and Cr alone or in combination. The stress of metals in plants is related to the chemical nature of the HMs (Anjum *et al*., 2012). In was found that phenolic contents started to increase in both wheat cultivars with an increase in the concentration of Cd, Cr and their combinations. According to Márquez-García *et al*., 2012 phenolics, flavonoids and antioxidants started to increase, when concentration of Cd was increased from 0, to 50 µg/g. In one other study, phenolic contents tend to increase at 50ppm of Cd in *Zea mays* plant (Kısa *et al*., 2016). A significant difference was noted between control and heavy metal treated wheat seedlings as for as total phenolics are concerned. The different doses of Cd (80 µM and 100 µM) considerably increased the concentration of phenolics in leaves of both cultivars as compared to increased concentrations of Cr (120 µM and 140 µM). Higher synthesis of phenolic contents was observed in wheat in response to metal toxicity. An increase of phenolics correlated to the increase in activity of enzymes involved in phenolic compounds metabolism was reported, under heavy metal stress (Mallick *et al*., 2006). Under control conditions, both cultivars displayed the lowest phenolic contents. the lowest value is noted at level Cr+Cd:140+80 in case of cultivar SH-13 (14.32±1.39) followed by FA-08 (17.52±1.27). HMs affects polyphenol level in *Albizia procera* decreased at 5ppm and increased at 10ppm in case of Cd (Preeti and Tripathi, 2011).

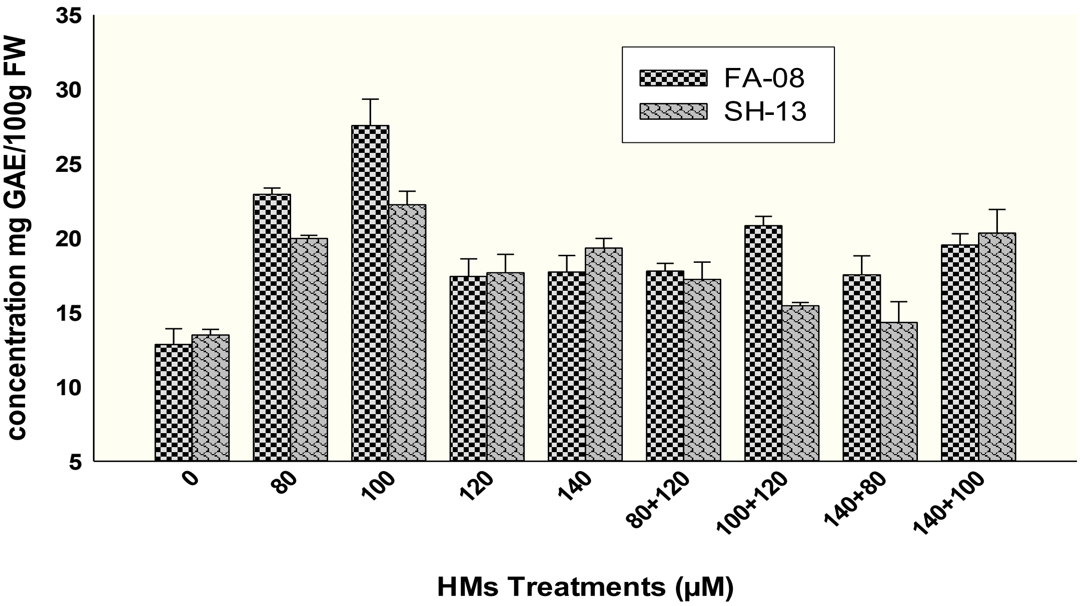

Figure 5. Quantification of total phenolic contents (mg GAE/100 g, FW) in the leaves of wheat cultivars via spectrophotometer.

Gallic acid was used as a standard for quantification of phenolics in leaves of wheat cultivars, FA-08, SH-13. Different concentrations of gallic acid ranging from 0 to 100 ppm were used to construct the standard curve. Data are expressed as the mean ± SD of three replicates. Bars show significant difference between control and treatments by *Tukey*-test at P=0.05. The equation used for polyphenols quantification was $y = 121x-1.2638$. Where x is the absorbance of sample and y is the concentration of gallic acid and R^2 value determined using gallic acid as a standard was 0.9764 (Fig. 5).

3.3. Effect of HMs on total flavonoids contents

Flavonoid contents in two wheat cultivars (FA-08 and SH-13) under control conditions were ranged from 16.5153 to 16.7222 mg/100 g FW (Fig. 6). The concentration of flavonoids was increased in both wheat cultivars as the concentration of Cr and Cd was increased individually or in combination. Higher concentration of flavonoids was recorded in cultivar SH-13 as compared to FA-08, when Cd and Cr were applied separately. At a concentration of Cd 100 µM, the flavonoid contents (19.01±1.57) in FA-08 is significantly different from SH-13 (27.34±1.60) whereas at level Cr: 140 µM the flavonoids in FA-08 was significantly lower (27.60±1.45) than cultivar SH-13 (34.49±0.38) and also showed a significant difference from the control FA-08 (16.71±1.61), SH-3 (16.88±0.87) respectively. Total flavonoid content was observed highest in FA-08 (42.94 ± 1.03 mg/100 g) followed by SH-13 (37.77 ± 1.83) at level Cr + Cd (140 µM + 80 µM) while the control of FA-08 (16.71±1.16) and SH-13 (16.88±0.87) were lowest in flavonoid content. The cultivar FA-08 had higher flavonoids concentration (42.94±1.03) when of Cd (80 µM) and Cr (140 µM) were applied in combination followed by SH-13 (37.77±1.83) and at level Cr + Cd : 140 µM +100 µM where flavonoids contents were low in FA-08 as compared to SH-13. At level Cd + Cr : 80 µM + 120 µM, and 100 µM +120 µM showed a significant difference between FA-08 and SH-13 and all values in combine form of stress showed a significant difference from the control. It was concluded that flavonoids could rescue the growth inhibition of seedling at different doses of HMs and when stress of Cd, Cr was given in combine form. Flavonoids commonly found in aerial part of plants and usually accumulate in vacuole as glycosides. The flavonoid contents tend to increase under biotic and abiotic stress (Gill & Tuteja, 2010). In reported study, phenolics and flavonoids tend to increase at 2mM of Boron (B) in tomato plant (Cervilla *et al.*, 2012). In present study leaf part was used for phytochemical analysis because most of the bioactive compounds were present in leaf part of wheat.

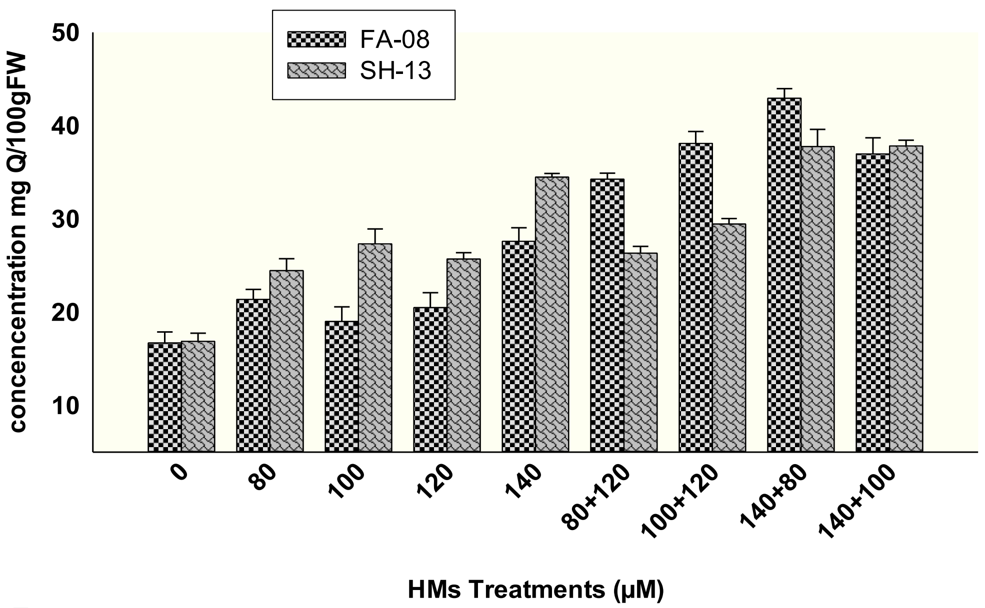

Figure 6. Concentration of total flavonoid contents (mg CE/100 g, FW) in the leaves of wheat cultivars by spectrophotometer. Data are expressed as the mean ± SD of three replicates. Bars show significant difference between cultivars and between control and treatments by *Tukey*-test at *P*=0.05.

For the estimation of total flavonoids by spectrophotometer among the two wheat cultivars quercetin was used as a standard and then standard curve was made by using different concentration of quercetin ranging from 0 to 100 ppm. The equation used for quantification of flavonoids was $y = 492.72x-5.2677$ and $R^2 = 0.9878$. Where x is the absorbance of sample and y is concentration of quercetin and R^2 value determined using quercetin as a standard was 0.9878 (Fig. 6).

3.4. Effect of HMs on antioxidant activity

Ascorbic acid is abundant in photosynthetic cells of mature leaves (Gill & Tuteja, 2010). Present results indicated that cultivar SH-13 had highest antioxidant activity (47.06% ± 0.11) followed by cultivar FA-08 (40.34%± 0.20) without HMs treatment. Both cultivars exhibited a trend in lower antioxidant activities with an increase in the application of Cd and Cr. Antioxidant capacity of any plant fluctuates due to the presence of antioxidant compounds like phenolic acid and tannins, which enhance its capacity to overcome ROS (reactive oxygen species) and comprises of high content of ascorbic acid, vitamin E and vitamin A (Santos *et al*., 2014). The antioxidant activity was found higher when Cd was applied singly in a concentration of 80μM in cultivar SH-13 (61.78% ± 0.83) followed by cultivar FA-08 (54.53%± 0.40). When Cr was applied in a concentration of 120 μM, antioxidant activity was higher in cultivar FA-08 (58.20±0.43) followed by SH-13 (50.72±0.30). On the other hand, high dose of Cr (140 μM) had reduced the antioxidant considerably and showed a significant difference between both wheat cultivars FA-08 (30.46±0.68); SH-13 (29.0±0.80) as compared to other Cd treated and controlled wheat seedlings in both cultivars. Ascorbic acid plays an important role in protection of cellular compartments against the ROS stress, but they cannot cope with the reducing radicals such as superoxide's (Michalak, 2006). There was more reduction in the antioxidant activity at high dose of Cr:140 μM, FA-08 (30.46±0.68); SH-13 (29.0±0.80) as compared to the high dose of Cd:100 μM, FA-08 (37.87±0.57), SH-13 (50.90±0.22). Ascorbic acid found significantly lower in tomato fruit when grown in heavy metal contaminated soil as compared to virgin soil, so nutritional values are greatly affected (Hashem *et al*., 2018). The significant difference was noted between cultivars, FA-08 and SH-13 at level Cd:100 and stress in combine form Cd + Cr: 100:120. FA-08 at control level showed a significant difference from Cd:80; Cr:120 and Cd + Cr: 80+120. SH-13 showed a significant difference between control and Cd:80, Cd + Cr: 80+120, (Fig. 7).

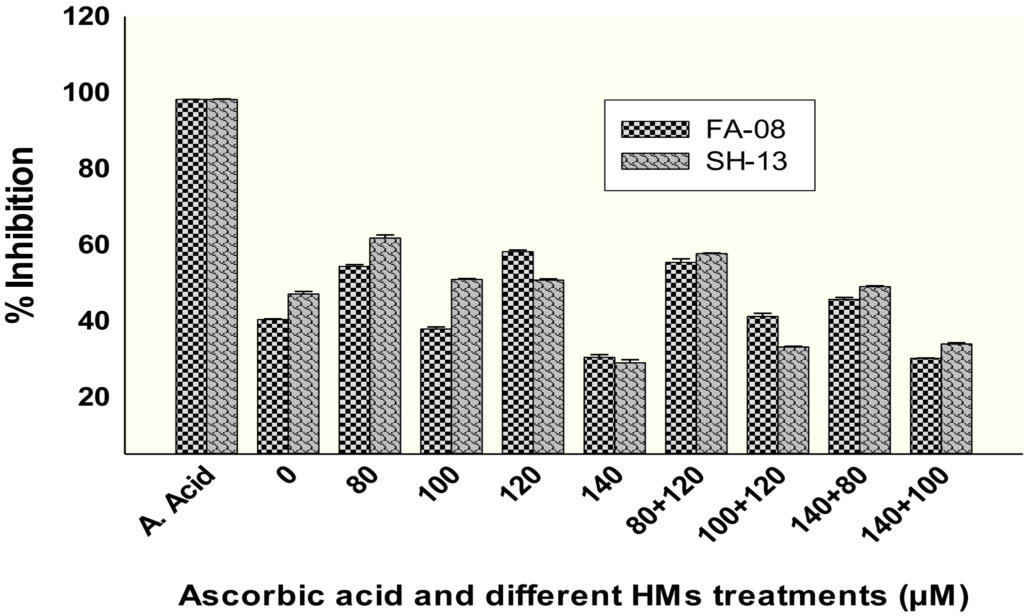

Figure 7. Analysis of DPPH scavenging potential of wheat leave extracts both cultivars.

Antioxidant activity of the leaves of two wheat cultivars was checked against DPPH. The absorbance of DPPH and its decolorized form was measured at 517 nm by spectrophotometer. Ascorbic acid was used as a positive internal control which yielded 98% ± 0.1256 antioxidant activities. Data are expressed as the mean ± SD of three replicates. Bars show significant difference between cultivars and between control and treatments by *Tukey*-test at *P*=0.05.

Table 2. The R values (correlation coefficients) of phytochemical compounds of the leaves of wheat exposed to heavy metal applications.

Treatments(µM)/L	FA-08			SH-13		
	TPC	TFC	DPPH	TPC	TFC	DPPH
80	0.99^{**}	0.97^{**}	0.99^{**}	0.99^{**}	0.99^{**}	0.96^{**}
100	0.99^{**}	0.98^{**}	-0.93^{**}	0.99^{**}	0.77^{*}	0.83^{*}
120	0.98^{**}	0.95^{**}	0.98^{**}	0.99^{**}	0.99^{**}	0.83^{*}
140	0.99^{**}	0.98^{**}	-0.99^{**}	0.99^{**}	0.98^{**}	-0.95^{**}
80+120	0.98^{**}	1^{**}	0.92^{**}	0.99^{**}	0.99^{**}	0.93^{**}
100+120	0.99^{**}	0.99^{**}	-0.55	0.93^{**}	0.99^{**}	-0.91^{**}
140+80	0.96^{**}	0.95^{**}	0.67	0.87^{**}	0.99^{**}	0.67
140+100	0.99^{**}	1^{**}	-0.98^{**}	0.99^{**}	0.99^{**}	-0.91^{**}

Correlation is significant at the 0.01 (**) and 0.05 (*) level (2-tailed).

The correlation analysis among the biochemical compounds of wheat leaves in the growth medium containing heavy metals was performed with bivariate (Pearson's) correlation. We demonstrate a positive correlation with the total phenolics and flavonoids when the wheat is exposed to Cd and Cr, especially ($p<0.01$). Likewise, there are negative correlations between antioxidants and some treatments in wheat leaves exposed to all heavy metal applications except a few showed a positive correlation when heavy metal concentration was low (Table 2).

In correlation results, it is shown that in cultivar FA-08 at a concentration of Cd:100 and Cr:140 antioxidant decreased showed a negative correlation (Table 2) and in the same way Cd high concentration in combination form also decreased the antioxidant contents and in cultivar SH-13, the Chromium high concentration Cr:140µM decreased the antioxidant and showed negative value Cd showed positive correlation with antioxidant and in combination form showed decreased in contents of antioxidants as concentration of cadmium is 100µM and Cd+Cr:100+120, Cr+Cd:140+100µM.

5. Conclusion

From this research study it can be concluded; wheat is an important cereal crop and is widely cultivated in Pakistan, exposure of HM pollution at any stage of plant growth is a threat to living organisms when consumed. The tested cultivars FA-08, SH-13, accumulated the HMs Cd, Cr at different concentrations in their tissues, applied separately and in combination and caused physiological changes. Visual observations depicted those morphological parameters are less affected in cultivar SH-13 as compared to cultivar FA-08. The phenolic and antioxidant contents of cultivar SH-13 were higher as compared to cultivar FA-08 in the control condition. The contents of phenolics and flavonoids decreased as the concentration of HMs increased in wheat cultivars. The content of chlorophyll, carotenoids and other biochemicals can be used as indicators under heavy metal stress conditions or nutritional deficiencies and combine form of metals less affected the plants as compared to when they applied separately so these parameters can be further studied in wheat cultivars. The study also prompts to launch an analysis of plant which also helps to suggest a better cultivar like SH-13 to be used in daily uptake.

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Conflict of interest

Authors don’t have any conflict of interest.

References

Adrees M., Ali S., Rizwan M., Zia-ur-Rehman M., Ibrahim M., Abbas F. and Irshad, M. K. (2015), Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. *Ecotoxicology and Environmental Safety*, **119**, 186-197.

Al Mahmud J., Hasanuzzaman M., Nahar K., Rahman A., Hossain M.S. and Fujita M. (2017), Maleic acid assisted improvement of metal chelation and antioxidant metabolism confers chromium tolerance in *Brassica juncea L*. *Ecotoxicology and Environmental Safety*, **144**, 216-226.

Ali S., Farooq M. A., Yasmeen T., Hussain S., Arif M. S., Abbas F., Bharwana S.A. and Zhang G. (2013), The influence of silicon on barley growth, photosynthesis and ultra-structure under chromium stress. *Ecotoxicology and Environmental Safety*, **89**, 66-72.

Ali Z., Razi-ul-Hasnain R., Quraishi U.M. and Malik R.N. (2018), Heavy metal tolerance and biochemical attributes of selected wheat genotypes on irrigation with industrial wastewaters. *Pakistan Journal of Agricultural Sciences*, **55**(4).

Alves R.C., Costa A.S., Jerez M., Casal S., Sineiro J., Núñez M.J. and Oliveira B. (2010). Antiradical activity, phenolics profile, and hydroxymethylfurfural in espresso coffee: influence of technological factors. *J. Agric. Food Chem*. **58**, 12221–12229.

Anjum N. A., Ahmad I., Mohmood I., Pacheco M., Duarte A.C., Pereira E., Umar S., Ahmad, A., Khan N.A., Iqbal M. and Prasad M.N.V. (2012), Modulation of glutathione and its related enzymes in plants’ responses to toxic metals and metalloids—a review. *Environmental and Experimental Botany*, **75**, 307-324.

Aoshima H., Tsunoue H., Koda H. and Kiso Y. (2004), Aging of whiskey increases 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity. *J. Agric. Food Chem*, **52**, 5240–5244.

Athar R., and Ahmad M. (2002), Heavy metal toxicity: effect on plant growth and metal uptake by wheat, and on free living Azotobacter. *Water, Air, and Soil Pollution*, **138**(1-4), 165-180.

Barroso M.F., Noronha J.P., Delerue-Matos C. and Oliveira, M. (2011), Flavored waters: influence of ingredients on antioxidant capacity and terpenoid profile by HS-SPME/GC-MS. *J. Agric. Food Chem*. **59**, 5062–5072.

Bartwal A., Mall R., Lohani P., Guru S.K., and Arora S. (2013), Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. *Journal of Plant Growth Regulation*, **32**(1), 216-232.

Cervantes C., Campos-García J., Devars S., Gutiérrez-Corona F., Loza-Tavera H., Torres-Guzmán J.C., and Moreno-Sánchez R. (2001), Interactions of chromium with microorganisms and plants. *FEMS microbiology reviews*, **25**(3), 335-347.

Cervilla L.M., Blasco B., Rios J.J., Rosales M.A., Sánchez-Rodríguez E., Rubio-Wilhelmi M.M., Romero L., and Ruiz J. M. (2012), Parameters symptomatic for boron toxicity in leaves of tomato plants. *Journal of Botany*, **2012**.

Chandio A.A., Jiang Y., Joyo M.A., and Rehman A. (2016), Impact of area under cultivation, water availability, credit disbursement, and fertilizer off-take on wheat production in Pakistan. *Journal of Applied Environmental and Biological Sciences*, **6**(10), 10-18.

Chaudhry H., Nisar N., Mehmood S., Iqbal M., Nazir A., and Yasir M. (2020), Indian Mustard Brassica juncea efficiency for the accumulation, tolerance and translocation of zinc from metal contaminated soil. *Biocatalysis and Agricultural Biotechnology*, **101489**.

da Conceicao Gomes M. A., 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.

Dubey S., Shri M., Gupta A., Rani V. and Chakrabarty D. (2018), Toxicity and detoxification of heavy metals during plant growth and metabolism. *Environmental chemistry letters*, **16**(4), 1169-1192.

Ghani A., Khan I., Umer S., Ahmed I., Mustafa I. and Mohammad N. (2015), Response of wheat (*Triticum aestivum*) to exogenously applied chromium: effect on growth, chlorophyll and mineral composition. *Journal of Environmental & Analytical Toxicology*, **5**(3),1.

Ghosh M. and Singh S. P. (2005), A comparative study of cadmium phytoextraction by accumulator and weed species. *Environmental Pollution*, **133**(2), 365-371.

Gill S. S. and Tuteja N. (2011), Cadmium stress tolerance in crop plants: probing the role of sulfur. *Plant Signaling & Behavior*, **6**(2), 215-222.

Gill S.S., Khan N.A., and Tuteja N. (2012), Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (*Lepidium sativum* L.*). Plant Science*, **182**, 112-120.

Guarino F., Ruiz K.B., Castiglione S., Cicatelli A., and Biondi S. (2020), The combined effect of Cr (III) and NaCl determines changes in metal uptake, nutrient content, and gene expression in quinoa (Chenopodium quinoa Willd.). *Ecotoxicology and Environmental Safety*, **193**, 110345.

Hashem H.A., Shouman A. I., and Hassanein R. A. (2018), Physico–biochemical properties of tomato (*Solanum lycopersicum*) grown in heavy–metal contaminated soil. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, **68**(4), 334-341.

Hakeem K. R. (Ed.). (2015), *Crop production and global environmental issues*. Springer.

Hithamani G. and Srinivasan K. (2014), Bioaccessibility of polyphenols from wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), green gram (*Vigna radiata*), and chickpea (*Cicer arietinum*) as influenced by domestic food processing. *Journal of agricultural and food chemistry*, **62**(46), 11170-11179.

Hoagland D.R., and Arnon D.I. (1950), The water-culture method for growing plants without soil. *Circular. California agricultural experiment station*, **347**(2nd edit).

Jamali N., Ghaderian S. M., and Karimi N. (2014), Effects of cadmium and zinc on growth and metal accumulation of Mathiola flavida Boiss. *Environmental Engineering & Management Journal (EEMJ)*, **13**(12).

Khan A., Khan S. K. M.A., Khan M. A., Aamir M., Ullah H., Nawab J., Rehman I. U. and Shah J. (2019). Heavy metals effects on plant growth and dietary intake of trace metals in vegetables cultivated in contaminated soil. *International journal of Environmental Science and Technology*, **16**(5), 2295-2304.

Kısa D., Elmastaş M., Öztürk L., and Kayır Ö. (2016), Responses of the phenolic compounds of *Zea mays* under heavy metal stress. *Applied Biological Chemistry*, **59**(6), 813-820.

Koleva I.I., Van Beek T.A., Linssen J.P., Groot A.D., and Evstatieva L. N. (2002), Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, **13**(1), 8-17.

Kumar A., and Maiti S. K. (2013), Availability of chromium, nickel and other associated heavy metals of ultramafic and serpentine soil/rock and in plants. *International Journal of Emerging Technology and Advanced Engineering*, **3**(2), 256-268.

Liangli L. Y. (2008), *Wheat antioxidants*. John Wiley & Sons. Mac Farlance, G.R. (2002). Leaf biochemical parameters in *Avicennia marina* (Forsk) vierh as potential biomarkers of heavy metal stress in esturine ecosystem. *Marine Pollution Bulletin*, **44**:244-256.

Lin L., Cui C., Wen L., Yang B., Luo W., and Zhao M. (2011), Assessment of in vitro antioxidant capacity of stem and leaf extracts of Rabdosia serra (MAXIM.) HARA and identification of the major compound. *Food Chemistry*, **126**(1), 54-59.

Maleva M.G., Nekrasova G.F., Borisova G.G., Chukina N.V., and Ushakova O. S. (2012), Effect of heavy metals on photosynthetic apparatus and antioxidant status of Elodea. *Russian Journal of Plant Physiology*, **59**(2), 190-197.

Mallick S., Sinam G., Mishra R. K., and Sinha S. (2010), Interactive effects of Cr and Fe treatments on plants growth, nutrition and oxidative status in *Zea mays* L. *Ecotoxicology and Environmental Safety*, **73**(5), 987-995.

Márquez-García B., Fernández-Recamales M., and Córdoba F. (2012), Effects of cadmium on phenolic composition and antioxidant activities of *Erica andevalensis*. *Journal of botany*, **2012**.

Michalak A. (2006), Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies*, **15**(4).

Mohamed A.A., Castagna A., Ranieri A., and di Toppi L.S. (2012), Cadmium tolerance in *Brassica juncea* roots and shoots is affected by antioxidant status and phytochelatin biosynthesis. *Plant Physiology and Biochemistry*, **57**, 15-22.

Nagajyoti P.C., Lee K. D., and Sreekanth T. V. M. (2010), Heavy metals, occurrence and toxicity for plants: a review. *Environmental Chemistry Letters*, **8**(3), 199-216.

Omoloye O.A., Adedapo A.A., and Ohore O.G. (2007), Studies on the toxicity of an aqueous extract of the leaves of *Abrus precatorius* in rats. *Onderstepoort Journal of Veterinary Research*, **74**(1), 31-36.

Parlak K.U. (2016), Effect of Nickel on growth and biochemical characteristics of Wheat (*Triticum aestivum* L.) seedlings. *NJAS-Wegeningen Journal of life Sciences*, **76**, 1-5.

Preeti P. and Tripathi A.K. (2011), Effect of HMs on morpholpogical and biochemical characteristics of Albizia procera (Roxb). Benth.seedlings. *International Journal of Environmental Science*, **1**(5), 1009-1018.

Shahid M., Pourrut B., Dumat C., Nadeem M., Aslam M. and Pinelli E. (2014), Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. In *Reviews of Environmental Contamination and Toxicology,* 232, 1-44. Springer, Cham.

Sharma D.C., Chatterjee C., and Sharma C.P. (1995), Chromium accumulation and its effects on wheat (*Triticum aestivum* L. cv. HD 2204) metabolism. *Plant Science*, **111**(2), 145-151.

Shewry P. R. (2009), Wheat. *Journal of Experimental Botany*, 60(6), 1537-1553.

Vajravel S., and Saravanan P. (2013), Accumulation of chromium and its effects on physiological and biochemical parameters of *Alternanthera philoxeroides* seedlings. *Journal of Pharmacy Research*, **7**(7), 633-639.

Venkateswaran S., and Pari L. (2003), Effect of Coccinia indica leaves on antioxidant status in streptozotocin-induced diabetic rats. *Journal of ethnopharmacology*, **84**(2-3), 163-168.

Wilkins D.A. (1957), A technique for the measurement of lead tolerance in plants. *Nature*, **180**(4575), 37.

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. *International Scholarly Research Network (ISRN) Ecology*, **20**.

Yu L., Haley S., Perret J., and Harris M. (2002), Antioxidant properties of hard winter wheat extracts. *Food chemistry*, **78**(4), 457-461.

Žilić S., Serpen A., Akıllıoğlu G., Janković M., and Gökmen V. (2012), Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. *Journal of Cereal Science*, **56**(3), 652-658.

Žilić S., Šukalović V.H.T., Dodig D., Maksimović V., Maksimović M. and Basić, Z. (2011), Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. *Journal of Cereal Science*, **54**(3), 417-424.

Zulfiqar F., and Hussain A. (2014), Forecasting wheat production gaps to assess the state of future food security in Pakistan. *Journal of Food and Nutritional Disorders*, **3**(3), 2.