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Effects of chromium contamination on membrane and resistance capacity in spinach (*Spinacia oleracea*)

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ABSTRACT

Environmental contamination caused by chromium poses serious threat to both plants and animals. This engrossed devotion of environmental and medical scientists and plant nutritionists in recent years because of its toxicity and mobility in the soil-plant continuum and food chain as well. This study was conducted to investigate the cytotoxic effect of Cr on membrane and resistance level in Spinacia oleracea. Four different Cr concentrations denoted as T0 (0 mg), T1 (25 mg), T3 (50 mg) and (100 mg) was used to contaminate the soil (1 kg). Using Completely Randomize Design (CRD) under 8 replications, the membrane electrolyte leakage (MEL), membrane stability index (MSI), malondialdehyde (MDA), protein oxidation (carbonyl), Total antioxidant assay (TAA), Reducing power ability (RPA), H_2O_2 scavenging, catalase (CAT) and ascorbate peroxidase (APX) activities were determined. A significant $P \leq 0.005$ increased MEL, MSI, MDA, carbonyl, TAA, RPA, H_2O_2 scavenging, CAT and APX were noted under T2 and T3 compared to the control. The Cr accumulation was higher in root than edible parts of S. oleracea where lower translocation of Cr was observed. Hence, Cr has serious effect in S.oleracea membrane and it developed antioxidant to cater with reactive oxygen species.

Keywords: *Chromium, Spinacia oleracea, toxicity, antioxidants*

INTRODUCTION

Rare earth elements (REEs) have been widely used in industry, agriculture, energy, medicine, and military due to their special properties (Yang *et al.*, 2014). Heavy metal contamination has disastrous impacts on terrestrial as well as aquatic life (Pushkar *et al.*, 2021) and it has significantly disrupted the natural ecosystem (Zulfiqar *et al.*, 2022 & 2023).

The unplanned urban and industrial development that disregards the value of a healthy environment is the main cause of environmental pollution (Wei *et al.*, 2022). These actions have greatly increased the pollution from heavy metals, which upsets the natural balance (Qianqian *et al.*, 2022). More than 1.7 million deaths were reported by World Health Organization (WHO) because of exposure to harmful contaminants, such as heavy metals (Xu *et al.*, 2018). Chromium (Cr) has become more prevalent as an environmental pollutant due to its increased industrial uses (Wei *et al.*, 2022). Cr is a pervasive contaminant with significant environmental hazards, particularly for soil-plant ecosystem (Ao *et al.*, 2022). Hexavalent Cr is known to be a dangerous metal relative to the trivalent form because of its carcinogenic, mutagenic, and oxidizing properties (Wei *et al.*, 2022).

Chromium is one of the seventh most toxic heavy metals which threaten the food chain (Jaishankar *et al.*, 2014). It is ingested by human beings and animals with the edible parts of agricultural and horticultural crops or derived products. Many of these effects can be interrelated through a general action on membrane biogenesis and integrity, which in turn can occur because lipid metabolism is altered. Plant membrane structure may be regarded as the first "living" structure that is a target for heavy metal toxicity (Hall, 2002). There is no known biological function of Cr in plants (Srivastava *et al.*, 2021). To combat the elevated amounts of Cr-mediated ROS, plants also have a secondary mechanism for generating antioxidant enzymes (Srivastava *et al.*, 2021; Ao et al., 2022). Many studies have suggested that excessive Cr in the soil can be toxic to plants, causing growth reduction and leaf necrosis (Dai *et al.*, 2013), a disturbance to mineral elements and carbohydrate metabolism (Wu *et al.*, 2007), and the production of reactive oxygen species (ROS), thus strongly reducing biomass production (Chen *et al.*, 2011). Generally, ROS in plants is continuously produced as by-products of various metabolic pathways that are localized in different cellular compartments (Gratão *et al.*, 2005). However, once stressed by a heavy metal, ROS formation may exceed the antioxidant scavenging capacity, thus creating oxidative stress and damage to the bio-molecules (Davies, 2003).

Spinach (Spinacia oleracea), belongs to Chenopodiaceae family (Cai *et al.*, 2021). It is one of the most important vegetables. It is a leafy cool-season vegetable with global cultivation usually consumed after boiling either fresh or frozen leaves or raw consumed in salad (Alessa *et al.*, 2017). It has the potential to absorb a higher quantity of heavy metals and toxic elements from the rhizosphere and translocate them into edible parts compared to fruits and root vegetables (Bashir *et al.,* 2020). This study investigates the cytotoxic effect of Cr on membrane and the ability of *Spinacia oleracea* to cater with stress.

Methodology

Experimental design and treatment plan

S. oleracea seeds were first sown on germination trays filled with cocoa peat and kept for 12 days. The top soil was collected and sieved to remove unwanted materials. About 1 kg of soil in pot was contaminated with 25, 50 and 100 mg Cr. Using completely randomized design, the pots were grouped into T0 (control), T1 (25 $mgkg^{-1}$), T2 (50 $mgkg^{-1}$), and T3 (100 $mgkg^{-1}$) with five replications respectively. The healthy four leaved seedlings with same height were selected and then transplanted into already contaminated soil 1 seedling pot^{-1}. The seedling were water ones every morning for 5 weeks.

Determination of Cytotoxic Effect of Cr on Membrane

Malondialdehyde

MDA was quantified using a Li *et al.* (2000) method with minor alteration. About 0.2 g fresh leaves in 2 mL 5% (w/v) TCA were homogenized in a mortar and pestle and then spun for 20 min at 13,000 g. A reaction mixture containing 0.5 ml supernatant and 1 mL TCA 20% (w/v) and TBA (0.5%) was incubated at 95°C in a water bath for 25 min. Optical density of the mixture was read at 450, 532 and 600 nm, respectively.

MDA C(µm/l) = 6.45(A532 - A600) - 0.56 A450.

Protein oxidation

The reaction of carbonyls with 2,4-dinitrophenylhydrazine (DNPH) was used to determine the amount of protein oxidation, as described by Gonçalves et al. (2007) with slight modification. *S. oleracea* leaves were homogenized in a 25 mmol L^{-1} K-phosphate buffer containing 10 mL L^{-1} Triton X-100, pH 7.0, at a proportion of 1:5 (w/v). The homogenate was centrifuged at 13,000 g for 30 min at 4°C. After the DNPH-reaction, the carbonyl concentration was calculated by absorbance at 370 nm, using the molar extinction coefficient 21 x 103 mM cm^{-1}.

Electrolyte Leakage and Membrane Stability Index

The method developed by Farooq et al. (2016) was used to assess membrane electrolyte leakage percentage. After six weeks of treatment, leaf samples were divided into 5mm-size pieces and placed in test tubes with 8ml deionized and distilled water. For two hours, the tubes were bathed in a water set at 32 °C. The medium initial electrical conductivity (EC1) was measured. Samples for second electrical conductivity (EC2) were autoclaved at 121 °C for 20 min to remove all electrolytes. The samples were chilled to 25 °C. The formula below was used to determine the total amount of electrolyte leakage:

$$MEL = \left(\frac{EC1}{EC2}\right) x\ 100 \qquad \text{eqn. 1}$$

Membrane stability (MSI) was determined according to the formular;

$$MSI = [1 - \left(\frac{C1}{C2}\right) x\ 100] \qquad \text{eqn. 2}$$

Antioxidant defence against absorbed reactive oxygen species

Non-Enzymatic antioxidant assay

Total antioxidant assay (TAA) by phosphomolybdate method

TAA was evaluated based on the method used by Jayaprakasha et al. (2002) with some alteration. Extract (1 ml) was added to 1.5 ml of reagent (28 mM sodium phosphate, 0.6 M sulfuric acid, and 4 mM ammonium molybdate). The tubes were covered and incubated for 90 minutes in a boiling water bath at 95°C. Using a UV spectrophotometer, the optical density of the samples was read at 695 nm against a blank after they had cooled to room temperature. Butylated hydroxytoluene (BHT) as the standard, TAA was expressed as mg equivalents of BHT by using the standard BHT graph.

Reducing power ability (RPA) of the extract

By using the Fe^{3+} to Fe^{2+} reduction with the fractions as per Fejes *et al.* (2000) description, the reducing power of the extract was examined. The production of Perl's Prussian blue at 700 nm can be used to detect the presence of Fe^{2+} (Meir *et al.,* 1995). One millilitre of the fraction, 2.5 ml of 1% potassium ferricyanide, and 2.5 ml of phosphate buffer (pH 6.6) was incubated at 50°C for 30 minutes before being combined with 2.5 ml of 10% trichloroacetic acid and centrifuged at 3000 g for 10 minutes. TAA was represented in mg equivalents of butylated hydroxytoluene (BHT), using the standard BHT graph

H_2O_2 scavenging activity

The Bhatti et al. (2015) technique was used to assess the extract's capacity to scavenge hydrogen peroxide (H_2O_2). After transferring an aliquot of 0.1 mL of extracts into Eppendorf tubes, their volume was increased to 0.4 mL with 50 mM phosphate buffer (pH 7.4), and then 0.6 mL of H_2O_2 (2 mM) solution was added. After 10 minutes reaction time, the reaction mixture was vortexed, and its absorbance at 230 nm was determined. The positive control utilized was ascorbic acid. The following equation was used to determine the extracts' capacity to scavenge H_2O_2:

$$H_2O_2 \text{ scavenging activity percentage} = \frac{[A_0 - A_1]}{A_0} \times 100 \qquad \text{eqn. 3}$$

Enzymatic antioxidant assay

Catalase assay (CAT) - CAT activity was measured by spectrophotometry at 28ºC in a final reaction mixture of 2 mL comprising 100 mmol L^{-1} potassium phosphate buffer (pH 7.0), 12.5 mmol L^{-1} hydrogen peroxide (H_2O_2), and water, as reported by Azevedo et al. (1998) with minor modifications. The activity was evaluated after the decomposition of H_2O_2, by monitoring changes in absorbance at 240 nm, with a molar extinction value of 0.0394 mmol L^{-1} cm^{-1}. The results were represented in µmol of H_2O_2 consumed per minute per mg protein.

Ascorbate peroxidase assay (APX) - The APX activity was measured by determining the ascorbate oxidation rate using a technique adapted by Soares (2011). The reaction mixture (2 mL) containing 100 mmol L^{-1} potassium phosphate buffer (pH 7.0), 0.5 mmol L^{-1} L-ascorbic acid, 0.1 mmol L^{-1} hydrogen peroxide (H_2O_2), and 30 µL of the extract was incubated at 28ºC. The ascorbate oxidation rate was monitored at 290 nm for 3 min and enzyme activity was expressed as µmol$min^{-1}$$mg^{-1}$protein (Extinction coefficient of 2.8 mmol L^{-1} cm^{-1}).

Superoxide dismutase (SOD) - The capacity of the enzyme extracts to prevent the photochemical reduction of nitroblue tetrazolium (NBT) was measured at 560 nm to quantify the activity of superoxide dismutase. An assay mixture (3 ml) containing 100 mM of phosphate buffer (pH 7.4), 1.0 mM of EDTA, 50 mM of riboflavin, 10 mM of methionine, 75 mM of NBT, and 100 mL of enzyme extract was incubated for 15 minutes under fluorescent light as described by Ahanger et al. (2020). The absorbance was measured at 560 nm and the activity was reported as µmol min^{-1} mg^{-1} protein.

Estimation of Cr Content

The amount of chromium in root, stem, and leaf tissues was measured as outlined by (Zhou et al., 2017) with little modifications Samples of leaves, stems, and roots were dried for 48 hours at 80 ºC, crushed into a fine powder, and then 0.2 grams of that powder were microwave-digested with concentrated HNO_3 Atomic Absorption Spectrophotometer (AAS) was used to assess the quantity of chromium in the tissues. The Translocation factor (TF) was calculated using the formula:

$$TF = \frac{[Chromium\ in\ Shoot]}{[Chromium\ in\ Root]} \qquad \text{eqn. 4}$$

Data analysis

The data collected were assembled for statistical analysis using Microsoft Excel. The Shapiro test was used to verify the normality of the data distribution. Because the data does not violate the assumption of normality at 0.05 % level. One Way Analysis of Variance (ANOVA) was performed using the IBM SPSS software package version 26.0. Means were separated using the Tukey's LSD test at a 5% significance level.

Results and Discussion

MDA

The concentration of MDA under various Cr concentrations are illustrated in Figure 1A. Cadmium cause membrane lipid peroxidation to increase significantly by 75.54%, 86.00% and 89.22% under T1, T2 and T3 respectively compared to the control. A progress increase in MDA was noted with increased level of Cr contamination. T2 and T3 increased by 42.73 and 55.93% over the T1 whereas an insignificant increase in MDA by 23.059% was recorded in T3 relative to T2 accordingly.

Protein Oxidation (Carbonyl)

Protein oxidation is determined by the available carbonyl in the extract. Figure 1B shows that the progressive increase in carbonyl is proportional to the amount of cadmium in the soil spiked. However, carbonyl concentration increased significantly by 1.37, 1.72 and 1.75-folds under T1, T2 and T3 respectively, relative to the T0. The significant increase in carbonyl concentration observed in this study is attributed to the effect of cadmium in the soil.

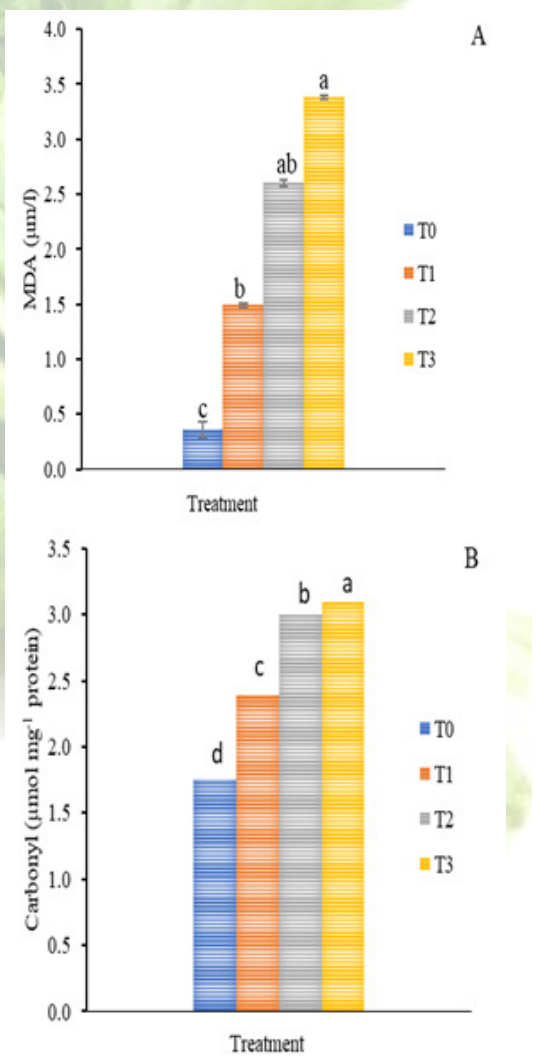

Figure 1: Effect of Cr on the level of malondialdehyde (A) and protein oxidation (B) in Spinacia oleracea. T0, control; T1, 25 $mgkg^{-1}$, T2, 50 $mgkg^{-1}$ and T3 100 $mgkg^{-1}$. Dissimilar letters denote significant difference at $P < 0.05$, LSD test. Data is the pentaplicate mean ± SE

Membrane Electrolyte Leakage and Membrane Stability Index

Membrane electrolyte leakage demonstrated in Figure 2A show significant increase in the leakage of electrolyte. The MEL increased by 43.68, 57.87 and 72.379% under T1, T2 and T3 respectively, relative to the T0. Membrane stability index decreased significantly in T1, T2 and T3 by 40.39, 64.76 and 75.38% respectively, compared to T0 (Fig. 2B).

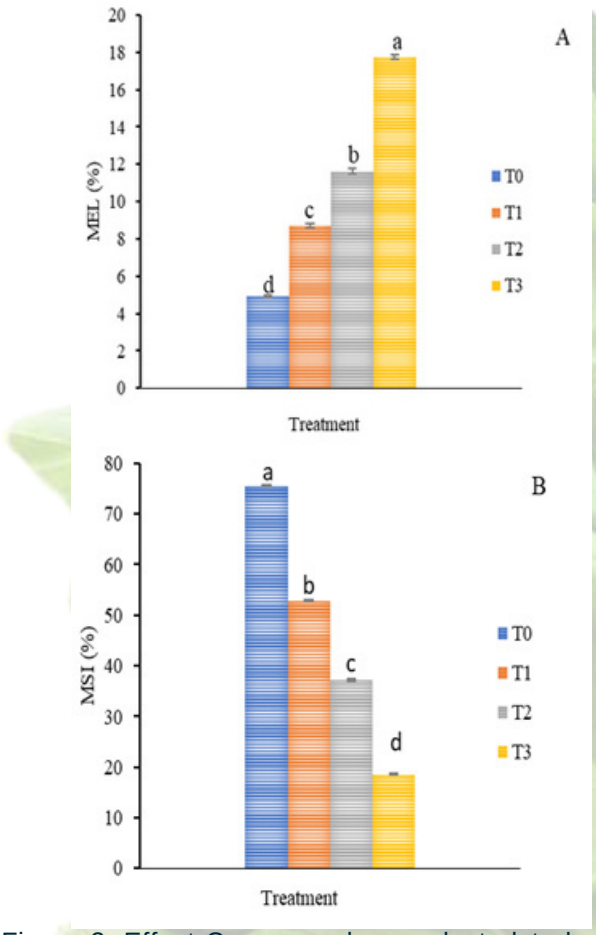

Figure 2: Effect Cr on membrane electrolyte leakage (A) and Membrane stability index (B). T0, control; T1, 25 mgkg^{-1} , T2, 50 mgkg^{-1} and T3 100 mgkg-1. Dissimilar letters denote significant difference at P < 0.05, LSD test. Data is the pentaplicate mean ± SE.

Reducing Power and Total Antioxidant Assay

The reducing power and total antioxidant assay of the extracts at various concentration of chromium were shown in Figure 3 below. The reducing power of extract increased significantly by 1.44, 2.00 and 2.41-fold under T1, T2 and T3 respectively compared to the control. Relative to T1, T2 and T3 increased by 1.39 and 1.67-fold accordingly. No significant increase in RP was recorded between T2 and T3 (Fig. 3A).

Total antioxidant assay (Fig. 3B) illustrated a significant fold increased in TAA under T1 (1.68), T2 (2.03-fold) and T3 (2.66-fold), relative to the T1. However, TAA increased significantly under T2 (1.21-fold) and T3 (1.58-fold) over the T1 whereas no significant increase was noted on comparing T2 and T3.

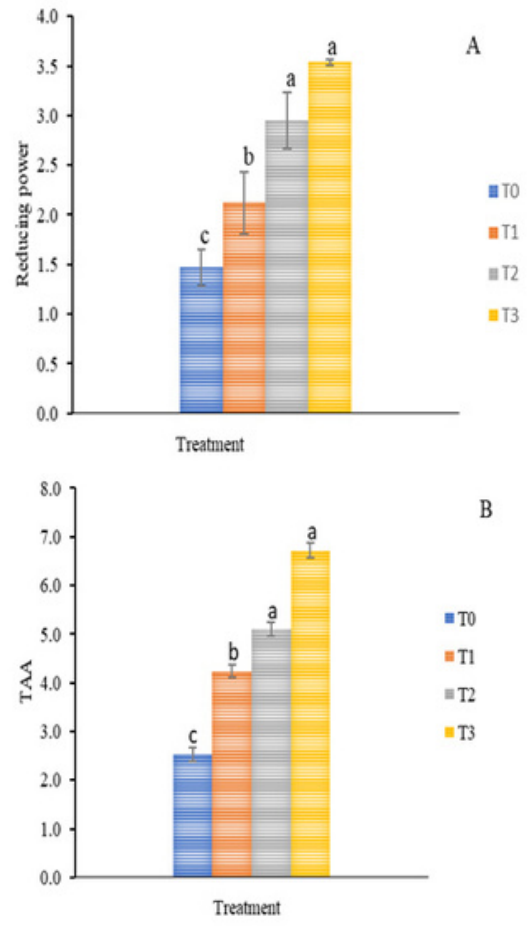

Figure 3: Influence cadmium on reducing power (A) and total antioxidant assay (B) on the S. oleracea leaf extract. T0, control; T1, 25 mgkg-1, T2, 50 mgkg, and T3 100 mgkg^{-1}. Dissimilar letters denote significant difference at P < 0.05, LSD test. Data is the pentaplicate mean ± SE

H_2O_2 scavenge activity

The ability of *S. oleracea* to scavenge H_2O_2 was determined and illustrated in Figure 4. The H_2O_2 scavenging activity of the extracts increased under T1 (9.43%), T2 (30.39%) and T3 (40.16%) compared to T0. Among the Cr treated groups T2 and T3 increased significantly by 21.09 and 32.16% respectively, over the T1 whereas T3 increased statistically significant by 14.03% relative to T2 (Fig. 4).

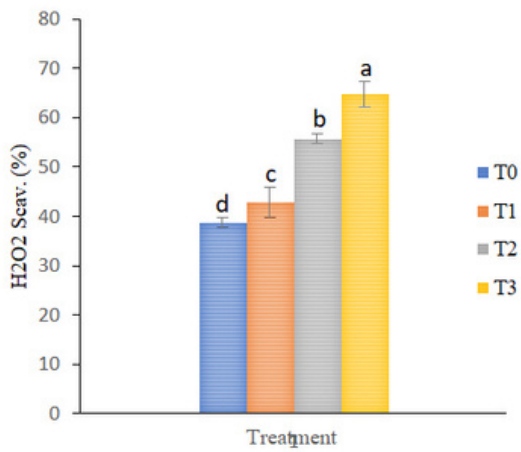

Figure 4: H_2O_2 scavenging ability of the *S. oleracea* extracts. T0, control; T1, 25 mgkg^{-1} , T2, 50 mgkg^{-1} and T3 100 mgkg^{-1} Dissimilar letters denote significant difference at P < 0.05, LSD test. Data is the pentaplicate mean ± SE

Enzymatic Antioxidant Activities of the Spinacia oleracea Extracts

CAT activity of the extract shows that the chromium at lower dose displays low activity whereas higher dose cause increased in CAT activities compared to the control (Fig. 5A). Compared to the control, T1 decreased insignificantly by 1.09-fold whereas it significant increase under T2 (1.57) and T3 (2.28-fold) respectively.

APX activity was illustrated in figure 5B. the activity is with increasing Cr dose in the soil. Over the T0, T1, T2 and T3 increased significantly by 1.37, 1.57 and 1.86-folds. On comparing among the Cr treated groups, T2 and T3 increased significantly by 1.14 and 1.36-folds relative to T1 whereas 1.19-fold under T3 was recorded compared to T2. SOD activity increased significantly by 1.17, 1.32 and 1.36-folds under T1, T2 and T3 respectively, compared to the T0 as illustrated in figure 5C. However, among the Cr spiked soil, T1 and T2 increased statistically significant over T1 by 1.13 and 1.17-folds.

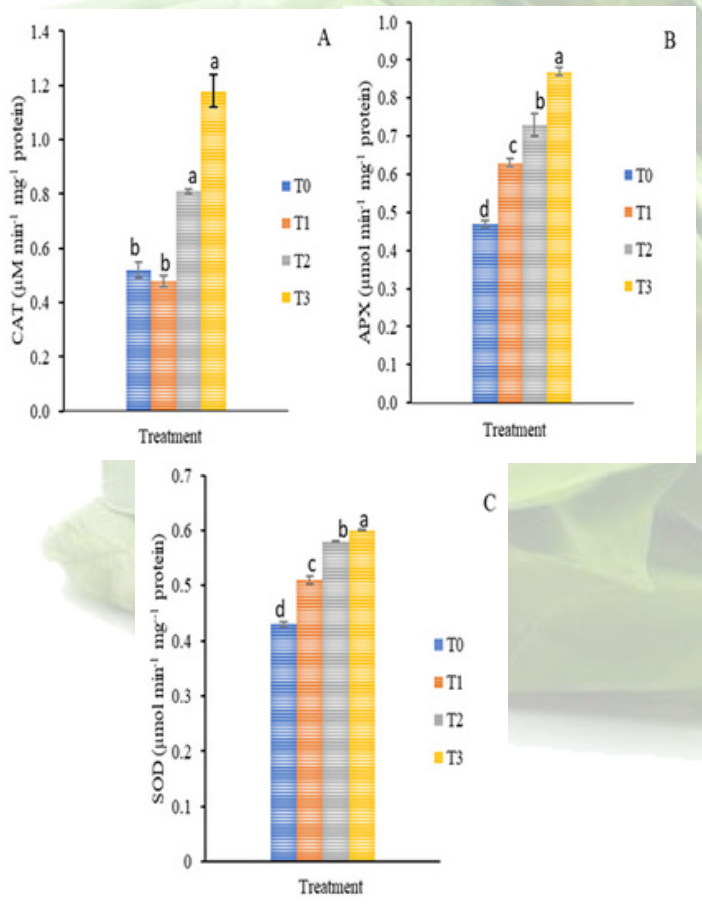

Figure 5: Antioxidant enzyme activities in the Cr stress *Spinacia oleracea*. T0, control; T1, 25 mgkg^{-1} Cr, T2, 50 mgkg^{-1}Cr, and T3 100 mgkg^{-1}Cr. Dissimilar letters denote significant difference at P < 0.05, LSD test. Data is the pentaplicate mean ± SE

Absorbed Cr and Translocation factor

The absorbed Cr in root, shoot and translocation factor were increasing with increase in Cr concentration in the soil (Fig. 6). The Cr absorbed was higher in root than the shoot (Fig. 6-A & B). However, the translocation factor shows no significant difference under T1 and T2 but statistically significant in T3 (Fig. 6C).

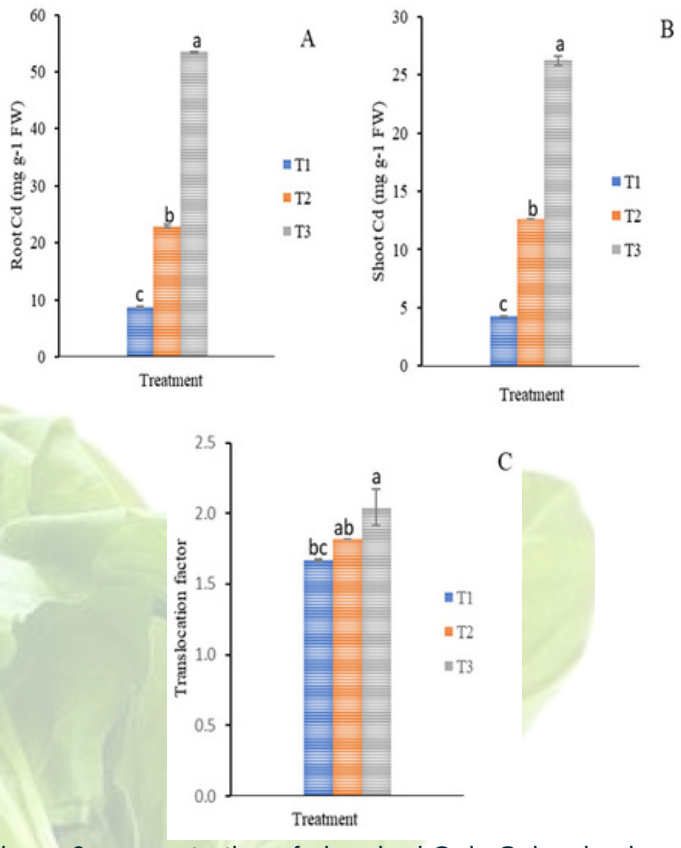

Figure 6: concentration of absorbed Cr in Spinacia oleracea root (A), shoot (B) and translocation factor (C). T1, 25 mgkg^{-1}, T2, 50 mgkg^{-1}, and T3 100 mgkg^{-1}. Dissimilar letters denote significant difference at P < 0.05, LSD test. Data is the pentaplicate mean ± SE

Discussion

MDA significant raised with increased level of Cr dose in spiked soil (Fig. 1A). The level of MDA is often used as an indicator of oxidative damage due to enhanced generation of ROS (Ayala et al., 2014). Membrane damage might be a result of initiated oxidative stress and the accumulation of "reactive oxygen species" leading to disturbances in membrane configuration (Foyer & Noctor, 2005) and oxidation of cell membrane fatty acids (DaCosta & Huang, 2007). Our study shows increased level of MDA with increased level of contamination illustrated in Figure 1A. This finding tallies with a number of studies (e.g. Abdullahi et., 2022; Rahman et al., 2010; Guo et al., 2004). MDA *increased significantly in Kandelia candel.Rahoui and co-workers (2010) reported that in Cr-stressed Pisum sativum* L. an increase in malondialdehyde (MDA) content was observed, owing to Cr-induced membrane lipid peroxidation. The present study is in consistent with effects of cadmium on malondialdehyde, glutathione levels and superoxide dismutase activity in the liver, kidneys, and testes of the male rats reported by Haouem and El Hani, (2013).

Protein oxidation (carbonyl)

Extreme environmental variation may cause deviation in gene expression, contributing to changes in protein diversity in cells (Kieffer *et al.*, 2009). The synthesis of healthy proteins and the creation of stress proteins (SP) can both be impacted by Cr stress. Carbonyl derivatives occur on the side chains of histidine, arginine, proline, lysine, and residues during these oxidative stress (Gonçalves *et al.*, 2007).

Protein oxidation in S. oleracea seedlings grown under absolute Cr experiences an increase in carbonyl formation (Fig. 1B). Substantial concentrations of ROS can be produced quickly under stressful circumstances, defeating the protective mechanism and causing numerous protein structural changes (Cargnelutti *et al.*, 2006). Our result shows progressive increase in carbonyl compound as chromium spike increased. This finding is in agreement with Gonçalves *et al*. (2007) who found accumulation of carbonyl in cucumber seedlings and Aravind and Prasad (2005) in *Ceratophyllum demersum.*

Membrane electrolyte leakage and stability index

MEL is an imperative index in cell stress physiology and is used to evaluate the leakage of cell components (Jawad *et al.*, 2020). Electrolyte leakage from damaged tissues was commonly used to assess cell membrane stability (Sikder & Paul, 2010). The oxidative stress caused due to Cr toxicity may lead to reduce membrane stability due to the over-accumulation of reactive oxygen species (ROS) that may also damage the morpho-physiological attributes in the plants (Azeez *et al.*, 2021). We reported increased level of electrolyte leakage and membrane instability with increased level of chromium contamination (Fig. 2). Under heavy metal stress, oxidative damage evokes cell membrane rupture hence, electrolyte the leakage (Abdullahi *et al.*, 2021). This study corresponded with significant increase in electrolyte leakage occurred following storage of mature melons reported by (Lester *et al.*, 1993). This study corroborated with the findings on significant variation in membrane stability index in wheat genotype reported by (Singh *et al.*, 2020). Demidchik and Maathuis (2007) coined out that both membrane electrolytes leakage and stability index are affected with increase in metal concentration in *Phaseolus vulgaris plant.*

Non-enzymatic antioxidant activities

Non-enzymatic antioxidants that control the levels of ROS in cells, such as tocopherols, carotenoids, GSH, proline, and AsA are regarded as moderators of oxidative damage (Adrees *et al.*, 2015). However, their antioxidants' activity and availability are dependent on secondary metabolites' capacity to synthesize specific compounds, which varies widely among different plant species (Akyol *et al.*, 2020).

Reducing Power and Total Antioxidant Assay

Cr, although not being a redox active metal, causes the production of reactive oxygen species (ROS), such as superoxide radicals ($O^{2\bullet}$), hydrogen peroxides (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$) (Gill & Tuteja, 2010). Antioxidants fight against free radicals and protect various plants from detrimental effects of free radicals (Umamaheswari & Chatterjee, 2008). They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. Plants use ROS-detoxifying antioxidant defense machinery, which includes nonenzymatic antioxidants, to reverse the inhibitory effects of ROS caused by Cr (Gill & Tuteja, 2010). In the present investigation, the reducing power capacity (RPC), total antioxidant activity (TAA) (Fig. 3) were increased in response to Cr stress conditions in all treatment. This study is consisted with findings on reducing power by aqueous than methanolic extracts reported by (González -Palma *et al.,* 2016). Our study corresponded with findings on antioxidant activity of aqueous methanol extracts of *Pleurotus ostreatus* in different growth stages reported by (González -Palma *et al.*, 2016). Fan *et al.* (2013) reported increased level of antioxidant activity in *S. oleracea.* Jibril *et al.* (2017) recorded an increase in antioxidant activities as cadmium concentration increased in *Lactuca sativa* L. Márquez-García *et al.* (2012) reported the significant increase of antioxdant in *Erica andevalensis*.

H_2O_2 scavenging activity of the extract

This study revealed the ability of *S. oleracea* extract antioxidant capacity based on the H_2O_2 scavenging activity (Fig. 4). Our study corresponded with findings on hydrogen peroxide scavenging activity of some phenolic acids reported by Rahman et al. (2015). Hundu *et al.* (2018) reported increased level of scavenging activities in *Plantago ovata* upon exposure to Cr. Romero-Puertas *et al*. (2004) reported that pea accumulate H_2O_2 in leaves under abiotic stress.

Enzymatic Antioxidant Activities of the Extracts

Trace metals generally cause oxidative stress to plants, either directly or indirectly through the formation of ROS (Nagajyoti *et al.*, 2010; Zaheer *et al.*, 2015). To ensure survival, plants have developed efficient antioxidant enzymatic machinery like catalase (CAT) ascorbate peroxidase (APX) and superoxide dismutase (SOD) under Cr toxicity to minimized the counter effects of ROS production in plant metabolic processes (Jan *et al.,* 2020). In the present study, the obtained results revealed that of APX, SOD and CAT significant increases compared to T0 in all treatments illustrated in Figure 5. This study is consistent with the findings on antioxidant activity of biological active compounds reported by (Ligor et al., 2013). Irfan *et al.* (2014) reported that activity of antioxidant enzymes; "peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD)" in the leaves of plants increased significantly in response to Cr in a concentration dependent manner. Reported increased level of POX. SOD, APX and CAT activities were increased under absolute Cr significantly higher than T0. Increased in SOD and APX activities in the leaves of *S. oleracea* was reported previously in absolute Cr treatment by Zhu *et al*. (2019).

Chromium concentration and Translocation factor

In this study, Cr concentration absorbed by *S. oleracea* is higher in roots than the aerial parts (stem and leaves) demonstrated in Figure 6. However, the significant increase in both the tissue concentration and translocation factor as the level of chromium contamination increased was noted. The higher concentrations in roots than in other plant tissues imply that plants do not translocate all Cr absorbed

by the plant to the above-ground biomass (Das *et al.*, 2022). Poor translocation of Cr to the shoots could be due to sequestration of most of the Cr in the vacuoles of the root cells to render it non-toxic which may be a natural toxicity response of the plant. In plant tissues, the Cr (VI) is converted to Cr (III) that has the tendency to bind to the cell walls, which hinders the further transport of Cr within plant tissues (Kabata-Pendias & Szteke, 2015; Sharma *et al.,* 2019)

Conclusion

Chromium was absorbed by *S. oleracea* which is seriously affecting the membrane lipid and protein composition and stability of the membrane hence, membrane lost its function. Chromium transfer in leafy vegetable tissues was significant, with the highest accumulation being recorded in roots. The translocation and accumulation in edible parts of *S. oleracea* affirm the chromium shuttling effects into food chain.

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