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Foliar application of silver nanoparticles differentially intervenes remediation statuses and oxidative stress indicators in *Abelmoschus esculentus* planted on gold-mined soil

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ABSTRACT

The study assessed the intervention of foliar application of silver nanoparticles (AgNPs) on heavy metal toxicity and phytoremediation status of *Abelmoschus esculentus* planted in gold-mined soil. The green synthesized AgNPs absorbed maximally at 425 nm, had an average particle size of 55 ± 2.3 nm and peaks at 3,443 and $1,636 \text{ cm}^{-1}$. *A. esculentus* seeds were grown in gold-mined soil and its seedlings were wetted with water and different concentrations of AgNPs (0.75, 0.50 and 0.25 mg/mL). Foliar applications of AgNPs significantly improved percentage heavy metal remediation and reduced contamination intensity by 60% and 44%, respectively in *A. esculentus*. Heavy metals induced oxidative stress in *A. esculentus* wetted with water which manifested in the reduction of growth performance and photosynthetic pigments by 43% and 15% in that order. Significant overexpression of superoxide dismutase activity and malondialdehyde by 70% and 86%, respectively together with a significant reduction in carotenoid contents and antioxidant activity by 92% and 15%, respectively were obtained for *A. esculentus* in control. The intervention of foliar application considerably protected *A. esculentus* with improved physiology, enzymic and non-enzymic antioxidant activities. These results conclude that foliar application AgNPs beneficially mediated toxicities of heavy metals in plants.

Novelty statement

Gold mining is an economic venture but contamination of ecological matrixes by heavy metals usually accompanies it. Farming on either an active or abandoned gold site can predispose residents to the toxicity of heavy metals. Therefore, remediation before or during cultivation is key to ensuring safety. Silver nanoparticles have proved effective in remediating heavy metals and improving biochemical activities in plants due to their intrinsic properties and adsorptive potentials.

KEYWORDS

AgNPs; enzymic activities; foliar application; gold-mined soil; toxicity

Introduction

Gold mining sites are typically characterized by widespread heavy metal pollution (Adeoye 2016; Eludoyin *et al.* 2017). In many communities where gold mining is preponderant, public health disasters have been reported ranging from lead poisoning, neurological disorders in children, deformities, lower life expectancy, land degradation, water pollution, erosion and loss of biodiversity (Amoakwah *et al.* 2020; Rabiun *et al.* 2020). Usually, settlements in and around gold mining sites contend with contamination of environmental and ecological media (Adewumi *et al.* 2020; Basir-Cyio *et al.* 2020). Ironically, the major occupation of the inhabitants of these settlements is farming and with uncontrolled artisanal mining, residents are susceptible to toxic pollutants that affect lives and livelihood. Heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr) and arsenic (As) at low

concentrations do not assist in any biological activities but toxic to plants and humans (Liu *et al.* 2019; Adewumi *et al.* 2020). Exposure to heavy metals has been linked to disruption in the electron transport chain of the nervous system, oxidative stress, and cancers of blood, liver, kidney and skin. They distort plant physiology, generate free radicals that hinder cell proliferation, nutrient uptake and formation of photosynthetic pigments in plants (Singh *et al.* 2017; Ogunkunle *et al.* 2018, 2020; Azeez, Adejumo, *et al.* 2019; Raklami *et al.* 2021). Due to the lack of available farmlands, residents of gold mining communities cultivate active and abandoned gold mining sites. The possibilities of exposure to heavy metals through the consumption of vegetables planted on gold-mined soil are worrying.

To prevent this, different remediation systems ranging from chelation/complexation, soil flushing, phytoremediation,

chemical immobilization, microbial degradation, chemical treatment, bioremediation and adsorption among others are good techniques but not without limitations (Galdames *et al.* 2017; Lawal *et al.* 2021; Raklami *et al.* 2021). In addition, nutrient management and mobilization for plants including strengthening their mechanisms against heavy metals' toxicities are lacking in these systems. Therefore, an effective heavy metal remediation system without being a source of pollution itself is a promising alternative (Salem *et al.* 2018).

The use of nanoparticles for remediating heavy metal-polluted sites is effective, cost-efficient, integrative, environmental-friendly and less arduous (White and Gardea-Torresdey 2018; Azeez, Adejumo, *et al.* 2019). Metal nanoparticles enjoy excellent morphological properties and reactive adsorptive sites required for remediating heavy metals. They have been found to effectively adsorb heavy metals such as Cd, Pb, As, Ni and Cr, reduce toxicities and translocation rate. Additionally, they offer nutrient support and pesticidal actions against recalcitrant phytopathogens. They were equally found to promote morphological and biochemical indices in plants by stimulating enzymes desirable for ideal performance (Guerra *et al.* 2018; Hasanuzzaman *et al.* 2018; Seleiman, Almutairi, *et al.* 2020; Seleiman, Alotaibi, *et al.* 2020; Zare *et al.* 2020). Metal nanoparticles can either be applied to soil and via foliage for soil remediation. Studies have reported foliar and soil applications of nanoparticles aided plant growth, improved enzyme activities, adsorbed heavy metals and modulated essential nutrients levels (Sharma 2017; Singh *et al.* 2017; White and Gardea-Torresdey 2018; Ogunkunle *et al.* 2018). Silver nanoparticles (AgNPs) biologically synthesized using extracts from plants' parts, cobweb and cell-free enzymes have found immeasurable applications in agriculture as nanofertilizers, nanopesticides, and adsorbents for pollutants in soils due to their unequaled reactive surfaces, adsorption efficiency, phytomodulating properties and nutrient mobilization (Azeez, Lateef, *et al.* 2019; Lawal *et al.* 2021).

Okra (*Abelmoschus esculentus* L. Moench) is an economically cheap, heat-tolerant and drought-resistant flowering vegetable with a good nutritional profile for human consumption (Durazzo *et al.* 2018). It is a medicinal plant containing health-promoting bioactive compounds for treating ulcers, diabetes and neurodegenerative diseases. It contains polyphenols, flavonoids, alkaloids, vitamins, essential minerals and high dietary fiber but it is highly perishable due to its moisture content (Liu *et al.* 2019; Xu *et al.* 2020).

Different studies have reported the applications of various nanoparticles for heavy metal remediation from simulated soil contamination and were conducted with premediated heavy metal concentration that does not reflect the true status of field contamination. This study, therefore, considered exposure to heavy metals from the consumption of vegetables planted on gold-mined soil (source contamination) and its attendant consequences on health. Consequently, silver nanoparticles through foliar application were used as mediating agents against toxicities of heavy metals found in gold-mined soil. An assessment of their effects on biochemical parameters in *A. esculentus* was also determined using

different treatment conditions to achieve the highest remediation status, and best protection on biochemical parameters.

Materials and methods

Green synthesis of AgNPs

Green synthesis of AgNPs was mediated with the extract of *Prunus dulcis* (almond) leaves using the method reported by Lateef *et al.* (2016). Precisely 1 mL of *P. dulcis* leaf extract was added to 40 mL of AgNO₃ solution and this was monitored for some minutes for color change. The green synthesized AgNPs were characterized using UV-visible spectrometer (Biobase BK-UV1900 PC spectrometer, China), Fourier transform infrared spectrometer (FTIR) from 400 to 4,000 cm⁻¹ (SHIMADZU) and Scanning Electron Microscope with energy dispersive X-Ray (Jeol JSM-6400).

Soil sampling and planting experiments

Soil samples were collected from Ijana Wasare on latitude 7.607549 N and longitude 4.740614°E. Ijana Warase is a town near Ilesa in Atakumosa Local Government. It is an agrarian settlement and one of the gold-rich communities where land degradation is a common occurrence arising from unprofessional artisanal gold mining (Adeoye 2016).

Representative soil samples were collected at three places between 0 and 25 cm depth, mixed thoroughly, air-dried, pulverized and sieved with 600 and 300 µm wire meshes.

Exactly 500 g of soil was filled into sixteen (16) buckets; four (4) buckets for each group. Ten seeds of *A. esculentus* were randomly planted in each bucket and wetted with water until they sprouted to seedlings. Foliar application of AgNPs on *A. esculentus* seedlings was done with 0.75, 0.5 and 0.25 mg/mL for groups B, C, and D, respectively; group A had only water. The planting was for twenty (20) days with 100 mL solution of each concentration of AgNPs and watered daily. The *A. esculentus* vegetables were harvested and washed thoroughly. A portion of the harvested samples was used freshly for biochemical analyses including enzyme assay, malondialdehyde, chlorophyll pigments, and the remaining portion was dried at 80 °C to constant weight.

Analysis of mineral and heavy metal contents in gold-mined soil and *A. esculentus*

Precisely 1 g of both pre-and post-planting soil was digested with HNO₃:HCl (7:3) while 0.5 g of *A. esculentus* was digested with HNO₃:HClO₄. Mineral and heavy metal contents were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES) as reported by Azeez *et al.* (2020).

Remediation of heavy metal and contamination intensity

The heavy metals contamination intensity (CI) and phytoremediation status were calculated with Equations (1) and (2), respectively. When CI > 1, *A. esculentus* has accumulated

high concentrations of heavy metals. When $CI = 1$, accumulation is minimal and *A. esculentus* activities may not be influenced by heavy metals. When $CI < 1$, *A. esculentus* avoided uptake of heavy metals.

$$\text{Contamination intensity} = \frac{\text{heavy metal contents in } A. \text{esculentus shoot}}{\text{heavy metal content in corresponding soil}} \quad (1)$$

$$\text{Remediation status} = \frac{\text{heavy metal content in pre-planting soil} - \text{heavy metal content in post-harvest soil}}{\text{heavy metal content in pre-planting soil}} \times 100 \quad (2)$$

Determination of germination indices

After harvesting and thorough washing, germination parameters such as lengths of roots and shoots, percentage germination and the number of leaves were determined as reported by Azeez, Adejumo, *et al.* (2019).

Determination of biochemical parameters, lipid peroxidation and photosynthetic pigment contents

The enzymic activities of superoxide dismutase (SOD), catalase (CAT) and total protein (TP) were assayed following the extraction methods of Ogunkunle *et al.* (2020). One gram of fresh *A. esculentus* leaf was extracted by homogenizing in cold 100 mM potassium phosphate buffer (pH 7.8) that included 0.1 M EDTA (ethylenediamine-tetraacetic acid), 1% PVP (polyvinylpyrrolidone), and 0.5% Triton X-100 and centrifuged at $18,000 \times g$ for 20 min at 4°C . The resulting supernatant was used for analyses of SOD, CAT and TP. The activities of CAT, SOD and TP were done according to the methods of Aebi (1974), Beyer and Fridovich (1987) and Bradford (1976), respectively.

The activity of glutathione (GSH) was assayed by homogenizing 0.5 g of fresh *A. esculentus* leaf with 5% m-phosphoric acid that contained 1 mM EDTA and centrifuged at $11,500 \times g$ for 15 min at 4°C . The resulting supernatant was analyzed for GSH using the method reported by Hasanuzzaman *et al.* (2018).

The free radical scavenging ability of *A. esculentus* subjected to different treatments was evaluated by extracting 2 g of dried leaves with 70% aqueous methanol twice, filtered and concentrated. The ability of the extract to bleach 2,2-diphenyl-1-picrylhydrazyl (DPPH) was recorded at 517 nm as reported by Azeez, Adejumo, *et al.* (2019).

The malondialdehyde (MDA) level (a product of lipid peroxidation) was done by homogenizing 0.2 g of fresh *A. esculentus* with 5 mL ice-cold ethanol followed by centrifugation at 3,500 rpm for 20 min. 1 mL of the supernatant was added to 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 in ice and centrifuged at 3,500 rpm for 30 min. The absorbance of the supernatant

was recorded at 440, 532 and 600 nm as reported by Gupta *et al.* (2018).

Photosynthetic pigment contents were measured as reported by Azeez, Adejumo, *et al.* (2019). The extraction was done by homogenizing 0.1 g of fresh *A. esculentus* in 5 mL chilled acetone (80%), centrifuged (5,000 rpm for 5 min) and re-extracted twice with 5 mL acetone (80%). The measurement of absorbance values was done at 470, 645, 663 nm for the determination of chlorophyll a, b and

carotenoids.

Statistical analysis

The results of metal content and biochemical parameters are expressed as mean $\pm$ standard error of mean (SEM) of three concordant triplicate values. Data on lengths of root and shoot and germination percentage are expressed as the number of germinated seedlings. These results were subjected to one-way ANOVA followed by Turkey's HSD Post Hoc test for comparison of means and correlation matrix at $p < 0.05$ using SPSS 2020 (IBM Corp. Released 2020 IBM SPSS statistics for Windows, Version 27.0, Armonk, NY).

Results and discussion

Green synthesis of AgNPs

Green synthesized AgNPs mediated with extract of *P. dulcis* leaves had a dark brown color with maximum absorption at 425 nm (Figure 1a). This peak is characteristic of AgNPs and it occurred within the range of 391–460 nm mostly reported for AgNPs (Lateef *et al.* 2016). It was a colloidal solution characterized by intense peaks at 3,443 and $1,636 \text{ cm}^{-1}$ (Figure 1b) with an average particle size of $55 \pm 2.3 \text{ nm}$ (SEM: Figure 2). The presence of these peaks indicates N-H of aromatic amine and C=O of amide of protein and polyphenolic biomolecules in *P. dulcis* responsible as reducing and capping agents for the mediation of AgNPs. The plasmonic appearance and the prominence of Ag in EDX indicate the conversion of Ag^+ to zerovalent Ag (Figure 2) (Lateef *et al.* 2016; Lawal *et al.* 2021).

Elemental concentrations of gold-mined soil and *A. esculentus*

The gold-mined soil samples were basically clay (clay: 70.02%, silt: 18.74% and sand: 11.24%), slightly acidic (pH

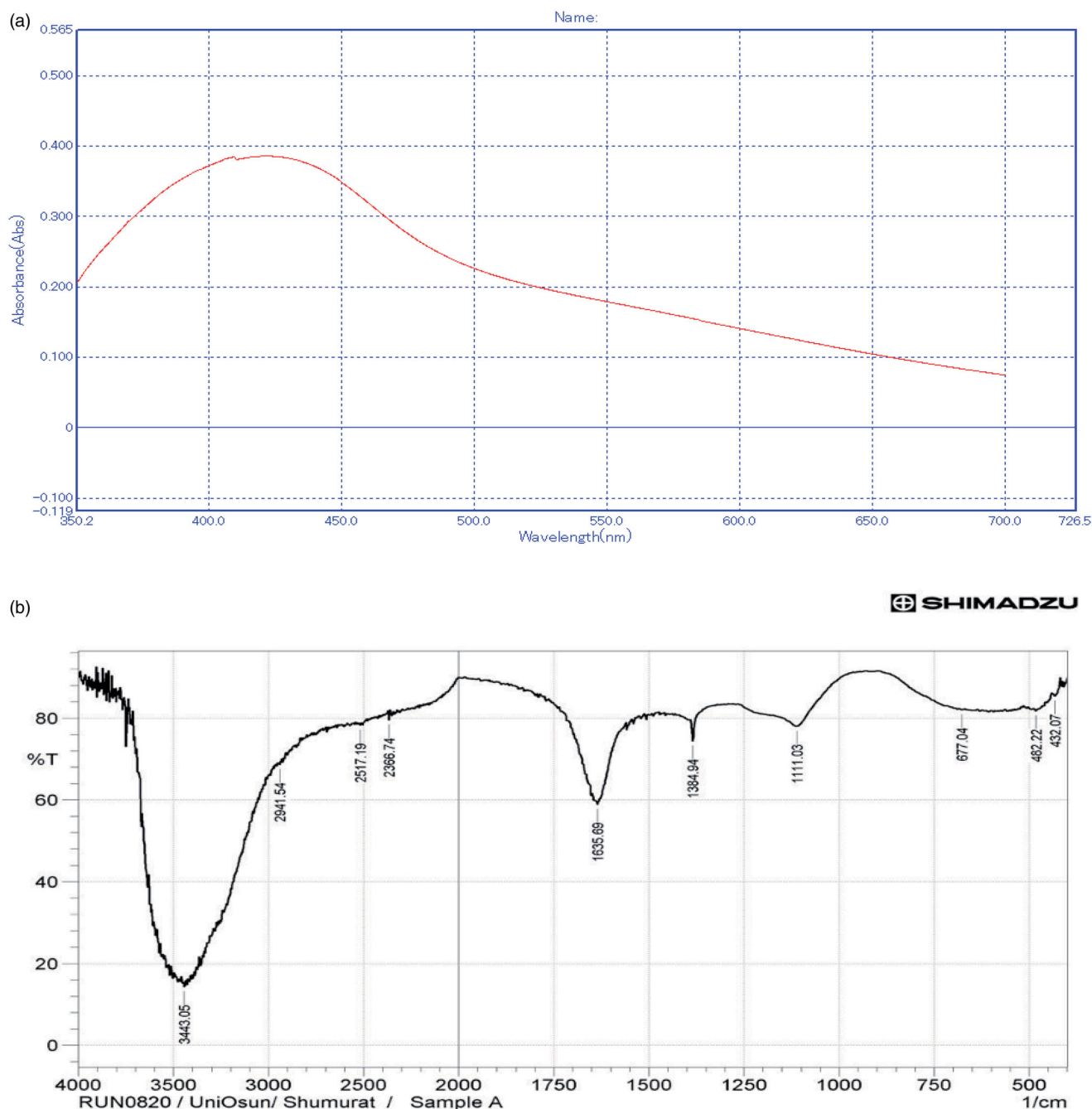


Figure 1. (a) UV-Visible spectrum of green synthesized AgNPs mediated with leaf extract of *P. dulcis*. (b) FTIR spectrum of green synthesized AgNPs mediated with leaf extract of *P. dulcis*.

6.3), averagely fertile (organic matter: 6.79 mg kg^{-1}) and had Fe ($638.195 \pm 2.531 \text{ mg kg}^{-1}$), K ($17.236 \pm 0.129 \text{ mg kg}^{-1}$), Mg ($30.286 \pm 0.394 \text{ mg kg}^{-1}$), Al ($103.252 \pm 0.688 \text{ mg kg}^{-1}$), Na ($21.468 \pm 0.391 \text{ mg kg}^{-1}$), P ($19.67 \pm 0.686 \text{ mg kg}^{-1}$), Ca ($12.716 \pm 0.042 \text{ mg kg}^{-1}$), S ($4.69 \pm 0.235 \text{ mg kg}^{-1}$), Mn ($8.745 \pm 0.000 \text{ mg kg}^{-1}$), Cu ($0.519 \pm 0.000 \text{ mg kg}^{-1}$), Ni ($0.403 \pm 0.000 \text{ mg kg}^{-1}$), Ag ($0.010 \pm 0.000 \text{ mg kg}^{-1}$), Au ($0.266 \pm 0.001 \text{ mg kg}^{-1}$), Cd ($0.036 \pm 0.001 \text{ mg kg}^{-1}$), Co ($0.084 \pm 0.000 \text{ mg kg}^{-1}$), Cr ($1.738 \pm 0.003 \text{ mg kg}^{-1}$), Hg ($0.007 \pm 0.000 \text{ mg kg}^{-1}$), Pb ($0.732 \pm 0.001 \text{ mg kg}^{-1}$), Zn ($1.592 \pm 0.001 \text{ mg kg}^{-1}$) and As ($0.020 \pm 0.000 \text{ mg kg}^{-1}$) (Table 1). The elemental profiles of gold-mined soil were characterized by high concentrations of essential metals and

minimal contents of heavy metals. Although, the concentrations of heavy metals were below the acceptable limit, yet their presence is undesirable especially the highly hazardous metals (Rinklebe *et al.* 2019; Adewumi *et al.* 2020). The biologically non-essential metals such as Pb, Cd, As, Hg and Cr at small concentrations have been linked with kidney damage, sore throat, disruption of hemoglobin, developmental thinking prowess, reproductive, cardiovascular and immune systems. These metals are harmful to plants disrupting their metabolisms (Hasanuzzaman *et al.* 2018; Salem *et al.* 2018; Azeez, Adejumo, *et al.* 2019; Amoakwah *et al.* 2020).

Significant correlation coefficients existed between Au and heavy metals especially with Cr (0.977), Fe (0.922), Ni

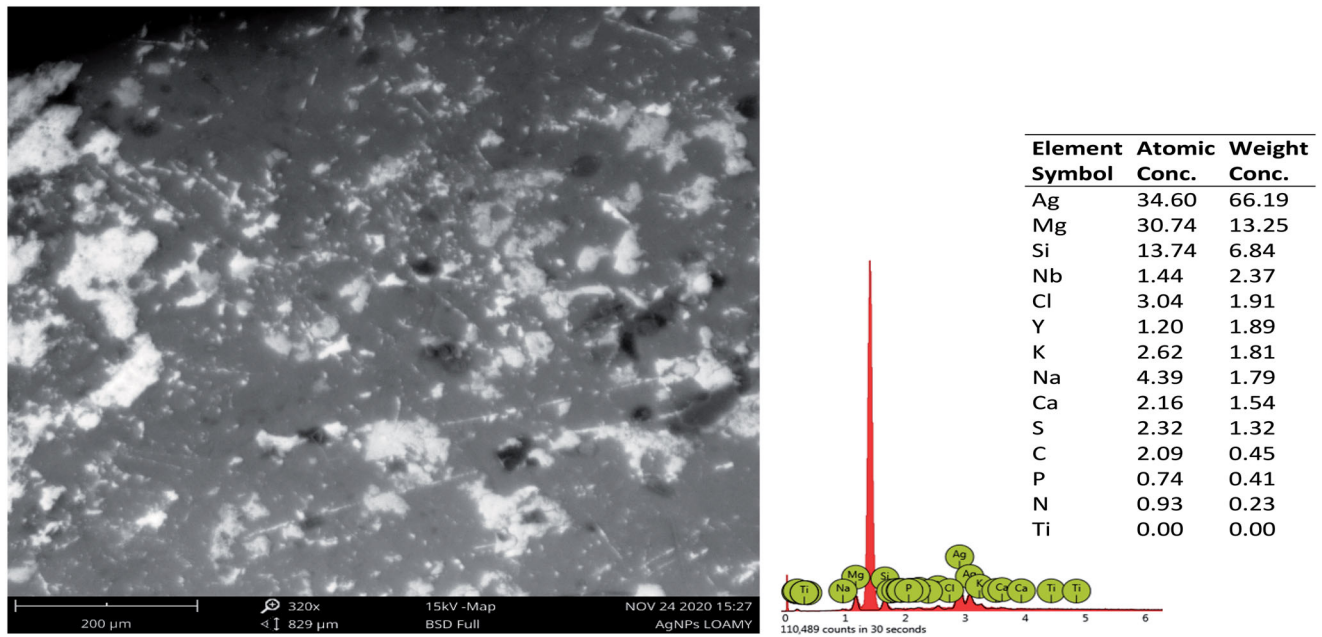


Figure 2. SEM and EDX images of green synthesized AgNPs mediated with leaf extract of *P. dulcis*.

Table 1. Concentrations of heavy metals and minerals analyzed in gold-mined soil and *A. esculentus*.

Metals	Soil					Plants			
	RS	AS	BS	CS	DS	AP	BP	CP	DP
Au	0.266 ± 0.001 ^a	0.264 ± 0.000 ^a	0.171 ± 0.001 ^b	0.166 ± 0.001 ^b	0.206 ± 0.001 ^c	0.206 ± 0.007 ^a	0.055 ± 0.000 ^b	0.107 ± 0.000 ^c	0.054 ± 0.000 ^b
Cd	0.036 ± 0.001 ^a	0.023 ± 0.001 ^b	0.015 ± 0.000 ^c	0.016 ± 0.000 ^c	0.016 ± 0.001 ^c	0.016 ± 0.001 ^a	0.008 ± 0.000 ^b	0.003 ± 0.000 ^{bc}	0.005 ± 0.000 ^b
Co	0.084 ± 0.000 ^a	0.071 ± 0.000 ^b	0.062 ± 0.000 ^c	0.076 ± 0.000 ^b	0.078 ± 0.000 ^b	0.055 ± 0.001 ^a	0.014 ± 0.002 ^b	0.006 ± 0.000 ^c	0.013 ± 0.002 ^b
Cr	1.738 ± 0.003 ^a	1.574 ± 0.001 ^b	0.734 ± 0.001 ^c	0.874 ± 0.000 ^d	1.096 ± 0.000 ^e	0.074 ± 0.000 ^a	0.074 ± 0.000 ^a	0.086 ± 0.001 ^b	0.086 ± 0.000 ^c
Fe	638.195 ± 2.531 ^a	595.071 ± 1.290 ^b	431.516 ± 2.577 ^c	470.496 ± 1.291 ^d	547.289 ± 1.354 ^e	150.770 ± 7.611 ^a	180.653 ± 7.202 ^b	160.110 ± 1.860 ^c	110.228 ± 2.547 ^d
Hg	0.007 ± 0.000 ^a	0.004 ± 0.000 ^a	0.004 ± 0.000 ^a	0.012 ± 0.000 ^b	0.005 ± 0.000 ^a	0.003 ± 0.000 ^a	0.002 ± 0.000 ^a	0.002 ± 0.000 ^a	0.002 ± 0.000 ^a
Mn	8.745 ± 0.000 ^a	6.517 ± 0.000 ^b	8.724 ± 0.000 ^a	6.132 ± 0.000 ^c	8.035 ± 0.000 ^d	0.001 ± 0.000 ^a	0.006 ± 0.000 ^b	0.002 ± 0.000 ^c	0.002 ± 0.000 ^c
Ni	0.403 ± 0.000 ^a	0.334 ± 0.000 ^b	0.233 ± 0.000 ^c	0.243 ± 0.001 ^c	0.315 ± 0.000 ^b	0.025 ± 0.001 ^a	0.016 ± 0.000 ^b	0.015 ± 0.000 ^c	0.023 ± 0.001 ^a
Pb	0.732 ± 0.001 ^a	0.727 ± 0.000 ^a	0.423 ± 0.001 ^b	0.564 ± 0.000 ^c	0.615 ± 0.001 ^d	0.334 ± 0.001 ^a	0.051 ± 0.000 ^b	0.062 ± 0.000 ^c	0.145 ± 0.001 ^d
Sn	0.556 ± 0.000 ^a	0.214 ± 0.001 ^b	0.486 ± 0.000 ^c	0.264 ± 0.000 ^d	0.333 ± 0.001 ^e	0.145 ± 0.001 ^a	0.353 ± 0.000 ^b	0.252 ± 0.000 ^c	0.284 ± 0.001 ^d
Zn	1.592 ± 0.001 ^a	1.492 ± 0.001 ^b	1.313 ± 0.000 ^c	1.355 ± 0.001 ^d	1.404 ± 0.001 ^e	0.006 ± 0.000 ^a	0.004 ± 0.000 ^a	0.005 ± 0.000 ^a	0.014 ± 0.000 ^b
As	0.020 ± 0.000 ^a	0.013 ± 0.000 ^b	0.008 ± 0.000 ^c	0.007 ± 0.000 ^c	0.013 ± 0.000 ^b	0.004 ± 0.000 ^a	0.002 ± 0.000 ^a	0.004 ± 0.000 ^a	0.002 ± 0.000 ^a
Cu	0.519 ± 0.000 ^a	0.286 ± 0.000 ^b	0.225 ± 0.001 ^c	0.214 ± 0.000 ^c	0.304 ± 0.000 ^d	0.034 ± 0.000 ^a	0.023 ± 0.000 ^b	0.063 ± 0.001 ^c	0.034 ± 0.001 ^a
Al	103.252 ± 0.688 ^a	88.047 ± 0.285 ^b	67.530 ± 0.336 ^c	90.953 ± 0.428 ^d	57.033 ± 0.460 ^e	30.077 ± 0.484 ^a	10.793 ± 0.232 ^b	10.340 ± 0.073 ^b	10.537 ± 0.645 ^b
Ca	12.716 ± 0.042 ^a	11.160 ± 0.114 ^b	9.652 ± 0.000 ^c	10.684 ± 0.000 ^b	6.423 ± 0.000 ^d	5.984 ± 0.001 ^a	8.943 ± 0.062 ^b	6.105 ± 0.015 ^c	11.124 ± 0.009 ^d
K	17.236 ± 0.192 ^a	15.477 ± 0.032 ^b	13.502 ± 0.449 ^b	16.317 ± 0.259 ^b	13.616 ± 0.341 ^b	19.606 ± 0.394 ^a	23.407 ± 1.043 ^b	21.044 ± 0.829 ^a	27.184 ± 0.62 ^c
Mg	30.286 ± 0.394 ^a	29.819 ± 0.407 ^a	21.942 ± 0.320 ^b	28.755 ± 0.352 ^a	22.764 ± 0.337 ^b	13.720 ± 0.131 ^a	17.908 ± 0.485 ^b	16.181 ± 0.043 ^c	13.255 ± 0.919 ^d
Na	21.468 ± 0.391 ^a	22.685 ± 0.323 ^a	18.972 ± 0.084 ^b	20.598 ± 0.013 ^c	17.833 ± 0.258 ^b	15.214 ± 0.104 ^a	12.895 ± 0.335 ^b	18.237 ± 0.492 ^c	17.983 ± 0.262 ^c

Results: mean ± standard error of mean (SEM). Results with different superscripts are significantly different ($p < 0.05$). RS: Raw soil (Gold soil); AS: Control (Water) – gold-mined soil foliar wetted with water; BS: gold-mined soil with foliar applications of 0.75 mg/ml AgNPs; CS: gold-mined soil with foliar applications of 0.50 mg/ml AgNPs; DS: gold-mined soil with foliar applications of 0.25 mg/ml AgNPs. AP (Control) – *A. esculentus* planted on gold-mined soil foliar wetted with water, BP, CP and DP – *A. esculentus* planted on gold-mined soil with foliar applications of AgNPs at 0.75, 0.50 and 0.25 mg/ml AgNPs, respectively.

(0.910), Zn (0.958), As (0.847) and Pb (0.816) whereas a non-significant correlation occurred between Au and essential minerals in gold-mined soil. Average correlation coefficients were obtained between some biologically non-important heavy metals and essential minerals such as Cd-Mg (0.569), Cd-Na (0.693), Cr-Mg (0.598), Cr-Na (0.656), Pb-Mg (0.685) and Pb-Na (0.597). High correlation coefficients were recorded among all essential metals (Supplementary material). The significant correlation matrices obtained between Au and other heavy metals imply that with continuous artisanal gold mining activities, heavy metals contamination would accompany it (Eludoyin *et al.* 2017; Amoakwah *et al.* 2020; Basir-Cyio *et al.* 2020). Also, noticeable in the results are the high occurrences of Al and Fe

beyond the permissible limit that could be injurious to man and plants. The prominent presence was worrisome as it has been implicated in nervous systems imbalances and in the overproduction of reactive radicals that are toxic to plants (Nahar *et al.* 2016; Rinklebe *et al.* 2019; Chijioke *et al.* 2020; Rabiou *et al.* 2020).

Elemental abundance in *A. esculentus* planted on gold-mined soil and exposed to different treatments follows Fe > Al > K > Na > Ca > Pb > Au > Sn > Cr > Co > Cu > Ni > Cd > Zn > As > Sn = Hg > Mn (Table 1). Essential minerals were principally more abundant in *A. esculentus* intervened with AgNPs whereas heavy metal concentrations were substantially reduced due to interventions of foliar treatment with AgNPs. The improvements in essential

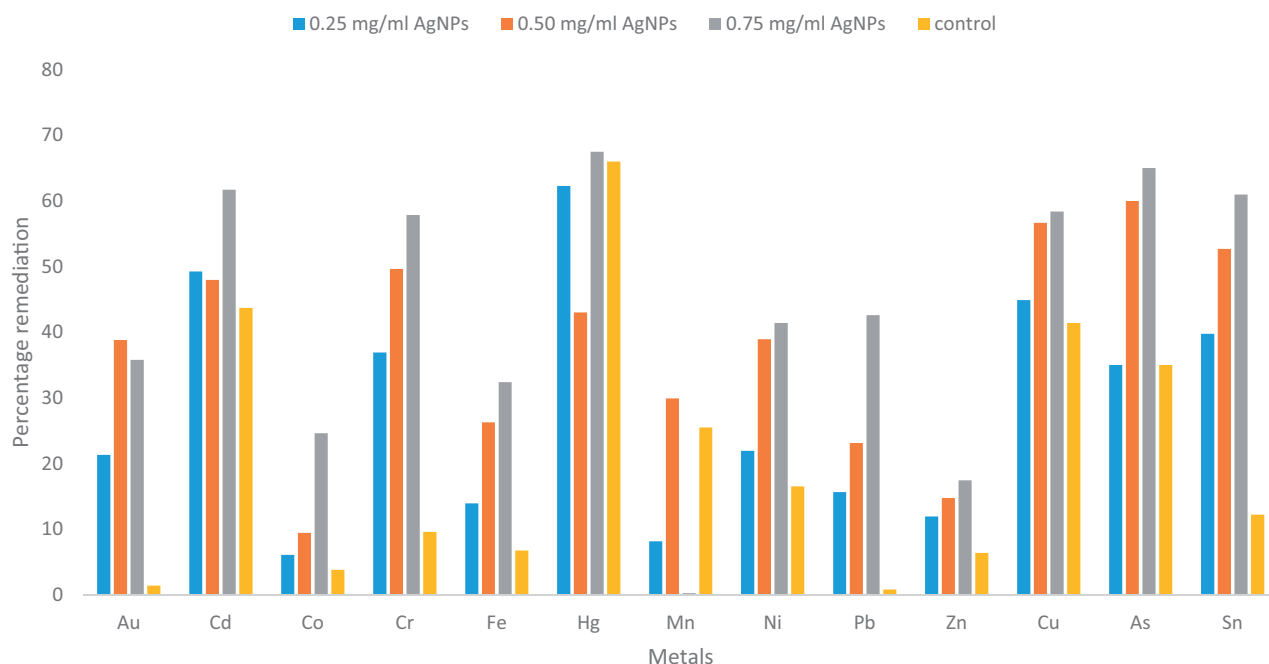


Figure 3. Remediation percentage of heavy metals under foliar application of different concentrations of AgNPs.

mineral contents range from 33.23 to 65.03% for Na and Mg, respectively. The consumption of *A. esculentus* is nutritionally beneficial to humans due to its rich contents of essential minerals, bioactive compounds, polysaccharides and vitamins needed for boosting immunity and preventing diseases (Durazzo *et al.* 2018; Liu *et al.* 2019; Xu *et al.* 2020). Interestingly, the intervention of foliar application of AgNPs increased the transfer of essential minerals but a contrasting reduction was obtained for heavy metals. The lower amounts of heavy metals analyzed in *A. esculentus* intervened with AgNPs compared to control was due to high adsorptive efficiency of AgNPs and their reactive surfaces and special affinity for heavy metals (Azeez, Adejumo, *et al.* 2019, 2020; Ogunkunle *et al.* 2020).

Enhancement of phytoremediation and contamination intensity

The reductions in the concentrations of heavy metals analyzed in gold-mined soil under foliar applications of different concentrations of AgNPs were significant (Table 1). Percentage remediation potentials were generally highest in soil with foliar application of 0.75 mg/mL AgNPs followed 0.25 mg/mL AgNPs, 0.50 mg/mL AgNPs and then control for heavy metals (Figure 3). Remediation percentages recorded for Cd, As and Hg were higher than 60%; Cr, Cu and Sn were more than 50% remediated while others had between 10% and 40% remediation percentages (Figure 3). Heavy metals bioaccumulate in plants and therefrom get passed to humans through consumption (Liu *et al.* 2019; Amoakwah *et al.* 2020). Thus, the remediation of these metals is indispensable to adequately protect against the toxic effects of heavy metals on health. Remediation of heavy metals in gold-mined soil with foliar-applied AgNPs enhanced

phytoremediation performances of *A. esculentus* with more than 50% better quality for all heavy metals except Hg. This ability of AgNPs to enhance phytoremediation is due to their morphological characteristics, effective sorption capacity and strong affinity for heavy metals (Guerra *et al.* 2018; Seleiman, Almutairi, *et al.* 2020; Seleiman, Alotaibi, *et al.* 2020).

Contamination intensity (CI) from the bioaccumulation of heavy metals into *A. esculentus* follows control (4.99) > 0.75 mg/mL AgNPs (3.15) > 0.50 mg/mL AgNPs (2.81) > 0.25 mg/mL AgNPs (2.76). The contamination intensity drastically reduced by 34.63, 44.17 and 44.99% in 0.75, 0.50 and 0.25 mg/mL AgNPs, respectively. As obtained in this study, the CI was noticeably greater than 1 in all groups implying there was a dangerous accumulation of heavy metals in the plant. The effects of which would strikingly affect *A. esculentus* activities. Foliar application of AgNPs considerably reduced the CI in *A. esculentus* to ensure safe consumption. The intrinsic characteristics of AgNPs stemming from adjustable size, shape, reactive surfaces, plasmon resonance and effective adsorption sites are crucial for remediating heavy metals. Similar results were reported for adsorption of Cd, Al, Cu, Fe and Pb with metal nanoparticles (Singh and Lee 2016; Venkatachalam *et al.* 2016; Galdames *et al.* 2017; Nahar *et al.* 2017; Azeez, Adejumo, *et al.* 2019).

Physiological parameters of *A. esculentus* on gold-mined soil

The differential effects of gold-mined soil were evident in the stunted germination percentage, reduced lengths of roots and shoots and number of leaves in *A. esculentus* wetted with water (Figure 4). The mediating interventions of foliar

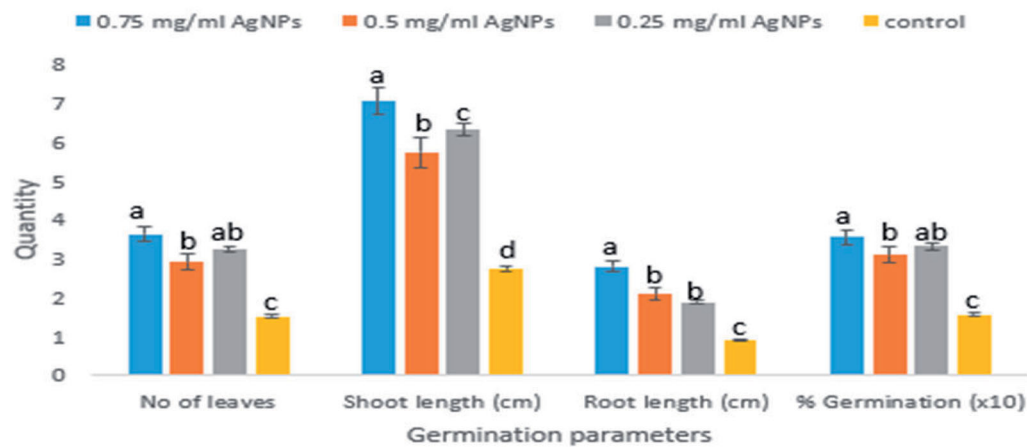


Figure 4. Physiological parameters of *A. esculentus* planted on gold-mined soil under foliar application of AgNPs. Bars with different superscripts for each physiological parameter are significantly different at $p < 0.05$.

applications of AgNPs were obvious in the improvements of these indices by 56.02, 49.52 and 52.85% in *A. esculentus* treated with 0.75, 0.50 and 0.25 mg/mL AgNPs, respectively.

The disruption of plant growth and metabolism indicates excessive exposure of a plant to highly dangerous pollutants such as heavy metals (Liu *et al.* 2019). These effects manifested in the stunted growth, reduced percentage germination, shorter roots and shoots in *A. esculentus* planted in gold-mined soil without any intervention. Heavy metals have been severally reported to disrupt growth mechanisms and interrupt enzyme activities in plants. The prominence of Al could also be a factor responsible for reductions in physiological appearances of *A. esculentus* as this has been previously reported (Nahar *et al.* 2017; Rinklebe *et al.* 2019; Raklami *et al.* 2021). Due to the inherent quality of AgNPs, it stimulated enzymes to modulate the growth of *A. esculentus* to overcome the deleterious effects of heavy metals. AgNPs are known to mediate phytohormones and regulate microRNA for cell proliferation and division in plants (Azeez, Lateef, *et al.* 2019; Azeez, Adejumo, *et al.* 2019; Ogunkunle *et al.* 2018, 2020; Zare *et al.* 2020).

Oxidative stress in *A. esculentus* and defense mechanisms

Oxidative stress biomarker

A significant increase in MDA contents of *A. esculentus* exposed to gold-mined soil without any interventions was obtained (Figure 5). However, these gradually reduced in a concentration-dependent trend with the highest foliar concentration of AgNPs (0.75 mg/mL) having the highest modulation of oxidative stress induced by pollutants in gold soil. The level of MDA is an indicator of a suppressed immune system in the plant. It establishes the phytotoxicity of heavy metals. In this study, the MDA level in *A. esculentus* exposed to gold-mined without intervention was far higher implying the oxidative stress status in the plant. The high level of MDA obtained for *A. esculentus* without foliar application of AgNPs could be due to a succession of generations of reactive radicals by heavy metals and Al. Heavy metals

like Pb, Cd, Cr and also Al are known to be associated with phytotoxicity while AgNPs are renowned to ameliorate phytotoxicity (Singh and Lee 2016; Venkatachalam *et al.* 2017; Sharma 2017; Ogunkunle *et al.* 2018, 2020; Azeez, Lateef, *et al.* 2019; Azeez, Adejumo, *et al.* 2019; Azeez *et al.* 2020; Kushwaha and Singh 2020; Raklami *et al.* 2021).

Enzymic antioxidant activities

Disruption in defense systems was observed with a significant increase in superoxide dismutase (SOD) activities in *A. esculentus* planted on gold-mined soil compared to treated *A. esculentus* (Figure 5). The activity of SOD in *A. esculentus* treated with foliar applications of 0.25, 0.50 and 0.75 mg/mL AgNPs reduced to 10.78, 33.36 and 69.79%, respectively. Catalase (CAT) activities were significantly improved in *A. esculentus* treated with 0.75 mg/mL AgNPs over control whereas other concentrations had significantly reduced activities compared to control (Figure 5).

Plants are protected by a collection of antioxidant compounds and enzymes such as SOD, CAT, GSH and carotenoids (Sohag *et al.* 2020). Therefore, their levels of activities either increase or decrease signal the disturbance or ease in the mechanisms of plants' activities. Exposure of *A. esculentus* to heavy metals in gold-mined soil created stress imbalance in the vegetable that led to an increase in the activities of SOD as the first line of defense. When exposed to pollutants, plants activate SOD for scavenging and converting superoxide radicals O_2^- to H_2O_2 . The increased activity of SOD was a response to tolerating stress induced by heavy metals (Nahar *et al.* 2016, 2017; Hasanuzzaman *et al.* 2018; Ogunkunle *et al.* 2018, 2020). The foliar intervention of AgNPs alleviated metal stress by reducing the SOD activities. This is connected to the inherent antioxidative properties of AgNPs (Lateef *et al.* 2016; Azeez, Adejumo, *et al.* 2019; Liu *et al.* 2020).

The CAT activity is noted for enzymic elimination and subsequent conversion of reactive radicals such as H_2O_2 to innocuous products. The reduced CAT activities in *A. esculentus* grown in gold-mined soil without intervention suggest that the continuous production of radicals and conversion

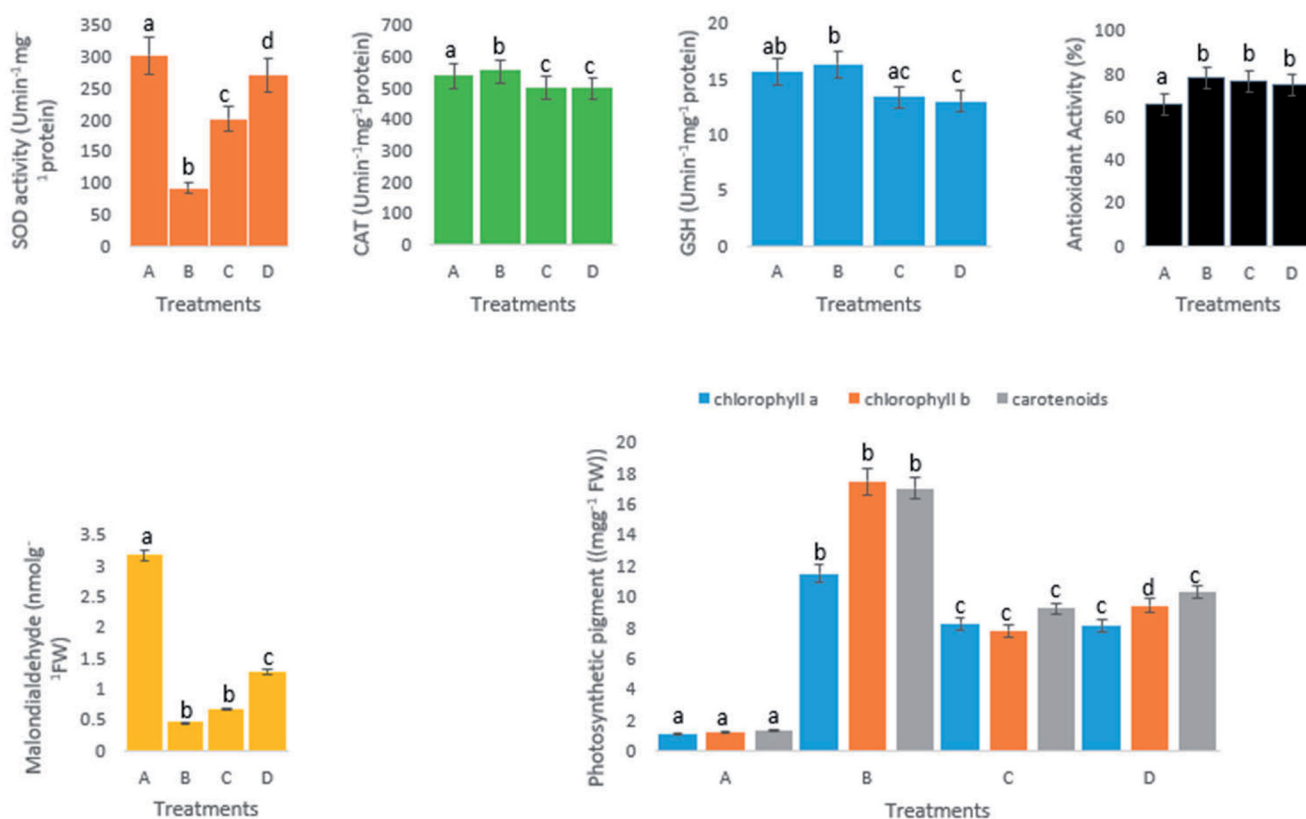


Figure 5. Contents of SOD, CAT, GSH, antioxidant activity, MDA level and photosynthetic parameters in *A. esculentus* planted on gold-mined soil exposed to different treatments. Results: mean $\pm$ standard error of mean. Bars with different superscripts are significantly different ($p < 0.05$). SOD: Superoxide dismutase; CAT: catalase; GSH: reduced glutathione; A – Control – *A. esculentus* planted on gold-mined soil wetted with water, B, C and D – *A. esculentus* planted on gold-mined soil with foliar applications of AgNPs at 0.75, 0.50 and 0.25 mg/ml AgNPs respectively.

of $O_2^{\cdot-}$ to H_2O_2 by SOD were overwhelming. The foliar application of AgNPs promoted CAT activities to detoxify heavy metals toxicity in *A. esculentus*. AgNPs modulated the activities of enzymes to overcome the burdens of radicals. Many studies have reported enhanced activities of enzymes such as CAT in metal-stressed plants exposed to metal nanoparticles (Kushwaha and Singh 2020; Sohag *et al.* 2020).

Non-enzymic antioxidant activities

The contents of GSH in *A. esculentus* were improved by foliar application of 0.75 mg/mL AgNPs as compared to control while other treatments had reduced activities compared to control (Figure 5). The free radical scavenging abilities of *A. esculentus* planted on gold-mined soil and subjected to different treatment protocols were determined with DPPH and carotenoid contents. Both activities were significantly improved by foliar applications of AgNPs with 0.75 mg/mL AgNPs having the highest impact.

Plants enjoy protection against the onslaught of reactive radicals from non-enzymic antioxidant compounds such as GSH, carotenoids and vitamins. They offer a dependable defense to counter the toxicity of metals by chelation, breaking the propagation chains of reactive radicals and acting as a cellular redox buffer (Nahar *et al.* 2016, 2017; Hasanuzzaman *et al.* 2018). Reduced glutathione is renowned for metal chelation using its thiol (SH) group and as an effective scavenger for $\cdot OH$ and singlet oxygen. The

reduced GSH level is an indication of cytotoxicity of gold-mined soil containing heavy metals and Al (White and Gardea-Torresdey 2018). The intervention of AgNPs upwardly regulated its activities. Metal nanoparticles protect plants by mitigating the generation of reactive radicals via stimulation of enzymic and non-enzymic antioxidants (Sharma 2017; Singh *et al.* 2017; Azeez, Lateef, *et al.* 2019). Similarly, carotenoids as antioxidant compounds act as scavengers and quenchers of reactive radicals and synergistically associate with other antioxidant compounds to mitigate the onslaught of free radicals (Liu *et al.* 2020). Significantly improved carotenoid contents are attestations to the amelioration of toxicity by AgNPs and its ability to trigger defense mechanisms to fight off cellular damage in plants (Singh *et al.* 2017).

Photosynthetic pigment impairment

Impairment of photosynthetic pigments is a known index for understanding the effects of pollutants on their formation. Significant stimulation of chlorophyll a and b contents were recorded for *A. esculentus* treated with foliar application of AgNPs over control (Figure 5). The improvements in chlorophyll parameters were concentration-dependent; the highest and the lowest concentrations (0.75 and 0.25 mg/mL AgNPs) had the best and minimum influence.

Photosynthetic pigments are biochemical components used as assessors of plant vulnerabilities to environmental toxins. Reductions in contents are signals of exposure to

phytotoxicity (Ogunkunle *et al.* 2020; Lawal *et al.* 2021). As obtained in this study, chlorophylls a and b were significantly reduced following exposure to heavy metals and Al in gold-mined soil. Many studies have reported the degradation of photosynthetic pigments as an expression of phytotoxicities of heavy metals on plants (Azeez, Adejumo, *et al.* 2019). However, the intervention of AgNPs ameliorated and modulated their contents upwardly for optimum photosynthesis. AgNPs attenuate alteration in photosynthetic pigments by scavenging radicals that distort their formations (Azeez, Adejumo, *et al.* 2019; Ogunkunle *et al.* 2020).

Conclusions

The exposure of *A. esculentus* to heavy metals and Al in gold-mined soils could predispose consumers to heavy metals toxicity. This is explained by the high contamination intensity of heavy metals recorded in *A. esculentus* and the prominent presence of Al. *Abelmoschus esculentus* exposed to gold-mined soil was significantly affected, showed disrupted physiological parameters and reduced antioxidant activities. Foliar application of synthesized AgNPs ameliorated physiological parameters, enhanced percentage phytoremediation and reduced contamination intensity in *A. esculentus*. Enzymic and non-enzymic antioxidant activities were significantly improved by AgNPs to suppress the effects of heavy metals on *A. esculentus*. This study showed that AgNPs can intervene and reduce the toxicities of heavy metals.

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