Original Research Paper

Phytoremedial Potential of *Tagetes Erecta* Under Mycorrhizal Inoculation in Heavy Metal Polluted Soil

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Abstract: Investigation of the growth of *Tagetes erecta* under different levels of contamination of Copper (Cu) and Lead (Pb) in the soil and effects of mycorrhizal species (*Glomus mosseae*) on Cu and Pb to identify hyperaccumulator plants in tropical environment that could be used in remediation of heavy metal from contaminated soils. It was a greenhouse experiment consisted of factorial combination of two heavy metal (Cu and Pb) treatments which were replicated three times in Complete Ramdomized Design (CRD) with two mycorrhizal (*Glomus mosseae* and non-mycorrhizal) treatments. Metal solutions of Cu and Pb at the concentrations of Cu are 0, 125, 250, 500, 1000 mg kg^{-1} and concentrations of Pb are 0, 25, 50, 75, 100 mg kg^{-1} were prepared using $CuCl_2$ and $PbCl_2$ soluble compounds respectively. Soil mycorrhizal inocula of *Glomus mosseae* was applied at the rate of 20 g per pot. Five seeds of *Tagetes erecta* were planted per pot and were thinned to two stands per pot at two weeks after planting. Pre- and post- soil tests were carried out to determine soil physical and chemical properties, using standard methods. At 12 Weeks After Planting (WAP), *Tagetes erecta* was harvested and analyzed for Copper and Lead uptake using Atomic Absorption Spectrophotometer. The data collected were analyzed using descriptive and inferential statistics. The results showed that Arbuscular Mycorrhizal Fungi enhanced the number of leaves, height and stem girth of *Tagetes erecta* in high concentration (1000 mg kg^{-1}) of Cu contaminated soil. *Glomus mosseae* inoculation did not enhance the growth parameters of *Tagetes erecta* irrespective of the levels of Pb contamination *Glomus mosseae* inoculation significantly ($p \leq 0.05$) increased Cu uptake of *Tagetes erecta* at 500 mg kg^{-1} of Cu concentration of a polluted soil. In contaminated soil, containing 50 mg kg^{-1} Pb *Glomus mosseae* significantly ($p \leq 0.05$) increased Pb uptake in *Tagetes erecta*.

Keywords: Arbuscular Mycorrhiza, Heavy Metals, *Tagetes Erecta*, *Glomus Mosseae*, Hyperaccumulation

Introduction

In many parts of the world, the problem of environmental pollution has assumed an unprecedented proportion (Giuffré *et al*., 2012). Industrial revolution and increase in world population have inevitably been responsible for environmental pollution. Many events of coming decades already were predetermined with past and present activities of man on planet earth. Major pollution sources include point sources such as emissions, waste discharge from industries, vehicle exhaustion and non-point sources such as soluble salts (natural and artificial), insecticides/pesticides, disposal of industrial and municipal waste and excessive use of fertilizers (Olarenwaju *et al*., 2009). About 60% of these pollutants are referred to as heavy metals (Kamnev, 2003). Heavy metals are the most insidious pollutants because of their non-biodegradable nature and properties that affect all forms of ecological system (Saba *et al*., 2013). Despite the toxicity potentials of heavy metals, some are essential for normal healthy growth and reproduction at low but critical concentration. Unfortunately, developing countries of the world such as Nigeria could not afford the cost of remediation because of lack of adequate fund, misplacement of priorities, poor management strategy and low level of technology advancement to handle problems of pollution. To address these challenges, the application of a method that offers a cost effective and an environmentally friendly approach that utilizes bio-agents with appropriate techniques such as phytoextraction should be employed. Although, over 500 plants species had been identified as metalliferous or hyperaccumulators of metals from polluted sites, majority of which are exotic species with adaption to a species natural habitat (Krämer, 2010). Thus, the need to also access the phytoremedial potentials of some indigenous plant species or weeds whose potential are yet to be known, as hidden potentials of some commonly grown weeds needs to be unveiled for human benefit particularly as it relates to improving the quality of the environment.

Hence, this study investigate the ability of the weed plants as African Marigold (*Tagetes erecta*) to extract heavy metals from polluted soils.

Material and Methods

Experimental Site

The greenhouse experiment was carried out at the Faculty of Agriculture, Obafemi Awolowo University (OAU).

Experimental Procedure

A total of 120 plastic pots with drainage holes at the bottom with 3 kg each of air-dried soil. The soil that was collected was air dried and sterilized at 121°C for 2 h using autoclave to eliminate native mycorrhiza fungi propagules as well as other microorganisms present in the soil and sieved using 2mm mesh. These concentrations were used to contaminate the soil in the pots. The soil was left for four days for equilibration before mycorrhiza treatments were applied. The mycorrhiza treatment consisted of 30 pots each of *Glomus mosseae* (mycorrhiza) and non-mycorrhiza respectively. Soil mycorrhiza inoculum of *Glomus mosseae* was applied at the rate of 12 g per pot of 100 spores. Five seeds of *Tagetes erecta* were planted per pot and were thinned to two stands per pot at two weeks after planting and replicated 3 times in a completely randomized design. The pots were maintained weed free and pots were watered regularly with deionized water to moisture capacity to maintain soil moisture at 70% of field water holding capacity.

Parameters Determined

At the end of twelve weeks after planting, plant growth, percentage of root colonization by Arbuscular Mycorrhizal fungi, soil analysis, N, K, Ca, Mg, Zn, Pb, Fe, Cd, Cu, K and Na were all determined based on series of methods used at the end of twelve weeks after planting.

Statistical Analyses

A statistical comparison of means was made with analysis of variance (ANOVA) and treatment means were separated using Duncan Multiple Range Test at $p<0.05$ available in the SPSS. 16 statistical packages version.

Results and Discussion

The results of the pre-experiment analysis conducted on the soil samples used for this study indicated that the soil was sandy loam (84.4% and 15.4%, sand and silt composition respectively) and were characterized by mean pH of 6.5, available P 12.8 mg kg^{-1}, 2.1 mg kg^{-1} Organic carbon, Nitrogen 0.22 mg kg^{-1} and Ca^{2+} was 2.02 mg kg^{-1}. The Pb and Cu contents of the soil were 0.22 mg kg^{-1} and 0.74 mg kg^{-1}. The physical and chemical characteristics of soil used in the experiment is presented in the Table 1 above and Fig. 1 shows the weed plants of African Marigold (*Tagetes erecta*) planted on the soil.

Growth of T. erecta Different Concentrations of Cu in Soil with Glomus Mosseae Inoculation.

Number of Leaves

The response of leaves of *T. erecta* to different levels of Cu concentration varied with *Glomus mosseae* inoculation. In an uncontaminated Cu (0 mg kg^{-1}), the effect of *Glomus mosseae* inoculation on the number of leaves in *T. erecta* was evident at 6, 10 and 12 Weeks After Planting (WAP). However, at 125 mg kg^{-1} of Cu contamination, the effect of *Glomus mosseae* became evident at 12 weeks after planting on leaf development *T. erecta.*

Table 1: Physical and chemical characteristics of soil used in the experiment

Characteristics	Value
Sand (gkg^{-1})	760
Silt (gkg^{-1})	140
Clay (gkg^{-1})	100
pH in water (H_2O)	6.50
Organic Carbon (gkg^{-1})	2.06
Available phosphorus (gkg^{-1})	12.81
Nitrogen (gkg^{-1})	0.22
Exchangeable cations (cmol kg^{-1})	
Na^+	0.56
K^+	0.51
Ca^{2+}	2.02
Mg^{2+}	0.54
Textural class	Sandy loam
Cu ($mgkg^{-1}$)	0.74
Pb ($mgkg^{-1}$)	0.22

Fig. 1: *Tagetes erecta*

With the increase in the level of Cu contamination to 250 mg kg^{-1}, the number of leaves in *T. erecta* was enhanced by *Glomus mosseae* inoculation at 4-12 WAP, (Fig. 2).

The response of leaf of *T. erecta* to different levels of Pb contamination under the influence of *Glomus mosseae* varied with the concentrations of Pb. In the 0 mg Pb kg^{-1} contaminated soil, *Glomus mosseae* inoculation significantly ($p \leq 0.05$) enhanced the number of leaf (Fig. 5). When the soil was contaminated with 25 mg kg^{-1} of Pb, *Glomus mosseae* improved leaf production *T. erecta*. At 100 mg Pb kg^{-1}, there was no effect of *Glomus mosseae* on the effect of *Glomus mosseae* on number of leaves.

Plant Height

In an uncontaminated Cu soil (0 mg kg^{-1}), *Glomus mosseae* enhanced the plant height of *T. erecta* at 6 to 12 WAP. At 125 mg kg^{-1} of Cu contamination, plant height of *T. erecta* was enhanced by *Glomus mosseae* inoculation except at 12 weeks after planting in *T. erecta*, there was no marked effect of mycorrhiza inoculation on the plant height. Similar, trend was also observed for *T. erecta* 250 mg kg^{-1} Cu contamination.

When the Cu contamination was doubled (500 mg kg^{-1}) the effect of *Glomus mosseae* significantly ($p \leq 0.05$) enhanced the plant height in both plants, particularly at 10 to 12 WAP (Fig. 3d). At a higher concentration (1000 mg kg^{-1}) the effects of mycorrhizal inoculation on the plant height of *T. erecta* significantly ($p \leq 0.05$) increase the plant height at early stage of development (2 to 4 weeks after planting) (Fig. 3).

Stem Girth

At 0 mg kg^{-1} of Cu soil contamination, mycorrhizal inoculation did not have appreciable effect on the stem girth of *T. erecta* except at 4 and 10 WAP, where *Glomus mosseae* was more pronounced (Fig. 4a). Similar trend was also observed at the level of 125 mg kg^{-1} of Cu contamination (Fig. 4b). Mycorrhizal inoculation increased the stem girth of *T. erecta* at 8 to 12 Weeks After Planting (WAP) under 500 mg kg^{-1} of Cu contaminated soil. At 1000 mg kg^{-1} concentration of Cu, *Glomus mosseae* inoculation significantly ($p \leq 0.05$) enhanced the stem girth of both *T. erecta*, at 8 to 12 Weeks After Planting (WAP) in *T. erecta*.

Growth of T. erecta Different Concentrations of Pb in Soil with Glomus mosseae Inoculation

Number of Leaves

The response of leaf of *T. erecta* to different levels of Pb contamination under the influence of *Glomus mosseae* varied with the concentrations of Pb. In the 0 mg Pb kg^{-1} contaminated soil, *Glomus mosseae* inoculation significantly ($p \leq 0.05$) enhanced the number of leaf. When the soil was contaminated with 25 mg kg^{-1} of Pb, *Glomus mosseae* improved leaf production in both *T. erecta.* At 100 mg Pb kg^{-1}, there was no effect of *Glomus mosseae* on the effect of *Glomus mosseae* on number of leaves.

In an uncontaminated (0 mg kg^{-1}) soil under Pb treatment experiment, the height of *Glomus mosseae* inoculated *T. erecta* was higher than those with non-inoculated plants.

Plant Height

However, at 50 mg Pb kg^{-1} soil contamination, the influence of *Glomus mosseae* on plant height of *T. erecta* was reduced throughout the period of plant growth. The response of plant height of *T. erecta* to 75 mg Pb kg^{-1} contaminated soil was significantly ($p \leq 0.05$) enhanced by *Glomus mosseae* inoculation at 12 WAP. At a higher concentration of 100 mg kg^{-1} of Pb, the plant plants height did not follow a definite pattern with respect to mycorrhizal inoculation (Fig. 6).

Stem Girth

At 25 mg Pb kg^{-1}, *Glomus mosseae* inoculation significantly ($p \leq 0.05$) enhanced the stem girth of *T. erecta* 4 WAP, 10 WAP. The influence of *Glomus*

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mosseae on stem girth of *T. erecta* was observed at 50 mg Pb kg^{-1} soil contamination. *Glomus mosseae* significantly ($p \leq 0.05$) enhanced the stem girth of *T. erecta* at 75 mg Pb kg^{-1} contamination (Fig. 7).

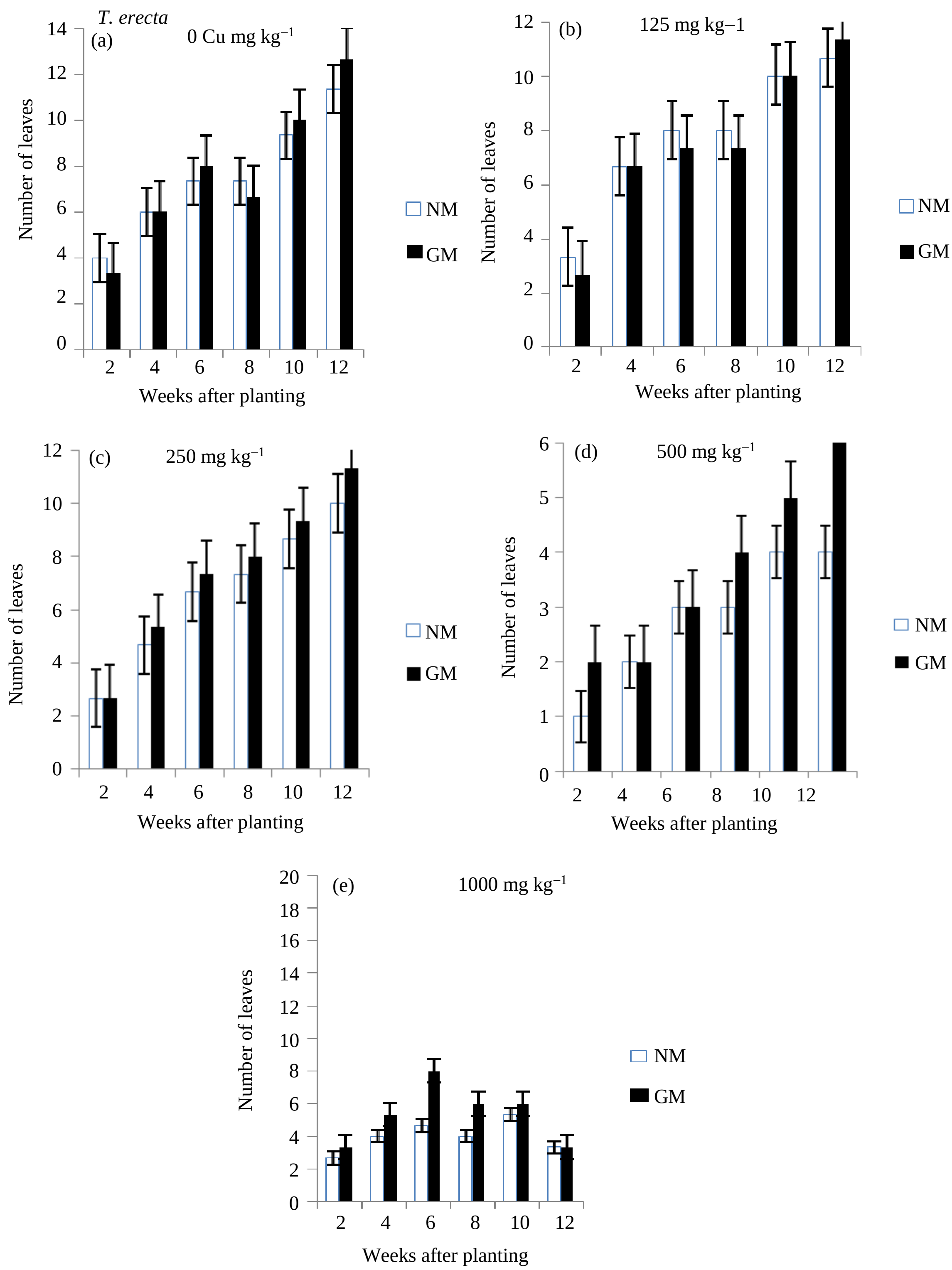

Fig. 2: Plant leave of *T. erecta* inoculated with *Glomus Mosseae* (GM) in 0, 125, 250, 500, 1000 mg kg^{-1} Copper contamination soil. Legend: NM-------Non Mycorrhiza; GM-------Glomus Mossae (with Mycorrhiza)

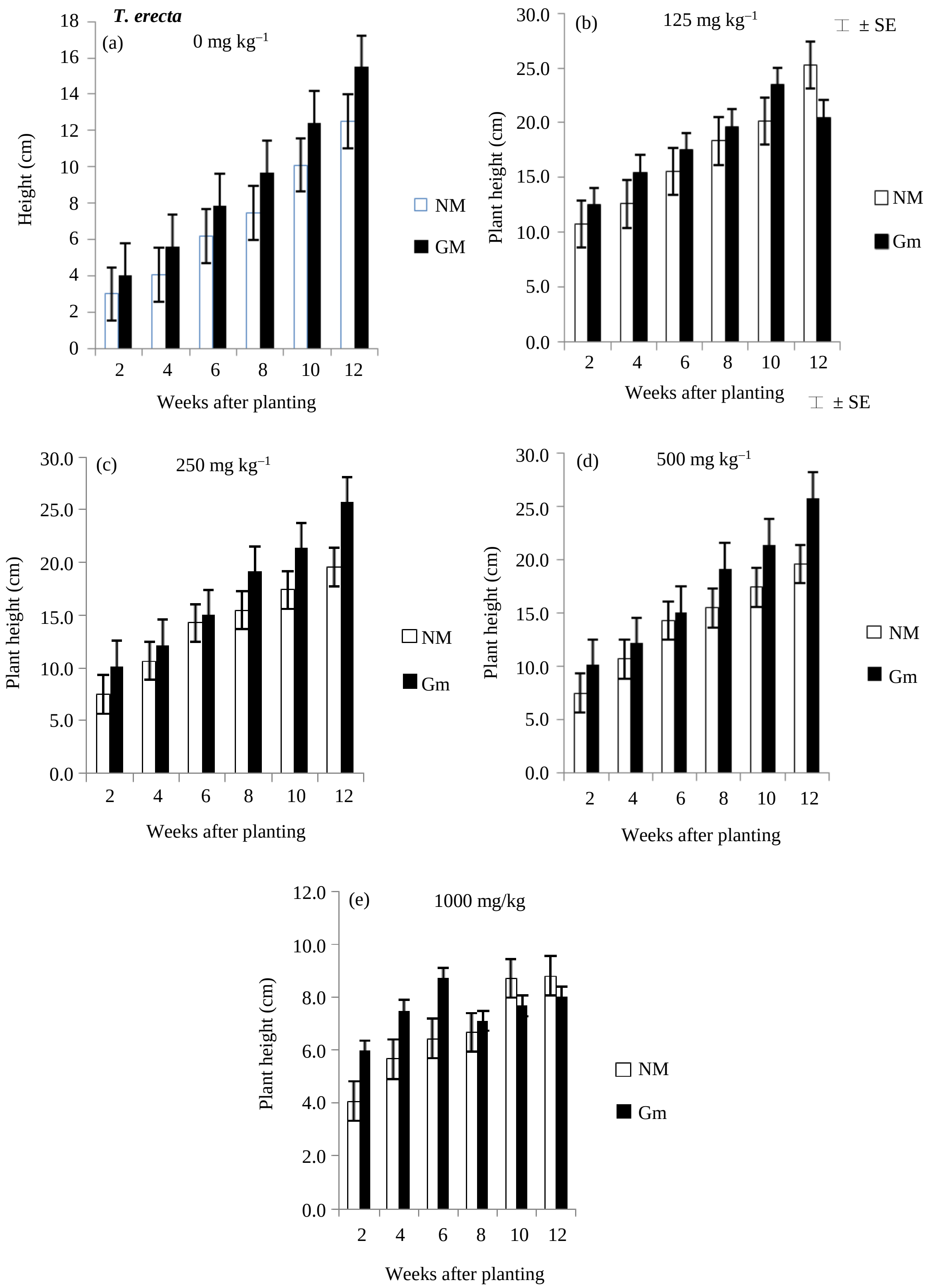

Fig. 3: Plant Height of *T. erecta* inoculated with *Glomus mosseae* (GM) in 0, 125, 250, 500, 1000 mg kg^{-1} Copper contamination soil. Legend: NM- Non-mycorrhizal; GM- *Glomus mosseae* (with Mycorrihiza)

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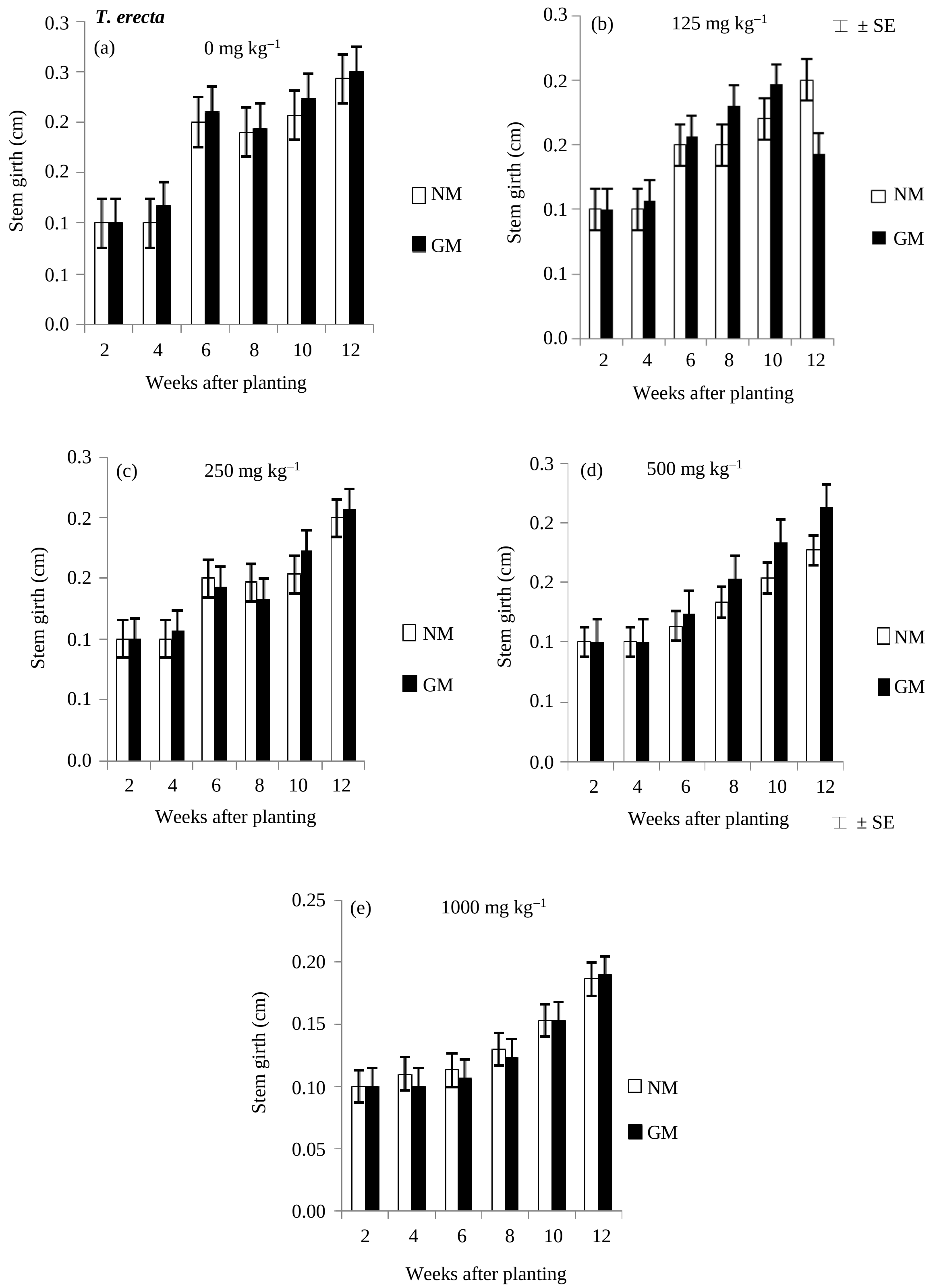

Fig. 4: Stem Girth of *T. erecta* inoculated with *Glomus mosseae* (GM) in 0, 125, 250, 500, 1000 mg kg^{-1} Copper contamination soil. Legend: NM- Non-mycorrhizal; GM- *Glomus mosseae* (with Mycorrihiza)

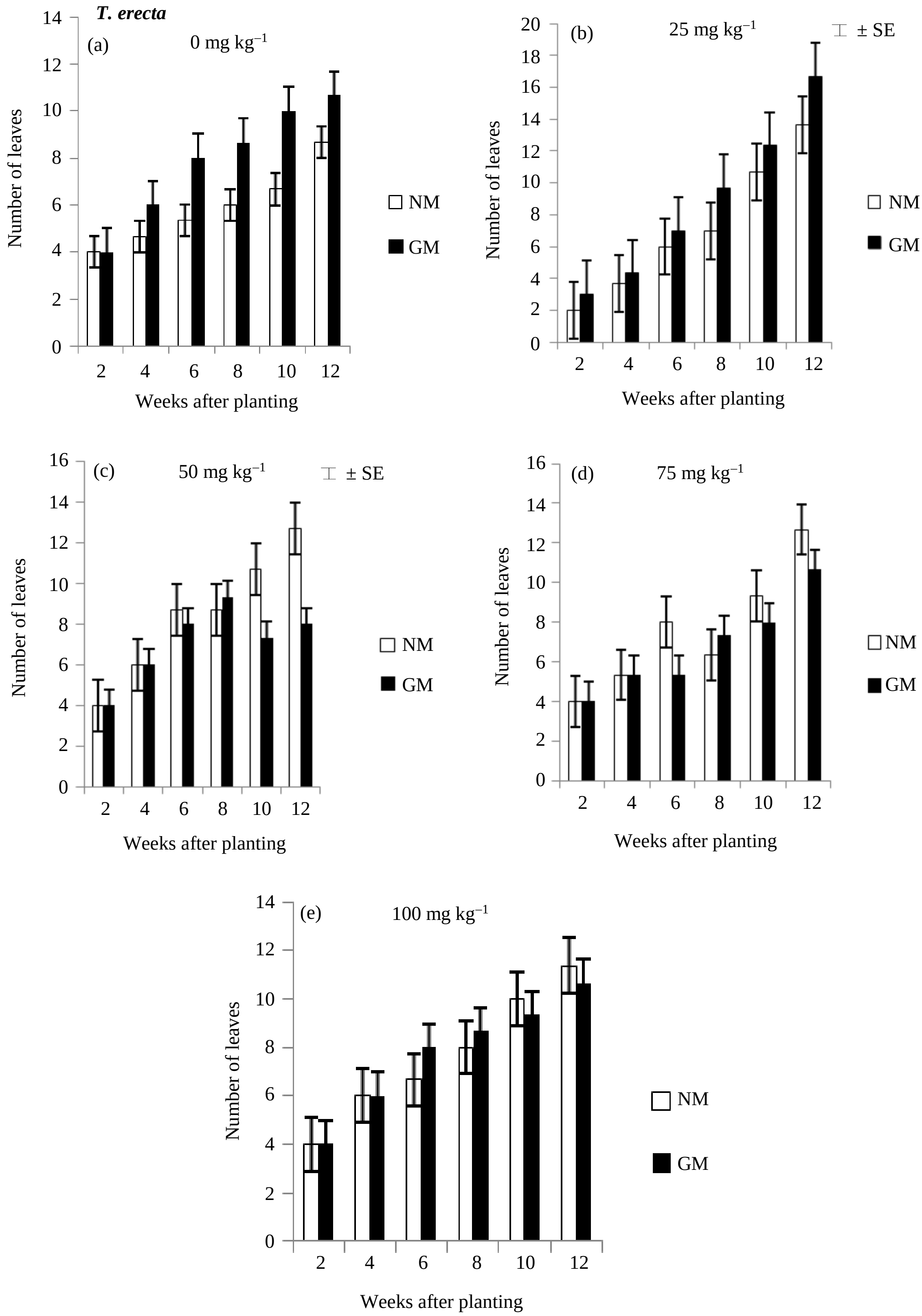

Fig. 5: Number of Leaves of *T. erecta* inoculated with *Glomus mosseae* (GM) in 0, 25, 50, 75, 100 mg kg^{-1} Lead contamination soil. Legend: NM- Non-mycorrhizal; GM- *Glomus mosseae.* (with Mycorrihiza)

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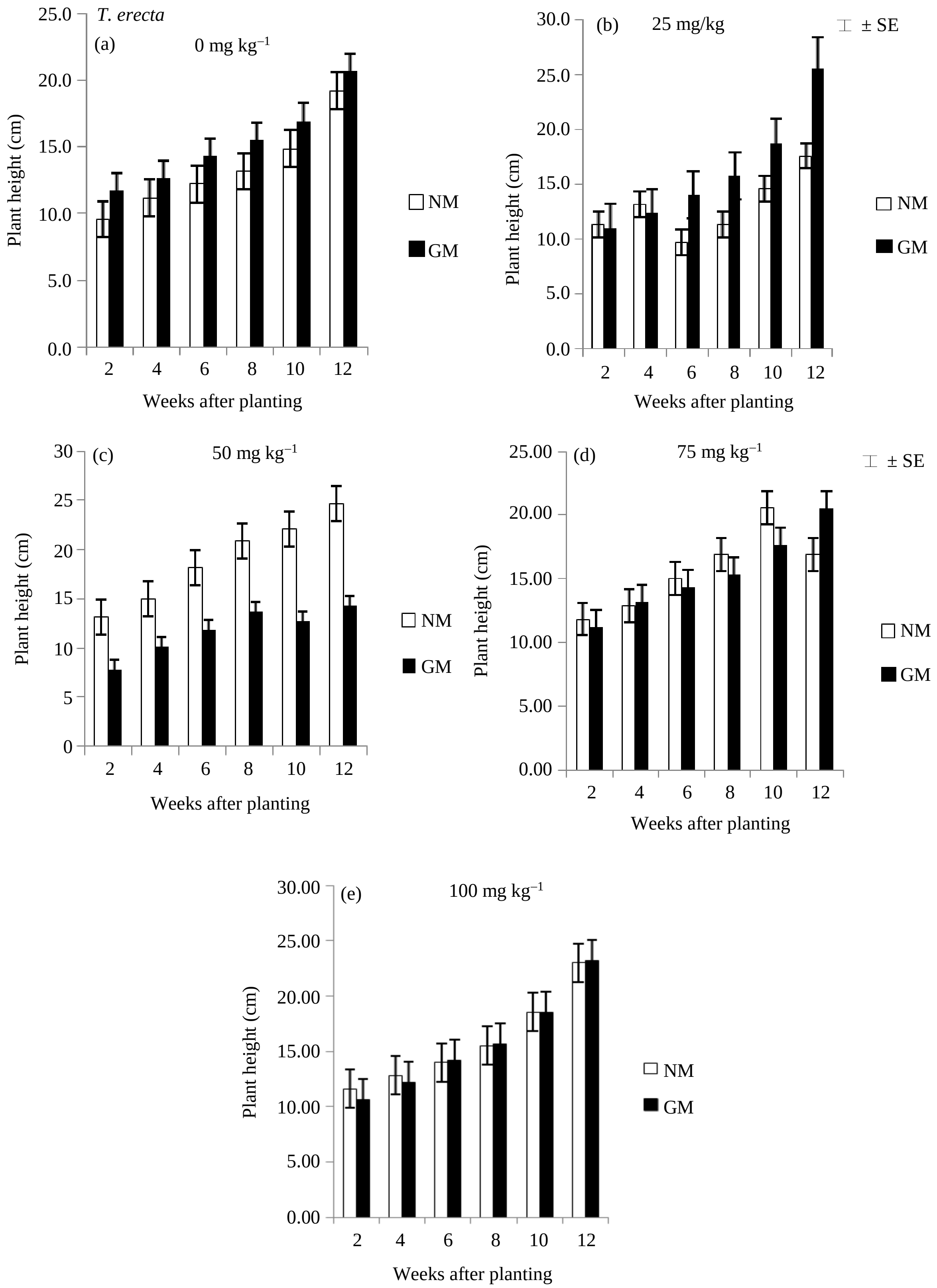

Fig. 6: Plant Height of *T. erecta* inoculated with *Glomus mosseae* (GM) in 0, 25, 50, 75, 100 mg kg^{-1} lead contamination soil. Legend: NM- Non-mycorrhizal; GM- *Glomus mosseae.* (with Mycorrihiza)

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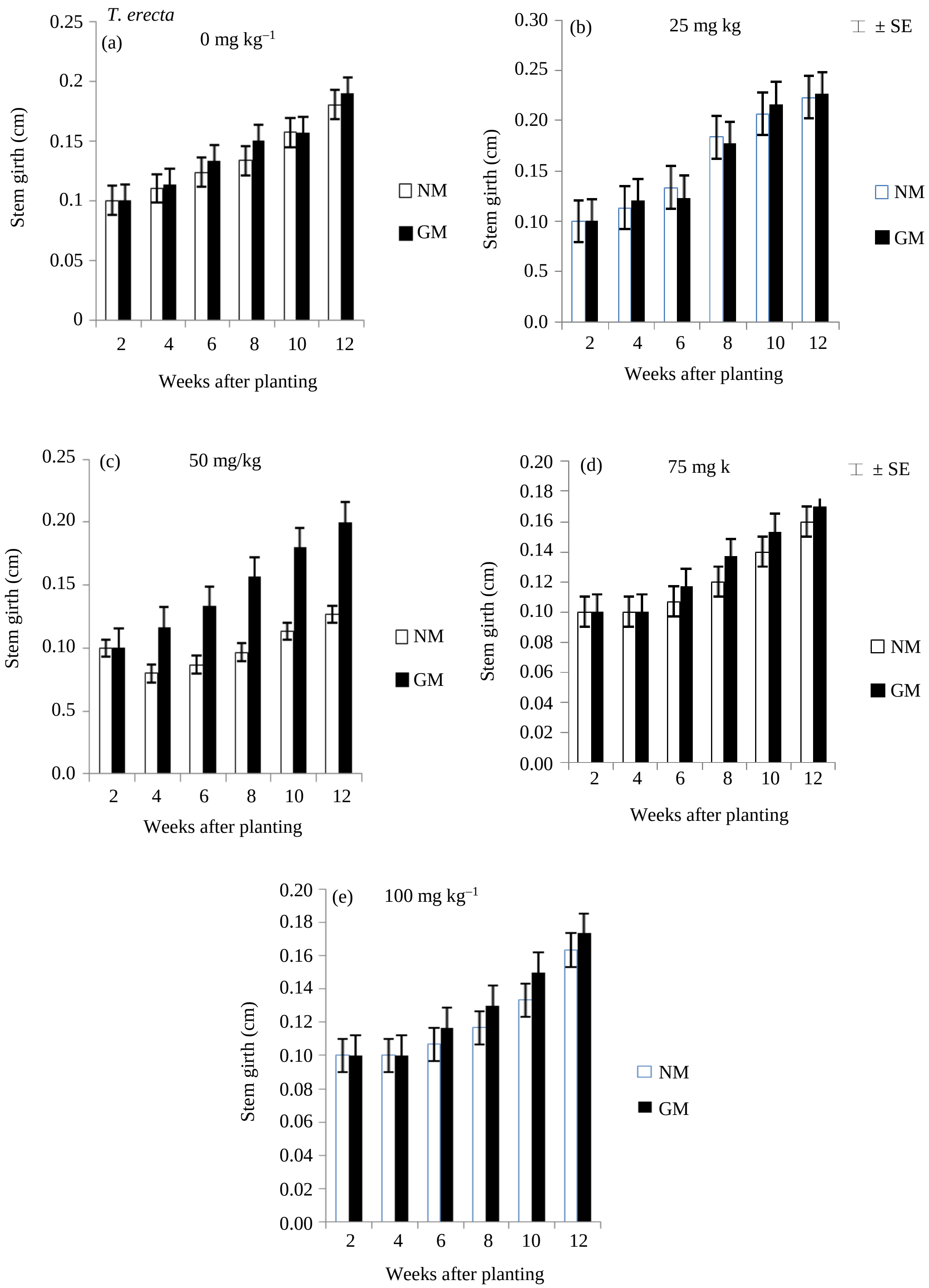

Fig. 7: Stem Girth of *T. erecta* inoculated with *Glomus mosseae* (GM) in 0, 25, 50, 75, 100 mg kg^{-1} Lead contamination soil. Legend: NM- Non-mycorrhizal; GM- *Glomus mosseae.* (with Mycorrihiza)

Influence of Arbuscular Mycorrizal (AM) Fungi on the Nutrient Uptake of T. erecta in Cu and Pb in Contaminated Soil

Glomus mosseae inoculation enhanced the N uptake of *T. erecta* in Cu polluted soil, the highest N uptake of *T. erecta* in Cu polluted soils occurred at 250 mg Cu kg^{-1} contamination. The P uptake decreased as the concentration of contaminant increased in *Glomus mosseae* inoculated plants. There was a marked effect of *Glomus mosseae* inoculation on the P uptake of *T. erecta* compared with non-inoculated plants. The Zn uptake decreased as the Cu concentration increased in *Glomus mosseae* inoculated plant. *Glomus mosseae* had significantly ($p \leq 0.05$) enhanced the uptake of Fe in *T. erecta*. Fe uptake by *T. erecta* decreased as the Cu concentration increases in the soil (Table 2).

In the uncontaminated soil, *Glomus mosseae* inoculation improved the N, P, K uptakes However, in the 25 and 50 Pb kg^{-1} contaminated soil, mycorrhizzal inoculation enhanced N, P and K contents of *T. erecta*. Mycorrhizal inoculation enhanced Zn uptake at 250 mg Pb kg^{-1} contamination. Fe uptake was significant at 50 mg Pb kg^{-1} under *Glomus mosseae* inoculation (Table 3).

Influence of Arbuscular Mycorrizal (AM) Fungi on the Plant Dry Weight, Cu and Pb uptakes in T. erecta

Glomus mosseae inoculation enhanced plant dry weight in Cu contaminated soil irrespective of the level of the concentrations except at 1000 mg kg^{-1} Cu. The uptake of Cu by *T. erecta* as influenced by *Glomus mosseae* is shown in Cu uptake increased with increasing level of soil contamination to a threshold level and then declined. Threshold level of Cu uptake with non-inoculated plants was at 250 mg kg^{-1} contamination, while with *Glomus mosseae* was at 500 mg kg^{-1} (Table 4).

Similar to Cu contaminated soil, the *Glomus mosseae* inoculation influenced the plant dry weight of *T. erecta* in Pb contaminated soils (Table 5). *Glomus mosseae* increased Pb uptake in *T. erecta* with increased level of contamination to a threshold before it declined. Plant optimum uptake for lead was at 75 mg kg^{-1} contamination level with *Glomus mosseae*.

Table 2: Effect of *Glomus mosseae* on Plant nutrient of *T. erecta* under different concentrations of Cu Contamination

Trt	Cu Contamination Conc $mgkg^{-1}$	N	P	K	Ca $mgkg^{-1}$	Mg	Na	Fe	Mn	Zn
NM	0	1.16c	0.23b	0.22c	1.17c	0.40b	0.16c	5.39d	77.9c	2.82b
	125	0.77b	0.15c	0.16e	0.83e	0.35c	0.11d	5.47c	68.5d	3.03a
	250	1.04c	0.22b	0.17e	1.15c	0.42b	0.12d	7.42b	72.65c	3.10a
	500	0.45d	0.13d	0.15e	1.03d	0.37d	0.21c	3.47e	42.6d	1.48d
	1000	0.72c	0.10e	0.23c	1.30b	0.24d	0.81a	8.16b	10.2b	1.79c
GM	0	1.26b	0.27b	0.22c	0.57e	0.51b	0.20c	5.47c	98.35c	3.02a
	125	2.29b	0.44a	0.31b	1.48b	0.51b	0.33b	10.27b	113.50a	3.52a
	250	2.82a	0.52a	0.37a	1.61a	0.64a	0.44b	10.88a	107.65b	3.23a
	500	0.69c	0.26b	0.18e	1.02d	0.37d	0.20c	3.88e	40.48d	2.33b
	1000	0.45d	0.13d	0.16e	0.37e	0.39c	0.20c	9.16b	31.92e	2.04c

Values in the same group followed by the same letter did not differ significantly at $p \leq 0.05$ using Duncan Multiple Range Test.
Legend: Trt- Treatment, NM-Non-mycorrhiza; GM- *Glomus mossea*

Table 3: Effect of *Glomus mosseae* on Plant nutrient of *T.erecta* under different concentrations of Pb contamin

Trt	Pb Contamination Conc $mgkg^{-1}$	N	P	K	Ca $mgkg^{-1}$	Mg	Na	Fe	Mn	Zn
NM	0	0.25d	0.16d	0.16c	0.39e	0.29d	0.14d	7.02d	2.03b	86.50b
	25	0.49c	0.18d	0.23b	0.45c	0.34c	0.19c	7.98c	2.02b	82.55b
	50	0.50c	0.28c	0.14d	0.47c	0.33c	0.27b	6.27d	1.93c	86.89b
	75	1.07d	0.24c	0.13d	0.10e	0.54a	0.21c	2.98e	1.73c	27.00c
	100	1.85b	0.13e	0.17c	0.40c	0.28d	0.20c	4.94e	1.63d	25.50c
GM	0	1.18c	0.35b	0.22b	0.39e	0.34c	0.20c	7.02d	2.08b	101.75a
	25	2.49a	0.38b	0.24b	0.73d	0.33c	0.29b	8.08b	2.13a	107.20a
	50	1.95b	0.47a	0.98a	1.24a	0.40b	0.31a	8.53a	2.13a	85.56b
	75	1.07d	0.28c	0.19b	1.07b	0.41b	0.20c	6.27d	1.87c	83.55b
	100	1.11d	0.17d	0.16c	0.45c	0.25d	0.16d	3.17e	1.73c	86.75b

Values in the same group followed by the same letter did not differ significantly at $p \leq 0.05$ using Duncan Multiple Range Test.
Legend: Trt- Treatment, NM-Non-mycorrhiza; GM- *Glomus mossea*

Table 4: Effect of copper concentration uptake on the dry weight of *T. erecta*

Treatment	Concentration Heavy metal (Cu) Levels (mg kg^{-1})	Dry weight of uptake (mg/plant)	plant (g/plant)
NM	0	9.32e	0.10e
	125	10.25d	0.12e
	250	12.02a	0.14e
	500	11.00c	0.11e
	1000	11.90b	0.13e
GM 0	9.50e	0.37d	
	125	10.26d	0.40c
	250	10.86b	0.47b
	500	12.34a	0.57a
	1000	10.57d	0.42c

Values in the same group followed by the same letter did not differ significantly at $p \leq 0.05$ using Duncan Multiple Range Test.
Legend: Trt- Treatment, NM-Non-mycorrhiza; GM- *Glomus mosseae*

Table 5: Effect of lead concentration uptake on the dry weight of *T. erecta*

Treatment	Concentration Levels (mg kg^{-1})	Heavy metal (Pb) uptake (mg/plant)	Dry weight of plant (g/plant)
NM	0	1.13e	0.33e
	25	1.80e	0.38e
	50	4.05c	0.59c
	75	2.34c	0.47d
	100	3.75b	0.33e
GM	0	1.70e	0.40d
	25	2.80d	0.60c
	50	3.81b	1.23b
	75	4.56a	1.50a
	100	2.47c	0.23e

Values in the same group followed by the same letter did not differ significantly at $p \leq 0.05$ using Duncan Multiple Range Test.
Legend: Trt- Treatment, NM-Non-mycorrhiza; GM- *Glomus mosseae*

Table 6: Assessment of mycorrhizal colonization of Roots of *T.erecta*

Metal	mg kg^{-1}	Mycorrhiza colonization (%)	
		T. erecta	
		NM	GM
Cu	0	3.22a	34.13a
	125	2.00b	23.34b
	250	1.33c	14.51c
	500	1.30c	11.03d
	1000	1.20d	9.27e
Pb	0	1.26a	23.34a
	25	0.83b	19.78a
	50	0.72b	11.71c
	75	0.50c	6.91d
	100	0.42d	5.94d

Values in the same group followed by the same letter did not differ significantly at $p \leq 0.05$ using Duncan Multiple Range Test.
Legend: NM – Non- Mycorrhizal; GM-*Glomus mossea*

Percentage Arbuscular Mycorrhizal Root Colonization

Perecentage mycorrhizal root colonization, for AM inoculated and non-inoculated plants are shown in Table 6 Inoculated and non-inoculated plants were both infected by AM fungi. Percentage root infection however was significantly higher at $P<0.05$ in plants inoculated with *Glomus Mosseae* (GM) than in the non-mycorrhizal (GM). Greater colonization occurred between 0-250 mg Cu kg^{-1} and 0-50 mg Pb kg^{-1} than at higher concentrations of contaminations.

Discussion

This study assessed the potential of indigenous plant species (*Tagetes erecta*) in removing Cu and Pb from contaminated soil using *Glomus mosseae* as an enhancer. In a view to achieve this, *T. erecta* was planted in greenhouse with *Glomus mosseae* inoculation under different concentration levels of Cu and Pb soil contamination. The knowledge of AM associations with *T. erecta* exposed to different levels of Cu and Pb contamination could provide a basis for alleviating the stress of heavy metal toxicity in weeds studied. Specific criteria had been put into cognizance when screening, selecting and identifying weed species with, tropical climate, primary goal of identifying potential hyperaccumulators high potential for phytoremediation of Cu and Pb.

The result revealed that the growth parameters (number of leaves, plant height and stem girth) were enhanced by *Glomus mossea* in *T. erecta.* Leaf production of *T. erecta* was sustained at highest level of (1000 mg kg^{-1}) of Cu concentration, similar observation had been made on the effect of AM inoculation on *Solenostemon monostachyus* grown in Cu and Cd contaminated soil (Awotoye *et al*., 2012). This suggests that AM infection offers protection against heavy metal toxicity.

The study also observed the toxicity effect of Pb which was noticed on the leaf production of AM inoculated *T. erecta* at 50-100 mg kg^{-1} concentrations. This indicates that the effectiveness of AM inoculation in a polluted environment may be controlled primarily by the concentration level of the contaminant. This supports the findings of Awotoye *et al.* (2013) on mycorrhizal inoculation of *Amaranthus spinosus, Synedralla nodiflora, Sida acuta* and *Euphorbia heterophylla* where increase Pb concentration had pronounced negative effects on the plant growth parameters.

Arbuscular Mycorrizal fungi are able to enhance heavy metal uptake through their external mycelium, which support a wider exploration of soil volumes by spreading beyond root exploration zone (Malcova *et al*., 2003), thus providing access to greater volume of metals present in the rhizosphere. Another relevance of AM fungi symbiosis is that it can increase plant establishment and growth despite high levels of soil heavy metal (Enkhtuya *et al*., 2002), due to better nutrition (Fewtrell *et al*., 2003) and water availability (Augé, 2001) and soil aggregation properties (Rillig and Steingerg, 2002). It has been suggested that mycorrhizal status is related to environmental elements, especially contamination levels in soil (Smith and Read, 1997). Cu and Pb addition decreased the percent of root colonization in the present study and root colonization decreased with increasing soil Cu and Pb concentrations. This result demonstrates that high levels of soil Cu and Pb inhibited the growth of AMF and then inhibited the formation of mycorrhiza. Andrad *et al*. (2004) reported that the increase of Pb concentrations decreased the root colonization and the spore numbers, which is confirm insistent with the present study.

Conclusion

Arbuscular mycorrhiza fungi improved the growth of *Tagetes erecta* under high toxicity of Copper (1000 mg kg^{-1}). AM fungi also improved the uptake of Cu and Pb *Tagetes erecta*. This study shows that AM fungi are beneficial to the phytoremediation process and that *Tagetes erecta* and *Crotalaria juncea* are suitable to grow and rehabilitate a Cu and Pb contaminated site.

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Author's Contributions

Aina, I.O.: Participated in all experiments, coordinated the data-analysis and contributed to the writing of the manuscripts.

Amusa, F.L.: Contributed to the writing of the manuscript and designated to the research.

Olasupo, A.D.: Designed the research plan, organized the study and contributed to the mouse work.

Olagoke, O.V.: Organized the study and coordinated the mouse work.

Awodiran, T.P.: Contributed to the mouse work.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Andrad S.A., M.F. Abreu and A.P. Silveira, 2004. Influence of lead additions on arbuscular mycorrhiza and rhizobium symbioses under soybean plants. Applied Soil Ecol., 2: 151-156.

Augé, R.M., 2001. Water relations, drought and vesicular mycorrhizal symbiosis. Mycorrhiza, 11: 3-42.

Awotoye, O.O., M.B. Adewole and E. Dada, 2013. Assessment of the phytoremediation potentials of selected West African weeds in cadmium and lead contaminated soil. J. Sci. Res.

Awotoye, O.O., M.B. Adewole and O. Olofinjana, 2012. Assessment of *Solenostemon monostachyus* for phytoremediation potential under the influence of mycorrhizal inoculation in heavy metal pouted soil. J. S. Res.

Enkhtuya, B., J. Rydlová and M. Vosátka, 2002. Effectiveness of indigenous and non-Indigenous Isolates of arbuscular mycorrhizal Fungi in soils from degraded ecosystems and man-made habitats. Applied Soil Ecol., 14: 201-211.

Fewtrell, L, R. Kaufmann and A. Prüss-Üstün, 2003. Lead: Assessing the environmental burden of disease at national and local levels. Geneva, World Health Organization Environmental Burden of Disease Series, No. 2.

Giuffré, L., R.I. Romaniuk, L. Marbán, R.P. Ríos and T. P. García Torres, 2012. Public health and heavy metals in urban and periurban horticulture. Emir. J. Food Agric., 24: 148-154.

Kamnev, A.A., 2003. Phytoremediation of Heavy Metals: An Overview. In: Recent Advances in Marine Biotechnology, Fingerman, M. and R. Nagabhushanam (Eds.), Science Publishers Inc., Enfield (NH), Bioremediation. USA, pp: 269-317.

Krämer, K., 2010. Metal hyperaccumulation in plants, annual revolutional. Plant Biol. Palo Alto, 61: 517-534.

Malcova, R., M. Vosatka and M. Gryndle, 2003. Effects of inoculation with *Glomus intraradices* on lead uptake by *Zea mays* L. and *Agrotis capillaries* L. Applied Soil Ecol., 239: 55- 67.

Olarenwaju, O.E., M.T. Adetunji, C.O. Adeofun and I.M. Adekunle, 2009. Nitrate and phosphate loss from agricultural land: Implication for non-point pollution. J. Nutrient Cycle Agroeco. Syst.

Rillig, M.C. and P.D. Steinberg, 2002. Glomalin production by an arbuscular mycorrhizal fungus: A mechanism of habitat modification. Soil Biol. Biochem., 34: 1371-1374.

Saba, H., P. Jyoti and S. Neha, 2013. Mycorrhiza and phytochelators as remedy in heavy metal contaminated land remediation. Int. Resources J. Environ. Sci., 2: 74-78.

Smith, S.E. and D.J. Read, 1997. Mycorrhizal symbiosis, academic press, San Diego, USA. phytoremediation-a novel and promising approach for environmental clean-up. Biotechnology, 24: 97-124.