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GREEN SYNTHESIZED SILVER NANOPARTICLES ALLEVIATE LEAD TOXICITY IN MAIZE AND WHEAT

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Abstract

Lead (Pb) toxicity detrimentally impacts crop growth. Its toxicity in plants results in stunted growth, chlorosis, reduced photosynthesis, disrupted nutrient uptake, oxidative stress, and altered root morphology, compromising overall plant health. Silver nanoparticles (AgNPs) demonstrate high surface area and reactivity in this regard. It can facilitate the efficient adsorption of lead ions in the contaminated rhizosphere, which makes it a promising remediation strategy. Therefore, a current pot experiment was conducted on wheat and maize cultivated in Pb-contaminated and non-contaminated soil. There were 2 levels of AgNPs (0 and 100 mg kg^{-1}) were applied with and without Pb toxicity (400 mg kg^{-1}). Results showed that applying 100 mg kg^{-1} AgNPs+Pb caused improvement in shoot length (~30 and ~26%), chlorophyll a (~31 and ~33%), chlorophyll b (~44 and ~57%), total chlorophyll (~36 and ~40%) over Pb (400 mg kg^{-1}) in wheat and maize respectively. A significant improvement in wheat and maize leaves N, P, K, photosynthetic rate, transpiration rate, and stomatal conductance also validated the effectiveness of AgNPs+Pb over Pb. In conclusion, 100 mg kg^{-1} AgNPs have the potential to regulate antioxidant activity and improve nutrient concentration to alleviate Pb toxicity in wheat and maize. More investigations are suggested at the field level to declare 100 mg kg^{-1} AgNPs as the best application rate and treatment for mitigating Pb toxicity in different cereal crops under variable agro climates.

Key words: Lead toxicity, Nanoparticles, Maize, Wheat, Growth attributes, Antioxidants, Nutrient concentration.

Introduction

Heavy metal toxicity has been found to negatively impact wheat and maize crops' growth, yield, and quality (Xiang *et al.*, 2021; Dawar *et al.*, 2023a; Sheikh *et al.*, 2023; Sana *et al.*, 2024). Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), and arsenic (As) can enter the soil through various sources, including mining, industrial activities, and the use of contaminated fertilizers. They can accumulate in the plant tissue (Clemens, 2006; Dawar *et al.*, 2023b; Haider *et al.*, 2023; Qian *et al.*, 2023). Among different heavy metals, Pb toxicity in wheat and maize plants is the center of attention. The negative impacts of Pb toxicity have significant implications for human nutrition and food security, particularly in developing countries where people rely heavily on these crops as a primary source of calories (Duruibe *et al.*, 2007). Consuming heavy metal-contaminated wheat and maize can lead to adverse health effects in humans, including neurological disorders, renal failure, and cancer (Duruibe *et al.*, 2007). Pb mainly causes toxicity in plants due to its interference with essential metabolic processes such as photosynthesis, respiration, and nutrient uptake (Feleafel & Mirdad, 2013). It can also induce oxidative stress, leading to cellular damage and reduced crop productivity (Hu *et al.*, 2020). Therefore, monitoring and managing Pb contamination in wheat and maize is crucial to ensure the safety and quality of food for human consumption (Lamhamdi *et al.*, 2013; Abedi *et al.*, 2022).

Silver nanoparticles (AgNPs) have been found to have the potential for mitigating lead (Pb) toxicity in plants (Haq *et al.*, 2022). Several studies have demonstrated the ability of AgNPs to reduce the negative effects of Pb on plant growth and productivity (Kim *et al.*, 2012; Premkumar *et al.*, 2018). AgNPs can interact with Pb in soil, forming complexes that reduce Pb uptake by plants and enhance the availability of essential nutrients (Chen *et al.*, 2022). Additionally, AgNPs have been shown to enhance plant growth and development by increasing the activity of antioxidant enzymes and improving nutrient uptake (Liu *et al.*, 2020). The application of AgNPs has been found to mitigate Pb toxicity and improve plant growth and productivity (Liu *et al.*, 2020; Chen *et al.*, 2022). Nanoparticles have the potential to absorb and transform heavy metals, thus decreasing their mobility and bioavailability (Sebastian *et al.*, 2019). They also make stable complexes (Levard *et al.*, 2012), which minimize heavy metal's potential toxic effects on plants (Azeez *et al.*, 2019). On the other hand, silver ranks as the second most harmful metal to aquatic organisms, following mercury (Moreno-Garrido *et al.*, 2015). AgNPs can release silver ions (Ag^{+}), which exhibit persistence, bioaccumulation, and significant toxicity to organisms. Therefore, introducing AgNPs into ecosystems raises substantial concerns regarding their safety and environmental impact (Ratte, 1999).

Furthermore, nanoparticles synthesized through green methods, such as using plant extracts, bacteria, or enzymes as precursors, offer a convenient, economical, and eco-friendly alternative (Hussain *et al.*, 2016). Green synthesized silver nanoparticles (AgNPs) have attracted interest due to their potential uses in agriculture and environmental fields. These AgNPs have been found to enhance seed germination, plant growth, and the activity of antioxidant enzymes in various plant species (Gupta *et al.*, 2018). Additionally, they contribute to bolstering the defense mechanisms of economic plants, mitigating the toxic effects of metals and alleviating stress induced by environmental factors on plant growth and productivity (Konate *et al.*, 2017).

Wheat and maize are two of the most important cereal crops in the world and are widely consumed as a staple food by millions of people. Wheat is a primary source of carbohydrates, proteins, vitamins, and minerals, while maize is rich in carbohydrates, dietary fiber, and essential nutrients such as vitamins A and C (Shewry & Hey, 2015; Rouf Shah *et al.*, 2016). Both crops are critical to human nutrition and food security, particularly in developing countries where they are a primary source of calories (Shewry & Hey, 2015; Rouf Shah *et al.*, 2016).

As the co-occurrence of Pb and nanoparticles in the soil can have complex effects on plants, their potential interactions with soil nutrients and their concentration in plant growth are not yet fully understood; the current study is covering the knowledge gap regarding the impact of AgNPs on maize and wheat growth with and without Pb toxicity. The study aimed to investigate the impact of AgNPs effects on wheat growth, chlorophyll contents, gas exchange attributes, and nutrient concentration when cultivated under Pb stress. It is hypothesized that AgNPs might interact with soil nutrients to alleviate the stress caused by Pb toxicity in plants by improving the availability of N, P, and K and reducing Pb uptake.

Material and Methods

Soil sampling and preparation: Soil samples were collected from a lead-contaminated field near a lead-acid battery factory. The soil was air-dried, passed through a 2-mm sieve, and analyzed for physicochemical properties (Petersen & Calvin, 1986). The texture of sandy loam (30% clay, 50% sand and 20% silt) (Gee & Bauder, 2018). The characteristics of soil and irrigation water are provided in Table 1.

Synthesis of AgNPs: In an erlenmeyer flask, a 100 mL solution containing 1 mM of silver nitrate ($AgNO_3$) was prepared. Silver nanoparticles (AgNPs) were synthesized by combining 10 mL of neem leaf extract with 50 mL of the 1 mM aqueous $AgNO_3$ solution. The mixture was stirred continuously for 20 minutes at room temperature. To prevent auto-oxidation of the silver nitrate, the resulting mixture was incubated in a dark chamber at room temperature. The reduction of silver ions to silver nanoparticles was verified by observing a colour change in the solution, transitioning from reddish to dark brown. After 24 hours, the solution containing Ag-NPs underwent centrifugation at 2000 rpm for 15 minutes. The resulting pellets were carefully separated and subsequently dried in an oven at 100°C for 24 hours (Kumari *et al.*, 2022).

Table 1. Pre-experimental soil, irrigation and *A. fabrum* characteristics.

Soil	Values	References
pH	8.37	(Page *et al.*, 1983)
ECe (dS/m)	5.14	(Rhoades, 1996)
SOM (%)	0.40	(Nelson & Sommers, 1982)
TN (%)	0.025	(Bremner, 1996)
AP (µg/g)	2.47	(Kuo, 2018)
EK (µg/g)	71	(Pratt, 2016)
ENa (µg/g)	216	(Donald & Hanson, 1998)
EPb (µg/g)	0.25	(Estefan *et al.*, 2013)
Texture	Sandy loam	(Gee & Bauder, 2018)
Irrigation	**Values**	**References**
pH	7.15	(Estefan *et al.*, 2013)
EC (µS/cm)	519	
Carbonates (meq./L)	0.00	
Bicarbonates (meq./L)	5.09	
Chloride (meq./L)	0.05	
Ca+Mg (meq./L)	4.99	
Sodium (mg/L)	32	
Pb (µg/g)	0.05	

TN = Total nitrogen; EK = Extractable potassium; AP = Available phosphorus; ENa = Extractable sodium; EPb = Extractable lead

Plant material and pots dimension: Seeds of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) were obtained from a certified seed dealer in Multan. Initially, broken seeds were screened out manually. After that, surface sterilization was done using sodium hypochlorite (5%) (Ahmad *et al.*, 2014). For experimental purposes, plastic pots were used with dimensions, i.e., 20 cm in diameter and 30 cm deep for wheat while 30 cm in diameter and 45 cm deep for maize. For wheat cultivation, 10 kg of soil was filled in each pot, while for maize, 15 kg of soil was filled.

Treatment plan and experimental design: There were 2 levels of lead (Pb), i.e., control (no Pb toxicity) and (400 mg kg^{-1}) (Awashthi, 2000; WHO/FAO, 2007). On the other hand, 2 levels of AgNPs (0 and 100 mg kg^{-1}) were used as treatment (Elshazly *et al.*, 2022). The treatments include control (no Pb and no AgNPs), Pb (400 mg kg^{-1}), AgNPs (100 mg kg^{-1}), and AgNPs +Pb. All the treatments were applied in 4 replicates following a completely randomized design (CRD) in wheat and maize.

Fertilizer and irrigation: The plants were watered twice a week with tap water based on soil moisture (65% field capacity as maintained) content and nutrient solution, i.e., Hoagland's solution (Hoagland & Arnon, 1950). During the experiment, the soil moisture level was consistently regulated at 65% of its field capacity, employing a soil moisture meter (Cubilan 4 in 1 Soil Moisture Meter).

Data collection and analysis: After 60 days of growth, the plants were harvested, and various growth parameters, such as shoot and root length, biomass, chlorophyll content, and nutrient uptake, were measured. The concentration of Pb and other essential nutrients in the plant tissue was determined using atomic absorption spectroscopy.

Morphological attributes: After 60 days of growth, morphological attributes such as plant height (cm), root length (cm), and fresh and dry shoot weight (g) were measured. Plant height was measured using a ruler, and shoot and root length were measured using a measuring tape. Samples were dried in an oven at 70°C for 48 hours to determine the dry weight.

Chlorophyll contents: 0.1 g of fresh leaf tissue was homogenized with 10 ml of 80% acetone solution to extract chlorophyll pigments. The homogenate was centrifuged, and the supernatant was collected. The absorbance of the supernatant was measured at 663 nm and 645 nm using a spectrophotometer (Arnon, 1949).

Antioxidants: For SOD (Giannopolitis & Ries, 1977) and POD activity assays (Hammerschmidt *et al.*, 1982) standard methods were used. For CAT analysis, Aebi (1984) protocol was followed. For MDA analysis, 0.5 g of fresh leaf tissue was homogenized in 5 mL of 10% trichloroacetic acid (TCA) and then centrifuged at 10,000 g for 10 minutes at 4°C. The supernatant was mixed with 0.6% thiobarbituric acid (TBA) and heated at 95°C for 30 minutes. Absorbance was measured at 532 and 520nm to assess the concentration of MDA (Uchiyama & Mihara, 1978).

Leaves nutrients analysis: The nitrogen (N), phosphorus (P), and potassium (K) contents in the leaves of wheat and maize plants were determined after the digestion of samples (Mills & Jones, 1991; Miller, 1997). Leaf samples were collected at the grain-filling stage and oven-dried at 65°C for 72 hours. Then, 0.5 g of dried leaf tissue was ground into fine powder and digested with a mixture of H_2SO_4 and H_2O_2. The N content was determined by the Kjeldahl method (Campbell & Plank, 1998), while the P and K contents were determined by spectrophotometer (HITACHI U-2000, Beijing, China) and flame photometer (PFP 7, Jenway) (Mills & Jones, 1991). For the analysis of Pb, an atomic absorption spectrophotometer was used.

Gas exchange attributes: Expanded and mature leaves from wheat and maize plants were used to measure gas exchange attributes. Measurements were taken between 9:00 am and 11:00 am on a clear day. The photosynthetic rate (Pn), stomatal conductance (gs) and transpiration rate (E) using IRGA (Danish *et al.*, 2020).

Statistical analysis

For standard statistical analysis (Steel *et al.*, 1997), the data obtained were analyzed using OriginPro 2021 (OriginLab Corporation, Northampton, MA, USA) (OriginLab Corporation, 2021). One-way analysis of variance (ANOVA) was performed to determine the effects of treatments on wheat and maize. The significance of the differences between means was evaluated using the Tukey Test at $p \leq 0.05$. Paired comparison graphs were made using OriginPro 2021.

Results

Growth attributes: Results showed that AgNPs and AgNPs + Pb caused improvement in plant height (~6 and ~30%) over control and Pb, respectively, in wheat. AgNPs and AgNPs + Pb showed enhancement of ~5 and ~26% in plant height of maize compared to control and Pb, respectively (Fig. 1A). Pb exhibited a ~43% decrease in root length over control for wheat. Applying AgNPs and Pb+AgNPs resulted in a ~1% and ~43% increase in wheat from the control and Pb, respectively. For Maize, Pb led to a ~45% decrease compared to control. However, treatments AgNPs and Pb+AgNPs improved ~1% and ~54% in root length over the control and Pb, respectively (Fig. 1B). The shoot fresh weight of wheat and maize plants significantly ~32% and ~34% decrease with Pb compared to their respective control. Shoot fresh weight showed a ~8% and ~35% increase with AgNPs and Pb+AgNPs in wheat while ~7% and ~35% in maize over control and Pb, respectively (Fig. 1C). It was noted that AgNPs and AgNPs + Pb caused improvement in shoot dry weight (~13 and ~34%) over control and Pb, respectively, in wheat. Treatments AgNPs and AgNPs + Pb showed enhancement of ~13 and ~33% in shoot dry weight of maize when compared to control and Pb, respectively. However, Pb showed a decline, i.e., ~36 and ~32% in shoot dry weight over control in wheat and maize, respectively (Fig. 1D).

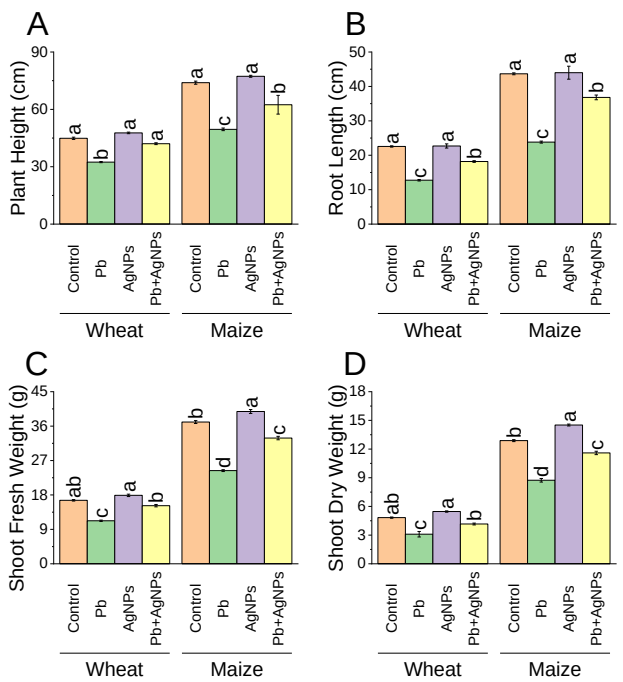
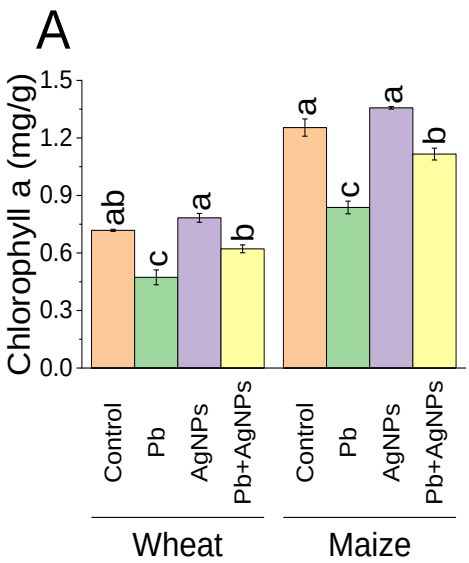
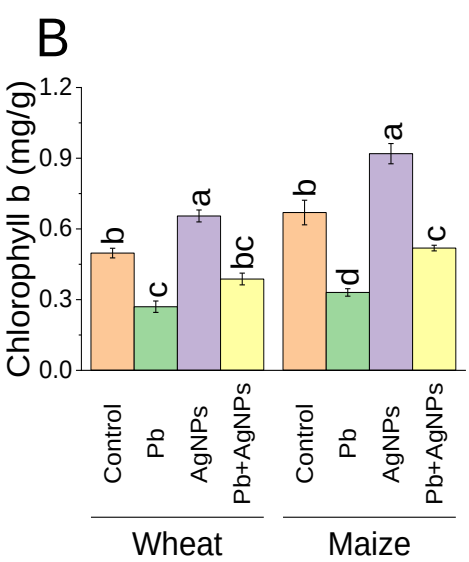


Fig. 1. The impact of AgNPs on shoot length (A), root length (B), shoot fresh weight (C), and shoot dry weight (D) of wheat and maize cultivated with and without Pb stress. Bars are means of 4 replicates ± SE. Different letters showed significant changes at $p \leq 0.05$; Tukey Test.

Chlorophyll contents: For wheat, Pb treatment showed a decrease in chlorophyll a (~34), chlorophyll b (~46%), and total chlorophyll (~39%) content over control. This decrease was also noted in maize chlorophyll a (~33), chlorophyll b (~51%) and total chlorophyll (~39) compared to control where Pb was applied. Adding AgNPs and AgNPs + Pb increased from ~9 and ~31% in chlorophyll a, ~32 and ~44% in chlorophyll b, and ~18 and ~36% in total chlorophyll in wheat than the control and Pb, respectively. In the case of maize treatments AgNPs and AgNPs + Pb showed an enhancement of ~8 and ~33% in chlorophyll a (Fig. 2A), ~37 and ~57% in chlorophyll b (Fig. 2B), and ~18 and ~40% in total chlorophyll (Fig. 2C) in maize than the control and Pb respectively.





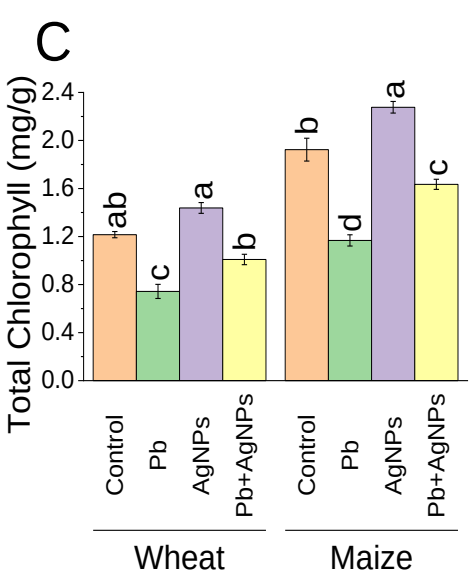
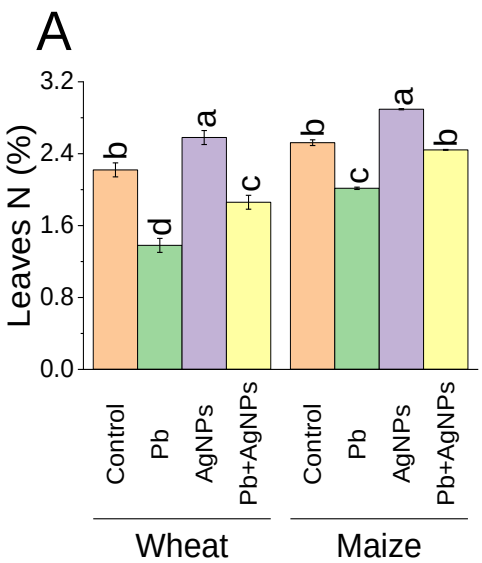
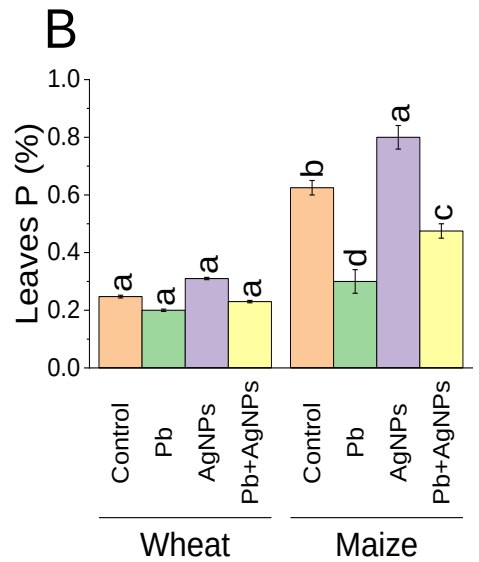


Fig. 2. The impact of AgNPs on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) of wheat and maize cultivated with and without Pb stress. Bars are means of 4 replicates ± SE. Different letters showed significant changes at p≤0.05; Tukey Test.





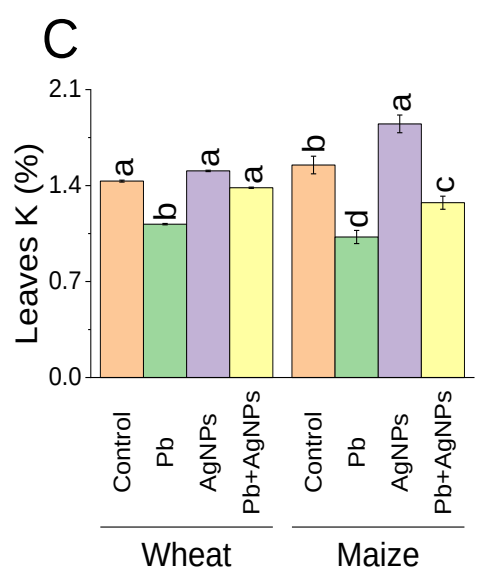


Fig. 3. The impact of AgNPs on leaves N (A), leaves P (B), and leaves K (C) of wheat and maize cultivated with and without Pb stress. Bars are means of 4 replicates ± SE. Different letters showed significant changes at p≤0.05; Tukey Test.

Leaves N, P, and K: Adding Pb in wheat resulted in a ~38% decrease in leaves N, ~19% in leaves P and ~22% in leaves K over the control. For maize, this decrease due to Pb was ~20% in leaves N, ~52% in leaves P, and ~34% in leaves K compared to control. Application of AgNPs and Pb+AgNPs in wheat showed a significant enhancement in leaves N (~16 and ~35%), leaves P (~25 and ~15%), and leaves K (~5 and ~24%) than the control. In maize, AgNPs showed an enhancement in leaves N (~15%), P (~28%), and leaf K (~19%) over control. Adding Pb+AgNPs showed a ~21% increase in leaves N, ~58% in leaves P, and ~24% in leaves K concentration than control (Fig. 3A, B, and C).

Antioxidant activity: In wheat, SOD activity ~31% increased with Pb, ~15% with AgNPs, and ~40% with Pb+AgNPs over the control. In maize, SOD activity showed a ~29% increase with Pb, ~12% with AgNPs, and ~44% with Pb+AgNPs in comparison to the control (Table 2). The results showed that adding Pb in wheat exhibited a ~22% increase in POD activity, while AgNPs and Pb+AgNPs resulted in a ~9% and ~41% increase over the control. In maize, adding Pb resulted in a ~58% increase, AgNPs ~9.00% decrease, and Pb+AgNPs ~29% increase in POD activity compared to the control (Table 2). Regarding CAT activity in wheat, Pb led a ~48% increase, AgNPs showed a ~25% increase, and Pb+AgNPs exhibited a ~72% increase from the control (Table 2). Adding Pb in maize resulted in a ~6% increase, AgNPs and Pb+AgNPs exhibited a ~14% and ~9% decrease in CAT activity compared to the control (Table 2). Compared to the control, adding Pb in wheat showed a ~75% increase in MDA activity, AgNPs ~30% increase, and Pb+AgNPs ~127% increase. (Table 2). In maize, applying Pb resulted in a ~21% decrease in MDA activity, AgNPs ~13% decrease, and Pb+AgNPs ~38% decrease over the control (Table 2).

Gass exchange attributes, shoot and root Pb uptake: In wheat, the photosynthetic rate is a ~46% decrease with Pb, a ~4% increase with AgNPs, and a ~29% decrease with Pb+AgNPs than the control. In maize, adding Pb resulted in a ~67% decrease in photosynthetic rate, a ~16% increase with AgNPs, and a ~24% decrease with Pb+AgNPs over the control (Table 3). The transpiration rate of wheat was assessed in Pb, resulting in a ~12% decrease; AgNPs showed a ~13% increase, and Pb+AgNPs resulted in a 28% decrease compared to the control. In maize, Pb resulted in a ~37% decrease in transpiration rate, AgNPs and Pb+AgNPs showed a ~12% and ~28% increase above the control (Table 3). Adding Pb in wheat resulted in a ~33% decrease in stomatal conductance, a ~21% increase with AgNPs, and a ~14% decrease with Pb+AgNPs from the control. In maize, Pb led a ~40% decrease in stomatal conductance, AgNPs and Pb+AgNPs exhibited a ~46% and ~29% increase compared to the control (Table 3). In wheat, adding Pb increased Pb uptake in the shoot (~211%) and root (~246%) over the control. With AgNPs, Pb uptake in shoot (~64%) and root (~212%) decreased compared to the control, and Pb+AgNPs resulted in a ~116% and ~137% increase over the control in wheat. In maize, Pb increased Pb uptake in shoot (~175%) and root (~244%) over the control. With AgNPs, Pb uptake in shoot (~4%) and root (~26%) decreased in comparison to the control, and with Pb+AgNPs resulted in a ~94% and ~144% increase over the control in maize (Table 3).

Table 2. The impact of treatments on the measured parameters, i.e., SOD (A), POD (B), CAT (C), MDA (D) for both wheat and maize.

Crop	Treatments	SOD (U/mg protein)	POD (U/mg protein)	CAT (U/mg protein)	MDA (nmol/mg protein)
Wheat	Control	9.35 ± 0.13c	6.75 ± 0.13d	13.45 ± 0.13d	1.65 ± 0.13d
	Pb	12.28 ± 0.17ab	8.25 ± 0.21b	19.85 ± 0.21b	2.88 ± 0.17b
	AgNPs	10.73 ± 0.17bc	7.35 ± 0.13c	16.75 ± 0.13c	2.15 ± 0.13c
	Pb+AgNPs	13.10 ± 0.18a	9.50 ± 0.18a	23.15 ± 0.13a	3.75 ± 0.13a
Maize	Control	21.05 ± 1.14d	2.35 ± 0.13d	15.25 ± 0.22b	4.69 ± 0.03a
	Pb	27.10 ± 1.01b	3.70 ± 0.17a	16.10 ± 0.22a	3.88 ± 0.03c
	AgNPs	23.48 ± 0.56c	2.15 ± 0.13c	13.33 ± 0.17d	4.17 ± 0.03b
	Pb+AgNPs	30.28 ± 0.92a	3.03 ± 0.13b	14.03 ± 0.17c	3.43 ± 0.03d

Statistical analysis using Tukey Test revealed that significant changes ($p \leq 0.05$) existed between treatments, as indicated by different letter labels on the mean values ± SD (n=4)

Table 3. The impact of treatments on the measured parameters, i.e., photosynthetic rate, transpiration rate, stomatal conductance, and Pb uptake in shoot and root for both wheat and maize.

Crop	Treatments	Photosynthetic rate (μmol $CO_2/m^2/s$)	Transpiration rate (mmol $H_2O/m^2/s$)	Stomatal conductance (mol $H_2O/m^2/s$)	Pb uptake in shoot (μg/g)	Pb uptake in root (μg/g)
Wheat	Control	11.73 ± 0.17a	4.60 ± 0.18a	0.24 ± 0.036b	1.80 ± 0.082c	5.15 ± 0.129c
	Pb	8.05 ± 0.21c	4.10 ± 0.18b	0.18 ± 0.013d	5.60 ± 0.082a	17.80 ± 0.258a
	AgNPs	12.25 ± 0.26a	5.20 ± 0.47a	0.29 ± 0.034a	1.10 ± 0.082d	1.65 ± 0.129d
	Pb+AgNPs	9.13 ± 0.30b	3.60 ± 0.18c	0.21 ± 0.008c	3.88 ± 0.171b	12.18 ± 0.222b
Maize	Control	18.15 ± 0.29b	4.85 ± 0.13c	0.35 ± 0.013c	2.35 ± 0.129c	6.35 ± 0.129c
	Pb	10.90 ± 0.32d	3.55 ± 0.13d	0.25 ± 0.013d	6.45 ± 0.129a	21.83 ± 0.275a
	AgNPs	21.13 ± 0.17a	5.45 ± 0.13b	0.51 ± 0.017a	2.25 ± 0.129c	5.05 ± 0.129d
	Pb+AgNPs	14.45 ± 0.57c	6.25 ± 0.13a	0.45 ± 0.013b	4.55 ± 0.208b	15.50 ± 0.258b

Statistical analysis using Tukey Test revealed that significant changes ($p \leq 0.05$) existed between treatments, as indicated by different letter labels on the mean values ± SD (n=4)

Discussion

Lead (Pb) toxicity: Lead (Pb) is a highly toxic heavy metal that can accumulate in the soil and negatively affect plant growth and development (Ghafoor *et al.*, 2023). The toxicity of Pb is attributed to several mechanisms, such as the inhibition of enzymatic activities, oxidative stress, and alteration of nutrient uptake and transport (Sharma & Dubey, 2005). The present study observed that Pb treatment significantly reduced the growth of both maize and wheat crops and decreased the photosynthetic rate, transpiration rate, and stomatal conductance over control. This is consistent with previous studies that reported Pb-induced growth and physiological parameter reductions in various plant species (Islam *et al.*, 2008; Irfan *et al.*, 2021). Similar results were also noted in the current study, where Pb toxicity caused a significant decline in gas exchange attributes of wheat and maize, i.e., photosynthetic rate, transpiration rate and stomatal conductance (Table 3). The toxic effects of Pb on plant growth are mainly due to the inhibition of enzymatic activities involved in photosynthesis, respiration, and nutrient metabolism (Collin *et al.*, 2022). Pb can bind to sulfhydryl groups in enzymes, thereby inhibiting their activity and reducing photosynthetic and respiratory rates (Flora *et al.*, 2012). Additionally, Pb can induce oxidative stress by generating reactive oxygen species (ROS), which can cause damage to cellular components such as lipids, proteins, and DNA. The accumulation of ROS can also disrupt the balance of antioxidant defense mechanisms, leading to further damage (Małkowski *et al.*, 2020).

Several studies have reported that silver nanoparticles (AgNPs) can improve plant growth parameters under heavy metal stress, such as Pb toxicity (Kim *et al.*, 2012; Premkumar *et al.*, 2018; Elshazly *et al.*, 2022). The addition of AgNPs has been found to enhance shoot and root length, as well as the fresh and dry weight of shoots while reducing the level of antioxidants (Ojemaye *et al.*, 2021). One possible mechanism for the beneficial effects of AgNPs is their ability to scavenge free radicals and reactive oxygen species (ROS) produced under Pb stress. ROS can cause oxidative damage to plant cells and lead to a decrease in growth parameters. AgNPs can act as antioxidants by directly scavenging ROS or by upregulating the expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). Such arguments are also in line with our findings where AgNPs caused significant enhancement in SOD, POD and CAT over control and Pb treatments (Table 2). The mechanism of AgNPs on antioxidant activity involves multiple pathways, i.e., modulation of gene expression, Nrf2-Keap1 pathway, influencing their redox status of proteins, lipids, and DNA, contributing to the enhancement of the antioxidant defense system in plants. The AgNPs exhibit intrinsic antioxidant properties, directly scavenging ROS like superoxide radicals (O_2^-) and hydroxyl radicals (OH^-), thereby preventing oxidative damage to cellular components (Zare *et al.*, 2020).

Additionally, AgNPs can upregulate the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which are crucial for detoxifying ROS and maintaining cellular redox balance. Furthermore, AgNPs may stimulate the synthesis of non-enzymatic antioxidants like glutathione (GSH) and ascorbic acid, along with phenolic compounds, which act as ROS scavengers, fortifying plant cells against oxidative stress (Hafez & Fouad, 2020). Overall, AgNPs bolster the antioxidant defense system in plants through direct scavenging of ROS, upregulation of antioxidant enzymes, and enhancement of non-enzymatic antioxidant synthesis, promoting plant health and resilience against environmental stresses (Yang *et al.*, 2021).

In addition to their antioxidant activity, AgNPs can improve plant nutrient uptake. Pb toxicity can cause a decrease in the absorption and transport of essential nutrients such as nitrogen, phosphorus, and potassium, leading to a decrease in plant growth. AgNPs can enhance nutrient uptake by increasing the permeability of the cell membrane and activating nutrient transporters (Guzmán-Báez *et al.*, 2021). In the current experiment, a significant enhancement in N, P and K of leaves validates the effectiveness of AgNPs against Pb toxicity in wheat and maize (Fig. 3). This also justified the significant enhancement in fresh and dry weight of shoot, shoot length and root length. It is a fact that better uptake of N, P and K promotes the morphological growth attributes of crops (Ding *et al.*, 2016). The mechanism underlying the improvement in photosynthetic pigments with the use of AgNPs involves several factors that positively impact the photosynthetic process in plants (Tejada-Alvarado *et al.*, 2023). The AgNPs have been shown to enhance chlorophyll biosynthesis by upregulating the expression of genes involved in chlorophyll synthesis pathways. This leads to increased chlorophyll content in plant tissues, which is essential for efficient light absorption during photosynthesis (Verma *et al.*, 2020).

Furthermore, AgNPs can also chelate Pb in the soil, preventing its uptake by the plant. This reduces the accumulation of Pb in the leaves and roots, which can cause toxicity and reduce growth parameters. AgNPs can form complexes with Pb ions through electrostatic interactions, which can reduce the bioavailability of Pb in the soil and prevent its uptake by the plant (Khan *et al.*, 2020). Elaborating on the specific mechanisms through which AgNPs enhance nutrient uptake can significantly strengthen the argument. AgNPs have been shown to increase membrane permeability, allowing for improved passage of nutrients across cell membranes (Mikhailova, 2020).

Additionally, AgNPs can activate nutrient transporters, facilitating the uptake of essential nutrients into plant cells. Collectively, these mechanisms enhance the efficiency of nutrient absorption and utilization by plants, leading to improved growth and productivity (Siddiqi & Husen, 2022). Adding AgNPs can improve plant growth parameters under Pb stress by reducing oxidative damage, enhancing nutrient uptake, and chelating Pb in the soil. These mechanisms can ultimately improve the yield and quality of crops grown in Pb-contaminated soil.

Conclusion

In conclusion, adding AgNPs to Pb-contaminated soil can improve nutrient uptake and reduce Pb uptake in leaves and roots, leading to improved plant growth and physiological parameters. The mechanisms underlying these effects include enhanced enzymatic activity, nutrient uptake, and chelation of Pb ions by AgNPs. These findings have significant practical implications for remediation strategies in Pb-contaminated agricultural soils, highlighting the potential of AgNPs to mitigate the adverse effects of heavy metal pollution on crop productivity and plant health. Further research is needed to explore the potential of AgNPs as a safe and effective means of reducing the toxic effects of heavy metals on plant growth and development.

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