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Exploiting the synergistic influence of AgNPs-TiO₂NPs: enhancing phytostabilization of Pb and mitigating its toxicity in *Vigna unguiculata*

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ABSTRACT

In this study, a composite of silver and titanium dioxide nanoparticles (AgNPs-TiO₂NPs) was examined for its synergistic effects on phytostabilization of lead (Pb) and mitigation of toxicity in cowpea (*Vigna unguiculata* (L) Walp). Seeds of *V. unguiculata* were wetted with water, 0.05 and 0.1 mgL⁻¹ Pb and 25 mgmL⁻¹ each of AgNPs, TiO₂NPs, and AgNPs-TiO₂NPs. Root lengths of *V. unguiculata* were reduced by 25% and 44% at 0.05 and 0.1 mgL⁻¹ Pb, respectively, while shoot lengths were reduced by 2% and 7%. In *V. unguiculata*, AgNPs and TiO₂NPs significantly improved physiological indicators and mitigated Pb effects, with TiO₂NPs modulating physiological parameters more effectively than AgNPs. The composite (AgNPs-TiO₂NPs) synergistically regulated *V. unguiculata* physiology better than individual nanoparticles. Compared to individual AgNPs and TiO₂NPs, the composite (AgNPs-TiO₂NPs) synergistically increased antioxidant activity by 12% and 9%, and carotenoid contents by 88%. Additionally, AgNPs-TiO₂NPs effectively reduced malondialdehyde levels by 29%, thereby mitigating the effects of Pb on *V. unguiculata* better than individual nanoparticles. AgNPs-TiO₂NPs enhanced Pb immobilization by 57%, reducing its translocation from soil to shoots compared to *V. unguiculata* wetted with water. The bioconcentration and translocation factors of Pb indicate that phytostabilization was most effective when the composite was used.

NOVELTY STATEMENT

A synergetic interaction between silver nanoparticles and titanium dioxide nanoparticles (AgNPs-TiO₂NPs) was investigated in this study for their capability to promote phytostabilization of Pb pollution in cowpea (*Vigna unguiculata* (L) Walp). This study shows a notable improvement in plant physiological indices, antioxidant activity, and the immobilization of Pb when AgNPs-TiO₂NPs are employed in synergy, as opposed to using them separately. Additionally, the study demonstrates how nanoparticles can synergize to enhance phytostabilization, offering promising prospects for sustainable phytoremediation strategies in the environment.

KEYWORDS

Immobilization; Malondialdehyde level; metal toxicity; silver nanoparticles; titanium dioxide nanoparticle; translocation factor

Introduction

Globally, environmental contamination is increasing dramatically due to factors such as urbanization, chemical fertilizers, burning fossil fuels, waste disposal, sewage discharges, mining operations and application of pesticides among others (Collin *et al.* 2022; Xu *et al.* 2024). Anthropogenic activities are the primary sources of toxic metals (TMs) contamination in the environment (Ozyigit *et al.* 2022). Toxic metals are environmental contaminants that are regularly identified in both aquatic and terrestrial environments (Azeez *et al.* 2023a; Naqqash *et al.* 2024). As a TM, lead (Pb) causes oxidative stress, physiological and morphological changes in plants (Gashi *et al.* 2024; Ur Rahman *et al.* 2024; Xu *et al.* 2024). Plant growth and development are substantially altered by oxidative stress induced by Pb, which affects

biochemical and photosynthetic pigment levels, resulting in phytotoxicity (Zhou *et al.* 2018; Akinci *et al.* 2019; Rasheed *et al.* 2022). This manifests in various aspects of plant growth and development, including germination, growth, fruiting, nutrient uptake, and quality (Zhao *et al.* 2019; Ghouri *et al.* 2024; Saleem *et al.* 2024).

Plants that accumulate TMs may experience a decrease in yield, low nutrient absorption, root browning, growth inhibition, chlorosis, and other physiological traits that ultimately result in plant death (Dinu *et al.* 2021; Gupta *et al.* 2022). Although plants can regulate TMs uptake, high concentrations of TMs can cause malformations, yellowing leaves, stunted growth, and death (Dinu *et al.* 2021; Ghouri *et al.* 2024; Gashi *et al.* 2024). Secondary metabolites such as polyphenols, carotenoids, glutathione,

and vitamins strengthen plants to regulate and resist harmful effects of TMs while free radicals induced by TMs can be scavenged by antioxidant enzymes such as superoxide dismutase, catalase, glutathione, and reductase (Deepika *et al.* 2020; Ghouri *et al.* 2024). However, when plants' defense system is overwhelmed, they need other strategies to combat excessive radical production by TMs, particularly Pb, which is highly toxic (Novák *et al.* 2023; Saleem *et al.* 2024). Phytoremediation represents one strategy to lessen the influence of TM on plant properties (Xu *et al.* 2024). It is a plant strategy that helps control the mobilization and accumulation of TM by eliminating them, therefore addressing their toxicities (Anum *et al.* 2022; Ebrahimbabaie *et al.* 2020). It has been demonstrated that phytoremediation is cost-effective and environmentally friendly (Hadeer *et al.* 2024). It entails utilizing plants to adsorb TMs and reduce their harmful impact on the environment and in plants (Anum *et al.* 2022). Phytoremediation mechanisms mainly involve phytoextraction, phytostabilization, phytodegradation, and phytovolatilization (Mouniera *et al.* 2024).

In comparison with conventional phytoremediation process, metallic nanoparticles such as silver nanoparticles (AgNPs) and titanium dioxide nanoparticles (TiO₂NPs) have gained popularity as adsorbents for TM immobilization and as enhancers of phytoremediation because of their distinctive morphology, ease of administration, rapid soil distribution, strong affinity, and high sorption capacity for target TMs (Adejumo *et al.* 2023, Nie *et al.* 2023; Ganie *et al.* 2024). These nanoparticles can protect plants from environmental stresses by stimulating and modulating enzyme activities, hormonal signaling pathways and induction of phytochemicals (Ma *et al.* 2024; Mounier *et al.* 2024). Additionally, these nanoparticles can immobilize TM in soil, preventing their accumulation in plants. This immobilization improves the resilience of crops against environmental stressors such as Pb, increasing their yields and resistance with further modulation of antioxidant activity (Kovendan *et al.* 2024; Kumari *et al.* 2024). Among metal nanoparticles, AgNPs and TiO₂NPs stand out for their distinct physical, chemical, and biological properties. As a result of their effectiveness in adsorbing and photocatalyzing pollutants, they are widely used in pollution remediation and TM immobilization (Ogunkunle *et al.* 2020; Azeez *et al.* 2023). The two types of nanoparticles have been shown to regulate plant growth and inhibit TM accumulation in plants (Gupta *et al.* 2018). In recent studies, metallic nanoparticles have also been shown to form chelates with TMs before they are absorbed by plants thereby reducing their toxicity (Naqqash *et al.* 2024). Moreover, these nanoparticles bind to intracellular enzymes and proteins to promote normal plant growth and alter soil properties to support beneficial bacteria (Ma *et al.* 2024; Naeem *et al.* 2024).

Cowpea (*Vigna unguiculata* (L) Walp) is an economically viable legume that serves as a staple food for many people in developing countries. This crop is thermophilic and drought-resistant, with a protein content ranging from 22% to 24% (Alabdallah and Alzahrani 2020). It thrives on

infertile soil more effectively than other crops. It flourishes in both hot and arid regions (Gerrano *et al.* 2022).

Nanoparticles of titanium dioxide (TiO₂), magnesium oxide (MgO), calcium (Ca), selenium (Se), zinc oxide (ZnO), silver (Ag) and silicon dioxide (SiO₂) have been shown to have beneficial impacts on agronomic characteristics, physiology regulation, and antioxidant activity modulation of different plants (Alabdallah and Alzahrani 2020; Azeez *et al.* 2023; Kamal and Mogazy 2023; Koçak *et al.* 2023; Okeke *et al.* 2023; Upadhayay *et al.* 2023; Ganie *et al.* 2024; Kumari *et al.* 2024; Naeem *et al.* 2024) whereas copper (II) oxide (CuO) nanoparticles, as reported by Tauseef *et al.* (2021), had a negative impact on its properties.

The separate characteristics of AgNPs and TiO₂NPs for immobilizing Pb and their stimulating plant characteristics have been extensively studied. By applying AgNPs-TiO₂NPs dual composite, it is expected to offer two advantages: first, it may effectively mitigate the toxic effects of TM such as Pb beyond the effects of individual nanoparticles; second, it may improve plant growth and contribute to more efficient and sustainable agriculture by suppressing diseases. Hence, in this study, the composite (AgNPs-TiO₂NPs) was used to demonstrate and decipher the synergistic influence of the dual nanoparticles on phytostabilization (a form of phytoremediation) as well as mitigation of Pb toxicity in *V. unguiculata* compared with individual nanoparticles.

Materials and methods

Collection of soil samples

Soil samples were collected at Osun State University, Osun State, Nigeria (Latitude: 7.760816N; Longitude: 4.604552 E) as previously described by Adejumo *et al.* (2023). After air-drying, crushing, and sieving the soil samples with a 600 µm wire screen, soil analysis including pH, organic matter content, soil texture, nutrient contents and were determined using the method described by Page *et al.* (1982). Metal contents were determined as described by Adejumo *et al.* (2023) by digesting 0.5 g of soil with a 10 mL acid mixture of HNO₃:HCl (7:3) and subsequently analyzed with an inductively coupled plasma optical emission spectrometer (ICP-OES, Agilent 720-ES, USA). The instrument has method detection limits of 0.8, 0.1, 0.4, 0.4, 0.8, 0.6, 0.5, 1.0, 0.3, 1.0, 10.0, 3.0, 2.0 and 1.0 µg L⁻¹ for Ag, Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn, Na, K, Mg, Ca and Fe respectively.

Biosynthesis of AgNPs and TiO₂NPs

An earlier study by Azeez *et al.* (2022b, 2023) reported the synthesis of TiO₂NPs and AgNPs from *Terminalia catappa* leaf extract and their characterization using spectroscopic techniques (UV-Visible and Fourier Transformed Infra-Red), microscopic technique (Scanning Electron microscopy) diffractometric analysis (X-ray diffraction and Energy dispersive X-ray) and textural properties (BET). Briefly, titanium dioxide nanoparticles (TiO₂NPs) were synthesized by adding 100 mL of 1 mM TiO₂ solution to 20 mL of *T. catappa* leaf

extract while AgNPs were mediated with 1 mL of *T. catappa* leaf extract mixed with 40 mL of AgNO₃ solution. Green synthesized TiO₂NPs and AgNPs had stable dark brown and deep golden solutions, respectively. Dual composite (AgNPs-TiO₂NPs) was prepared by mixing 25 mgmL⁻¹ of each nanoparticle, while Pb solution was prepared using Pb(NO₃)₂ salt.

V. Unguiculata planting and analysis

Twenty-four buckets of soil samples were divided randomly into twelve sets of two buckets each containing 500 g of soil for each treatment. Every bucket contained three (3) seeds, making six (6) *V. unguiculata* seeds per group (Figure 1). The groups were labeled as follows: A- water, B- soil+Pb (0.05 mgL⁻¹), C- soil+Pb (0.1 mgL⁻¹), D- AgNPs+Soil, E- AgNPs+Soil+Pb (0.05 mgL⁻¹), F- AgNPs+Soil+Pb (0.1 mgL⁻¹), G- TiO₂NPs+Soil, H- TiO₂NPs+Soil+Pb (0.05 mgL⁻¹), I- TiO₂NPs+Soil+Pb (0.1 mgL⁻¹), J- AgNPs+TiO₂NPs+Soil, K- AgNPs+TiO₂NPs+Pb (0.05 mgL⁻¹) + Soil, L- AgNPs+TiO₂NPs+Pb (0.1 mgL⁻¹) + Soil. Seeds/seedlings of *V. unguiculata* were watered/wetted daily with 70 mL of appropriate solutions for twenty-one days. On day 21, *V. unguiculata* plants were harvested and a number of physiological/growth parameters were determined as reported by to Azeez *et al.* (2019a).

Determination of physiological and biochemical parameters

To determine the MDA level, fresh *V. unguiculata* shoots weighing 0.2 g were homogenized and extracted with cold 80% ethanol. The extract was centrifuged at 3,500 rpm for 20 min and the supernatant was added to a solution containing 4 mL of 20% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA). The resulting solution was incubated at 90°C for 30 min, cooled on ice, and centrifuged again at 3,500 rpm for 30 min. MDA level was determined by measuring absorbance at 440, 532, and 600 nm, according to Gupta *et al.* (2018).

V. unguiculata shoots (0.1 g) were homogenized in 80% acetone, centrifuged for 5 min at 5,000 rpm, and extracted twice with 80% acetone to determine the photosynthetic pigment contents (chlorophyll a, b, and carotenoids) as reported by Adejumo *et al.* (2023). The absorbance of the supernatant was determined at 470, 645 and 663 nm.

The ability of the extract to scavenge free radicals by bleaching 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured at 517 nm after the dried *V. unguiculata* sample was extracted twice with 70% methanol, filtered, and concentrated as previously described by Azeez *et al.* (2023).

For moisture contents, exactly 10 g of *V. unguiculata* shoots were dried in a laboratory oven (UNISCOPE SM9053, Surgifriend Medicals, England) at 105°C and reweighed after

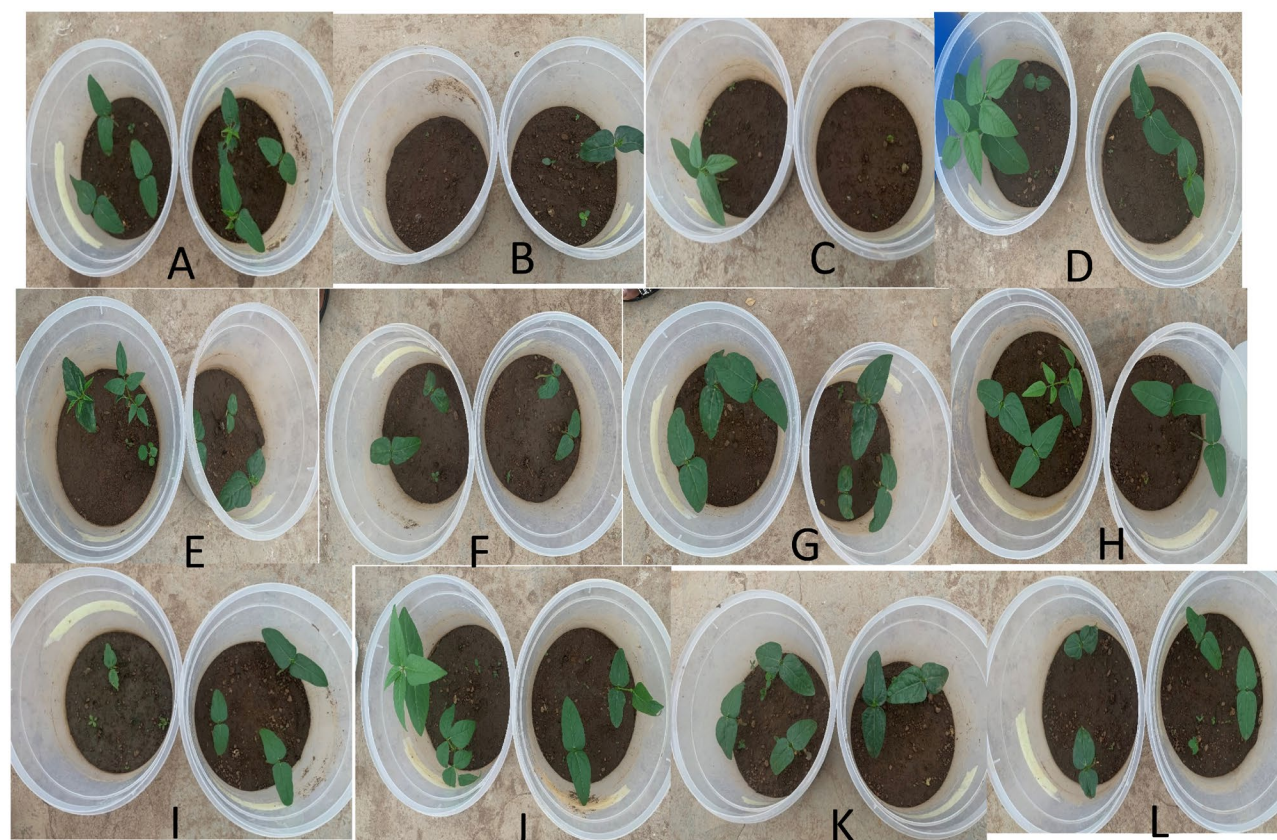


Figure 1. Physiological appearance of *V. unguiculata* planted with Pb, AgNPs, TiO₂NPs and AgNPs-TiO₂NPs. Control group A- water+soil, group B- soil+Pb (0.05 mgL⁻¹), group C- soil+Pb(0.1 mgL⁻¹), group D- AgNPs+Soil, group E- AgNPs+Soil+Pb(0.05 mgL⁻¹), group F- AgNPs+Soil+Pb(0.1 mgL⁻¹), group G-TiO₂NPs+Soil, group H- TiO₂ NPs+Soil+Pb(0.05 mgL⁻¹), group I- TiO₂NPs+Soil+Pb(0.1 mgL⁻¹), group J-AgNPs+TiO₂NPs+Soil, group K - AgNPs+TiO₂ NPs+Pb (0.05 mgL⁻¹) + Soil, group L-AgNPs+TiO₂ NPs+Pb (0.1 mgL⁻¹) +Soil.

3h. The weight loss was continuously monitored until a constant weight was reached, and the moisture content was calculated by expressing the weight loss during drying as a percentage of the original weight using the method of Azeez *et al.* (2019a).

Pb immobilization and phytoremediation type

Immobilization of Pb by nanoparticles and their composite was calculated using equation 1. Phytoremediation factors were examined to identify the specific phytoremediation mechanism responsible for Pb removal from soil samples, including the translocation factor (TF, equation 2), bio-extraction factor (BEF, equation 3) and bioconcentration factor (BCF, equation 4).

$$\frac{Pb\text{immobilization / phytoremediation}(\%) = \text{Initial Pb concentration} - \text{Final Pb concentration}}{\text{Initial Pb concentration}} \times 100 \quad (1)$$

$$TF = \frac{Pb\text{content in } V.\text{unguiculata shoot}}{Pb\text{content in } V.\text{unguiculata root}} \quad (2)$$

$$BEF = \frac{Pb\text{content in } V.\text{unguiculata shoot}}{Pb\text{content in soil}} \quad (3)$$

$$TF = \frac{Pb\text{content in } V.\text{unguiculata root}}{Pb\text{content in soil}} \quad (4)$$

Statistical analysis

Results are presented as mean \pm standard error of the mean (SEM). Shapiro-Wilk Normality test was used to verify the normal distribution of the data obtained from the experiment. The variance among the various experimental groups was analyzed using a parametric test. An analysis of variance (ANOVA) was conducted, followed by a Tukey's honest significance test (HSD) post-hoc test at $p < 0.05$ to compare means of different treatments using IBM statistical package for social sciences (SPSS) version 25.

Results and discussion

Soil analysis and metal contents

The raw soil sampled from Osun State University exhibited a dark coloration and had a pH of 6.88 ± 0.28 . It contained 415.08 ± 30.43 mg/kg of organic matter as well as 9.69% silt, 22.15% clay and 68.16% sand. The pH of 6.88 ± 0.28 indicates a slightly acidic soil, which is generally suitable for a wide variety of plants (Razmi *et al.* 2021). This pH level would generally support plant growth, while the high organic matter content and soil texture suggest good soil fertility and organic nutrient availability (Ogunkunle *et al.* 2020). Growth of plants relies heavily on organic nutrients containing P, K, and N being the primary macronutrients needed for optimal performance (Hong *et al.* 2022). The raw soil also contained N (227.17 ± 2.68 mg/kg),

P (7.18 ± 0.17 mg/kg), K (23.89 ± 0.95 mg/kg), Na (1.05 ± 0.003 mg/kg), Ca (5.78 ± 0.004 mg/kg), Mg (4.09 ± 0.006 mg/kg), Fe (66.56 ± 0.34 mg/kg) and Pb (0.48 ± 0.02 mg/kg). As, Cr, Pb, and Cd levels were below the ICP-OES detection limits of 0.1, 0.4, and $0.1 \mu\text{gL}^{-1}$, respectively. Arsenic (As), Cr and Cd contents were below detection limits, indicating minimal contamination and making the soil safe for planting (Razmi *et al.* 2021).

Effects of Pb, AgNPs, TiO₂NPs and AgNPs-TiO₂NPs on physiological parameters of V. unguiculata

Physiological attributes, such as root and shoot lengths are used to assess the health of a plant. This study demonstrated that Pb contamination significantly impacted *V. unguiculata* physiological parameters, decreasing shoot length by 2% and root length by 44% at 0.05 mgL^{-1} , and by 7% and 25% at 0.1 mgL^{-1} , respectively, compared to water-wetted (control) *V. unguiculata* (Table 1). This is consistent with previous reports that Pb is toxic to plants, causing them to lose healthiness because of reductions in beneficial parameters (Zhou *et al.* 2018; Saleem *et al.* 2024; Ur Rahman *et al.* 2024; Xu *et al.* 2024). The results suggest that the toxicity of Pb increased with concentration, with 0.1 mgL^{-1} being particularly more harmful to *V. unguiculata*. Physiological parameters examined in this study showed that Pb weakened/compromised *V. unguiculata* physiological characteristics. This is in consonance with reports of Deepika *et al.* (2020), Dinu *et al.* (2021), Collin *et al.* (2022) and Gashi *et al.* (2024) on the effects of Pb on *Mentha Piperita* and *Zea mays*. As against Pb, AgNPs and TiO₂NPs significantly enhanced shoot and root lengths, increasing shoot elongation by 53% and 56%, respectively, and root extension by 3% and 20%, respectively (Table 1). Notably, TiO₂NPs exhibited superior modulation of shoot and root lengths than AgNPs by 5% and 87%, respectively. Both AgNPs and TiO₂NPs have been reported to promote shoot and root growth against TM toxicity (Ebrahimbabaie *et al.* 2020; Ghouri *et al.* 2024; Saleem *et al.* 2024). Similarly to this study, Ma *et al.* (2024) found that TiO₂NPs mitigated chromium toxicity, a TM like Pb to

Table 1. Physiological parameters of *V. unguiculata* (L) grown with different concentrations of Pb, AgNPs, TiO₂NPs and AgNPs-TiO₂NPs.

| Groups | Moisture content | Shoot length(cm) | Root length(cm) |
|--------|------------------------|--------------------|-----------------------|
| A | 43.26 ± 0.60^a | 7.30 ± 1.19^a | 5.63 ± 0.12^a |
| B | $53.75 \pm 0.88^{b,f}$ | 7.15 ± 1.42^a | 5.22 ± 1.04^b |
| C | 69.67 ± 0.87^c | 4.09 ± 0.83^b | 4.22 ± 0.84^c |
| D | 35.04 ± 0.42^d | 15.53 ± 0.72^c | $5.78 \pm 0.17^{a,g}$ |
| E | 48.59 ± 0.57^e | 7.33 ± 0.86^a | 6.12 ± 0.81^d |
| F | $55.12 \pm 0.58^{b,g}$ | 6.82 ± 0.85^d | 5.24 ± 0.65^b |
| G | 32.14 ± 0.56^d | 16.50 ± 0.96^e | 7.06 ± 0.48^e |
| H | 52.29 ± 0.57^f | 8.93 ± 0.57^f | 6.68 ± 0.62^f |
| I | 57.19 ± 0.58^g | 7.47 ± 1.15^g | 5.90 ± 0.46^g |
| J | 51.01 ± 0.88^f | 15.70 ± 0.37^c | 7.80 ± 0.68^b |
| K | 73.13 ± 0.57^h | 9.25 ± 2.50^i | 6.25 ± 0.07^d |
| L | 81.48 ± 0.88^i | 8.10 ± 2.12^j | 5.95 ± 0.35^g |

Results - mean \pm standard error of mean (SEM) of three replicates. Data with different subscript down the row for each parameter are significantly different at $p < 0.05$. Control group A-water+soil, group B- soil+Pb (0.05 mgL^{-1}), group C- soil+Pb(0.1 mgL^{-1}), group D-AgNPs+Soil, group E- AgNPs+Soil+Pb(0.05 mgL^{-1}), group F- AgNPs+Soil+Pb(0.1 mgL^{-1}), group G-TiO₂NPs+Soil, group H-TiO₂ NPs+Soil+Pb(0.05 mgL^{-1}), group I- TiO₂NPs+Soil+Pb(0.1 mgL^{-1}), group J-AgNPs+TiO₂NPs+Soil, group K - AgNPs+TiO₂ NPs+Pb (0.05 mgL^{-1}) + Soil, group L-AgNPs+TiO₂ NPs+Pb (0.1 mgL^{-1}) +Soil.

promote *Trachyspermum ammi* L. biomass. Furthermore, AgNPs-TiO₂NPs enhanced shoot length by 54% and root length by 28% compared to water-wetted *V. unguiculata* (Table 1). Remarkably, the dual composite outperformed the individual nanoparticles in terms of root elongation. This improved root elongation would enable *V. unguiculata* to absorb more nutrients as demonstrated in the modulation of physiological indices. Enhanced shoot and root lengths may be attributed to intrinsic properties of AgNPs, TiO₂NPs, and their composite which are known to alter gene expression involved in several cellular pathways (Gupta *et al.* 2018; Hong *et al.* 2022; Ganie *et al.* 2024). Moreover, the ability the nanoparticles to modulate hormones, suppress diseases, modify growth, regulate enzymes, and immobilize pollutants are frequently evidenced in the enhancement of physiological parameters. AgNPs and ZnO nanoparticles were found by Ganie *et al.* (2024) and Alabdallah and Alzahrani (2020) to improve cowpea resistance to salt stress and enhance its physiological parameters by modulating anti-stress factors, including hormones, just as found in this study. In groups E and F containing 0.05 and 0.1 mgL⁻¹ Pb with AgNPs, AgNPs decreased the toxicity of Pb on shoot and root lengths by 2% and 40%, respectively, compared to *V. unguiculata* planted on Pb soil without any additions. In contrast to those planted without nanoparticles but exposed to Pb, TiO₂NPs watered *V. unguiculata* had 19% and 45% modulation of shoot lengths while root lengths were elongated by 21% and 28% (Table 1). Pb toxicity was reduced by TiO₂NPs more effectively than AgNPs on physiological parameters as observed in the results. In addition, AgNPs-TiO₂NPs were found to have greater modulation than individual nanoparticles, elongating shoot lengths by 22% and 50% in 0.05 and 0.1 mgL⁻¹ Pb solution wetted *V. unguiculata*, while increasing root lengths by 16% and 29%. According to these results, AgNPs-TiO₂NPs synergy performed much better than individual nanoparticles applied

separately. In some studies, nanoparticles loaded on biochar or augmented with genome doubling performed better just as dual composite AgNPs-TiO₂NPs were more effective than individual nanoparticles. However, AgNPs combined with cow dung adversely affected plant growth (Ghouri *et al.* 2024; Kumari *et al.* 2024; Verma *et al.* 2024). When grown with both nanoparticles separately and their composite, *V. unguiculata* exposed to Pb showed significant growth improvements due to the immobilizing ability of these nanoparticles (Rehman *et al.* 2023; Upadhyay *et al.* 2023). Besides adsorbing TMs with their vast reactive sites, nanoparticles stimulate plant growth by counteracting the harmful effects of TMs. As a result, plants experience an increase in cell proliferation and division and hence better growth (Ogunkunle *et al.* 2020; Kamali and Magazy, 2023; Okeke *et al.* 2023).

The moisture content that determines how easily fruits and vegetables rot was increased by Pb, while it was significantly ($p < 0.05$) decreased in AgNPs- and TiO₂NPs-grown *V. unguiculata* (Table 1). Individual nanoparticles, however, exhibited lower moisture contents than composite-watered *V. unguiculata*. As a result of moisture content reduction by nanoparticles, it means that the perishability of *V. unguiculata* could be reduced, leading to less microbial degradation after harvest and a slower spoilage rate, thereby enabling them to extend shelf-life and improve their storage stability for longer periods of time (Azeez *et al.* 2023).

Effects of Pb, AgNPs, TiO₂NPs and AgNPs-TiO₂NPs on antioxidant activity, malondialdehyde and photosynthetic pigment contents of *V. unguiculata*

In this study, MDA levels and DPPH radical scavenging ability of *V. unguiculata* were assessed following exposure to Pb at 0.05 and 0.1 mgL⁻¹ and the modulatory properties of AgNPs, TiO₂NPs, and AgNPs-TiO₂NPs synergy (Figures 2 and 3).

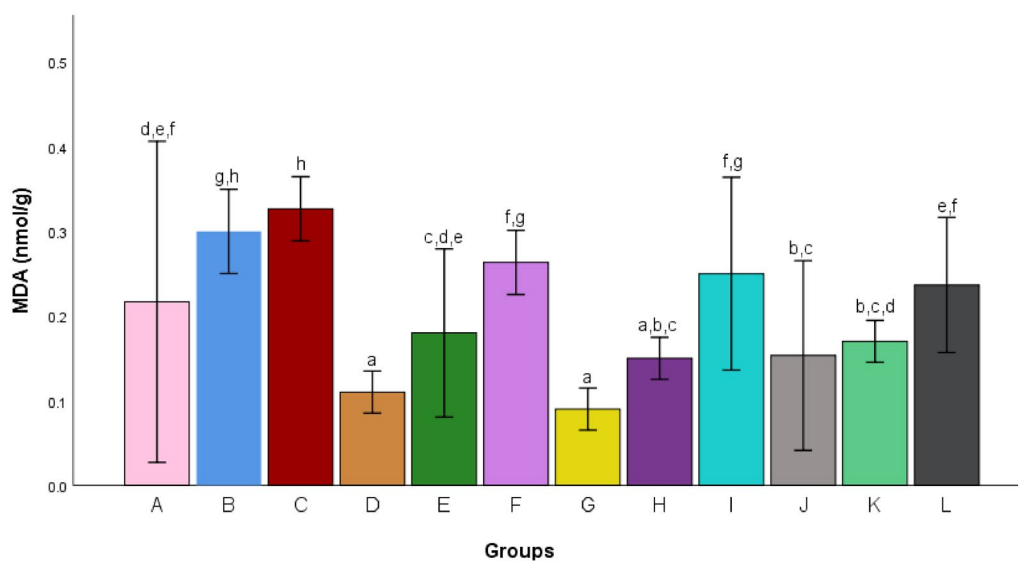


Figure 2. Malondialdehyde (MDA) content of *V. unguiculata* (L) grown with different concentrations of Pb, AgNPs, TiO₂NPs and AgNPs-TiO₂NPs. Bars represent mean \pm standard error of the mean (SEM) of three concordant triplicate values. Bars sharing different colors and featuring distinct superscripts indicate statistically significant differences at $p < 0.05$. Control group A-water+soil, group B- soil +Pb (0.05 mgL⁻¹), group C- soil+Pb(0.1 mgL⁻¹), group D-AgNPs+Soil, group E-AgNPs+Soil+Pb(0.05 mgL⁻¹), group F- AgNPs+Soil+Pb(0.1 mgL⁻¹), group G-TiO₂NPs+Soil, group H- TiO₂ NPs+Soil+Pb(0.05 mgL⁻¹), group I- TiO₂NPs+Soil+Pb(0.1 mgL⁻¹), group J-AgNPs+TiO₂NPs+Soil, group K - AgNPs+TiO₂ NPs+Pb (0.05 mgL⁻¹) + Soil, group L-AgNPs+TiO₂ NPs+Pb (0.1 mgL⁻¹) +Soil.

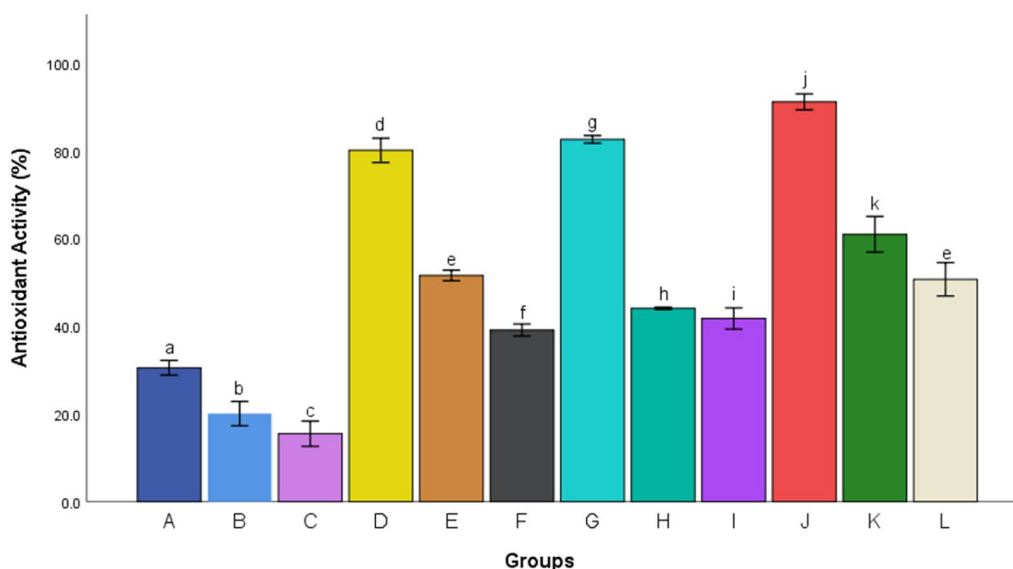


Figure 3. Antioxidant activity of *V. unguiculata* (L) grown with different concentrations of Pb, AgNPs, TiO₂NPs and and AgNPs-TiO₂NPs. Bars represent mean \pm standard error of the mean (SEM) of three concordant triplicate values. Bars sharing different colors and featuring distinct superscripts indicate statistically significant differences at $p < 0.05$. Control group A-water+soil, group B- soil+Pb (0.05 mgL⁻¹), group C- soil+Pb(0.1 mgL⁻¹), group D- AgNPs+Soil, group E- AgNPs+Soil+Pb(0.05 mgL⁻¹), group F- AgNPs+Soil+Pb(0.1 mgL⁻¹), group G-TiO₂NPs+Soil, group H- TiO₂ NPs+Soil+Pb(0.05 mgL⁻¹), group I- TiO₂NPs+Soil+Pb(0.1 mgL⁻¹), group J-AgNPs+TiO₂NPs+Soil, group K - AgNPs+TiO₂ NPs+Pb (0.05 mgL⁻¹) + Soil, group L-AgNPs+TiO₂ NPs+Pb (0.1 mgL⁻¹) +Soil.

Exposure to 0.05 and 0.1 mgL⁻¹ Pb concentrations resulted in increased MDA levels by 28% and 34% in *V. unguiculata*, respectively (Figure 2). Pb, like TMs, produces reactive and peroxide radicals, among other disease-causing radicals (Ur Rahman *et al.* 2024). As a result of the elevated levels of MDA in the Pb solution-watered groups (B and C), it is evident that the *V. unguiculata* defense system was compromised and demonstrates the harmful effects of Pb on *V. unguiculata* even at trace amount (Zhou *et al.* 2018; Akinci *et al.* 2019). A similar report was presented by Rasheed *et al.* (2022) and Gashi *et al.* (2024) stating that TM including Pb increased MDA levels in *Zea mays*. The application of AgNPs to *V. unguiculata* resulted in a 49% decrease in malondialdehyde levels compared to the control group (Figure 2). Furthermore, AgNPs reduced the toxic response of *V. unguiculata* to Pb in groups E and F by 40% and 19%, respectively, over *V. unguiculata* planted in soil watered with Pb without nanoparticles. AgNPs reduced the toxicity of Pb more effectively at 0.05 mgL⁻¹ compared to the control, but less effectively at 0.1 mgL⁻¹ when compared to the control (Figure 2). Malondialdehyde levels in *V. unguiculata* grown with TiO₂NPs were reduced by 58% and 18%, respectively, in comparison with those grown with AgNPs and the control group (Figure 2). However, the MDA level was higher in *V. unguiculata* watered with TiO₂NPs after exposure to Pb 0.05 mgL⁻¹ than in those watered with AgNPs after exposure to the same Pb concentration but was significantly ($p < 0.05$) lower than the control and those watered with Pb without any addition. In comparison to the control, AgNPs-TiO₂NPs synergized to significantly decrease MDA levels in *V. unguiculata* by 29% and to limit the effects of Pb more effectively than individual AgNPs or TiO₂NPs as demonstrated by further reductions in MDA levels (Figure 2). Several factors could contribute to the significant decrease in MDA level influenced by nanoparticles, such as their inherent

antioxidant properties and ability to neutralize free radicals (Hong *et al.* 2022; Adejumo *et al.* 2023; Azeez *et al.* 2023; Ghouri *et al.* 2024; Ma *et al.* 2024). In addition to decreasing MDA levels, AgNPs-TiO₂NPs also exhibited synergistic properties to mitigate Pb toxicity on *V. unguiculata*. As modulators of toxicity, the ability of nanoparticles to modulate toxicity is related to their ability to stimulate the production of phytochemicals and enzymes that protect plants from Pb-mediated toxicity and their inherent radical-scavenging abilities (Zand *et al.* 2020; Kamal and Mogazy 2023; Naem *et al.* 2024; Verma *et al.* 2024; Xu *et al.* 2024).

V. unguiculata planted on soil with Pb at of 0.05 and 0.1 mgL⁻¹ exhibited decreased antioxidant activity by 34% and 49% compared to the control, indicating that Pb contaminations impacted the antioxidant activity of *V. unguiculata* significantly (Figure 3). In *V. unguiculata* wetted with different concentrations of Pb, the antioxidant activities of *V. unguiculata* were significantly ($p < 0.05$) reduced in a concentration-dependent proportion. Accordingly, 0.1 mgL⁻¹ of Pb had a more detrimental effect and greater reduction on antioxidant activity of *V. unguiculata* than 0.05 mgL⁻¹ (Figure 3). Previous studies have demonstrated that Pb decreased antioxidant activity and antioxidant component levels, exposing plants to adverse radical attacks (Azeez *et al.* 2019a; Ghouri *et al.* 2024; Saleem *et al.* 2024; Ur Rahman *et al.* 2024). AgNPs enhanced antioxidant activity of *V. unguiculata* by 62% compared to the control and improved antioxidant activity of Pb-mediated toxicity by 75% and 85% in groups B and C, respectively (Figure 3). The antioxidant-modulating effect of AgNPs on *V. unguiculata* could be responsible for this improvement. Moreover, *V. unguiculata* watered with TiO₂NPs showed 3% and 63% more antioxidant activity than *V. unguiculata* watered with AgNPs and control, respectively (Figure 3). In contrast to

groups B and C, TiO₂NPs improved antioxidant activity of Pb-impacted *V. unguiculata* by 54% and 63%, although at lower percentages than AgNPs. The composite (AgNPs-TiO₂NPs) significantly enhanced the antioxidant activity of *V. unguiculata* by 12% and 9%, compared to using each nanoparticle separately (Figure 3). In addition, AgNPs and TiO₂NPs combined effectively to reduce the toxicity of Pb, resulting in 15% and 23% modulations of antioxidant activity compared to AgNPs, and 28% and 18% stimulation compared to TiO₂NPs in *V. unguiculata* plants exposed to 0.05 and 0.1 mgL⁻¹ Pb, respectively. The effectiveness of plants in preventing damage is largely determined by their capacity to produce phytochemicals and neutralize free radicals produced by toxic metals such as Pb (Hong *et al.* 2022; Adejumo *et al.* 2023; Kumari *et al.* 2024). It has also been found in different studies that some nanoparticles, including AgNPs and TiO₂NPs have inherent abilities to scavenge radicals (Gupta *et al.* 2018; Zand *et al.* 2020). This is particularly more appealing when both nanoparticles are combined to synergistically neutralize free radicals produced by Pb and thereby enhancing the antioxidant activities of *V. unguiculata*. Previous studies have demonstrated that the antioxidant capacity of plants could be improved with the application of several nanoparticles such as AgNPs, MgONPs, TiO₂NPs, FeONPs and SiO₂NPs (Gupta *et al.* 2022; Koçak *et al.* 2023; Okeke *et al.* 2023; Rehman *et al.* 2023; Upadhayay *et al.* 2023; Gashi *et al.* 2024; Kamal and Mogazy, 2023; Mounier *et al.* 2024; Naeem *et al.* 2024; Saleem *et al.* 2024; Xu *et al.* 2024).

Plant function is strongly influenced by carotenoids, chlorophylls a and b, which are photosynthetic factors. When carotenoids and chlorophyll a and b are impaired, it probably indicates that the plant's health has been compromised (Gupta *et al.* 2018; Ogunkunle *et al.* 2020). In comparison to the control, exposure to Pb at 0.05 and 0.1 mgL⁻¹ decreased carotenoids by 80% and 88%, chlorophyll a by 50% and 53%, and chlorophyll b by 31% and 73% in *V. unguiculata* (Table 2). The compromised photosynthetic parameters of *V. unguiculata* demonstrates the harmful

effects of even trace amounts of Pb similarly to what was found for Pb, As and Cr on impairment of photosynthesis pigments by Dinu *et al.* (2021), Hong *et al.* (2022), Rashid *et al.* (2023) and Ma *et al.* (2024). Previous studies have shown that Pb inhibited chlorophyll biosynthesis and it hindered their contents (Azeez *et al.* 2019a,2019b; Adejumo *et al.* 2023; Gashi *et al.* 2024; Ur Rahman *et al.* 2024). Applying AgNPs to *V. unguiculata* led to a 33% rise in carotenoids, a 34% increase in chlorophyll a, and a 28% increase in chlorophyll b, compared to the control group (Table 2). In addition, the presence of AgNPs resulted in significant modulations of carotenoid, chlorophyll a, and chlorophyll b levels in *V. unguiculata* exposed to 0.05 mgL⁻¹ Pb in group E. The enhancements were 82%, 43%, and 19% respectively. Similarly, in group F exposed to 0.1 mgL⁻¹ Pb, AgNPs led to an increase of 61%, 37%, and 57% for carotenoids, chlorophyll a, and chlorophyll b respectively, compared to *V. unguiculata* watered with Pb alone (Table 2). TiO₂NPs considerably enhanced photosynthetic indices in *V. unguiculata*, resulting in a 27% increase in carotenoids, a 29% increase in chlorophyll a, and a 27% increase in chlorophyll b compared to the control group (Table 2). However, the improvement was not as pronounced as with AgNPs. Furthermore, TiO₂NPs exhibited a lesser ability to reduce the toxicity of Pb on photosynthetic parameters compared to AgNPs. AgNPs-TiO₂NPs exhibited a notable ability to enhance the levels of carotenoids, chlorophyll a, and chlorophyll b in *V. unguiculata*. Specifically, there was a 37% increase in carotenoids, a 26% increase in chlorophyll a, and a 21% increase in chlorophyll b compared to the control (Table 2). However, the modulation of chlorophyll a and b contents was not as significant as that observed with individual AgNPs or TiO₂NPs (Table 2). Compared to individual nanoparticles, AgNPs-TiO₂NPs showed synergistic effects in reducing Pb toxicity by increasing carotenoid levels by 88%, however, this synergy had a weaker effect on chlorophyll a and b induction. The increase in carotenoid, an antioxidant molecule, is similar to that reported in the antioxidant activity of DPPH obtained for nanoparticles, with the composite exhibiting better modulation. As demonstrated in this study, previous research has indicated that nanoparticles enhance photosynthetic pigment parameters (Rehman *et al.* 2023; Kumari *et al.* 2024; Verma *et al.* 2024). This is achieved by activating redox properties in plants, leading to better photosynthetic pigments through processes such as water splitting and electron exchange (Rasheed *et al.* 2022; Adejumo *et al.* 2023; Azeez *et al.* 2023). Moreover, nanoparticles possess the capability to remove reactive radicals that disrupt the production of photosynthetic pigments (Zand *et al.* 2020; Ghouri *et al.* 2024).

Influence of AgNPs, TiO₂NPs and AgNPs-TiO₂NPs on Pb contents, immobilization and phytoremediation type

The absorption and translocation patterns of Pb influence its phytotoxicity and are critical for evaluating phytoremediation mechanisms for its removal (Razmi *et al.* 2021; Rasheed *et al.* 2022). Accumulation of Pb varied in the soil from raw to contaminated and treated with nanoparticles. Moreover,

Table 2. Photosynthetic pigment contents of *V. unguiculata* (L) grown with different concentrations of Pb, AgNPs, TiO₂NPs and AgNPs-TiO₂NPs.

| Groups | Carotenoids | Chlorophyll a | Chlorophyll b |
|--------|-------------------------------|----------------------------|------------------------------|
| A | 942.47 ± 53.072 ^a | 10.15 ± 0.51 ^a | 3.98 ± 0.20 ^{a,d} |
| B | 186.27 ± 45.37 ^b | 5.07 ± 0.59 ^b | 2.72 ± 0.88 ^b |
| C | 105.04 ± 57.45 ^c | 4.71 ± 1.00 ^b | 1.09 ± 0.50 ^c |
| D | 1413.73 ± 141.60 ^d | 15.32 ± 1.42 ^c | 5.51 ± 0.41 ^f |
| E | 1047.61 ± 94.198 ^e | 8.82 ± 0.94 ^d | 3.35 ± 0.44 ^{b,a} |
| F | 269.47 ± 10.05 ^h | 7.44 ± 0.08 ^e | 2.61 ± 0.02 ^b |
| G | 1289.41 ± 304.04 ^g | 14.21 ± 1.05 ^c | 5.45 ± 0.17 ^f |
| H | 252.56 ± 13.75 ^f | 6.38 ± 1.66 ^{b,e} | 4.41 ± 1.25 ^{d,e} |
| I | 270.15 ± 25.15 ^h | 6.22 ± 0.43 ^{b,e} | 4.56 ± 0.15 ^{d,e,f} |
| J | 1496.41 ± 341.87 ⁱ | 13.78 ± 2.51 ^c | 5.05 ± 0.04 ^{e,f} |
| K | 1172.78 ± 252.37 ^j | 9.92 ± 1.97 ^a | 3.21 ± 0.57 ^b |
| L | 886.02 ± 169.41 ^k | 9.77 ± 1.18 ^a | 3.52 ± 0.47 ^a |

Results with different superscripts are significantly different at $p < 0.05$ down the row for each parameter. Control group A-water + soil, group B- soil + Pb (0.05 mgL⁻¹), group C- soil + Pb(0.1 mgL⁻¹), group D-AgNPs + Soil, group E- AgNPs + Soil + Pb(0.05 mgL⁻¹), group F- AgNPs + Soil + Pb(0.1 mgL⁻¹), group G-TiO₂NPs + Soil, group H- TiO₂ NPs + Soil + Pb(0.05 mgL⁻¹), group I- TiO₂NPs + Soil + Pb(0.1 mgL⁻¹), group J-AgNPs + TiO₂NPs + Soil, group K - AgNPs + TiO₂ NPs + Pb (0.05 mgL⁻¹) + Soil, group L-AgNPs + TiO₂ NPs + Pb (0.1 mgL⁻¹) + Soil.

Pb contents varied significantly ($p < 0.05$) in plant tissues under different experimental conditions. Groups B and C contaminated with 0.05 and 0.1 mgL⁻¹ Pb, respectively had significantly ($p < 0.05$) higher Pb contents than water-wetted soil (Table 3a). Pb content was significantly ($p < 0.05$) reduced in soils treated with AgNPs, TiO₂NPs, and AgNPs-TiO₂NPs compared to water-wetted soil (Table 3a). As a result of the application of these nanoparticles, including their composites the Pb contents in contaminated soil significantly decreased further (Table 3a). The roots and shoots of *V. unguiculata* in groups B and C showed the highest Pb contents whereas applications of AgNPs, TiO₂NPs, and AgNPs-TiO₂NPs considerably reduced Pb absorption and translocation, particularly the composite, preventing functional impairment in *V.*

Table 3a. Pb concentrations in soil and *V. unguiculata*'s parts.

| Groups | Soil (mgkg ⁻¹) | Root (mgkg ⁻¹) | Shoot (mgkg ⁻¹) |
|--------|----------------------------|----------------------------|-----------------------------|
| A | 0.48 ± 0.02 ^b | 0.17 ± 0.01 ^b | 0.12 ± 0.01 ^a |
| B | 3.51 ± 0.68 ^c | 1.81 ± 0.02 ^f | 1.37 ± 0.02 ^b |
| C | 7.68 ± 1.05 ^d | 4.30 ± 0.04 ^g | 3.48 ± 0.03 ^g |
| D | 0.33 ± 0.07 ^e | 0.16 ± 0.00 ^b | 0.09 ± 0.01 ^a |
| E | 1.04 ± 0.03 ^f | 0.37 ± 0.02 ^c | 0.25 ± 0.00 ^{bc} |
| F | 2.65 ± 0.08 ^g | 1.19 ± 0.05 ^d | 0.76 ± 0.01 ^f |
| G | 0.22 ± 0.02 ^a | 0.15 ± 0.01 ^{ab} | 0.09 ± 0.01 ^a |
| H | 1.26 ± 0.09 ^h | 0.33 ± 0.02 ^c | 0.22 ± 0.01 ^b |
| I | 1.99 ± 0.04 ⁱ | 0.65 ± 0.02 ^e | 0.46 ± 0.01 ^e |
| J | 0.19 ± 0.01 ^a | 0.12 ± 0.01 ^a | 0.08 ± 0.01 ^a |
| K | 1.16 ± 0.03 ^j | 0.34 ± 0.01 ^c | 0.19 ± 0.01 ^b |
| L | 1.73 ± 0.09 ^k | 0.61 ± 0.06 ^e | 0.36 ± 0.01 ^d |

Results - mean ± standard error of mean (SEM) of three replicates. Data with different subscript down the row for each parameter are significantly different at $p < 0.05$. Control group A-water + soil, group B- soil + Pb (0.05 mgL⁻¹), group C- soil + Pb(0.1 mgL⁻¹), group D-AgNPs + Soil, group E- AgNPs + Soil + Pb(0.05 mgL⁻¹), group F- AgNPs + Soil + Pb(0.1 mgL⁻¹), group G-TiO₂NPs + Soil, group H-TiO₂ NPs + Soil + Pb(0.05 mgL⁻¹), group I- TiO₂NPs + Soil + Pb(0.1 mgL⁻¹), group J-AgNPs + TiO₂NPs + Soil, group K - AgNPs + TiO₂ NPs + Pb (0.05 mgL⁻¹) + Soil, group L-AgNPs + TiO₂ NPs + Pb (0.1 mgL⁻¹) + Soil.

unguiculata. These results highlight the differences in uptake patterns of Pb in roots and translocation to shoots across experimental groups and reveal the influence of nanoparticles on distribution and accumulation of Pb in *V. unguiculata*. The reactive surfaces and morphological characteristics of nanoparticles used in this study make them well suited for adsorbing TMs and pollutants as previously reported by Zand *et al.* (2020), Okeke *et al.* (2023), Upadhaya *et al.* (2023). Nanoparticles have notable impacts on metal toxicity by functioning as immobilizing agents that prevent plants from being exposed to metal toxicity or, in certain situations, alleviate toxicity after it has been induced (Azeez *et al.* 2019a,2019b; Rasheed *et al.* 2022; Upadhayay *et al.* 2023). Furthermore, reduction in germination parameters, increased MDA, and decreased photosynthetic parameters are all evidence of the adverse effects of Pb on the soil-plant system, as previously noted and discussed in this study.

The phytoremediation status of *V. unguiculata* exposed to 0.05 and 0.1 mgL⁻¹ Pb, as well as the ability of AgNPs, TiO₂NPs, and AgNPs-TiO₂NPs to immobilize Pb, were examined. Percentage immobilization significantly increased with the addition of nanoparticles and declined the translocation of Pb from root to shoot (Figure 4 and Table 3). In groups containing 0.05 and 0.1 mgL⁻¹ of Pb, *V. unguiculata* demonstrated a phytoremediation efficiency of 28% and 23%. Moreover, AgNPs resulted in increased phytoremediation percentages (Pb immobilization) of 79% and 74%, TiO₂NPs showed percentages of 73% and 80%, and AgNPs-TiO₂NPs showed percentages of 77% and 83%. This explains the stunted characteristics of *V. unguiculata* grown on 0.05 and 0.1 mgL⁻¹ Pb as compared to nanoparticles-supported growth (Figure 4). Furthermore, the enhanced immobilization of Pb by the composite of

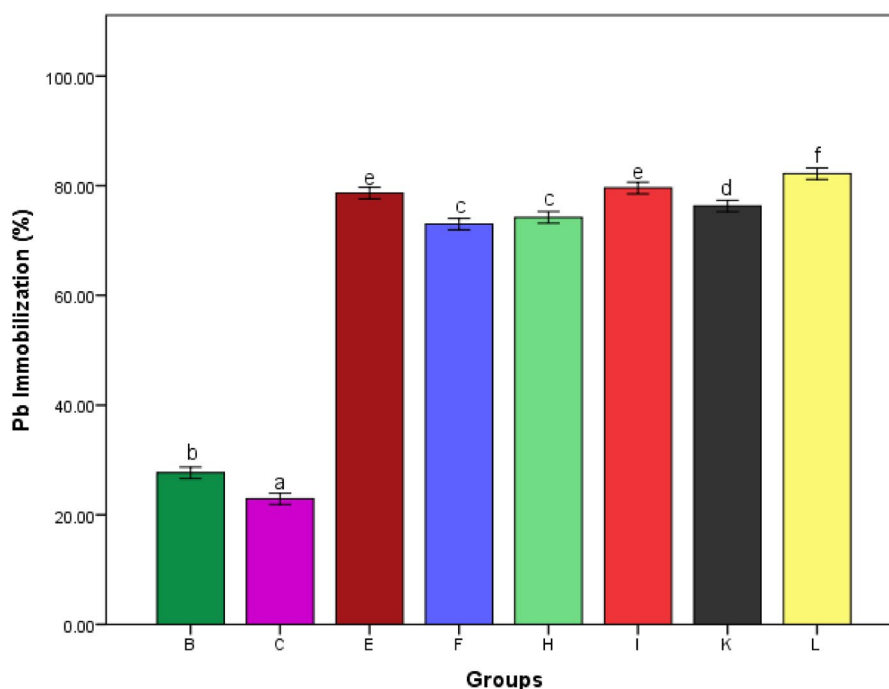


Figure 4. Percentage immobilization of Pb by AgNPs, TiO₂NPs and AgNPs-TiO₂NPs. Group B- soil+Pb (0.05 mgL⁻¹), group C- soil+Pb(0.1 mgL⁻¹), group E- AgNPs + Soil + Pb(0.05 mgL⁻¹), group F- AgNPs + Soil + Pb(0.1 mgL⁻¹), group H- TiO₂ NPs + Soil + Pb(0.05 mgL⁻¹), group I- TiO₂NPs + Soil + Pb(0.1 mgL⁻¹), group K - AgNPs + TiO₂ NPs + Pb (0.05 mgL⁻¹) + Soil, group L-AgNPs + TiO₂ NPs + Pb (0.1 mgL⁻¹) + Soil.

Table 3b. Translocation efficiency, translocation factor, bio-extraction factor and bioconcentration factor of lead exposed to different treatments.

| Group | TF | BEF | BCF |
|-------|------|------|------|
| B | 0.76 | 0.38 | 0.50 |
| C | 0.81 | 0.45 | 0.56 |
| E | 0.66 | 0.24 | 0.36 |
| F | 0.64 | 0.29 | 0.45 |
| H | 0.67 | 0.18 | 0.26 |
| I | 0.69 | 0.22 | 0.38 |
| K | 0.55 | 0.16 | 0.30 |
| L | 0.58 | 0.20 | 0.35 |

Group B- soil+Pb (0.05 mgL⁻¹), group C- soil+Pb(0.1 mgL⁻¹), group E- AgNPs+Soil+Pb(0.05 mgL⁻¹), group F- AgNPs+Soil+Pb(0.1 mgL⁻¹), group H- TiO₂ NPs+Soil+Pb(0.05 mgL⁻¹), group I- TiO₂NPs+Soil+Pb(0.1 mgL⁻¹), group K - AgNPs+TiO₂ NPs+Pb (0.05 mgL⁻¹) + Soil, group L-AgNPs+TiO₂ NPs+Pb (0.1 mgL⁻¹) +Soil.

AgNPs-TiO₂NPs represents the superior immobilizing capability of the synergy over the individual nanoparticles.

Nanoparticles significantly reduced the translocation factor (TF) of Pb from root to shoot (Table 3b), with AgNPs-TiO₂NPs having the greatest impact. Studies have frequently employed the values of TF, BCF, and BEF to identify the predominant phytoremediation mechanism (Al-Mur, 2021; Razmi *et al.* 2021, Gajic *et al.* 2020). When both Transfer Factor (TF) and Bioconcentration Factor (BCF) are < 1, phytostabilization is the primary phytoremediation mechanism. Conversely, when the bio-extraction Factor (BEF) and TF are > 1, phytoextraction becomes the major phytoremediation method (Azeez *et al.* 2022a). The TF values for Pb in B, C, E, F, H, I, K, and L are < 1 with decreasing order of water > TiO₂NPs > AgNPs > AgNPs-TiO₂NPs (Table 3b). Additionally, both BCF and BEF for Pb in these groups are < 1 following the similar trend as expressed for TF (Table 3b). These findings clearly indicate that phytostabilization, a phytoremediation mechanism, was responsible for immobilizing Pb, thereby reducing its toxicity in *V. unguiculata* (Adejumo *et al.* 2023; Saleem *et al.* 2024). The TF quantifies the ability of a plant to translocate Pb from its roots to its shoots for storage, with a value below 1 indicating restricted movement (Akinci *et al.* 2019; Dinu *et al.* 2021). Through the application of nanoparticles, both TF and BCF are significantly reduced, while the combination further reduces its (Pb) presence in the edible part of *V. unguiculata* synergistically. The results are consistent with a report by Mouniera *et al.* (2024) on the improvement of multi-metal phytoextraction by *Helianthus annuus* enhanced by iron oxide nanoparticles.

Conclusion

The results of this study demonstrate that Pb contamination had a significant impact on *V. unguiculata*'s physiological parameters and antioxidant activity. Due to Pb-induced toxicity, shoot and root lengths were reduced, antioxidant activity was compromised, photosynthetic pigment content was impaired, and malondialdehyde content increased. Additionally, AgNPs, TiO₂NPs, and their composite effectively mitigated these adverse effects, demonstrating their potential in phytoremediation, with the composite having the greatest effect. In addition, the AgNPs-TiO₂NPs synergy demonstrated promising performance in immobilizing Pb

and reducing its translocation, thus enhancing phytostabilization. The findings in this study highlight the potentials of nanoparticle-synergy approaches for alleviating toxic metal-induced stress in plants and advancing sustainable phytoremediation.

Disclosure statement

Authors declare no conflict of interest.

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References

- Adejumo AL, Azeez L, Kolawole TO, Aremu HK, Adedotun IS, Oladeji RD, Adeleke AE, Abdullah M. 2023. Silver nanoparticles strengthen *Zea mays* against toxic metal-related phytotoxicity via enhanced metal phytostabilization and improved antioxidant responses. *Int J Phytoremediation*. 25(12):1676–1686. doi:10.1080/15226514.2023.2187224.
- Akinci G, Gok G, Bilgin M. 2019. Heavy Metals Bioconcentration and Translocation in Plants: the Influence of a Thermal Power Site. *Environ Eng Manag J*. 18(8):1625–1637. doi:10.30638/eemj.2019.153.
- Alabdallah NM, Alzahrani HS. 2020. Impact of ZnO Nanoparticles on Growth of Cowpea and Okra Plants under Salt Stress Conditions. *Biosci Biotechnol Res Asia*. 17(2):329–340. doi:10.13005/bbra/2836.
- Al-Mur BA. 2021. Assessment of heavy metal contamination in water, sediments, and Mangrove plant of Al-Budhai region, Red Sea Coast, Kingdom of Saudi Arabia. *J Taibah Uni Sci*. 15(1):423–441. doi:10.1080/16583655.2021.1985871.
- Anum W, Riaz U, Murtaza G, Raza MU. 2022. Sustainable agroecosystems: recent trends and approaches in phytoremediation and rhizoremediation. In: Malik JA, Goyal MR, editors. *Bioremediation and phytoremediation technologies in sustainable soil management*. United Kingdom: Taylor and Francis. Chapter 8. p. 185–204. doi:10.1201/97810032806211.
- Azeez L, Adebisi SA, Adetoro RO, Oyediji A, O, Agbaje WB, Olabode OA. 2022a. Foliar application of silver nanoparticles differentially intervenes remediation statuses and oxidative stress indicators in *Abelmoschus esculentus* planted on gold-mined soil. *Int J Phytoremediation*. 24(4):384–393. doi:10.1080/15226514.2021.1949578.
- Azeez L, Adejumo AL, Lateef A, Adebisi SA, Adetoro RO, Adewuyi SO, Tijani KO, Olaoye S. 2019b. Zero-valent silver nanoparticles attenuate Cd and Pb toxicities on *Moringa oleifera* via immobilization and induction of phytochemicals. *Plant Physiol Biochem*. 139:283–292. doi:10.1016/j.plaphy.2019.03.030.
- Azeez L, Adetoro RO, Busari HK, Aremu HK, Adeleke JT, Adewinbi S, Olabode OA, Ayandayo I. 2022b. AgNPs-TiO₂NPs doped calcined hydroxyapatite for effective removal of ibuprofen and Acetaminophen. *Int J Environ Anal Chem*. 1–25. doi:10.1080/03067319.2022.2106434.
- Azeez L, Aremu HK, Busari HK, Adekale I, Olabode OA, Adewinbi S. 2023. Biofabrication of titanium dioxide nanoparticles with *Terminalia catappa* bark extract: characterization, antioxidant activity and plant modulating ability. *Inorg Nano-Met Chem*. 1–8. doi:10.1080/24701556.2023.2184386.
- Azeez L, Lateef A, Wahab AA, Rufai MA, Salau AK, Ajayi EIO, Ajayi M, Adegbite MK, Adebisi B. 2019a. Phytomodulatory effects of silver nanoparticles on *Corchorus olitorius*: its antiphytopathogenic and hepatoprotective potentials. *Plant Physiol Biochem*. 136:109–117. doi:10.1016/j.plaphy.2018.12.006.
- Collin MS, Venkatraman SK, Vijayakumar N, Kanimozhi V, Arbaaz SM, Stacey RGS, Anusha J, Choudhary R, Lvov V, Tovar GI, *et al.* 2022. Bioaccumulation of lead (Pb) and its effects on human: a review. *J Hazard Mater Adv*. 7:100094. doi:10.1016/j.hazadv.2022.100094.
- Deepika G, Yadav A, Prasad M, Singh TB, Shrivastav P, Ali A, Dantu PK, Mishra S. 2020. Effect of heavy metals on plant growth : an

- overview. In: Naeem M, Ansari A, Gill S, editors. Contaminants in agriculture. Switzerland: Springer, p. 79–101. doi:10.1007/978-3-030-415525_4.
- Dinu C, Gheorghe S, Tenea AG, Stoica C, Vasile G-G, Popescu RL, Serban EA, Pascu LF. 2021. Toxic metals (As, Cd, Ni, Pb) impact in the most common medicinal plant (*Mentha Piperita*). Int J Environ Res Public Health. 18(8):3904. doi:10.3390/ijerph18083904.
- Ebrahimbabaie P, Meeinkuirt W, Pichtel J. 2020. Phytoremediation of engineered nanoparticles using aquatic plants: Mechanisms and practical feasibility. J Environ Sci (China). 93:151–163. doi:10.1016/j.jes.2020.03.034.
- Gajic G, Djurdjevic L, Kostic O, Jaric S, Stevanovic B, Mitrovic M, Pavlovic P. 2020. Phytoremediation potential, photosynthetic and antioxidant response to arsenic-induced stress of *Dactylis glomerata* L. sown on fly ash deposits. Plants. 9:657. doi: 10.3390/plants9050657.
- Ganie IB, Hussain MA, Shahzad A. 2024. Application of silver nanoparticles synthesized from the calli of *Centella asiatica* for the management of *Meloidogyne arenaria* infection in cowpea plants. S Afr J Bot. 171:323–336. doi:10.1016/j.sajb.2024.06.014.
- Gashi B, Buqaj L, Vataj R, Tuna M. 2024. Chlorophyll biosynthesis suppression, oxidative level and cell cycle arrest caused by Ni, Cr and Pb stress in maize exposed to treated soil from the Ferronikel smelter in Drenas, Kosovo. Plant Stress. 11:100379. doi:10.1016/j.stress.2024.100379.
- Gerrano AS, Lubinga MH, Bairu MH. 2022. Genetic resources management, seed production constraints and trade performance of orphan crops in Southern Africa: a case of Cowpea. S Afr J Bot. 146:340–347. doi:10.1016/j.sajb.2021.11.007.
- Ghouri F, Shahid MJ, Zhong M, Zia MA, Alomrani SO, Liu J, Sun L, Ali S, Liu X, Shahid MQ. 2024. Alleviated lead toxicity in rice plant by co-augmented action of genome doubling and TiO₂ nanoparticles on gene expression, cytological and physiological changes. Sci Total Environ. 911:168709. doi:10.1016/j.scitotenv.2023.168709.
- Gupta N, Singh PM, Sagar V, Pandya A, Chinnappa M, Kumar R, Bahadur A. 2022. Seed priming with ZnO and Fe₃O₄ nanoparticles alleviate the lead toxicity in *Basella alba* L. through reduced lead uptake and regulation of ROS. Plants (Basel). 11(17):27. doi:10.3390/plants11172227.
- Gupta SD, Agarwal A, Pradhan S. 2018. Phytostimulatory effect of silver nanoparticles (AgNPs) on rice seedling growth: an insight from antioxidative enzyme activities and gene expression patterns. Ecotoxicol Environ Saf. 161:624–633. doi:10.1016/j.ecoenv.2018.06.023.
- Hadeer I, Ghada SMI, Masarrat MM, Ghazy MA, Mahmoud N. 2024. Dual phytoremediation and biochar production by *Eichhornia crassipes* in hydroponic system receiving different 1,4-dioxane dosages. Int J Phytoremediation. 26(4):546–556. doi:10.1080/15226514.2023.2253915.
- Hong Y, Li D, Xie C, Zheng X, Yin J, Li Z, Zhang K, Jiao Y, Wang B, Hu Y, et al. 2022. Combined apatite, biochar, and organic fertilizer application for heavy metal co-contaminated soil remediation reduces heavy metal transport and alters soil microbial community structure. Sci Total Environ. 851(Pt 1):158033. doi:10.1016/j.scitotenv.2022.158033.
- Kamal R, Mogazy AM. 2023. Effect of doping on TiO₂ nanoparticles characteristics: studying of fertilizing effect on cowpea plant growth and yield. J Soil Sci Plant Nutr. 23(1):325–337. doi:10.1007/s42729-021-00648-0.
- Koçak R, Okcu M, Haliloğlu K, Türkoğlu A, Pour-Aboughadareh A, Jamshidi B, Janda T, Alaylı A, Nadaroğlu H. 2023. Magnesium oxide nanoparticles: an influential element in Cowpea (*Vigna unguiculata* L. Walp.) tissue culture. Agronomy. 13(6):1646. doi:10.3390/agronomy13061646.
- Kovendan K, Fabiola M, Jebanesan A, Rajaganesh R. 2024. Green synthesis of *Malvastrum coromandelianum* fabricated AgNPs: anti-dengue and mosquitocidal studies. Inorg Chem Commun. 161:112067. doi:10.1016/j.inoche.2024.112067.
- Kumari K, Rani N, Hooda V. 2024. Silver nanoparticles and silver/silica nanocomposites: impacts on *Z. mays* L. growth, nutrient uptake and soil health. Plant Nano Biol. 7:100064. doi:10.1016/j.plana.2024.100064.
- Ma J, Hua Z, Zhu Y, Saleem MH, Zulfiqar F, Chen F, Touqeer Abbas T, El-Sheikh MA, Yong JWH, Adil MF. 2024. Interaction of titanium dioxide nanoparticles with PVC microplastics and chromium counteracts oxidative injuries in *Trachyspermum ammi* L. by modulating antioxidants and gene expression. Ecotoxicol Environ Saf. 274:116181. doi:10.1016/j.ecoenv.2024.116181.
- Mounier L, Pédrot M, Bouhnik-Le-Coz M, Cabello-Hurtado F. 2024. Iron oxide nanoparticles improving multimetal phytoextraction in *Helianthus annuus*. Chemosphere. 353:141534. doi:10.1016/j.chemosphere.2024.141534.
- Naeem MA, Shabbir A, Imran M, Ahmad S, Shahid M, Murtaza B, Amjad M, Khan W-u-D. 2024. Silicon-nanoparticles loaded biochar for soil arsenic immobilization and alleviation of phytotoxicity in barley: implications for human health risk. Environ Sci Pollut Res. 31(16):23591–23609. doi:10.1007/s11356-024-32580-y.
- Naqash T, Aziz A, Gohar M, Khan J, Ali S, Radicetti M, Babar M, Manzer H, Ghulam H. 2024. Heavy metal-resistant rhizobacteria fosters to alleviate the cadmium toxicity in *Arabidopsis* by upregulating the plant physiological responses. Int J Phytoremediation. 26(4):557–568. doi:10.1080/15226514.2023.2253923.
- Nie P, Zhao Y, Xu H. 2023. Synthesis, applications, toxicity and toxicity mechanisms of silver nanoparticles: a review. Ecotoxicol Environ Saf. 253:114636. doi:10.1016/j.ecoenv.2023.114636.
- Novák M, Zemanová V, Lhotská M, Pavlík M, Klement A, Hnilička F, Pavlíková D. 2023. Response of Carrot (*Daucus carota* L.) to Multi-Contaminated Soil from Historic Mining and Smelting Activities. Int J Mol Sci. 24(24):45. doi:10.3390/ijms242417345.
- Ogunkunle CO, Odulaja DA, Akande FO, Varun M, Vishwakarma V, Fatoba PO. 2020. Cadmium toxicity in cowpea plant: effect of foliar intervention of nano-TiO₂ on tissue Cd bioaccumulation, stress enzymes and potential dietary health risk. J Biotechnol. 310:54–61. doi:10.1016/j.jbiotec.2020.01.009.
- Okeke ES, Nweze EJ, Ezike TC, Nwuche CO, Ezeorba TPC, Nwankwo CEI. 2023. Silicon based nanoparticles for mitigating the effect of potentially toxic elements and plant stress in agroecosystems: a sustainable pathway towards food security. Sci Total Environ. 898:165446. doi:10.1016/j.scitotenv.2023.165446.
- Ozyigit II, Karahan F, Yalcin IE, Hocaoglu-Ozyigit A, Ilcim A. 2022. Heavy metals and trace elements detected in the leaves of medicinal plants collected in the southeast part of Turkey. Arab J Geosci. 15(1):27. doi: 10.1007/s12517-021-09264-9.
- Page A, Miller RH, Keeney DR. 1982. Soil analysis part 2. Chemical and microbiological properties. Madison (WI): ASA, SSSA.
- Rasheed A, Li H, Tahir MM, Mahmood A, Nawaz M, Shah AN, Aslam MT, Negm S, Moustafa M, Hassan MU, et al. 2022. The role of nanoparticles in plant biochemical, physiological, and molecular responses under drought stress: A review. Front Plant Sci. 13:976179. doi:10.3389/fpls.2022.976179.
- Rashid A, Schutte BJ, Ulery A, Deyholos MK, Sanogo S, Lehnhoff EA, Beck L. 2023. Heavy metal contamination in agricultural soil: environmental pollutants affecting crop health. Agronomy. 13(6):1521. doi:10.3390/agronomy13061521.
- Razmi B, Ghasemi-Fasaei R, Ronaghi A, Mostowfizadeh-Ghalmfarsa R. 2021. Investigation of factors affecting phytoremediation of multi-elements polluted calcareous soil using Taguchi optimization. Ecotoxicol Environ Saf. 207:111315. doi:10.1016/j.ecoenv.2020.111315.
- Rehman A, Khan S, Sun F, Peng Z, Feng K, Wang N, Jia Y, Pan Z, He S, Wang L, et al. 2023. Exploring the nano-wonders: unveiling the role of nanoparticles in enhancing salinity and drought tolerance in plants. Front Plant Sci. 14:1324176. doi:10.3389/fpls.2023.1324176.
- Saleem Y, Ali A, Naz S, Jamil M, Naveed NH. 2024. A comparative *in vitro* study of Pb tolerance in three sugarcane (*Saccharum officinarum* L.). J Agric Res. 15:100953. doi:10.1016/j.jafr.2023.100953.
- Tauseef A, Gupta J, Rehman A, Uddin I, Hisamuddin. 2021. Differential response of cowpea towards the CuO nanoparticles under *Meloidogyne incognita* stress. S Afr J Bot. 139: 175–182. doi: 10.1016/j.sajb.2021.02.017.
- Upadhyay VK, Chitara MK, Mishra D, Jha MN, Jaiswal A, Kumari G, Ghosh S, Patel VK, Naitam MG, Singh AK, et al. 2023. Synergistic impact of nanomaterials and plant probiotics in agriculture: A tale of two-way strategy for long-term sustainability. Front Microbiol. 14:1133968. doi:10.3389/fmicb.2023.1133968.
- Ur Rahman S, Qin A, Zain M, Mushtaq Z, Mehmood F, Riaz L, Naveed S, Ansari MJ, Saeed M, Ahmad I, et al. 2024. Pb uptake,

- accumulation, and translocation in plants: plant physiological, biochemical, and molecular response: A review. *Heliyon*. 10(6):e27724. doi:[10.1016/j.heliyon.2024.e27724](https://doi.org/10.1016/j.heliyon.2024.e27724).
- Verma KK, Joshi A, Song X-P, Singh S, Kumari A, Arora J, Singh SK, Solanki MK, Seth CS, Li Y-R. 2024. Synergistic interactions of nanoparticles and plant growth promoting rhizobacteria enhancing soil-plant systems: a multigenerational perspective. *Front Plant Sci*. 15:1376214. doi:[10.3389/fpls.2024.1376214](https://doi.org/10.3389/fpls.2024.1376214).
- Xu C, Liu C, Li Z, Zu Y, Wang J. 2024. Response of growth and Pb accumulation characteristics of plants with intercropping *Arabis alpina*-*Zeamays* to exogenous oxalic acid. *Int J Phytoremediation*. 26(4):472–480. doi:[10.1080/15226514.2023.2248268](https://doi.org/10.1080/15226514.2023.2248268).
- Zand AD, Tabrizi AM, Heir AV. 2020. Application of titanium dioxide nanoparticles to promote phytoremediation of Cd-polluted soil: contribution of PGPR inoculation. *Bioremediat J*. 24(2-3):171–189. doi:[10.1080/10889868.2020.1799929](https://doi.org/10.1080/10889868.2020.1799929).
- Zhao Y, Hu C, Wang X, Qing X, Wang P, Zhang Y, Zhang X, Zhao X. 2019. Selenium alleviated chromium stress in Chinese cabbage (*Brassica campestris* L. ssp. *Pekinensis*) by regulating root morphology and metal element uptake. *Ecotoxicol Environ Saf*. 173:314–321. doi: [10.1016/j.ecoenv.2019.01.090](https://doi.org/10.1016/j.ecoenv.2019.01.090).
- Zhou J, Zhang Z, Zhang Y, Wei Y, Jiang Z. 2018. Effects of lead stress on the growth, physiology, and cellular structure of privet seedlings. *PLoS One*. 13(3):e0191139. doi:[10.1371/journal.pone.0191139](https://doi.org/10.1371/journal.pone.0191139).