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CHANGE IN LETTUCE NUTRIENTS, SOIL ENZYMES, SOIL MICROBIAL BIOMASS, AND ACTIVITIES UNDER CHROMIUM TOXICITY

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Abstract

The toxicity of heavy metals in the soil plays a detrimental role in microbial activities. Higher concentrations of Cr causes restriction of enzyme secretion in soil by disturbing the microbial population and proliferation. On the other hand, the cultivation of crops in such Cr-contaminated soils also caused a significant decline in nutrient uptake in the plants. That's why the current pot study was conducted to explore the impacts of different Cr application rates on soil enzymes activities and lettuce plants' nutrient concentration. A total of 6 treatments were applied in 5 replicates. The treatments include T0 =control (no Cr), T1=50 mg, T2=100 mg, T3=150 mg, T4=200 mg, and T5=250 mg Cr/kg soil. Results showed that increasing concentration of Cr toxicity caused a significant decrease in SMBP, SMBS, alkaline phosphatase activity, and arylsulphatase over control. A significant decline in macronutrient N, P, K, Ca, and Mg concentration in lettuce validated the negative impacts of Cr on lettuce when cultivated in Cr toxicity, i.e., 50, 100, 150, 200, and 250 mg Cr/kg soil. The increasing level of Cr also significantly minimized the concentration of Zn, Cu, Mn, Fe, and S in lettuce when grown in Cr-contaminated soil. In conclusion, increasing Cr toxicity is inversely related to soil enzymes activities and lettuce nutrient uptake. More in-depth investigations are required to explore the impacts of Cr on nutrient uptake in crops and enzyme activities in soil under different soil textures and cultivated crops type.

Key words: Heavy metal, Micronutrients, Macronutrients, Lettuce, Enzymes activities.

Introduction

The continuous discharge of industrial wastewater to ariculture soils elevates the probability of heavy metal contamination in the soil (Bashir *et al*., 2021). The toxicity of chromium among heavy metals is particularly acute in the rhizosphere (Dhal *et al*., 2013). The high crystallization of chromium in industrial processes, particularly in leather tanning, has resulted in it becoming a prevalent contaminant in water, soil, and sediment, ranking as the second most hazardous heavy metal (Singh *et al*., 2013). It usually exists in two forms, i.e., Cr^{3+} and Cr^{6+} but also has other unstable forms (Tripathi *et al*., 2016, Godinho *et al*., 2019). The consistent utilization of chromium-contaminated industrial wastewater exacerbates the enrichment of chromium concentration within the soil profile (Oubane *et al*., 2021). When plants update chromium beyond the threshold limit, their physiological and metabolic processes become disturbed (Mushtaq *et al*., 2021). This enrichment also leads to an imbalance in the ionic concentration of the soil, and the toxicity of chromium has a detrimental impact on microbial proliferation in the soil (Shuaib *et al*., 2021, Yang *et al*., 2021).

Conversely, the microbial population and their activities in the soil are considered a key factor in determining soil health. The majority of microbial communities in the rhizosphere are vulnerable to the toxicity of heavy metals, including chromium (Terekhova *et al*., 2021). However, research has also identified the presence of rhizobacteria in the rhizosphere that exhibit tolerance to the toxicity and stress imposed by heavy metals. These tolerant rhizobacteria can be utilized as an effective strategy for alleviating heavy metal stress in plants (Danish *et al*., 2019, Zafar-ul-Hye *et al*., 2020). Such microbes play an imperative role in the nutrient cycling and decomposition of organic matter for better availability of nutrients to the plants and maintenance of soil fertility (Zafar-ul-Hye *et al*., 2018a; b; Danish *et al*., 2020; Zafar-ul-Hye *et al*., 2020; Wahid *et al*., 2020).

Enzymes play a crucial role in the proper functioning of microbes with regards to nutrient cycling and organic matter decomposition. These enzymes are essential for maintaining soil ecosystem health and regulating nutrient cycles (Bautista-Cruz & Ortiz-Hernández, 2015). It is important to note that the component and structures of microbial communities in the soil are key factors that decide the secretion of enzymes for the maintenance of soil health. Any alteration in the secretion of these enzymes is considered one of the natural indicators for assessing stress (Pradhan *et al*., 2020).

A significant increase in chromium concentration in the soil profile also minimizes the uptake of nutrients in plants (Chigonum *et al*., 2019). Most chromium makes complexes with the essential nutrients, decreasing their update when plants are cultivated in chromium-contaminated soils (Osu Charles & Onyema, 2016). It is well documented that higher chromium concentrations decrease the uptake of calcium, phosphorus, and iron in plants by making complexes. Moreover, the deficiency of sulfur, copper, and zinc in plants is another drawback of chromium toxicity in the soil (Kabata-Pendias & Szteke, 2015, Osu Charles & Onyema, 2016).

Given the significance of essential nutrient bioavailability and soil microbial enzyme secretion, the current study was designed to investigate the effects of chromium (Cr) on soil enzymes (alkaline phosphatase and arylsulfatase) and lettuce nutrient concentrations under toxic soil conditions. The study aimed to examine the impact of Cr on these factors to gain a better understanding of its effects on soil and plant health. It was hypothesized

that the exposure of lettuce plants to chromium-contaminated soil might result in less uptake of essential nutrients and poor activity of soil enzymes (alkaline phosphatase and arylsulfatase), which might negatively impact the growth and health of the lettuce plants.

Material and Methods

Experimental soil site: The soil collection (Sultanpur series of aridisol soil order) was done from 0 to 15 cm depth. The site of experimental soil collection was in the research area of the Faculty of Agriculture, Bahauddin Zakariya University Multan (30°16′40″N, 71°30′79″E).

Preparation of soil: For the preparation of the soil sample, it was initially sieved from a 2 mm sieve. After that, a composite soil sample was made for the pre-experimental characterization of soil (Petersen & Calvin, 1986).

Experimental design: The design of the experiment was completely randomized. All the treatments were applied in a 2-factorial arrangement while conducting the pot experiment.

Pots type and preparation: Clay pots were used for the experimental purpose having the capacity to carry 5 kg of soil. To apply the toxicity of chromium as per the treatment plan salt of chromium (Potassium dichromate) was mixed in soil at the time of preparation for the achievement of homogenized condition.

Treatments: A total of 6 treatments were applied in 5 replicates. The treatments include T0 =control (no Cr), T1=50 mg, T2=100 mg, T3=150 mg, T4=200 mg, and T5=250 mg Cr/kg soil.

Seed sowing and thinning: A total of six seeds were sown in each pot. After 10 days of seed sowing, thinning was performed to maintain two healthy plants.

Samples collections for microbial biomass enzyme activity changes analysis: From each experimental pot, soil samples were collected every 15 days interval to assess soil microbial biomass and enzyme activity changes. The collection of samples was continued for 90 days.

Harvesting of lettuce: The lettuce crop was grown in all experimental pots for 90 days (05 Nov 2019 to 02 Feb 2020). Plant samples were collected twice. Lettuce mature leaves were harvested after 45 days of the experiment. After 90 days, lettuce was harvested from pots to record lettuce nutrients.

Soil microbial biomass phosphorus (SMB-P): Estimation of soil microbial biomass phosphorous was done by using the chloroform fumigation extraction method. Moist soil samples (2.5 g) on oven-dry basis was weighed and fumigated with alcohol-free chloroform. At the same time, the non-fumigated soil sample was incubated at 25°C. Fumigated and incubated soil samples was extracted with $NaHCO_3$ (0.5 M, pH 8.5) for 30 minutes. After extraction and filtration, 10 ml of extract was pipetted into a 50 ml flask, color developing reagent (8 ml), and addition of distilled water to make the volume 50 ml. After 15 minutes of color development, absorbance was noted on a spectrophotometer at 882 nm wavelength (Brookes *et al*., 1982). The microbial biomass phosphorous was calculated by using the formula given below:

Microbial biomass P = (P fumigated – P unfumigated) x Factor i.e. 0.40

Soil microbial biomass sulphur (SMB-S): For SMB-S determination, the chloroform fumigation extraction method was applied. Fumigated and non-fumigated soil samples (20 grams) was extracted for 30 minutes with $CaCl_2$ solution (20 ml, 10 mM) (Houba *et al*., 2000). The turbidity in filtrate was measured at 420 nm wavelength. The SMB-S was estimated by using the following calculation:

Soil microbial biomass S = (S fumigated – S unfumigated): kS i.e. 0.31

Soil alkaline phosphatase activity: To determine soil alkaline phosphatase activity, toluene (1.5 ml) was added in moist sieved soil (10 grams) in a 100 ml flask and kept for 15 minutes at room temperature. Di-sodium phenyl phosphate substrate solution (10 ml) and borate buffer (20 ml) were added. Flask was stoppered; contents were mixed and incubated for 3 hours at 37°C. After 3 hours of incubation, soil suspension was diluted to 100 ml with warm distilled water, mixed, and filtered immediately. Distilled water (10 ml) was added instead of the substrate solution to perform blank. To determine phenol, the buffer solution (5 ml) and the filtrate (1-8 ml) were pipetted in a 100 ml flask, and the contents were diluted to 25 ml. Then, 2,6-dibromo-quinonechloromide solution (1 ml) was added and kept at room temperature for 20-30 minutes. The final contents were diluted to 100 ml with distilled water, and optical density was measured at 600 nm.

For the preparation of the calibration curve, 0, 5, 10, 15, and 20 ml of standard phenol solution (0-200 µg phenol/ml) was taken, and the rest of the procedure for solution preparation was followed. Soil Alkaline Phosphatase activity was measured by measuring phenol through the following calculations.

$$\text{Phenol}\left(\frac{\frac{\mu g}{g\ dwt}}{hour}\right) = C \times \frac{100}{dwt} \times t \times 10$$

Soil arylsulfatase activity: To measure the arylsulfatase activity in soil, 1 gram of sieved (< 2 mm) moist soil was placed in a 50 ml Erlenmeyer flask. Then, 0.25 ml of toluene, 4 ml of acetate buffer, and 1 ml of p-nitrophenol sulfate solution were added, and the mixture was stirred. Flask was capped and incubated at 37°C for 1 hour. After incubation, $CaCl_2$ (1 ml, 0.5 M) and NaOH (4 ml, 0.5 M) were added and filtered. A spectrophotometer was set at 400 nm to measure the optical density of the filtrate. For the preparation of control, 1 ml of *p*-nitrophenol sulfate solution was added after the addition of $CaCl_2$ and NaOH (immediately before filtration of soil suspension) (Tabatabai & Bremner, 1970).

For the preparation of the calibration curve, 1 ml of the standard p-nitrophenol solution was diluted to 100 ml with distilled water, and 0, 1, 2, 3, 4, and 5 ml of aliquot was pipetted to 50 ml Erlenmeyer flask, and volume was adjusted to 5 ml by addition of distilled water. After dilution, 01 ml of 0.5 M $CaCl_2$ and 4 ml of NaOH were added and mixed, and absorbance was measured at 400 nm (Tabatabai & Bremner, 1970).

The calculation of p-nitrophenol was as follows:

$$\text{p} - \text{nitrophenol} \left(\frac{\frac{\mu g}{g} \text{dwt}}{\text{hour}} \right) = \text{C x} \frac{\text{V}}{\text{dwt}} \text{x SW x t}$$

Microbial transformation of chromium: The microbial transformation of chromium from Cr(VI) to Cr(III) was determined indirectly. At the start of the experiment, the soil sample was collected from the experiment soil to determine trivalent and hexavalent chromium. Hexavalent chromium was introduced to the soil as a source, and the changes in its chemical form from hexavalent (more toxic) to trivalent (less toxic) were observed at various time intervals.

Statistical analysis

A standard procedure for the statistical analysis was done (Steel *et al*., 1997). The data was analyzed statistically by standard analysis of variance (ANOVA) by using the statistical software Origin 2021, and variations was analyzed by the least significant difference (LSD at $P \leq 0.05$) test (OriginLab Corporation, 2021).

Results

The effect of treatments and different time intervals was significant on SMBP and SMBS. Results showed that T2, T3, T4, and T5 differed significantly compared to T0 at 15, 45, 60, 75, and 90 days for the decrease in SMBP. No significant change was noted between T0 and T1 for SMBP at 15, 45, 60, 75, and 90 days (Fig. 1A). For SMBS, T2, T3, T4, and T5 caused a significant decline at 15, 45, 60, 75, and 90 days compared to T0. Treatment T1 also remained significantly different for SMBS at 45 days over T0. However, T0 and T1 did not differ significantly at 15, 30, 60, 75, and 90 days (Fig. 1B). Maximum decline in SMBP and SMBS was noted in T5 compared to T0 at 15, 30, 60, 75, and 90 days.

The impact of different time intervals and treatments was significantly different on phosphatase and arylsulfatase. It was observed that T4 and T5 were significantly different compared to T0 at 15, 45, 60, 75, and 90 days for the decrease in phosphatase and arylsulfatase. No significant change was noted between T0 and T1 for phosphatase and arylsulfatase at 15 and 45, yet T1 caused a significant decrease at 60, 75, and 90 days (Fig. 2A and 2B). Maximum decline in phosphatase and arylsulfatase was noted in T5 compared to T0 at 15, 30, 60, 75, and 90 days.

The influence of different time intervals and treatments differed significantly for Cr. Trans (Cr-III) and Cr. Trans (Cr-VI). It was observed that T1, T2, T3, T4, and T5 remained significantly better for an increase in Cr. Trans (Cr-III) and over T0 at 15, 45, 60, 75, and 90 days (Fig. 3A). A significant decrease was also noted in T1, T2, T3, T4, and T5 for Cr. Trans (Cr-VI) at 15, 30, 45, 60, and 75, yet T1 did not cause significant change 90 days than T0 (Fig. 3B). Maximum increase and decline in Cr. Trans (Cr-III) and Cr. Trans (Cr-VI) was noted in T5 over T0 at 15, 30, 60, 75, and 90 days respectively.

It was observed that nitrogen (N), phosphorus (P), and potassium in lettuce were significantly different where different Cr levels (T0 to T5) were applied. No significant change was noted between T0 and T1 for N in lettuce. However, T2, T3, T4, and T5 remained significant for the decrease in N in lettuce. A maximum decrease in N in lettuce was noted where T5 was applied over T0 (Fig. 4A). For P in lettuce, all the treatments differed significantly from T0. No significant change was noted between T4 and T5 for the decrease in P in lettuce. A maximum decrease in P in lettuce was noted where T5 was applied over T0 (Fig. 4B). In the case of K in lettuce, T2, T3, T4, and T5 caused a significant decline over T0. However, T0 and T1 remained statistically alike to each other for K in lettuce (Fig. 4C).

For calcium (Ca) and magnesium (Mg) in lettuce significant difference was noted where different Cr levels (T0 to T5) were applied. No significant change was noted between T0 and T1 for Ca and Mg in lettuce. However, T2, T3, T4, and T5 remained significant for the decline in Ca and Mg in lettuce. A maximum decrease in Ca and Mg in lettuce was observed where T5 was applied over T0 (Figs. 5A & 5B).

For sulfur (S), iron (Fe), and copper (Cu) in lettuce significant difference was observed where different Cr levels (T0 to T5) were applied. No significant change was noted between T0 and T1 for S, however, a significant change existed for Fe and S in lettuce. Treatment T1 caused a significant decrease in Fe while the increase in Cu than T0. However, T2, T3, T4, and T5 remained significant for the decline in S, Fe, and Cu in lettuce. A maximum decrease in S, Fe, and Cu in lettuce was observed where T5 was applied over T0 (Figs. 6A, 6B, and 6C).

For sulfur (S), iron (Fe), and copper (Cu) in lettuce significant difference was observed where different Cr levels (T0 to T5) were applied. No significant change was noted between T0 and T1 for S, however, a significant change existed for Fe and S in lettuce. Treatment T1 caused a significant decrease in Fe while an increase in Cu than T0. However, T2, T3, T4, and T5 remained significant for a decline in S, Fe, and Cu in lettuce. A maximum decrease in S, Fe, and Cu in lettuce was observed where T5 was applied over T0 (Figs. 6A, 6B, and 6C).

In the case of zinc (Zn) and manganese (Mn) in lettuce, a significant difference was noted where different Cr levels (T0 to T5) were applied. No significant change was noted between T0 and T1 for Zn and Mn in lettuce. Treatments T2, T3, T4, and T5 differed significantly for the decline in Zn and Mn in lettuce. A maximum decrease in Zn and Mn in lettuce was observed where T5 was applied over T0 (Figs. 7A and 7B).

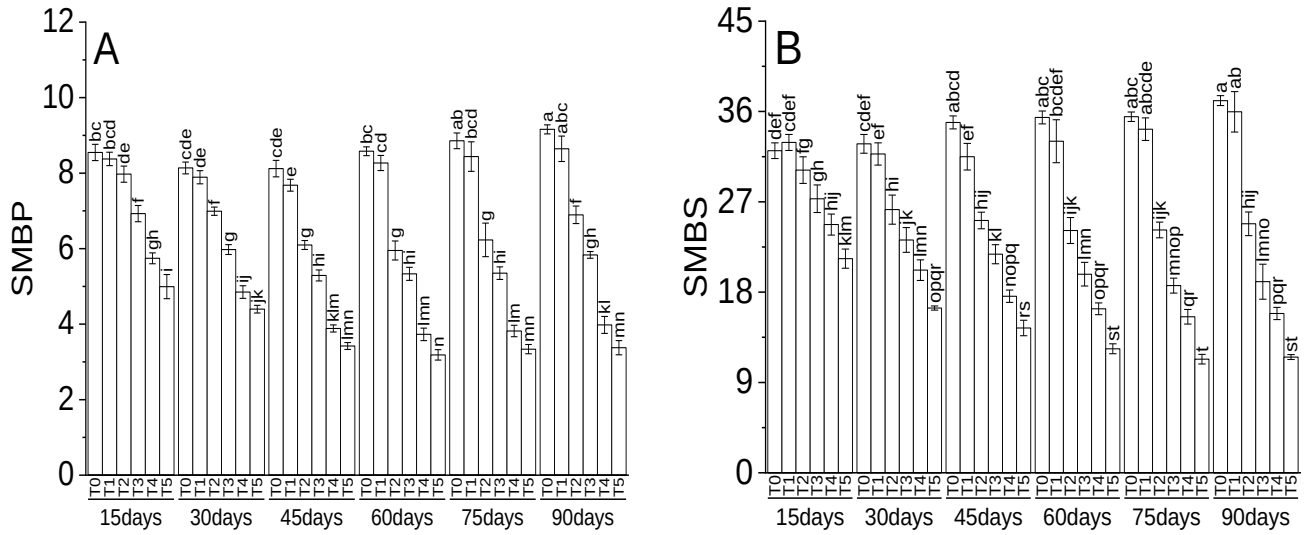

Fig. 1. Effect of treatments and different time intervals on SMBP (µg P/g soil) (A) and SMBS (µg S/g soil) (B). Bars are means of 5 replicates ± SE. Different bar values show significant differences at $p \leq 0.05$ compared to Fisher LSD.

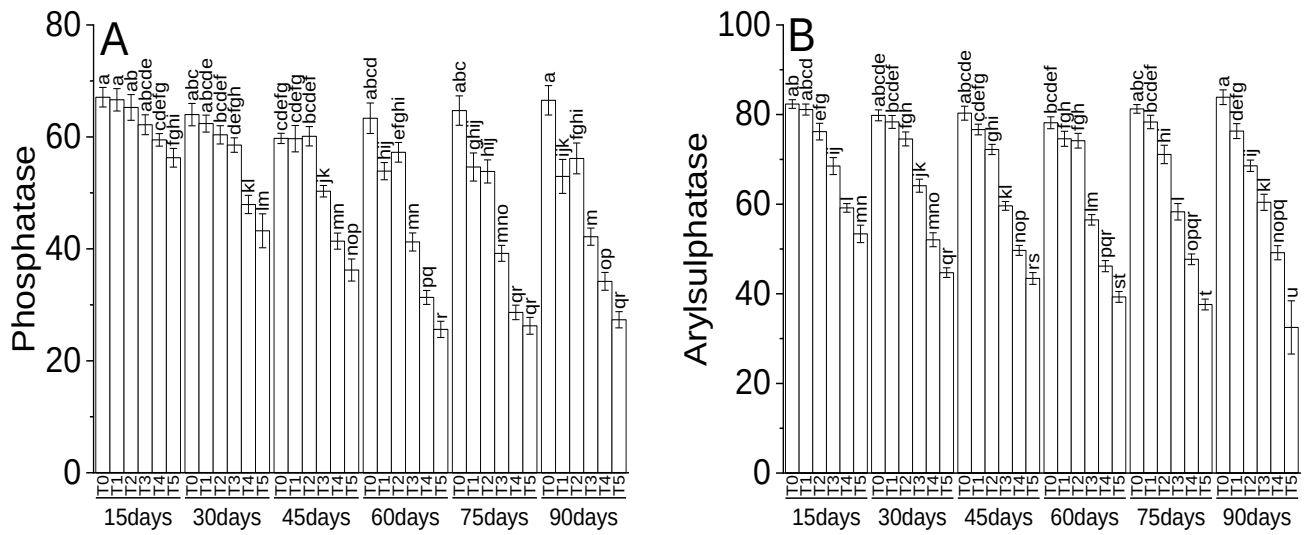

Fig. 2. Effect of treatments and different time intervals on alkaline phosphatase activity (Phenol µg/gdwt/hour) (A) and Arylsulphatase (*p*-nitrophenol µg/gdwt/hour) (B). Bars are means of 5 replicates ± SE. Different values on bars are showing significant differences at $p \leq 0.05$ compared to Fisher LSD.

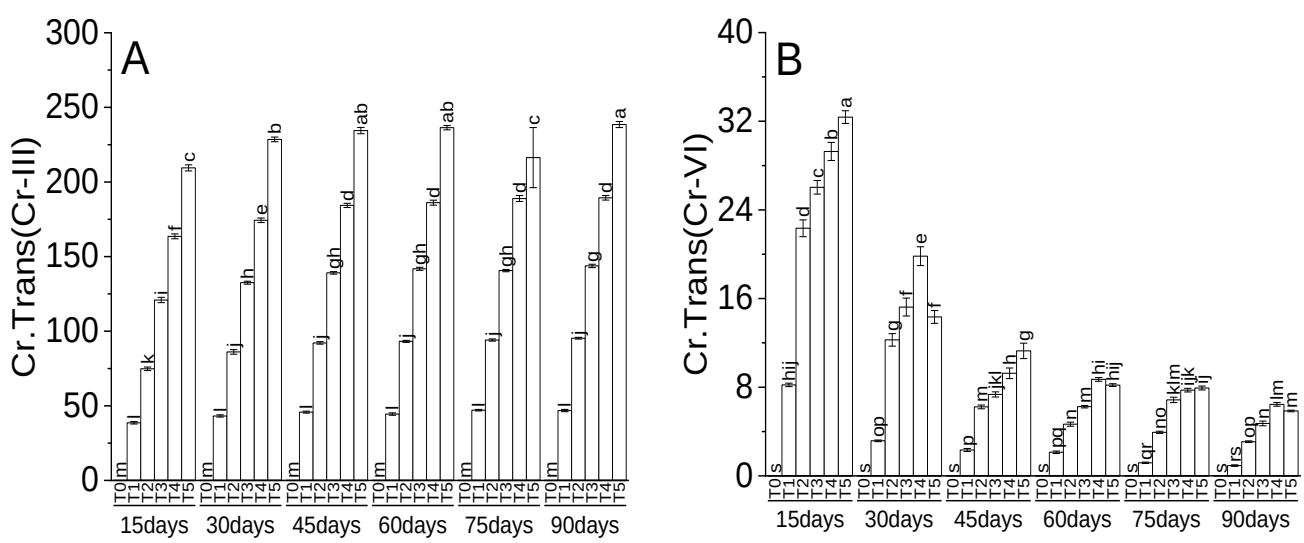

Fig. 3. Effect of treatments and different time intervals on Cr Transformation (Cr-III) (µg/g) (A) and Cr Transformation (Cr-VI) (µg/g) (B) in soil. Bars are means of 5 replicates ± SE. Different values on bars are showing significant differences at $p \leq 0.05$ compared to Fisher LSD.

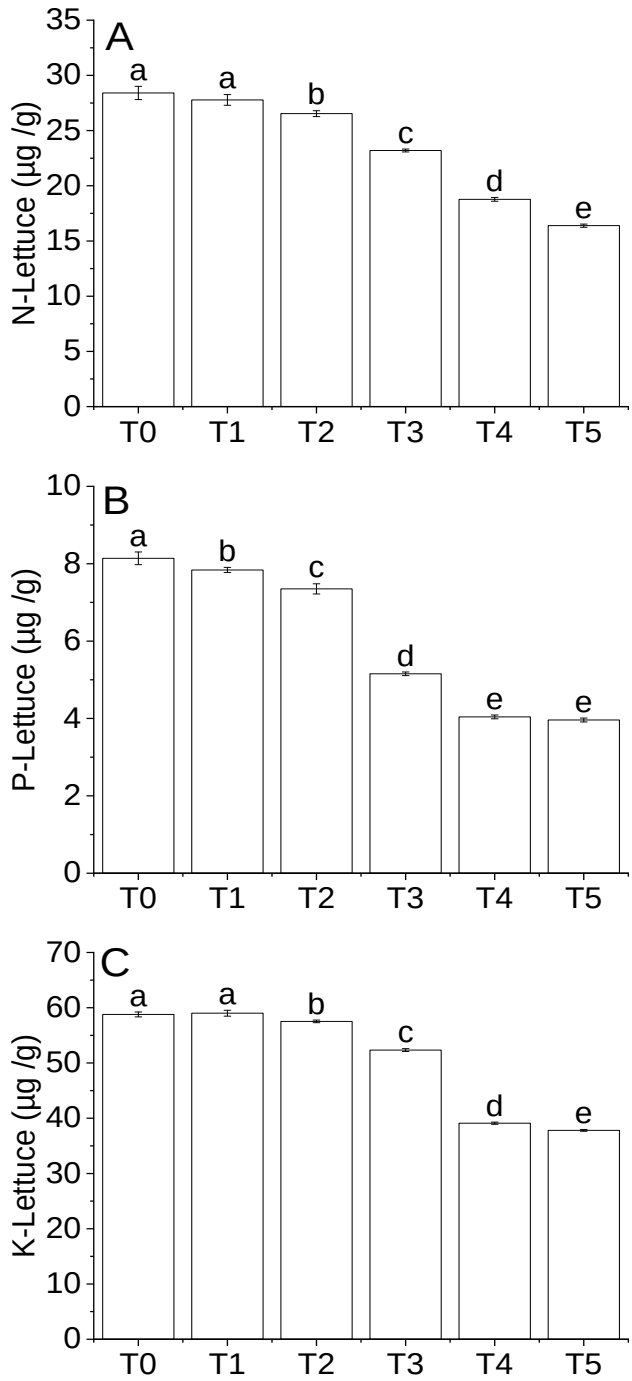

Fig. 4. Effect of treatments on nitrogen (µg/g dry weight) (A), phosphorus (µg/g) (B), and potassium (C) in lettuce. Bars are means of 5 replicates ± SE. Different values on bars are showing significant differences at $p \leq 0.05$ compared to Fisher LSD.

Discussion

Increasing chromium toxicity caused a significant decrease in SMBP and SMBS. It was toxicity induced by chromium in the microbial population, which caused a disturbance in their proliferation. The limited proliferation of microbes in the soil may be one of the major causes of the decrease in SMBP and SMBS. The disturbance in the microbial population also played an important role in the disturbance of nitrogen and carbon cycles in the soil (Shi *et al*., 2002). Aerobic microbes suffer from a deficiency of oxygen, which plays an imperative role in decreasing microbial proliferation under the toxicity of Cr (Kharab & Singh, 1987, Henderson, 1989). All the enzymes are specific in action and catalyze the reaction by decreasing the activation energy (Choi *et al*., 2015). The presence of Cr beyond the threshold limit increases the chances of its interaction with the active site of enzymes. This interaction blocks the active site, and activity of enzymes become restricted (Fopase *et al*., 2019).

In the current study, similar findings were also noted where increasing Cr concentration decreases the activities of soil enzymes. The sulfhydryl groups are present in the active sites of enzymes. These sulfhydryl groups played an imperative role in binding heavy metals that destroyed an enzyme structure (Zaborska *et al*., 2001). A significant change in soil nutrient dynamics due to a change in the ionic concentration of the labile pool also caused the restriction of enzyme activities (Dotaniya & Pipalde, 2018). These heavy metals also changed soil pH and EC by disturbing the chemical reactions, i.e., precipitation and complex formation. A sudden change in pH resulted in the inactivation of enzymatic activities in the soil because of the specific nature of enzymes (Neina, 2019). In the current experiment, the restricted activities of alkaline-phosphatase and arylsulphatase might also be associated with the change in soil chemical attributes after the addition of Cr beyond threshold limits.

It was also noted that Cr caused a significant decrease in the lettuce growth attributes in calcareous soils. Higher uptake of Cr in plants decreases the optimum uptake of nutrients. This decrease in nutrient uptake is majorly associated with poor root growth when plants are cultivated in Cr toxic soils (Danish *et al*., 2019). Accumulation of H_2O_2, lipid peroxidation and superoxide production due to Cr toxicity restrict the photosynthetic activity in the plants. It also disables the chloroplast structure and blocks the electron transport chain in plants. Ultimately restricted electron transport chain diverts the electrons from PSI (electron-donating side) towards Cr(IV), which significantly minimizes photosynthesis and plant growth (Shanker *et al*., 2005). The relationship between K and Cr is antagonistic in soil (Sinha *et al*., 2005). A higher concentration of Cr in soil decreases the bioavailability of K in plants. Less K uptake disturbed the water uptake and stomatal conductance, thus resulting in a poor gain in the fresh weight of plants (Xia *et al*., 2019). Our findings are also in line with this argument. A significant decrease in the fresh weight of the root was noted due to increased Cr toxicity.

Higher concentration of Cr in soil can decrease the uptake of micronutrients (such as Zn, Cu, Mn, Fe, and S) in plants because it can cause root growth inhibition and imbalances in plasma membrane function. Cr toxicity in soil can cause a decrease in nutrient exchange through a number of mechanisms (Sharma *et al*., 2020). Firstly, it can limit root growth, leading to a decrease in the surface area available for nutrient uptake. Secondly, Cr toxicity can alter soil pH and electrical conductivity (EC), which can impact the solubility and availability of essential nutrients in the soil (Chigonum *et al*., 2019). Thirdly, the toxicity of Cr can limit cell division and alter the pattern of mineral ion movement across the plasma membrane, leading to imbalances in nutrient ion concentrations within the plant and decreased nutrient uptake. These factors contribute to a decrease in nutrient exchange in soil when Cr toxicity is present.

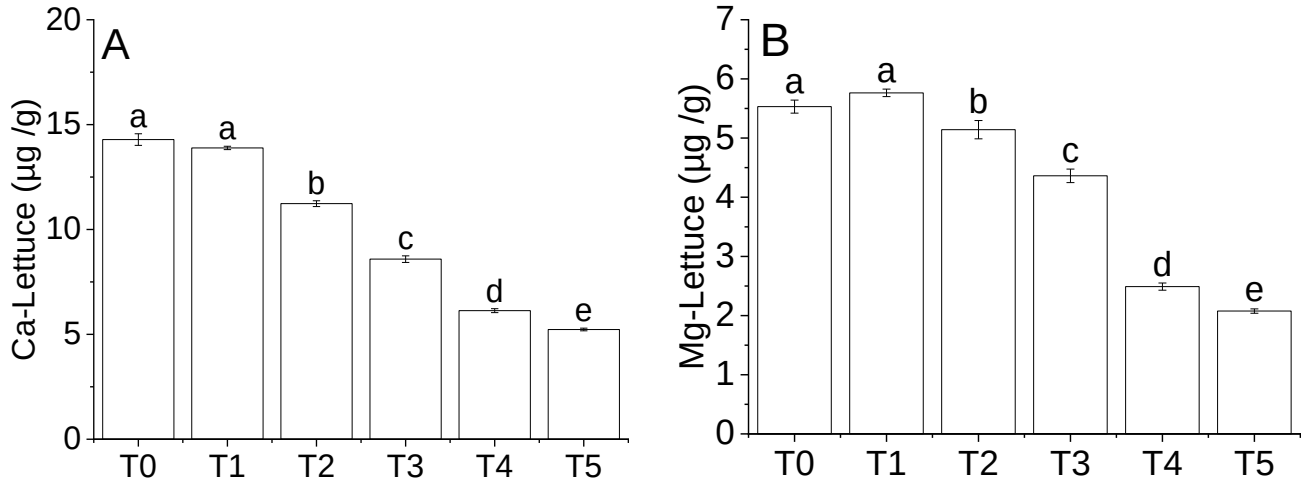

Fig. 5. Effect of treatments on calcium (µg/g dry weight) (A), magnesium (µg/g dry weight) (B), and potassium (C) in lettuce. Bars are means of 5 replicates ± SE. Different values on bars are showing significant differences at $p \leq 0.05$ compared to Fisher LSD.

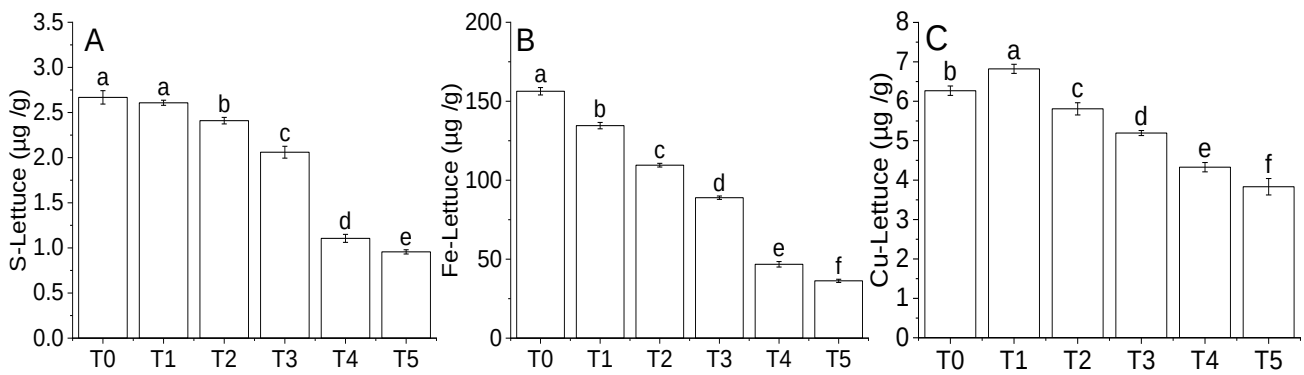

Fig. 6. Effect of treatments on sulfur (µg/g dry weight) (A), iron (µg/g dry weight) (B), and copper (C) in lettuce. Bars are means of 5 replicates ± SE. Different values on bars are showing significant differences at $p \leq 0.05$ compared to Fisher LSD.

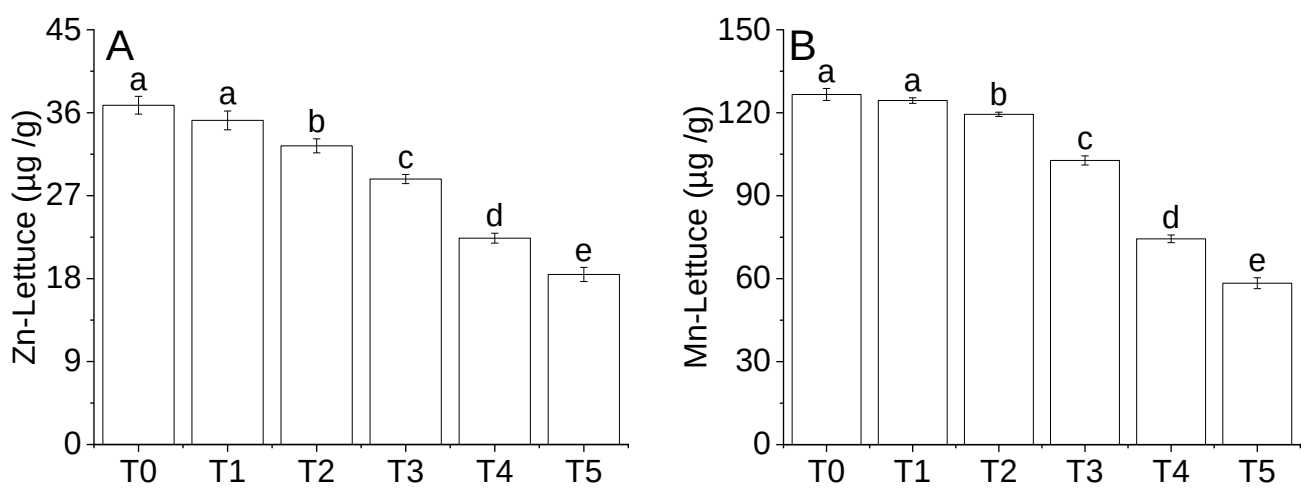

Fig. 7. Effect of treatments on zinc (µg/g dry weight) (A) and manganese (µg/g dry weight) (B) in lettuce. Bars are means of 5 replicates ± SE. Different bar values show significant differences at $p \leq 0.05$ compared to Fisher LSD.

Conclusion

In conclusion, the increasing toxicity of chromium (Cr) has detrimental effects on various aspects of soil and plant health. The presence of excessive levels of Cr in the soil leads to a decrease in soil biochemical properties, such as soil microbial biomass (SMBP and SMBS), as well as a decrease in enzyme activities such as alkaline phosphatase and arylsulphatase. This, in turn, negatively impacts the nutrient uptake of crops such as lettuce, reducing the concentration of essential macro- and micronutrients. The limited nutrient uptake by plants is due to several factors, including hampered root growth, altered soil pH and electrical conductivity (EC), and imbalanced calcium concentration caused by limited cell division. All of these factors contribute to the overall

decline in soil and plant health in the presence of excessive Cr. To gain a deeper understanding of the impact of Cr on soil and plant health, further research is recommended, particularly studies that examine the effects of Cr under different soil textures and with different crops. This will provide a more comprehensive understanding of the issue and allow for the development of effective strategies to mitigate the negative effects of Cr on soil and plant health.

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