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Unraveling the Physiological and Growth Impacts of Heavy Metal Toxicity on Maize

Abdul Basit[1], Muhammad Atif[2], Altaf Hussain[3], Aftab Aslam[3], Mushtaq Hussain[4], Rida Altaf [4], Tehreem Fatima[4] and Sehrish Ameer[4]

[1]Department of Botany, Abdul Wali Khan University Mardan, Pakistan
[2]Department of Botany, The Islamia University of Bahawalpur, Pakistan
[3]Department of Botany, University of Agriculture Faisalabad, Pakistan
[4]Department of Botany, Government College University Faisalabad, Pakistan

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ABSTRACT

Commercial, Industrial and agricultural wastes are major soil pollutants, which contain inorganic, organic compounds and heavy metals. When these heavy metals accumulate in agricultural lands, they adversely affect on growth and physiology of crops ultimately causing decrease in food productivity. In this experiment maize was used to investigate the effects of heavy metals on plant physiology and growth. Maize is cultivated throughout the world for its importance as cereal. It is the third most important cereal crop, following wheat and rice. Maize is a significant food source for humans providing essential minerals, vitamins, carotene and ascorbic acid. Apart from its importance as a cereal source, it also serves as important raw material for various products such as syrup, starch and oil. Various concentrations of Cr and Mn were applied individually as well as in combination to examine their effects on growth and physiology of maize. Analysis of recorded data showed that the treatment 0.5 mM $MnCl_2$ + 0.25 mM $CrCl_3$ had the most harmful effect and caused significant decrease in height, number of leaves and chlorophyll 'a' and 'b' in the stems and leaves. While less harmful effects were seen when heavy metals were applied individually such as 0.25 mM $CrCl_3$, 0.25 mM $MnCl_2$ and 0.5 mM $MnCl_2$ as these treatments did not significantly impact on height, leaf area and chlorophyll content in stems and leaves. So, from these results it can be concluded that. when chromium was applied separately, it had minimal impact on plant physiology and growth, but in combination with manganese, it severely affected on maize plants. Similarly, a small concentration of manganese alone did not affect plant growth, but combined with chromium, it negatively impacted photosynthetic pigments and growth traits.

Keywords: Maize, heavy metals, chromium, manganese, photosynthetic pigments.

1. Introduction

Maize, an annual cereal crop from the Gramineae family, was introduced to Europe at the close of the 15th century and subsequently spread throughout the old world. Today, maize is known as the most vital and extensively cultivated cereal crops globally. Maize is a significant food source for humans providing essential minerals, vitamins, carotene and ascorbic acid. Apart from its importance as a cereal source, it also serves as important raw material for various products such as syrup, starch and oil. Consequently, global demand for maize products is anticipated to increase in the future (Revilla *et al.,* 2022). To meet the increasing demand for maize products, farmers employ various agricultural practices, including the extensive use of fertilizers and chemical pesticides, which can cause soil, atmospheric as well as ground water pollution (Tudi *et al.,* 2021). Heavy metals are released through various natural as well as anthropogenic practices. Natural sources such as soil parent materials, weathering and volcanic eruptions can cause significant heavy metal pollution in soils. Major soil pollutants, including heavy metals, non-metals, phenolics and organic compounds, often arise from industrial processes, mining and other human activities. These enhanced levels of heavy metals in soils

Corresponding Author: Abdul Basit, Department of Botany, Abdul Wali Khan University Mardan, Pakistan
E-mail: absit056@gmail.com

can cause reduction in food growth and quality, ultimately posing serious health risks to humans through the food chain (Alengebawy *et al.,* 2021).

Heavy metals like manganese, lead, chromium, cadmium, mercury, iron and copper are significant environmental pollutants. These metals can accumulate in animals and human tissues via inhalation. Among these heavy metals certain are essential for plant growth, their excess amounts become toxic. When the concentration of heavy metals exceeds safe levels, they accumulate in plant parts, leading to oxidative stress and damaging cell structures, as plants are unable to break them (Assche *et al.,* 1990; Jadia *et al.,* 1999). High levels of heavy metals negatively impact on soil fertility by affecting soil microorganisms, which lead to decrease in the decomposition of organic matter (Schaller & Diez, 1991). Heavy metal toxicity in maize plants causes numerous physio-morphological changes. Research indicates that the impact of heavy metals on plant growth and development is significantly influenced by the soil's physicochemical properties as well as the form and concentration of heavy metals present in the soil (AL-Huqail *et al.,* 2022; Xu *et al*., 2022).

Chromium is a harmful metal which induces oxidative stress to rupture the cell membranes of plant and animal tissues. It hinders plant growth by degrading photosynthetic pigments. High concentrations of chromium can affect seed germination and impair the photosynthetic process by disrupting chloroplast structure (Peralta *et al.,* 2001). Previous studies showed that concentration of 200 µM Cr inhibited the germination of the weed Echinochloa colona. Similarly high levels of hexavalent chromium reduced germination rates in *Phaseolus vulgaris* (Parr & Taylor, 1982). Chromium stress impacts photophosphorylation, CO_2 fixation and electron transport chain. On the other hand, manganese is crucial for plant growth, but excessive levels can lead to inhibited chlorophyll synthesis, necrosis, induce chlorosis, and result in reduced root and shoot length (Loneragan, 1988). Manganese toxicity typically occurs under low pH conditions (below 6.0). Previous studies showed that high levels of manganese lead to a decrease in photosynthetic pigments in *Mentha spicata* and *Pisum sativum* (Doncheva *et al.,* 2005). However, manganese also reduces plant growth in *Lycopersicon esculentum* and leads to a decrease in chlorophyll content (Shenker *et al.,* 2004).

This experiment was conducted with aim to investigate the impact of the accumulation of heavy metals on soil characteristics and heavy metal content of soil and detoxification mechanisms in maize plants. A deeper comprehension of these mechanisms can assist farmers and scientists in predicting and elucidating the effects of heavy metals on maize production. Furthermore, understanding these mechanisms can guide decision-making regarding the feasibility of maize cultivation on soils contaminated by heavy metals.

2. Materials and Methods

2.1. Plant Material

This experiment was conducted to study the impact of heavy metals such as manganese (Mn) and chromium (Cr) on both plant growth and physiology of maize crop. This experiment was conducted during summer season of 2021, according to CRD by using three replications. Maize seeds of Pak-afghoi cultivar and pots were purchased from a seed market. The pots were filled with 7 kg of clean, porous soil and were labeled according to their assigned treatments. To prevent contamination of seeds, maize seeds were rinsed with distilled water. Each pot was sown with ten maize seeds, and adequate water was provided.

2.2. Treatments

Each treatment was replicated three times to ensure accuracy. Additionally, a separate set of pots without heavy metals was designated as the control group. Following treatments were used in this experiment:

T0	Control	T5	0.5 mM $MnCl_2$ and 0.5 mM $CrCl_3$
T1	0.5 mM $MnCl_2$	T6	0.5 mM $MnCl_2$ and 0.25 mM $CrCl_3$
T2	0.25 mM $MnCl_2$	T7	0.25 mM $MnCl_2$ and 0.5 mM $CrCl_3$
T3	0.5 mM $CrCl_3$	T8	0.25 mM $MnCl_2$ and 0.25 mM $CrCl_3$
T4	0.25 mM $CrCl_3$		

2.3. Data Collection

The plants were harvested every ten days, and precise data was recorded. Pots were shielded from any biotic influences by placing them under shelter. Plant height and leaf area were measured using a meter rod, while chlorophyll contents were determined using Arnon's method, which involves crushing 1 g of fresh leaf sample in a mortar and pestle with 90% ethanol, followed by centrifugation at 10,000 rpm for 15 minutes. Absorbance was measured at 600 nm using a spectrophotometer. Carotenoids value was calculated using the following formula (Witham *et al.,* 1971).

$$\text{Carotenoids} \quad = \quad 1000 - \text{chl(a)} - \text{chl(b)} / 246$$

2.4. Biometrical Approaches

The collected data on maize was subjected to analysis of variance (ANOVA) following the procedure outlined by reference (Steel *et al.,* 1997). Post hoc pairwise mean comparison tests using the LSD method were conducted to determine significant differences among the treatments for sunflower hybrids. Standard error of each treatment and percentage increase/ decrease effect of each treatment were also calculated.

3. Results and Discussion

3.1. Analysis of Variance

In this experiment eight treatments of chromium and manganese were used. ANOVA showed significant differences for treatments of cadmium and treatments × exposure duration for all physiological and growth parameters. Mean squares of ANOVA for all traits are represented in Table 1.

Table 1: Mean Square values from ANOVA

Source	Df	Plant Height	No. of Leaves	Leaf Area	Chl. a in Leaves	Chl. b in Leaves	Carotenoids in Leaves	Chl. a in Stem	Chl. b in Stem	Carotenoids in Stem
Treatment	8	14.234	0.2437	10.779	0.2892	0.3371	0.2891	0.0973	0.0989	0.0991
Error	18	0.065	0.0047	0.147	0.0116	0.0043	0.0116	0.0001	0.0001	0.0001
Total	26									

3.2. Least Significance Difference Test

3.2.1. Plant Height (cm)

Table 2 shows the plant height was greatest with 0.5 mM $MnCl_2$ treatment alone, which causes a significant increase in plant height, whereas combined treatment of 0.5 mM $MnCl_2$ and 0.25 mM $CrCl_3$ showed the shortest plant height, measuring 5 cm. The application of a high concentration of Mn alone did not impact plant height. However, when Mn was combined with a small concentration of Cr, it causes a significant decrease in plant height. The reduced plant height under heavy metal treatments shows the adverse effects of toxicity on maize plants. Nevertheless, the plants that survived higher concentrations of these metals might be valuable for developing stress-tolerant maize hybrids and varieties (Asrar *et al.,* 2005; Haseeb *et al.,* 2020).

3.2.2. Number of Leaves Per Plant

Table 2 indicates that Cr or Mn alone didn't affect on number of leaves. In contrast, under the 0.5 mM $MnCl_2$ + 0.25 mM $CrCl_3$ treatment a lower number of leaves were found. The number of leaves per plant was unaffected by high concentrations of Mn and Cr when applied individually, but when applied in combined form, these metals showed significant effect and reduced the number of leaves. This decrease suggests that heavy metals have adverse effects on maize plants, leading to a reduction in the photosynthetic rate. Plants which survived higher concentrations of these metals could be utilized to develop maize hybrids and varieties with tolerance to such abiotic stress (Asif *et al.,* 2020).

3.2.3. Leaf Area (cm2)

Leaf area is an important trait which is directly related to absorption of sunlight for photosynthetic processes. Table 2 shows the largest leaf area was observed under the 0.25 mM $MnCl_2$ treatment, while the smallest leaf area was found under treatment 7. Which showed that heavy metals in combined form are more dangerous to plants. The reduced leaf area in plants exposed to heavy metals suggests adverse effects on maize, likely leading to a decreased photosynthetic rate (Mazhar *et al.,* 2020).

Table 2: LSD mean values ± Standard Error for plant height, number of leaves and leaf area

Treatment	Plant height	No. of leaves	Leaf area
T0	9.89 ± 0.117 ^{C}	3.13 ± 0.035 ^{AB}	20.08 ± 0.340 ^{CD}
T1	12.95 ± 0.091 ^{A}	2.99 ± 0.059 ^{BC}	19.07 ± 0.292 ^{DE}
T2	10.20 ± 0.058 ^{C}	2.80 ± 0.009 ^{D}	23.62 ± 0.249 ^{A}
T3	9.63 ± 0.180 ^{C}	2.69 ± 0.053 ^{D}	20.01 ± 0.119 ^{CD}
T4	8.27 ± 0.025 ^{D}	3.31 ± 0.049 ^{A}	18.83 ± 0.156 ^{E}
T5	10.97 ± 0.282 ^{B}	2.85 ± 0.044 ^{CD}	22.80 ± 0.199 ^{AB}
T6	5.35 ± 0.088 ^{F}	2.38 ± 0.019 ^{E}	22.04 ± 0.217 ^{B}
T7	7.51 ± 0.208 ^{E}	3.15 ± 0.032 ^{AB}	18.04 ± 0.098 ^{E}
T8	10.25 ± 0.086 ^{BC}	2.72 ± 0.029 ^{D}	20.27 ± 0.205 ^{C}

3.2.4. Chlorophyll Contents

Table 3 and Table 4 indicate that the highest chlorophyll a levels in leaves and stems were found with the 0.25 mM $CrCl_3$ treatment, while the lowest levels were observed with the 0.5 mM $MnCl_2$ + 0.25 mM $CrCl_3$ treatment. When low concentrations of Cr were applied alone, they did not affect chlorophyll a levels in leaves and stems, but when combined with Mn, they reduced chlorophyll a and b levels. This suggests that heavy metal toxicity decreases the photosynthetic rate and can lead to plant death.

Table 3 and Table 4 show that the highest chlorophyll b levels in leaves and stems were observed with the 0.25 mM $MnCl_2$ treatment, while the lowest levels were found with the 0.5 mM $MnCl_2$ + 0.25 mM $CrCl_3$ treatment. Low concentrations of Mn alone did not affect chlorophyll b levels, but in combination with Cr, they reduced these levels. This reduction in chlorophyll b under heavy metal stress also leads to a decreased photosynthetic rate and potential plant death (Ali *et al.,* 2020; Khalil *et al.,* 2020).

Table 3: LSD mean values ± Standard Error for chlorophyll a, b and carotenoids in leaves

Treatment	Chlorophyll a in leaves	Chlorophyll b in leaves	Carotenoids in leaves
T0	2.06 ± 0.096 ^{BC}	2.20 ± 0.012 ^{DE}	997.93 ± 0.096 ^{AB}
T1	2.34 ± 0.077 ^{B}	2.19 ± 0.077 ^{DE}	997.65 ± 0.077 ^{B}
T2	2.21 ± 0.064 ^{BC}	2.37 ± 0.037 ^{CD}	997.78 ± 0.064 ^{AB}
T3	2.66 ± 0.029 ^{A}	2.67 ± 0.015 ^{B}	997.33 ± 0.029 ^{C}
T4	2.85 ± 0.075 ^{A}	2.92 ± 0.058 ^{A}	997.14 ± 0.075 ^{C}
T5	2.29 ± 0.053 ^{BC}	2.39 ± 0.029 ^{C}	997.70 ± 0.053 ^{AB}
T6	2.00 ± 0.070 ^{C}	2.09 ± 0.052 ^{E}	997.99 ± 0.07 ^{A}
T7	2.68 ± 0.018 ^{A}	2.69 ± 0.039 ^{B}	997.31 ± 0.018 ^{C}
T8	2.71 ± 0.032 ^{A}	3.03 ± 0.049 ^{A}	997.28 ± 0.032 ^{C}

3.2.5. Carotenoides (mg/g)

Table 4 shows the highest carotenoid levels in stems were observed under the 0.5 mM $MnCl_2$ + 0.25 mM $CrCl_3$ treatment, while the lowest levels were found with the 0.25 mM $MnCl_2$ treatment. Similarly, Table 3 indicates that the highest carotenoid levels in leaves were also under the 0.5 mM

$MnCl_2$ + 0.25 mM $CrCl_3$ treatment, and the lowest were with the 0.25 mM $CrCl_3$ treatment. Carotenoids were the only trait under study that were increased by the treatment of combined heavy metals. Carotenoids protect chlorophyll under stress, so their concentration increases under such conditions. This indicates that heavy metal toxicity decreases the photosynthetic rate, potentially leading to plant death (Ali *et al.,* 2012; Naseem *et al.,* 2020).

Table 4: LSD mean values ± Standard Error for chlorophyll a, b and carotenoids in stem

Treatment	Chlorophyll a in Stem	Chlorophyll b in Stem	Carotenoids in Stem
T0	0.21 ± 0.014 C	0.19 ± 0.008 C	999.79 ± 0.012 D
T1	0.13 ± 0.004 EF	0.11 ± 0.005 FG	999.88 ± 0.003 A
T2	0.67 ± 0.004 A	0.66 ± 0.011 A	999.33 ± 0.006 F
T3	0.17 ± 0.005 D	0.17 ± 0.004 D	999.83 ± 0.006 C
T4	0.14 ± 0.002 EF	0.13 ± 0.002 EF	999.86 ± 0.000 AB
T5	0.14 ± 0.004 EF	0.12 ± 0.002 EFG	996.86 ± 0.003 AB
T6	0.12 ± 0.003 F	0.10 ± 0.0015 G	999.88 ± 0.003 A
T7	0.15 ± 0.005 DE	0.15 ± 0.003 DE	999.85 ± 0.003 BC
T8	0.37 ± 0.004 B	0.36 ± 0.004 B	999.63 ± 0.006 E

3.3. Percentage effect of Treatments

3.3.1 Percentage effect of Treatments on Plant Height, Number of Leaves and Leaf Area

Figure1 demonstrated that the application of different doses of chromium and manganese when applied individually didn't cause much decrease in values of plant height, number of leaves and leaf area. But when applied in combined form 0.5 mM $MnCl_2$ and 0.25 mM $CrCl_3$ cause significant reduction in plant height (46%) and number of leaves (24%). While treatment 0.25 mM $MnCl_2$ and 0.5 mM $CrCl_3$ significantly affected on leaf area (11%).

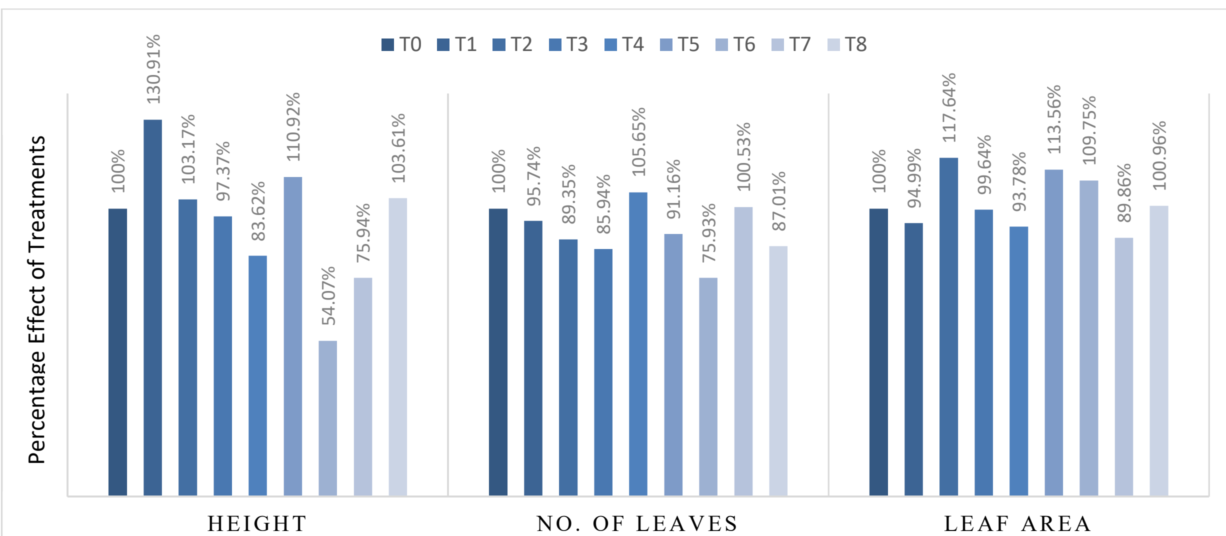

Fig. 1: Graphical Representation of Percentage effect of Treatments on Plant Height, Number of Leaves and Leaf Area

3.3.2 Percentage effect of Treatments on Chlorophylls

Figure 2 and Figure 3 reveal that the different levels of chromium and manganese when applied individually caused an increase in chlorophylls content in leaves. Which reveals that small concentrations of these metals are beneficial for plants. But when applied in higher concentrations or in combined form these metals became toxic for plants. Such as, 0.5 mM $MnCl_2$ and 0.25 mM $CrCl_3$ cause significant reduction in both chlorophyll a and chlorophyll b in leaves. While chlorophylls content in stem were significantly affected in both individual and combined forms.

3.3.3 Percentage effect of Treatments on Carotenoids

Figure 2 and Figure 3 illustrate that all treatments showed opposite effects on carotenoids as compared to chlorophylls. Such as various levels of chromium and manganese when applied individually caused an increase in chlorophylls content and decrease in carotenoids in leaves. While treatment of 0.5 mM $MnCl_2$ and 0.25 mM $CrCl_3$ cause a small increase in carotenoids content in leaves. While carotenoids content in stem were not affected much in both individual and combined forms.

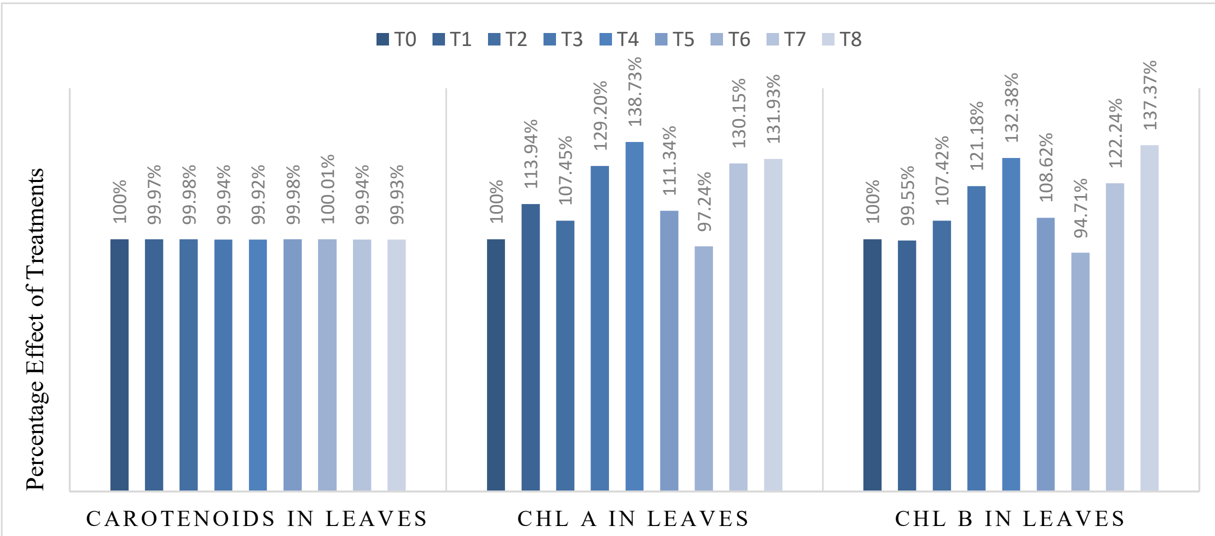

Fig. 2: Graphical Representation of Percentage effect of Treatments on Chlorophyll a, b and arotenoids in Leaves

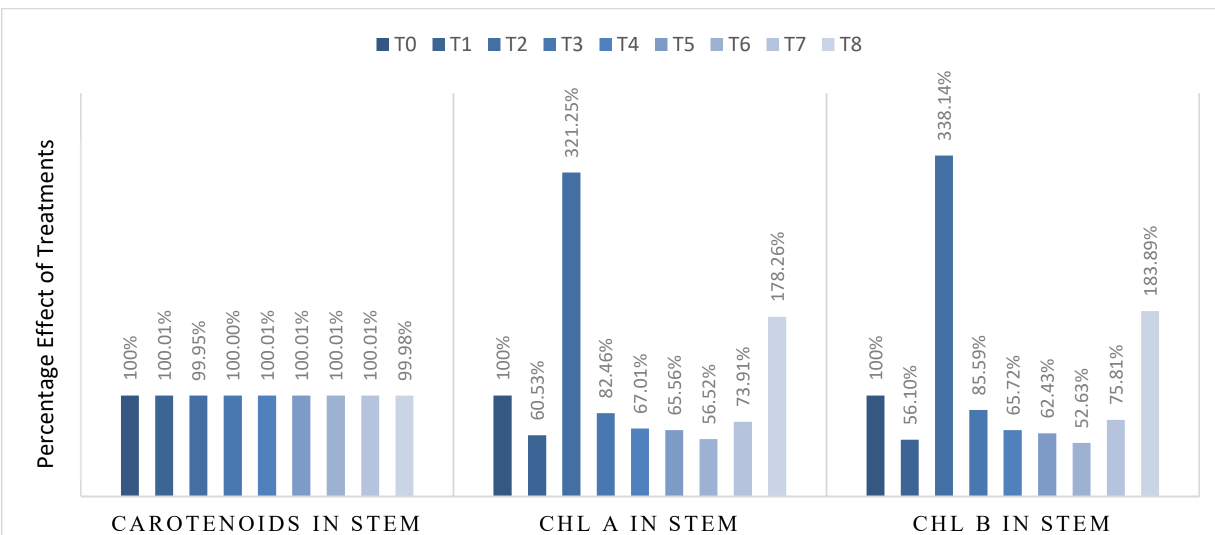

Fig. 3: Graphical Representation of Percentage effect of Treatments on Chlorophyll a, b and Carotenoids in Stem

4. Conclusion

Results of this study showed that 0.5 mM $MnCl_2$ + 0.25 mM $CrCl_3$ treatment was the most toxic to plant growth. So, from these results it can be concluded that. when chromium was applied separately, it had minimal impact on plant physiology and growth, but in combination with manganese, it severely affected maize plants. Similarly, a small concentration of manganese alone did not affect plant growth, but when combined with chromium, it negatively impacted photosynthetic pigments and growth traits. Carotenoid levels increased under stress conditions, as carotenoids help enhance plant tolerance to metal stress. Consequently, as chlorophyll content decreased under metal stress, carotenoid levels rose.

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