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RESEARCH ARTICLE

Chromium induced changes in growth and physiological attributes of Chicory (*Cichorium intybus* L), an important medicinal plant

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ABSTRACT
This study was conducted to determine the impact of different concentrations (25, 37.5 and 50 mg kg^{-1} soil) of chromium (Cr) on growth, photosynthetic pigments, protein content, proline content, activities of antioxidant enzymes and seed yield of *Cichorium intybus* in a pot experiment. The results revealed that all the Cr treatments significantly ($P \leq 0.05$) reduced the growth, photosynthetic pigments (chlorophyll a, chlorophyll b and total chlorophyll and carotenoids contents), protein content and seed yield in *C. intybus*. The activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and proline content in *C. intybus* leaves increased significantly ($P \leq 0.05$) with increasing levels of Cr treatments. Cr accumulation was found greater in the roots than in the shoots and enhanced with increasing Cr concentrations. Hence, *C. intybus* may serve as a bio-meter of Cr in Cr contaminated soils.

Introduction

Heavy metals are the elements having a specific gravity of more than 5g/cm^{-3} that often accumulate in the soil because of uncontrolled waste dumping, mining, widespread use of pesticides and chemical fertilizers (1, 2). Heavy metals such as cadmium, arsenic, chromium, lead, mercury, vanadium and cobalt are non-essential and toxic to plant; in contrast, metals, such as copper, iron, manganese, zinc, copper and iron (3). Accumulation of heavy metals in plants, aquatic animals, human and micro-organisms poses a serious threat to biota and the environment (4). Due to the extensive release from domestic, chemical, agricultural, industrial and technological sources, the toxicity from heavy metals is continuously increasing, which in turn contaminate the soil, water and air (3).

In recent years, chromium (Cr), especially hexavalent Cr, has become a major area of concern in environmental contamination. Among the most hazardous substances, Cr has been ranked 17th by the agency for toxic substances and disease registry (ATSDR) and also the number one carcinogen (5). In plant physiology, Cr does not have any known biological role to date (6). It is believed that the accumulation of excessive Cr levels in plant tissues affects plant growth and impedes various plant morpho-physiological and biochemical processes (7, 8, 9). Typically, the toxicity of Cr affects plant growth by inducing ultrastructural changes of the chloroplast and cell membrane, damaging root cells, decreasing photosynthetic pigments, affecting mineral nutrition and water relations, disturbing nitrogen assimilation and transpiration and altering the activities of various enzymes (10, 11). All these toxic effects of Cr have been attributed to the overproduction of a massive amount of reactive oxygen species (ROS), ultimately disrupting the redox balance in plants (11). The translocation and distribution of Cr within plants depend on its concentration in the growth medium, the oxidation state of the Cr ions and also on the plant species (12).

Cichorium intybus L. (Chicory) belongs to the family Asteraceae and is widely distributed in Asia and Europe (13). This is an introduced species in the present Indian Territory. It is a rich source of compounds like sesquiterpene lactones, flavonoids, unsaturated sterols, insulin, alkaloids, tannins, saponins, vitamins and coumarins (14, 15). The fresh chicory typically contains inulin (68%), sucrose (14%), cellulose (5%), protein (6%), ash (4%) and other compounds (3%), while dried chicory contains approximately 98% inulin and 2% other compounds (13, 16). It has been traditionally used for the treatment of fever, diarrhea, jaundice and gallstones (17) and has been found as a useful bio-monitoring of heavy metals such as Pb, Cd, Cu and Zn (18).

The aim of the present study was to investigate the effects of different doses of Cr on growth,

photosynthetic pigments and protein and proline contents, as well as the activities of antioxidant enzymes (SOD, CAT and POD) in *C. Intybus* L.

Materials and Methods

Plant material and growth conditions

Before starting the experimental work, soil samples were collected randomly from different field beds for analyzing the characteristics of the soil. The samples were analyzed in the Soil Testing Laboratory, Government Agriculture Farm, Quarsi, Aligarh, India. The physicochemical properties of the soil used for the experiment are given in Table 1. Seeds of chicory (*Cichorium intybus* L.) procured from the central institute of medicinal and aromatic plants (CIMAP), Lucknow, were surface decontaminated with 0.01% $HgCl_2$ solution, followed by three times washing with double distilled water (DDW). Earthen pots of 25 x 25 cm were autoclaved at 20 1b pressure/inch^2 for 20 minutes after filling with 4 kg of soil and compost

Table 1. Physiochemical characteristics of experimental soil.

Characteristics	Soil
Soil texture	Sandy loam
CEC (meq 100 g^{-1} soil)	2.87
Ph	7.7
Porosity (%)	40.67
Water holding capacity (%)	35.78
Organic carbon (%)	0.756
NO_3-N (g kg^{-1} soil)	0.293
Chromium (mg kg^{-1})	0.46
Phosphorus (g kg^{-1} soil)	0.135
Potassium (K)	21.00
Magnesium (Mg)	31.44
Calcium (Ca)	19.33
Sodium (Na)	11.92
Bicarbonate	19.22
Carbonate	78.43
Sulphate	17.58
Chloride	28.18

mixture (3:1). Ten seeds of nearly uniform size and weight were sown with uniform distance in each pot. After germination, three plants of equal height and leaf number were maintained in each pot. The pots were kept in the naturally illuminated greenhouse of the Department of Botany, Aligarh Muslim University, Aligarh, India with average day/night temperatures 20 ± 3 and 10 ± 2 °C, respectively. Plants were treated with 0, 25, 37.5 and 50 mg kg^{-1} soil of chromium (Cr) and was applied in the form of dilute aqueous solution of Chromium nitrate [(Ni $(NO_3)_2 4H_2O$]. Each treatment was replicated three times. Irrigation was done with tap water as and when required. The experiment was conducted in a randomized complete block design. The plants were cultivated until full maturity (120 days). Sampling of plants was done 60 days after sowing (DAS) and at maturity (120 DAS) to record different parameters. All decreases or increases described in the results section are in comparison to control.

Estimation of growth attributes

Growth parameters viz., root and shoot length, root and shoot fresh mass and root and shoot dry mass were studied. Plants were harvested and cut at the root-shoot junction and the length of their shoot and root were determined with a metric scale and expressed in centimeters. The fresh mass of shoot and root were recorded on an analytical balance and expressed in gm per plant. To determine plant dry mass, samples were dried in an oven at 60 °C for 48 hrs.

Estimation of chlorophyll and carotenoids contents

The photosynthetic pigments (chlorophyll and carotenoid) in the fresh leaf samples was determined according to the methodology of (19). One gm of freshly cut leaves was ground to fine pulp in 80% acetone using a mortar and pestle. The mixture was centrifuged at 5000 rpm for 5 minutes. The supernatant was collected, and the residue was washed thrice, using 80% acetone. The absorbance of the sample was read on spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) at 645 and 663 nm for chlorophyll and 480 and 510 nm for carotenoids against the blank (80% acetone).

Assay of antioxidant enzymes

To determine the activities of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), and POD (EC 1.11.1.7), fresh leaf tissue was ground in liquid nitrogen and homogenized on ice bath in 3 ml of a solution containing 0.1 mM EDTA (Ethylene diamine tetraacetate), 50 mM potassium phosphate buffer (pH 6.8) and 1% polyvinylpyrrolidone (PVP). For ascorbate peroxidase assay extraction buffer was supplemented with 1.0 mM ascorbic acid. The homogenate was centrifuged at 15000×gm for 15 min at 4 ^{0}C and the supernatant was used as a crude enzyme extract.

Estimation of superoxide dismutase (SOD) activity

SOD activity was determined by its ability to catalyze nitro blue tetrazolium (NBT) to formazan at 560 nm according to the method of (20). Five ml of reaction mixture containing 75 mm NBT, 13 mm methionine, 50 mm phosphate buffer (pH 7.8), 0.1 mm EDTA, 2 mm riboflavin and the enzyme extract. The absorbance of the sample was read at 560 nm using a UV-visible spectrophotometer (UV-1700, Shimadzu, Japan). The difference of percentage reduction of color development in blank and the sample was calculated. Fifty percent reduction in the color was taken as one unit of enzyme activity and was expressed in enzyme units per mg protein (U mg^{-1} protein).

Catalase (CAT) activity

With slight modifications, catalase activity was assayed according to the method of (21) by monitoring the H_2O_2 disappearance at 240 nm. The 3 ml reaction mixture consisted of 3 mM EDTA, 0.1 mM of H_2O_2, 0.1M potassium phosphate buffer and enzyme extract. The reaction was allowed to run for 3 min. The enzyme activity was calculated by using extinction coefficient 0.036 mM^{-1} cm^{-1} at 25 °C and

expressed as enzyme units per ml protein (U mg^{-1} protein) in which one unit enzyme determines the amount necessary to decompose one µmol of H_2O_2 per minute.

Peroxidase (POD) activity

The POD activity was assayed following the methodology described by (22), with slight modifications. The 3 ml reaction mixture contained 20 mM pyrogallol, 20 mM H_2O_2, 25 mM potassium phosphate (pH 6.8) and enzyme extract. The reaction was started after the addition of the enzyme extract and the enzyme activity was determined through the absorbance of colored purpurogallin recorded at 420 nm using a UV-visible spectrophotometer (UV-1700, Shimadzu, Japan) for 1 min at 25 °C. An extinction coefficient of 2.47 mM^{-1} cm^{-1} 25^0C was used to calculate the POD activity and was expressed as enzyme units per milligram protein (U mg^{-1} protein).

Estimation of proline content

The proline content in fresh leaves was estimated following the method of (23), Fresh leaf sample (300 mg) was homogenized in 3 ml of 3 % sulphosalicylic acid. The homogenate was filtered, and the filtrate was then reacted with 1 ml each of ninhydrin and glacial acetic acid for 1 hr in a test tube placed in a water bath at 100 °C. Finally, the sample was transferred to the ice bath and the mixture was extracted with toluene and read at 520 nm using L-proline as a standard.

Protein estimation

The estimation of protein was done using the method of (24). One gm of leaf sample was weighed and macerated in 5 ml of phosphate buffer (0.1 M, pH 7.5) using mortar and pestle. The homogenate obtained was centrifuged at 8000 rpm for 20 min. and the supernatant collected was extracted the process was repeated 4-5 times. Supernatants were then combined and the final volume was made to 50 ml with phosphate buffer. To 1 ml of the above extract, 1 ml of 20% trichloroacetic acid (TCA) was added and centrifuged again at 8000 rpm for 20 min. Pellet was washed twice with acetone and centrifuged again. The supernatant was discarded and the pellet was dissolved in 1 ml of NaOH (0.1N). To 1 ml of aliquots, 5 ml of Bradford reagent was added and mixed thoroughly. Absorbance was recorded at 595 nm against blank. The standard curve was plotted using varying concentrations of Bovine Serum Albumin. Soluble protein content was expressed as mg g^{-1} DW.

Heavy metal analysis

Metal levels in root and shoot were determined at 60 DAS and 120 DAS. Root and shoot samples were dried at 80 °C for 48 hrs in an oven, and the dried tissue was weighed, ground to a fine powder. The powdered samples were then digested with concentrated HNO_3/ $HClO_4$ (3:1, v/v). The Cr content was estimated through atomic absorption spectrophotometer (GBC, 932 plus; GBC Scientific Instruments, Braeside, Australia).

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using R (3.6.1) statistical software (package library, agricolae). Duncan's multiple range test (DMRT) was performed to determine the significance of the difference of means at $P \leq 0.05$.

Results

Plant growth parameters

All treatments except 25 mg kg^{-1} caused significant reduction in shoot and root length, shoot and root fresh and dry weight both at 60 DAS and 120 DAS (Table 2). 50 mg kg^{-1} treatment highest reduction of 28.96% and 39.26% in root length, 20.02% and 17.03% in shoot length, 29.01% and 37.74% in root fresh weight, 29.07% and 23.20% in shoot fresh weight, 29.01% and 37.74% in root dry weight and 29.07% and 23.20% in shoot dry weight over control at 60 DAS and 120 DAS respectively (Table 2).

Photosynthetic pigments, protein and proline content

All treatments caused significant reduction in photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, carotenoid,) and protein content and increase in proline content both at 60 DAS and 120 DAS (Fig. 1). 50 mg kg^{-1} treatment highest reduction of 48.15% and 38.94% in chlorophyll a, 39.53% and 62.28% in chlorophyll b, 46.67% and 48.60% in total chlorophyll content,

Table 2. Effects of different doses of chromium (25, 37. 5 and 50 mg kg^{-1} soil) on the growth attributes of *Cichorium intybus* at 60 and 120 DAS.

	Treatments	Root length (cm)	Shoot length (cm)	Root fresh weight (gm)	Shoot fresh weight (gm)	Root dry weight (gm)	Shoot dry weight (gm)
60 DAS	Control	10.11 ± 0.31a	29.86 ± 0.62a	2.98 ± 0.08a	36.45 ± 0.79a	0.24 ± 0.01a	2.95 ± 0.06a
	25 mg kg^{-1} soil	8.91 ± 0.30b	26.4 ± 0.59b	2.16 ± 0.08b	34.55 ± 0.71a	0.17 ± 0.01b	2.80 ± 0.05a
	37.5 mg kg^{-1} soil	6.63 ± 0.29c	23.63 ± 0.62c	1.62 ± 0.08c	27.62 ± 0.69b	0.13 ± 0.01c	2.24 ± 0.05b
	50 mg kg^{-1} soil	4.71 ± 0.33d	18.9 ± 0.54d	1.15 ± 0.08d	19.59 ± 0.69c	0.09 ± 0.01d	1.59 ± 0.05c
	LSD p ≤ 0.05	**1.03**	**1.98**	**0.27**	**2.41**	**0.02**	**0.19**
120 DAS	Control	21.11 ± 0.49a	44.21 ± 0.77a	8.12 ± 0.16a	73.48 ± 1.18a	0.66 ± 0.01a	5.95 ± 0.09a
	25 mg kg^{-1} soil	18.23 ± 0.44b	39.69 ± 0.60b	7.81 ± 0.15a	70.67 ± 1.19a	0.63 ± 0.01a	5.63 ± 0.10a
	37.5 mg kg^{-1} soil	15.74 ± 0.47c	31.47 ± 0.69c	5.67 ± 0.16b	53.71 ± 1.17b	0.46 ± 0.01b	4.35 ± 0.09b
	50 mg kg^{-1} soil	9.56 ± 0.44d	26.11 ± 0.69d	3.53 ± 0.15c	41.25 ± 1.10c	0.29 ± 0.01c	3.34 ± 0.08c
	LSD p ≤ 0.05	**1.54**	**2.29**	**0.52**	**3.89**	**0.04**	**0.31**

Data are presented as treatments mean ± SE (n = 3). Mean values within a column followed by different letters are statistically significant at p ≤ 0.05 by Duncan's multiple range test.

37.84% and 33.70% in carotenoids content, and 34.72% and 41.15% in protein content over control at 60 DAS and 120 DAS respectively. Same treatment caused highest increase of 69.92 and 75.89% in proline content over control at 60 DAS and 120 DAS (Fig. 1).

Antioxidative defense enzymes

All treatments caused increase in the activities of SOD, CAT and POD at 60 DAS and 120 DAS (Fig. 2). 50 mg kg^{-1} caused maximum increase of 179.23% and 124.1% in SOD activity, 21.88% and 32.53% in CAT activity and 39.28% and 68.44% in POD activity over control at 60 DAS and 120 DAS respectively (Fig. 2).

Seed yield per plant

Plants treated with 37.5 and 50 mg kg^{-1} Cr showed a significant ($P \leq 0.05$) reduction in seed yield per plant as compared to control (Fig. 3). Plants treated with 25 mg kg^{-1} Cr resulted in a statistically non-significant ($P \leq 0.05$) reduction in seed yield per plant compared to control. Plants treated with 50 mg kg^{-1} Cr caused a maximum significant ($P \leq 0.05$) reduction of 54.76% in seed yield per plant over control (Fig. 3).

Cr uptake in root and shoot under Cr stress

As compared to the control, Cr concentrations in roots and shoots of *C. intybus* were significantly ($P \leq 0.05$) increased with increasing levels of Cr both at 60 DAS and 120 DAS (Fig 4). Accumulation of Cr was higher in roots of *C. intybus* as compared to shoots. Plants treated with 50 mg kg^{-1} Cr showed a maximum significant ($P \leq 0.05$) increase of 620.41% and 746.88% in root and 378.57% and 621.62% in the shoot of *C. intybus* over control at 60 DAS and 120 DAS, respectively (Fig. 4).

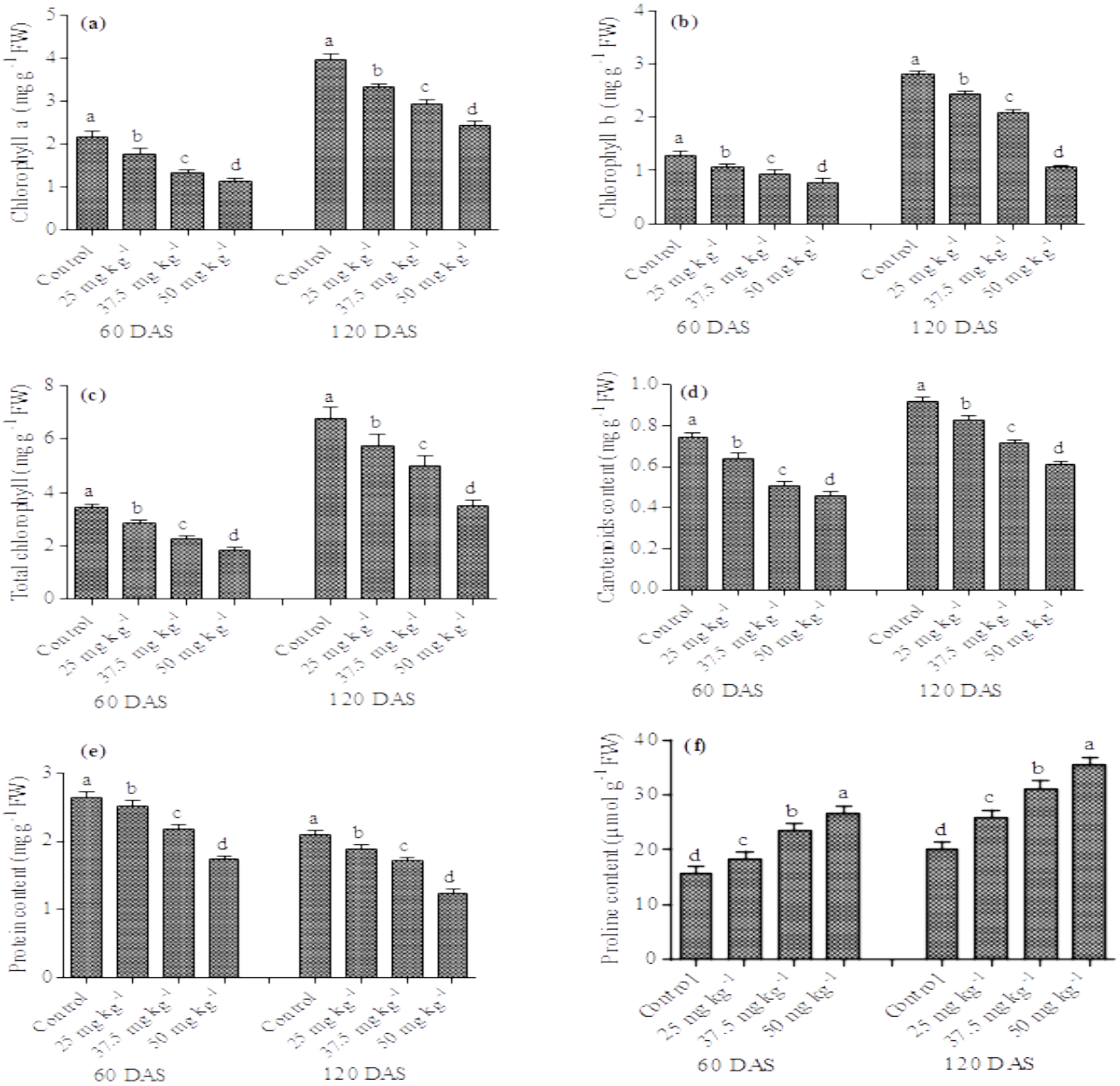

Fig. 1. Effects of different doses of chromium (Cr) on chlorophyll a **(a)** chlorophyll b **(b)** total chlorophyll **(c)** carotenoids content **(d)**, protein content **(e)** and proline content **(f)** of *Cichorium intybus* at 60 and 120 days after sowing (DAS), treated with 25, 37. 5 and 50 mg kg^{-1} soil of Cr. Data are presented as treatments mean ± SE (n=3). The different letter above the bars shows that data are significantly different at $P \leq 0.05$ by Duncan's multiple range test.

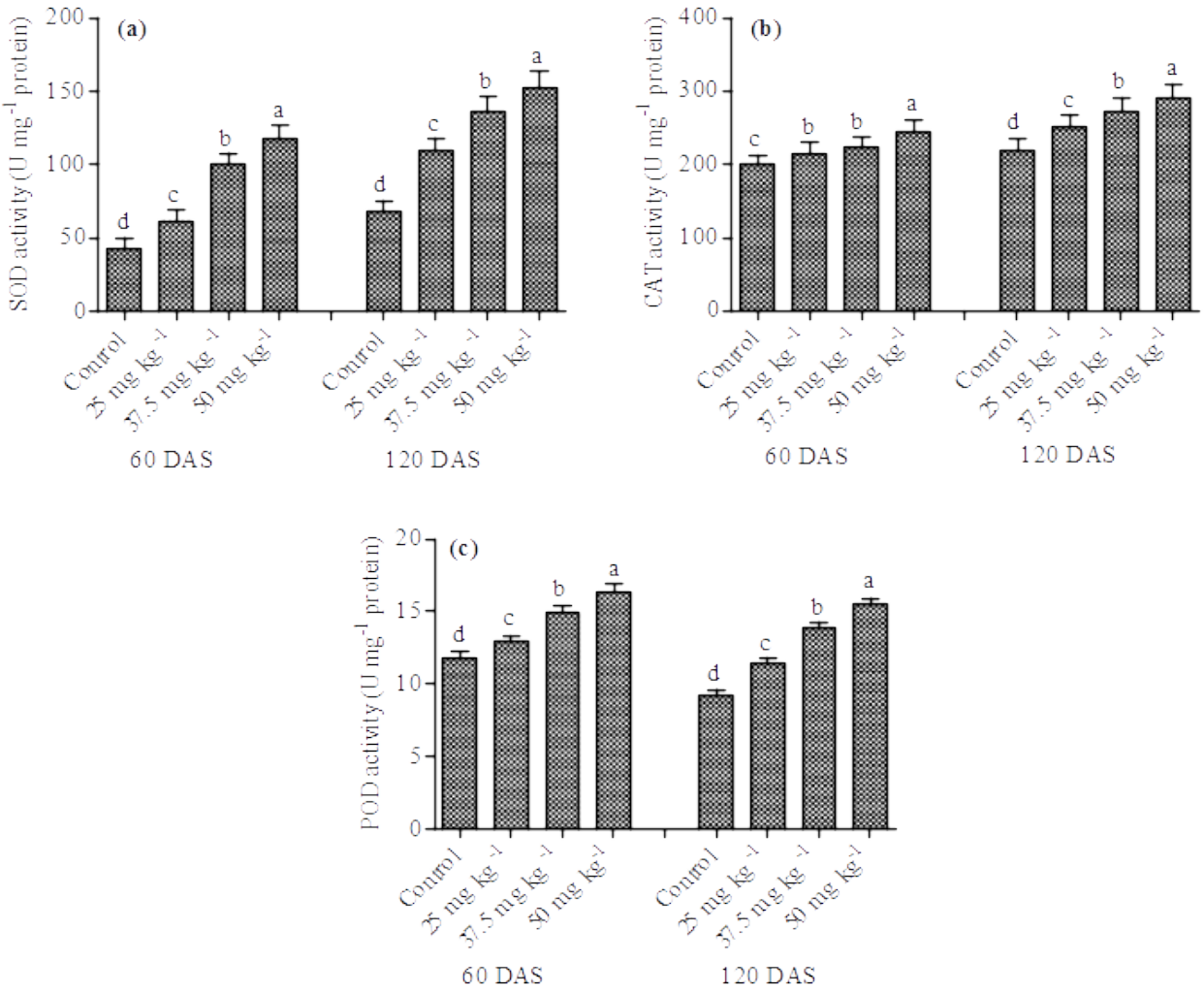

Fig. 2. The activity of SOD **(a)** CAT **(b)** and POD **(c)** in *Cichorium intybus* treated with 25, 37. 5 and 50 mg kg^{-1} soil of Cr at 60 and 120 DAS. Data are presented as treatments mean ± SE (n=3). The different letter above the bars shows that data are significantly different at $P \leq 0.05$ by Duncan's multiple range test. SOD = superoxide dismutase, CAT = catalase, POD = peroxidase, Cr = chromium, DAS = days after sowing.

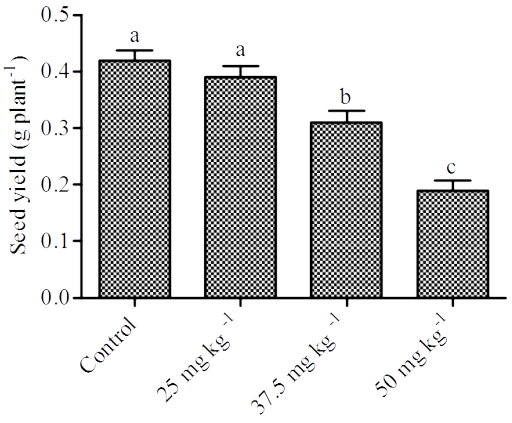

Fig. 3. Seed yield of *Cichorium intybus* treated with Cr at 25, 37. 5 and 50 mg kg^{-1} soil. Data are presented as treatments mean ± SE (n=3). The different letter above the bars shows that data are significantly different at $P \leq 0.05$ by Duncan's multiple range test. Cr = chromium.

Discussion

Plant growth parameters showed a significant decline in response to all treatments of Cr. Previous studies demonstrated that, Cr at low concentration promotes the growth and yield of crop plants (25). Our different results could be due to the genotypes differences, as this study wild plant was used, which

may respond differently to Cr stress than crop plants (7). In contrast, a linear reduction in maize biomass was recorded with increasing Cr concentrations (26), which is in accordance with the findings of this investigation. Cr toxicity is reported to impede the essential metabolic processes which affect the growth of plants (9). Moreover, Cr toxicity induces ultrastructural modifications of the chloroplast and cell membrane, affecting transpiration and nitrogen assimilation, damaging root cells, altering the activities of several enzymes, disturbing water relations and mineral nutrition and hence, reducing plant growth (11, 27). These Cr-induced toxic effects may be attributed to the massive production and release of reactive oxygen species (ROS), which ultimately interrupt the plant's redox balance (11).

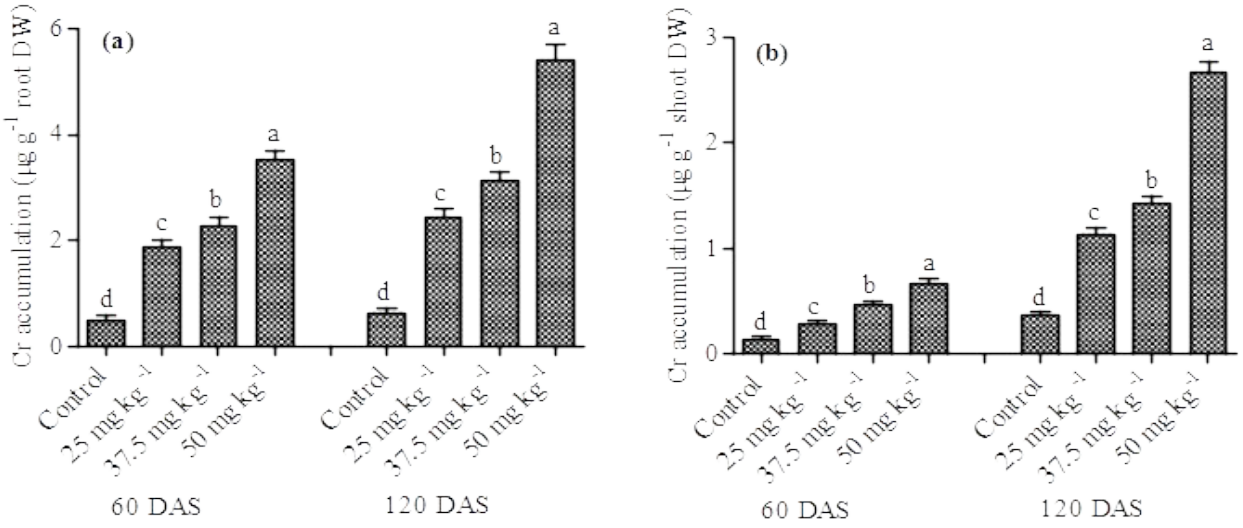

Fig. 4. Root Cr content **(a)** and shoot Cr content **(b)** in *C. intybus* treated with 25, 37. 5 and 50 mg kg^{-1} soil of Cr at 60 and 120 DAS. Data are presented as treatments mean ± SE (n=3). The different letters above the bars show that data are significantly different at $P \leq 0.05$ by Duncan's multiple range test. Cr = chromium, DAS = days after sowing.

In the present study, all the Cr treatments significantly reduced chlorophyll and carotenoids contents in *C. intybus*. The decrease in photosynthetic pigments due to Cr-induced toxicity has been reported in various plant species such as *Ocimum tenuiflorum* (28), *Pistia stratiotes* (29), *Camellia sinensis* (30), *Zea mays* (31) and *Citrus limonia* and *C. reshni* (32). The decrease in photosynthetic pigments under Cr toxicity could be due to the impairment of biosynthetic enzymes of chlorophyll (27, 33) and degradation of δ-aminolevulinic acid dehydratase (ALAD), leading to a decrease in photosynthetic pigments (34). Therefore, the photosynthetic performance of plants is compromised under Cr stress as a result of interaction with the biosynthesis of chlorophyll molecules.

In the current investigation, all the treatments of Cr reduced the leaf protein content. It has been reported that Cr toxicity induces the degradation of proteins which also results in the inhibition of nitrate reductase activity (35). In response to Cr toxicity, the activities of various antioxidative enzymes change drastically. Numerous reports are available concerning the response of plant antioxidative defense system to various abiotic stresses (36). It has been reported that heavy metal-induced oxidative stress enhances SOD, CAT, POD and free proline contents of the plant (7, 37). In the present study, in response to Cr toxicity, the activities of SOD, CAT and POD increased. These results are in line with (38), who observed an increase in SOD and POD activity with increasing concentrations of Cr. However, under any stress condition, the activities of these enzymes may vary with the duration, crop species and tissues (11). For instance, *Echinochloa colona* plants showed enhanced activities of CAT and POD in tolerant calluses than in non-tolerant ones (39). Similarly, during stress, proline makes a metal-poly-chelatins complex which induces the tolerance to heavy metal stress in plants (37). An increase in the contents of proline upon Cr stress has been attributed to enhanced uptake of certain elements like Cl (7). At higher Cr levels, an increase in proline was recorded, which correlates with enhanced activity of POD in leaves of *C. intybus*, suggesting a strong interaction between proline content and POD.

In this study, Cr accumulation was found higher in roots than in shoots. Higher concentrations of Cr in roots may be due to the presence of Cr exclusion strategy in *C. intybus,* as reported previously in *Elodea canadensis* (40). It has been reported earlier that plants accumulate more Cr in roots as compared to shoots (41). The minimum aerial translocation of Cr has been reported in maize (42), cauliflower (43), pea (44) and *Solanum nigrum* and *Parthenium hysterophorus* (7). Cr is accumulated in the roots, possibly through the action of the reductase enzyme, which causes the reduction of Cr (VI) to Cr (III) (7, 42). Moreover, the higher accumulation of Cr in the roots than in shoots could be due to Cr immobilization in root cortex cells (45) to prevent the higher Cr translocation to the aerial parts, which may be a natural toxicity response of *C. intybus* (46).

Conclusion

All the Cr treatments significantly reduced the growth and various physiological attributes of *C. intybus*. The activities of antioxidative defense enzymes and proline content in *C. intybus* leaves increased substantially with increasing levels of Cr treatments. The accumulation of Cr was found higher in the roots than in the shoots of *C. intybus*. Therefore, *C. intybus* from Cr contaminated soils should not be used for medicinal purposes due to higher Cr content.

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Authors' contributions

AQ performed the experiment, analyzed the data, and wrote the manuscript; AAK designed the experiment, edited the manuscript and supervised the overall work.

Conflict of interests

The authors declare that they have no conflict of interest.

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