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Original Article

Effects of Chromium on Enzymatic/Nonenzymatic Antioxidants and Oxidant Levels of *Portulaca oleracea* L.

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Abstract

Purslane (*Portulaca oleracea* L.) is a local plant in Iran that can easily breed. Chromium is a heavy metal that causes toxicity for the growth and development of plants. To study the effects of potassium dichromate on growth, efficiency of photosystem II (Fv/Fm), cell membrane stability index (MSI), indexes of oxidative stress and antioxidant properties of purslane, an experiment was done in a completely randomized design of five levels (0, 25, 50, 75 and 100 ppm Cr per kg of soil) in a greenhouse condition. After 60 days, the effect of different levels of chromium on growth parameters, Fv/Fm, MSI, total phenolic compound, flavonoid, H_2O_2 and malondialdehyde content and antioxidants enzymatic activity (catalase, peroxidese and ascorbate peroxidase) was assayed. Along With increasing concentrations of chromium, the malondialdehyde (MDA) and H_2O_2 was increased, and followed by the enzymatic and nonenzymatic antioxidant compounds (except APX) was increased to compensate the effect of oxidant compounds. But, Heavy metal negatively affected growth parameters such as dry weight and length of root and shoot, Fv/Fm and MSI in contaminated plants. Therefore, the destructive effects due to chromium stress with increases the activity of enzymatic and non-enzymatic antioxidant system was minimized. It was noted that accumulation of chromium in the roots was higher than the shoots of the plants under treatment, significantly. Based on these results, purslane can be introduced as a good candidate for tolerance to chromium.

Keywords: Chromium, Enzymatic antioxidants, Oxidants level, Phenolic compounds, Purslane

Introduction

Chromium (Cr) is a heavy metal that has potential hazardous to the environment [1]. In plants, Cr is found in the forms of trivalent (Cr [III]) and hexavalent (Cr [VI]), where the former is of lower toxicity than the latter [2]. Cr (VI) forms chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) and is highly soluble in water. On the other hand, Cr (III) is less soluble in water and thus is bioavailable in soils, it has reduced to Cr (VI) by organic matter or humus [3,4]. The sources of Cr in environment are both natural and anthropogenic. Cr is used on a large scale in many industries, including electroplating, metallurgy, wood preservation, production of paints, leather industry and Cr chemicals production [5]. Chromium interferes with several metabolic processes and causes to decrease growth and biomass, chlorosis, photosynthetic impairing and finally plant death [6,7]. Amin *et al.*, reported that chromium metal affects the growth of *Hibiscus esculentus* L. by reducing seed germination and decreasing seedling growth [8]. Also, Akinci and Akinci, showed that dry weight and length of *Lycopersicon esculentum* Mill. was negatively

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affected by the increasing of chromium concentrations at the seedling stage [9].

Fv/Fm ratio is a maximum quantum efficiency of PSII photochemistry, which allows detection of any damage to PSII and possible photoinhibition [10]. Based on studies conducted in 2013, Cd negatively was affected the chlorophyll content and Fv/Fm [11]. Leaf membrane stability index (MSI) was measured as ion leakage [12]. Sanchez-viveros *et al.*, reported that Cu-induced stress was caused membrane damage in *Azolla* Lam.[13].

Cr induce production and accumulation of Reactive Oxygen Species (ROS) in cells and tissues includes superoxide ($O2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$), which could lead to severe oxidative damage in plants [14]. The lipid peroxidation is the most prominent symptom of oxidative stress. Malondialdehyde (MDA) is the product of lipid peroxidation in plants under stress, and it is often used as an indicator of oxidative stress [15]. Ahmad *et al.*, reported that a positive correlation was between of heavy metals content in the soil and the level of MDA and H_2O_2 in plant [16].

Plant cells are equipped with enzymatic and nonenzymatic antioxidants [17]. Non-enzymatic antioxidants are ascorbate, glutathione, tocopherol, phenolic compound especially flavonoids and carotenoids. Flavonoids have been suggested to act as antioxidants, protecting plants from oxidative stress. Phenolics, especially flavonoids, can be oxidized by peroxidase and act in the H_2O_2-scavenging, phenolic/ASC/POX system against heavy metal contamination [19]. The induction of phenolic compound biosynthesis was observed in wheat in response to nickel toxicity [20]. Plants defend themselves from oxidative stress by making use of antioxidant enzymes, such as catalase (CAT, EC 1.11.1.6), peroxidase (POX, EC 1.11.1.7) and ascorbate peroxidase (APX, EC 1.11.1.11), have the ability to scavenge reactive oxygen species and prevent oxidative damage [21]. Thus, the balance between ROS generation and scavenging determines the survival of the system [22]. Enhancement the activity of POX observed in several metal stresses by other researches [23]. Devi Chinmayee *et al.*, by examining the *Jatropha curcas* L. treated with heavy metals, showed a strong correlation between antioxidant enzyme activity and concentrations of heavy metals [22].

Portulaca oleracea L. commonly known as purslane, is an annual succulent plant and an herbaceous weed in the family Portulacaceae. Purslane is a reasonable selection due to its high nutritious and antioxidant properties as human food, animal feed and medical utilization. Purslane comprises more nutritive values than other vegetable due to its fatty acid (Like omega 3), α-tocopherol, ascorbic acid, β-carotene and glutathione-rich shoots [24]. Acute reaction will only occur at above the chromium LD 50 level (20–250 mg Cr (VI) and 185–615 mg Cr (III) per kg body weight) [25].

Also, Azizi *et al.*, reported that bioaccumulation factor in *P. oleracea* reached a value more than 1, which confirmed the role of *P. oleracea* as an effective Cr^{+6} accumulator in soils [26], On the other hand, due to the medicinal properties of this plant and the possibility of accumulation of heavy metals in its root, the use of its aerial parts will be used for pharmaceutical purposes [27]. The aim of current study was determining chromium concentrations in soil, root and shoot of *P. oleracea*, and analyze the effect of Cr stress on growth parameters (dry weight and length of shoots and roots), Fv/Fm, MSI, oxidant compounds and the mechanism of stress tolerance (enzymatic and non enzymatic antioxidants) of *P. oleracea* in the soil contaminated with chromium, finally, it is probability introduced *P. oleracea* as a suitable candidate for phytoremediation of chromium contaminated soils.

Material and Methods

Plant Materials

This study was conducted based on completely randomized design with three replications in control condition at the research Greenhouse of Ferdowsi University of Mashhad. 10 Seeds of *Portulaca oleracea* were planted in each pot containing 3 Kg of sandy soil. Each pot was considered as an experimental unit. The greenhouse conditions were maintained at 24-28 °C, night temperature 16-20 °C, and humidity 50% with 16 hours of light and 8 hours of darkness. Characteristics of the soil used in the experiment were listed in Table 1. During the period of growth, pots were irrigated as much as field capacity with a solution of potassium dichromate at different levels (25, 50, 75 and 100 ppm) and control pots were irrigated with distilled water. After 60 days, the samples were collected and the biochemical measurements were performed on the shoots.

Table 1 Physico-chemical properties of the soil before start of the experiment

Soil texture	EC ($dS.m^{-1}$)	pH	Nutrient level (ppm)			
			Total N	Available P	Available K	Available Cr
Loam- silty	1.14	7.75	298	7.1	118	3.47

Growth Parameters

After 60 days, length of shoots and roots was measured. In addition, dry weight of shoots and roots was determined with after drying the sample for 48 h at 75 °C.

Determination Cr (VI) Content in Soil

The Cr (VI) content in soil was determined according to diphenylcarbazide method. In this method, it was used the mixture of sodium carbonate 0.28 M and NaOH 0.5 M for extraction of soil and then they was centrifuged. Finally, the amount of Cr (VI) content was measured at 540 nm with a spectrophotometer [28].

Determination Cr (VI) Content in the Plant

Samples of shoot and root were dried individually at a temperature of 75 ° C for 48 hours in the oven. The Cr (VI) content in plants from alkaline digestion was determined according to EPA method [29]. After digestion, the samples were reacted with the reagent 1 and 5-diphenyl Krbazayd and then the amount of hexavalent chromium were measured by using a spectrophotometer at 540 nm.

Chlorophyll Fluorescence

Photosystem photochemical efficiency (Fv/Fm) was measured using a portable chlorophyll fluorometer (OS5-FL modulated chlorophyll fluorometer, ADC Bio Scientific Ltd. Hoddesdon, Hert, EN11 0DB England). Minimal fluorescence (Fo) was determined by applying weak modulated light (0.4 µmol m-2s-1) and maximal fluorescence (Fm) was induced by a short pulse (0.8 s) of saturating light (8000 µmol $m^{-}2s^{-}1$). Measurements were made from the same leaf used for gas exchange determination, after 20 min dark adaptation [30]. All physiological measurements used four or more plants from each treatment under drought stress and control conditions.

Membrane Stability Index (MSI)

Leaf samples (0.1 g) of leaf material were taken in 10 mL double-distilled water in glass vials and kept at 40°C for 10 min. Initial conductivity (C1) was recorded with a conductivity meter after bringing the sample to 25 °C. The samples were kept at 100 °C for 30 min and cooled to 25 °C, and final conductivity (C2) was recorded according to Premachandra *et al.*, [31] as modified by Sairam 1994 . The membrane stability index (MSI) was calculated as

$$MSI = [1– (C1/C2)]×100$$

Determination of Malondialdehyde (MDA) Content

The MDA content was determined by the reaction of thiobarbituric acid (TBA), as described by Heath and Packer [32], with minor modifications by Zhang and Shin (1994). After trials, the optical density value of the supernatant was measured at 532 and 600 nm. The MDA content was calculated according to molar extinction coefficient of the MDA (156 $mM^{-1}cm^{-1}$).

Determination of Peroxide Hydrogen (H_2O_2) Content

The H_2O_2 content of both control and chromium purslane shoot was determined according to Sagisaka [33]. One gram of shoot tissue was homogenized in 5% trichloroacetic acid (TCA) and the homogenate was centrifuged at 12,000 x g at 4°C for 15 min. The reaction mixture contained 0.5 ml supernatant of shoot extract, 0.5 ml of 10 mM potassium phosphate buffer with pH 7 and 1 ml of 1 mM potassium iodide KI. The absorbance was recorded at 390 nm**.**

Determination of Total Phenolic Content

The total phenolic compound of purslane shoot was extracted with distilled water and 1:20 ratio. For this purpose sample centrifuged for 45 min at 10.000 rpm and supernatants were collected. A suitable aliquot of the solution under test was diluted with water to about 7 ml in a 10-ml test tube. The content was well mixed with 0.5 ml of the Folin-Ciocalteu reagent. After three minutes, 1 ml of saturated sodium carbonate solution was added and the mixture made up to 10 ml. After 45 min the absorption was determined in 760 nm by spectrophotometer [34].

Determination of Total Flavonoid Content

The total flavonoids content was determined by aluminium trichloride method using quercetin as a reference compound [35]. The total flavonoid of purslane shoot was extracted with distilled water and 1:20 ratio. This method was done based on the formation of a complex flavonoid-aluminum having the absorptivity maximum at 415 nm, after remained react at room temperature for 30 min.

Determinations of Antioxidant Enzymes Activities

For the assays of CAT, POX, APX, frozen leaves was extracted with KH_2PO_4 and K_2HPO_4. After centrifuged at 15000×g for 20 min in a refrigerated centrifuge, the supernatant was collected in a bottle for the determination of enzymes activity. Peroxidase activity was measured according to Holy [36]. The reaction mixture contained 0.2 M acetate buffer (pH 5) with H_2O_2 (3%), benzidin 0.2 mM in methanol (50%) and enzyme extract. The reaction rate was then identified by increasing in absorbance at 530 nm. One unit of POX and CAT was defined as μmol ml^{-1} H_2O_2 decomposed per min at 25°C. Catalase activity was measured according to Chandlee and Scandalios [37] with modification. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. Ascorbate peroxidase activity was measured according to Asada and Takahashi [38]. The reaction mixture contained 50 mM phosphate buffer (pH 6.5), 5 μM ascorbate, H_2O_2 (3%) and enzyme extract. Therefore, the H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 256 nm. One unit of this enzyme was defined as the amount of enzyme required to hydrolyze 1 mmol of the substrate per min at 25 °C.

Statistical Analysis

The analysis of the variance was conducted on the data, and significant differences among treatment means were calculated by using duncan multiple range tests ($p \leq 0.05$).

Results

Content of Cr (VI) in Soil and Plant

Based on the results with increasing chromium concentration in the medium, concentrations of Cr (VI) in the shoot, root and soil significantly increased ($p \leq 0.01$) (Fig. 1). Increasing trend was more significant in soil and root. So that, the amount of chromium in the soil, shoot and root of cultured plants in 100 ppm, about 11, 2 and 6.5 times compared with control, respectively. Also, results showed that the concentration of Cr (VI) in the root was more than shoot and soil. In 100 ppm chromium, content of Cr (VI) in the roots was about 5.2 and 5 times compared with shoot and soil, respectively (Fig. 1).

Growth Parameters of Shoots and Roots

Analysis of variance showed that the dry weight and length in treated plants was significantly decreased compared with control ($P \leq 0.05$) (Fig. 2). The maximum and the minimum of length of shoots and roots was observed in control sample and plants treated with 75 ppm chromium respectively. So that, the length of shoots and roots in plant treated with 100 ppm chromium was close to the control plants (Fig. 2). According to Fig. 2, dry weight had similar decreasing trend with length. Shoot dry weight of plants planted in the 75 and 100 ppm was decreased 56 and 2 times compared with control plants respectively.

Content of Efficiency of Photosystem II (Fv/Fm) and Membrane Stability Index (MSI)

Results revealed that chromium had significant effect on the Fv/Fm and MSI (Table 2). Linear decrease occurred in Fv/Fm and MSI with the increasing concentration of chromium ($p \leq 0.01$). But, in the plants treated with 50 and 75 ppm, together showed no significant differences. (Table 2).

Table 2 Average changes of efficiency of photosystem II (Fv/Fm) and cell membrane stability index (MSI) purslane under different levels of chromium.

Cr (ppm)	Fv/Fm	MSI
control	0.930[a]	60.223[a]
25	0.850[b]	52.840[b]
50	0.710[c]	48.090[c]
75	0.700[c]	46.883[cd]
100	0.620[d]	44.040[d]

Content of Oxidants (MDA and H_2O_2)

This study revealed that chromium had significantly effected on the MDA and H_2O_2 content of *P. oleracea* ($P \leq 0.05$) (Table 3). The content of MDA and H_2O_2 treated plants with 100 ppm was increased 5.5 and 1.2 times compared with control plants, respectively (Fig. 3).

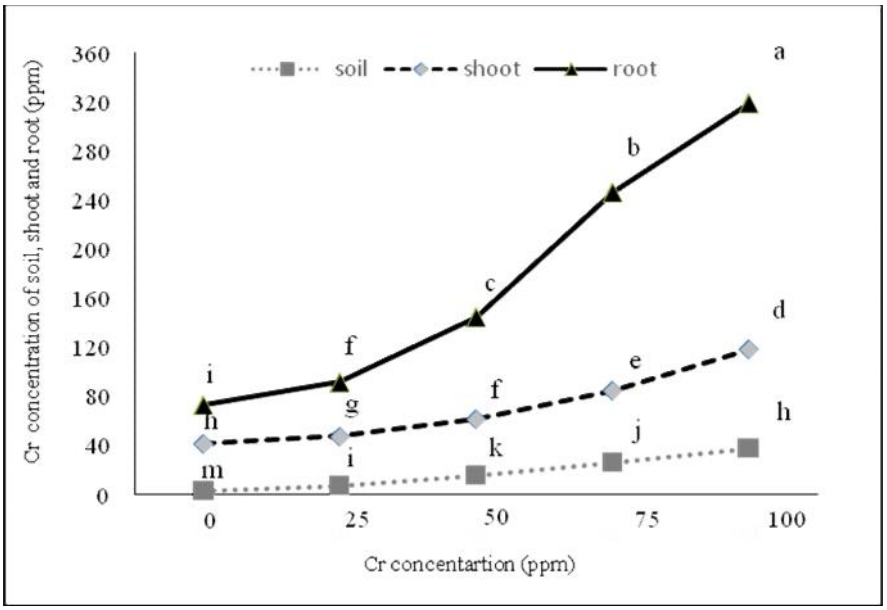
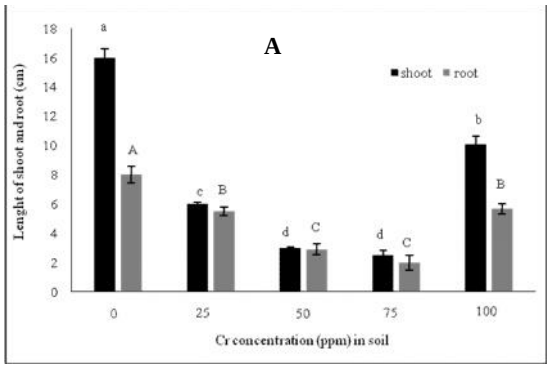


Fig. 1 The concentration of Cr (VI) in soil, shoot and root of *Portulaca oleracea* L. treated with different concentrations of chromium after 60 days.



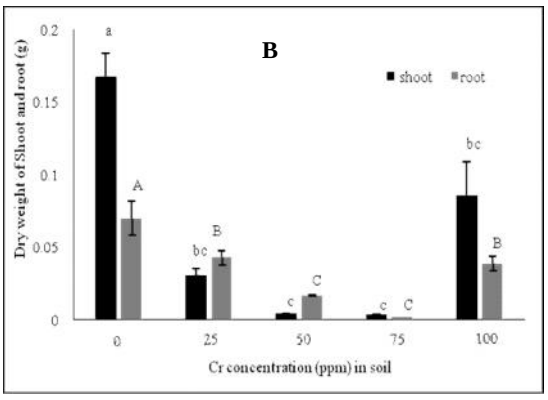


Fig. 2 Effect of Cr stress on (A) length and (B) dry weight of shoot and root of *Portulaca oleracea* L.. Columns with the same letters are not significantly different (P≤0.05)

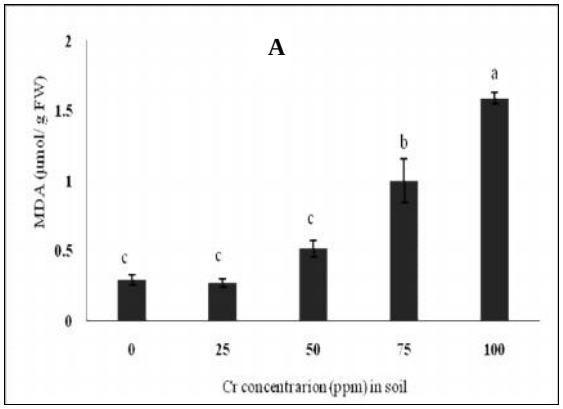
According to Fig. 3 (A and B), diagram of MDA had most changes and significant trend in the concentration of chromium in comparison of diagram of hydrogen peroxide. In addition to, comparison between chromium levels showed that with increasing chromium from 0 to 50 ppm, no significant differences were observed content of MDA and H_2O_2. Also, the content of H_2O_2 in treated plants of 100 ppm compared with 75 ppm of chromium was increase, non significantly.

Content of Nonenzymatic Antioxidants (Phenolic Compound and Flavonoid)

The results showed that levels of phenolic compound and flavonoid contents in all contaminated plants were significantly increased as compared to control plants (Table 3). According to the Fig. 4 (A and B), the amount of phenolic compounds, flavonoids and chromium concentration of treated plants were increased with the same trends. Phenolic compounds and flavonoid content in the concentration of 100 ppm Cr and control were the highest and the lowest amount respectively (Fig. 4).

Content of Enzymatic Antioxidants (POX, CAT and APX)

The Fig. 5 showed that chromium had significant (P≤0.05) effects on the POX, CAT and APX activity compared with the control (Table 3). POX and CAT activity significantly (P≤0.05) increased in parallel with increasing chromium concentration in treated purslane (Fig. 5 A and B). In contrast these results, APX activity significantly (P≤0.05) decreased with increasing Cr stress (Fig. 5, C).



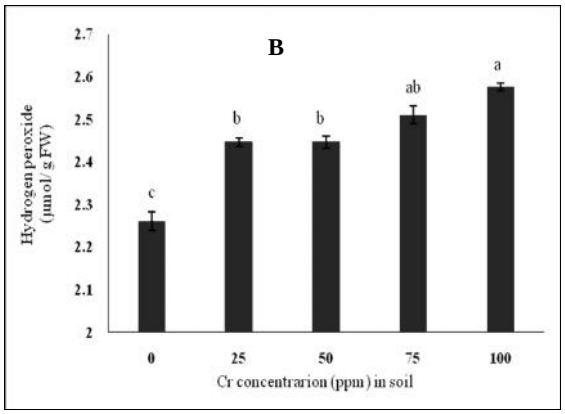
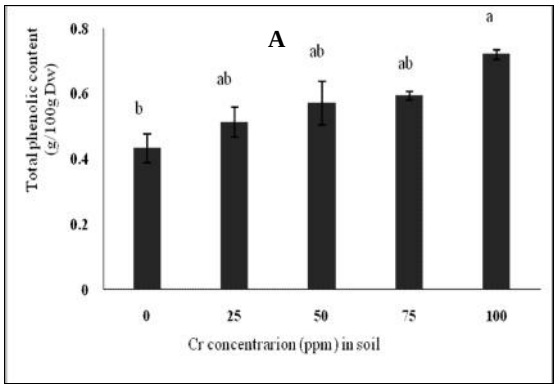


Fig. 3 Effect of Cr stress on (A) MDA and (B) H_2O_2 content in the shoot of *Portulaca oleracea* L. Columns with the same letters are not significantly different (P≤0.05).



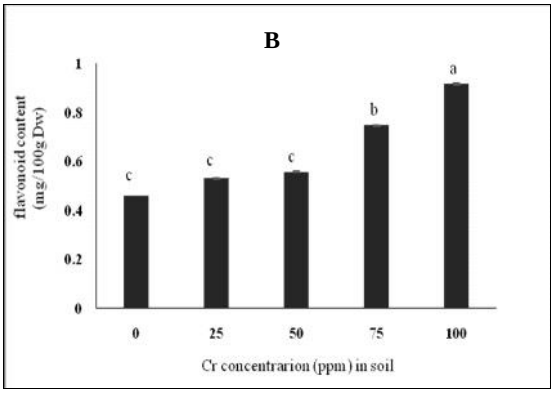
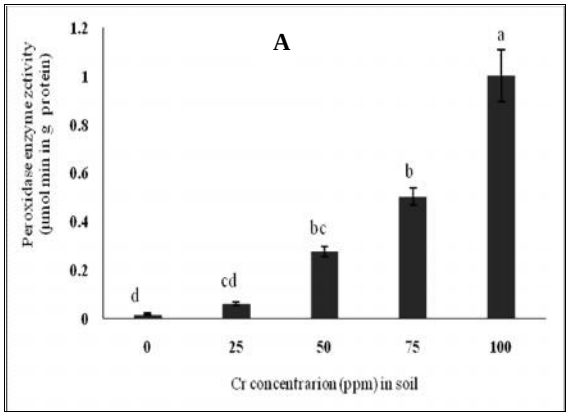
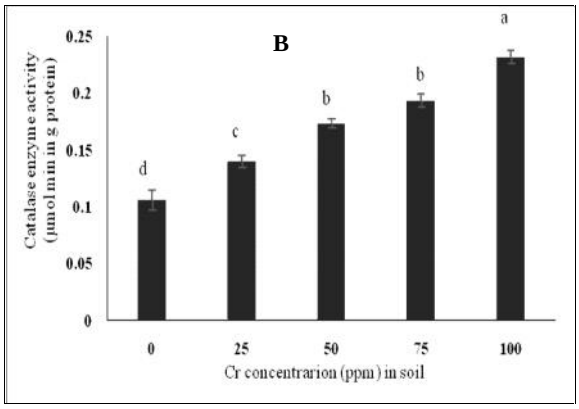


Fig. 4 Effect of Cr treatment on (A) total phenolic compounds and (B) flavonoids content in the shoot of *Portulaca oleracea* L.. Columns with the same letters are not significantly different (p<0.05).





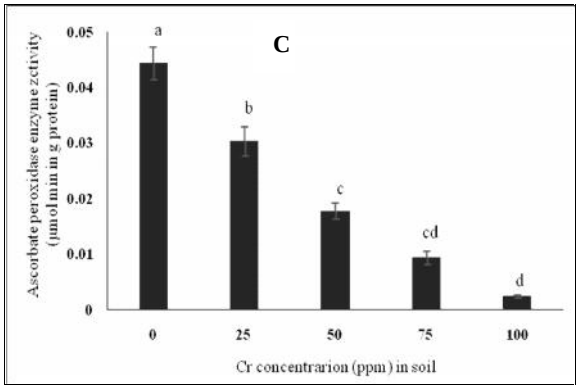


Fig. 5 Effect of Cr treatment on (A) peroxidase enzyme (POX), (B) Catalase enzyme (CAT) and (C) Ascorbate peroxidase enzyme (APX) activity in the leaf of *Portulaca oleracea* L.. Columns with the same letters are not significantly different (P≤0.05).

Table 3 Analysis of variances (ANOVA) of measured parameters in *Portulaca oleracea* L. under different treatments of Cr stress.

Mean square								Sources of Change
CAT	POX	APX	Flavonoids	Phenolic compound	H_2O_2	MDA	df	
0.007**	0.485 **	0.001**	0.001**	0.034*	0.042**	0.943**	4	Cr
0.000	0.008	0.000	0.000	0.006	0.001	0.018	10	Error
							14	total

ns, * and ** indicate non significant, Significant at 5% and 1% respectively.

Discussion

According to the results, by increasing the concentration of chromium in the medium, the amount of Cr (VI) in purslane was increased significantly. The effect of heavy metals on plants is directly related to the availability of metal in the soil [3]. These results are consistent with the results reported in *Zea mays* L. [39], and *Allium cepa* L. [40]. Generally, the amount accumulation of chromium in various parts of plants is different [42,43]. Among these parts, the highest amount of accumulation of Cr (VI) was in roots of *Portulaca oleracea* L.. Similar to the results, Abdussalam *et al.*, showed that considerable accumulation of chromium was in root>stem>leaf of *Boerhavia diffusa* L. [42]. Cr was absorbed by root system from the soil and root tissue is stored at high concentrations. There are restrictions on the transfer of chromium from the root to the apex of the plants, which probably due to binding of this ion form at the place of cationic exchange in the root and it is immobilized in the vacuoles of the root cells [44]. Thus, the accumulation of Cr (VI) in roots is much higher than aerial parts of the purslane that is probably due to chelate and compartment of chromium ions in the roots, so it can be a mechanism of resistance to stress of Cr (VI) in purslane.

According to study, chromium reduced length and dry weight of purslane. Decrease in root and shoot lengths are well documented effects by different heavy metal toxicity in *Caesalpinia pulcherrima* (L.) Sw. [3], *Sorghum bicolor* (L.) Moench [45] and *Glycine max* (L.) Merr. [5]. In general, the root system is the first place which can be influenced by heavy metal [8]. The decrease in root growth in the presence of Cr could be due to inhibition of root cell division and elongation, which might have occurred as a result of inability of the roots to absorb water from the medium [46,47]. Decreased of water uptake can effect on the physiological processes such as transpiration, respiration and photosynthesis and eventually leads to decrease growth in all the parts of the plant [39,40]. In addition, the presence of Cr in soil can disturb the pattern of nutrient uptake in plant because of nutrient metal interaction [8]. Sundaramoorthy *et al.*, observed that Cr (VI) caused an extension in cell cycle, leading to inhibition of cell division, and thus root growth [47]. The nutrient elements N, P, K, Na, Ca and Mg concentrations in shoots were significantly affected by the Cr treatments (50 and 100 mg L^{-1}) in tomato [3]. Also, Pande *et al.*, reported that supply of Cr (10, 20, and 40 ppm) decreased Fe, Cu and Zn concentration in *Bacopa monnieri* (L.) Wettst. [48]. The altered root growth also affected the shoot growth and biomass since there was limited water and nutrient supply to shoot tissues [46]. In addition to this, Cr transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction in plant growth [46]. On the other hand it was indicated that the high concentrations of chromium, effects on the nitrate reductase enzyme activity, reduced nitrogen uptake and nitrate fixation [40]. The overall adverse effect of Cr on growth and development of plants could be serious impairment in uptake of mineral nutrients and water leading to deficiency in shoots [41]. Therefore, the results seem that purslane has tolerated Cr (VI) up to 75 ppm and afterwards, its defense mechanisms have been activated, So that not only the adverse effects compensated, but also it partly relieved stress.

Result of this study showed that MSI and Fv/Fm was decreased with parallel increase of chromium concentration. Vernay *et al.*, showed that in plants under stress with Cr (VI), a decrease in the the quantum yield of PSII electron transport Fv/Fm was observed [49]. The declining slope of Fv/Fm is a good indicator to evaluate photo-inhibition of

plants exposed to environmental stresses [50]. Cr stress is one of the important factors that affect on photosynthesis in terms of CO_2 fixation, electron transport, photophosphorylation, chlorophyll *a* fluorescence parameters and pigment contents and enzyme activities [41,51]. Measuring parameters such as Fv/Fm and Fv/F0 ratios) are an important tool to evaluate disturbances in PSII caused by heavy metals in plants [51]. A gradual decline in FV/FM ratio suggested that Cr decreased the quantum efficiency of PSII photochemistry either by causing a decrease in the rate of primary charge separation or by disconnection of some minor antenna from PSII [41].

Leaf membrane stability index (MSI) was measured as ion leakage [12]. Chaparzadeh and Ghodrati [50] reported that *Allium cepa* exposed to Cu stress showed a statistically decrease in MSI of leaves compared with control plants. Membrane stability was expressed as % relative injury [53]. It was reported that much of injury to plants caused by various stresses is associated with oxidative damage at cellular level such as cell membrane damage [54]. Moreover, heavy metals stimulated lipid peroxidation and damaged the chloroplast membranes plants through the formation of ROS [55]. Janmohammadi *et al.*, indicated H_2O_2 and MDA negatively correlated with MSI [12].

Cr stress increased significantly MDA and H_2O_2 content in *P. oleracea*. Similarly, a frequently reported effect of Cr stress is membrane injury [56]. Chatterjee *et al.*, showed that exposure of Cr induced oxidative stress in turnip, as indicated by an increased accumulation of MDA and H_2O_2 [7]. Moreover, Hao *et al.*, showed that there was a direct relation between heavy metal and increase in oxidative stress as a result of an enhanced level of lipid peroxidation and hydrogen peroxide production in both roots and leaves of contaminated plants [57]. The observed increase in lipid peroxidation and, an indicator of increased oxidative damage severely affected the functioning of plasma membrane and finally lead to the death of the cells [7]. Cr can inhibit uncoupled electron transport, indicating the electron transport chain to be a common site of Cr binding in plants, Also, Cr may be transferred by cytochrome in the mitochondria to reduce it or the reduced heme group of cytochrome may act as a site for Cr binding, blocking electron transport. The sever inhibition of cytochrome oxidase activity may be due to the binding of Cr to complex IV. Moreover, the reduction of Cr (VI) to the less toxic Cr (III) results in the formation of reactive intermediates leading to oxidative damage and cellular injury [58,46]. High production of H_2O_2 and O_2 radicals were reported in many plant species exposed to Cr and the metals has been implicated in the generation of oxidative stress [4, 7]. plants growing under Cr-stressed also stimulate the formation of reactive oxygen species (ROS), which can harm the production of biomolecules [41]. Chromium-induced oxidative stress may cause lipid peroxidation and severe damage to cell membranes [59]. Panda and Choudhury, reported that in wheat, Cr exposed to 1, 10 and 100 mM concentrations initiated the process of lipid peroxidation and increased the amounts of MDA [4].

According to this study, results showed that significant increase in phenolic compound and flavonoid contents of contaminated plants. Similarly, Ghelich *et al.*, reported that total phenols and flavonoids in Pb-treated plants was increased [14]. Recent results showed that flavonoids can facilitate heavy metal tolerance in *Arabidopsis thaliana* [60]. An enhancement of phenylpropanoid metabolism and the amount of phenolic compounds can be observed under different environmental factors and stress conditions [19]. Within heavy metal stress phenolic compounds can act as metal chelators and on the other hand phenolics can straightly scavenge molecular species of active oxygen [17]. Flavonoids also may sequester metal ions by chelating and preventing metal-mediated generation of free radicals and, accordingly, may protect the biological targets from oxidative stress. Also flavonoids can play an important role in limiting metal bioavailability and suppressing metal toxicity [14]. As a result, increasing phenol and flavonoid in purslane can lead to heavy metal stress tolerance.

This survey illustrated that Cr had significant effects on increasing of the POX and CAT activity, but the enzyme APX activity was decreased compared to the control. Similarly to our results, heavy metal increased the CAT and peroxidase activities in *Medicago sativa* L. and *Zea mays* [61]. The activities of antioxidant enzymes were altered by Cr exposure [7]. Furthermore, Kaur *et al.*, showed that a significant increase in POX activity of *Vicia faba* was observed with increasing Cr concentration [46]. Farrag *et al.*, have proved the increase in heavy metal concentrations in the contaminated plants causes parallel increase of

enzyme antioxidant defenses like catalase and glutathione peroxidase which are used as biomarkers of oxidative damage [62]. According to study conducted by Patnaik *et al.*, the antioxidant enzymes such as peroxidases play an important role in protecting plants against Cr toxicity via scavenging ROS production [63]. Thus alterations in the activities of enzymes like SOD, CAT, POD and APX was due to Cr stress induced accumulation of ROS, like superoxide and H_2O_2 [23]. H_2O_2 is scavenged directly by peroxidase and catalase, converting it to H_2O and O_2 [21,23]. Peroxidase is one of the principle enzymes involved in the elimination of ROS under stress. Peroxidase has a higher affinity for H_2O_2 than CAT [23]. CAT is an indispensable enzyme required for ROS detoxification in plants [45]. Also, ascorbate peroxidase enzymes play a key role catalyzing the conversion of H_2O_2 into H_2O, using ascorbate as a specific electron donor [21]. Duhan, reported that decrease in the activity of ascorbate peroxidase with increase in cadmium concentration in radicle and plumule of pea seedling [64]. The decline in activity of APX enzyme has been ascribed to inhibition of enzyme biosynthesis and the denaturation of enzyme proteins [64]. Furthermore, APX can break down H_2O_2, reduced activity of APX probably related to the amount of hydrogen peroxide. Based on the results obtained in plants treated, hydrogen peroxide greatly was increased less than the MDA [64]. Pandy and Choudhury, reported that Cr can degraded protein [4].

Conclusion

Based on the results of this study, it has been found that *P. oleracea* have antioxidative defense mechanism (enzymatic and non enzymatic) that can counteract with the damaging effects of ROS and lipid peroxidation. Imbalance in ROS generation and antioxidant activity results in damage to vital physiological functions, resulting in decrease in growth and development. Moreover, in high concentrations of chromium (100ppm), purslane not only can minimize destructive effects of chromium stress by activating the antioxidant system and increasing level of antioxidants. So, it prevents sever decrease in growth. Therefore, the cultivation of purslane in chrome contaminated soils is justified, due to its medicinal properties, resistance to various concentrations of chromium and high Cr (VI) accumulation in the roots. Considering the high accumulation of chromium was in the root of P. oleracea and increased Bioaccumulation factor was reached a value greater than 1, it is introduced P. oleracea as a suitable candidate for phytoremediation of chromium contaminated soils.

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