Water Air Soil Pollut (2024) 235:738
https://doi.org/10.1007/s11270-024-07551-1

A Comparative Study About Physical Properties of Copper Oxide and Zinc Oxide Nanoparticles on *Fagus orientalis* L. as Bioindicator

Kaan Isinkaralar · Oznur Isinkaralar · Halil Barış Özel · Hakan Şevik

Received: 23 July 2024 / Accepted: 28 September 2024 / Published online: 8 October 2024

Abstract Nanoparticles (NPs) have recently begun to be used extensively in many areas of our lives. It is stated that the resulting NPs pollution will affect even the most remote ecosystems on the Earth. Therefore, determining the effects of NPs on diverse ecosystems is a research topic of interest. From this perspective, current researches on NPs affecting forest ecosystems and forest trees are very scarce. The aim of this investigation was to reveal the inhibitory and toxic properties of CuO and ZnO-NPs on oriental beech seed germination parameters and identify the toxic threshold values of CuO and ZnO-NPs. Therefore, the oriental beech (*Fagus orientalis* L.) seeds obtained from ten populations (P1 to P10) were treated with CuO and ZnO-NPs as single compounds at concentrations of 200, 400, 600, 800 and 1000 mg/L and germination rate (GR), germination percentage (GP), root collar diameter (RCD), plumula length (PL), radicle thickness (RT) and radicle diameter (RD) were determined for 35 days. The water containing CuO-ZnO NPs as a single compound at 1000 mg/L negatively affected all growth in general. The significant decrease in our study occurred at P9. The CuO-ZnO NPs were reduced in GP, RCD, PL, RT, and RD as 3.5–4.2%; 11.2–0.1%; 9.1–36.4%; 23.07–38.46%; and 39.29–17.86%, while GR enhanced as 21.46–20.09%. It was found that water with a low concentration of NP (200 mg/L) is suitable for irrigation of seeds grown in soil media and does not have a significant toxic effect on the growth and uptake of metal ions. The findings and limitations of the present research allow us to assess the feasibility of reusing NP-contaminated water in agriculture. Nevertheless, further research is needed to understand the toxic effects of NP mixtures on growth and absorption mechanisms.

Keywords Irrigation · Nanoparticle pollution · Wastewater use · Germination parameters · Seeds grown

K. Isinkaralar · H. Şevik
Department of Environmental Engineering, Faculty of Engineering and Architecture, Kastamonu University, 37150 Kastamonu, Türkiye

O. Isinkaralar (✉)
Department of Landscape Architecture, Faculty of Engineering and Architecture, Kastamonu University, 37150 Kastamonu, Türkiye
e-mail: obulan@kastamonu.edu.tr

H. B. Özel
Faculty of Forestry, Department of Forestry, Bartın University, Bartın, Türkiye

1 Introduction

In the last century, dizzying speed developments have been experienced in the field of technology and industry, and these developments have caused permanent and extreme changes around the world. Population mobility to supply the workforce needed in factories has caused the population to gather in certain regions, and urbanization has become a global problem in the long term (Sun et al., 2020). The current urbanization rate, around 9% in the early twentieth century, has exceeded 50% today and is estimated to reach 90% by 2030 (Li et al., 2021). Thus, urbanization has become an irreversible problem on a global scale (Cai et al., 2021).

Fossil fuels primarily meet the energy needed in the industrial field, and global climate change, which is emphasised to occur mainly due to carbon emissions, has become another problem that is considered irreversible worldwide (Dong et al., 2021; Fuller et al., 2022). It is worth noting that global climate change will directly or indirectly affect all living things worldwide in the long term (Isinkaralar, 2024). Along with industrial activities, mining activities have also accelerated, and the elements extracted from underground reserves and released into nature have caused pollution, one of the biggest problems worldwide (Zheng et al., 2023). In particular, air pollution has reached such a level that today, it is stated that approximately 92% of the world's population lives in areas with poor air quality, one in every eight deaths is related to air pollution, and air pollution causes premature births-death and serious illnesses for millions of people annually (Fisher et al., 2021). Air pollution is so threatening and harmful because of heavy metals, which are components of air pollution (Goossens et al., 2021; Isinkaralar, 2022). Because heavy metals are pollutants that can be harmful and fatal even at low concentrations, they can remain intact for a long time, and their concentrations are constantly increasing due to human origin (Pacheco et al., 2023).

Recent developments in the technological field have brought the threat level of trace metal pollution to another dimension, and nanoparticles (NPs) pollution has come to the world agenda (Latif et al., 2020). NPs are used during the production of devices in all areas of life, especially chip and information technologies, which have domestic and industrial use (Medici et al., 2021). CuO-NPs, one of the most widely used NPs, are widely used for nanomedical purposes due to their antimicrobial activities and their use as potential disinfectants against nosocomial infections (Intisar et al., 2022; Nair et al., 2022). Apart from this, it is widely used in different fields, such as catalysts, gas sensors, detectors, semiconductors, plastics, metallic coatings, lubricants, inks, skin products, heat transfer nanofluids, and antimicrobial agents (Bhatt et al., 2022). However, although it is essential for human metabolism, as with all heavy metals, excessive Cu consumption is harmful to health and can cause nausea, vomiting, diarrhea, liver and kidney damage, nose, mouth, and eye irritation, headache, dizziness, jaundice, hemolytic anemia, and It can eventually cause death (Francis et al., 2022; Rajput et al., 2020). Therefore, Cu is on the priority pollutant list of ATSDR (2004) and USEPA (2016).

In summary, exposure to NPs has been caused by toxicity in the respiratory system, gastrointestinal tract, and skin problems when certain levels are exceeded (Baskar et al., 2021). However, the increasing production and widespread use of CuO-NPs may cause concerns for living things and ecosystems because they are highly toxic compared to other metal oxide NPs (Sajjad et al., 2023). Moreover, it is stated that nanoparticle pollution will damage all biological life cycles, making them inoperable very slowly and will become an unsolvable problem. NPs pollution makes its effects felt in all living systems day by day, damages essential vitality functions in animal, plant, and human societies, causes serious losses, and destroys the ecological balance, causing irreversible systemic problems (Singh & Kumar, 2020). It is reported that previously uncommon life disruptions, diseases, natural degenerations, and sudden losses in natural resources have started to be observed due to NPs contamination reaching higher values, particularly in less developed or developing nations. In the next 20–40 years, annual losses in plant, animal, and human populations due to NPs pollution will reach up to 33%, and new virological elements that have never been seen before will take shape. The plants will be among the living communities most affected by NPs pollution. It is emphasized that the effects of global warming, considered irreversible today, combined with NPs pollution, will reach even higher levels of damage very shortly. Different types of environmental pollution, especially in plants and their natural habitats, are very effective in this regard (Velusamy et al., 2024). Some of the most critical effects of NPs are seen on plants. Over the last few decades, various studies on the effects of NPs on forest ecosystems and forest elements have been carried out *M. officinalis* (Bordbar et al., 2018), *A. glabra* (Nguyen et al., 2023), and *T. officinale* (Abrica-González et al., 2023). However, NPs pollution will affect even the most remote ecosystems on Earth, reaching even the so-called unreachable points and causing serious damage to life (Kolesnikov et al., 2024).

This study aimed to examine the effect on *Fagus orientalis* L. seeds grown in soil culture of irrigation with water containing CuO and ZnO-NPs applied as single compounds. Studies were not carried out over the entire lifetime of spinach, and the effect of exposure to NP mixtures on seed germination was not assessed. The strength of this study will contribute to defining the suitability of irrigation of *F. orientalis* seeds with water containing CuO and ZnO-NPs for future research.

2 Materials and Methods

2.1 Study Area and Preparation Sampling

Oriental beech seeds were selected from the forests of Türkiye in the current research. Details of the populations from different seeds are presented in Table 1.

All collected seeds were evaluated for germination, and oriental beech seeds with typical growth performance showed no problems in the embryo and endosperm parts utilized in the germination experiments.

2.2 Germination Tests

The primary objectives of the study were to determine the effects of CuO and ZnO-NPs on the germination characteristics of oriental beech seeds and to identify the toxic levels. Therefore, five different concentrations of NPs (200, 400, 600, 800, and 1000 mg/L) were specified in the laboratory in sterile and hygienic conditions and preserved in sterile concentration bottles to be transferred to the seeds. The oriental beech seeds treated with the concentrations prepared in five different doses were placed in disposable sterile petri dishes with quantitative filter papers for germination tests. Five replicates were performed for each dose, and NPs were applied to 150 seeds, 30 healthy seeds in each replicate. In the germination experiments, a total of 900 seeds were utilized, including the control group. The germination assays were carried out in a 3M Climacell brand germination chamber. The temperature of the germination environment in the

Table 1 Populations of the research field at the sites

No	Location	Coordinate		Height (m)	Direction	Stand type
1	Adapazarı-Karasu	41º 01′ 13"	30º 44′ 45"	300–500	Northwest	Knd_2
		41º 00′ 38"	30º 45′ 01"			
2	Balıkesir-Dursunbey	39º 27′ 35"	28º 33′ 54"	800–1000	North	Knd_1
		39º 27′ 30"	28º 32′ 44"			
3	Bartın-Kumluca	41º 29′ 43"	32º 26′ 32"	450–700	North	Knd_3
		41º 29′ 55"	32º 27′ 54"			
4	Bursa-İnegöl	39º 58′ 44"	29º 29′ 04"	700–1200	Northwest	Knd_3
		39º 58′ 02"	29º 28′ 46"			
5	Çanakkale-Kalkım	39º 46′ 56"	27º 09′ 46"	400–600	North	Knd_2
		39º 46′ 11"	27º 09′ 10"			
6	Düzce-Yığılca	31º 25′ 35"	31º 25′ 35"	700–1100	Northwest	Knd_3
		31º 25′ 35"	31º 25′ 35"			
7	Kahramanmaraş-Andırın	37º 46′ 12"	36º 22′ 10"	1400–1800	North	Knd_3
		37º 44′ 52"	36º 23′ 34"			
8	Karabük-Yenice	41º 09′ 08"	32º 16′ 37"	600–900	North	Knd_1
		41º 08′ 52"	32º 15′ 53"			
9	Ordu-Akkuş	40º 47′ 25"	36º 58′ 50"	1100–1400	North	Knd_2
		40º 46′ 56"	36º 59′ 05"			
10	Zonguldak-Devrek	41º 12′ 27"	31º 53′ 15"	500–700	Northwest	Knd_1
		41º 16′ 13"	32º 12′ 52"			

Table 2 Change of GR depending on population and nanoparticle application

Population	Reference	200 mg/L		400 mg/L		600 mg/L		800 mg/L		1000 mg/L		F value		Mean	
		CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO
P1	19.1abcdA	27.2bcdB	26.5abcB	26.6abcB	25.3B	25.9abcdB	24.9bB	24.9abB	23.9bAB	24.9abcdB	22.5bAB	8.1***	2.7*	24.8bcd	23.7bcd
P2	20.2bcdeA	28.6cdeC	26.2abc	27.4abcC	26.2	26.9abcdC	26.4b	26.9abC	24.8b	24.0abcdB	24.0	15.1***	2.5ns	25.7cde	24.6cde
P3	18.1abA	24.7abB	23.2ab	24.5aB	23.2	23.8aB	22.6ab	23.9aB	21.8ab	22.5aB	21.4ab	3.8*	1.5ns	22.9a	21.7b
P4	21.1deA	29.8deC	27.7bcB	29.2cBC	27.2B	28.1dBC	27.3bB	27.2abB	26.7bB	27.5dBC	26.3bB	16.5***	3.1*	27.1e	26.0e
P5	18.7abcA	26.9bcB	25.3abc	25.7abB	26.2	24.9abcdB	24.2b	23.8aB	24.7b	23.4abcB	23.8b	5.7**	2.6ns	23.9abc	23.8bcd
P6	19.8abcdeA	27.4bcdC	26.2abcB	27.8abcC	26.1B	25.8abcdBC	25.7bB	25.1abBC	25.5bB	24.1abcdB	24.9bB	10.8***	3.5*	25.0bcd	24.7cde
P7	17.8aA	23.1aB	21.7a	24.9abB	21.6	23.9abB	18.4a	23.7aB	17.6a	21.6aAB	17.5a	3.6*	1.4ns	22.5a	19.1a
P8	20.7cdeA	28.7cdeC	27.8bc	28.1bcBC	26.3	27.4bcdBC	26.5b	26.9abB	26.0b	26.8cdB	25.5b	27.5***	2.4ns	26.4de	25.5de
P9	21.9eA	30.2eC	30.1c	29.7cC	28.4	27.5cdBC	27.0b	27.4bBC	26.2b	26.6bcdB	26.3b	10.5***	2.1ns	27.2e	26.7e
P10	18.5abA	25.2abB	24.6ab	25.7abB	25.3	24.3abcB	23.2ab	23.9aB	22.7ab	22.9abB	21.9ab	7.2***	2.4ns	23.4ab	22.7bc
F value	4.1**	7.1***	2.2*	2.8*	1.2ns	2.1*	2.6*	2.2*	2.3*	2.8*	2.8*			7.9***	9.9***
Mean	19.6A	27.2D	25.9C	27.0D	25.6C	25.9C	24.6BC	25.4BC	24.0B	24.4B	23.4B	55.8***	17.4***		

*** Correlation is significant at the 0.001 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). Duncan's test represents the members of the group with the letters a, b, c, etc. They are statistically different from the corresponding values in the other groups; a numerical value grows starting with the letter

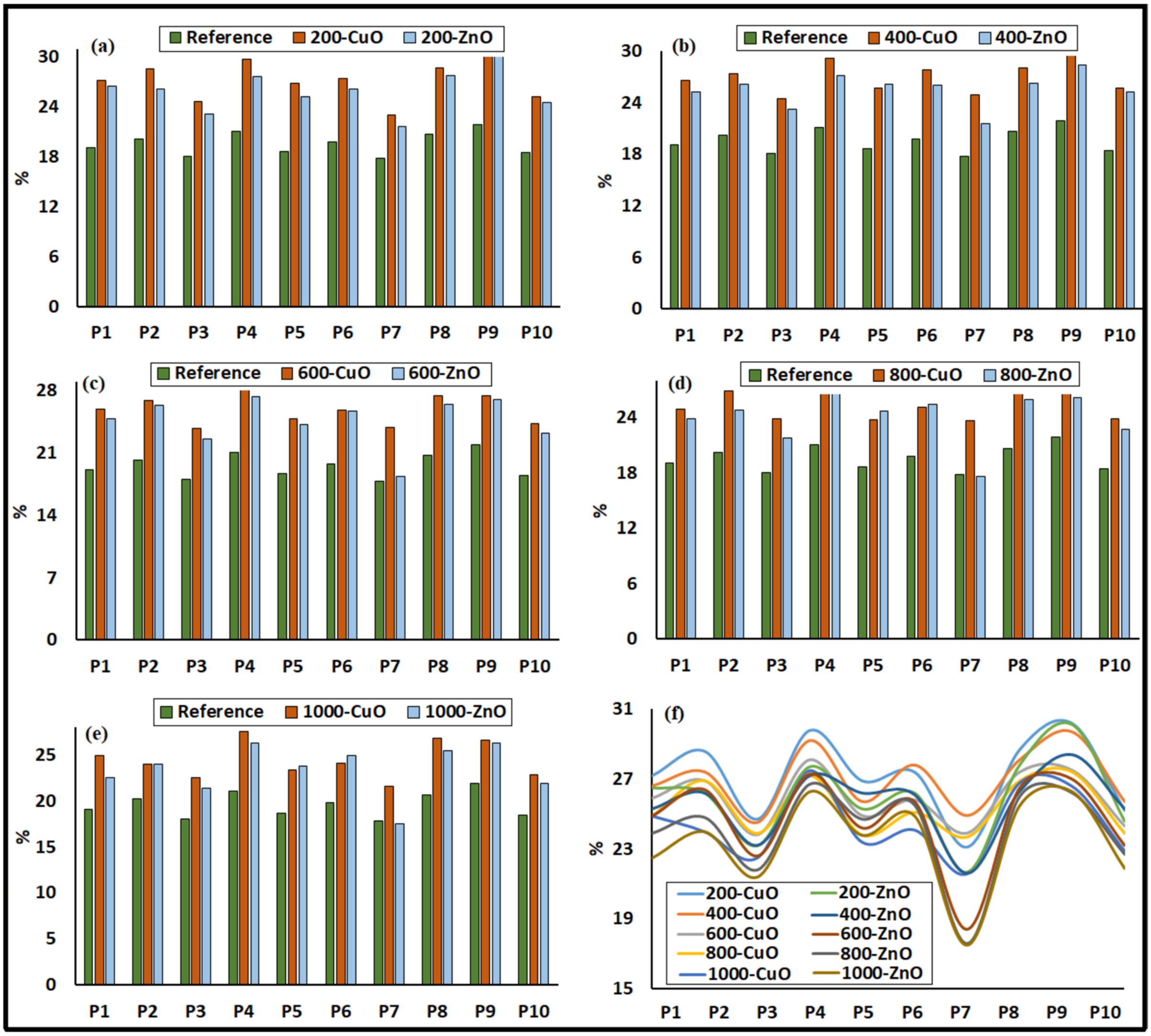

Fig. 1 Variation loading (mg/L) of (**a**) 200-CuO and 200-ZnO, (**b**) 400-CuO and 400-ZnO, (**c**) 600-CuO and 600-ZnO, (**d**) 800-CuO and 800-ZnO, (**e**) 1000-CuO and 1000-ZnO, (**f**) all concentration in all populations for CH

germination chamber was adjusted to 20ºC, relative humidity to 70%, and exposure time to 12 h.

Growth was monitored by applying 10 mL of NPs daily to the beech seeds placed in 100 mL petri dishes without touching each other. The number of emerging seeds was counted on the seventh day of the treatments, and the germination rate (GR) was analyzed. The application continued for 35 days, and seedling length (SL), root collar diameter (RCD), and plumula length (PL) were appointed in all germinated seeds. Radicle diameter (RD) and Radicle thickness (RT) were measured using a digital micro compass. Non-germinating seeds were excised to ensure they were still germinated, and the germination percentage (GP) was calculated by dividing the total germinated seeds by the total healthy seeds. Similarly, the germination rate was used to ratio the number of germinated seeds to the number of healthy seeds on day 7.

2.3 Statical Analysis

The data were assessed by using software SPSS 23.0 (IBM Corp., USA) with a significance level of

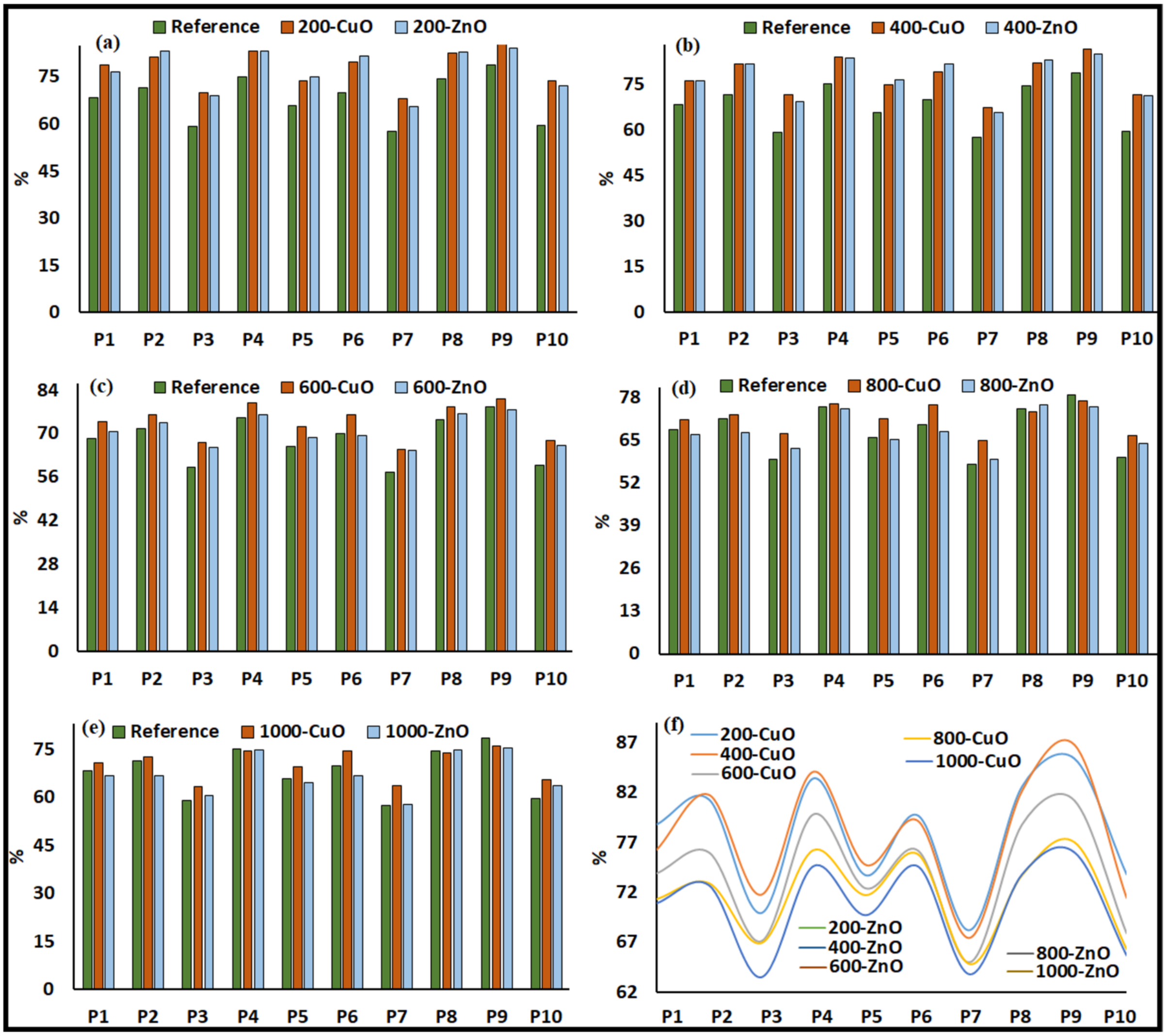

Fig. 2 Variation loading (mg/L)of (**a**) 200-CuO and 200-ZnO, (**b**) 400-CuO and 400-ZnO, (**c**) 600-CuO and 600-ZnO, (**d**) 800-CuO and 800-ZnO, (**e**) 1000-CuO and 1000-ZnO, (**f**) all concentration in all populations for GP

$p < 0.05$, one-way analysis of variance (ANOVA), and Duncan's multiple range test were performed on the values. In recent years, this method has been widely applied in similar research in the field (Ahmed et al., 2018; Chen et al., 2022).

3 Results

Findings regarding GR are summarized in Table 2. To test the CuO values, the change in GR concentration was examined to ensure that it was statistically significant at all doses and in all populations. While pondering, the highest value at the lowest dose was obtained at P9, and the highest value at the highest dose was acquired at P4. When we look at the doses according to the average values, it is seen that the highest value is in the application of 200 and 400 mg/L NPs. When looking at the populations according to the average values the highest value was in P4 and P9. The ZnO results showed that the GR effect of treatment doses was not statistically significant for all populations, excluding the P1 and the P6. At the same time, the

Table 3 Variation of GP concentration based on population and dose

Population	Reference	200 mg/L		400 mg/L		600 mg/L		800 mg/L		1000 mg/L		F value		Mean	
		CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO
P1	68.4bcA	78.8bcC	76.5cdeB	76.3bcdC	76.2bB	73.9cdeBC	70.7bcdAB	71.3abcdAB	66.6bA	70.9cdAB	66.9cA	5.7**	5.3**	73.3bc	70.9de
P2	71.5bcdA	81.3cB	83.2efB	81.8defB	81.8cdB	76.0defAB	73.7cdeA	72.9bcdA	67.3bA	72.7cdA	66.9cA	3.6*	8.8***	76.0cd	74.1e
P3	59.2aA	69.9aC	69.0abC	71.8abC	69.4aC	67.1abBC	65.5abBC	66.9abcBC	62.6abAB	63.5aAB	60.5abAB	5.0**	5.7**	66.4a	64.4ab
P4	75.2cd	83.4c	83.3efB	84.1ef	83.6dB	79.8ef	76.0deA	76.2d	74.5cA	74.6cd	74.9dA	2.1 ns	3.2*	78.9de	77.9f
P5	65.9abA	73.7abB	75.0bcdB	74.8bcB	76.6bcB	72.4bcdB	68.9abcA	71.7bcdB	65.1bA	69.7bcAB	64.7bcA	2.9*	7.7***	71.4b	69.4cd
P6	69.9bc	79.7bc	81.7defB	79.2cde	81.7cdB	76.2def	69.5abcA	75.8d	67.7bA	74.6cd	66.7cA	2.4 ns	8.3***	75.9cd	72.9e
P7	57.6aA	68.2aB	65.6aB	67.5aB	65.9aB	65.0aB	64.7aB	64.8aB	59.1aAB	63.8aAB	57.7aA	2.6*	3.3*	64.5a	61.8a
P8	74.5bcdA	82.6cB	83.0efB	82.2defB	83.1dB	78.8defAB	76.4eA	73.7cdA	75.8cA	73.8cdA	74.8dA	3.7*	5.2**	77.6d	77.9f
P9	78.7dA	85.4cB	84.1fB	86.8fB	85.0dB	81.2fAB	77.6eA	77.0dA	75.3cA	76.0dA	75.4dA	4.6**	4.1**	80.8e	79.4f
P10	59.7aA	73.8abC	72.1abcC	71.5abBC	71.3abC	67.9abcBC	66.3abB	66.3abAB	63.9abAB	65.7abAB	63.8bcAB	4.6**	8.4***	67.5a	66.2bc
F value	7.0***	6.7***	8.3***	8.5***	14.0***	7.2***	6.7***	4.0**	10.5***	7.7***	12.6***			27.0***	27.8***
Mean	68.1A	77.7D	77.4C	77.6D	77.5C	73.8C	70.9B	71.7BC	67.8A	70.5AB	67.2A	14.9***	21.2***		

*** Correlation is significant at the 0.001 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). Duncan's test represents the members of the group with the letters a, b, c, etc. They are statistically different from the corresponding values in the other groups; a numerical value grows starting with the letter

change in GR on a population basis is statistically significant at all doses except the 200 mg/L dose of NPs application.

In general, the lowest GR was underscored in the control group in all populations, while the highest GR value was achieved in the low-dose (200 mg/L) NPs. However, Fig. 1 demonstrates that the GR value decreased as the nanoparticle application increased. Based on population, the lowest point value was recorded in P7, while the highest values were obtained in P4 and P9. In the control group, the lowest value was gained in P7 (17.8%), and the highest was found in P9 (21.9%). GR, which increased to 21.7% with 200 mg/L NPs in P7, decreased negatively with the dose increase and decreased to 17.5% in 1000 mg/L NPs. In P9, GR, which increased to 30.1% at 200 mg/L NPs, decreased to 26.3% at 1000 mg/L NPs due to dose increase.

The change in GP levels as a function of population and dose is presented in Table 2. Considering CuO values, the change in GP content was statistically significant on a dose-dependent scale in all populations except P4 and P6. It was stated to be statistically significant on a population basis at all doses. At the lightest dose, the lowest value was observed in P3, P7, and P10, while at the highest dose, the lowest value was observed in P3 and P7. The highest value was found in 200 and 400 mg/L dose nanoparticle application when the doses were analyzed according to the mean values. Again, when the populations were analyzed in terms of mean levels, it was seen that the maximum value was obtained in P9. According to the results of variance analysis of ZnO values, treatment doses were statistically significant in GP in almost all populations. Similarly, the population-based change attributed to GP at all doses was statistically significant.

In general, the minimum GP was achieved in the high-dose (1000 mg/L) NPs, and the maximum GP was recorded in the 400 mg/L NPs in all populations. Nevertheless, Fig. 2 illustrates that the GP value decreased as the NPs increased. Generally, the lower level was achieved in the P7 population, while the maximum values were observed in the P4 and P8 populations. Meanwhile, in the control group, the lowest value was recorded in P7 (57.6%), while the highest value was determined in

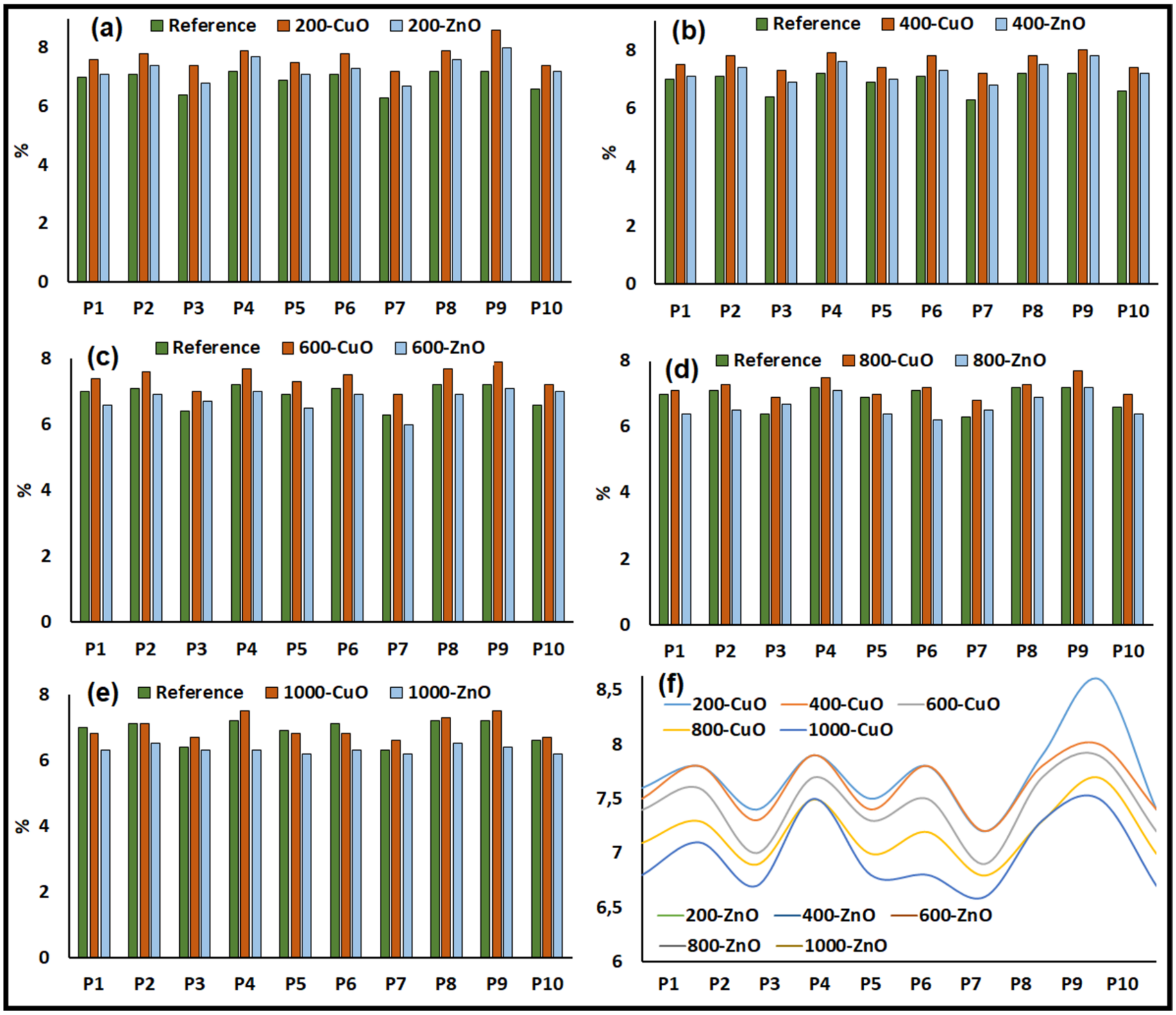

Fig. 3 Variation loading (mg/L) of (**a**) 200-CuO and 200-ZnO, (**b**) 400-CuO and 400-ZnO, (**c**) 600-CuO and 600-ZnO, (**d**) 800-CuO and 800-ZnO, (**e**) 1000-CuO and 1000-ZnO, (**f**) all concentration in all populations for SL

P9. In P7, GP increased to 65.6% in the 200 mg/L NPs and to 65.9% in the 400 mg/L NPs and then started to decrease inversely proportional to the dose increase. In P9, GP increased to 84.1% at 200 mg/L NPs but decreased with increasing dose to 75.4% at 1000 mg/L NPs.

Population and dose-dependent variation of SL concentration is presented in Table 3. The analysis of variance results of CuO values indicated that the change in SL concentration was not statistically significant in all populations on a dose basis. At doses of 200 and 400 mg/L NPs, the highest values were obtained in P4, P8, and P9. Considering the ZnO results, it was verified that the treatment doses were not statistically significant on SL in all populations except P9. Likewise, the change in SL by population was not statistically significant at all doses except for the control group. Generally speaking, the lowest SL value in all populations was recorded in the 1000 mg/L NPs, while the highest SL value was recorded in the 200 and 400 mg/L NPs.

Figure 3 provides that the SL value decreases as the nanoparticle concentration increases. On a population basis, the lowest level was achieved in P7, while the maximum level was achieved in P9.

Table 4 Variation of SL concentration based on population and dose

Population	Reference	200 mg/L		400 mg/L		600 mg/L		800 mg/L		1000 mg/L		F value		Mean	
		CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO
P1	7.0bc	7.6	7.1	7.5	7.1	7.4	6.6	7.1	6.4	6.8	6.3	0.7ns	1.1ns	7.2abcde	6.8abc
P2	7.1bc	7.8	7.4	7.8	7.4	7.6	6.9	7.3	6.5	7.1	6.5	0.9ns	1.3ns	7.4cdef	7.0bcd
P3	6.4ab	7.4	6.8	7.3	6.9	7.0	6.7	6.9	6.7	6.7	6.3	1.9ns	0.4ns	6.9ab	6.6ab
P4	7.2c	7.9	7.7	7.9	7.6	7.7	7.0	7.5	7.1	7.5	6.3	0.5ns	1.8ns	7.6ef	7.1cd
P5	6.9abc	7.5	7.1	7.4	7.0	7.3	6.5	7.0	6.4	6.8	6.2	0.9ns	1.3ns	7.2abcd	6.7abc
P6	7.1bc	7.8	7.3	7.8	7.3	7.5	6.9	7.2	6.2	6.8	6.3	1.2ns	1.2ns	7.3bcde	6.8abcd
P7	6.3a	7.2	6.7	7.2	6.8	6.9	6.0	6.8	6.5	6.6	6.2	1.7ns	0.9ns	6.8a	6.4a
P8	7.2c	7.9	7.6	7.8	7.5	7.7	6.9	7.3	6.9	7.3	6.5	0.7ns	1.1ns	7.5def	7.1bcd
P9	7.2c	8.6	8.0B	8.0	7.8B	7.9	7.1AB	7.7	7.2AB	7.5	6.4A	1.4ns	3.5*	7.8f	7.3d
P10	6.6abc	7.4	7.2	7.4	7.2	7.2	7.0	7.0	6.4	6.7	6.2	0.9ns	1.8ns	7.1abc	6.8abc
F value	2.3*	1.2ns	1.3ns	0.6ns	0.8ns	0.7ns	0.5ns	0.5ns	0.8ns	0.9ns	0.0ns			5.0***	3.0**
Mean	6.9A	7.7C	7.3C	7.6C	7.3C	7.4BC	6.8B	7.2AB	6.6B	7.0A	6.3A	9.8***	12.0***		

*** Correlation is significant at the 0.001 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). Duncan's test represents the members of the group with the letters a, b, c, etc. They are statistically different from the corresponding values in the other groups; a numerical value grows starting with the letter

Among the control group, the minimum value was found in P7 (6.3%), while the maximum value was reached in P4, P8 and P9 (7.2%). SL, which reached 6.7% with 200 mg/L NPs in P7, started to decline inversely proportional to the dose increase and decreased to 6.2% in 1000 mg/L NPs. In P9, SL increased to 8.0% with 200 mg/L NPs, decreased with dose increase, and decreased to 6.4% with 1000 mg/L NPs.

The change in RCD concentration based on population and dose is given in Table 4. Similarly, CuO values are examined, and the change in RCD concentration on a dose basis in all populations and on a population basis at all doses is not statistically significant. When the populations were examined according to average values, the highest value was obtained in P9. Considering the ZnO results, the application doses were not statistically significant in all populations on RCD. Additionally, the population-based change in RCD at all doses is not statistically significant.

Overall, the lowest RCD value in all populations was gained in the control group, while the highest RCD value was obtained in the 200 and 400 mg/L treatments. However, Fig. 4 presents that the RCD value decreased as the NPs increased. Regarding population, the minimum value was found in P7, whereas the maximum value was found in P9.

In Table 5, there were CuO values in the PL concentration changed in all populations or on a population basis at all doses except reference samples. It was determined that the lowest value in the control dose, reference, was seen in P7. When we look at the doses considering the average values, the highest values were obtained in the control group and low-dose nanoparticle applications. In contrast, the highest values were obtained in high-dose nanoparticle applications. According to the ZnO analysis of variance results, application doses on PL were statistically significant in all populations except P3 and P7. Except for the control dose, the population-based change in PL at all doses is not statistically significant.

While the lowest PL value was achieved in the 1000 mg/L NPs in all populations, the highest value was gained in the control group. Figure 5 exhibits that the PL value decreases as the NPs increase. Whereas the lowest value on a population basis was recorded in P7, the highest value was achieved

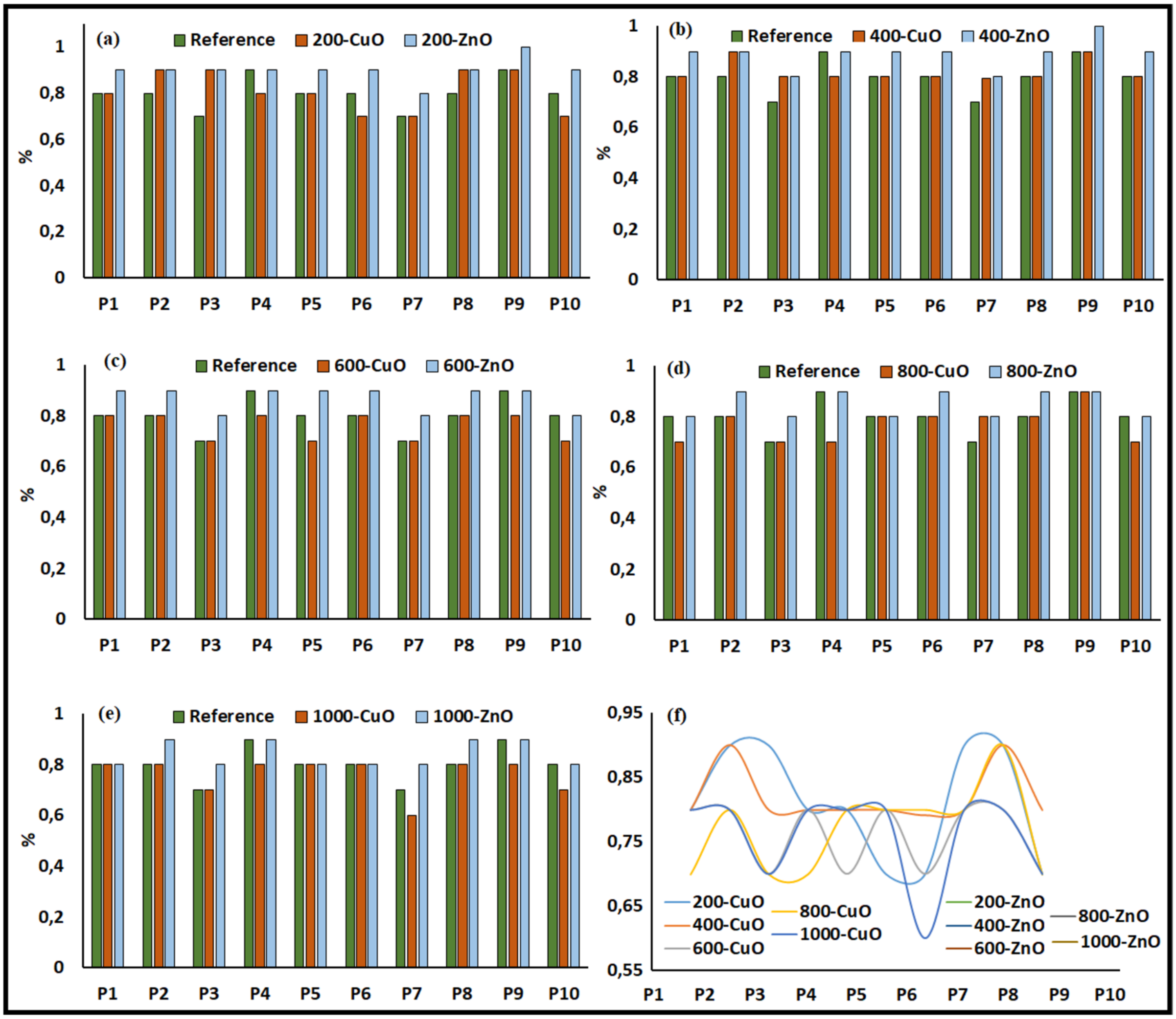

Fig. 4 Variation loading (mg/L) of (**a**) 200-CuO and 200-ZnO, (**b**) 400-CuO and 400-ZnO, (**c**) 600-CuO and 600-ZnO, (**d**) 800-CuO and 800-ZnO, (**e**) 1000-CuO and 1000-ZnO, (**f**) all concentration in all populations for KBC

in P9. In the control group, the lowest value was obtained in P7 (0.8%) and the highest in P9 (1.1%).

The change in RT concentration based on population and dose is given in Table 6. Considering the variance analysis results, CuO values determined that the RT concentration change was not statistically significant on a dose basis in all populations except P2, P4, P6, and P9. Again, it is not statistically significant on a population basis at all doses except reference. Notably, the highest value was obtained at reference in P2, P4, P6, and P9. As previously mentioned, the highest value was seen in the control group, and the lowest was seen in the highest dose nanoparticle application. When looking at the populations according to the average values, it was determined that the highest value was in P9. When the ZnO results were examined, it was stated that the application doses were statistically significant on RT in all populations except P7. At the same time, the population-based change in RT was not statistically significant at all doses except the control group.

On the whole, the minimum RT was obtained in the 1000 mg/L NPs in all populations, while the maximum value was found in the control group. Figure 6

Table 5 Variation of RCD concentration based on population and dose

Population	Reference	200 mg/L		400 mg/L		600 mg/L		800 mg/L		1000 mg/L		F value		Mean	
		CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO
P1	0.8	0.8	0.9	0.8	0.9	0.8	0.9	0.7	0.8	0.8	0.8	0.5ns	0.5ns	0.8abcd	0.9abcd
P2	0.8	0.9	0.9	0.9	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.5ns	0.5ns	0.8de	0.9bcde
P3	0.7	0.9	0.9	0.8	0.8	0.7	0.8	0.7	0.8	0.7	0.8	0.7ns	0.6ns	0.8abc	0.8ab
P4	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.7	0.9	0.8	0.9	0.8ns	0.3ns	0.8bcde	0.9de
P5	0.8	0.8	0.9	0.8	0.9	0.7	0.9	0.8	0.8	0.8	0.8	0.3ns	0.6ns	0.8abc	0.8abc
P6	0.8	0.7	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8	0.8	0.3ns	0.7ns	0.8abcd	0.9abcd
P7	0.7	0.7	0.8	0.7920	0.8	0.7	0.8	0.8	0.8	0.6	0.8	0.5ns	0.6ns	0.7a	0.8a
P8	0.8	0.9	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.3ns	0.5ns	0.8cde	0.9cde
P9	0.9	0.9	1.0	0.9	1.0	0.8	0.9	0.9	0.9	0.8	0.9	0.4ns	0.4ns	0.9e	0.9e
P10	0.8	0.7	0.9	0.8	0.9	0.7	0.8	0.7	0.8	0.7	0.8	0.2ns	0.6ns	0.7ab	0.8abc
F value	0.6ns	1.3ns	0.8ns	0.6ns	0.6ns	1.0ns	0.5ns	0.6ns	0.6ns	0.6ns	0.8ns			3.6***	4.1***
Mean	0.8	0.8	0.9C	0.8	0.9C	0.8	0.9BC	0.8	0.9BC	0.8	0.8AB	1.3ns	5.7***		

*** Correlation is significant at the 0.001 level (2-tailed). Duncan's test represents the members of the group with the letters a, b, c, etc. They are statistically different from the corresponding values in the other groups; a numerical value grows starting with the letter

determines that the RT value decreases as the NPs increases.

The change in RD concentration based on population and dose is given in Table 7. The CuO results show that the RD concentration change is not statistically significant on a population basis at all doses. It was appointed that the RD concentration change was statistically significant on a dose basis in all populations. When the average values are examined, considering the doses, the highest value was seen in the control group, while the lowest value was obtained in the highest dose nanoparticle application. Again, when the populations are examined by looking at the average values, it is quite striking that the highest value is given in P9, and the lowest value is seen in P7. Considering the ZnO results, the application doses were not statistically significant on RD in all populations except P1, P3, P5, and P7. Likewise, the population-based change in RD at all doses is not statistically significant Table 8.

The lowest RD value in all populations was reached in the 1000 mg/L NPs, while the maximum RD value was found in the control group. As depicted in Fig. 7, the RD value decreases as the NPs increase. In terms of population, the lowest value was achieved in P7, while the highest value was obtained in P9.

4 Discussion

The interaction of genetic structure and environmental factors shapes all phenotypic characteristics of plants. Another critical factor affecting the development of plants is the stress factors that the plant is affected by NPs (Zoufan et al., 2020). Stress factors such as drought, frost, and pollution are among the stress factors that can significantly negatively affect plant development. Trace metal pollution is a stress factor that significantly affects plant development. CuO and ZnO are also one of the important and widespread NPs, and even though it is necessary as a plant nutrient element, it is fundamental to note that the baseline heavy metals are toxic and harmful to living things in high concentrations (Koç et al., 2024; Pelegrino et al., 2020). Therefore, it can be predicted that CuO pollution will cause heavy metal stress in plants. It suggests that CuO and ZnO NPs

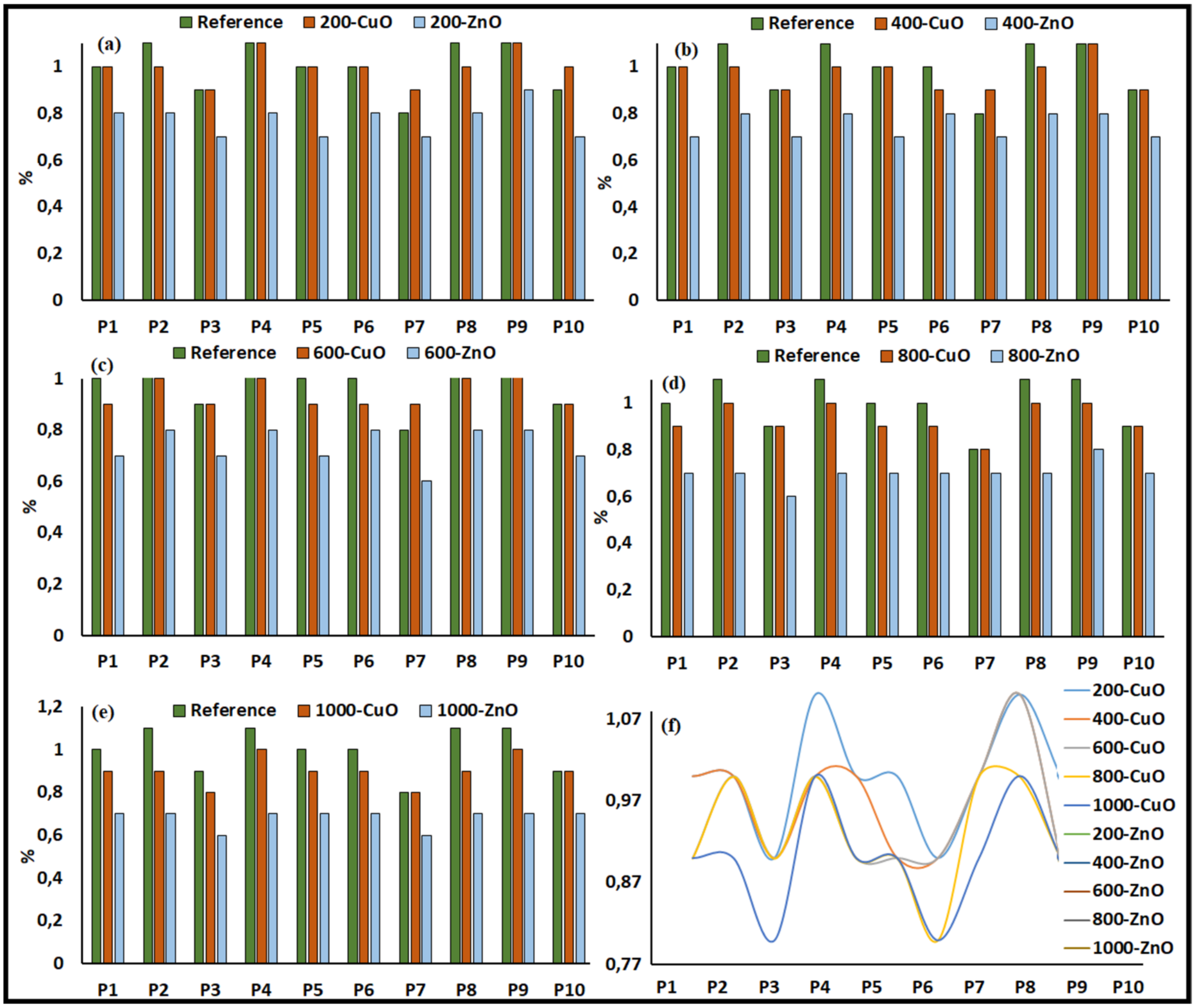

Fig. 5 Variation loading (mg/L) of (**a**) 200-CuO and 200-ZnO, (**b**) 400-CuO and 400-ZnO, (**c**) 600-CuO and 600-ZnO, (**d**) 800-CuO and 800-ZnO, (**e**) 1000-CuO and 1000-ZnO, (**f**) all concentration in all populations for PL

applications affect the development of seeds (Gbair & Alshamsi, 2022; Thounaojam et al., 2021). Generally, germination and seedling characteristics are positively affected at low CuO and ZnO nanoparticle doses, but the parameters decrease with increasing doses. In high-dose CuO and ZnO nanoparticle applications, the values obtained are generally much lower than the control group, and this decrease can exceed 25% compared to the average values (Hidouri et al., 2022; Mousa et al., 2021). Therefore, low nanoparticle applications positively affected germination and seedling characters but had a negative effect as the dose increased. This reveals that individuals will be significantly affected by CuO and ZnO nanoparticle pollution, especially in the early period. This situation is compatible with the information obtained in the literature by Da Costa et al. (2020), Fedorenko et al. (2021), Yadav et al. (2023), and Mahawar et al. (2024).

Metal oxide nanoparticles such as CuO and ZnO offer promising perspectives for the development of new agrochemical formulations of pesticides and fertilizers (Simonin et al., 2018). However, CuO and ZnO may have detrimental effects on microbial activity. Studies reveal that CuO and ZnO have harmful effects on soil microbial activities, and the

Table 6 Change of PL concentration based on population and dose

Population	Reference	200 mg/L		400 mg/L		600 mg/L		800 mg/L		1000 mg/L		F value		Mean	
		CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	CuO	ZnO	CuO	ZnO	CuO
P1	1.0bcd	1.0	0.8A	1.0	0.7A	0.9	0.7A	0.9	0.7A	0.9	0.7A	0.5ns	4.8**	1.0cde	0.8bcd
P2	1.1cd	1.0	0.8	1.0	0.8	1.0	0.8	1.0	0.7	0.9	0.7	0.7ns	8.8***	1.0def	0.8cd
P3	0.9ab	0.9	0.7	0.9	0.7	0.9	0.7	0.9	0.6	0.8	0.6	0.3ns	2.4 ns	0.9ab	0.7ab
P4	1.1cd	1.1	0.8A	1.0	0.8A	1.0	0.8A	1.0	0.7A	1.0	0.7A	0.6ns	6.9***	1.0ef	0.8cd
P5	1.0abcd	1.0	0.7A	1.0	0.7A	0.9	0.7A	0.9	0.7A	0.9	0.7A	0.4ns	4.1**	0.9bcd	0.7abc
P6	1.0cd	1.0	0.8A	0.9	0.8A	0.9	0.8A	0.9	0.7A	0.9	0.7A	1.1ns	5.9**	1.0cde	0.8cd
P7	0.8a	0.9	0.7	0.9	0.7	0.9	0.6	0.8	0.7	0.8	0.6	0.6ns	2.4ns	0.9a	0.7a
P8	1.1cd	1.0	0.8A	1.0	0.8A	1.0	0.8A	1.0	0.7A	0.9	0.7A	1.0ns	7.3***	1.0def	0.8cd
P9	1.1d	1.1	0.9A	1.1	0.8A	1.1	0.8A	1.0	0.8A	1.0	0.7A	0.6ns	6.2**	1.0f	0.8d
P10	0.9abc	1.0	0.7A	0.9	0.7A	0.9	0.7A	0.9	0.7A	0.9	0.7A	0.4ns	3.6*	0.9abc	0.7abc
F value	3.3**	1.0ns	1.2ns	1.0ns	1.2ns	0.7ns	0.5ns	1.1ns	0.6ns	0.7ns	0.4ns			6.8***	3.4***
Mean	1.0B	1.0B	0.8B	1.0B	0.7AB	0.9AB	0.7AB	0.9A	0.7AB	0.9A	0.7A	5.0***	47.3***		

*** Correlation is significant at the 0.001 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). Duncan's test represents the members of the group with the letters a, b, c, etc. They are statistically different from the corresponding values in the other groups; a numerical value grows starting with the letter

adverse effects increase over time (Nie et al., 2020; Sepasgozar et al., 2021). This situation increases the extent of anxiety. Studies have given that NPs significantly weaken germination even in the seeds of species that are short-lived and have very rapid germination ability and energy and that they stop entirely after a particular toxicological threshold value (García-Gómez & Fernández, 2019; Liu et al., 2016). In xerophyte plant species living in arid and semi-arid areas, the particles of very low natural nanomaterials support the plant up to a specific vital cycle against the danger of drought. After a certain level, they also cause toxic effects in such plants (Burachevskaya et al., 2021; Kohatsu et al., 2021). For example, Cu-NPs accumulate in *C. sativus* plant tissues, indicating a higher accumulation level in root tissues, and the genotoxic effect causes genomic DNA modifications. In addition, Cu-NPs have been found to cause a significant decrease in chlorophyll a and b contents, an increase in H_2O_2 and MDA contents, as well as an increase in electrolyte leakage, which causes damage to the root plasma membrane, and induce oxidative stress (Mohebian et al., 2022; Sarkar et al., 2021). In our study, a significant decrease occurred at P9. Although it negatively affected all growth in general, growth was observed only in GR, and these values were 21.46% and 20.09% for CuO and ZnO. 3.5% and 4.2% in GP; 11.2% and 0.1% in RCD; 9.1% and 36.4% in PL; 23.07% and 38.46% in RT; There was a decrease in RD to 39.29% and 17.86%. Therefore, it can be expected that the impact of CuO pollution will be higher, especially in arid areas. As standard in the literature, global climate change's most important and destructive effect will manifest in temperature increase and drought (Sheteiwy et al., 2021; Zong et al., 2022). It is almost certain that this situation will cause the effects of drought stress to be felt to a greater extent through the effect of NPs in plants.

5 Conclusion and Future Aspects

The germination and seedling parameters gathered from various populations vary significantly depending on the population. The lowest values in all parameters were obtained in P3, P7, and P10 populations, while the highest values were obtained in P4 and P9 populations. This shows that the effects of

Fig. 6 Variation loading (mg/L) of (**a**) 200-CuO and 200-ZnO, (**b**) 400-CuO and 400-ZnO, (**c**) 600-CuO and 600-ZnO, (**d**) 800-CuO and 800-ZnO, (**e**) 1000-CuO and 1000-ZnO, (**f**) all concentration in all populations for RT

CuO-NP applications on the populations were at different levels. The fact that the population's genetic structure and diversity are at varying levels leads to different responses to environmental stress factors. The phytotoxicity of NPs on plant seed germination and seedling development depends on the properties and concentration of NPs, the plant species, and their tolerance to stress. However, the number of studies on the long-term effects of NPs, especially on forest trees and seeds, is not yet sufficient, and many unknowns remain.

In addition, our results also suggest that environmental pollution is highly associated with the rapid increase in the use of NPs. However, there is not enough research on the impacts of NPs on ecosystems and forests yet. Future investigations should prioritize the identification and focus on the extent to which the concentrations of NPs in nature increase and at which concentrations they pose a hazard. Moreover, the effects of NPs on species and ecosystems, such as how they affect fruit yield and quality, species and thus biodiversity,

Table 7 Variation of RT concentration based on population and dose

Population	Reference	200 mg/L		400 mg/L		600 mg/L		800 mg/L		1000 mg/L		F value		Mean	
		CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO			CuO	ZnO	CuO	ZnO	CuO
P1	1.1bcd	1.0	0.9A	1.0	0.8A	0.9	0.8A	1.0	0.8A	0.9	0.7A	2.2ns	5.2**	1.0abc	0.9
P2	1.2cdeB	1.1AB	0.9A	1.0AB	0.9A	0.9A	0.8A	1.0A	0.8A	0.9A	0.8A	3.1*	4.8**	1.0bcd	0.9
P3	1.0ab	1.0	0.9BC	0.9	0.8AB	0.9	0.8AB	1.0	0.8AB	0.9	0.7A	1.1ns	4.7**	1.0ab	0.8
P4	1.2de C	1.1B	1.0A	1.1B	0.9A	1.1B	0.9A	0.9A	0.9A	1.0A	0.7A	12.3***	5.6**	1.1cd	0.9
P5	1.1abc	1.0	0.9AB	1.0	0.9A	1.0	0.8A	0.9	0.8A	0.9	0.7A	1.4ns	3.9*	1.0abc	0.9
P6	1.2cdeB	1.0AB	0.9A	1.0 AB	0.9A	0.9A	0.9A	1.0A	0.9A	0.9A	0.8A	2.7*	4.6**	1.0bc	0.9
P7	1.0a	1.0	0.8	0.9	0.8	0.8	0.7	0.9	0.7	0.8	0.7	2.0ns	1.8ns	0.9a	0.8
P8	1.2cde	1.1	1.0B	1.0	1.0B	1.1	0.8AB	1.0	0.9B	0.9	0.7A	2.1ns	6.8***	1.1cd	0.9
P9	1.3eB	1.1A	1.0A	1.1A	1.0A	1.1A	0.9A	1.1A	0.9A	1.0A	0.8A	4.3**	10.2***	1.1d	1.0
P10	1.0ab	1.0	0.8A	1.0	0.9A	1.0	0.8A	1.0	0.8A	0.9	0.8A	0.8ns	2.9*	1.0ab	0.9
F value	6.4***	0.7ns	0.6ns	0.5ns	0.3ns	1.5ns	0.4ns	0.4ns	1.0ns	0.6ns	0.4ns			4.1***	1.9ns
Mean	1.1D	1.0C	0.9C	1.0BC	0.9C	1.0B	0.8B	1.0B	0.8B	0.9A	0.8A	19.2***	45.9***		

*** Correlation is significant at the 0.001 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). Duncan's test represents the members of the group with the letters a, b, c, etc. They are statistically different from the corresponding values in the other groups; a numerical value grows starting with the letter

Table 8 Variation of RD concentration based on population and dose

Population	Reference	200 mg/L		400 mg/L		600 mg/L		800 mg/L		1000 mg/L		F value		Mean	
		CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO	CuO	ZnO
P1	5.8B	3.5A	4.7A	3.6A	4.7A	3.4A	4.2A	2.9A	4.0A	2.8A	3.8A	11.3***	4.7**	3.7abc	4.5abc
P2	5.3B	3.8A	5.0	3.7A	4.9	3.3A	4.6	3.3A	4.5	3.1A	4.1	5.2**	2.2ns	3.7abc	4.7bcd
P3	5.6C	3.3B	4.5B	3.2B	4.4B	3.1AB	4.3B	2.8AB	3.9AB	2.2A	3.3A	14.8***	5.9**	3.4ab	4.3ab
P4	5.2B	4.0A	5.2	3.9A	5.0	3.8A	5.0	3.5A	4.6	3.3A	4.4	3.6*	0.8ns	4.0bc	4.9cd
P5	5.7B	3.4A	4.6A	3.4A	4.6A	3.1A	4.3A	2.9A	4.0A	2.6A	3.7A	9.8***	4.0**	3.5abc	4.5abc
P6	5.3C	3.7B	4.8	3.8B	4.8	3.6AB	4.4	3.0AB	4.4	2.7A	4.0	8.1***	1.3ns	3.7abc	4.6abcd
P7	5.4B	3.1A	4.2A	3.0A	4.3A	2.6A	3.8A	2.4A	3.8A	2.0A	3.4A	8.1***	3.4*	3.1a	4.1a
P8	5.2B	3.8A	4.9	3.7A	5.0	3.4A	4.6	3.3A	4.4	3.0A	4.3	4.0**	0.7ns	3.7abc	4.7bcd
P9	5.6B	4.1A	5.2	4.1A	5.2	3.8A	5.0	3.6A	4.9	3.4A	4.6	8.6***	0.7ns	4.1c	5.1d
P10	5.2B	3.3A	4.6	3.3A	4.7	2.6A	4.0	3.1A	4.0	2.8A	3.9	7.0***	2.1ns	3.4ab	4.4abc
F value	0.4ns	0.9ns	0.6ns	1.5ns	0.8ns	1.0ns	1.2ns	1.2ns	1.0ns	1.4ns	1.3ns			2.0*	
Mean	5.4E	3.6D	4.8C	3.6CD	4.7C	3.3BC	4.4B	3.1AB	4.2AB	2.8A	3.9A	72.4***	21.2***		

*** Correlation is significant at the 0.001 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). Duncan's test represents the members of the group with the letters a, b, c, etc. They are statistically different from the corresponding values in the other groups; a numerical value grows starting with the letter

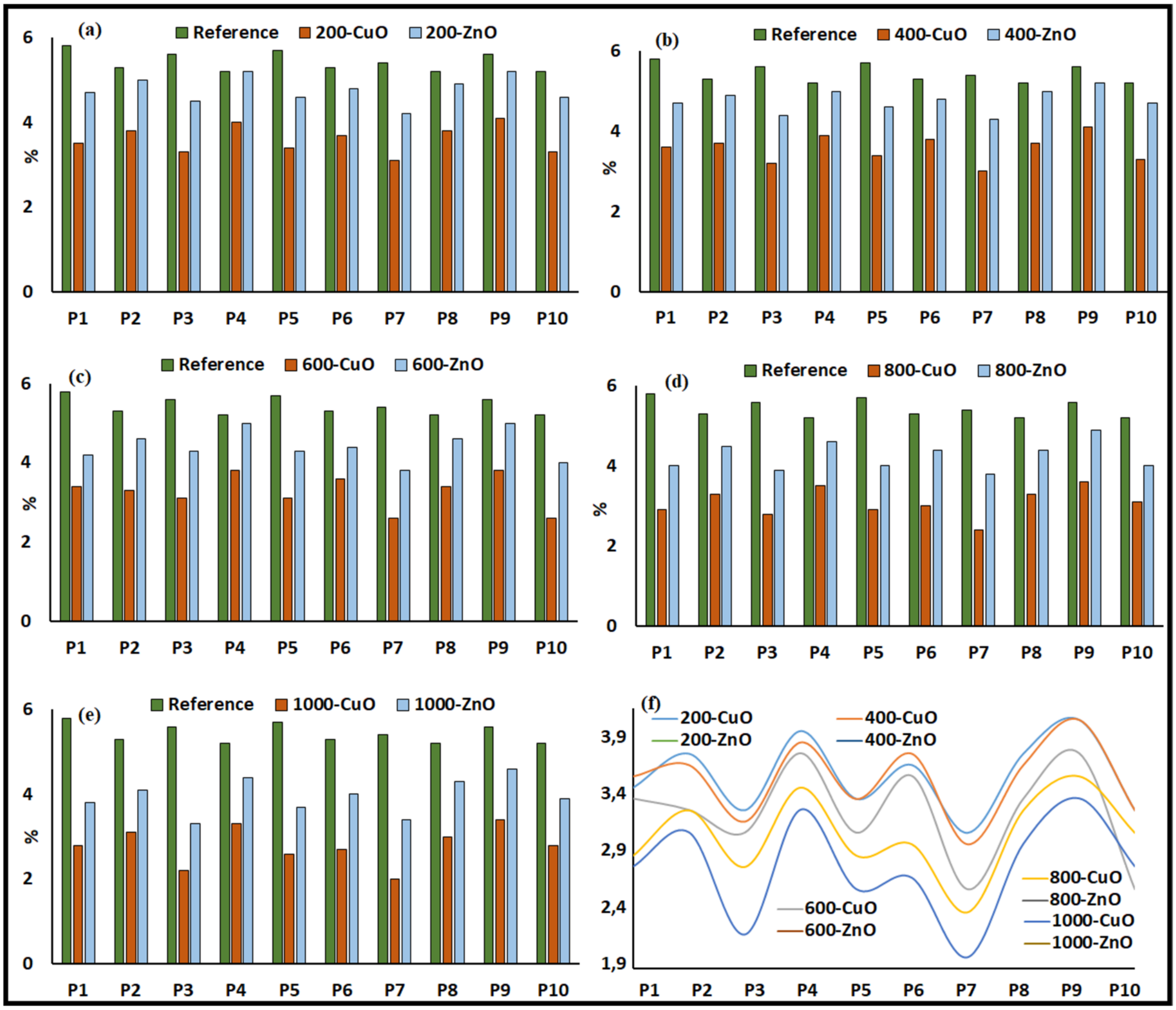

Fig. 7 Variation loading (mg/L) of (**a**) 200-CuO and 200-ZnO, (**b**) 400-CuO and 400-ZnO, (**c**) 600-CuO and 600-ZnO, (**d**) 800-CuO and 800-ZnO, (**e**) 1000-CuO and 1000-ZnO, (**f**) all concentration in all populations for RD

and their effects on fauna and microfauna should also be investigated in detail. Although considered a significant problem, there is insufficient information on the increase in concentrations of NPs in nature and at what concentrations they pose a hazard. The toxicity limits of NPs and the variation of this limit according to plant species and the current situation should be examined as a priority. Furthermore, the outcomes can guide future research, and the effects of NPs on plant species and their ecosystems should be explored in a multidimensional manner. The findings of the present studies show that some populations are less severely affected by contamination. These populations can be used as a primary seed source for seedling production. However, before this stage, studies on the subject should be conducted at a more advanced stage, such as the seedling stage.

Acknowledgements Not applicable.

Authors' Contributions Kaan Isinkaralar: Processing analysis, interpretation, writing original draft. Oznur Isinkaralar: Processing analysis, interpretation, writing original draft, data curation. Halil Barış Özel: Raw material collection, review and editing. Hakan Şevik: Review and editing.

Funding There is no financial support and commercial support.

Data Availability The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare that they have no conflict of interest.

References

Abrica-González, P., Gómez-Arroyo, S., Jazcilevich-Diamant, A., Sotelo-López, A., Flores-Márquez, A. R., & Cortés-Eslava, J. (2023). Evaluation of Toxicological Effects of ZnO and CuO Nanoparticles with Taraxacum officinale as Bioindicator. *Water, Air, & Soil Pollution, 234*(7), 443. https://doi.org/10.1007/s11270-023-06432-3

Ahmed, B., Khan, M. S., & Musarrat, J. (2018). Toxicity assessment of metal oxide nano-pollutants on tomato (Solanum lycopersicon): A study on growth dynamics and plant cell death. *Environmental Pollution, 240*, 802–816. https://doi.org/10.1016/j.envpol.2018.05.015

ATSDR (Agency for toxic substances and disease registry) (2004) Toxicological profile for copper Available at: https://www.atsdr.cdc.gov/toxprofiles/tp132.pdf

Baskar, V., Safia, N., Sree Preethy, K., Dhivya, S., Thiruvengadam, M., & Sathishkumar, R. (2021). A comparative study of phytotoxic effects of metal oxide (CuO, ZnO and NiO) nanoparticles on in-vitro grown Abelmoschus esculentus. *Plant Biosystems-an International Journal Dealing with All Aspects of Plant Biology, 155*(2), 374–383. https://doi.org/10.1080/11263504.2020.1753843

Bhatt, P., Pandey, S. C., Joshi, S., Chaudhary, P., Pathak, V. M., Huang, Y., ... & Chen, S. (2022). Nanobioremediation: A sustainable approach for the removal of toxic pollutants from the environment. Journal of Hazardous Materials, 427, 128033. https://doi.org/10.1016/j.jhazmat.2021.128033

Bordbar, M., Negahdar, N., & Nasrollahzadeh, M. (2018). Melissa Officinalis L. leaf extract assisted green synthesis of CuO/ZnO nanocomposite for the reduction of 4-nitrophenol and Rhodamine B. *Separation and Purification Technology, 191*, 295–300. https://doi.org/10.1016/j.seppur.2017.09.044

Burachevskaya, M., Minkina, T., Mandzhieva, S., Bauer, T., Nevidomskaya, D., Shuvaeva, V., ... & Rajput, V. (2021). Transformation of copper oxide and copper oxide nanoparticles in the soil and their accumulation by Hordeum sativum. Environmental Geochemistry and Health, 43, 1655–1672. https://doi.org/10.1007/s10653-021-00857-7

Cai, J., Li, X., Liu, L., Chen, Y., Wang, X., & Lu, S. (2021). Coupling and coordinated development of new urbanization and agro-ecological environment in China. *Science of the Total Environment, 776*, 145837. https://doi.org/10.1016/j.scitotenv.2021.145837

Chen, F., Aqeel, M., Maqsood, M. F., Khalid, N., Irshad, M. K., Ibrahim, M., ... & Lam, S. S. (2022). Mitigation of lead toxicity in Vigna radiata genotypes by silver nanoparticles. Environmental Pollution, 308, 119606. https://doi.org/10.1016/j.envpol.2022.119606

Da Costa, M. V. J., Kevat, N., & Sharma, P. K. (2020). Copper oxide nanoparticle and copper (II) ion exposure in Oryza sativa reveals two different mechanisms of toxicity. *Water, Air, & Soil Pollution, 231*, 1–16. https://doi.org/10.1007/s11270-020-04592-0

Dong, H., Xue, M., Xiao, Y., & Liu, Y. (2021). Do carbon emissions impact the health of residents? Considering China's industrialization and urbanization. *Science of the Total Environment, 758*, 143688. https://doi.org/10.1016/j.scitotenv.2020.143688

Fedorenko, A. G., Minkina, T. M., Chernikova, N. P., Fedorenko, G. M., Mandzhieva, S. S., Rajput, V. D., ... & Soldatov, A. V. (2021). The toxic effect of CuO of different dispersion degrees on the structure and ultrastructure of spring barley cells (Hordeum sativum distichum). Environmental Geochemistry and Health, 43, 1673–1687. https://doi.org/10.1007/s10653-020-00530-5

Fisher, S., Bellinger, D. C., Cropper, M. L., Kumar, P., Binagwaho, A., Koudenoukpo, J. B., ... & Landrigan, P. J. (2021). Air pollution and development in Africa: impacts on health, the economy, and human capital. The Lancet Planetary Health, 5(10), e681-e688. https://doi.org/10.1016/S2542-5196(21)00201-1

Francis, D. V., Sood, N., & Gokhale, T. (2022). Biogenic CuO and ZnO nanoparticles as nanofertilizers for sustainable growth of Amaranthus hybridus. *Plants, 11*(20), 2776. https://doi.org/10.3390/plants11202776

Fuller, R., Landrigan, P. J., Balakrishnan, K., Bathan, G., Bose-O'Reilly, S., Brauer, M., et al. (2022). Pollution and health: A progress update. *The Lancet Planetary Health, 6*(6), e535–e547. https://doi.org/10.1016/S2542-5196(22)00090-0

García-Gómez, C., & Fernández, M. D. (2019). Impacts of metal oxide nanoparticles on seed germination, plant growth and development. In Comprehensive analytical chemistry (Vol. 84, pp. 75–124). Elsevier. https://doi.org/10.1016/bs.coac.2019.04.007

Gbair, G. A., & Alshamsi, H. A. (2022). Facile green synthesis of CuO-ZnO nanocomposites from Argyreia nervosa leaves extract for photocatalytic degradation of Rhodamine B dye. Biomass Conversion and Biorefinery, 1–16. https://doi.org/10.1007/s13399-022-03408-x

Goossens, J., Jonckheere, A. C., Dupont, L. J., & Bullens, D. M. (2021). Air pollution and the airways: Lessons from a century of human urbanization. *Atmosphere, 12*(7), 898. https://doi.org/10.3390/atmos12070898

Hidouri, S., Karmous, I., Kadri, O., Kharbech, O., & Chaoui, A. (2022). Clue of zinc oxide and copper oxide nanoparticles in the remediation of cadmium toxicity in Phaseolus vulgaris L. via the modulation of antioxidant and redox systems. *Environmental Science and Pollution Research, 29*(56), 85271–85285. https://doi.org/10.1007/s11356-022-21799-2

Intisar, A., Ramzan, A., Sawaira, T., Kareem, A. T., Hussain, N., Din, M. I., ... & Iqbal, H. M. (2022). Occurrence, toxic effects, and mitigation of pesticides as emerging environmental pollutants using robust nanomaterials–A review. Chemosphere, 293, 133538. https://doi.org/10.1016/j.chemosphere.2022.133538

Isinkaralar, K. (2022). Some atmospheric trace metals deposition in selected trees as a possible biomonitor. *Romanian Biotechnological Letters, 27*(1), 3227–3236.

Isinkaralar, O. (2024). QGIS-based modeling and analysis of urban dynamics affecting land surface temperature towards climate hazards in coastal zones of Portugal. *Natural Hazards, 120*(8), 7749–7764. https://doi.org/10.1007/s11069-024-06519-y

Koç, İ, Canturk, U., Isinkaralar, K., Ozel, H. B., & Sevik, H. (2024). Assessment of metals (Ni, Ba) deposition in plant types and their organs at Mersin City Türkiye. *Environmental Monitoring and Assessment, 196*(3), 282. https://doi.org/10.1007/s10661-024-12448-x

Kohatsu, M. Y., Pelegrino, M. T., Monteiro, L. R., Freire, B. M., Pereira, R. M., Fincheira, P., ... & Lange, C. N. (2021). Comparison of foliar spray and soil irrigation of biogenic CuO nanoparticles (NPs) on elemental uptake and accumulation in lettuce. Environmental Science and Pollution Research, 28, 16350–16367. https://doi.org/10.1007/s11356-020-12169-x

Kolesnikov, S., Timoshenko, A., Varduni, V., Minnikova, T., Tsepina, N., Mandzhieva, S., ... & Rajput, V. D. (2024). Comparative estimation of ecotoxicity of nano-and microparticles of CuO, ZnO, NiO, Co3O4 by the integral biological indicator of soil state. Nanotechnology for Environmental Engineering, 1–11. https://doi.org/10.1007/s41204-024-00358-9

Latif, A., Sheng, D., Sun, K., Si, Y., Azeem, M., Abbas, A., & Bilal, M. (2020). Remediation of heavy metals polluted environment using Fe-based nanoparticles: Mechanisms, influencing factors, and environmental implications. *Environmental Pollution, 264*, 114728. https://doi.org/10.1016/j.envpol.2020.114728

Li, X., Zhou, Y., Hejazi, M., Wise, M., Vernon, C., Iyer, G., & Chen, W. (2021). Global urban growth between 1870 and 2100 from integrated high resolution mapped data and urban dynamic modeling. *Communications Earth & Environment, 2*(1), 201. https://doi.org/10.1038/s43247-021-00273-w

Liu, R., Zhang, H., & Lal, R. (2016). Effects of stabilized nanoparticles of copper, zinc, manganese, and iron oxides in low concentrations on lettuce (Lactuca sativa) seed germination: Nanotoxicants or nanonutrients? *Water, Air, & Soil Pollution, 227*, 1–14. https://doi.org/10.1007/s11270-015-2738-2

Mahawar, L., Živčák, M., Barboricova, M., Kovár, M., Filaček, A., Ferencova, J., ... & Brestič, M. (2024). Effect of copper oxide and zinc oxide nanoparticles on photosynthesis and physiology of Raphanus sativus L. under salinity stress. Plant Physiology and Biochemistry, 206, 108281. https://doi.org/10.1016/j.plaphy.2023.108281

Medici, S., Peana, M., Pelucelli, A., & Zoroddu, M. A. (2021). An updated overview on metal nanoparticles toxicity. In Seminars in cancer biology (Vol. 76, pp. 17–26). Academic Press. https://doi.org/10.1016/j.semcancer.2021.06.020

Mohebian, H., Jalali, M., & Merrikhpour, H. (2022). The effect of zeolite, ZnO, and CuO nanoparticles on heavy metals retention in a sandy loam soil: A column leaching and batch study. *Environmental Earth Sciences, 81*(16), 407. https://doi.org/10.1007/s12665-022-10507-5

Mousa, S. A., El-Sayed, E. S. R., Mohamed, S. S., Abo El-Seoud, M. A., Elmehlawy, A. A., & Abdou, D. A. (2021). Novel mycosynthesis of Co 3 O 4, CuO, Fe 3 O 4, NiO, and ZnO nanoparticles by the endophytic Aspergillus terreus and evaluation of their antioxidant and antimicrobial activities. *Applied Microbiology and Biotechnology, 105*, 741–753. https://doi.org/10.1007/s00253-020-11046-4

Nair, G. M., Sajini, T., & Mathew, B. (2022). Advanced green approaches for metal and metal oxide nanoparticles synthesis and their environmental applications. *Talanta Open, 5*, 100080. https://doi.org/10.1016/j.talo.2021.100080

Nguyen, T. T. T., Nguyen, Y. N. N., Tran, X. T., Nguyen, T. T. T., & Van Tran, T. (2023). Green synthesis of CuO, ZnO and CuO/ZnO nanoparticles using Annona glabra leaf extract for antioxidant, antibacterial and photocatalytic activities. *Journal of Environmental Chemical Engineering, 11*(5), 111003. https://doi.org/10.1016/j.jece.2023.111003

Nie, G., Zhao, J., He, R., & Tang, Y. (2020). CuO nanoparticle exposure impairs the root tip cell walls of Arabidopsis thaliana seedlings. *Water, Air, & Soil Pollution, 231*, 1–11. https://doi.org/10.1007/s11270-020-04676-x

Pacheco, P., Mera, E., & Fuentes, V. (2023). Intensive Urbanization, Urban Meteorology and Air Pollutants: Effects on the Temperature of a City in a Basin Geography. *International Journal of Environmental Research and Public Health, 20*(5), 3941. https://doi.org/10.3390/ijerph20053941

Pelegrino, M. T., Kohatsu, M. Y., Seabra, A. B., Monteiro, L. R., Gomes, D. G., Oliveira, H. C., ... & Lange, C. N. (2020). Effects of copper oxide nanoparticles on growth of lettuce (Lactuca sativa L.) seedlings and possible implications of nitric oxide in their antioxidative defense. Environmental Monitoring and Assessment, 192, 1–14. https://doi.org/10.1007/s10661-020-8188-3

Rajput, V., Minkina, T., Sushkova, S., Behal, A., Maksimov, A., Blicharska, E., ... & Barsova, N. (2020). ZnO and CuO nanoparticles: a threat to soil organisms, plants, and human health. Environmental Geochemistry and Health, 42, 147–158. https://doi.org/10.1007/s10653-019-00317-3

Sajjad, H., Sajjad, A., Haya, R. T., Khan, M. M., & Zia, M. (2023). Copper oxide nanoparticles: In vitro and in vivo toxicity, mechanisms of action and factors influencing their toxicology. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *271*, 109682. https://doi.org/10.1016/j.cbpc.2023.109682

Sarkar, N., Sharma, R. S., & Kaushik, M. (2021). Innovative application of facile single pot green synthesized CuO and CuO@ APTES nanoparticles in nanopriming of Vigna radiata seeds. *Environmental Science and Pollution Research, 28*(11), 13221–13228. https://doi.org/10.1007/s11356-020-11493-6

Sepasgozar, S. M. E., Mohseni, S., Feizyzadeh, B., & Morsali, A. (2021). Green synthesis of zinc oxide and copper oxide nanoparticles using Achillea Nobilis extract and evaluating their antioxidant and antibacterial properties. *Bulletin of Materials Science, 44*, 1–13. https://doi.org/10.1007/s12034-021-02419-0

Sheteiwy, M. S., Shaghaleh, H., Hamoud, Y. A., Holford, P., Shao, H., Qi, W., ... & Wu, T. (2021). Zinc oxide nanoparticles: potential effects on soil properties, crop production, food processing, and food quality. Environmental Science and Pollution Research, 28(28), 36942–36966. https://doi.org/10.1007/s11356-021-14542-w

Simonin, M., Cantarel, A. A., Crouzet, A., Gervaix, J., Martins, J. M., & Richaume, A. (2018). Negative effects of copper oxide nanoparticles on carbon and nitrogen cycle microbial activities in contrasting agricultural soils and in presence of plants. *Frontiers in Microbiology*, *9*, 3102. https://doi.org/10.3389/fmicb.2018.03102

Singh, D., & Kumar, A. (2020). Quantification of metal uptake in Spinacia oleracea irrigated with water containing a mixture of CuO and ZnO nanoparticles. *Chemosphere, 243*, 125239. https://doi.org/10.1016/j.chemosphere.2019.125239

Sun, L., Chen, J., Li, Q., & Huang, D. (2020). Dramatic uneven urbanization of large cities throughout the world in recent decades. *Nature Communications, 11*(1), 5366. https://doi.org/10.1038/s41467-020-19158-1

Thounaojam, T. C., Meetei, T. T., Devi, Y. B., Panda, S. K., & Upadhyaya, H. (2021). Zinc oxide nanoparticles (ZnO-NPs): A promising nanoparticle in renovating plant science. *Acta Physiologiae Plantarum, 43*, 1–21. https://doi.org/10.1007/s11738-021-03307-0

USEPA (United States environmental protection agency) (2016) Lead and copper rule revisions white paper Available at: https://www.epa.gov/sites/production/files/2016-10/documents/508_lcr_revisions_white_paper_final_10.26.16.pdf

Velusamy, S., Kandasamy, K., Kuppusamy, M. R., Eswaramoorthy, D., Shanmugam, M., & Murugesan, M. (2024). Green-synthesized CuO and ZnO nanoparticles derived from Calotropis gigantea (Apple of Sodom): Enhancing plant growth, efficient dye removal, and potent antibacterial applications. *Environmental Science and Pollution Research, 31*, 44995–45010. https://doi.org/10.1007/s11356-024-34053-8

Yadav, S., Rani, N., & Saini, K. (2023). Coupling ZnO with CuO for efficient organic pollutant removal. *Environmental Science and Pollution Research, 30*(28), 71984–72008. https://doi.org/10.1007/s11356-022-24139-6

Zheng, F., Guo, X., Tang, M., Zhu, D., Wang, H., Yang, X., & Chen, B. (2023). Variation in pollution status, sources, and risks of soil heavy metals in regions with different levels of urbanization. *Science of the Total Environment, 866*, 161355. https://doi.org/10.1016/j.scitotenv.2022.161355

Zong, X., Wu, D., Zhang, J., Tong, X., Yin, Y., Sun, Y., & Guo, H. (2022). Size-dependent biological effect of copper oxide nanoparticles exposure on cucumber (Cucumis sativus). *Environmental Science and Pollution Research, 29*(46), 69517–69526. https://doi.org/10.1007/s11356-022-20662-8

Zoufan, P., Baroonian, M., & Zargar, B. (2020). ZnO nanoparticles-induced oxidative stress in Chenopodium murale L, Zn uptake, and accumulation under hydroponic culture. *Environmental Science and Pollution Research, 27*, 11066–11078. https://doi.org/10.1007/s11356-020-07735-2