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Research article

Zero-valent silver nanoparticles attenuate Cd and Pb toxicities on *Moringa oleifera* via immobilization and induction of phytochemicals

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ABSTRACT

Potentials of zero-valent extract of cocoa pod mediated silver nanoparticles (AgNPs) for heavy metals (cadmium and lead) immobilization, attenuation of induced toxicities and influence on phytochemical contents in *Moringa oleifera* were investigated. *M. oleifera* seeds were planted in soil spiked and watered with water (control), 0.2 mg AgNPs, 0.5 mg $CdCl_2$, 0.5 mg $PbCl_2$, 0.2 mg AgNPs + 0.5 mg $CdCl_2$, 0.2 mg AgNPs + 0.5 mg $PbCl_2$, 0.2 mg AgNPs + 0.75 mg $CdCl_2$ and 0.2 mg AgNPs + 0.75 mg $PbCl_2$ per g soil designated as groups A, B, C, D, E, F, G and H respectively. Significant ($p < 0.05$) repression in shoot and root lengths, percentage germination, number of leaves, vigour and growth tolerance indices, relative water contents with attendant inhibition of photosynthetic pigments, total carotenoid contents, total flavonoid contents and total phenolic contents were obtained for *M. oleifera* planted on Cd and Pb spiked soil. There were marked decrease in ferric reducing, hydrogen peroxide scavenging and free radical scavenging activities with resultant significant increase in lipid peroxidation (MDA) levels for *M. oleifera* grown on Cd and Pb treated soil compared to control with Pb having more deleterious effects. Conversely, AgNPs significantly enhanced both physiological and biochemical parameters in *M. oleifera* over control and considerably attenuated suppressions of these parameters in *M. oleifera* induced by Cd and Pb. Results in this study have shown AgNPs as excellent immobilizing agents and outstanding modulators of heavy metal induced toxicities.

1. Introduction

Considering the increasing demands for beneficial, healthy and functional foods rich in phytochemicals to lower incidences of degenerative diseases, *Moringa oleifera* Linn belongs to the class of nutritional and medicinal plant which is consumed due to its anti-inflammatory, anti-diabetic, antioxidant, antimicrobial and anticancer properties. It is rich in polyphenols, flavonoids, β-carotene, linoleic acid, protein and has negligible anti-nutritional compounds. It is commonly eaten as food and taken as supplement in medicine, food and cosmetics (Santos et al., 2012; Fitriana et al., 2016; Alves et al., 2017; Castillo-Lopez et al., 2017; Desoky et al., 2018, 2019; Rehman et al., 2018). However, these properties can be greatly affected by both biotic and abiotic stresses (heavy metals, salinity, heat, drought, pathogen infection) with consequent effects on soil nutrients, fertility and fruit quality (Zhu, 2016; Olowolaju et al., 2018; Rizwan et al., 2018). Accumulation of heavy metals in soil resulting from continuous anthropogenic activities (mining, vehicle exhaust, sewage disposal) is of global concerns due to their detrimental toxicities on plants with attendant reduction in soil quality and fertility. It equally constitutes burdens on human health when eventually transferred into food chain through plant uptake (Lamhamdi et al., 2013; Liu et al., 2015; Singh and Prasad, 2015; Ashfaque et al., 2016). Cadmium (Cd) and lead (Pb) are heavy metals which are not known or needed for any essential cell activities in plants but rather are highly toxic causing oxidative stress in plant with consequent morphological, and physiological changes affecting their germination, growth, fruiting, quantity, quality and translocation of nutrients. Additionally, they cause chronic anaemia, central nervous system disorder, kidney, skin, brain and lung malfunctions in humans (Azeez et al., 2015; Liu et al., 2015; Pierart et al., 2015; Pattnaik, and

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Equeenuddin, 2016; Xiong et al., 2016; Khalid et al., 2017).

Cd and Pb toxicities are associated with oxidative damage in plants through generation of free radicals which inhibit photosynthetic pigments formation, nutrient uptake, germination and can cause cell damage. They are not easily degraded and are difficult to remediate in soil (Wińska-Krysiak et al., 2015; Rizwan et al., 2016a,b, 2018; Khalid et al., 2017; Minnikova et al., 2017; Zhou et al., 2018). Though, plants are imbued with mechanisms to tolerate and modulate heavy metal induced toxicities through induction of secondary metabolites (polyphenols, carotenoids, glutathione, vitamins) and secretion of antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase) which scavenge free radicals produced by heavy metals. However, these could be overwhelmed by excessive heavy metal contamination resulting in plant deformation, yellowing of leaves, stunted growth and eventual death of plant (Yin et al., 2016; Desoky et al., 2018, 2019; Rady et al., 2018; Rehman et al., 2018).

Different management systems to remediate heavy metal contaminated soil such as surface capping, soil replacement, chemical immobilization, vitrification. phytoremediation, encapsulation, electrokinetic extraction, phytostabilization, soil flushing and bioremediation have been applied with contrasting results (Puga et al., 2015; Singh and Prasad, 2015; Venegas et al., 2015; Ashraf et al., 2016; Niazi et al., 2016; Tajudin et al., 2016; Yin et al., 2016; Younis et al., 2016; ZiaurRehman et al., 2016; Ali et al., 2017; Khalid et al., 2017; Seshadri et al., 2017; Liu et al., 2018; Rizwan et al., 2018).

Chemical immobilization is one of the effective management mechanisms for heavy metals removal. It involves the introduction of chemical agents into contaminated soil to decrease heavy metal mobility, bioavailability, leaching and translocation into plant parts. This occurs via complexation, sequestration, stabilization, adsorption, precipitation and co-precipitation (Tajudin et al., 2016; Khalid et al., 2017; Liu et al., 2018; Rizwan et al., 2018). Application of various immobilizing agents such as biochar, manure, compost, hydroxyapatite, bone, activated carbon, zeolite, chitosan and clay have been reported with each having selective mechanism for specific metal species. They act as soil conditioners which improve soil physicochemical properties and enhance physiological tolerance of plants against heavy metal induced stress owing to their adsorbing sites, porousity, surface areas and retention capacity. (Zhang et al., 2013; Rehman et al., 2018; Younis et al., 2016; Ali et al., 2017; Seshadri et al., 2017; Desoky et al., 2018; Liu et al., 2018; Rizwan et al., 2016a,b, 2018).

However, influence of these immobilizing agents on biochemical parameters and control of plant pathogens has been scarcely reported.

Nanoparticles offer unparalleled and unequalled edge as effective and efficient adsorbents for heavy metal remediation over conventional bulk-sized and macro-aggregated immobilizing agents due to their morphological characteristics, easy delivery, fast dispersal in soil, strong affinity and high sorption capacity for target metals. More so, their phytostimulatory, phytomodulatory and phytopathogenic attributes vis-à-vis promotion of physiological tolerance, enhancement of growth, photosynthesis, nitrogen metabolism, seed germination and antifungal properties have prominently increased their applications (Liu et al., 2015; Manikandan and Sathiyabama, 2015; Azeez et al., 2017, 2019; Galdames et al., 2017; Li et al., 2017; Praveen et al., 2017; Gong et al., 2018; Ochoa et al., 2018). Germination potentials of AgNPs are connected to their ability to alter physiological processes such as regulation of genes responsible for cell proliferation, seed germination, water utilization and nitrogen metabolism vis-à-vis their ability to promote photosynthesis via translocation to the leaves to enhance it (Syu et al., 2014; Hernández-Hernández et al., 2017; Yang et al., 2017; Gupta et al., 2018).

Nanoparticles are effective adsorbents with promising results. Nanoparticles such as carbon nanotube, multi-walled carbon nanotube, graphene oxide, fullerene, titanium oxide, silver, magnetite, iron-phosphate, magnesium oxide, nickel oxide, zero-valent iron, copper oxide, silicon, zinc and aluminium oxide nanoparticles have been used as efficient adsorbents and immobilizing agents for removing naphthalene, phenanthrene, pesticides, As, Cd, Cr, Pb, Ni, Cu, Zn, Al, Fe, rhodamine B and antibiotics in soil and water (Li et al., 2013, 2016; Liu et al., 2015; Singh and Prasad, 2015; Tripathi et al., 2015; Singh and Lee, 2016; Venkatachalam et al., 2016; Yuan et al., 2016; Zhao et al., 2016; Ma et al., 2017; Praveen et al., 2017; Galdames et al., 2017; Azeez et al., 2019; Gong et al., 2018; Ochoa et al., 2018).

These properties of nanoparticles are dependent on the modes of synthesis. Different synthetic modes namely conventional (physio-chemical) and green (biosynthesis) have been reported. However, biosynthesis using plant extracts which act as reductants, stabilizing and capping agents offers comparative advantages over conventional methods due to its eco-friendliness and less toxicity unlike conventional method which involves the use of hazardous chemicals that are highly toxic (Lateef et al., 2016a, b, c, d; Kasithevar et al., 2017; Saravanana et al., 2018; Anandan et al., 2019; Azeez et al., 2017, 2018, 2019).

Application of silver nanoparticles (AgNPs) as excellent adsorbents for removal of contaminants owing to their higher adsorptive capacity and affinity compared with other nanoparticles have been reported with applause. These together with their eco-friendliness, facile synthesis, catalytic, antifungal, antimicrobial and antioxidant potentials make them very promising (Azeez et al., 2017, 2019; Lateef et al., 2016a, b, c). Consequent upon these properties of AgNPs, this study was aimed at using silver nanoparticles (AgNPs) synthesized from extract of cocoa pods as immobilizing agents to decontaminate, demobilize, adsorb Cd and Pb in addition to their phytostimulatory influence on *M. oleifera* for attenuation of toxicity induced by heavy metal.

2. Materials and methods

2.1. Biosynthesis of AgNPs using cocoa pod extract

AgNPs used in this study were biosynthesized from extract of cocoa pod and characterized as reported by Lateef et al. (2016a).

2.2. Soil sampling, analysis, treatment and planting of M. oleifera seeds

Soil samples were collected from a farm beside botanical garden situated on latitude 7°78′25.3″N and longitude 4°58′7.06″E in Osun State University at a depth between 0 and 25 cm, air dried, ground and sieved with 2 mm wire mesh. Soil components were determined using method of Page et al. (1982). 0.3 g of soil was digested for metal contents with concentrated HNO_3 and HCl (7:3), filtered, made up to 20 ml and their concentrations were determined with microwave plasma coupled with atomic emission spectrometer (MP-AES – model MY14280004).

Soil samples in each bucket were spiked with 0.1 mg AgNPs/g soil (group B), 0.25 mg $CdCl_2$/g soil, 0.25 mg $PbCl_2$/g soil, 0.5 mg $CdCl_2$/g soil, 0.5 mg $PbCl_2$/g soil, 0.75 mg $CdCl_2$/g soil, 0.75 mg $PbCl_2$/g soil while the control (group A) was exposed to water only. These buckets (each filled with 250 g soil) were watered for three weeks with 450 ml of solution meant for each group; water for group A, 250 mg/l $CdCl_2$ for group C, 250 mg/l $PbCl_2$ for group D, 75 mg/l AgNPs for group B and 100 mg/l AgNPs for groups E, F, G and H to achieve final concentrations of 0 (control), 0.2 mg AgNPs, 0.5 mg $CdCl_2$, 0.5 mg $PbCl_2$, (0.2 mg AgNPs + 0.5 mg $CdCl_2$), (0.2 mg AgNPs + 0.5 mg $PbCl_2$), (0.2 mg AgNPs + 0.75 mg $CdCl_2$), (0.2 mg AgNPs + 0.75 mg $PbCl_2$)/g soil respectively.

The planting experiments were conducted at temperature (30.23 ± 1.43 °C), relative humidity (21.07 ± 2.37%), UV index (6.05 ± 0.05) and light intensity (11216 ± 60.42 illuminance).

2.3. Determination of physiological parameters and relative water contents of M. oleifera

M. oleifera were harvested after 3 weeks of planting to maturity.

Different physiological parameters namely germination percentage (eq. (1)), root and shoot length (measured with meter rule), vigour index (eq. (2)), number of leaves and growth tolerance index (eq. (3)) were determined. Relative water contents were calculated using equation (4) by determining fresh, turgid and dry weights of 0.5 g of leaf sample. Turgid weight was determined by placing 0.5 g (fresh weight) of leaves in a 100 ml deionized-distilled water for 24 h and thereafter its weight recorded. The leaves were thereafter dried at 70 °C for 48 h and weight recorded.

$$\% \text{ Germination} = \left(\frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds}\right) \times 100 \quad (1)$$

$$\text{Vigour index} = (root\ length + shoot\ length) \times \%\ germination \quad (2)$$

$$\%\ Growth\ tolerance\ index = \frac{M.\ \ oleifera\ growth\ in\ heavy\ metal\ treated\ soil}{M.\ oleifera\ growth\ without\ heavy\ metal} \quad (3)$$

$$Relative\ water\ content = \frac{(leaf\ fresh\ weight - leaf\ dried\ weight)}{leaf\ turgid\ weight - leaf\ dried\ weight)}\ x\ 100 \quad (4)$$

2.4. Concentration, immobilization and translocation of Cd and Pb in soil and plant parts of M. oleifera

Concentrations of Cd and Pb were determined in soil samples by digesting 0.3 g of air dried samples with concentrated HNO_3 and HCl mixture (7:3). Also, 0.2 g of shoot and root samples dried at 70 °C for 2 h were digested with 5 ml HNO_3. The metal concentrations were analysed using microwave plasma coupled with atomic emission spectrometer (MP-AES – model MY14280004).

Immobilization (adsorption) and translocation parameters of Cd and Pb were calculated using equation (5) -7.

$$Percentage\ Root\ or\ Shoot\ concentration\ factor = \frac{heavy\ metal\ in\ shoot\ or\ root}{heavy\ metal\ in\ soil} x100 \quad (5)$$

$$Percentage\ Translocation\ factor = \frac{heavy\ metal\ in\ shoot}{heavy\ metal\ in\ root} x100 \quad (6)$$

$$Heavy\ metal\ immoblization(adsoption) = \left(\frac{Initial\ Concentration - Final\ concentration}{Initial\ Concentration}\right) x\ 100 \quad (7)$$

2.5. Determination of malondialdehyde (MDA) level in M. oleifera

The method as reported by Gupta et al. (2018) was used. Exactly, 0.2 g of fresh leaves was homogenized and extracted with 5 ml of chilled 80% ethanol, and centrifuged at 3500 rpm for 20 min. Thereafter, 1 ml of the extract was mixed with 4 ml of 20% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA), incubated at 90 °C for 30 min, cooled in ice and centrifuged at 3500 rpm for 30 min. The absorbance of the supernatant was recorded at, 440, 532 and 600 nm and concentration of MDA (nmol/g) was calculated using equation (8).

$$MDA\ (nmolg^{-1}) = \frac{[Abs_{532} - Abs_{600} - 0.05714\ x\ (Abs_{440} - Abs_{600})]}{0.157\ x\ FW} \quad (8)$$

Where FW is the fresh weight of leaf sample per unit volume.

2.6. AgNPs retention, translocation, dissociation in soil and M. oleifera parts

Soil samples were air dried, pulverised and sieved for AgNPs determination while roots and shoot were oven dried. AgNPs concentrations in soil, roots and shoots of *M. oleifera* were determined by dissolving 0.3 g of each sample in 10 ml of deionized-distilled water, shaken vigorously, centrifuged at 5000 rpm for 30 min and carefully decanted. The supernatant was extracted once more following the procedure. The absorbance of the doubly centrifuged supernatant was read at 428.5 nm (Lateef et al., 2016d) and concentrations extrapolated from calibration curve of stock solution.

To ascertain this procedure, AgNPs concentration in control sample of 0.2 mg AgNPs/g soil without planting was determined following same procedure.

Equally, concentrations of Ag^+ in stock solution and soil were determined using MP-AES.

AgNPs retention in soil and translocation to roots and shoots were calculated using equation (9)–11.

$$Nanoparticle\ retention = \frac{AgNPs\ in\ soil}{Total\ AgNPs\ applied} x100 \quad (9)$$

$$Nanoparticle\ uptake\ by\ root = \frac{AgNPs\ in\ root}{AgNPs\ in\ soil} x100 \quad (10)$$

$$Percentage\ Translocation\ factor = \frac{AgNPs\ in\ shoot}{AgNPs\ in\ root + shoot} x100 \quad (11)$$

2.7. Determination of antioxidant activities and phytochemical contents of M. oleifera

Two grams of dried *M. oleifera* sample was extracted twice with 150 ml of 70% aqueous methanol, filtered and concentrated using rotary evaporator. Absorbance of free radical scavenging ability of each extract and control using 2,2-diphenyl-1- picrylhydrazyl (DPPH) was read at 517 nm according to the method reported by Azeez et al. (2017), while ferric reducing and hydrogen peroxide scavenging activities were done using procedures described by Lateef et al. (2016b, 2017). Total phenolic and flavonoid contents were determined using the methods reported by Azeez et al. (2017).

2.8. Determination of photosynthetic pigments in M. oleifera

The photosynthetic pigments parameters namely chlorophyll *a*, *b* and carotenoid contents were quantified using the method reported by Arnon (1949) by homogenising 0.1 g of fresh root and shoot with 5 ml ice-cold 80% acetone. The extract was centrifuged at 5000 rpm for 5 min and re-extracted with 2.5 ml of ice-cold 80% acetone twice. Absorbance of the extract was measured at 470, 663, 645 nm respectively and their quantification calculated using equation (12)–14 and expressed as mg/g FW.

$$Chlorophyll\ a = 12.25\ x\ A_{663} - 2.79\ x\ A_{645} \quad (12)$$

$$Chlorophyll\ b = 21.50\ x\ A_{645} - 5.10\ x\ A_{663} \quad (13)$$

$$Carotenoid = \frac{(1000\ x\ A_{470} - 1.82\ x\ Chl\ a - 85.02\ x\ Chl\ b)}{198} \quad (14)$$

Chl a and Chl b – chlorophyll a and b.

2.9. Statistical analysis

Data of antioxidant activities, phytochemical contents, photosynthetic pigments, MDA, heavy metals and AgNPs are expressed as mean ± standard deviation of three replicates. Results of percentage germination, root length, shoot length, relative water contents, vigour index and growth tolerance index are expressed as mean ± standard deviation of eight replicates while number of leaves was expressed as mean ± standard deviation of leaves on eight replicates. These results were subjected to one-way ANOVA followed by Duncan's multiple

Table 1
Analysis of soil used in this study.

Soil characteristics	Value
pH	6.05 ± 0.05
Organic carbon	380.75 ± 26.65 mgkg^{-1}
Nitrogen	328.17 ± 2.27 mgkg^{-1}
Phosphorus	12.82 ± 0.62 mgkg^{-1}
Potassium	244.14 ± 2.86 mgkg^{-1}
Sodium	106.78 ± 3.56 mgkg^{-1}
Calcium	29.61 ± 1.51 mgkg^{-1}
Magnesium	2662.27 ± 10.97 mgkg^{-1}
Cadmium	Not detected
Lead	Not detected
Clay	10.46%
Silt	27.56%
Sand	61.98%

range test (DMRT) for comparison of means. Significant difference and correlation coefficient tests were performed at $p < 0.05$ using SPSS 17 version.

3. Results

3.1. Biosynthesis of AgNPs

The characteristics of AgNPs synthesized are as reported by Lateef et al. (2016a). The brownish colloidal AgNPs absorbed maximally at 428.5 nm, fairy spherical-shaped and with dimensions of 4–32 nm. The nanoparticles have shown potent antimicrobial, larvicidal, antioxidant and cell-growth arresting activities (Lateef et al., 2016a; Yekeen et al., 2017). The concentration of stock AgNPs synthesized was 100 mg/l with average size of 15.5 ± 4.2 nm.

3.2. Soil characteristics and composition

Basic properties of soil (Table 1) for this study were dark coloration, sandy loamy, averagely neutral (pH 6.05 ± 0.05), moderately high contents of organic carbon (380.75 ± 26.65 mgkg^{-1}), nitrogen (328.17 ± 2.27 mgkg^{-1}), phosphorus (12.82 ± 0.62 mgkg^{-1}), potassium (244.14 ± 2.86 mgkg^{-1}), sodium (106.78 ± 3.56 mgkg^{-1}), calcium (29.61 ± 1.51 mgkg^{-1}), magnesium (2662.27 ± 10.97 mgkg^{-1}) while cadmium and lead were not detected.

3.3. AgNPs modulation of M. oleifera growth under Cd and Pb contamination

Strikingly noticeable deleterious effects of Cd and Pb (groups C and D) were observed on the inhibition of growth of *M. oleifera* (Fig. 1). *M. oleifera* in these groups had conspicuous stunted growth and reduced number of leaves especially in Pb treated soil.

Treatment of soil with Cd significantly ($p < 0.05$) inhibited percentage germination, number of leaves, relative water contents, vigour index, root and shoot lengths by 14.14, 15.32, 12.97, 37.89, 39.01 and 26.45% respectively while soil spiked with Pb significantly ($p < 0.05$) retarded these parameters by 60.20, 36.56, 21.21, 87.09, 51.63 and 46.83% respectively compared with control in *M. oleifera* (Table 2).

Contrarily, AgNPs significantly ($p < 0.05$) improved percentage germination, root and shoot lengths by 15.04, 12.71 and 30.71% respectively in addition to non-significant ($p > 0.05$) albeit increase in number of leaves and vigour index by 3.00 and 7.83% respectively over control (Table 2).

Considerably, the addition of AgNPs significantly ($p < 0.05$) modulated physiological tolerance of *M. oleifera* by increasing shoot length, root length, number of leaves, percentage germination and vigour index by 12.26, 34.34, 11.08, 14.89 and 36.14% respectively over *M. oleifera* grown on 0.5 mg Cd treated soil. There were 0.02, 13.35, 9.29% elongation in shoot, root and increase number of leaves respectively in AgNPs wetted soil containing 0.75 mg $CdCl_2$. Meanwhile, AgNPs also significantly ($p < 0.05$) protected physiological parameters against 0.5 mg $PbCl_2$ toxicity by enhancing shoot length, root length, number of leaves, percentage germination and vigour index by 19.80, 47.19, 32.04, 84.49% respectively (Table 2). Extension in lengths of root (26.74%), shoot (7.99%), number of leaves (24.37%) and 52.87% invigoration of vigour were obtained in *M. oleifera* grown on AgNPs supplemented 0.75 mg $PbCl_2$ treated soil.

Growth tolerance indices of *M. oleifera* under Cd and Pb toxicities were significantly ($p < 0.05$) hindered than what were obtained for *M. oleifera* grown on Cd and Pb contamination followed by treatment with AgNPs (Table 2). There were 14.28 and 9.01% growth enhancement in root and shoot respectively of *M. oleifera* grown on AgNPs watered soil over Cd treated soil without AgNPs. Also, 17.32 and 14.53% growth improvement in root and shoot respectively of *M. oleifera* grown on AgNPs wetted soil over Pb treated soil without AgNPs were obtained.

Growth tolerance in 0.75 mg Pb treated soil was remarkably reduced, however, addition of AgNPs boosted it in root and shoot by 22.57 and 24.30% respectively.

3.4. Immobilization (adsorption) of Cd and Pb by AgNPs in soil and their translocation into M. oleifera parts

The concentrations of Cd and Pb (Fig. 2a) available in soil for uptake by *M. oleifera* significantly ($p < 0.05$) reduced when AgNPs was added to soil compared to Cd and Pb treated soil as obtained for percentage immobilization (Fig. 2b). There were 55.8 and 50.4% improved immobilizing performances by AgNPs on 0.5 mg Cd and 0.5 mg Pb respectively compared to remediation of Cd and Pb without AgNPs (control). Uptake/absorption of Cd and Pb in roots of *M. oleifera* significantly ($p < 0.05$) declined by 42.54 and 37.71% respectively while translocation rates of Cd and Pb were diminished by 12.52 and 15.01% respectively following AgNPs addition.

In addition, AgNPs raised immobilization of 0.75 mg Cd and 0.75 mg Pb by 51.36 and 66.67% (Fig. 2b) along with significant ($p < 0.05$) reduction in the uptake and translocation rates of 0.75 mg Cd by 26.02 and 31.12% respectively as well as by 5.00 and 37.16% respectively for 0.75 mg Pb.

Availability of AgNPs to *M. oleifera* and their dispersal in soil without aggregation were determined by evaluating their retention in soil, concentrations in roots and shoots in addition to concentrations of Ag^+ in soil.

Concentration of Ag^+ found in soil (0.186 mg Ag^+/g soil) was 4.62% lower than AgNPs concentration (0.195 mg AgNPs/g soil) (Fig. 3) for control sample. Likewise, there were 3.57, 13.06, 8.48, 5.77 and 7.90% reduction in concentrations of Ag^+ compared with AgNPs concentrations in soil for groups B, E, F, G and H.

Percentages of AgNPs retained in soil followed group B (22.75%) > group H (21.32%) > group G (20.28%) > group E (14.35%) > group F (12.03%) while 55.32, 34.52, 17.59, 29.14 and 27.58% were absorbed by *M. oleifera* in groups B, E, F, G and H respectively (Fig. 3). Translocation rates were in the range group B (21.79%) > group E (17.36%) > group F (15.25%) > group G (12.97%) > group H (11.93%).

Strong correlation coefficients (R^2) exist between AgNPs percentage uptake by root and total phenolic contents (0.89), DPPH scavenging activity (0.94), percentage germination (0.81) and total carotenoids (0.77).

3.5. Attenuation effects of AgNPs on MDA level, antioxidant activities and phytochemical contents in M. oleifera under Cd and Pb contamination

Malondialdehyde (MDA) content was determined as the index of phytotoxicity of Cd and Pb in *M. oleifera*. MDA contents were significantly ($p < 0.05$) altered by Cd and Pb (Table 3). Its levels were

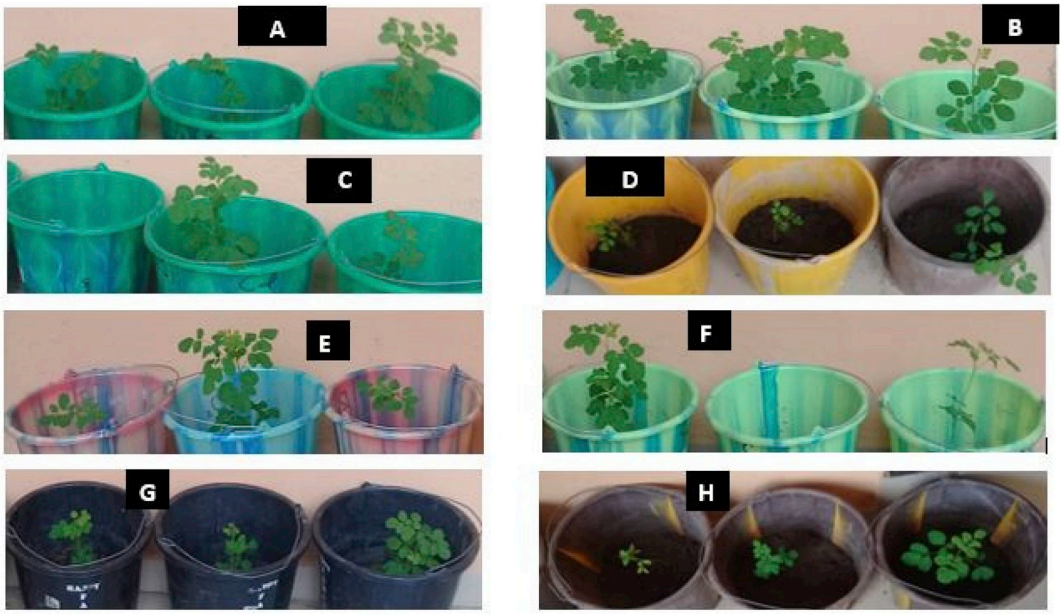

Fig. 1. Group A water (control), group B: 0.2 mg AgNPs/g soil group C: 0.8 mg $CdCl_2$/g soil, group D: 0.8 mg $PbCl_2$/g soil, group E: (0.2 mg AgNPs + 0.5 mg $CdCl_2$)/g soil, group F: (0.2 mg AgNPs + 0.5 mg $PbCl_2$)/g soil, group G: (0.2 mg AgNPs + 1 mg $CdCl_2$)/g soil, group H: (0.2 mg AgNPs + 1 mg $PbCl_2$)/g soil.

significantly ($p < 0.05$) increased by 55.95 and 58.79% in *M. oleifera* grown on Cd and Pb treated soil respectively over control. A non-significant ($p < 0.05$) increase was obtained for MDA in AgNPs treated soil compared to control. AgNPs significantly ($p < 0.05$) attenuated the toxicity induced by Cd and Pb by 40.17% (0.5 mg Cd), 42.5% (0.5 mg Pb), 27.24% (0.75 mg Cd) and 26.53% (0.75 mg Pb) compared with *M. oleifera* grown on Cd and Pb treated soil respectively.

Antioxidant activities were assayed using DPPH, ferric chloride and hydrogen peroxide methods. AgNPs significantly ($p < 0.05$) enhanced free radical and hydrogen peroxide scavenging activities by 13.66 and 13.59% respectively over control but had comparable ferric reducing activity to control (Table 3). Cd and Pb induced-toxicities led to significant ($p < 0.05$) reduction of 41.34 and 61.51% respectively in DPPH scavenging activity, 44.24 and 50.59% respectively in ferric reducing ability and 40.32 and 45.22% respectively in hydrogen peroxide scavenging ability (Table 3). AgNPs ameliorated decrease in antioxidant activities induced by 0.5 mg Cd and 0.5 mg Pb by enhancing significantly ($p < 0.05$) these activities in *M. oleifera* by 40.38 and 60.88% respectively in DPPH scavenging activity, 30.55 and 38.46% respectively in ferric reducing ability and 29.85 and 35.61% respectively in hydrogen peroxide scavenging ability over *M. oleifera* grown on Cd and Pb treated soil without AgNPs (Table 3). Moreover, AgNPs modulated 0.75 mg Cd and 0.75 mg Pb induced toxicities by boosting with 19.64 and 47.02% respectively in DPPH scavenging activity, 15.73 and 26.32% respectively in ferric reducing ability and 10.12 and 17.35% respectively in hydrogen peroxide scavenging ability compared with *M. oleifera* grown Cd and Pb treated soil without AgNPs (Table 3).

Both total phenolic and flavonoid contents were significantly repressed by Cd and Pb contamination. Their trends of abundance in *M. oleifera* followed group B > group A > group E > group F > group G > group H > group C > group D (Table 3). A non-significant ($p > 0.05$) rise of 6.46% in total phenolic content with 20.66% significant enrichment in total flavonoids were obtained for *M. oleifera* grown on AgNPs treated soil over control. However, 74.48 and 77.47% decline in total phenolic contents in addition to 42.34 and 55.80% in total flavonoid contents in *M. oleifera* grown on Cd and Pb were obtained respectively. Interestingly, 66.31 and 68.58% improvement in total phenolic contents together with 41.73 and 52.86% boost in total flavonoid contents in *M. oleifera* grown on AgNPs supplemented 0.5 mg Cd and 0.5 mg Pb contaminated soil respectively. Total phenolic and flavonoid contents were significantly enhanced by AgNPs by 59.17 and 32.91% over *M. oleifera* planted on 0.75 mg Cd treated soil while boost of 56.92% in total phenolic content and 43.43% in total flavonoid contents were obtained over *M. oleifera* on 0.75 mg Pb contaminated soil.

Table 2
Germination and physiological indices of *M. oleifera* grown under different soil conditions.

	Root length	Shoot length	number of leaves	Percentage germination	Relative water content	Vigour index	Growth tolerance index	
							Shoot	Root
A	5.51 ± 0.25^{a}	11.80 ± 0.52^{a}	47.28 ± 1.57^{a}	62.82 ± 7.12^{a}	74.43 ± 7.19^{a}	1038.75 ± 82.92^{a}		
B	6.31 ± 0.15^{b}	17.05 ± 1.15^{b}	48.75 ± 3.34^{a}	73.94 ± 8.38^{b}	73.28 ± 6.68^{a}	1127.01 ± 55.11^{b}		
C	3.36 ± 0.19^{c}	8.67 ± 0.08^{c}	40.04 ± 1.36^{b}	53.94 ± 6.78^{c}	64.78 ± 3.74^{b}	645.12 ± 44.54^{c}	33.72 ± 0.88^{a}	29.96 ± 0.18^{a}
D	2.66 ± 0.07^{d}	6.27 ± 0.45^{d}	30.02 ± 2.82^{c}	25.70 ± 4.78^{d}	58.64 ± 4.98^{c}	134.11 ± 12.76^{d}	19.50 ± 1.84^{b}	17.12 ± 1.87^{b}
E	5.12 ± 0.02^{a}	9.89 ± 1.01^{e}	45.04 ± 3.72^{a}	63.38 ± 5.52^{a}	67.81 ± 2.72^{b}	1010.34 ± 16.72^{a}	48.10 ± 0.35^{c}	39.01 ± 3.07^{c}
F	5.05 ± 0.05^{a}	7.82 ± 0.38^{f}	44.14 ± 2.86^{a,b}	48.60 ± 4.86^{e}	65.17 ± 0.67^{b}	992.43 ± 19.04^{e}	36.82 ± 0.62^{a}	31.62 ± 0.15^{a}
G	3.88 ± 0.14^{c}	8.68 ± 0.86^{c}	44.14 ± 3.06^{a,b}	42.82 ± 1.82^{e}	65.55 ± 3.85^{b}	490.71 ± 18.72^{f}	28.39 ± 0.02^{d}	27.70 ± 1.34^{a}
H	3.64 ± 0.12^{c}	6.82 ± 0.42^{d}	39.66 ± 1.67^{b}	30.04 ± 2.54^{f}	67.71 ± 0.97^{b}	284.57 ± 11.44^{g}	25.52 ± 0.05^{d}	22.61 ± 1.15^{b}

Group A water (control), group B: 0.2 mg AgNPs/g soil group C: 0.8 mg $CdCl_2$/g soil, group D: 0.8 mg $PbCl_2$/g soil, group E: (0.2 mg AgNPs + 0.5 mg $CdCl_2$)/g soil, group F: (0.2 mg AgNPs + 0.5 mg $PbCl_2$)/g soil, group G: (0.2 mg AgNPs + 0.75 mg $CdCl_2$)/g soil, group H: (0.2 mg AgNPs + 0.75 mg $PbCl_2$)/g soil.
Results of percentage germination, root length, shoot length, relative water contents, vigour index and growth tolerance index are expressed as mean ± standard deviation of eight replicates while number of leaves was expressed as mean ± standard deviation of leaves on eight replicates.
Data having different superscripts are along the column are significantly different ($p < 0.05$).

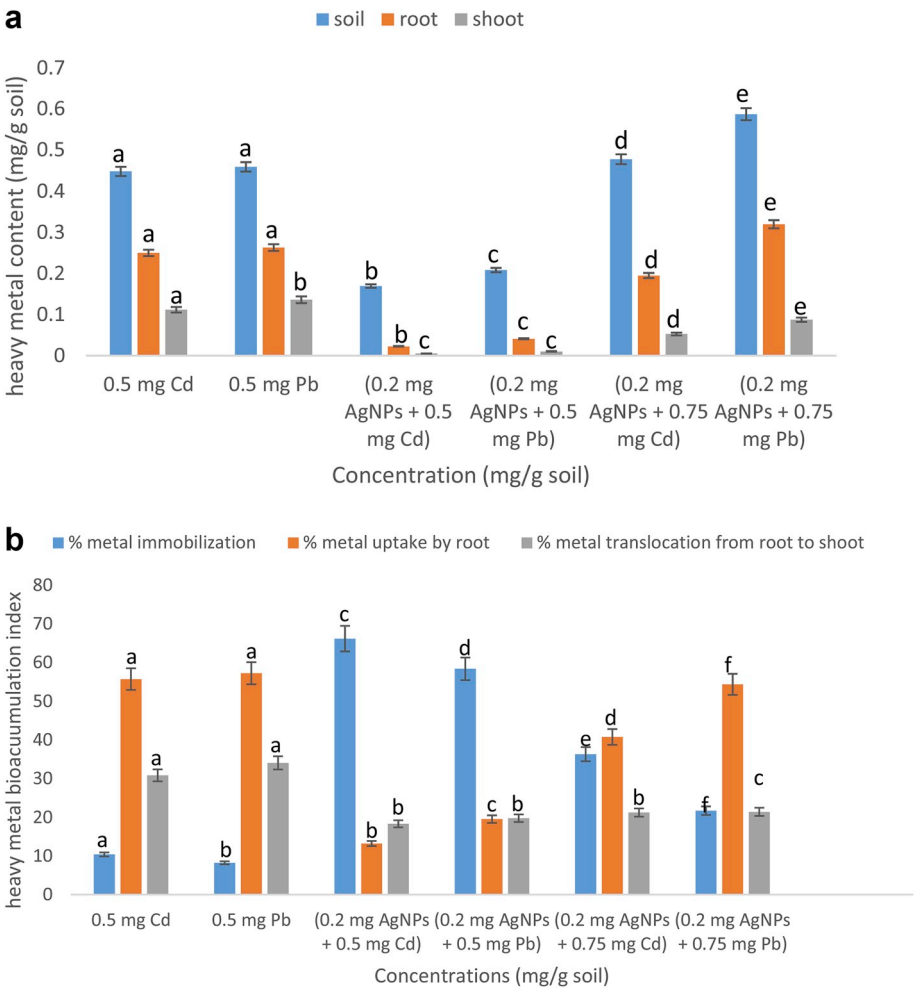

Fig. 2. a: Concentrations of Cd and Pb in soil, root and shoot. Bars with the same colour having different superscripts are significantly different ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)**b**: Heavy metal bioaccumulation index of *M. oleifera* grown on water (control), AgNPs, $CdCl_2$ and $PbCl_2$ spiked soil. Bars with the same colour having different superscripts are significantly different ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.6. *Effects of AgNPs on photosynthetic pigments and carotenoid contents in M. oleifera under Cd and Pb contamination*

Chlorophyll *a*, *b* and total carotenoid contents were significantly altered by Cd and Pb toxicities (Table 3). Cd and Pb significantly impaired formation of photosynthetic pigments in *M. oleifera* while there were improved contents in *M. oleifera* grown on AgNPs wetted Cd and Pb treated soil (Table 3). There were 17.43, 20.52 and 47.78% repressions in chlorophyll *a*, *b* and carotenoid contents respectively induced by Cd compared with control. Pb induced toxicity lowered

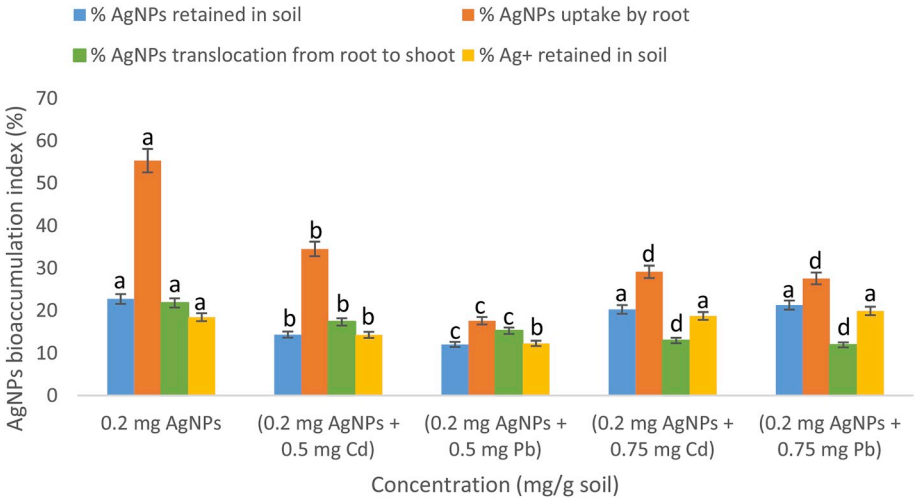

Fig. 3. Heavy metal bioaccumulation index of *M. oleifera* grown on water (control), AgNPs, $CdCl_2$ and $PbCl_2$ spiked soil. Bars with the same colour having different superscripts are significantly different ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Antioxidant activity, phytochemical, photosynthetic pigment contents and lipid peroxidation levels of *M. oleifera* grown under different soil conditions.

	DPPH (%)	Ferric chloride reducing (%)	Hydrogen peroxide scavenging (%)	Total phenolic contents (mg quercetin/g)	Total flavonoid contents (mg quercetin/g)	chlorophyll *a* (mg/g of fresh weight)	chlorophyll *b* (mg/g of fresh weight)	Carotenoids (mg/g of fresh weight)	MDA (nmol/g)
A	70.34 ± 3.42^{a}	67.46 ± 3.02^{a}	62.68 ± 2.24^{a}	486.07 ± 52.77^{a}	137.14 ± 11.29^{a}	6.53 ± 1.93^{a}	3.72 ± 0.02^{a}	2.69 ± 0.01^{a}	5.11 ± 0.21^{a}
B	81.47 ± 4.88^{b}	68.45 ± 3.28^{a}	72.54 ± 3.51^{b}	519.62 ± 30.08^{b}	172.85 ± 15.75^{b}	6.55 ± 0.06^{a}	3.73 ± 0.03^{a}	2.86 ± 0.05^{b}	5.38 ± 0.22^{a}
C	41.26 ± 5.23^{c}	37.61 ± 5.09^{b}	37.41 ± 3.02^{c}	124.05 ± 15.06^{c}	79.08 ± 8.63^{c}	5.39 ± 0.92^{a,b}	2.96 ± 0.02^{b}	1.40 ± 0.01^{c}	11.60 ± 0.40^{b}
D	27.07 ± 1.64^{d}	33.33 ± 3.13^{b}	34.34 ± 4.08^{c}	109.49 ± 9.37^{d}	60.61 ± 2.24^{d}	1.81 ± 0.05^{c}	1.05 ± 0.01^{c}	0.87 ± 0.00^{d}	12.20 ± 0.48^{b}
E	69.21 ± 3.26^{a}	54.16 ± 6.67^{c}	53.33 ± 1.73^{d}	368.22 ± 30.98^{e}	135.71 ± 12.43^{a}	5.97 ± 0.37^{a,b}	3.68 ± 0.02^{a}	1.46 ± 0.00^{c}	6.94 ± 0.28^{c}
F	67.04 ± 5.04^{a}	50.87 ± 3.02^{c}	53.88 ± 4.44^{d}	348.44 ± 42.54^{f}	128.57 ± 14.02^{a}	5.98 ± 0.42^{a,b}	3.69 ± 0.03^{a}	1.57 ± 0.01^{c}	7.13 ± 0.29^{c}
G	51.34 ± 8.45^{e}	44.64 ± 2.86^{d}	41.62 ± 2.72^{e}	303.79 ± 40.75^{g}	117.87 ± 10.76^{e}	5.45 ± 0.59^{a,b}	3.44 ± 0.02^{a,b}	1.97 ± 0.01^{d}	8.44 ± 0.24^{d}
H	51.10 ± 4.16^{e}	45.23 ± 1.81^{d}	41.55 ± 4.38^{e}	254.21 ± 22.94^{h}	107.14 ± 7.26^{f}	3.51 ± 0.04^{d}	2.02 ± 0.01^{d}	1.63 ± 0.00^{e}	9.21 ± 0.31^{e}

Group A water (control), group B: 0.2 mg AgNPs/g soil group C: 0.8 mg $CdCl_2$/g soil, group D: 0.8 mg $PbCl_2$/g soil, group E: (0.2 mg AgNPs + 0.5 mg $CdCl_2$)/g soil, group F: (0.2 mg AgNPs + 0.5 mg $PbCl_2$)/g soil, group G: (0.2 mg AgNPs + 0.75 mg $CdCl_2$)/g soil, group H: (0.2 mg AgNPs + 0.75 mg $PbCl_2$)/g soil.
MDA - Malondialdehyde.
Results in the table are expressed as mean ± standard deviation of three replicates. Data having different superscripts are along the column are significantly different ($p < 0.05$).

chlorophyll *a*, *b* and carotenoid contents by 72.24, 71.79, 67.55% respectively.

AgNPs ameliorated the toxicities of 0.5 mg Cd and 0.5 mg Pb by enhancing chlorophyll *a* by 43.10 and 69.67%, chlorophyll *b* by 46.81 and 71.57% and carotenoid contents by 3.91 and 44.38 in *M. oleifera* respectively.

Similarly, AgNPs mediated increase in chlorophyll *a* by 1.04 and 48.35%, chlorophyll *b* by 13.89 and 48.17% and carotenoid contents by 28.78 and 46.36 in *M. oleifera* grown on 0.75 mg Cd and 0.75 mg Pb contaminated soil supplemented with AgNPs respectively.

4. Discussion

The deleterious effects of heavy metals on quality and quantity of plants in addition to their transfer through food chain to humans cannot be underestimated. Although, different management mechanisms have been reported for heavy metal removal, nanoparticles especially those biologically synthesized offer an efficient remediation along with promotion of plant physiological, anatomical and biochemical parameters. Consequently, this study presents results of investigation on the potentials of cocoa pod extract mediated AgNPs on immobilization of Cd and Pb as well as attenuation of their toxicities induced on *M. oleifera*.

Growth parameters in *M. oleifera* were significantly suppressed as a result of exposure to Cd and Pb. Decreased root length, shoot length, number of leaves and percentage germination are common features attributable to toxicities of Cd and Pb which could have resulted from water imbalance, inhibition of enzymes and nutrient uptake (Praveen et al., 2017; Desoky et al., 2019). This is in consonance with the results of relative water contents which were significantly lowered by Cd and Pb. The decreased relative water contents in *M. oleifera* grown on Cd and Pb treated soil point to a compromised water positions in *M. oleifera* as a result of exposure to heavy metal. Indicators of *M. oleifera* seed viabilities (vigour and growth tolerance indices) were negatively affected by Cd and Pb with Pb having the more damaging effect suggesting the seeds under their influence were less viable and thus reduced growth. This is in agreement with reports of Lamhamdi et al. (2013), Singh and Lee (2016), Rizwan et al. (2016a,b) and Venkatachalam et al. (2016) that reported shorter root, shoot and decreased percentage germination for plant grown on Cd and Pb contamination.

AgNPs promoted seed viability as observed in the results of vigour and growth tolerance indices confirming the ability of AgNPs to enhance seed germination and strengthen *M. oleifera* growth. These were evidenced in longer root and shoot, higher percentage germination and more number of leaves. Moreso, higher water status in *M. oleifera* grown on AgNPs treated soil could be responsible for improved seed germination. This is consistent with the results of Liu et al. (2015), Tripathi et al. (2015), Praveen et al. (2017), Xiong et al. (2018), Azeez et al. (2017, 2019). These influence of AgNPs might be connected to their involvement in enhancement of some enzymes responsible for proliferation of cell and promotion of plant regulators leading to better physiological parameters (Syu et al., 2014; Shaheb et al., 2016; Wang et al., 2016; Cvjetko et al., 2017; Tripathi et al., 2015).

Higher germination potentials recorded for *M. oleifera* grown on AgNPs wetted Cd and Pb treated soil indicate the protective and modulative abilities of AgNPs against heavy metal induced toxicity in plants. It was noted with interest that AgNPs substantially alleviated Cd and Pb contamination by promoting germination and tolerance indices. These are related to the results of Venkatachalam et al. (2016), Galdames et al. (2017) and Gong et al. (2018). This could possibly be due to the abilities of AgNPs to strengthen *M. oleifera* against deleterious stresses of the heavy metals by decreasing their absorption and mobility.

M. oleifera had about 10% remediation action on Cd and Pb availability which could not prevent them from inducing their toxicity via absorption and translocation. However, this was greatly enhanced by AgNPs which immobilized more than 50% of the metals and reduced their translocation rates in both roots and shoot by about 40%. The ability to reduce mobility and bio-accessibility perhaps is dependent on the adsorptive affinity of AgNPs due to their morphological characteristics such as large surface area, electrostatic attraction and high reactivity which decrease mobility, bio-accessibility and bioavailability of the heavy metals (Shipley et al., 2011; Tripathi et al., 2015; López-Luna et al., 2016; Venkatachalam et al., 2016; Azeez et al., 2018; Gong et al., 2018).

Large portion of AgNPs absorbed by the roots of *M. oleifera* indicate soil bioavailability, non-aggregative and non-agglomerative attributes of AgNPs which were responsible for their ability to play roles in root enlargement for higher uptake of nutrients, promote regulators needed for cell division and enhance photosynthetic pigment formation. This is equally evident in the linear relationship (strong correlation coefficients) between the improvement of these parameters and percentage of AgNPs absorbed. This is in agreement with reports of Gupta et al. (2018) that AgNPs had phytostimulatory influence on rice seedling germination.

Malondialdehyde level (a biomarker of lipid peroxidation) is used to assess and estimate the oxidative stress status induced by stress factors. Higher MDA levels in *M. oleifera* grown on Cd and Pb treated soil showed they induced excessive free radical generation and suppressed antioxidant system that led to oxidative damage which injured root, shoot and leaves with manifestation in stunted growth and chlorosis. Similar reports have identified heavy metals of having potential of damaging stress in plant leading to increased MDA (Singh and Lee, 2016; Praveen et al., 2017). AgNPs addition positively influenced *M.*

oleifera ability to scavenge free radicals via induction of phytochemicals leading to significant decrease in MDA levels. This shows that AgNPs have the ability to inhibit the production of free radical causing oxidative stress stemming from their antioxidative properties (Lateef et al., 2015a,b, c). Equally, to cope with stresses and for defence, plants are imbued with phytochemicals for survival which can be overwhelmed by excessive production of free radicals but AgNPs prevented further effects of the metals by boosting antioxidant defence system to fight against oxidative damage of the metals (Azeez et al., 2017, 2019).

Decreased contents of total phenols, flavonoid and scavenging activities are indicative of heavy metals toxicities on *M. oleifera* because these parameters are stress modulators and assessors which reveal the extent of damage done to cell signalling pathway, cell proliferation and disruption of plant mechanism of survival. Scavenging abilities of free radical, super oxide ion radical, hydrogen peroxide radical and conversion of Fe^{3+} to a more useful Fe^{2+} were adversely hampered by the induced toxicities thereby exposing *M. oleifera* to oxidative injuries. Induction of polyphenols by AgNPs mitigated against oxidative damage because they play significant role in alleviating toxicities of heavy metals thus significant increase in the free radical and hydrogen peroxide scavenging activities in *M. oleifera* (Sharma et al., 2012; Kole et al., 2013; Raliya et al., 2015; Azeez et al., 2017). This is a confirmation of AgNPs potentials to attenuate heavy metal-induced toxicities via induction of polyphenol.

Developmental and healthy functions of plant depend on photosynthetic pigment contents and their reductions indicate an induced stress in the system (Gupta et al., 2018). Lowered contents of these parameters obtained for *M. oleifera* grown on heavy metal treated soil are suggestive of disruption of photosynthetic mechanism and inhibition of enzymes responsible for photosynthetic pathway (Praveen et al., 2017; Zhou et al., 2018). Chlorosis was more pronounced in *M. oleifera* grown on Pb treated soil showing the effects of heavy metals (Lamhamdi et al., 2013). Addition of AgNPs mopped up the toxicities of the metal by improving the concentrations of the pigment to support *M. oleifera* growth. This is similar to reports that nanoparticles attenuate alteration on photosynthetic pigment contents induced by heavy metals (Tripathi et al., 2015; López-Luna et al., 2016; Singh and Lee, 2016).

5. Conclusion

It is evident from the present study that AgNPs possess capacity for immobilizing (adsorbing) heavy metals by decreasing their absorption and mobility. It also has ability to attenuate oxidative damage on plants induced by heavy metals. *M. oleifera* exposed to Cd and Pb had compromised ability to scavenge free radicals and repressed physiological tolerance towards stress noticeable in their root and shoot lengths, relative water contents, growth tolerance index, antioxidant activities, polyphenolic contents and photosynthetic pigment contents. These were significantly enhanced by addition of AgNPs via induction of polyphenols indicating their protective and modulative abilities against injuries of heavy metals on *M. oleifera*. Therefore, the study has shown that the biosynthesized AgNPs can be valuable in agrosystems to mitigate the deleterious effects of heavy metals in crop production, and may assist in the bioremediation of environments that are contaminated with heavy metals by enhancing the growth of remediating plants.

Contribution

Professor Lateef A. synthesized and characterized silver nanoparticles using extract of cocoa pod. Dr. Azeez L. coordinated the planting, antioxidant and photosynthetic pigment assays together with Adetoro Raheed, Adewuyi Sulaimon and Olaoye Samuel. Dr. Adebisi Segun and Dr. Adejumo Ayoade coordinated heavy metal and nanoparticle analyses in soil and plant. Mr. Tijani handled all laboratory reagents preparation and sample drying.

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