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Mercuric Oxide Nanoparticles Deferred Germination and Devastated Root Anatomy of Maize

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ABSTRACT

Given the widespread use of mercuric oxide NPs (HgO-NPs), they have become increasingly prevalent in the soil ecosystem. Consequently, it is important to promptly evaluate their phytotoxic impacts. To this end, we have investigated the effects of HgO-NPs (0-200 mg/L) on germination and early growth of maize. Moreover, we have evaluated the interactive influences of HgO-NPs toxicity on the elongation and anatomical structures of primary roots. Relative to control, HgO-NPs decreased the germination percentage, speed and rate, but increased the mean germination time, mean daily germination time and time to 50% germination. The length and biomass of root and shoot and seedling vigour indices have significantly deteriorated. The inhibitory impacts of HgO-NPs on growth parameters were more pronounced in root than in shoot. The response of root was concomitant with dose and time-dependent inhibitions in root elongation and significant drops in root diameter, stele size, cortex size, and cortical cells count. The consequences of HgO-NPs were dose-dependent. For instance, the decrease of maize germination, growth, root elongation, and anatomy were more evident at 200 mg/L HgO-NPs compared to other doses and control. Overall, this study suggests that the presence of HgO-NPs leads to phytotoxic effects on germination and growth of young seedlings, highlighting a noteworthy challenge and environmental concern.

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INTRODUCTION

Mercury (Hg) is one of the most toxic heavy metals threating plants during all developmental stages (Deng *et al*., 2016; Lima *et al*., 2019). Unfortunately, when brought to nanoscale, heavy metals exhibit stronger phytotoxicity relative to their bulk forms (AbdElgawad *et al*., 2020). Owing to their exceptional physicochemical characteristics compared to bulk-counterparts, heavy metal nanoparticles (HM-NPs) such as nano-sized mercuric oxide (HgO-NPs) have been extensively manufactured ($26x10^4$ tons/year) and applied in different fields (Gao *et al*., 2023). The huge production of HM-NPs is certainly releasing large amounts to our ecosystems either during the process of manufacturing, transportation or through the utilization of HMNPs-based products (Nowack and Bucheli, 2007). Being an essential component of our ecosystem, soil receives the largest percentage (8–28%) of the produced HM-NPs (Liu *et al*., 2020; Peng *et al*., 2020). Consequently, they impose deleterious impacts on soil biota. Plants are a vital component

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of the soil ecosystem, and they are essential sources for human nourishment. Therefore, evaluation of plant growth and development under HMNPs is highly needed (Antisari *et al*., 2015).

Unfortunately, HM-NPs impose negative effects on seed germination, root elongation and seedling growth in many plants. For example, exposure to lead sulfide (PbS) NPs (Ullah *et al*., 2020), copper oxide (CuO) NPs (Ibrahim *et al*., 2022), yttrium (III) oxide (Y_2O_3) NPs (Zhao *et al*., 2021) and zinc oxide (ZnO) NPs (Yang *et al*., 2023) delayed seed germination and inhibited root elongation and seedling growth in maize, wheat, rice and *Arabidopsis thaliana*, respectively.

Although seed germination and root elongation are the commonly used standard phytotoxic indicators (Ahmad *et al*., 2022), their analyses are not sufficient for a comprehensive understanding of the HM-NPs toxicity (Yanık &Vardar, 2015).Therefore, it is recommended to investigate the changes in anatomical structures, as they distinctly reflect the development of the root system (Shao *et al*., 2008; Zhao *et al*., 2023). In this context, the damages of root structures upon exposure to HM-NPs e.g., Al_2O_3-NP (Yanık &Vardar, 2015) and GO-NPs (Zhao *et al*., 2023) have been reported. The distortion of root structures would reduce the efficiency of absorbing water and nutrients and hence, retards growth and performance of treated seedlings (Pandey *et al*., 2022).

Previous studies have not addressed the impacts of HgO-NPs on seed germination and growth of maize seedlings. Furthermore, the correlation between root growth and root anatomical criteria under HgO-NPs application has not been previously documented. Therefore, we aim to assess the interactive effects of different HgO-NPs doses (0.0 - 200 mg/L) on the maize germination and growth of germinating seedlings. Additionally, root anatomical structures as well as time and dose-dependent impacts of HgO-NPs on root elongation were investigated.

MATERIAL & METHODS

Different doses HgO-NPs (25, 50, 100 and 200 mg/L) were prepared by suspending HgO-NPs powder in water, then placed in ultrasonic bath for 30 minutes at 40°C. Details of synthesis method and characteristic features of HgO-NPs have been described previously (AbdElgawad *et al*., 2020; Saleh *et al*., 2021). Seeds of *Zea mays* L. (Single hybrid 30 K 08 white, lot no 11) were surface sterilized and germinated following the method reported in Ullah *et al*. (2020).

Germination of maize seeds under HgO-NPs treatments was detected by calculating the following parameters:

Final germination percentage (FGP; %) = $\frac{\sum n_i}{N} \times 100$, where *"**n**"* is the number of seeds germinated every day, "***N***" is the total number of sown seeds (ISTA, 1999).

Mean daily germination percentage (MDGP; %) = $\frac{FGP}{D}$, where *"**FGP**"* is the final germination percentage, "***D***" is total number of days (Scott *et al*., 1984).

Germination rate index (GRI) = $\sum \frac{Gi}{di}$, where *"**G**$_i$"* is the percentage of germination on day the "***d***$_i$" (Esechie, 1994).

Coefficient velocity of germination (CVG; %) = $\frac{N}{\sum n_i di} \times 100 \frac{N}{\sum n_i di} \times d$, where *"**N**"* is the total number of germinated seeds, "***n***$_i$" is the number of seeds germinated on the day "***d***$_i$" (Jones & Sanders, 1987).

Germination speed (GS; Seed/day) = $\Sigma \frac{n_i}{d_i}$, where *"**n**$_i$"* is the number of seeds germinated on the day "***d***$_i$" (AOSA, 1983).

Mean germination time (MDGT; Day) = $\frac{\sum n_i di}{N}$, where *"**N**"* is the total number of germinated seeds, "***n***$_i$" is the number of seeds germinated on the day d_i (Matthews & Khajeh-Hosseini, 2007).

Time to 50% germination (T_{50}; Day) = $d_i + \frac{\left(\left(\frac{N}{2}\right) - n_i\right)(d_i - d_j)}{(n_i - n_j)}$, where "***N***" is the total number of germinated seeds, "***n***$_j$" and "***n***$_i$" are represent the cumulative number of seeds germinated at

times "d_j" and "d_i", respectively, when $n_i < N/2 < n_j$ (Mirosavljević *et al*., 2013).

Promptness index (PI) = n_1d_1 (1.00) + n_2d_2 (0.75) + n_3d_3 (0.5) + n_4d_4 (0.25), where "n_1d_1", "n_2d_2", "n_3d_3" and "n_4d_4" are the number of seeds germinated on the **1st**, **2nd**, **3rd** and **4th** day, respectively (Sagar *et al*., 2018).

Germination stress tolerance index (GSTI) = $\frac{PI_S}{PI_C} \times 100$, where "$PI_S$" and "$PI_C$" are the promptness index of stressed and control seeds, respectively (Idrees *et al*., 2015).

Relative seed germination inhibition (RSGI) = $\frac{FGP_C - FGP_S}{FGP_C} \times 100$, where "$FGP_C$" and "$FGP_S$" are the final germination percentage of control and stressed seeds, respectively (EPA, 1996).

Root elongation was determined by following up the root length increase every day (3 roots/dish), from day two (after radical emergence) up to 7 days. Data were collected and the daily increase in root length was calculated as below:

Root length increase (Cm) = Length on day$_x$ - Length on day$_i$, where, "**x**" is the number of days (2-7), "**i**" denotes the initial day (day 2).

After seven days, 10 random seedlings were collected from both control and HgO-NPs treatments and separated to roots and shoots. The length, biomass (fresh and dry weights) and water content of root and shoot were quantified. Based on the magnitudes of length and weight, seedling vigour indices and seedling (root and shoot) toxicity and tolerance were calculated according to the formulas reported in Vashisth and Nagarajan (2010):

Seedling length vigour index (SLVI) = $\left[\left(Shoot\ length + Root\ length\right) \times FGP\right]$

Seedling weight vigour index (SWVI) = $\left[\left(Shoot\ dry\ weight + Root\ dry\ weight\right) \times FGP\right]$

The shoot and root toxicity were measured according to Idrees *et al*. (2015):

Shoot or root toxicity (%) = $\frac{L_{Stress} - L_{Control}}{L_{Control}} \times 100$, where "**L**" is the length of shoot or root.

Stress tolerance indices of shoot and root were calculated by applying the equations of Shah *et al*. (2020):

Shoot (ShLTI) or **root** (RLTI) **length tolerance index** = $\frac{L_{Stress}}{L_{Control}} \times 100$, where "$L$" is the length of shoot or root.

Shoot (ShWTI) **or root** (RWTI) **weight tolerance index** (ShWTI) = $\frac{DW_{Stress}}{DW_{Control}} \times 100$, where "$DW$" is the dry weight of shoot or root.

The anatomical structures of primary roots were assessed, at the end of the day seven, for control and HgO-NPs-treated seedlings. Preservation of samples, sectioning and staining were processed according to the method of Karahara *et al*. (2004). ImageJ software calibrated with a stage micrometer was used to determine the anatomical features.

All experiments were carried out as a full factorial experiment with a completely randomized design. Two-way analysis of Variance (two-way ANOVA) was performed to study the interactions between HgO-NPs treatment and organs (shoot and root) on the growth of maize seedlings as well as between doses of HgO-NPs and exposure time on root elongation. Moreover, all results were subjected to one-way ANOVA and expressed as mean ± SE (standard error). Duncan's Multiple Range Test (Duncan, 1955) was used to compare means at level of significance $P \leq 0.05$. The statistical analyses were performed using SPSS program (version 22) and the graphs were designed by GraphPad Prism software (Version 9).

RESULTS AND DISCUSSION

HgO-NPs application delayed maize seeds germination

Seed germination is the most critical stage for successful development and performance of plants (Asadi-Kavan *et al*., 2020). However, environmental pollutants such as HM-NPs cause

deleterious threats to germination events in many seeds (Jahani *et al*., 2019).

Following up the changes in seed germination characters is a fast and effective method for understanding the early response of plants to HM-NPs (Bezini *et al*., 2019; Faraji & Sepehri, 2019). Therefore, we have evaluated the influences of HgO-NPs (0.0, 25, 50, 100 and 200 mg/L) on seeds germination parameters including FGP, MDGP, GS, GRI, CVG, MGT and T_{50} (**Table 1**). PI, GSTI and RSGI were also measured as indicators for HgO-NPs toxicity and tolerance (**Fig. 1**). It is clear from the results that seeds exhibited the highest FGP (100%), MDGP (25%), GS (2.5), CVG (41.33) and GRI (43%) and the shortest MGT (2.42 days) and T_{50} (1.87 days) under control conditions. Similarly, higher values of PI (8.9) and GSTI (100) were noticed for control relative to HgO-NPs treated seeds (**Fig. 1 a** & **b**). On the other side, application of HgO-NPs decreased markedly FGP, GRI, CVG, MDGP, GS, PI and GSTI, and increased RSGI, MGT and T_{50}. Relative to control, PI, GSTI, GRI and CVG were reduced significantly in 25 (10.1%, 10.1%, 10% and 8.1%), 50 (20.1%, 20.1%, 20% and 12%), 100 (26.3%, 26.3%, 26% and 15%) and 200 mg/L (35.7%, 35.7%, 36% and 15.5%), respectively (**Table 1** & **Fig. 1**). Conversely, increasing HgO-NPs concentration increased RSGI, MGT and T_{50}, and the highest pronounced elevations by 26%, 19% and 40%, respectively were recorded for seeds treated with 200 mg/L HgO-NPs relative to other doses and control seeds (**Table 1** & **Fig. 1 c**).

To our knowledge, there have been no prior reports on seed germination delay due to application of HgO-NPs, while the effects induced by other HM-NPs have been studied. For example, declines of germination values were recorded in wheat treated with CuO-NPs (Ibrahim *et al*., 2022), rice under Y_2O_3-NPs (Zhao *et al*., 2021) and maize exposed to PbS-NPs (Ullah *et al*., 2020), and titanium dioxide NPs (TiO_2-NPS) (Karunakaran *et al*., 2016). All these studies

Table 1. The changes in germination parameters of maize seeds exposed to HgO-NPs.

	HgO-NPs concentration (mg/l)				
Parameters	***0***	***25***	***50***	***100***	***200***
FGP (%)	100±0c	96±2.45c	87.5±1.94b	84±2.45b	74±2.45a
MGT (day)	2.42±0.02c	2.64±0.07c	2.75±0.04bc	2.85±0.06b	2.88±0.11a
GRI	43±0.33e	38.75±0.56d	34.17±1.09c	31.67±0.7b	27.33±0.41a
T50 (day)	1.87±0.03c	2.14±0.15bc	2.24±0.08b	2.33±0.09ab	2.62±0.19a
CVG	41.33±0.33c	37.99±1.01b	36.36±0.52ab	35.12±0.79a	34.95±1.35a
MDGP (%)	25±0c	24±0.61c	22±0.5b	21±0.61b	18.5±0.61a
GS (seed/day)	2.5±0c	2.4±0.06c	2.2±0.05b	2.1±0.06b	1.85±0.06a

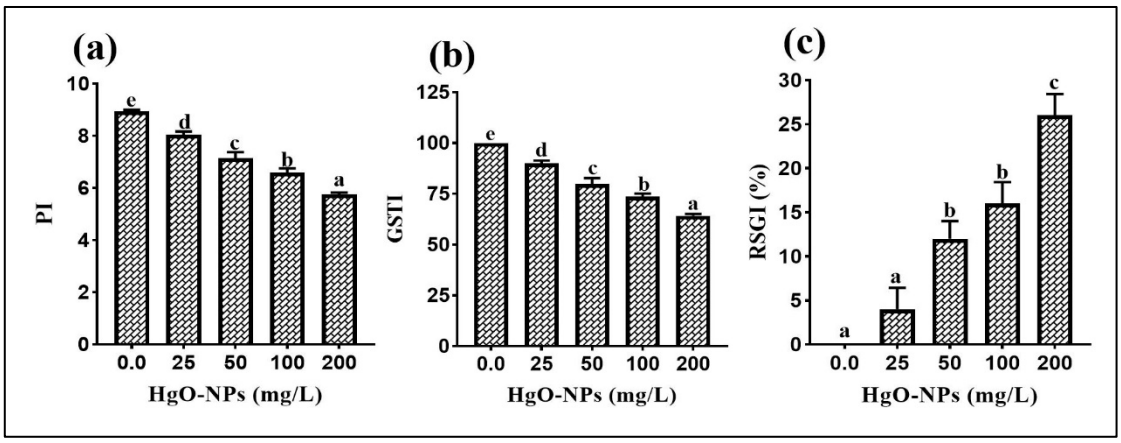

Fig. 1. The effects of HgO-NPs on promptness index (PI), germination stress tolerance index (GSTI) and relative seed germination inhibition (RSGI) of germinating maize seeds.

have consistently shown that the toxicity of the applied HM-NPs increases in tandem with the dose, which aligns with our data (**Table 1** & **Fig. 1**).

HgO-NPs exhibited a dose- and organ-dependent behavior, leading to the reduction in growth and vigour of maize seedling

Data of germination experiment gave a preliminary indication on the toxic impacts of HgO-NPs, but it was not sufficient for a comprehensive understanding of maize interaction with HgO NPs. Hence, we have assessed the differential interactions between doses of HgO-NPs and growth of roots and shoots. The achieved results revealed that the existence of HgO-NPs caused visible symptoms of toxicity on both roots and shoots, and these symptoms were aggravated as HgO-NPs concentration increased (**Fig. 2**).

Such visible reduction in growth was in consonance with the measured values of length, biomass, and water content (**Fig. 3 a - h**). Along with increasing HgO-NPs doses, the reduction in all measured parameters increased. At 200 mg/L, for instance, HgO-NPs induced the highest significant declines in the length (73% & 87%), fresh weight (76.3% & 75.6%), dry weight (63.4% & 64.5%) and water content (77.5% & 77.1%) of shoot and root, respectively compared to other doses and control (**Fig. 3**).

The relationship between seed germination and seedlings growth was evaluated by calculating the indices of seedling vigour. In this regard, SLVI and SWVI were significantly decreased by HgO-NPs. The maximum decline was observed in seedlings treated with 200 mg/L HgO-NPs i.e., 87% and 73.2% for SLVI and SWVI, respectively relative to other doses and control (**Fig. 4 a** & **b**).

The HgO-NPs-imposed inhibition on growth and vigour indices was supported by the toxicity and tolerance indices (**Fig. 5 a** – **f)**. All doses of HgO-NPs have significantly elevated the toxicity and decreased the tolerance indices in both shoot and root. Such impacts were intensified along with increasing the applied doses. The maximum induction of toxicity in shoot (72.3%) and in root (86.8%) was recorded for seedlings exposed to 200 mg/L compared to control (**Fig. 5 a** & **d**). Conversely, 200 mg/L decreased the length (27.7 % & 36.6%) and dry weight (13.23% & 35.76%) tolerance indices in shoot and root, respectively (**Fig. 5 b**, **c**, **e** & **f**).

The observed retardation and toxicity due to application of HgO-NPs may be attributed to the reduced internodal length and plant height (**Figs. 2** & **3**) as well as destruction of root apex (**Fig. 7**). Our findings were confirmed by results obtained in maize exposed to PbS-NPs (Ullah

Fig. 2. The visible modifications in growth of maize seedlings exposed to HgO-NPs.

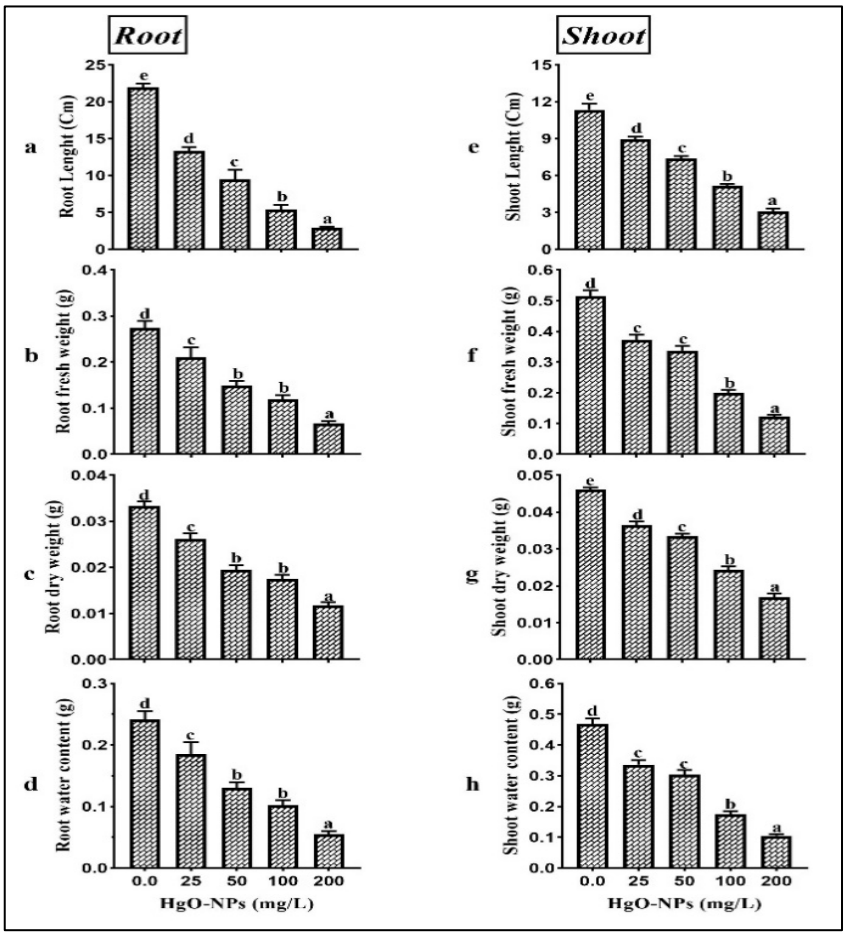

Fig. 3. The differential influences of HgO-NPs on growth features of maize root and shoot.

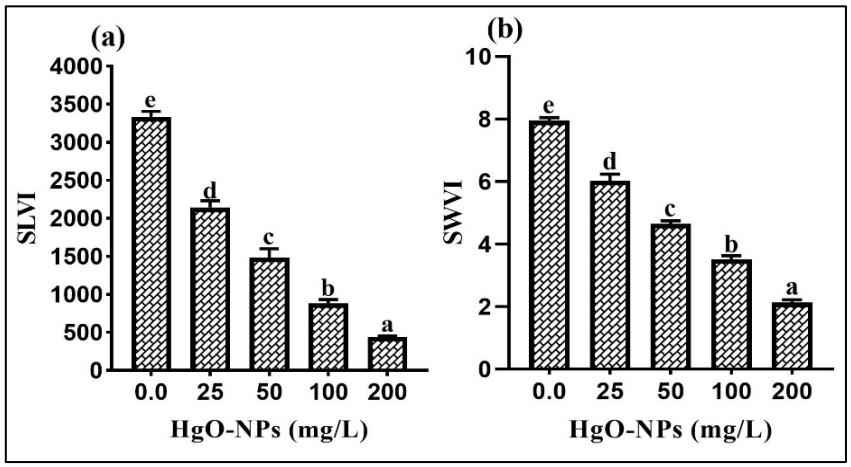

Fig. 4. The changes in seedling length (SLVI) and weight (SWVI) vigour indices of maize under HgO-NPs.

et al., 2020), alfalfa (*Medicago sativa* L.) treated with GO (Zhao *et al*., 2023) and *Arabidopsis thaliana* seedlings subjected to ZnO-NPs and multi-walled carbon nanotubes (MWCNTs) (Yang *et al*., 2023). Overall, it was clear from the two-way ANOVA analysis that, the individual impacts of HgO-NPs treatment and organ or their interactions were highly significant ($P \leq$

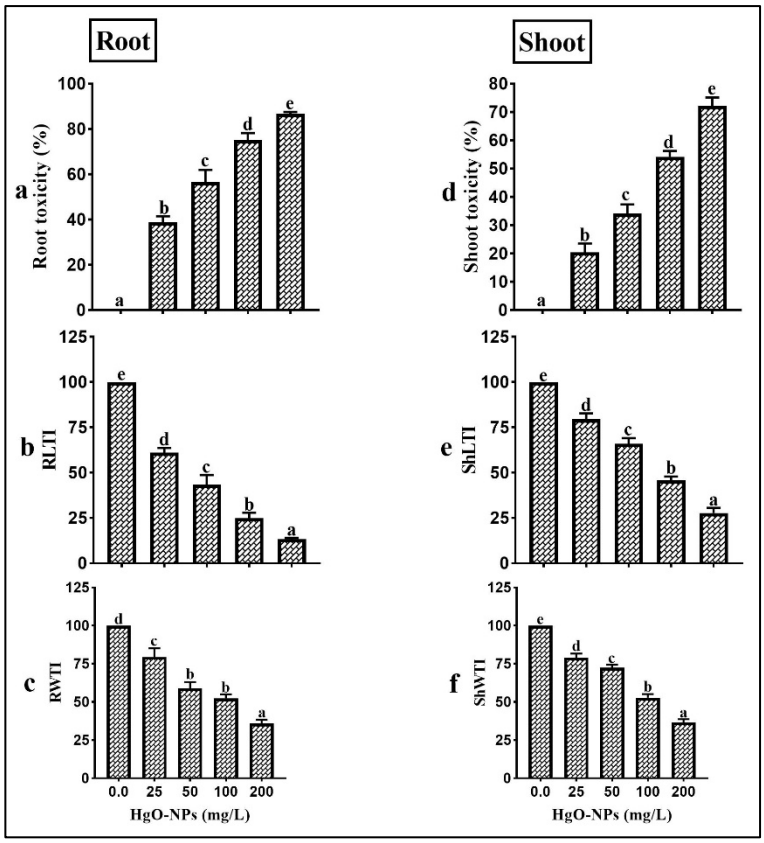

Fig. 5. The effects of HgO-NPs on toxicity and tolerance indices of maize root and shoot.

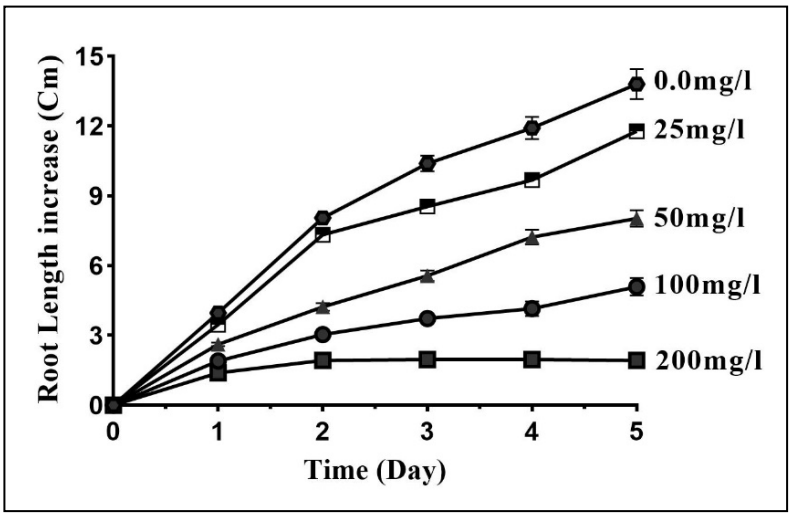

Fig. 6. Time and dose-dependent impacts of HgO-NPs on the elongation of maize root.

0.001) on the measured growth features (**Table 2**). All measured growth features revealed that the inhibitory impacts of HgO-NPs were more pronounced in root than shoot, indicating that root is the most sensitive organ to HgO-NPs exposure.

Time and dose interactive deterioration of HgO-NPs on maize root growth

Being the first organ inevitably exposed to toxic metals in the growing medium, inhibition of

Table 2. The interactive influences of organ and HgO-NPs dose on the early growth of maize.

Source		*df*	*F*
Organ	Length	1	100.899***
	Fresh weight	1	291.036***
	Dry weight	1	272.774***
	Water content	1	279.797***
	Length tolerance index	1	74.577***
	Dry weight tolerance index	1	2.399^{NS}
	Toxicity	1	74.577***
Dose	Length	4	193.549***
	Fresh weight	4	150.257***
	Dry weight	4	212.812***
	Water content	4	139.717***
	Length tolerance index	4	244.437***
	Dry weight tolerance index	4	142.161***
	Toxicity	4	244.437***
Organ * Dose	Length	4	32.956***
	Fresh weight	4	16.252***
	Dry weight	4	8.100***
	Water content	4	16.354***
	Length tolerance index	4	5.241***
	Dry weight tolerance index	4	2.050^{NS}
	Toxicity	4	5.241***

Table 3. The interactive impacts of time and HgO-NPs dose on the elongation of primary roots.

Source	*df*	*F*
Dose	4	684.900***
Time	4	292.796***
Dose * Time	16	28.351***

root elongation is the most obvious symptom in plants grown under HM-NPs (Yanık & Vardar, 2015; Liu *et al*., 2020; Ahmad *et al*., 2022). The daily measured values of root length increased significantly with time and decreased with raising HgO-NPs concentration. The maximum root lengths were recorded on day five i.e., 13.8 cm, 11.8 cm, 8 cm, 5.1cm and 1.94 cm for 0.0, 25, 50, 100 and 200 mg/L HgO-NPs suspensions, respectively (**Fig. 6**). The highest daily percent increase in root length was recorded in the second day by 103%, 111%, 61%, 59% and 39% for 0.0, 25, 50, 100 and 200 mg/L HgO-NPs, respectively. After day two, the increment was slightly reduced for all treatments except for 200 mg/L, there was no an increase in root length. The application of HgO-NPs induced marked reductions in root length under all experiment periods, with the most intensive reduction in 200 mg/L treated seedlings exhibiting an 86% decrease on day five compared to the control group (**Fig. 6**). It was obvious that the interaction between dose, time and their combination was highly significant at $P \leq 0.001$ (**Table 3**). Similar time and dose-dependent reduction in root elongation was observed in maize subjected to PbS-NPs (Ullah *et al*., 2020) and Al_2O_3-NPs (Ahmed *et al*., 2022). The attained inhibition in root elongation was consistent with the recorded declines in root growth features and overall growth of germinating seedlings (**Figs. 2 - 4**). Such decrease in growth of root could be due to the necrosis and finally cell death in apical tissues of root (**Fig. 7**) (Tripathi *et al*., 2017; Ullah *et al*., 2020).

HgO-NPs application destructs the anatomical structures in maize root

Although seed germination and root elongation are the commonly used standard phytotoxic

Fig. 7. Primary root tips of maize treated with HgO-NPs (0-200 mg/L).

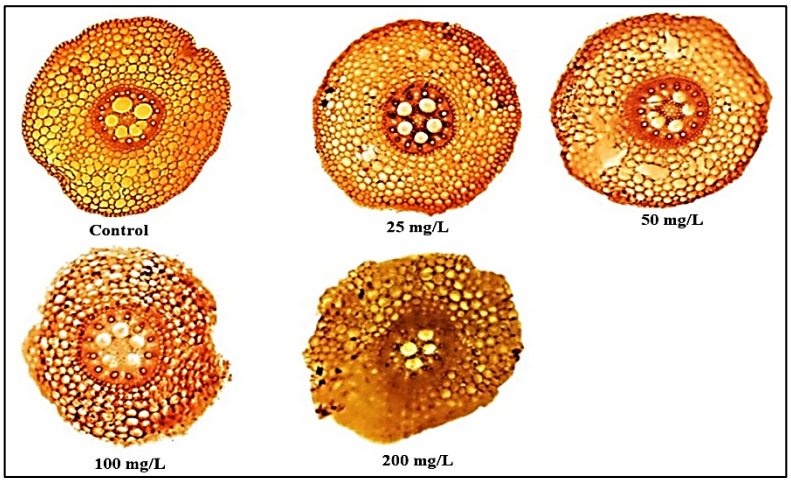

Fig. 8. Transverse sections in root tips of maize subjected to HgO-NPs (0-200 mg/L).

indicators (Ahmad *et al*., 2022), they may not be adequate for evaluating the mechanism of NPs toxicity (Yanık & Vardar, 2015). Accordingly, we have made semi-thin sections in the tip of primary roots to understand the reasons behind the inhibition in root growth and overall growth of early maize seedlings exposed to HgO-NPs. Studying the anatomical structures not only displays the growth and development of roots, but also their adaptation approaches to external cues such as HM-NPs (Shao *et al*., 2008; Zhao *et al*., 2023).

The obtained images of root tips and transverse sections made in roots revealed that HgO-NPs caused deformations and erosions in the apex of main roots (**Fig. 7**) as well as destruction of cortical and epidermal cells (**Fig. 8**).

Deformation and erosion of root tips were in line with a significant reduction in root diameter by 6.13%, 15.8%, 21% and 34% in 25, 50, 100 and 200 mg/L HgO-NPs, respectively relative to control (**Fig. 9 a**). Corresponding to control seedlings, all HgO-NPs treatments decreased other measured anatomical features (**Fig. 9 b - k**). The magnitude of reduction was intensified as HgO-NPs concentration elevated. At 200 mg/L concentration, the most significant reductions were recorded for the thickness of the epidermis (39.3%), endodermis (47.3%), cortex (41.7%), and stele (31.6%), as well as the diameter and number of metaxylem vessels (51.3% and 33.3%) and protoxylem vessels (40.2% and 28.6%) compared to other doses of HgO-NPs and the control group (**Fig. 9 b - k**).

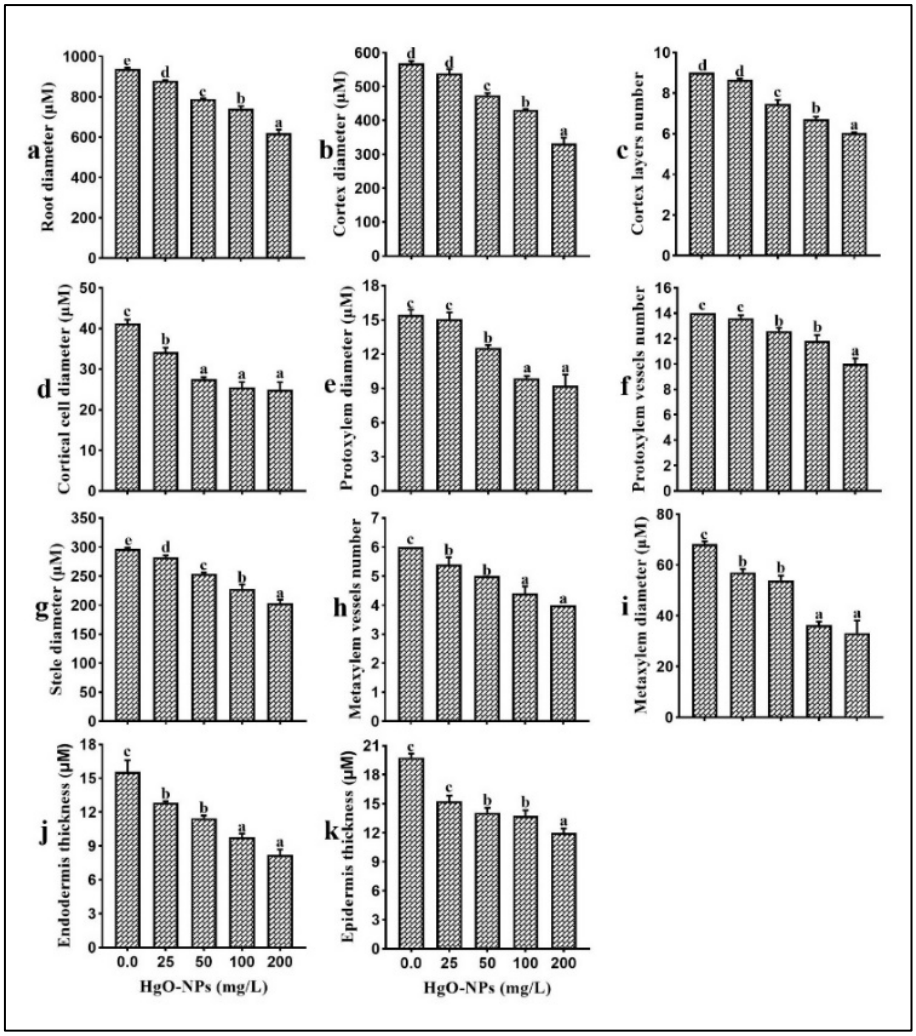

Fig. 9. The consequences of HgO-NPs on the calculated anatomical structures of maize root.

The observed and estimated reduction in root diameter could be explained by the breakdown of cortical cells and decrease in number of layers and cortical cells thickness (**Fig. 9 b - k**). Similarly, Yanık & Vardar (2015) reported that application of Al_2O_3-NPs reduced the growth of wheat due to the reduction of epidermal and the cortex cells diameter. They stated that NPs treatment caused cellular damage in epidermal and cortex cells due to vacuolization and shrinkage which is consistent with the present results (**Figs. 8** & **9**). Moreover, Chen *et al*. (2017) stated that the accumulation of GO in wheat roots induced destructions to the cortex. Furthermore, MWCNTs, carbon 60 (C60) and reduced GO induced decreased the root diameter and damaged the cortical cells (Hao *et al*., 2018).

Absorption and translocation of toxic agents such as HM-NPs through vascular tissues cause disintegration of xylem and phloem and hence, reduce the absorption efficiency of water and nutrients and finally induce growth inhibition (Pandey *et al*., 2022). Concomitantly, our results reveal significant decline in the number and diameter of xylem vessels under all HgO-NPs concentrations (**Fig. 9**). Align with our work, Zhao *et al*. (2023) observed a pronounced reduction in number and diameter of vascular tissues of GO treated alfalfa seedlings. They demonstrated that the magnitude of reduction aggravated as GO concentration increased, that typically fits with our results.

CONCLUSION

This is a novel study investigating the impacts of HgO-NPs suspensions on seed germination indicators and features of growth as well as tolerance indices of maize. Additionally, root elongation and anatomical structures were also reported under the treatment of different HgO-NPs doses. Results revealed that at all doses of HgO-NPs, germination and growth of maize were inhibited significantly. Moreover, root growth is markedly inhibited in a time and dose-dependent manner. Inhibition in growth and root elongation was in accordance with the measured reduction in anatomical parameters. This decline was further exacerbated with increasing concentrations of HgO-NPs. This work deciphered the relationship between growth inhibition and alterations of root anatomical structures due to application of HgO-NPs, highlighting a noteworthy challenge and environmental concern.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

AUTHORS' CONTRIBUTIONS

YMH: Planned and designed the research; performed the experiments; analysed and interpreted the data and wrote and revised a draft version of the manuscript.

HA, AHZ, OH and SEA: Supervised the work; revised and edited the manuscript.

All authors read and approved the final manuscript.

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