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Choline Chloride Mediates Chromium Tolerance in Spinach (*Spinacia oleracea* L.) by Restricting its Uptake in Relation to Morpho-physio-biochemical Attributes

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Abstract

In the current industrial scenario, chromium (Cr) as a metal is of great importance but poses a major threat to the ecosystem. In the present study, the effect of different levels of Cr, i.e., 0 (no Cr), 50, and 100 µM in the soil on growth, photosynthetic pigments, gas exchange characteristics, oxidative stress biomarkers, antioxidants machinery (enzymatic and non-enzymatic antioxidants), ions uptake, organic acids exudation, and Cr uptake in different parts of plant were investigated with and without the exogenous application of choline chloride i.e., 0 (no choline chloride), 2–5 mM in Cr-stressed spinach (*Spinacia oleracea* L.). Our results depicted that Cr addition to the soil significantly ($P < 0.05$) decreased plant growth and biomass, gas exchange attributes, and minerals uptake by *S. oleracea* as compared to the plants grown without addition of Cr. However, Cr toxicity boosted the production of reactive oxygen species (ROS) by increasing the contents of malondialdehyde (MDA), which is the indication of oxidative stress in *S. oleracea* and was also manifested by hydrogen peroxide (H_2O_2) contents and electrolyte leakage to the membrane-bounded organelles. Although activities of various antioxidative enzymes like superoxidase dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) and non-enzymatic antioxidants like phenolic, flavonoid, and ascorbic acid, anthocyanin contents initially increased up to a Cr level of 50 µM but decreased gradually with the further increased in the Cr level of 100 µM in the medium, compared to those plants which were grown in the control treatment. Results also revealed that the soluble sugar, reducing sugar, and non-reducing sugar were decreased in plants grown under elevating Cr levels but increased the Cr accumulation in the roots and shoots of *S. oleracea*. Although results also illustrated that the application of choline chloride also decreased Cr toxicity in *S. oleracea*

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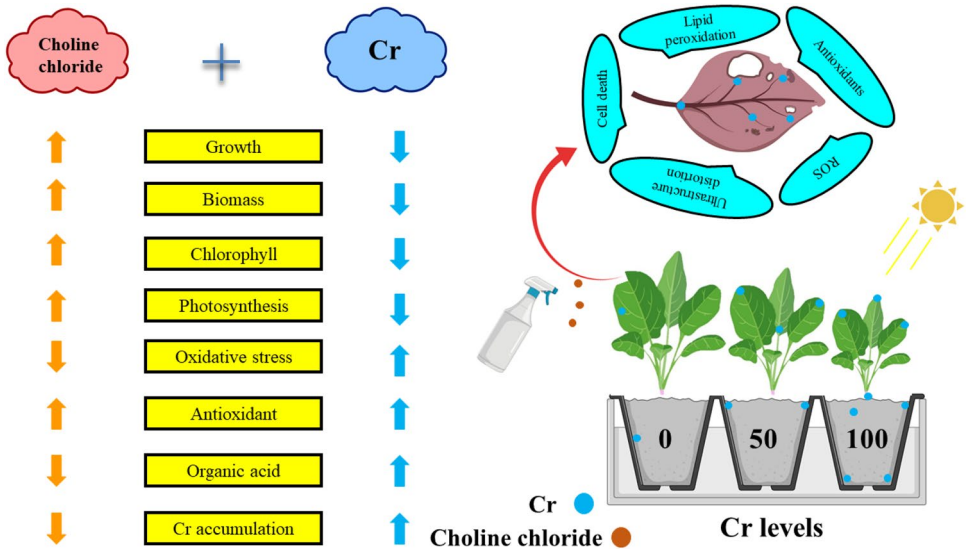
seedlings by increasing antioxidant capacity and, thus, improved the plant growth and biomass, photosynthetic pigments, gas exchange characteristics, and decrease oxidative stress in the roots and shoots of *S. oleracea* seedlings, compared to those plants which were not artificially supplied by choline chloride. Research findings, therefore, suggested that the choline chloride application can ameliorate Cr toxicity in *S. oleracea* seedlings and resulted in improved plant growth and composition under metal stress as depicted by balanced exudation of organic acids.

Graphic Abstract



Keywords Antioxidant compounds · Choline chloride · Heavy metal · Photosynthesis · Vegetative crop

Introduction

Contamination of agricultural soils with trace metals presents lethal consequences in terms of diverse ecological and environmental problems that entail entry of metal in food chain, soil deterioration, plant growth suppression, yield reduction, and alteration in microbial community (Handa et al. 2018b; Nagajyoti et al. 2010; Riaz et al. 2020; Saleem et al. 2020b). The buildup of toxic metals in various compartments of the environment is hazardous for biotic health including humans due to bioaccumulation and biomagnification of heavy metals in living organisms (Afzal et al. 2020; Ali et al. 2021; Hashmat et al. 2021; Javed et al. 2021). Chromium (Cr) is a potentially toxic metal which does not have any essential metabolic function in plants, and its excess concentration in the soil may cause toxic effects in plants and reduce the growth, photosynthesis, mineral nutrients, and quality of the crops (Gautam et al. 2020b; Handa et al. 2018a, 2019; Shahid et al. 2017; Zaheer et al. 2020c). Environmental contamination of Cr has gained substantial consideration worldwide because of its high levels in the water and soil originating from numerous natural and anthropogenic activities, and it is eventually accumulating in crops from contaminated soils and imparts severe health risks in humans via food chain contamination (Gautam et al. 2020a; Jan et al. 2020; Zaheer et al. 2020b). Higher Cr levels in plants cause ultra-structural alterations (Junaid et al. 2016; Zaheer et al. 2020d), oxidative stress in plants, and increased electrolyte leakage (EL) and malondialdehyde (MDA) concentrations, whereas induced alterations in antioxidant enzyme activities such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) and ascorbate peroxidase (APX) (Kamran et al. 2020; Saleem et al. 2020h; Sallah-Ud-Din et al. 2017; Tripathi et al. 2012). Previously, antioxidative enzymes played a significant role in the reduction of Cr phytotoxicity in *Lemna minor* (Sallah-Ud-Din et al. 2017), *Brassica napus* (Zaheer et al. 2020c), *Vigna radiata* (Gautam et al. 2020b), *Spinacia oleracea* (Zaheer et al. 2020a), and *Triticum aestivum* (Ali et al. 2015) grown under excessive Cr concentrations. Hence, it is immensely required to safeguard plant from Cr toxicity to counter the phytotoxicity and oxidative stress triggered by the uptake of Cr in plants.

Choline chloride is a multifunctional metabolite necessary for plant development and also acts as an antioxidant in many plant tissues (Radošević et al. 2015). Application of choline chloride gives freshness to the plant and its foliar spray enhances stress tolerance by altering various biological

and physical processes (Pavić et al. 2019). It was reported that, choline and its derivatives increase rooting and root growth in *Rehmannia glutinosa* seedlings, when subjected to abiotic stress (Zhao et al. 2007) and also increased the plant growth and biomass in *Triticum aestivum* when subjected to abiotic stress (Salama et al. 2011). Although it is notable point that choline chloride and its analogs have greater biological impacts as well as in vitro and in vivo appear aspromoter of process of photosynthesis affects cytokinin concentration and morphogenesis, to play a vital role in growth and morphogenesis in the various plant species (Radošević et al. 2015). Spinach (*S. oleracea* L.) belongs to family Chenopodiaceae that is a worldwide cultivated vegetable crop because of its relative high growth rate, its increased production of biomass, and its use of heavy metals and other important soil nutrients (Agarwal et al. 2018; Zaheer et al. 2020a). Furthermore, *S. oleracea* has been extensively investigated on the basis of these distinctive features to analyze its growth performance and stress responses to various heavy metals (Sehrish et al. 2019; Zaheer et al. 2020d). *S. oleracea* also can tolerate heavy metal stress, due to its strong antioxidant defense system and various other physiological mechanisms (Shahid et al. 2020; Younis et al. 2016). Since *S. oleracea* has the ability to tolerate various environmental stresses, due to its specific biological and physiological processes, it can also withstand Cr stress (Sehrish et al. 2019; Zaheer et al. 2020a).

During the recent years, certain heavy metals have received considerable attention on plant morphology and physiology owing to increasing environmental exposure which is also likely to have negative impact on vegetative crops including *S. oleracea*. Previously, many studies have been conducted on different plant species using various growth regulators or chelators (Kamran et al. 2020; Parveen et al. 2020; Saleem et al. 2020d, 2020f) in different soils (polluted with various heavy metals). But there are very few studies which have been conducted on the effect of choline chloride in any plant species under heavy metal-stressed soil/medium. So, we aimed to designed this study which will increase our knowledge about the effect of different concentrations of Cr in the soil on plant growth and biomass, photosynthetic pigments, gas exchange characteristics, oxidative stress biomarkers, antioxidants machinery (enzymatic and non-enzymatic antioxidants), ions uptake, organic acids exudation, and Cr uptake in different parts of plant under the application of choline chloride. The results from the present study gave a new insight that the use of choline chloride in heavy metals studies may be beneficial and can improve plant yield under Cr-contaminated soil.

Materials and Methods

Experimental Setup

The present study was conducted in the botanical garden under greenhouse environment belonging to the Department of Botany, Government College University, Faisalabad 38000, Punjab, Pakistan (31° 24/N, 73° 04/E). Healthy and mature seeds of Spinach (*S. oleracea* L.) were collected from Ayyub Agricultural Research Institute (AARI) Faisalabad, Punjab, Pakistan. The seeds of *S. oleracea* were surface sterilized with (0.1%) bleaching powder for 10–20 min and washed gently with deionized water before starting an experiment. All the pots were categorized into the three groups: (I), without any Cr treatment (II), addition of 50 µM of Cr, and (III) addition of 100 µM of Cr. Before sowing the plants, Cr was artificially supplied using potassium dichromate ($K_2Cr_2O_7$) at various concentrations. After adding the Cr, the pots were equilibrated for 2 months with four cycles of saturation with distilled water and air drying. Before seed sowing, the salt of choline chloride $[(CH_3)_3NCH_2CH_2OH]$ Cl was also added at various concentrations, i.e., 0 (no choline chloride), 2–5 mM and mixed vigorously with the contaminated soil. The soil used for this experiment was collected from experimental station of Government College University, Faisalabad and was air dried, passed through 5-mm sieve and was water saturated twice before being used in pots. The physio-chemical properties of the soil used in this study are presented in Table S1. The pots used in this study were rotated regularly in order to avoid environmental effects on the plants. All plants in the glass house territory received natural light, with day/night temperature of 35/40 °C and day/night humidity of 60/70%. The total duration of experimental treatments was two months under controlled conditions. Irrigation with Cd-free water and other intercultural operations were performed, when needed. All pots were placed in completely randomized design (CRD) having five plants in each pot with four replicates of each treatment. The same level of Cr was also used by previous studies (Sirirat et al. 2012; Zeng et al. 2011), and same levels of choline chloride were also used by Wang and Xiao (2009).

Sampling and Data Collection

After two months, three seedlings were up rooted and washed gently with the help of distilled water to eliminate the aerial dust and deposition. All plants were harvested on 15th October 2018 for the analysis of various morphological parameters. Leaves and root samples from each treatment group were picked after one month for chlorophyll,

carotenoid, and antioxidant analysis. The leaves were washed with distilled water, placed in liquid nitrogen, and stored at − 80 °C for further analysis. The plants from each treatment were washed with tap water to remove debris and waste and then with distilled water. Plant length (shoot and root) was measured using measuring scale. Number of leaves were measured by simple counting the leaves while leaf area was measured was also measured. For plant fresh weight (shoot and root) was determined by measuring the weight of plant with a digital weighting balance. Later, root and shoot were dried in an oven at 105 °C for 1 h, then at 70 °C for 72 h to determine their dry weight. Roots were immersed in 20 mM Na_2EDTA for 15–20 min to remove Cr adhered to the surface of roots. Then, roots were washed thrice with distilled water and finally once with deionized water and dried for further analysis. Although this experiment was conducted in pots, for the collection of organic acids, two seedlings were transferred to the rhizoboxes which consist of plastic sheet, nylon net, and wet soil (properties of soil are given in Table S1) (Javed et al. 2013; UdDin et al. 2015). After 48 h, plants were taken from the rhizoboxes and the roots were washed with redistilled water to collect the exudates from root surface. The samples were filtered through a 0.45-µm filter (MillexHA, Millipore) and collected in eppendorf tubes (Greger and Landberg 2008). The collected samples were mixed with NaOH (0.01 M) in order to analyze the organic acids. However, the samples used for analysis of oxalic acid were not treated with NaOH (Javed et al. 2013).

Photosynthetic Pigments and Gas Exchange Characteristics

Leaves were collected for the determination of chlorophyll and carotenoid contents. For chlorophylls, 0.1 g of fresh leaf sample was extracted with 8 mL of 95% acetone for 24 h at 4 °C in the dark. The absorbance was measured by a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan) at 646.6, 663.6, and 450 nm. Chlorophyll content was calculated by the standard method of (Arnon 1949).

Net photosynthesis (*Pn*), leaf stomatal conductance *(Gs)*, transpiration rate (*Ts*), and intercellular carbon dioxide concentration (*Ci*) were measured from 3 different plants in each treatment group. Measurements were conducted between 11:30 and 13:30 on days with a clear sky. Rates of leaf *Pn*, *Gs, Ts*, and *Ci* were measured with a LI-COR gas exchange system (LI-6400; LI-COR Biosciences, Lincoln, NE, USA) with a red-blue LED light source on the leaf chamber. In the LI-COR cuvette, CO_2 concentration was set as 380 mmol mol^{-1} and LED light intensity was set at 1000 mmol m^{-2} s^{-1}, which was the average saturation intensity for photosynthesis in *S. oleracea* (Austin 1990).

Oxidative Stress Indicators

The degree of lipid peroxidation was evaluated as MDA contents. Briefly, 0.1 g of frozen leaves were ground at 4 °C in a mortar with 25 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1% polyethene pyrrole. The homogenate was centrifuged at 10,000×*g* at 4 °C for 15 min. The mixtures were heated at 100 °C for 15–30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was recorded by using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad, United States) at wavelengths of 532, 600, and 450 nm. Lipid peroxidation was expressed as l mol g^{-1} by using the formula: 6.45 (A532-A600)-0.56 A450. Lipid peroxidation was measured by using a method previously published by (Heath and Packer 1968).

To estimate H_2O_2 content of plant tissues (root and leaf), 3 mL of sample extract was mixed with 1 mL of 0.1% titanium sulfate in 20% (v/v) H_2SO_4 and centrifuged at 6000×*g* for 15 min. The yellow color intensity was evaluated at 410 nm. The H_2O_2 level was computed by the extinction coefficient of 0.28 $mmol^{-1}$ cm^{-1}. The contents of H_2O_2 were measured by the method presented by (Jana and Choudhuri 1981).

Stress-induced EL of the uppermost stretched leaves was determined by using the methodology of (Dionisio-Sese and Tobita 1998). The leaves were cut into minor slices (5 mm length) and placed in test tubes having 8 mL distilled water. These tubes were incubated and transferred into a water bath for 2 h prior to measuring the initial electrical conductivity (EC_1). The samples were autoclaved at 121 °C for 20 min and then cooled down to 25 °C before measuring the final electrical conductivity (EC_2). EL was calculated by the following formula;

$$EL = \left(EC_1/EC_2\right) \times 100.$$

Antioxidant Enzyme Activities

To evaluate enzyme activities, fresh leaves (0.5 g) were homogenized in liquid nitrogen and 5 mL of 50 mmol sodium phosphate buffer (pH 7.0), including 0.5 mmol EDTA and 0.15 mol NaCl. The homogenate was centrifuged at 12,000×*g* for 10 min at 4 °C, and the supernatant was used for measurement of SOD and POD activities. SOD activity was assayed in 3 mL reaction mixture containing 50 mM sodium phosphate buffer (pH 7), 56 mM nitro-blue tetrazolium, 1.17 mM riboflavin, 10 mM methionine, and 100 µL enzyme extract. Finally, the sample was measured by using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad). Enzyme activity was measured by

using a method by (Chen and Pan 1996) and expressed as U g^{-1} FW.

POD activity in the leaves was estimated by using the method of (Sakharov and Ardila 1999) by using guaiacol as the substrate. A reaction mixture (3 mL) containing 0.05 mL of enzyme extract, 2.75 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2, and 0.1 mL of 4% guaiacol solution was prepared. Increases in the absorbance at 470 nm because of guaiacol oxidation were recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme.

CAT activity was analyzed according to (Aebi 1984). The assay mixture (3.0 mL) was composed of 100 µL enzyme extract, 100 µL H_2O_2 (300 mM), and 2.8 mL 50 mM phosphate buffer with 2 mM ETDA (pH 7.0). The CAT activity was measured from the decline in absorbance at 240 nm as a result of H_2O_2 loss ($\varepsilon = 39.4$ mM^{-1} cm^{-1}).

APX activity was measured according to Nakano and Asada (1981). The mixture containing 100 µL enzyme extract, 100 µL ascorbate (7.5 mM), 100 µL H_2O_2 (300 mM), and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0) was used for measuring APX activity. The oxidation pattern of ascorbate was estimated from the variations in wavelength at 290 nm ($\varepsilon = 2.8$ mM^{-1} cm^{-1}).

Non-enzymatic Antioxidants, Sugars, and Proline Contents

Plant ethanol extracts were prepared for the determination of non-enzymatic antioxidants and some key osmolytes. For this purpose, 50 mg of dry plant material was homogenized with 10 mL ethanol (80%) and filtered through Whatman No. 41 filter paper. The residue was re-extracted with ethanol, and the 2 extracts were pooled together to a final volume of 20 mL. The determination of flavonoids (Pękal and Pyrzynska 2014), phenolics (Bray and Thorpe 1954), ascorbic acid (Azuma et al. 1999), anthocyanin (Lewis et al. 1998), and total sugars (Dubois et al. 1956) was performed from the extracts.

Fresh leaf material (0.1 g) was mixed thoroughly in 5 mL aqueous sulphosalicylic acid (3%). The mixture was centrifuged at 10,000×*g* for 15 min, and an aliquot (1 mL) was poured into a test tube having 1 mL acidic ninhydrin and 1 mL glacial acetic acid. The reaction mixture was first heated at 100 °C for 10 min and then cooled in an ice bath. The reaction mixture was extracted with 4 mL toluene, and test tubes were vortexed for 20 s and cooled. Thereafter, the light absorbance at 520 nm was measured by using a UV–VIS spectrophotometer (Hitachi U-2910, Tokyo, Japan). The free proline content was determined on the basis of the standard curve at 520 nm absorbance and expressed as µmol $(g\ FW)^{-1}$ (Bates et al. 1973).

Nutrient Contents

For nutrient analysis, plant roots and shoots were washed twice in redistilled water, dipped in 20 mM EDTA for 3 s, and then, again, washed with deionized water twice for the removal of adsorbed metal on the plant surface. The washed samples were then oven dried for 24 h at 105 °C. The dried roots and shoots were digested by using a wet digestion method in HNO_3: $HClO_4$ (7:3 V/V) until clear samples were obtained. Each sample was filtered and diluted with redistilled water up to 50 mL. The root and shoot contents of Fe, Mg, and P and were analyzed by using Atomic Absorption Spectrophotometer (AAS) model Agilent 240FS-AA.

Root Exudates Analysis and Cr Contents

In order to determine the concentration of organic acids, freeze-dried exudates were mixed with ethanol (80%), and 20 µL of the solutions was injected into the C18 column (Brownlee Analytical C-183 µm; length 150 mm × 4.6 mm^2, USA). Quantitative analysis of organic acids in root exudates was executed with high-performance liquid chromatography (HPLC), having a Flexer FX-10 UHPLC isocratic pump (PerkinElmer, MA, USA). The mobile phase used in HPLC was composed of an acidic solution of aceto-nitrile containing aceto-nitrile:H_2SO_4:acetic acid in ratios of 15:4:1, respectively, and pH of 4.9. The samples were analyzed at a flow rate of 1.0 mL min^{-1} for a time period of 10 min. The inner temperature of the column was fixed at 45 °C, and quantification of organic acids was carried out at 214 nm wavelength with the help of a detector (UV–VIS Series 200, USA) as described by UdDin et al. (2015). Freeze-dried samples were dissolved in redistilled water, and the pH of the exudates was recorded with LL micro-pH glass electrode by using a pH meter (ISTEK Model 4005–08007 Seoul, South Korea).

Plant samples were vigilantly digested via di-acid (HNO_3-$HClO_4$) technique. 0.5 g dry sample of roots and shoots of the plants were taken into the flask having 10 mL of HNO3-HClO4 (3:1, v:v); this collection was then retained overnight. Final digestion of these plants' samples was completed after the addition of HNO_3 (5 mL) and then placed on the hot plate for complete digestion as described by Rehman et al. (2015). Atomic absorption spectrophotometer (AAS) was used to investigate the exact amount of Cr in shoots and roots of the plant.

Statistical Analysis

The normality of data was analyzed using IBM SPSS software (Version 21.0. Armonk, NY, USA: IBM Corp) through a multivariate post-hoc test, followed by a Duncan's test in

order to determine the interaction among significant values. Thus, the differences between treatments were determined by using ANOVA, and the least-significant difference test ($P < 0.05$) was used for multiple comparisons between treatment means where significant Tukey's HSD post-hoc test was used to compare the multiple comparisons of means. The analysis showed that the data in this study were almost normally distributed. The graphical presentation was carried out using Origin-Pro 2017. The Pearson correlation coefficients between the measured variables of *S. oleracea* were also calculated. The plots of principal component analysis on *S. oleracea* parameters were carried out using the RStudio software.

Results

Plant Growth and Photosynthetic Measurements

In this study, we elucidated various growth parameters, photosynthetic pigments, and gas exchange characteristics under the various levels of choline chloride in *S. oleracea* grown under Cr-polluted soil. We presented the various morphological traits of *S. oleracea* seedlings in Fig. 1 and photosynthetic pigments and gas exchange characteristics in Fig. 2, which were grown in Cr-polluted environment under the application of various levels of choline chloride. Our results depicted that shoot length, root length, number of leaves, leaf area, stem fresh weight, root fresh weight, shoot dry weight, root dry weight, chlorophyll contents, carotenoid contents, net photosynthesis, stomatal conductance, transpiration rate, and intercellular CO_2 were decreased with the increase in the Cr levels (50–100 µM) in the soil when compared with the plants grown without the addition of Cr in the soil in both *S. oleracea* seedlings (Figs. 1 and 2). We also noticed that various growth parameters, photosynthetic pigments, and gas exchange characteristics could be increased under the toxic concentration of Cr in the soil by the exogenous application of choline chloride (Figs. 1 and 2). In addition, results also showed that exogenous application with choline chloride increased all growth parameters, photosynthetic pigments, and gas exchange characteristics in *S. oleracea* seedlings, compared to those plants that were grown without the exogenous application with choline chloride in the natural soil. We have noticed that Cr toxicity did not significantly affected intercellular CO_2 (C*i*), and also application of choline chloride did not significantly influence C*i* in *S. oleracea* under all levels of Cr in the soil (Figs. 1 and 2).

Oxidative Stress Indicators

Oxidative stress markers, i.e., MDA contents, hydrogen peroxide (H_2O_2) initiation, and EL (%) in the roots and leaves of *S. oleracea* seedlings grown in toxic concentration of Cr in the soil, were also measured in the present study. The results regarding MDA, H_2O_2, and EL in the roots and leaves of *S. oleracea* seedlings grown under the application of choline chloride in Cr-polluted soil are presented in Fig. 3. From the given results, we also elucidated that increasing concentration of Cr in the soil induced a significant ($P < 0.05$) increased in the contents of MDA, H_2O_2 initiation, and EL (%) in the roots and leaves of *S. oleracea* seedlings, when compared with those plants, which were grown without the addition of Cr concentration in the soil (Fig. 3). Results also showed that the contents of MDA, H_2O_2 initiation, and EL (%) were significantly ($P < 0.05$) higher in the roots when compared to the leaves of *S. oleracea* seedlings at all levels of Cr in the soil. Results also illustrated that the application of choline chloride decreased, the contents of MDA, H_2O_2 initiation, and EL (%) in the roots and leaves of *S. oleracea* seedlings, compared with those plants, which were grown without the exogenous application with choline chloride in the soil. In addition, at all levels of Cr stress (50–100 µM), the contents of MDA, H_2O_2 initiation, and EL (%) were decreased with the increasing levels of choline chloride (2–5 mM) in the soil, compared with those plants, which were grown without the application of choline chloride.

Enzymatic Antioxidant Enzymes

In the present study, we also measured various enzymatic antioxidants, i.e., SOD, POD, CAT, and APX from the roots and leaves of *S. oleracea* seedlings grown under the application of choline chloride in Cr-polluted soil. The data regarding the activities of antioxidants (SOD, POD, CAT, and APX) in the roots and leaves of *S. oleracea* seedlings grown under the application of choline chloride in Cr-polluted soil are presented in Fig. 4. According to the given results, we elucidated that increasing concentrations of Cr in the soil increased the activities of antioxidants (SOD, POD, CAT, and APX) in the roots and leaves of *S. oleracea* seedlings, compared with those plants, which were grown without the addition of Cr in the soil. The activities of various antioxidants (SOD, POD, CAT, and APX) in the roots and leaves of *S. oleracea* seedlings initially increased up to a Cr level of 50 µM in the soil, but further increase in Cr concentration in the soil (100 µM) induced a significant ($P < 0.05$) decrease in antioxidants in the roots of leaves of *S. oleracea* seedlings (Fig. 4). Results also showed that the activities of antioxidants (SOD, POD, CAT, and APX) were significantly ($P < 0.05$) higher in the roots when compared to the

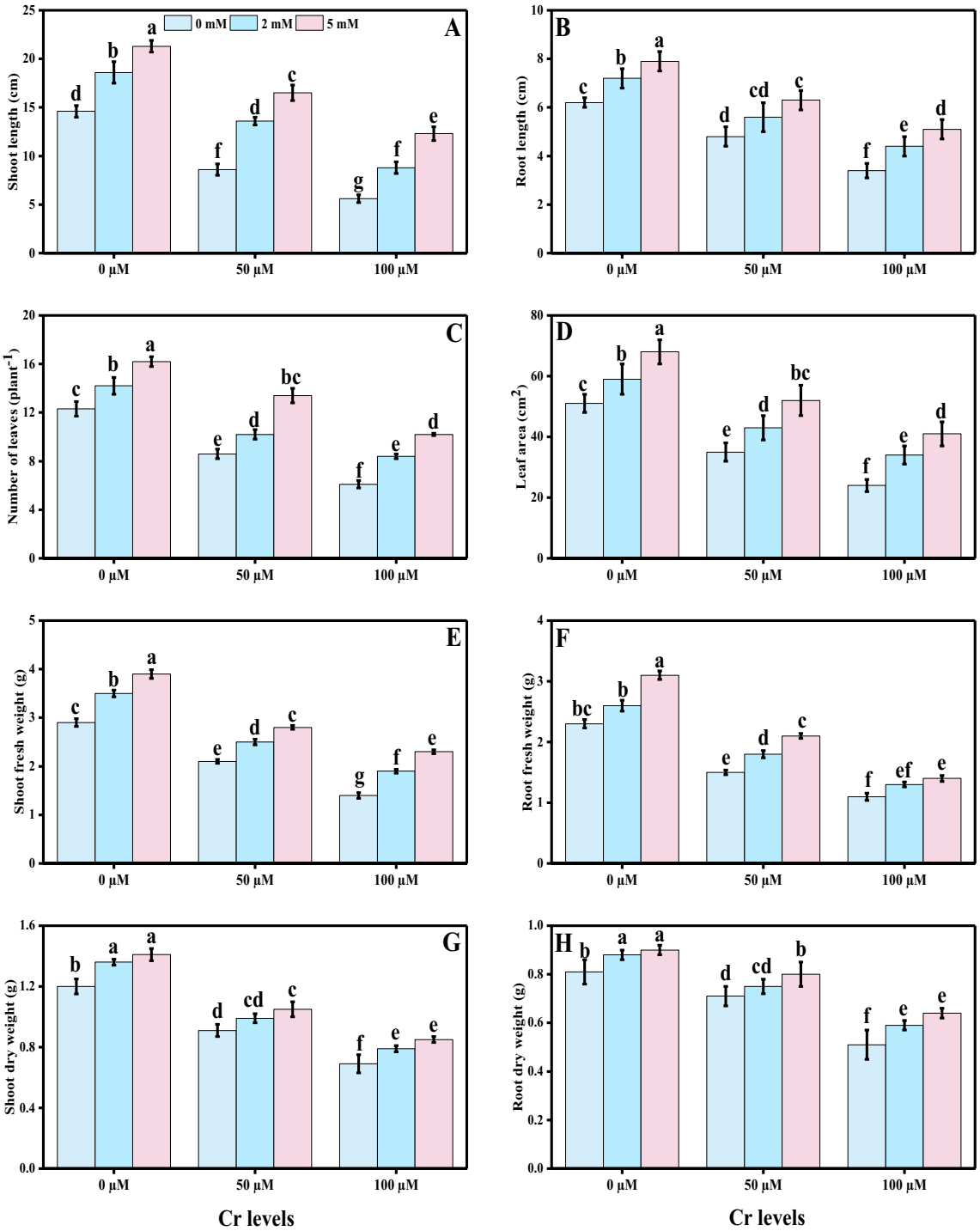


Fig. 1 Effect of foliar levels of choline chloride (0, 2, and 5 mM) on shoot length (**A**), root length (**B**), number of leaves (**C**), leaf area (**D**), stem fresh weight (**E**), root fresh weight (**F**), shoot dry weight (**G**), and root dry weight (**H**) on *S. oleracea* grown under various stress levels of Cr (0, 50 and 100 µM). Values are demonstrated as means of four replicates along with standard deviation (SD; $n=4$). Two-way ANOVA was performed and means differences were tested by HSD ($P<0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments

leaves of *S. oleracea* seedlings. Results also illustrated that the application of choline chloride increased non-significantly ($P<0.05$) the activities of antioxidants (SOD, POD, CAT, and APX) in the roots and leaves of *S. oleracea* seedlings, compared with those plants, which were grown without the exogenous application with choline chloride

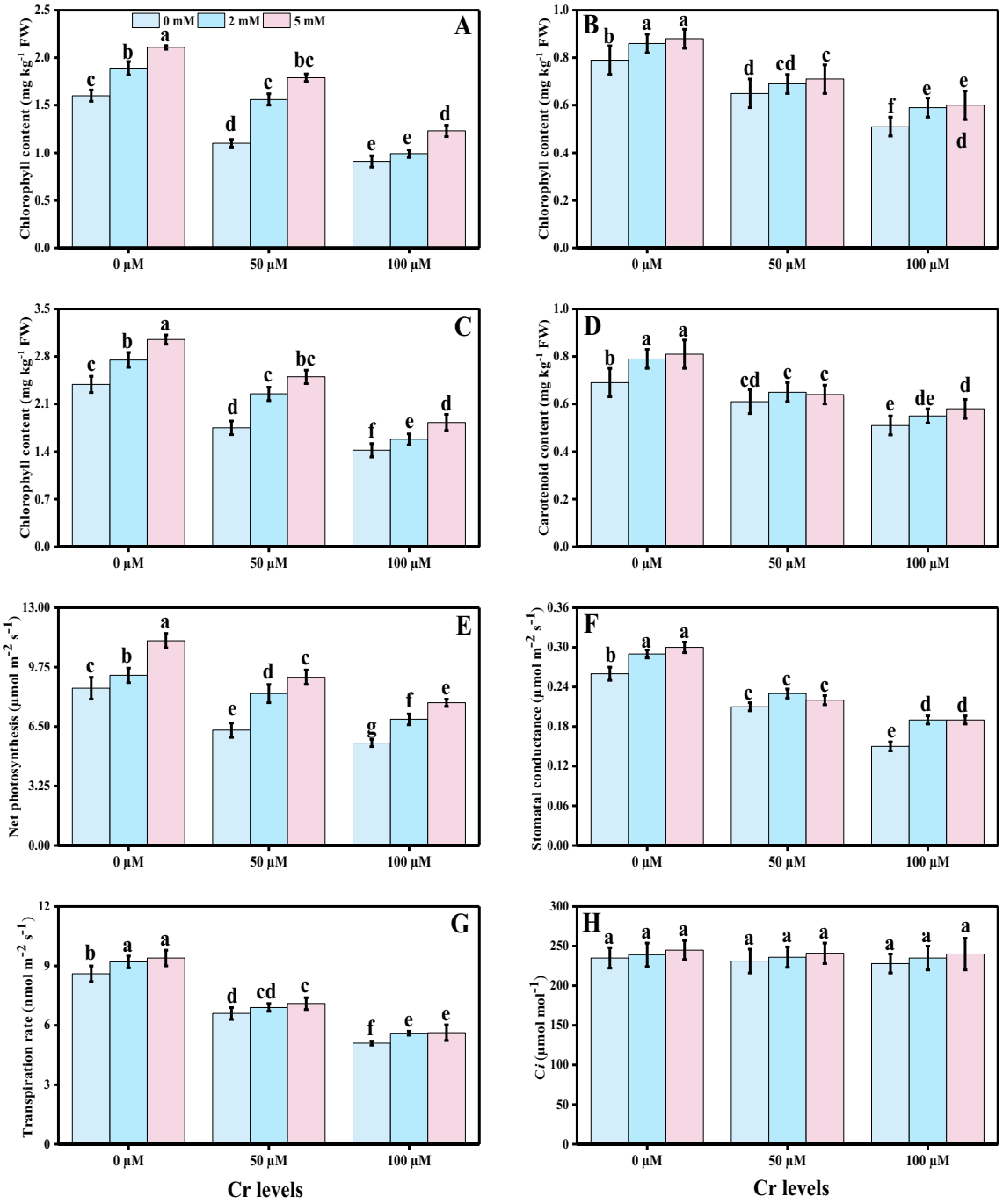


Fig. 2 Effect of foliar levels of choline chloride (0, 2 and 5 mM) on chlorophyll-a content (**A**), chlorophyll-b contents (**B**), total chlorophyll contents (**C**), carotenoid contents (**D**), net photosynthesis, (**E**) stomatal conductance (**F**), transpiration rate (**G**), and intercellular CO_2 (**H**) on *S. oleracea* grown under various stress levels of Cr (0, 50, and 100 µM). Values are demonstrated as means of four replicates along with standard deviation (SD; $n=4$). Two-way ANOVA was performed, and means differences were tested by HSD ($P<0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments

in the soil (Fig. 4). In addition, at all levels of Cr stress (50–100 µM), the activities of antioxidants (SOD, POD, CAT, and APX) were increased with the increasing levels of choline chloride (2–5 mM) in the soil, compared with those plants, which were grown without the application of choline chloride (Fig. 4).

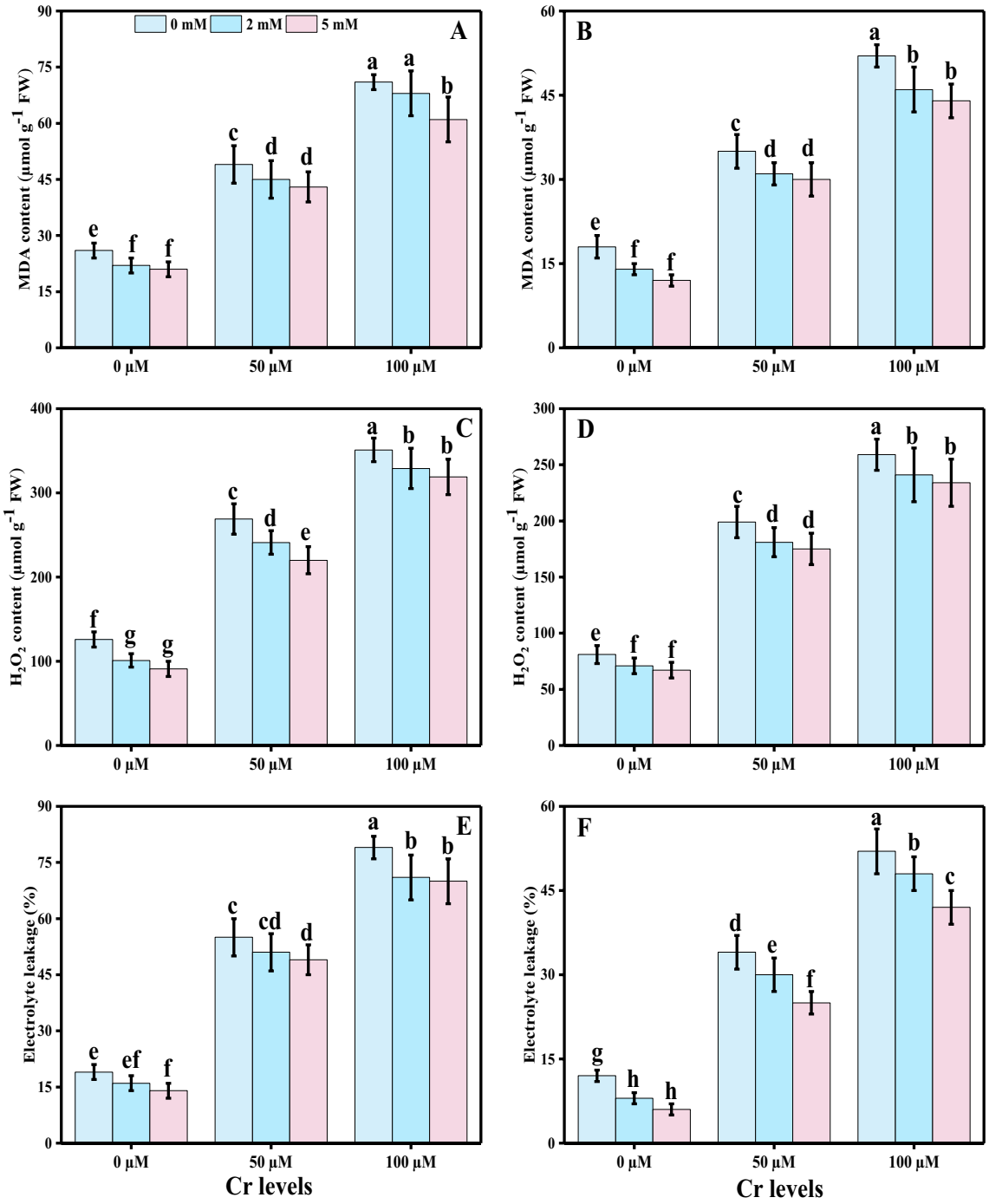


Fig. 3 Effect of foliar levels of choline chloride (0, 2 and 5 mM) on MDA contents in the roots (**A**), MDA contents in the leaves (**B**), H_2O_2 contents in the roots (**C**), H_2O_2 contents in the leaves (**D**), EL percentage in the roots (**E**), and EL percentage in the leaves (**F**) in the leaves of *S. oleracea* grown under various stress levels of Cr (0, 50, and 100 µM). Values are demonstrated as means of four replicates along with standard deviation (SD; $n=4$). Two-way ANOVA was performed, and means differences were tested by HSD ($P<0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments

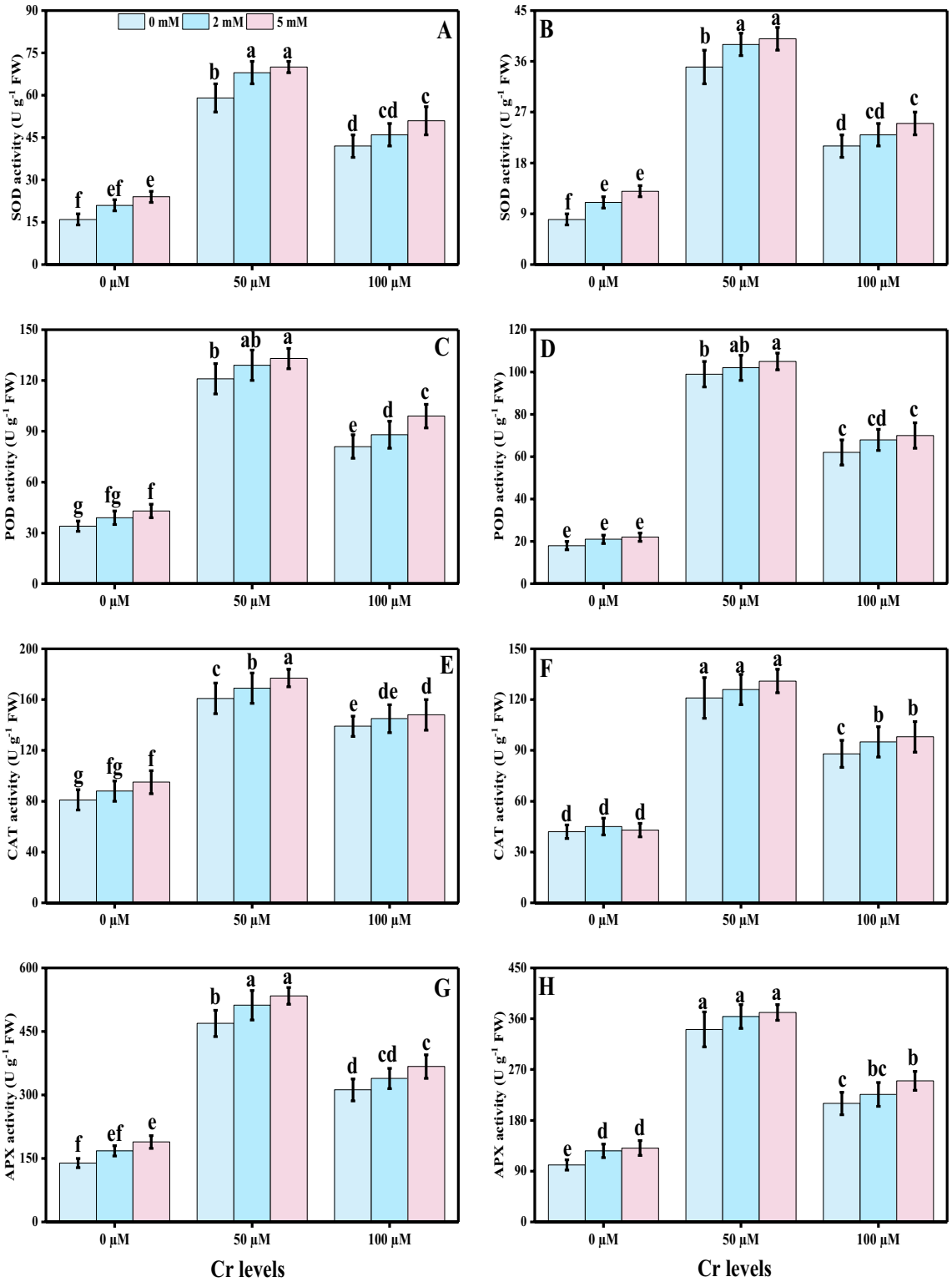


Fig. 4 Effect of foliar levels of choline chloride (0, 2, and 5 mM) on SOD activity in the roots (**A**), SOD activity in the leaves (**B**), POD activity in the roots (**C**), POD activity in the leaves (**D**) CAT activity in the roots (**E**), CAT activity in the leaves (**F**), APX activity in the roots, (**G**) and APX activity in the leaves (**H**) in the leaves of *S. oleracea* grown under various stress levels of Cr (0, 50, and 100 µM). Values are demonstrated as means of four replicates along with standard deviation (SD; $n=4$). Two-way ANOVA was performed and means differences were tested by HSD ($P<0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments

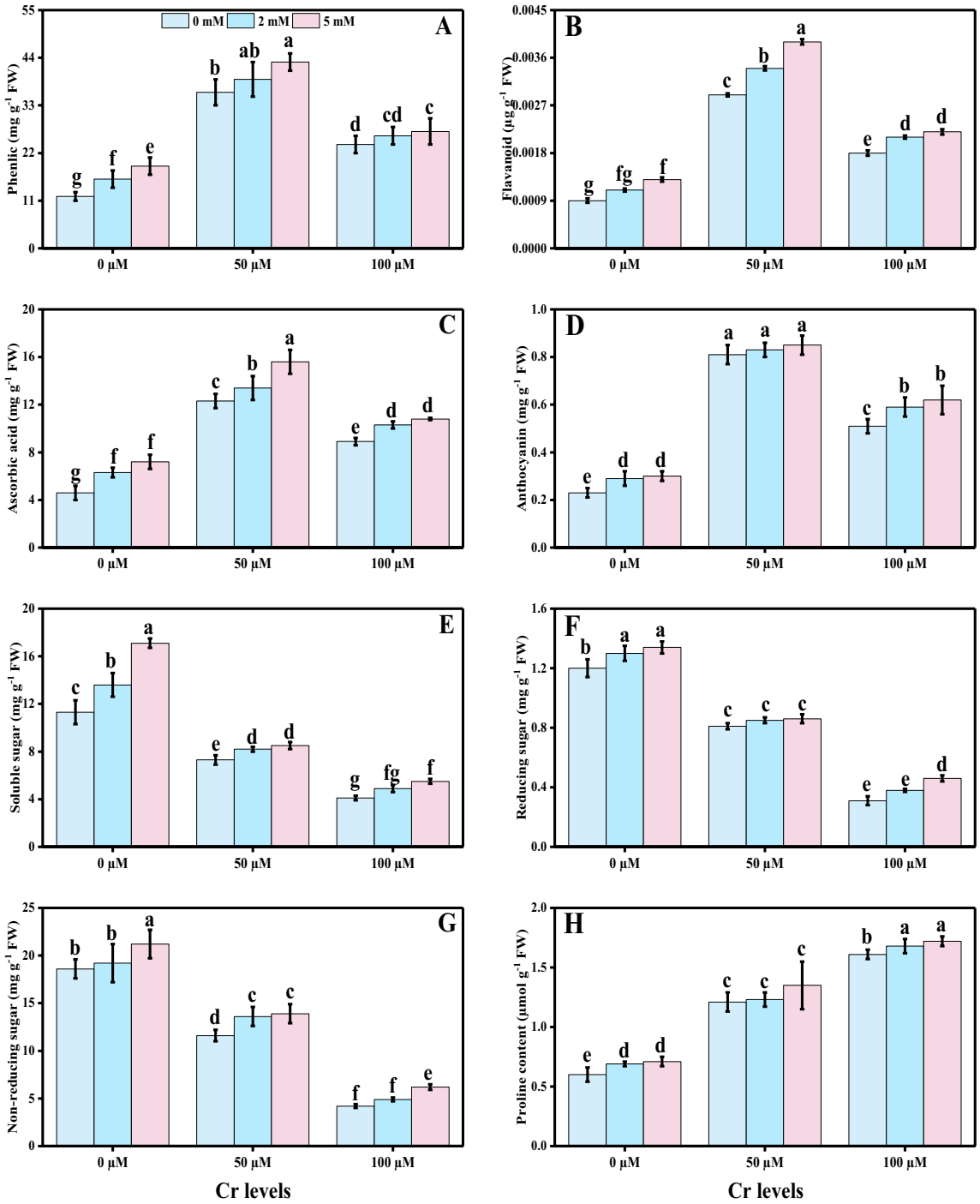


Fig. 5 Effect of foliar levels of choline chloride (0, 2, and 5 mM) on phenolic contents (**A**), flavonoid contents (**B**), ascorbic acid contents (**C**), anthocyanin contents (**D**), soluble sugar contents (**E**), reducing sugar contents (**F**), non-reducing sugar contents (**G**), and proline contents (**H**) of *S. oleracea* grown under various stress levels of Cr (0, 50, and 100 µM). Values are demonstrated as means of four replicates along with standard deviation (SD; $n=4$). Two-way ANOVA was performed and means differences were tested by HSD ($P<0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments

Non-enzymatic Antioxidants, Sugar, and Proline Content

In the present study, we also determined the content of non-enzymatic antioxidant compounds (phenolics, flavonoids, ascorbic acid, and anthocyanin) from the leaves of *S. oleracea* seedlings under the application of choline chloride, grown in Cr-polluted soil. The data regarding the non-enzymatic antioxidant compounds (phenolics, flavonoids, ascorbic acid, and anthocyanin) from the leaves of *S. oleracea* seedlings grown under the application of choline chloride in Cr-polluted soil are presented in Fig. 5. These results showed that the increasing concentration of Cr in the soil induced a significant ($P < 0.05$) increase in the contents of non-enzymatic antioxidants in the leaves of *S. oleracea* seedlings, with the application of choline chloride, grown in Cr-polluted soil (Fig. 5). The compounds of non-enzymatic antioxidant compounds (phenolics, flavonoids, ascorbic acid, and anthocyanin) in the leaves of *S. oleracea* seedlings initially increased up to a Cr level of 50 µM in the soil, but further increase in Cr concentration in the soil (100 µM) induced a significant ($P < 0.05$) decreased in antioxidants in the leaves of *S. oleracea* seedlings (Fig. 5). Results also illustrated that the application of choline chloride increased non-significantly ($P < 0.05$) the non-enzymatic antioxidant compounds (phenolics, flavonoids, ascorbic acid, and anthocyanin) in the leaves of *S. oleracea* seedlings, compared with those plants, which were grown without the exogenous application with choline chloride in the soil (Fig. 5). In addition, at all levels of Cr stress (50–100 µM), the non-enzymatic antioxidant compounds were increased with the increasing levels of choline chloride (2–5 mM) in the soil, compared with those plants, which were grown without the application of choline chloride (Fig. 4).

The contents of sugars (soluble, reducing, and non-reducing) and proline in the leaves of *S. oleracea* seedlings grown in the external application with choline chloride in the Cr-polluted soil are also presented in Fig. 5. It was also observed that the contents of soluble, reducing, and non-reducing sugars were decreased with the increasing concentration of Cr (50–100 µM) in the soil without the application of choline chloride. The contents of soluble, reducing, and non-reducing sugars were decreased significantly ($P < 0.05$) under all levels of Cr toxicity (50–100 µM) in the soil while increasing Cr concentration induced a significant ($P < 0.05$) increase in proline contents in the leaves of *S. oleracea* seedlings, compared to those plants, which were grown without the addition of Cr in the soil. Results also illustrated that the application of choline chloride increased non-significantly ($P < 0.05$) the contents of soluble, reducing, and non-reducing sugars and the contents of proline in the leaves of *S. oleracea* seedlings, compared with those plants, which were grown without the exogenous application with choline chloride in the soil (Fig. 5). In addition, at all levels of Cr stress (50–100 µM), the soluble, reducing, and non-reducing sugars and the contents of proline were increased with the increasing levels of choline chloride (2–5 mM) in the soil, compared with those plants, which were grown without the application of choline chloride (Fig. 5).

Nutrient Uptake

In the present study, the contents of essential minerals, i.e., iron (Fe^{2+}), calcium (Ca^{2+}) magnesium (Mg^{2+}), and phosphorus (P) were also determined from the roots and shoots of *S. oleracea* seedlings grown in different application levels of choline chloride (2–5 mM) under Cr-polluted soil. The contents of Fe^{2+}, Ca^{2+}, Mg^{2+}, and P from the roots and shoots of *S. oleracea* seedlings are presented in Fig. 6. Our results depicted that the concentrations of Fe^{2+}, Ca^{2+}, Mg^{2+}, and P in the roots and shoots of *S. oleracea* seedlings were decreased with the increase in the Cr levels (50–100 µM) in the soil, when compared with the plants grown without the addition of Cr in the soil in *S. oleracea* seedlings (Fig. 6). We also noticed that the concentrations of Fe^{2+}, Ca^{2+}, Mg^{2+}, and P in the roots and shoots of *S. oleracea* seedlings could be increased under the toxic concentration of Cr in the soil by the exogenous application of choline chloride (Fig. 6). In addition, results also showing that exogenous application with choline chloride increased the concentrations of Fe^{2+}, Ca^{2+}, Mg^{2+}, and P in the roots and shoots of *S. oleracea* seedlings, compared to those plants, which were grown without the exogenous application with choline chloride in the soil.

Organic Acids Exudation and Cr Uptake and Accumulation

The contents of fumaric acid, formic acid, acetic acid, citric acid, malic acid, and oxalic acid in the roots of *S. oleracea* seedlings grown under toxic levels of Cr in the soil, with or without the application of choline chloride are presented in Fig. 7. According to the given results, we have noticed that increasing the concentration of Cr in the soil (50–100 µM) induced a significant ($P < 0.05$) increased in the contents of fumaric acid, formic acid, acetic acid, citric acid, malic acid, and oxalic acid in the roots of *S. oleracea* seedlings, compared to those plants, which were grown in Cr level of 0 µM in the soil. Results also illustrated that the application of choline chloride decreased the contents of fumaric acid, formic acid, acetic acid, citric acid, malic acid, and oxalic acid in the roots of *S. oleracea* seedlings, compared with those plants, which were grown without the exogenous application with choline chloride in the soil. In addition, at all levels of Cr stress (50–100 µM), the contents of fumaric acid,

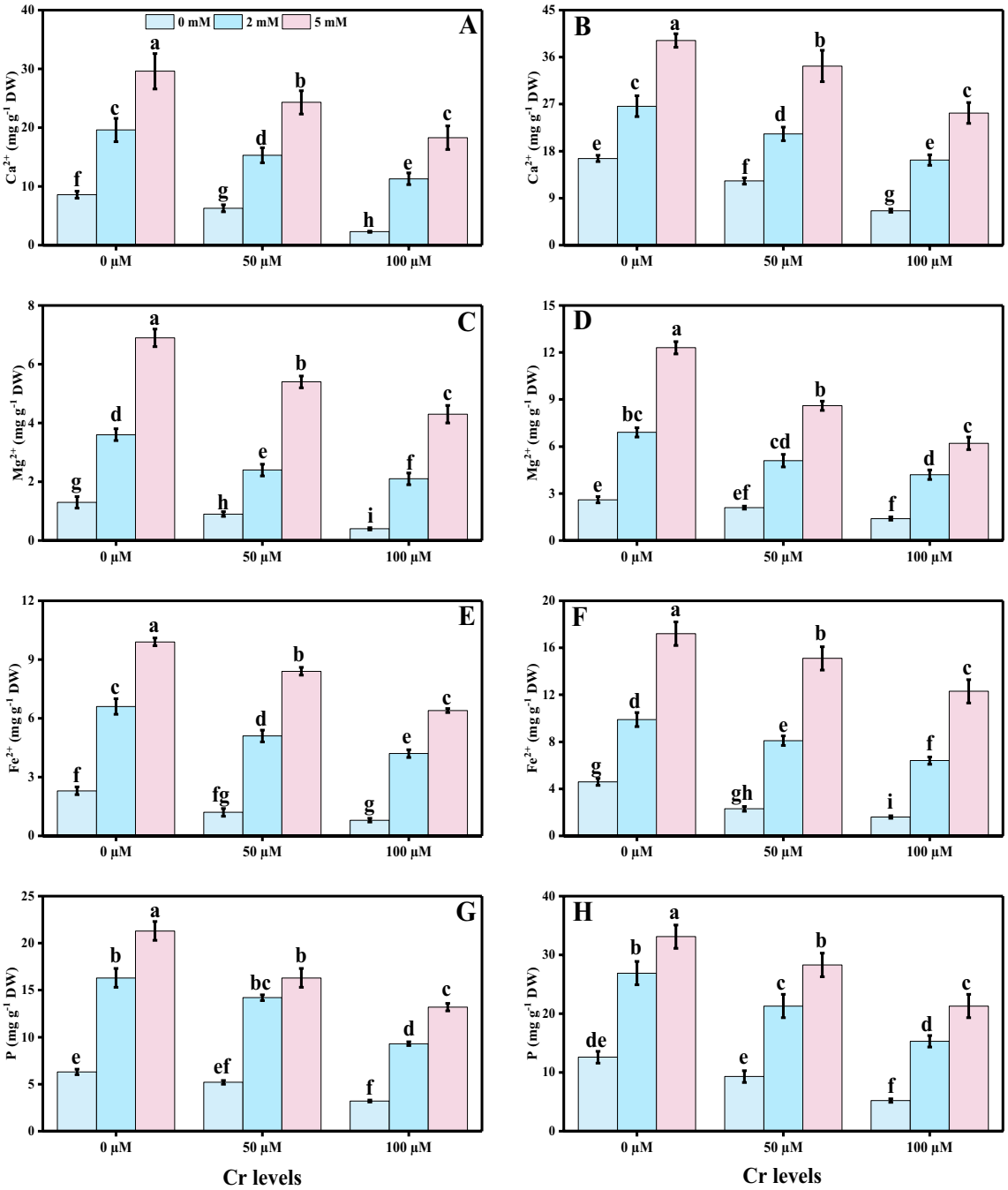


Fig. 6 Effect of foliar levels of choline chloride (0, 2, and 5 mM) on calcium contents in the roots (**A**), calcium contents in the leaves (**B**) in the shoots, magnesium contents in the roots (**C**), magnesium contents in the shoots (**D**), iron contents in the roots (**E**), iron contents in the shoots (**F**), phosphorus contents in the roots (**G**), and phosphorus contents in the leaves (**H**) in the shoots of *S. oleracea* grown under various stress levels of Cr (0, 50, and 100 µM). Values are demonstrated as means of four replicates along with standard deviation (SD; $n=4$). Two-way ANOVA was performed, and means differences were tested by HSD ($P<0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments

formic acid, acetic acid, citric acid, malic acid, and oxalic acid decreased with the increasing levels of choline chloride (2–5 mM) in the soil, compared with those plants, which were grown without the application of choline chloride.

We also manifested that the contents of Cr from the roots and shoots of *S. oleracea* seedlings grown under the toxic levels of Cr in the soil, with or without the application of choline chloride are presented in Fig. 7. Increasing levels of Cr in the soil induced a significant ($P<0.05$) increase in

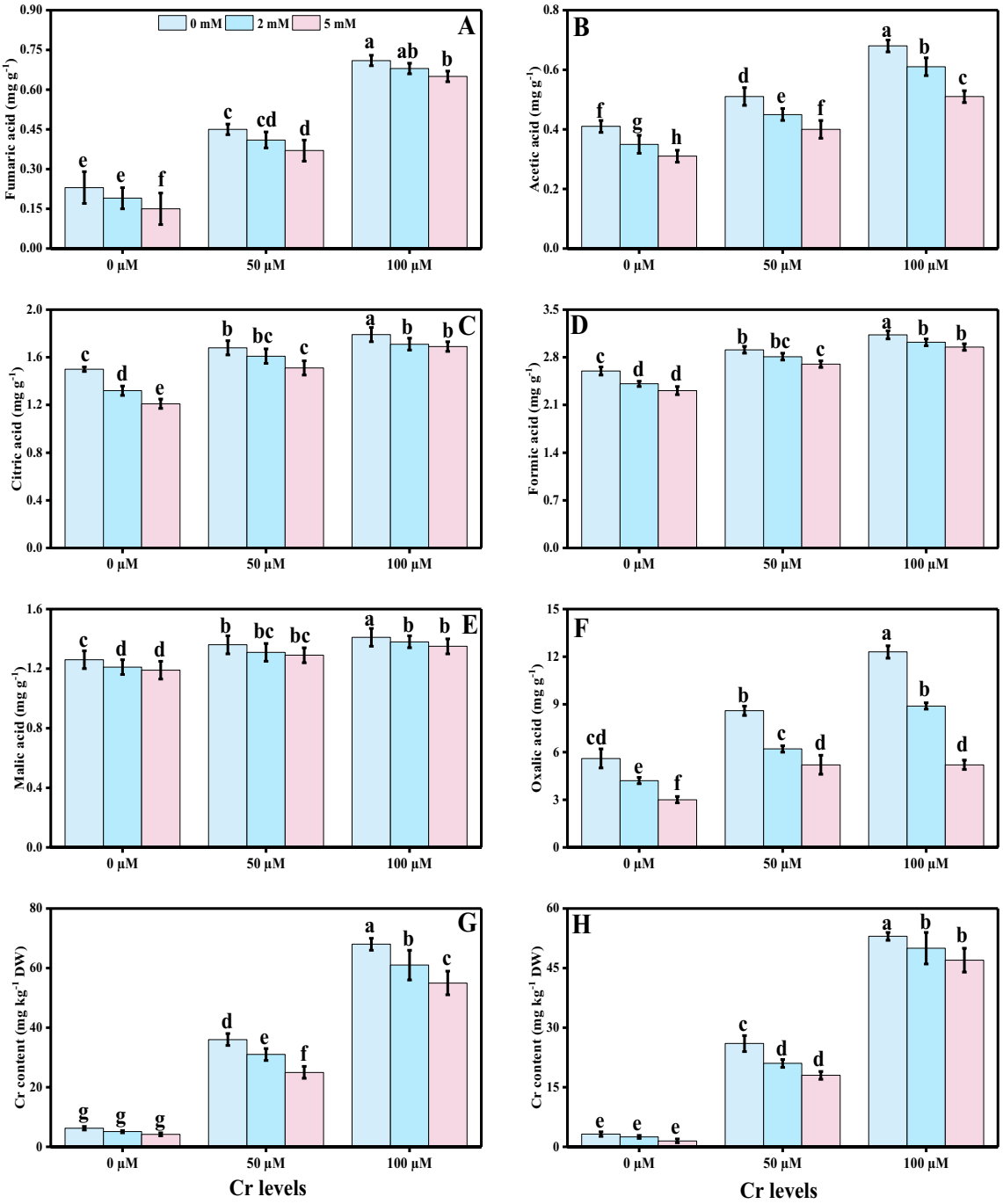


Fig. 7 Effect of foliar levels of choline chloride (0, 2, and 5 mM) on fumaric acid contents (**A**), acetic acid contents (**B**), citric acid contents (**C**), formic acid contents (**D**), malic acid contents (**E**), oxalic acid contents (**F**), in the roots and Cr contents in the roots (**G**), and Cr contents in the shoots (**H**) of *S. oleracea* grown under various stress levels of Cr (0, 50, and 100 µM). Values are demonstrated as means of four replicates along with standard deviation (SD; $n=4$). Two-way ANOVA was performed, and means differences were tested by HSD ($P<0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments

the Cr concentration in the roots and shoots of *S. oleracea* seedlings, compared to those plants which were grown in the control treatment. In addition, at all levels of Cr stress (50–100 µM), the contents of Cr were decreased with the increasing levels of choline chloride (2–5 mM) in the soil, compared with those plants, which were grown without the application of choline chloride.

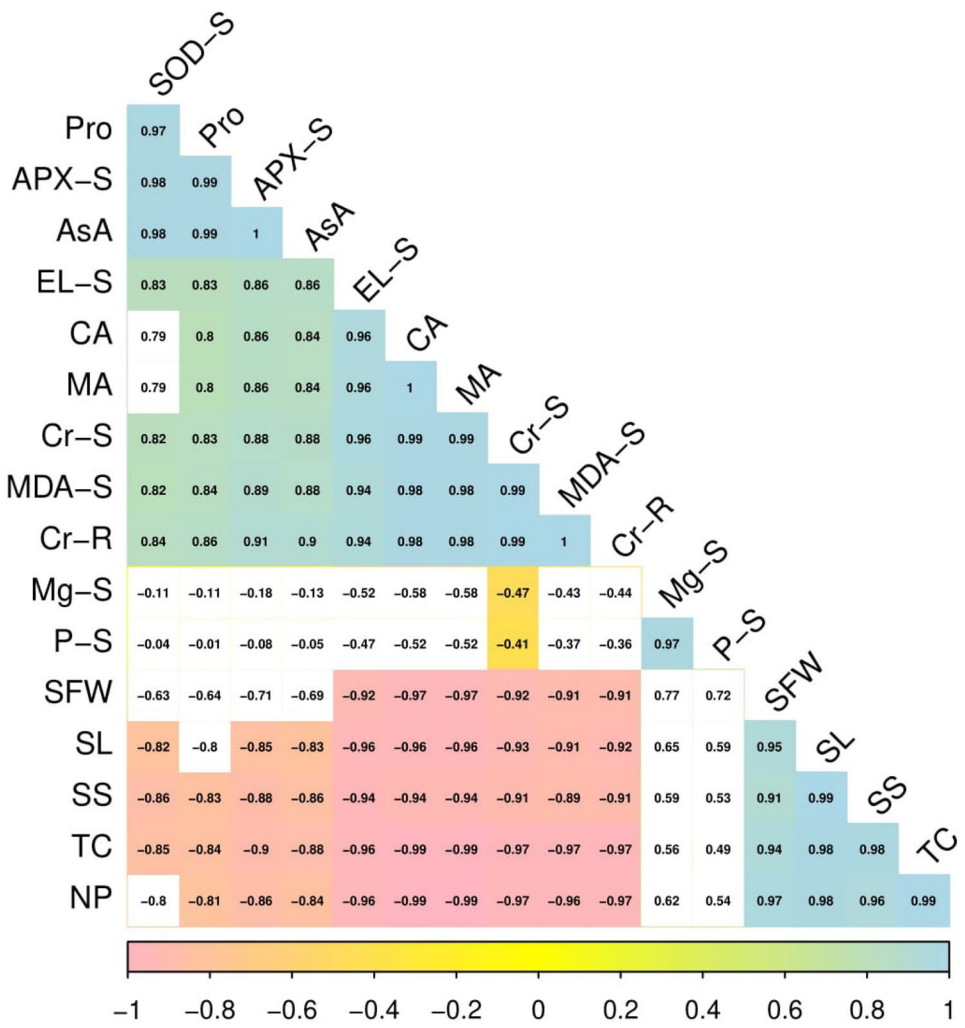


Fig. 8 Correlation between Cr uptakes in different parts of plant with some selected traits of morphological attributes, photosynthetic efficiency, oxidative stress and response of antioxidant enzymes, nutrients uptake, and organic acid exudation pattern. Different abbreviations used in the figure are as follows: *Pro* proline content, *APX-S* ascorbate peroxidase activity in the shoots, *EL-S* electrolyte leakage in the shoots, *CA* citric acid content in the roots, *MA* malic acid content in the roots, *Cr-S* Cr content in the shoots, *MDA-S* malondialdehyde content in the shoots, *Cr-R* Cr content in the roots, *Mg-S* magnesium content in the shoots, *P-S* phosphorus content in the shoots, *SFW* shoot fresh weight, *SL* shoot length, *SS* soluble sugar content, *TC* total chlorophyll content, and *NP* net photosynthesis

Relationship

A Pearson's correlation graph was constructed to quantify the relationship between various growth parameters with Cr uptake in different parts of the plant (Fig. 8). Cr concentration in the roots was positively correlated with Cr concentration in the shoots, MDA contents in the shoots, EL in the shoots, AsA content, malic acid content, CA content, proline content, and APX activity in the shoots while negatively correlated with Mg content in the shoots, P content in the shoots, shoot fresh weight, shoot length, soluble sugar content, total chlorophyll content, and net photosynthesis rate. Similarly, Cr concentration in the shoots was positively correlated with Cr concentration in the roots, MDA contents in the shoots, EL in the shoots, AsA content, malic acid content, CA content, proline content, and APX activity in the shoots while negatively correlated with Mg content in the shoots, P content in the shoots, shoot fresh weight, shoot length, soluble sugar content, total chlorophyll content, and net photosynthesis rate. This correlation is depicted a close connection between Cr uptake and growth in *S. oleracea*.

Principal Component Analysis

The loading plots of principal component analysis (PCA) to evaluate the effect of different levels of Cr in the soil with the exogenous application of choline chloride on different attributes of *S. oleracea* are presented in Fig. 9. Of all the main components, the first two components—Dim1 and Dim2—comprise more than 97% of the whole database and make up the largest portion of all components (Fig. 9). Among this, Dim1 contributes 83.2%, and Dim2 contributes 14% of the whole dataset. In addition, Fig. 9 also shows that Cr contents in roots and shoots, MDA contents in the shoots, EL in the shoots, AsA content, malic acid content, CA content, proline content, and APX activity in the shoots were positively correlated in the dataset from all the variables. In contrast, Mg content in the shoots, P content in the shoots, shoot fresh weight, shoot length, soluble sugar content, total

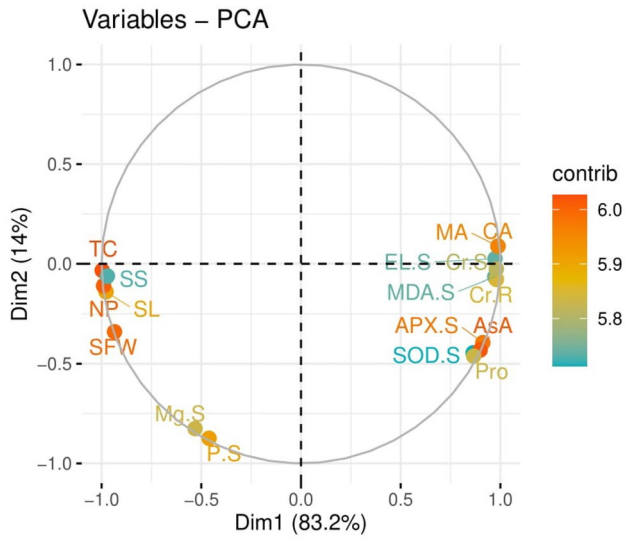


Fig. 9 Loading plots of principal component analysis (PCA) on different studied attributes of *S. oleracea* grown under various stress levels of Cr in the soil. Different abbreviations used in the figure are as follows: *Pro* proline content, *APX-S* ascorbate peroxidase activity in the shoots, *EL-S* electrolyte leakage in the shoots, *CA* citric acid content in the roots, *MA* malic acid content in the roots, *Cr-S* Cr content in the shoots, *MDA-S* malondialdehyde content in the shoots, *Cr-R* Cr content in the roots, *Mg-S* magnesium content in the shoots, *P-S* phosphorus content in the shoots, *SFW* shoot fresh weight, *SL* shoot length, *SS* soluble sugar content, *TC* total chlorophyll content, *NP* net photosynthesis

chlorophyll content, and net photosynthesis rate were negatively correlated in the dataset from all the variables (Fig. 9).

Discussion

Cr is among the most toxic trace elements present in agricultural soils and is being released through a variety of anthropogenic activities such as electroplating and leather tanning (Jobby et al. 2018; Kumar et al. 2016). In addition, Cr enters plant through roots exposed to Cr-contaminated soils and depends upon many soil factors such as soil pH, electrical conductivity (EC), competition between different metal species present in the soil, as well as the different plant-associated factors such as plant species, growth stages, and root system (Junaid et al. 2016, Madhu and Sadagopan 2020, Zaheer et al. 2020b). However, Cr is a well-known toxic metal and is harmful to the growth and development of the plant and it was also reported that it provokes adverse effects on biochemistry and physiology of various important crops (Ali et al. 2015; Gautam et al. 2020b; Qadir et al. 2020). Exposure to Cr may induce toxic effects in several biochemical processes in plants, such as plant germination, root growth and length, stem growth, and leaf development (Handa et al. 2019; Zaheer et al. 2019). Moreover, Cr stress is also known to negatively influence photosynthesis in terms of electron transport, CO_2 fixation, enzyme activities, and photophosphorylation and causes a decrease in chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoids which have been well established (Ahmad et al. 2019; Ertani et al. 2017; Sallah-Ud-Din et al. 2017; Tripathi et al. 2012). In the present study, we have noticed that Cr toxicity causes a significant decrease in the plant growth and biomass (Fig. 1), photosynthetic pigments, and gas exchange characteristics (Fig. 2), compared to those plants which were grown without the addition of Cr in the soil. It has been previously shown that Cr stress negatively affects the plant biomass and photosynthetic efficiency in different plant species which depends upon a number of factors including plant species, dose, and duration of Cr application (Gautam et al. 2020a; Jobby et al. 2018; Nafees et al. 2018; Ranieri et al. 2020; Zaheer et al. 2020a).

Stress conditions can disturb the dynamic equilibrium of reactive oxygen species (ROS) production and elimination under normal growth in plants (Kamran et al. 2019; Saleem et al. 2019; Shahid et al. 2020), which promote ROS accumulation and membrane lipid peroxidation, and disrupt the structure and function of cell membrane system (Mohamed et al. 2020; Nazar et al. 2020; Saleem et al. 2020a; Yaseen et al. 2020). It was reported that excess of Cr can increase lipid peroxidation and MDA, an oxidized product of membrane lipids, indicating the prevalence of oxidative stress and membrane damage (Ali et al. 2015; Gautam et al. 2020b; Handa et al. 2019; Sallah-Ud-Din et al. 2017). High concentration of Cr in the soil induced oxidative damage by increasing the contents of MDA, initiation of H_2O_2, and increased percentage of EL which was observed in *Brassica napus* (Afshan et al. 2015), *Arabidopsis thaliana* (Eleftheriou et al. 2015), and *Brassica juncea* (Handa et al. 2018a). This is because of the Cr has the capability of altering the K^+ efflux along with electron transport chain, which ultimately produces high level of OH and O radicals with a consequent increase in EL (Javed et al. 2021; Zaheer et al. 2020d). It is well documented that Cr toxicity directly caused oxidative injury in the plants through the Fetone and Haber–Weiss reactions which also helps in the generation of large amount of ROS which is toxic to the plant (Ertani et al. 2017, Madhu and Sadagopan 2020). This ROS accumulation in plants is removed by a variety of antioxidant enzymes such as SOD, POD, CAT, and APX (Fig. 4) and non-enzymatic antioxidant (Fig. 5). However, the expression of antioxidative enzymes, such as SOD, POD, CAT, and APX under Cr stressed environment plays a significant role in reducing Cr toxicity, which was reported in a number of studies under various plant species (Sallah-Ud-Din et al. 2017; Tripathi et al. 2012). It was also noticed that, severe Cr toxicity (100 µM) decreased the various antioxidants, possibly due to the severe concentration of Cr in soil which

induce alterations in gene expression and function of various proteins in plant tissues (Rehman et al. 2019b; Shahid et al. 2017). Plants produce a variety of secondary metabolites such as proline, flavonoids, and phenolics that improve tolerance against metal toxicity. Although, proline accumulation in plant tissue/organs is a response to metal toxicity, which might be associated with signal transduction and prevents membrane distortion, which has been observed in many plant species (Javed et al. 2020; Rehman et al. 2019a; Saleem et al. 2020g).

Essential nutrients are required for the normal growth of plants. Numerous reports demonstrated that the uptake and translocation of essential elements in plants were restricted under Cr stress (Ahmad et al. 2019; Handa et al. 2018b; Zaheer et al. 2020b). Excess Cr decreased the Fe^{2+}, Ca^{2+}, Mg^{2+}, and P contents in the roots and shoots of the plants, which was also noticed in the present study (Fig. 6). It is well known that Cr toxicity in crops depends on the bioavailability of Cr in soils and the concentration of elements, which can compete with Cr during plant uptake (Ali et al. 2015; Gautam et al. 2020a; Sallah-Ud-Din et al. 2017; Ulhassan et al. 2019). The reduction in Fe^{2+}, Ca^{2+}, Mg^{2+}, and P contents in different parts of the plants is directly linked with the failure of the plant to uptake essential micronutrient from the soil due to irrigation with excess amount of Cr in the soil. Previously, it was observed that under the toxic levels of Cr in the soil, it causes a significant increased in Cr contents in the roots and shoots of *S. oleracea* are (Zaheer et al. 2020a). Cr, being structurally similar to other essential ions, may interfere with plant mineral nutrition in a complex way. Several previous studies reported Cr (VI) and Cr (III) interference with essential nutrients: uptake of Fe^{2+}, Ca^{2+}, Mg^{2+}, and P in many plant species (Ertani et al. 2017; Jobby et al. 2018). Hence, the competitive binding of Cr to common carriers can decrease the uptake of many essential nutrients. Another reason behind Cr-induced decrease in nutrient uptake can be the decrease of the activity of plasma membrane H^+ ATPase (Madhu and Sadagopan 2020, Shahid et al. 2017). The antagonistic interaction between Cr and essential nutrients can be due to their interferences both within the soil and inside the plant tissues. The decrease in essential nutrients in the soil is the main factor that plants decrease their growth (Fig. 1) and photosynthesis (Fig. 2) and increasing oxidative damage (Fig. 3) to the membrane-bounded organelles, which was reported in the present study. Roots exclude especially organic acids, which are regarded as active ligands under the excess concentration of metals in the soil (Hashmat et al. 2021; Javed et al. 2021). Acidification of mucilage after uptake of Cr is likely due to the release of protons when plant roots release more cations than anions in order to maintain their charge balance (Kumar et al. 2016). The exudation of organic acids in the roots of *S. oleracea* seedlings (Fig. 7), accelerating metal transport from roots to the aboveground parts, is possibly due to the formation of metal-chelated ions as suggested by Javed et al. (2021), when they cultivated *Solanum lycopersicum Mill. cultivars* in Cr-polluted soil.

Heavy metals are natural components of the terrestrial ecosystem. However, their presence in excess is harmful to humans and the environment. Therefore, remediation is necessary to alleviate the negative effects caused by the heavy metals incorporated to ecosystems (Imran et al. 2020a; Kamran et al. 2020; Rehman et al. 2020; Saleem et al. 2020a, 2020e). Research has been continuing to develop effectual methods of remediation to treat contaminated lands (Rehman et al. 2019a; Saleem et al. 2020c). Remediation could be done by immobilization, removal, sequestration, active mixing, and phytoextraction. However, selection and applicability of the remediation methods depend on a number of factors including cost, duration of effectiveness, commercial level availability, general acceptance, applicability to high metal contents, and applicability to mixed metal and organic wastes as well as toxicity, mobility, and volume reduction (Imran et al. 2020b; Ullah et al. 2015). The use of choline chloride is a novel approach and it is an organic compound, white-soluble powder used commonly as animal fodder (Pavić et al. 2019). It can be used as feed for the animals like chicken to accelerate their growth and development (Radošević et al. 2015), and also an antigibberellin growth retardant and in- vitro used to enhance growth in many grasses and also plays role in morphogenesis in many other plant species (Yuniarti et al. 2019). Moreover, choline chloride may also serve as osmo-protectant in plants provided that plants are able to uptake large amount of nutrients from the soil, decrease oxidative damage, and induce due to abiotic stress environment (Salama et al. 2011). In addition, it has an important role as precursor for phosphatidylcholine and this characteristic of plant enhances ions uptake in plant organ which improves plant growth and biomass and increases overall plant performance, when plant is grown in the metal-stressed environment (Wang and Xiao 2009). In the present study, the application of choline chloride increased plant growth and biomass (Fig. 1), and regulated photosynthetic efficiency (Fig. 2) might be due to regulate the uptake of essential nutrients (Fig. 6) in the roots and shoots of the plant and decreased oxidative damage (Fig. 3) by regulating the activities of various antioxidants (enzymatic and non-enzymatic) (Figs. 4 and 5). This is because of application of choline chloride regulate nutritional status in the plants and gives a room to increase more nutrients from the soil in metal-polluted environment as showed by Salama et al. (2011). Although there are very few literature focusing on metal tolerance and maintenance in different plant organs with the application of choline chloride, results showed by Salama et al. (2011) depict that application of choline chloride regulates plant growth and biomass and decreases

oxidative stress by maintaining antioxidant activities and regulating the uptake of essential nutrients by decreasing the toxic nutrients (sodium and chloride) in different parts of *Triticum aestivum*, when it is grown in the saline soil (Salama et al. 2011). These findings indicated that the application of choline chloride is a practical approach which is appropriate for agricultural practices to combat metal stress environment.

Conclusion

On the basis of these findings, it can be concluded that the negative impact of Cr toxicity can be overcome by the application with choline chloride. Our results depict that Cr toxicity induced severe metal toxicity in *S. oleracea* seedlings by increasing the generation of ROS in the form of oxidative stress and also increased the concentration of Cr in the roots and shoots of the plants. Furthermore, Cr toxicity also increased organic acids exudation and imbalance the nutritional status of the plants, which ultimately decrease plant growth and yield and photosynthetic efficiency. Hence, Cr toxicity was eliminated by the external application of choline chloride, which also decreased the Cr concentration in the plant tissues, degenerated ROS, and organic acids exudation, and increased the activities of antioxidants and essential nutrients in the plants. Therefore, long-term field studies should be executed to draw parallels among plants/crops root exudations, metal stress, nutrients mobility patterns, and plant growth in order to gain insights into underlying mechanisms.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00344-021-10401-7.

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Author Contributions IH, SA, and SM conceived and designed the study and MHS and SA has executed the experiment and compiled data. RR help in critical editing and revised manuscript. MAA statistically analyzed the data and help in chemical analysis. FM interpreted the results. MR and MHS wrote the manuscript. HY and SA critically edited and revised the manuscript. MI helped in sample collection and chemical analysis.

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Data Availability Data and material are available for research purpose and for reference.

Declarations

Conflict of interest There is no competing interest in the publication of this manuscript.

Ethical Approval Ethical approval was taken from Department Ethical Review Committee to conduct study and no plants or experiments were harmed in this study.

Consent to Publish Written consent was sought from each author to publish the manuscript.

Consent to Participate Informed consent was taken from formers to conduct the study and to collect the samples. They were briefed about the research plan in details.

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