



## Effect of green-synthesized copper oxide nanoparticles on growth, physiology, nutrient uptake, and cadmium accumulation in *Triticum aestivum* (L.)

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### ABSTRACT

Cadmium (Cd) stress in crops has been serious concern while little is known about the copper oxide nanoparticles (CuO NPs) effects on Cd accumulation by crops. This study investigated the effectiveness of CuO NPs in mitigating Cd contamination in wheat (*Triticum aestivum* L.) cultivation through a pot experiment, presenting an eco-friendly solution to a critical agricultural concern. The CuO NPs, synthesized using green methods, exhibited a circular shape with a crystalline structure and a particle size ranging from 8 to 12 nm. The foliar spray of CuO NPs was applied in four different concentrations i.e. control, 25, 50, 75, 100 mg/L. The obtained data demonstrated that, in comparison to the control group, CuO NPs had a beneficial influence on various growth metrics and straw and grain yields of *T. aestivum*. The green CuO NPs improved *T. aestivum* growth and physiology under Cd stress, enhanced selected enzyme activities, reduced oxidative stress, and decreased malondialdehyde levels in the *T. aestivum* plants. CuO NPs lowered Cd contents in *T. aestivum* tissues and boosted the uptake of essential nutrients from the soil. Overall, foliar applied CuO NPs were effective in minimizing Cd contents in grains thereby reducing the health risks associated with Cd excess in humans. However, more in depth studies with several plant species and application methods of CuO NPs are required for better utilization of NPs in agricultural purposes.

### 1. Introduction

One of the most important problems facing modern civilization is the soil contamination caused by harmful trace elements, which is one of the key hurdles involved in feeding the people. Due to human activities like mining, industrialization, and agricultural practices, heavy metals may

accumulate in soils (Ahmed et al., 2023; Yadav et al., 2023; Xing et al., 2023; Gupta et al., 2020; Abeer and Dawood, 2020). Heavy metals are released into the soil as a result of increased industrialization, which over time has a negative effect on the physico-chemical characteristics of the soil. The excess of heavy metals may concentrate in edible parts of crops thereby transferring to humans via food chain (Adrees et al., 2020;

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Sarker et al., 2020; Abeer and Salama, 2022). The complex link between soil pollution caused by heavy metals and agricultural decline necessitates innovative solutions to safeguard both the environment and livelihoods globally (Kumar et al., 2022; Keller et al., 2015). Food security is seriously impacted by the excess of heavy metals in the soil and plants (Chen et al., 2023; Salama et al., 2022; Mazarji et al., 2021; Hossain, 2020).

Cadmium is particularly noteworthy among several heavy metals in soil due to its significant toxicity and high mobility, with the ability to induce cancer even at low concentrations. Additionally, Cd toxicity has a significant impact on a variety of physiological and metabolic processes, as well as plant development (Xing et al., 2023; Al-Huqail et al., 2023; Zhang et al., 2020; Rizwan et al., 2017a). Numerous researchers have shown that the provision of good quality food is considerably impacted by Cd toxicity in foodstuffs brought on by soil pollution (Alabdallah et al., 2023; Irshad et al., 2021; Ezedom et al., 2020; Rizwan et al., 2017b; Yang et al., 2016). Additionally, Cd toxicity has a significant impact on a variety of physiological and metabolic processes, as well as plant development. Cadmium buildup in soil reduces agricultural yields and fertility while also depleting essential resources. The increasing demand for food caused by the expanding world population puts more stress on ecosystems. *T. aestivum* has a large potential for Cd buildup in its above-ground components since it is one of the staple meals and the main cereal crop feeding more than 50% of the world's population (Al-Huqail et al., 2023; Sterckeman et al., 2020). Consequently, it is essential to put into action efficient measures aiming for the minimization of Cd uptake by *T. aestivum* plants in light of the prevalent irrigation practices involving sewage wastewater.

A viable answer to the expanding problems in agriculture is offered by nanotechnology. It provides a variety of techniques, including fertilizers in nano-form, to improve soil characteristics and decrease the absorption of hazardous substances for increasing crop yields (Hasan et al., 2020; Shang et al., 2019). Controlled release of substances is made feasible by nanoscale encapsulation, lowering the necessary dosages and minimizing environmental effects (Awan et al., 2023; Irshad et al., 2021). By using nano-sized fertilizers to improve soil fertility, nanotechnology increases agricultural output while lowering runoff water pollution. There has been a lot of interest in the use of organisms including fungus, bacteria, and plants in the biological production of NPs. Among other biological organisms, plants are being more used for the green and ecofriendly synthesis of metallic oxide NPs for the treatment of different environmental ailments (Masood et al., 2023; Alotaibi et al., 2023; Al-Huqail et al., 2023; Singh et al., 2021). The green synthesized NPs may help plants by enhancing growth, germination, and alleviate the metal toxicity (Jiang et al., 2021; Acharya and Pal, 2020; Irshad et al., 2020, 2021a; Pandey et al., 2018; Cai et al., 2017). These NPs aid in transferring heavy metals from contaminated soils to plants, lowering health risks from dietary exposure (Al-Huqail et al., 2023; Irshad et al., 2022, 2023; Irshad et al., 2019).

Copper oxide nanoparticles, recognized for their low cost, photocatalytic activity, and chemical stability, have significant potential as anti-infective agents. This is due to the huge surface area and beneficial crystal shapes. Cost-effectiveness, photocatalytic powers, and stability in both chemical and physical aspects are all displayed by Cu NPs. Due to their advantageous crystal morphologies and especially high surface areas, they have the potential to be anti-infective agents. Nano-copper is widely used in a variety of applications, such as antifungal agents, sensors, metallic inks, and colorimetric probes, due to its low cost and exceptional qualities including antifungal, electrical, optical, and catalytic characteristics (Ingle and Rai, 2017; Pournbeyram and Mehdizadeh, 2016; Tsai et al., 2015; Hatamie et al., 2014). The production of NPs through plant extracts is safer and efficient way than other techniques used for this purpose (Magudieswaran et al., 2019; Rajeshkumar et al., 2018; Walkey et al., 2015). The *A. indica* tree, valued for its medicinal and germicidal properties, has been integral to traditional medicine and hygiene practices for generations. With diverse medicinal compounds, it

also offers environmental benefits like watershed preservation. Studies suggested that powder obtained from *A. indica* leaves showed promise as an effective adsorbent for wastewater pollutants (Irshad et al., 2020; Irshad et al., 2023; Eid et al., 2017; Prashanth et al., 2014). This plant is common in Pakistan and other nations on the subcontinent, where it is frequently planted for agroforestry reasons in and around agricultural regions. It's noteworthy that this plant is underutilized for both Cd removal from soil via plant uptake and the synthesis of CuO nanoparticles. Therefore, it was postulated that examining this plant's potential would help reduce environmental pollution by resolving the problem of heavy metals in the soil. This study pioneers the green synthesis of CuO NPs, offering novel insights into their impact on the growth, physiology, and Cd accumulation in *T. aestivum*. This area of CuO NPs remains largely unexplored, marking a significant advancement in our understanding. The study aimed to test the hypothesis that these nanoparticles can reduce Cd accumulation in *T. aestivum* grains, thereby mitigating associated Cd health risks in humans. To address this gap, the research assessed the impact of CuO NPs derived from *A. indica* on *T. aestivum* growth, yield, leaf antioxidant activities, variation in photosynthetic contents, and Cd uptake by *T. aestivum*. Additionally, the investigation also studied Cd health risk assessment by the consumption of grains obtained from Cd-contaminated soil treated or not with CuO NPs. These findings may have significant implications for agricultural practices, highlighting the potential of CuO NPs in reducing metal uptake in crops, enhancing food security, and addressing public related health issues.

## 2. Materials and methods

### 2.1. Soil preparation and foliar application of Cu NPs

The soil employed for the pot study was sourced from a field that soil exhibited heightened levels of metals, particularly Cd, with the greatest accumulation observed within the soil zone influenced by root activity. Soil specimens were precisely collected from a depth of 0–20 cm, thoroughly cleansed to remove any extraneous matter such as roots, and subsequently subjected to controlled air-drying in shaded conditions. After this, samples were carefully passed through a 2.0 mm sieve to attain a uniform particle size distribution of soil. The soil samples underwent an initial analysis with the intention of assessing particular soil characteristics. These included the determination of organic matter content using the method outlined by Walkley and Black (1934), the assessment of soil texture according to Bouyoucos approach (1962), the measurement of electrical conductivity and pH levels, and the evaluation of various selected anions and cations as per methods of Page et al. (1982). The concentrations of total metals were also determined in accordance with the methodology by Soltanpour (1985). The soil was clay loam having sand, silt and clay values of 46%, 28%, and 26% with pH value of 7.79 and EC value of 1.73 dS/m. Total Cd level of the soil was 3.51 mg/kg and the soil organic material was 1.01%. The CuO NPs employed in this study were synthesized using a green method involving the leaf extract of *Azadirachta indica*. These green NPs possessed the round surface structure and have a porous structure with the crystallite grain sizes ranging from 8 to 12 nm. These nanoparticles were successfully synthesized and specifically employed for the purpose of removing chromium from wastewater by the batch experimental study (Irshad et al., 2023a).

### 2.2. Pot study and experimental setup

The pot study used 5.0 kg of processed soil that had been subjected to a series of tests and was found to be contaminated with Cd. The chosen application method for CuO NPs involved foliar treatments (25, 50, 75, and 100) mg/L, with a control group maintained without NPs for comparison. Surface-disinfected seeds, treated with 2.5% (v/v) H<sub>2</sub>O<sub>2</sub> for 20 min, were placed approximately 2 in. deep in the soil within the pots.

After seven days from the initial germination of the seeds, each pot was maintained with five young seedlings. The foliar treatment with CuO NPs was administered on days 20, 30, 40, and 60 days of post-germinations. A fresh solution of CuO NPs was prepared for each foliar application by combining a measured quantity of CuO NPs with distilled water, followed by thorough dispersion using ultra-sonication for approximately thirty minutes. A hand sprayer was utilized for the application of NPs, with the first spray conducted four weeks after sowing and the second spray was done two weeks after the first. In parallel, plants without NPs were foliar-sprayed with distilled water. The total volume of solution used for each treatment across all replications was four liters. The complete randomized design with 5 replications was applied to the experimental data. To minimize microenvironmental fluctuations, the pots were periodically rotated. Irrigation was conducted using high-quality water, maintaining soil water content at 65–75% of its total capacity throughout the growth period. Essential macronutrients (NPK) were applied for fertilization to ensure adequate nutrient supply. Harvesting of plants took place at 121 days after seed sowing in the pots.

### 2.3. Estimation of growth, yield, chlorophyll contents and gaseous exchange parameters

The determination of spike lengths and plant height in the pot study was conducted using a meter scale. Subsequently, the plants were divided into distinct tissues, encompassing roots, shoots, husks, and grains. These tissues were then subjected to drying prior to the recording of their respective dry weights. Measurement of leaf chlorophyll concentrations was performed 85 days after seed sowing. To extract the chlorophyll contents from leaves, a solution of 85% acetone was utilized, and the extraction process continued until the leaves lost their color. The resulting extract was employed to determine levels of chlorophyll *a*, *b*, and carotenoids, utilizing a spectrophotometer based on the methodology outlined by Lichtenthaler (1987). An infrared gas analyzer (IRGA) was employed to simultaneously assess the features of gas exchange, i.e. photosynthetic rate, stomatal conductance, as well as rate of leaf transpiration.

### 2.4. Determination of antioxidant enzymes of *T. aestivum*

The leaves were examined for various criteria of antioxidant enzymes at the 83th day after seed sowing. This comprised the amount of malondialdehyde (MDA), activities of the enzymes i.e. superoxide dismutase (SOD) and peroxidase (POD), and electrolyte leakage (EL), the amount of catalase as well as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Leaf samples were divided into uniform pieces and submerged for two hours in deionized water at a constant temperature of 32 °C to evaluate electrolyte loss. It was noted the first electrical conductivity (EC1). The ultimate electrical conductivity (EC2) of the solution (which had been heated to 121 °C for 20 min) was then recorded. In order to determine EL, Dionisio-Sese and Tobita's (1998) formula was used.

$$EL = \frac{EC1}{EC2} \times 100 \quad (1)$$

On the basis of Heath and Packer's (1968) approach, a modified procedure was used to determine the amount of MDA in leaves. A 0.1% thiobarbituric acid (TBA) solution was added to 5.0 mL of leaves, and the mixture was then centrifuged. A heating-cooling cycle was used to create a solution from this combination, which was then centrifuged once more. From this solution, 1.0 mL was amalgamated with 20% trichloroacetic-acid that contained 0.5% TBA. Then, at 532 nm, absorbance of MDA was recorded using an attenuation value of 155 mM per cm<sup>1</sup>. A 50 mg sample was added in 3 mL of 50 mM phosphate-buffer (pH 6.5) in order to detect hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). An extinction coefficient was used to calculate the concentration of H<sub>2</sub>O<sub>2</sub> after measuring the absorbance at 410 nm.

### 2.5. Determination of Cd contents in root, straw and grains

The *T. aestivum* plant samples were individually grounded to from a powder and were used for conducting Cd measurement. For this, a predetermined amount of each sample was combined in a mixture of 10 mL of nitric acid + perchloric acid (3:1, v/v) and allowed to stand for approximately 24 h. Mixed solutions were then subjected to digestion through heating process followed by preparation of specified volume of solution. Cd concentrations in the roots, straw and grains were determined using an atomic absorption spectrophotometer (Analytik Jena AG - novAA® 300, Germany).

### 2.6. Measurement of Zn, Fe and Mn contents in *T. aestivum*

The samples of plant tissues underwent digestion using an acid mixture consisting of a 3:1 v/v ratio of HNO<sub>3</sub> and HClO<sub>4</sub>, followed by the preparation of a specific volume for the analysis of micronutrients (Fe, Zn, and Mn). After digestion, the samples were treated with concentrated HNO<sub>3</sub> and HClO<sub>4</sub> in a 3:1 ratio and heated to 350 °C. This procedure enabled the measurement of specific micronutrients, namely Zn, Fe, and Mn, present in distinct tissues such as roots, shoots, and grains of *T. aestivum* plants. Quantification of these micronutrients were carried out by employing the Ohno and Zibilske (1991) technique by utilizing the atomic absorption spectrophotometer to achieve precise measurements of nutrient levels.

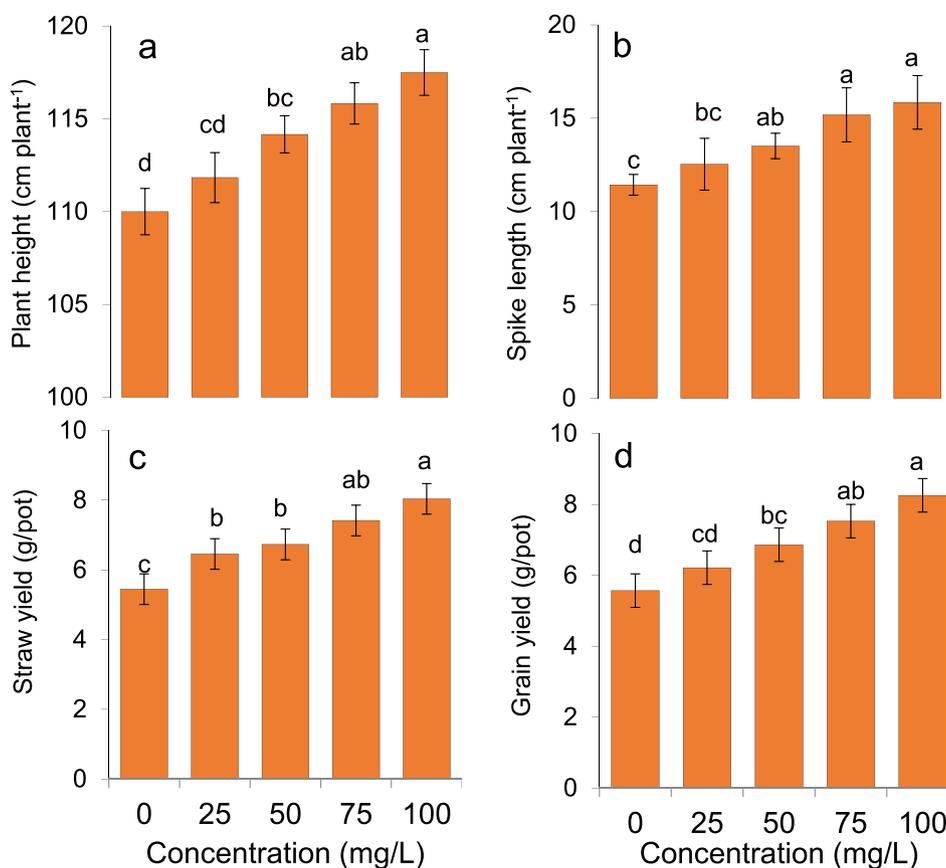
### 2.7. Statistical analysis

Using IBM SPSS Statistical software, version 21.0, a one-way analysis of variance was performed to evaluate the significant difference among the treatments.

## 3. Results and discussion

### 3.1. Growth and morphological assessment of *T. aestivum*

The application of CuO NPs via foliar spray led to a significant enhancement in both growth and morphological parameters across all concentrations when compared with control. These improvements encompassed plant heights, spike lengths, straw, and grain yields. The physio-morphological attributes of *T. aestivum* exposed to CuO NPs in the presence of Cd in the soil are depicted in Fig. 1. The results demonstrated consistent increases in morphological characteristics such as grain and straw yield for all concentrations, except control. Additionally, there were notable differences observed in the response to the various dosage levels across all parameters. The findings indicated that morphological traits and grain yields experienced significant enhancement with the applied CuO NPs (25, 50, 75, and 100 mg/L), while minimum values were depicted in control. Specifically, plant height exhibited enhancements of 12%, 24%, 35%, and 45% at 25, 50, 75, 100-mg/L, respectively, in comparison to control. Correspondingly, spike length increased by 10%, 18%, 33%, and 46% in these treatments relative to the control. Moreover, straw yield saw increments of 19%, 24%, 36%, and 44% at 25, 50, 75, 100-mg/L, respectively. The most notable rise in grain production occurred in the T3 treatment, reflecting an impressive 43% increase over the control. Excess level of CuO NPs frequently impede plant growths, and this effect varies with concentration. For instance, Ibrahim et al. (2022) shown that *T. aestivum* growth was enhanced by lower concentrations of CuO NPs, but that growth was inhibited by greater quantities. On the other hand, several research have demonstrated that increased CuO levels can in fact negatively affect *T. aestivum* development (Dimkpa et al., 2012; Kacziba et al., 2023). The growing medium also has an impact, with sand having a stronger inhibitory effect than soil. The impacts of CuO NPs on *T. aestivum* are more complicated and inconsistent since they depend on variables including concentration and growing circumstances. The



**Fig. 1.** The effect of CuO NPs on plant height (a), spike length (b), straw yield (c), and grain yield (d). Each dataset is representative of the mean values derived from three independent replicates, accompanied by their respective standard deviations. Variations of statistical significance among treatments are denoted by distinct letters displayed on the bars, adhering to a significance threshold of  $P \leq 0.05$ .

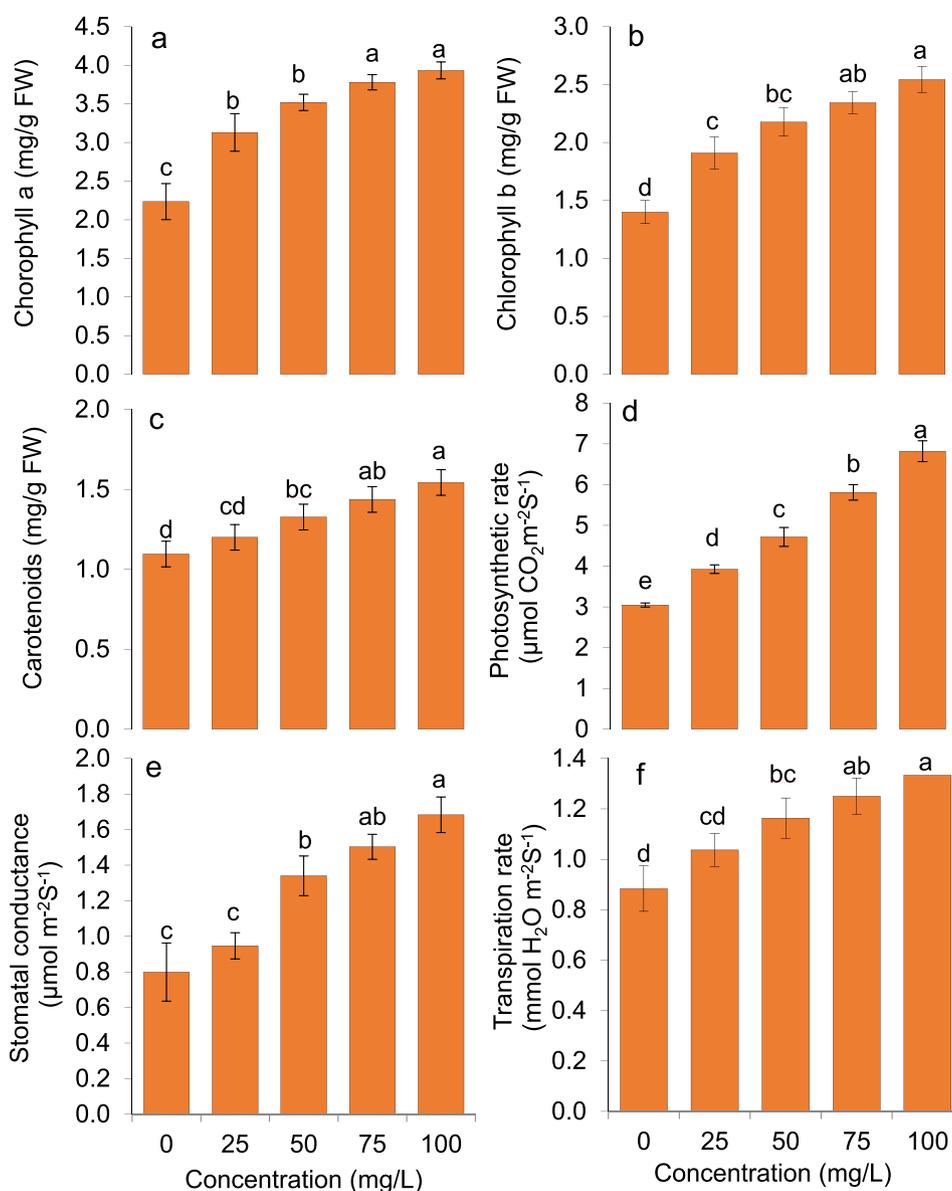
results of the present study align with the reported previous results that CuO NP having the positive impacts on *T. aestivum* crop. In the study conducted by Badawy et al. (2021), using a foliar spray of 50–100 ppm on *T. aestivum* resulted in a significant increase in growth parameters, with a maximum range. Interestingly, this method proved to be more effective than the soaking or seed priming approach for treatment. According to Adams et al. (2017), utilizing less than 50 mg/kg of CuO NPs in a sand medium had no discernible impact on root development in *T. aestivum*. Higher CuO NPs concentrations, however, clearly inhibited root elongation. The results of the current investigation show that, in comparison to lower concentrations (25 mg/kg) and the control plots, both growth and yield significantly increased at concentrations of CuO NPs of 50, 75, and 100 mg/kg. This shows that these eco-friendly CuO NPs, especially at high concentrations, work well when applied topically.

### 3.2. Effect of CuO NPs on the physiological and photosynthetic parameter

Similar to its impact on morphological parameters, foliar application of CuO NPs also influenced the physiological and photosynthetic aspects of *T. aestivum*. Fig. 2(a–f) illustrates a positive correlation between CuO NPs application and the increase in chlorophyll a and b, chlorides, photosynthetic rate, as well as transpiration and stomatal conductance of the *T. aestivum* plants. Results from the figure indicated that the lowest chlorophyll and gaseous exchange attributes were observed in control group, whereas the highest values were recorded with application of 100 mg/L of CuO NPs as a foliar treatment. Specifically, treatments (25, 50, 75, and 100) mg/L exhibited notable increases in chlorophyll concentration by 40%, 58%, 69%, and 76%, respectively, compared to the control at 0 mg/L. Chlorophyll b enhanced by 36%,

55%, 67%, and 73%, while carotenoid contents increased by 10%, 21%, 31%, and 49% at 25, 50, 75, 100-mg/L CuO NPs. Furthermore, gaseous exchange parameters showed significant improvement in all treatments when compared to the control. Treatments (25, 50, 75, and 100) led to 29%, 55%, 74%, and 61% increases in photosynthetic rate. Stomatal conductance also notably improved with increments of 18%, 68%, 78%, and 88% compared to the control. A similar trend was observed in transpiration rate, with increase of 17%, 32%, 39%, and 42% compared to the control plants. The results of current study highlighted the positive influence of CuO NPs on chlorophyll concentrations, photosynthetic parameters and gas exchange attributes in *T. aestivum* plants.

CuO NPs were applied to *T. aestivum* at a dosage of 50 mg/kg, and this caused a notable growth response. The average data showed that CuO NPs had a good impact on *T. aestivum* growth, photosynthesis, and physiological enhancement (Guan et al., 2020). In the study done by Yasmeen et al. in 2018, concentrations under 50 ppm were examined in a Petri dish with paper using the *T. aestivum* of different cultivars. Positive physiological reactions were found in the results, which were increased at the higher concentration such as 50 ppm. In a Petri dish experiment, *T. aestivum*, as reported by Kacziba et al. in 2023, showed an average improvement in growth and photosynthetic indices. Reactive oxygen species (ROS) increased noticeably, and these changes linked with the cultivar under study's reported growth and other photosynthetic and physiological factors. Studies reported the stunted growth of *T. aestivum* under excess Cd levels which might be due to limited supply of nutrients in the presence of Cd (Ahmed et al., 2023; Ahmad et al., 2020; Adhikari et al., 2012). This study confirms that effects of CuO NPs are highly dependent on the concentration of NPs dosage applied. The most favorable results were observed at higher concentrations of CuO NPs. Moreover, foliar application of CuO NPs proved to be more



**Fig. 2.** Effect of CuO NPs on chl. a (a), Chl. b (b), carotenoids (c), photosynthetic rate (d), stomatal conductance (e), and transpiration rate (f). Each dataset is representative of the mean values derived from three independent replicates, accompanied by their respective standard deviations. Variations of statistical significance among treatments are denoted by distinct letters displayed on the bars, adhering to a significance threshold of  $P \leq 0.05$ .

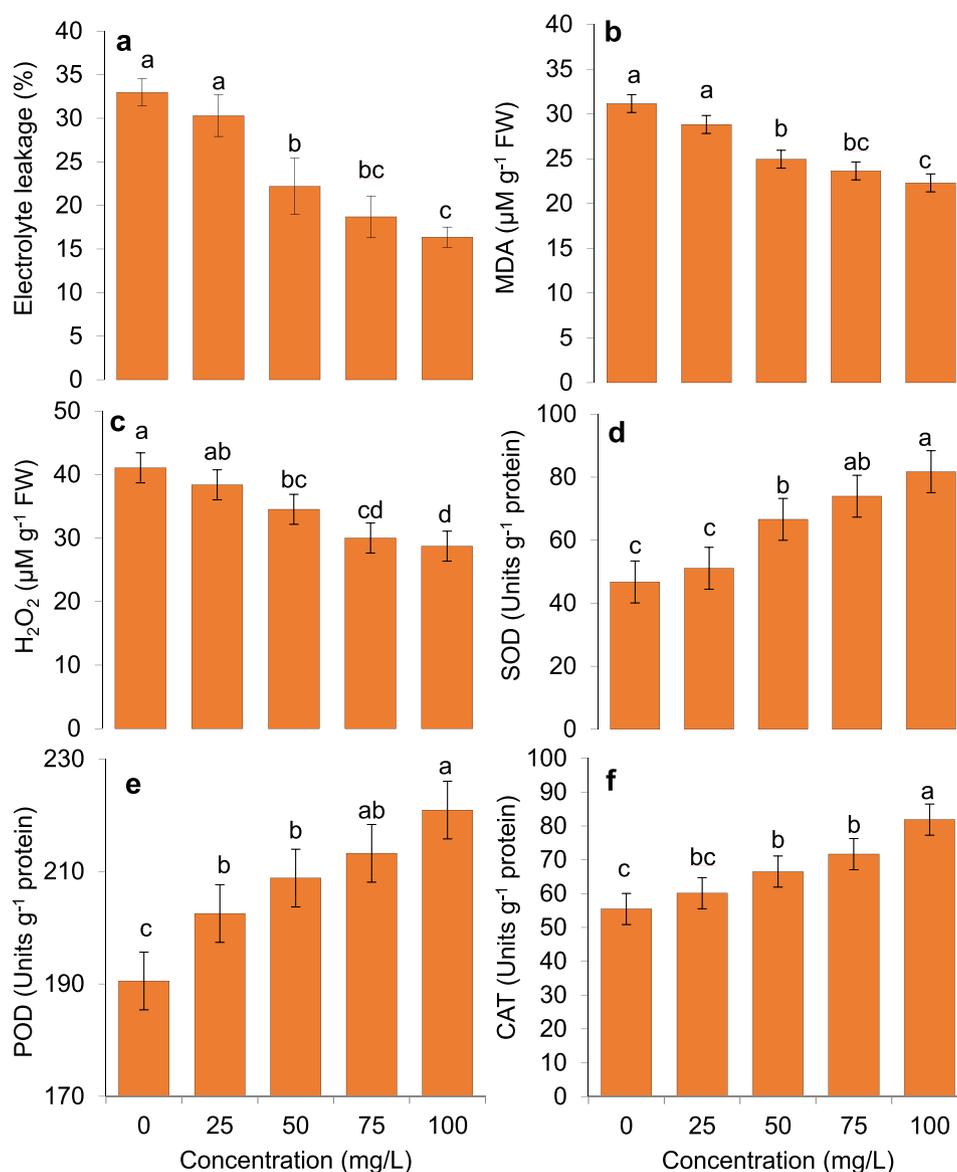
effective compared to other methods such as petri plates and soil application. The figure illustrates that chlorophyll content peaked at 2.5 mg/kg, while the photosynthetic rate reached  $6.7 (\mu\text{mol CO}_2 \text{ m}^{-2} \text{ S}^{-1})$  with 100 mg/L CuO NPs. Additionally, applying CuO NPs through foliar spray showed positive effects on plant development. The discovery has significant implications for regions struggling with soil contamination caused by Cd, a major cause of crop yield reductions.

### 3.3. Effects of CuO NPs on the antioxidant enzymes of *T. aestivum*

CuO NPs caused significant alterations in various antioxidant-enzyme activities, and EL, MDA, and  $\text{H}_2\text{O}_2$  concentrations. Fig. 3(a-f) visually presented the variations in these parameters following the use of CuO NPs at various levels. When comparing the NP-treated groups to control, noticeable minimization in EL values were found, with declines of 8%, 33%, 43%, 59%, respectively. Similarly, MDA levels exhibited a decrease of 7%, 20%, 24%, and 33% under the influence of CuO NPs at different concentrations. Moreover,  $\text{H}_2\text{O}_2$  concentration also showed

reductions of 6%, 16%, 27%, and 35%, respectively. Conversely, the activities of CAT, SOD, and POD enzymes demonstrated an increase in their respective values. Specifically, SOD values exhibited a rise of 9%, 42%, 58%, and 60% in comparison to the control, while POD values showed an increase of 6%, 10%, 12%, and 29%. Additionally, CAT activities were observed to increase by 8%, 20%, 29%, and 36% at all concentrations of CuO NPs, respectively. These findings indicated that CuO NPs contributed to a decrease in EL, MDA, as well as  $\text{H}_2\text{O}_2$  contents, while simultaneously increasing SOD, POD, and CAT enzyme activities in *T. aestivum* plants compared to their absence as in the control pots.

The current results emphasized the efficiency of CuO NPs in enhancing the defense system in leaves of *T. aestivum*. Proteins like molecular chaperones and/or antioxidant enzymes, particularly superoxide dismutase (SOD), are vital for defending plant cells against damage. SOD specifically converts harmful superoxide radicals into oxygen and  $\text{H}_2\text{O}_2$ . Reduced SOD activities can lead to the increase in the amounts of these damaging radicals in leaves of plants. Photosynthesis, a complex process, has sensitivity to external factors. Changes in MDA

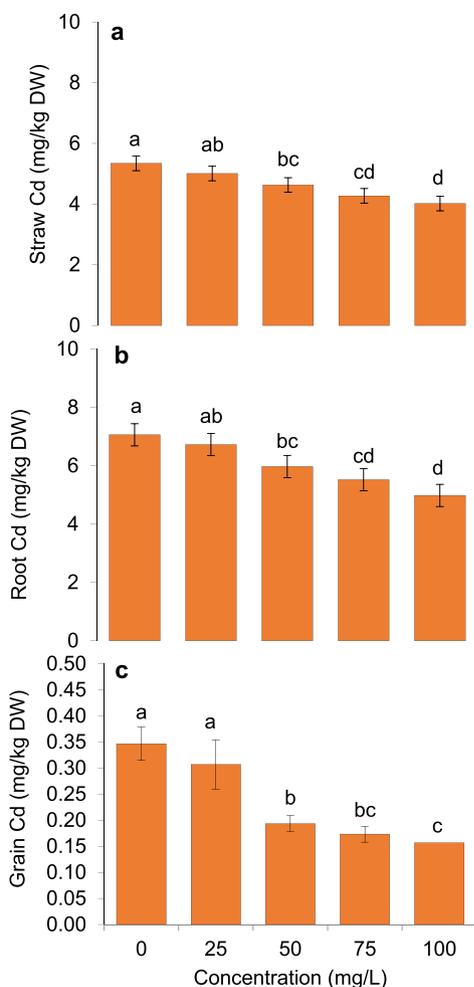


**Fig. 3.** Effect of CuO NPs on EL (a), MDA (b), H<sub>2</sub>O<sub>2</sub> (c), SOD (d), POD (e), CAT (f). Each dataset represents the mean values collected from three separate replicates, together with associated standard deviations. Statistically significant differences between treatments are marked by different letters displayed on the bars, with a significance level of  $P \leq 0.05$ .

levels (a marker for oxidative stress) and SOD and CAT activities impact seedling development, potentially causing growth disruptions. Overall, these processes and enzymes play a crucial role in safeguarding plants from oxidative stress and maintaining their overall health and vitality (Munawaroh et al., 2023; Rajput et al., 2021; Mishra et al., 2019). Although numerous reports have explored the interaction of CuO NPs with plants, their influence on agronomic traits, crop yield, and nutritional contents in crops remained insufficiently investigated. Cu NPs exerted a significant positive impact on both the growth and yield attributes of *Gossypium hirsutum* (L.), concurrently improving defense of plants which constitute stress tolerance mechanisms in plants. The application of CuO NPs via foliar route appears to enhance these mechanisms, facilitating its activation within plants. Applying CuO NPs through foliar application enhanced the physiological, photosynthetic, and antioxidant enzyme aspects of *T. aestivum* crops, leading to improved yield.

#### 3.4. Total accumulation and presence of Cd in *T. aestivum*

The data presented in Fig. 4(a, b, and c) provides information on the Cd concentration in straw, roots, and grains (mg/kg of DW) respectively. The results clearly indicated that foliar applications of CuO NPs reduced the Cd levels in straw, roots, and grains across all levels. Specifically, the Cd content in straw decreased by 6%, 13%, 20%, and 30% respectively, in comparison to the control, for all concentrations of CuO NPs. Similarly, there was a slight decrease of 5%, 15%, 22%, and 26% in the Cd content of the roots, while a more pronounced decline of 11%, 44%, 50%, and 59% was observed for grains across all concentrations. Additionally, plants Cd uptake was reduced in presence of CuO NPs across all concentrations, as shown in Fig. 5(a, b, c, and d). Specifically, the Cd uptake by straw decreased by 7%, 13%, 19%, and 21%, while grain Cd uptake was reduced by 6%, 34%, 38%, and 50%, respectively. Root to straw accumulation declined by 11%, 16%, 28%, and 33%, and straw to grain Cd content decreased by 14%, 18%, 33%, and 43%, for all concentrations of CuO NPs. These findings highlight the efficiency of CuO NPs in reducing Cd levels in various plant parts and uptake, which



**Fig. 4.** Effect of CuO NPs on straw Cd (a), root Cd (b), grain Cd (c). Each dataset represents the mean values collected from three separate replicates, together with associated standard deviations. Statistically significant differences between treatments are marked by different letters displayed on the bars, with a significance level of  $P \leq 0.05$ .

can have positive implications for the overall health of *T. aestivum* plants and the safety of agricultural products.

The harmful effects of Cd on numerous elements of plant physiology have been well investigated and recorded by researchers from all over the world (Rizwan et al., 2016). For instance, Shoeva and Khlestkina (2018) described how Cd buildup in tissues caused cellular oxidative damage, which reduced the development of *T. aestivum* seedlings. A different investigation discovered that *T. aestivum* plants growing in Cd-contaminated soil suffered from nutritional imbalances and photosynthetic disturbances (Venkatchalam et al., 2017). These observations are supported by our results, which show that soil-mixed Cd had a detrimental effect plant development even in the absence of Cu NPs. In addition to an enhanced upward translocation of Cd, this was demonstrated by decreased growth and nutrient absorption. Plant biomass loss can be related to a number of things, including oxidative stress, nutritional imbalances, and decreased photosynthesis in Cd-induced stress conditions. These results corroborate earlier who noted a decrease in plant dry weight in Cd-stressed *T. aestivum* seedlings that were not treated with silicon nanoparticles (Hussain et al., 2019).

Numerous kinds of NPs have been successfully used in the past to remediate heavy metal-contaminated soil (Feigl, 2023; Alotaibi et al., 2023; Dong et al., 2020). Our own research showed that green CuO NPs, primarily through limiting its translocation, significantly benefited *T. aestivum* plant development and nutritional content in soil polluted

with Cd. The *T. aestivum* plants' length and biomass increased significantly at the maximum concentration of CuO NPs (100 mg/kg of soil), partly because Cd translocation was minimized at this level. This is similar to the results of Shi et al. (2018) who noted biomass and increased activity of antioxidant enzymes in Cd-stressed *T. aestivum* treated with nano-silica. Accordingly, our research reveals that the enhanced development of *T. aestivum* plants can be related to Cd immobilization by adsorption onto surfaces of CuO NPs, which was achieved in order to hinder Cd absorption in the above-ground parts. When examining effect of various NPs on translocation of Cd, several researchers have already reported on this process (Chen et al., 2023; Irshad et al., 2021a). Several reported highlighted phytotoxicity of CuO NPs on plant growth, which contradicts our findings. Previous research has documented adverse effects of Cu-NPs on root development, seedling growth, and shoot growth in various plants, including *T. aestivum* (Azhar et al., 2023; Baskar et al., 2018; Adhikari et al., 2012). However, it is important to note that these phytotoxic effects were found to be concentration-dependent. Specifically, studies reporting toxic effects used Cu-NP concentrations exceeding 200 ppm (Lin et al., 2008). Elevated CuO NP levels in the environment can enhance their bioavailability to plants. It is probably that increased activities of chloroplasts, rubisco, antioxidant-enzyme systems, and nitrate reductase may underlie the mechanisms responsible for the increased growth and yield (Nekrasova et al., 2011; Lin et al., 2008). In contrast, other nanoparticles have been found to increase *T. aestivum* yield by 20–25%, such as nano-iron oxide particles, which boosted soybean grain yield by 48% than control (Batsmanova et al., 2013; Sheykhabaglou et al., 2010). Consequently, it appears that Cu-NPs have concentration-dependent effects on plants, with higher concentrations potentially posing harm to plants. Guan et al. (2020) reported that *T. aestivum* exposed to 50-mg/kg of a soil-borne substance showed no significant growth response, indicating stability. However, it was observed that photosynthetic processes were improved, despite the absence of notable growth changes, with an average increase of 28%. The current results also aligned somewhat that cupric oxide NPs have enormous beneficial effects on *T. aestivum* development and growth at the concentration of 50 mg/kg and above compared with the control plots. Plant growth exhibited positive correlations with chlorophyll contents and negative correlation with Cd contents in tissues (Table 1). Similarly, the parameters like plant height, spike length are visually correlated with the increase of some essential micronutrients such as Zn, Fe, and Mn (Table 2). This means that CuO NPs are very effective for addressing the HMs accumulation potential primarily when synthesized by green materials, such as the green synthesis of CuO NPs by using the plant extract of *A. indica* leaves.

### 3.5. Effects of CuO NPs on Zn, Fe and Mn in *T. aestivum* plants

The CuO NPs had a noteworthy influence on Zn, Fe, and Mn contents in *T. aestivum* tissues, as depicted in Fig. 6. The lowest Zn concentration in tissues was observed in the control treatment, while the highest Zn concentration occurred at 100 mg/L. As compared to control, the utilization of CuO NPs resulted in a substantial increase in straw Zn concentration, with increments of 68%, 75%, 85%, and 96% at 25, 50, 75, 100-mg/L CuO NPs, respectively. Similarly, root Zn concentration exhibited significant growth of 88%, 92%, 95%, and 97% at 25, 50, 75, 100-mg/L CuO NPs, respectively. Regarding Zn concentration in grains, CuO NPs induced increases of 79%, 90%, 92%, and 95% at the same concentrations, respectively, in comparison to the control. Likewise, the lowest Fe concentration in tissues was also registered in the control treatment, whereas the maximum Fe concentration was observed at 100 mg/L, aligning with the presence of Fe at this concentration. As compared to control, application of CuO NPs led to substantial enhancements in root Fe concentration, with increments of 12%, 18%, 22%, and 29% at 25, 50, 75, 100-mg/L CuO NPs, respectively. Root Fe concentration also experienced significant growth of 10%, 22%, and

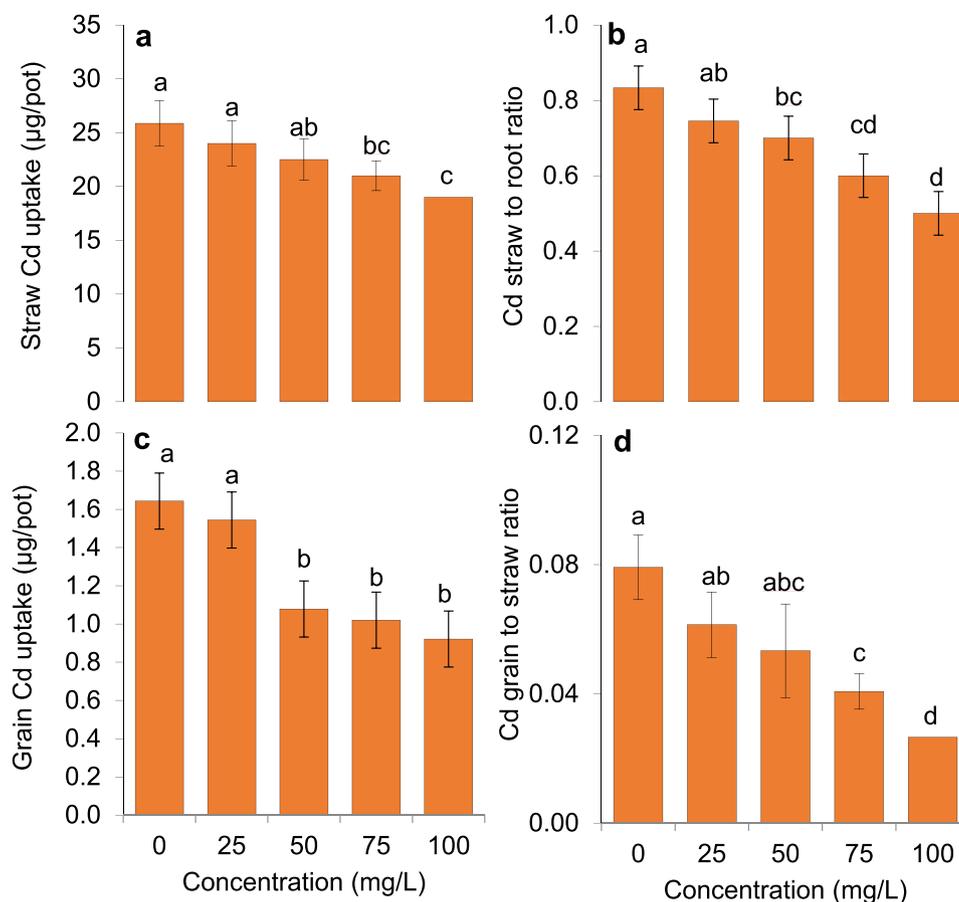


Fig. 5. Effect of CuO NPs on straw Cd uptake (a), grain Cd uptake (b), Cd straw to root ratio (c), Cd grain to straw ratio (d) are among the metrics measured. Each dataset represents the mean values collected from three separate replicates, together with associated standard deviations. Statistically significant differences between treatments are marked by different letters displayed on the bars, with a significance level of  $P \leq 0.05$ .

Table 1

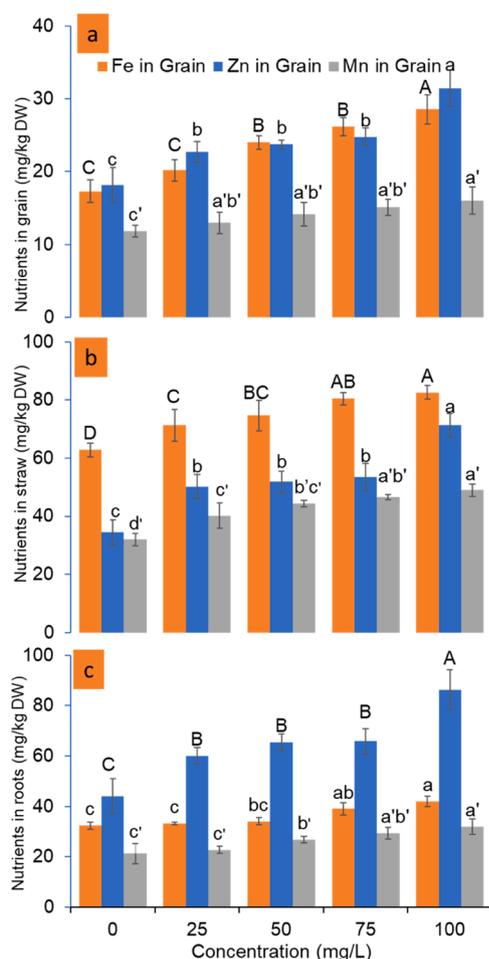
Correlation matrix of physio-biochemical, growth and micronutrients of wheat plants in response to foliar application of CuO NPs.

	PH	GY	Chl.a	Chl. b	PR	TR	Zn <sub>straw</sub>	Zn <sub>grain</sub>	Fe <sub>straw</sub>	Fe <sub>grain</sub>	Mn <sub>straw</sub>	Mn <sub>grain</sub>
PH												
GY	0.6942											
Chl. a	0.6822	0.8571										
Chl. b	0.6914	0.8786	0.9731									
PR	0.6918	0.945	0.9055	0.9288								
TR	0.6916	0.8126	0.9192	0.8909	0.8807							
Zn <sub>straw</sub>	0.6378	0.864	0.801	0.8238	0.8968	0.7181						
Zn <sub>grain</sub>	0.6788	0.8671	0.8648	0.8704	0.9204	0.8546	0.9397					
Fe <sub>straw</sub>	0.5319	0.5882	0.5914	0.5806	0.5935	0.4034	0.5837	0.4587				
Fe <sub>grain</sub>	0.7161	0.8663	0.9554	0.9499	0.9371	0.9258	0.8021	0.8541	0.6438			
Mn <sub>straw</sub>	0.6736	0.9052	0.9091	0.9356	0.9178	0.8319	0.8018	0.7833	0.581	0.9036		
Mn <sub>grain</sub>	0.3785	0.5878	0.6904	0.6921	0.6715	0.6645	0.642	0.5961	0.594	0.777	0.6303	

Table 2

Correlation matrix of antioxidant enzymes on the Cd uptake and HRI of wheat plants in response to foliar application of CuO NPs.

	EL	MDA	H <sub>2</sub> O <sub>2</sub>	SOD	POD	CAT	Cd <sub>root</sub>	Cd <sub>straw</sub>	Cd <sub>grain</sub>	HRI
EL										
MDA	0.8613									
H <sub>2</sub> O <sub>2</sub>	0.9018	0.8044								
SOD	-0.9159	-0.8636	-0.8567							
POD	-0.8326	-0.589	-0.7097	0.6344						
CAT	-0.8608	-0.78	-0.8708	0.9199	0.664					
Cd <sub>root</sub>	0.7818	0.8193	0.7422	-0.8343	-0.5799	-0.7987				
Cd <sub>straw</sub>	0.8536	0.6954	0.8534	-0.8146	-0.7039	-0.8667	0.7253			
Cd <sub>grain</sub>	0.8593	0.8458	0.7066	-0.9094	-0.631	-0.819	0.7375	0.6933		
HRI	0.8957	0.9126	0.7569	-0.8719	-0.7063	-0.7896	0.7779	0.7532	0.8335	



**Fig. 6.** Effect of CuO NPs on Zn, Fe, and Mn contents in grains (a), straw (b), and roots (c) of the wheat plant. Each dataset represents the mean values from three independent replicates, along with their corresponding standard deviations. Different letters on the bars indicate statistically significant differences between treatments, with a significance level set at  $P \leq 0.05$ .

30% at the same concentrations compared to the control. Concerning Fe concentration in grains, CuO NPs resulted in increases of 20%, 25%, 26%, and 30% at 25, 50, 75, 100-mg/L CuO NPs, respectively. Additionally, the Mn concentration exhibited variations under different concentrations of CuO NPs in *T. aestivum* plants. The lowest Mn concentration in tissues was found in the control treatment, with the maximum Mn concentration occurring at 100 mg/L, in line with the presence of Zn and Fe at this concentration. Comparatively, CuO NPs brought about significant increases in shoot Mn concentration, with increments of 15%, 28%, 35%, and 53% at the same concentrations, respectively, when compared to the control. Likewise, root Mn concentration exhibited significant growth of 12%, 23%, 32%, and 49%, and regarding Mn concentration in grains, CuO NPs led to increases of 12%, 19%, 25%, and 35% at 25, 50, 75, 100-mg/L CuO NPs, respectively, in comparison to the control. The results from the present study, it has been found that foliar CuO NPs were more effective means to enhance Zn, Fe, as well as Mn concentration in the *T. aestivum* plants. Different NPs have been reported that they have tendency to uptake essential nutrients from the soil to various plant tissues (Sun et al., 2023). Recent research has highlighted the nuanced response of nanoparticles (NPs) to heavy metal uptake and essential nutrient absorption, which is contingent on factors such as dosage, exposure method, NPs type, and the specific plant species involved (Hussain et al., 2019).

In general, the CuO nanoparticles produced through the eco-friendly method have the potential to address deficiencies in nutrients like Zn,

Fe, and Mn. They may also reduce the absorption of Cd, as Zn tends to interact with Cd during root uptake and subsequent translocation to other plant tissues. This interaction between Zn and Cd exhibits an antagonistic effect (Adrees et al., 2021; Rizwan et al., 2019; Usman et al., 2023). Green nanotechnology has the potential to revolutionize agriculture by improving production efficiency and minimizing losses. This technology promises to increase crop yields while ensuring environmental sustainability and economic stability. Its applications in agriculture have led to more efficient and innovative management practices (Jiang et al., 2021; Pandey et al., 2018; Acharya and Pal, 2020; Salama et al., 2021; Fincheira et al., 2021). Furthermore, green nanotechnology offers diverse opportunities for development and understanding compared to traditional agricultural materials. Hence, green CuO NPs applied as foliar could be an efficient way for nutrients biofortification and mitigation of Cd accumulation in *T. aestivum* crop. However, future studies are still needed to adjust the dose, size, and application time of the amendment. Furthermore, crop varieties and the effects of soil types are also needed to be explored before any final consideration.

#### 4. Conclusions

The research findings highlighted the positive impact of foliar-applied CuO NPs on *T. aestivum* yield, with notable improvements observed in plant height, spike length, chlorophyll levels, and straw biomass. This treatment also led to enhancement in activities of antioxidant enzymes in the leaves, thereby reducing oxidative stress. Moreover, foliar exposure to CuO NPs resulted in reduced concentrations of Cd and its overall uptake in the grains. The study may suggest that this approach could effectively bring down Cd levels in *T. aestivum* grains below the maximum permissible limit of 0.2 mg/kg for cereals, thus minimizing potential health risks associated with grain consumption. However, it is crucial to specify appropriate CuO NPs levels for different crops in large-scale field applications. These findings emphasized the potential of CuO NPs to improve *T. aestivum* plant physiology, boost stress resilience, and may provide a long-term agricultural solution.

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#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

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