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Alleviation of heavy metal stress by arbuscular mycorrhizal symbiosis in *Glycine max* (L.) grown in copper, lead and zinc contaminated soils

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ABSTRACT

There are few reports on the use of arbuscular mycorrhizal fungi (AMF) to promote growth and stress tolerance of soybean (*Glycine max* L.) in agricultural soils contaminated with heavy metals. The present study evaluated the role of AMF in promoting tolerance and growth, as well as uptake of heavy metals in shoots of soybean plants. Soybean plants were inoculated with AMF (*Funneliformis mosseae*) in a pot experiment polluted with different concentrations of heavy metals [copper (Cu), lead (Pb) and zinc (Zn)] as well as their combination. The tested AMF inoculum promoted the soybean growth and seed yield. Increased colonization of the soybean roots improved the soybean growth through increased phosphorus uptake and accumulation in the plant tissues by 68.8%. The results showed that soybean grown in the contaminated soils inoculated with AMF were more tolerant in alleviating the metals toxicity by retaining the heavy metals in the roots, thereby reducing translocation of Cu, Pb and Zn by 21.8, 57.6 and 67.3% respectively in the aerial part of the plant and improving the overall plant productivity by 59.1%. The findings provide evidence of the potential of AMF in phytoremediation of agricultural soils contaminated with toxic metals.

1. Introduction

In recent years, the pollution of agricultural soils with heavy metals by anthropogenic activities and agricultural practices, including excessive input of agrochemicals, has become a major concern for the sustainability of production in most crops (Li et al., 2014; Rafique and Tariq 2016). In most contaminated soils, heavy metals such as copper (Cu), lead (Pb) and zinc (Zn) are widespread and non-degradable (Ranjan et al., 2017). Considering the significance of some essential heavy metals such as Cu and Zn for plant structural and enzymes activation, their high soil concentrations can cause plant phytotoxicity and stunted growth (Anjum et al., 2015; Alongi 2017). In addition, ingestion of edible parts of plants such as fruits or grains may pose significant health risks to humans and animals (Toth et al., 2016). Plants have evolved many adaptive and tolerance strategies in alleviating toxicity to heavy metals in contaminated soils (Li et al., 2015). The formation of a symbiotic relationship in roots with arbuscular mycorrhizal fungi (AMF) was reported to be one of the most successful methods for enhancing the tolerance of host plants in soils contaminated with heavy metals (Joner and Leyval 1997; González-Chávez et al., 2019). Arbuscular mycorrhizal fungi are often found in heavy metal-polluted soils (Wang, 2017; Huang et al., 2018). Most plants form a symbiotic relationship with AMF (Smith and Read, 2008). The AMF hyphae acts as an intermediary between plants and fungi for nutrient uptake, in particular phosphorus (P) in exchange for carbohydrates from the host plant (Smith and Read, 2008). Several researchers have recently focused their attention on the use of AMF in contaminated soils to immobilize heavy metals (Gu et al., 2017; González -Chávez et al., 2019). Extra and intra-radical AMF mycelia, as well as vesicles and spores, act as an effective sink of heavy metals (Wang et al., 2017; Liu et al., 2018). In addition, P nutrition is reported to be an essential element in promoting plant tolerance through detoxification of heavy metals (Andrade et al., 2004). Inoculation with AMF improve plant growth and indirectly alleviate the stress induced by excess metals in the soil because of the boost of P uptake by AMF (Zhan et al., 2016). In some findings, AMF decreased plant uptake of heavy metals such as Zn, Cd, and Mn (Li and Christie 2000), whereas, in others

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increased uptake was reported (Jamal et al., 2002; Liao et al., 2003). Nevertheless, the reports on the impacts of AMF on host plant P absorption under heavy metal toxicity remain contradictory.

Most polluted soils naturally contain a mixture of various heavy metals at various concentrations (Versieren et al., 2016). Therefore, in predicting the phytotoxic effects of multiple metals and their absorption in plants, reports of single metal pollution may not be accurate, since the concentration of heavy metals in plants depend on their soil concentrations and interaction that may be antagonistic or synergistic (Wu et al., 2016). There are inconsistencies in previous studies on the ameliorative effects of AMF inoculation, which could be linked to the fugal species, host plant(Yang et al., 2015) heavy metal type and soil concentrations (Wu et al., 2015; Doubková and Sudová 2016). It is therefore important to provide a precise understanding of the impact of AMF on alleviating the toxicity of heavy metals in crops, as well as their mycoremediation strategies in both single and multi-metal soil contaminations.

Soybean (*Glycine max* L.) is an oilseed legume commonly cultivated in most parts of the world due to its high quality oil and protein content for livestock and human consumption, as well as for industrial purposes (Adeyemi et al., 2020). In spite of several reports on the growth promoting ability of AMF under heavy metal toxicity, there are limited findings on the effects of AMF on soybean in contaminated soils. Arbuscular mycorrhizal fungal isolate (*Funneliformis mosseae*) was chosen based on its beneficial effects on plant growth and P uptake in soybean (Thioub et al., 2019) as well as its reported predominance in heavy metal contaminated soils (Yang et al., 2015). Thus, this study presumed that (1) the AMF isolate would support the growth of soybean and P-nutrition in heavy metal contaminated soils, (2) the P-nutrition of AMF would lead to plant tolerance and alter the content, translocation and uptake of Cu, Pb and Zn in soybean tissues.

2. Materials and methods

2.1. Soil preparation

Top-layer soil samples were collected at depth of 0–20 cm from the Teaching and Research Farms of the Federal University of Agriculture, Abeokuta, Ogun state, Nigeria (Latitude 7° 15′ N, Longitude 3° 28′ E, 144 m a s l). The soil was classified as *kandic paleustalf* in the *Alfisol* order of the United States Department of Agriculture (USDA) soil taxonomy. The soil samples collected were bulked and sieved with 2 mm mesh prior to soil analysis. The particle size distribution (clay, silt, sand) was determined using the hydrometer method. Soil pH (1: 1 soil: water ratio) was determined using the electrometric method (Page et al., 1982). The organic carbon was measured using Walkley–Black wet oxidation method (Nelson and Sommers, 1982) and the result was multiplied by VanBenmelen's factor of 1.724 (Page et al., 1982) to estimate the soil organic matter content. Available phosphorus was measured using the Bray No. 1 method (Bray and Kurtz, 1945) and determined colometrically using the method of Murphy and Riley (1962). The total nitrogen was determined using modified micro-Kjeldahl digestion technique (Bremner and Mulvaney, 1982) and exchangeable cations was determined using ammonium acetate method (Moss, 1961). The modified wet sieving and sucrose techniques of Giovanetti and Mosse (1980) was used to determine the initial mycorrhizal spore density in the soil (48 spores/100 g of soil). The initial soil properties is shown in Table 1.

2.2. AMF inoculum

The AMF inoculum, *F. mosseae* (previously *Glomus mosseae*), was obtained from International Institute of Tropical Agriculture, Ibadan, Nigeria. The inoculum was propagated in maize (*Zea mays* L.) roots grown in autoclaved sand for 4 months. The crude inoculum used was mixture of soil, roots, hyphae and spores (approximately ~ 480 spores per 50 g of soil).

Table 1
Selected soil physical and chemical properties.

Soil property	value
Sand (g kg^{-1})	929
Silt (g kg^{-1})	55
Clay (g kg^{-1})	16
Texture	Sand
pH	6.8
Total nitrogen (g kg^{-1})	3.0
Organic matter (g kg^{-1})	6.88
Available P (mg kg^{-1})	8.6
Exchangeable Ca (cmol kg^{-1})	1.19
Exchangeable Mg (cmol kg^{-1})	2.06
Exchangeable K (cmol kg^{-1})	0.16
Exchangeable Na (cmol kg^{-1})	0.13
Cu (mg kg^{-1})	0.7
Zn (mg kg^{-1})	2.3
Pb (mg kg^{-1})	4.4
AMF spore density (spores/100 g of soil)	48

2.3. Pot experiment

The study was a 2 × 8 factorial on the basis of a completely randomized design, with three replicates. The first factor was AMF inoculation with or without *F. mosseae*. The second factor was eight levels of heavy metal contaminations [100 mg Cu kg^{-1}, 300 mg Cu kg^{-1}, 100 mg Pb kg^{-1}, 300 mg Pb kg^{-1}, 300 mg Zn kg^{-1}, 600 mg Zn kg^{-1}, multi-metal (100 mg Cu kg^{-1} + 100 mg Pb kg^{-1} + 300 mg Zn kg^{-1}) and control (C)]. The levels of the heavy metals were based on maximum allowable concentrations on agricultural and industrial soils respectively. The soil was air-dried and spiked with the heavy metals salts of $CuSO_4.5H_2O$, $PbSO_4$ and Zn $SO_4.7H_2O$ as source of Cu, Pb and Zn respectively. The pots filled with 10 kg of soil each were left for 15 days to bond the heavy metals with the matrix of the soil, and then subjected to saturation with water to balance the distribution of the heavy metals throughout the entire soil mass.

The seeds (5) of soybean (*Glycine max* L. cv. TGx 1448-2E) were sown directly in each pot and thinned to two plants 7 days after sowing. Each pot was inoculated with 20 g of AMF inoculum, applied to the base of the planting hole. The soil moisture was maintained at 70% water holding capacity (based on the weight of the water held in the sample). There was no fertilizer applied during the experiment. The whole plants (roots, shoots and seeds) were harvested at 120 days after sowing (four months).

2.4. Soybean growth and seed yield

The plant height and trifoliate leaf area (TLA) of the soybean plants were measured using metric ruler at 50 days after sowing. The leaf area was calculated using the derived equation (TLA = 0.411 + 2.008 LW), L and W represent the length and width of the terminal leaflet respectively (Weirsma and Bailey 1975). The soybean plants from each pot were harvested after four month of growth period.

2.5. Heavy metals (Cu, Pb and Zn) and phosphorus concentrations and uptake

The root and shoot samples were dried at 70 °C and then ground to fine particles. For the analyses, 0.5 g of each sample was used. The samples were mineralized in a mixture of concentrated HNO_3 and $HClO_4$ (4:1 v/v). The digestive was concentrated by evaporation to about 0.5 ml and then diluted with deionized water to a final volume of 10 ml. The concentrations of Cu, Pb and Zn in plant extracts were determined using Atomic Absorption Spectrometer. Total P was measured with the molybdate-blue colorimetric method (Murphy and Riley, 1962).

2.6. Bioabsorption, bioconcentration and translocation factors

The ability of plants to absorb, accumulate and translocate the heavy metals was measured by means of bioconcentration factor (BCF) and translocation factor (TLF) as described by Chaturvedi et al. (2018) using the equations below:

$$BCF = \frac{heavy\ metal\ content\ in\ shoot}{heavy\ metal\ content\ in\ soil}$$

$$TLF = \frac{heavy\ metal\ content\ in\ shoot}{heavy\ metal\ content\ in\ root}$$

2.7. AMF colonization

Thirty roots (approximately 1 cm segments) from each pot were cleared in hot KOH solution (10% w/v, at 90°C) for 1 h, before staining in acidic glycerol containing trypan blue lacto-glycerol (1:1:1:0.5g) at 90 °C for 30 min (Phillips and Hayman, 1970), then examined under a compound light microscope (Olympus Bx51, Japan) at 200× magnification. The root colonization of the soybean was calculated on percentage root length colonized (RC);

$$RC = \frac{Number\ of\ colonized\ roots}{Total\ number\ of\ roots} \times 100$$

2.8. Statistical analysis

The variables for the treatments (heavy metals and AMF) were subjected to a two-way analysis of variance (ANOVA) using a general linear model with mixed-effects. Prior to the ANOVA, Shapiro-Wilk test for normality and Bartlett's test for homogeneity of variances were performed. The means of treatment were compared by Fisher's protected LSD test at $p < 0.05$, using Genstat Release 12.1 statistical software package (Copyright, 2009; VSN International Ltd). Pearson's moment correlation coefficients were determined among the root colonization, P uptake and translocation of Cu, Pb and Zn in soybean tissues.

3. Results

3.1. Heavy metals (Cu, Pb and Zn) bioaccumulation and translocation in soybean plants

The effect of heavy metal concentrations, AMF inoculation and their interaction on bioaccumulation and translocation factor are summarized in Table 2. The results showed that the interactions of AMF inoculation and concentrations of the heavy metals significantly ($p < 0.01$) influenced the concentration of Cu, Pb and Zn in the soybean tissues (Fig. 1A, B and C). The BCF of Cu was found to be highest in Zn-polluted soil (300 mg kg^{-1}) inoculated with AMF compared to other treatments (Fig. 1A). The concentration of Pb was recorded to be highest in non-inoculated soybean plants grown in Cu-polluted soil (300 mg kg^{-1}), having BCF > 1 compared to other treatments (Fig. 2A). The BCF of Zn was reduced ($p < 0.01$) by AMF inoculation in Pb-polluted soil (300 mg kg^{-1}), Zn-polluted soil (600 mg kg^{-1}) and multi metals-polluted soil by 26.8, 30.8 and 64.5%, respectively (Fig. 1C). The lowest concentration of Zn was found in soybean inoculated with AMF under multi-metals contamination.

Similarly, interactions of AMF inoculation and concentrations of the heavy metals significantly ($p < 0.01$) influenced the translocation of heavy metals in the soybean tissues (Fig. 2A, B and C). The translocation factor (TLF) of Cu was significantly reduced in Cu-polluted soils (100 and 300 mg kg^{-1}) by 81.9 and 80.3% respectively, Zn-polluted soil at 300 mg kg^{-1} by 30.6% and multi metals-polluted soil by 75% when inoculated with AMF (Fig. 2A). The TLF of Pb was generally lower in all treatment combinations except in non-inoculated soybean plants grown in Pb-polluted soils (100 and 300 mg kg^{-1}) and multi metals-polluted soil (Fig. 2B). Furthermore, the results showed lower ($p < 0.01$) TLF of Zn in all treatment combinations except in non-inoculated soybean plants grown in Zn-polluted soils (300 and 600 mg kg^{-1}) and multi metals-polluted soil (Fig. 2C).

Table 2
Factorial ANOVA of treatment effects on dry plant weight, root colonization, P concentration, and bioconcentration and translocation factor in soybean.

	P-values		
	HM treatments	AMF inoculation	HM × AMF treatments
BCF			
Cu	<0.001	0.893	0.037
Pb	0.056	0.027	0.031
Zn	<0.001	0.082	0.045
TF			
Cu	<0.001	<0.019	<0.001
Pb	<0.001	<0.001	<0.001
Zn	<0.001	<0.001	<0.001
P concentration			
Shoot	<0.001	<0.001	<0.001
Root	<0.001	<0.001	<0.001
Root colonization	<0.001	<0.001	<0.001
Plant height	<0.001	0.004	0.038
Leaf area	<0.001	0.011	0.014
Dry weight			
Shoot	<0.001	<0.001	0.005
Root	<0.001	<0.001	0.003
Seed yield	<0.001	<0.001	0.045

BCF: bioconcentration factor; TF: translocation factor.

Table 3
Correlation coefficients (Pearson) among variables of heavy metals translocation, phosphorus uptake and root colonization.

Variables	Root colonization	TLF (Cu)	TLF (Pb)	TLF (Zn)
P content	0.96	−0.46	−0.43	Ns
Root colonization	–	−0.38	−0.39	Ns
TLF (Cu)		–	ns	0.41
TLF (Pb)			–	Ns
TLF (Zn)				–

TLF = translocation coefficient; ns = not significant.

3.2. Root colonization and phosphorus content

The effect of heavy metal concentrations, AMF inoculation and their interaction on root colonization and P concentration in shoot and root are summarized in Table 2. The root colonization of the soybean plants was increased ($p < 0.01$) by AMF inoculation in all the treatments (Fig. 3A). The highest root colonization was observed in non-polluted soil, while the lowest colonization rate was recorded in Zn (600 mg kg^{-1}) polluted soil. The P content in the shoots and roots were increased ($p < 0.01$) by AMF inoculation in all the treatments as well as the control. In AMF-inoculated plants, shoot P content was lower in Zn-polluted soil (600 mg kg^{-1}) compared to other treatments (Fig. 3B), while for the roots, P content was lower in Pb-polluted soil (300 mg kg^{-1}) as shown in Fig. 3C.

3.3. Growth and grain yield of soybean

The effect of heavy metal concentrations, AMF inoculation and their interaction on plant height, leaf area, shoot and root dry weight and seed yield are summarized in Table 2. The shoot height of the soybean plants was observed to significantly increase ($p < 0.01$) by AMF inoculation in all the treatments, except in soils polluted with higher dose of Pb (300 mg kg^{-1}) and Zn (600 mg kg^{-1}) (Fig. 4A). Similarly, AMF inoculation significantly increased the leaf area of the soybean plants (Fig. 4B), except in multi-metal polluted soil where no difference ($p > 0.05$) was

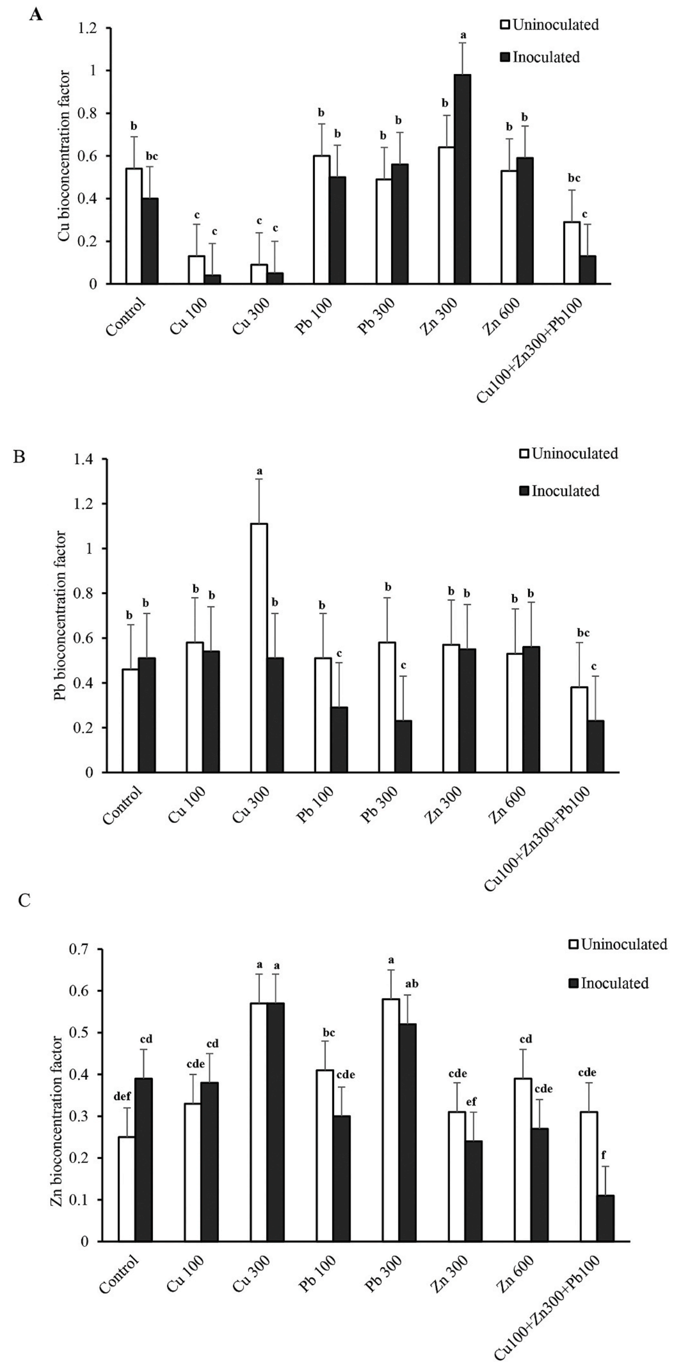

Fig. 1. Effect of AMF inoculation on bioconcentration factor of Cu (A) Pb (B) and Zn (C) in soybean plant under different heavy metals treatments. Bars represent means and error bars represent standard error of means (n = 5). Means followed by the same letter above columns do not differ significantly by the Fisher's protected test ($p \leq 0.05$).

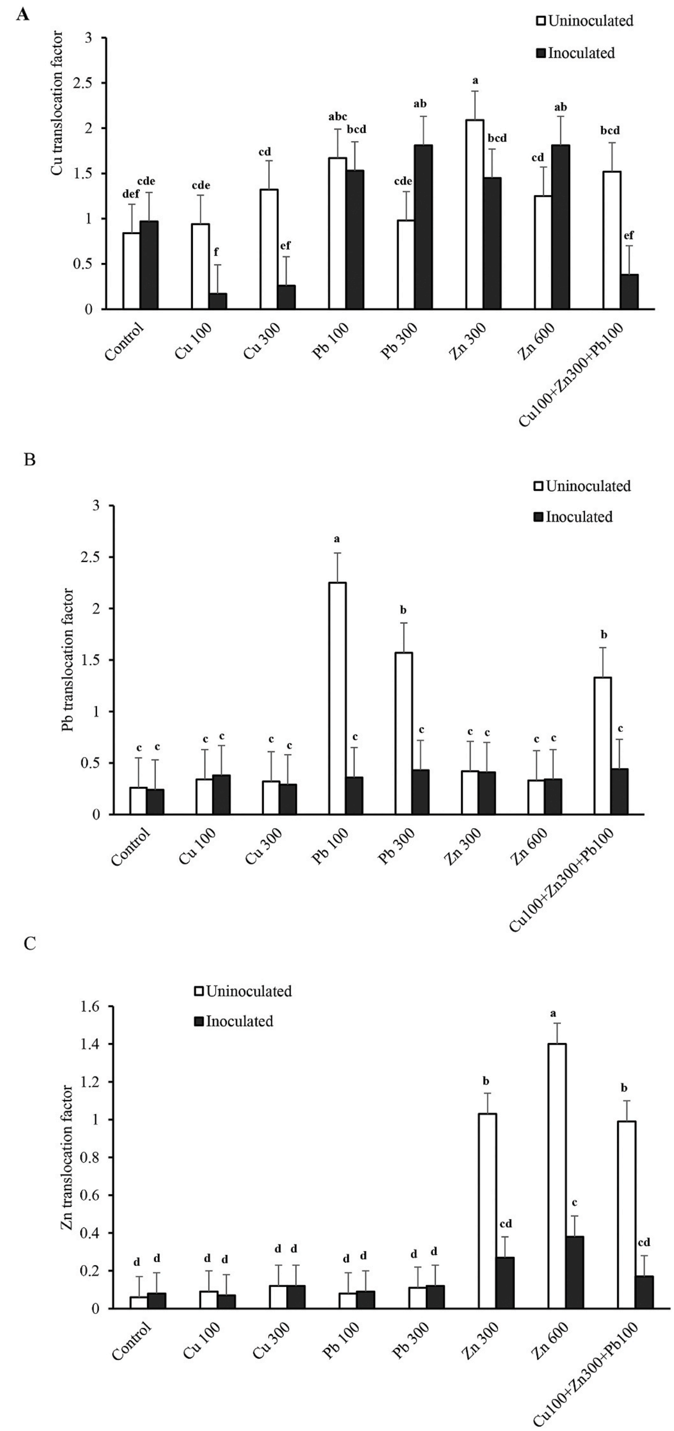

Fig. 2. Effect of AMF inoculation on translocation factor of Cu (A) Pb (B) and Zn (C) in soybean plant under different heavy metals treatments. Bars represent means and error bars represent standard error of means (n = 5). Means followed by the same letter above columns do not differ significantly by the Fisher's protected test ($p \leq 0.05$).

observed between inoculated and non-inoculated plants.

Dry matter accumulation in the shoots and roots of the soybean plants increased significantly by AMF inoculation across all the treatments and control as shown in Fig. 5A and B. However, in soils polluted with Zn (300 mg kg^{-1}) and multi-metals polluted soil, no significant difference was observed between inoculated and non-inoculated plants. The seed yield was observed to increase significantly by AMF inoculation in all the treatments, except in soils polluted with Pb (300 mg kg^{-1}), Zn (600 mg kg^{-1}) and multi-metals polluted soil (Fig. 5C).

3.4. Correlations among heavy metals translocation, phosphorus uptake and root colonization

The Pearson's moment correlation analysis showed significant ($p < 0.01$) and positive relationship ($r = 0.96$) between AMF root colonization and P accumulation in the soybean plants (Table 2). The increased P accumulation in the soybean reduced the translocation factor (TLF) of Cu and Pb in the shoots, which resulted in negative correlations between

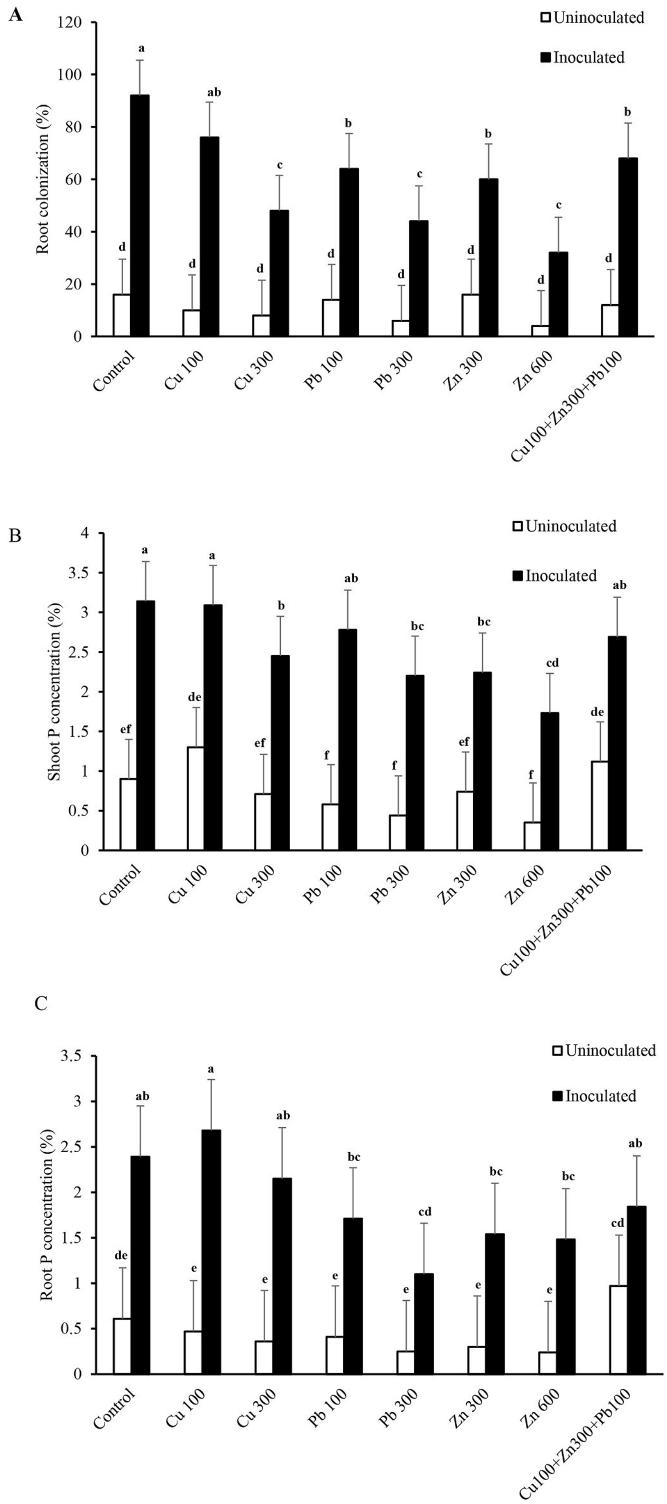

Fig. 3. Effect of AMF inoculation on root colonization (A) and P contents in shoot (B) and root (C) of soybean plant under different heavy metals treatments. Bars represent means and error bars represent standard error of means ($n = 5$). Means followed by the same letter above columns do not differ significantly by the Fisher's protected test ($p \leq 0.05$).

P content and TLF of the heavy metals (Table 2). Similar negative correlation was observed between root colonization and TLF of both Cu and Pb. There was no correlation between P content and TLF of Zn, as well as between root colonization and TLF of Zn.

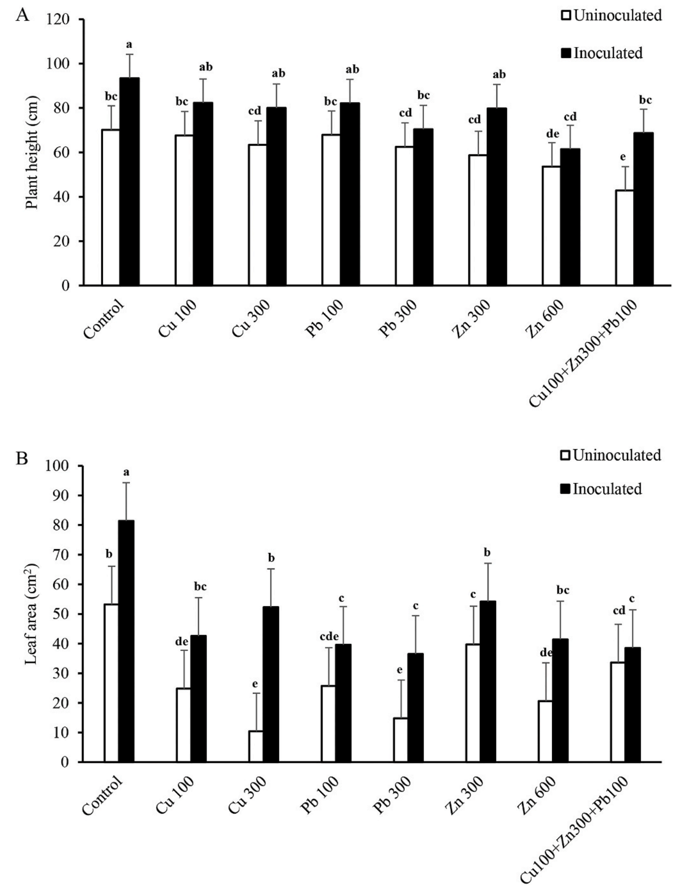

Fig. 4. Effect of AMF inoculation on plant height (A) and leaf area (B) of soybean plant under different heavy metals treatments. Bars represent means and error bars represent standard error of means ($n = 5$). Means followed by the same letter above columns do not differ significantly by the Fisher's protected test ($p \leq 0.05$).

4. Discussion

In the present study, phosphorus uptake, growth and yield responses to AMF inoculation and heavy metal contaminated soils showed a decrease in soybean P uptake, biomass and seed yield with an increase in soil heavy metal concentrations. The relationship between heavy metals and root growth could lead to metabolic responses include cell injury, mitochondrial dysfunction, and decreased absorption of water and nutrients. In general, high Zn exposure (600 mg kg^{-1}) was found to be the most toxic, contributing to a greater reduction in biomass, P uptake and soybean seed yield.

Several studies have shown that heavy metals can seriously inhibit the cell expansion, accumulation of biomass and absorption of mineral nutrients such as P, resulting in stunted growth (Zhou et al., 2018). This confirms the results of the present study. High Cu and Zn concentrations can directly alter different physiological and biochemical properties by hindering the absorption of nutrients, reducing water potential and disrupting photosynthesis (Anjum et al., 2015; Rizwan et al., 2016; Alongi 2017). Similarly, the study of Porrut et al. (2011) found that high Pb concentrations directly influenced the status of water supply and photosynthesis by changing the chloroplast structure. However, the findings of the study showed that, relative to single heavy metal stress, soybean growth under multi-metal stress of Cu, Pb and Zn was not significantly negatively affected. This may mean antagonistic interaction of these three metals on soybean development (Wu et al., 2016).

Extensive mycorrhizal colonization was observed in soybean inoculated with *F. mosseae*, however the proportions decreased marginally

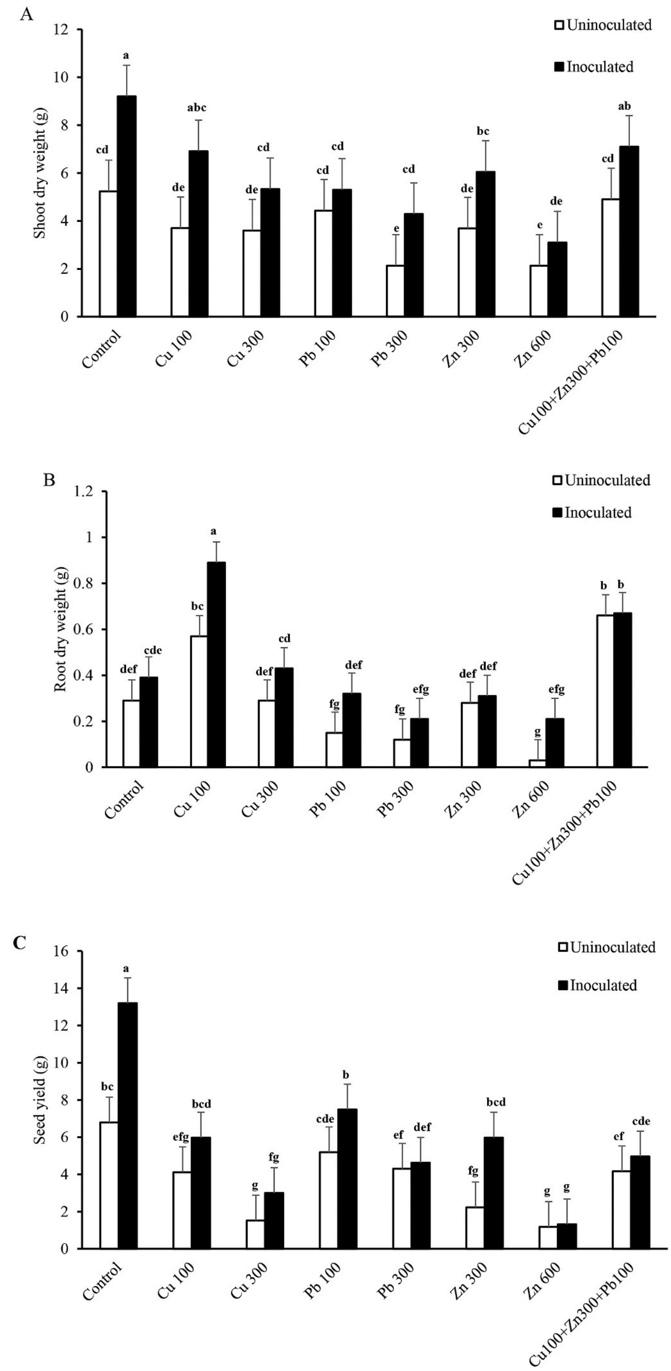

Fig. 5. Effect of AMF inoculation on shoot dry weight (A), root dry weight (B) and seed yield (C) of soybean plant under different heavy metals treatments. Bars represent means and error bars represent standard error of means (n = 5). Means followed by the same letter above columns do not differ significantly by the Fisher's protected test ($p \leq 0.05$).

with increased heavy metal concentration. Due to the capacity of the AMF isolate to inhabit contaminated soils as well as soybean mycorrhizal dependency, the mycorrhizal colonization observed can be partly explained (Wang, 2017; Huang et al., 2018). It should be noted that mycorrhizal colonization was exhibited by non-inoculated plants, supporting the resistance of native AMF in the soil used for the study. High concentrations of heavy metals also decrease the colonization of AMF in plant roots but such findings may differ due to a wide variety of reasons, such as the compatibility of AMF species and plants (Bahraminia et al., 2016; Sarkar et al., 2018), element concentration of metal and oxidation state, and environmental conditions (Yang et al., 2015; Dietterich et al., 2017). Our observations, however, are consistent with previous studies in which metal concentrations in plants such as sunflower, barley (Barcos-Arias et al., 2015) and soybean did not influence mycorrhizal colonization (Adeyemi et al., 2021).

An important aspect of this study was the retention of the applied heavy metals in the soybean roots and enhanced P uptake necessary for several physiological processes as well as plant tolerance to heavy metal toxicity. The findings in this study revealed that, under conditions of heavy metal contamination, inoculated soybean had more biomass, higher P uptake and seed yield compared to non-mycorrhizal plants explaining the symbiotic ability of the fungal isolate used. The symbiotic relationship with AMF has been reported to change plant responses to heavy metal stress and improve plant resistance in heavy metal contaminated soils (Joner and Leyval 1997; González-Chávez et al., 2019). Reduction of heavy metals toxicity induced by mycorrhization is due to the positive effect of AMF on heavy metal uptake in contaminated soil (Houben et al., 2013). The enhanced P uptake of the AMF isolate confirmed its high P uptake potential, which is consistent with the findings of Tan et al. (2015); Sakariyawo et al. (2017); Wang et al. (2017) and Adeye et al. (2020).

Several mechanisms have been developed by plants in heavy metal polluted soils to adapt or alleviate heavy metals toxicity (Li et al., 2015). The symbiosis of AMF can trigger some of changes in the host plants to cope with toxicity of the heavy metals. In the present study, AMF inoculation was found to enhance root colonization, thereby increasing absorptive surface area of the soybean roots through the extra-radical hyphae exploration of the rhizosphere beyond its root-hair zone. This is responsible for the enhanced P uptake with mycorrhization in the contaminated soils thus, resulting in greater photosynthetic activity. In many mycorrhizal plant types, Andrade et al. (2004) reported higher P/metal ratios, indicating that the greater P level of these plants may be responsible for alleviating metal stress by chelating metal ions within the cells with phosphate. The P uptake by plants with AMF inoculation is stored as polyphosphate granules in their vesicles and help in detoxification of metals (Zhan et al., 2016; Shi et al., 2019). In addition, the improved root and shoot growth through efficient P uptake by AMF could cause a decrease in heavy metal toxicity due to dilution effect, a potential mechanism by which AMF increases resistance to heavy metal stress in host plants (Leyval et al., 1997). However, the efficacy of this dilution effect could vary between plants, heavy metals and concentrations in the soil.

The induced morphological changes in the roots and rhizosphere by AMF could help plants to respond better to the adverse conditions caused by heavy metals. The root is the first and primary organ of plants that is in frequent contact with contaminated areas. Several plants have been reported as having substantial potential to impart resistance to AMF-related heavy metal stress by stabilizing heavy metals in the plant roots to reduce their translocation to shoots and leaves (Zhou et al., 2017). Mycorrhizal fungi can accumulate a large proportion of heavy metals across fungal structures and decrease their uptake by the host plant, resulting in less toxicity and fostering tolerance. Increased intra-radical hyphae has been reported to assist in binding processes of heavy metals (Huang et al., 2017; Liu et al., 2018).Mycorrhizal hyphae has been reported to accumulate more metals in the roots through their chelating ability (Nayuki et al., 2014; Wang et al., 2016), thereby limiting the translocation of the metals from root to the shoot. Several mechanisms through which AMF exclude or immobilize heavy metals have been reported. The extra and intra-radical hyphae in root cortex contain chelatin molecules such as glucan, chitin and galactosamine polymers that adsorb heavy metals to its surface (Wu et al., 2016). Organic acid exudation such as citric, malic and oxalic acids by the AMF hyphae helps in immobilization of metals through complexation (Shi et al., 2019).

In addition, glomalin secretion by AMF helps in heavy metal inactivation in soil. AMF secretes organic compounds with low molecular weight in conjunction with plants, which decreases the bioavailability of heavy metals (Ferrol et al., 2016). Several studies have provided clear proof of glomalin sequestration or chelation of heavy metals (Wu et al., 2014). Mycorrhizal inoculation was reported to show a significant decrease in shoot Cd, Pb, Zn, and Cu concentrations due to the release of glomalin chelating heavy metals in the soil (Nafady and Elgharably 2018). Hu (2019) recently indicated that symbiosis of *Rhizophagus intraradices*, *Glomus versiforme* and *F. mosseae* showed an increase in soil total-glomalin and decreased accumulation of Pb and Cd in maize due to increased soil pH.

The overall results of the inoculation of AMF on the soybean growth and tolerances under Cu, Pb and Zn stresses are interlinked to enhanced P uptake and sequestration of the heavy metals in the roots, thus reducing metals translocation to the shoots. Moreover, the results set the basis for future research, since this AMF isolate seems suitable for heavy metal phytostabilization strategies. However, it is necessary to carry out bioassays with contaminated soils as well as trials under field conditions to validate the phytoremediation potential of *F. mosseae* as well as other AMF species.

5. Conclusion

The results revealed the negative effects of Cu, Pb and Zn as well as their interactions on soybean growth, phosphorus uptake and yield responses grown under heavy metals (Cu, Pb and Zn) stresses. The cultivation of soybean plant under heavy metals stress resulted in reduced P uptake and biomass, which influenced overall growth and yield performance. Arbuscular mycorrhizal fungal inoculation enhanced soybean growth, P uptake and mitigate the toxicity caused by Cu, Pb and Zn and their multiple stress. Overall, the findings revealed that inoculation of AMF (*F. mosseae*) might be an important solution to improving the tolerance of soybean in contaminated agricultural soils.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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