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ASSESSMENT OF TITHONIA DIVERSIFOLIA WITH MYCORRHIZAL BIOAUGMENTATION IN PHYTOREMEDIATION OF LEAD AND ZINC POLLUTED SOILS

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ABSTRACT

Human activities cause soil contamination with heavy metals, with a negative impact on plant growth and productivity, and consequently animal and human health. Phytoremediation, a cost effective and eco-friendly remediation technique, requires fast growing large biomass plants naturally found in polluted soil. This research assessed the potential of Tithonia diversifolia with arbuscular mycorrhiza (AM) bio-augmentation in phytoremediation of heavy metal polluted soil. T. diversifolia seedlings were raised in potted soil contaminated with lead (PbSO4) or zinc ($ZnSO_4$) in separate experiments at 0, 20, 40, 60, 80 and 100 mg/kg. The potted soils were divided into two groups; the first group contained Pb/Zn contaminated potted soils with 5 g of AM fungus (Glomus clarum) each while the second group made up of potted soils with Pb/Zn contamination each without AM inoculation. Growth and biomass production reduced in Pb and Zn contaminated soils with/without AM, compared to the control. Heavy metal contamination decreased the number of leaves, plant height, stem girth, leaf area, fresh/dry mass of plant parts and total biomass, with variations depending on metal type, concentration applied, parameter determined and soil status (presence or absence of AM). Metal contamination led to more Pb and Zn contents in plant tissues than the control. Soil with AM caused increased plant metal content than those without it. More Zn was accumulated than Pb with a higher quantity of Pb in the root than shoot with/without AM while Zn was more in the shoot than root under AM inoculation and vice versa without it. Tithonia diversifolia is recommended for phytoremediation of Pb and Zn polluted soil, and bio-augmentation with Glomus clarum will bring about better performance.
Keywords: *Lead, Zinc, soil contamination, Glomus clarum, remediation.*

1.0 INTRODUCTION

Due to industrial emission, application of sewage sludge and phosphate fertilizers, heavy metal pollution is now a worldwide environmental problem; it contaminates soil, water and air. Heavy metal pollution of the soil is caused by various metals especially Cu, Ni, Cd and Pb (Karaca, Cetin, Turgay, and Kizilkaya, 2010). The concentration has increased beyond the natural limit to a toxic level (Wani, Khan and Zaidi, 2007), and metal concentration in the soil had risen from less than 1mg/kg (ppm) to as high as 100,000 (ppm) as a result of human activities, causing a negative effect

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to plant growth and productivity (Huang, Bing, Yang, Chai, and Zhou, 2009). Nicholls and Mal (2003) reported that combination of lead (Pb) and Copper (Cu) at both high concentrations (1000 mg/kg each) and low concentration (500 mg/kg) resulted in rapid and complete death of the leaves and stem of *Lythrum salicaria*. Excess zinc can cause chlorosis, inhibition of root growth and damage to plasma membrane permeability leading to ion leakage (Kennedy and Gonsalves, 1989). Kennedy and Gonsalves (1989) further documented that heavy metals from anthropogenic sources are more readily taken up by plants with a negative impact on their growth.

Heavy metal pollution does not only affect the production and quality of crops but may be absorbed by crops and enter the food chain through consumption and threatening health and reproduction of humans and animals (Ji, Wu, Zhang, Zhang, Zhou, Peng,... & Gao, 2018). Some of the heavy metals like Fe and Zn have been reported to be bio-important to man and their daily medicinal and dietary allowances had been recommended. However, some others like As, Cd, Pb and methylated forms of Hg have been reported to have no bio-importance in human biochemistry and physiology and consumption even at low concentration can be toxic. It is therefore of utmost importance to remove the heavy metals from soil.

The conventional methods of environmental remediation, though effective, some of the techniques require expertise, high maintenance costs and may cause secondary pollution or adverse effect on biological activities, soil structure and fertility (Haque, Peralta-Videa, Jones, Gill, and Gardea-Torresdey, 2008). There is, therefore, a need for a less expensive and environmentally friendly technique of phytoremediation. Phytoremediation refers to the technologies that use living plants to clean up soil, air and water contaminated with hazardous contaminants (Reicheinauer and Germida, 2008) and it has been identified as a promising alternative. For example, Blaylock, Salt, and Dushenkov, (1997) reported 50% to 65% saving of money when phytoremediation was used for the treatment of 1 acre of Pb polluted soil compared with the case when a conventional method (excavation and landfill) was used for the same purpose.

Most works on exploring the potentials of this emerging green technology were carried out in the temperate environment, while very little has been reported on identifying plants capable of hyper-accumulating metals or pollutants in the tropical region. Aransiola, Ijah, and Abioye, (2013) demonstrated the potential of *Glycine max* (Soybean) to remediate lead contaminated soil with the highest accumulation in the seed. They, however, stated that this could pose a great danger to the population who harvest its seeds for consumption. The way out therefore is to focus attention on non-crop plants like *Tithonia diversifolia* that are not used as livestock feed nor consumed by man. Besides, Ayesa, Chukwuka, and Odeyemi (2018) and Al-Jobori, and Kadhim, (2019) identified *Tithonia diversifolia* as a promising species for phytoextraction of heavy metals from contaminated sites without being affected. The luxuriant growth habits of *Tithonia diversifolia* in particular on nutrient poor soils and on roadsides exposed to frequent lead (Pb) emission from automobiles instigated the need to investigate this plant for its metal accumulation potentials. Besides, plants found growing naturally in polluted environments are suggestive of their potential for remediation purposes in such areas (Li, Lun, Zhao, Zhu, Gu, and Li, 2017).

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According to Morganwalp (2015), biological augmentation is the addition of archaea or bacterial cultures required to speed up the rate of degradation of contaminants. Arbuscular mycorrhizas help plants to capture nutrients such as phosphorus, sulfur, nitrogen and micronutrients from the soil. Bücking and Shachar-Hill (2005) reported that new approach to restoring land is to inoculate the soil with arbuscular mycorrhiza fungi when reintroducing vegetation in ecological restoration projects (phytoremediation); this has enabled host plants to establish themselves on degraded soil and improve soil quality and health. The benefits of mycorrhiza bio-augmentation are increased plant growth, increased phosphorus uptake and soil nitrogen content, higher soil organic matter content and soil aggregation, attributed to higher legume nodulation in the presence of arbuscular mycorrhiza fungi, better water infiltration and soil aeration due to soil aggregation. The objective of this research is to evaluate the potential of *T. diversifolia* in phytoremediation heavy metal contaminated soil and to examine the influence of bio-augmentation with arbuscular mycorrhiza.

2.0 MATERIALS AND METHOD

Source of Plant

Young uniform seedlings of *Tithonia diversifolia* were collected from farmland at Akungba-Akoko, Ondo State, Nigeria.

2.1 Source of salt

Salts of Lead ($PbSO_4$) and Zinc ($ZnSO_4$) (Sigma-Aldrich, Australia) were obtained from Plant Science and Biotechnology Laboratory, Adekunle Ajasin University, Akungba - Akoko, Ondo State.

2.2 Source of soil

Topsoil used for the experiment was collected from the experimental farm of Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba Akoko.

Source of arbuscular mycorrhiza

Glomus clarum was purchased from the University of Ibadan, Oyo State, Nigeria.

2.3 Study location

This research was carried out in the screen house of Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba-Akoko, Ondo State (Latitude $7^0 37^/ N$ and Longitude $5^0 44^/ E$) with an elevation of 336 to 390 cm.

2.4 Experimental set up

Polythene pots (8 cm by 26 cm) were filled with the topsoil of 4.5 kg and two young seedlings of *Tithonia diversifolia* were planted in each polythene pot. Three weeks after planting, the seedlings were thinned to one per pot. Heavy metal salts of Lead and Zinc ($PbSO_4$ and $ZnSO_4$ respectively) were applied to the soil at 0 (control), 20, 40, 60, 80 and 100 mg/kg of soil) in separate experiments. In the first experiment, $PbSO_4$ was applied to the soil at different rates while in the second

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experiment, $ZnSO_4$ was applied to the soil at the different concentrations. Each treatment was replicated five times in a completely randomized design. The pots were divided into two (2) groups with each containing 30 pots in each experiment. An arbuscular mycorrhiza *Glomus clarum* was inoculated on the soil at 20 g per pot in one group while the second group did not contain the mycorrhiza in each of the experiments.

2.5 Plant growth measurement

Plant height was measured using a meter rule from the soil surface to the apical bud; stem girth was measured by using digital Vernier caliper (Mitutoyo 530-123 Standard model 0-200 mm) at the 5 cm point from the base of the stem, and the leaf area was measured by the method of Eze (1965). The number of leaves and branches were counted and plants were harvested at 8 weeks after treatment and separated into roots, stems and leaves. The major roots were counted and their lengths measured. Freshly harvested plants were weighed immediately for fresh mass determination while the dry mass was measured after oven-drying at 80 ^{0}C to a constant weight.

2.6 Plant analysis

Dried plant samples were digested using 10 ml of 20% sulphuric acid. Pb and Zn were determined by Atomic Absorption Spectrophotometer (Buck 210) following the standard laboratory procedures by AOAC (1990). This was carried out at the Central Laboratory of the National Horticultural Institute (NIHORT) at Ibadan, Nigeria.

2.7 Statistical analysis

Data were subjected to One-way ANOVA and means were separated with Tukey HSD test at 5% level of probability using SPSS 24.0 version.

3.0 RESULTS

Growth was reduced in soil contamination with Pb both in the presence and absence of mycorrhiza relative to the control (Table 1). When mycorrhiza was added to the soil, plant height, stem girth, root length and the number of roots in contaminated soils did not differ significantly from the control. The number of leaves differed significantly from the control at 40-100 mg/kg while that of leaf area was at all concentrations applied. In contaminated soil without mycorrhiza, stem girth, root length and the number of roots did not differ significantly from the control but the number of leaves, plant height and number of branches showed significant differences at all concentrations of Pb while leaf area differed significantly only at 100 mg/kg.

Growth was also reduced in plants grown in Zn contaminated soil in the presence or absence of mycorrhizal fungus (Table 2). Mycorrhizal fungus inoculation did not significantly affect root growth in comparison with the control. However, plant height and number of leaves had significantly reduced values at all levels of contamination when compared with the control. The number of branches differed only at 80-100 mg/kg while leaf area did not significantly differ from the control. When mycorrhiza was not added to the soil, Zn contamination significantly reduced

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the number of leaves at all the concentrations used. Plant height differed at a significant level only at 60-100 mg/kg, stem girth at 40-100 mg/kg and leaf area at 80-100 mg/kg.

The fresh mass of plant parts as well as total biomass reduced in soil contaminated with Pb with or without mycorrhizal inoculation (Table 3). Generally, stem fresh and dry mass were not significantly reduced by Pb application compared to the control. When mycorrhiza was added to the soil, root fresh and dry mass decreased significantly at 40-100 mg/kg while that of the leaf significantly reduced at 20-100 mg/kg compared to the control. Plants grown in contaminated soil without mycorrhiza had root/leaf fresh and dry mass values that were significantly lower than the control at all levels of contamination. Also, total biomass was negatively affected by Pb contamination with or without mycorrhiza treatment. However, while a significant difference was obtained at all levels of contamination in soil without mycorrhiza, significant differences were recorded at 40-100 mg/kg when mycorrhiza was added to the soil.

Furthermore, in Zn contaminated soil, fresh/dry mass of plant parts and total biomass were significantly reduced at 40-100 mg/kg concentrations. When the soil was not treated with mycorrhiza fungus, root and stem fresh/dry mass differed significantly from the control at 40-100 mg/kg while reduction in leaf fresh/dry mass was significant at all contamination levels. Plant biomass also reduced significantly at 60-100 mg/kg in the presence of mycorrhiza but it occurred at 40-100mg/kg in its absence.

Tables 5 and 6 show that the quantity of Pb and Zn accumulated in plant tissues was significantly more in plants grown in contaminated soil than in the control. Plants grown in soil treated with mycorrhiza contained more metal content than those without it. Besides, a higher quantity of Zn was accumulated in plant tissues than Pb. Pb was generally higher in the root than shoot either with mycorrhizal inoculation or not (Table 5). Zn was higher in the shoot than the root of plants grown in soil inoculated with mycorrhizal fungus but higher in the root than shoot without the fungus (Table 6).

4.0 DISCUSSION

Both Pb and Zn had a negative impact on the growth of the plant but with variations depending on the metal. This corroborates the assertion that a plant differs in responses to heavy metal toxicity according to the particular heavy metal for that process (Kibra, 2008). Growth reduction by Pb and Zn as recorded in this research is similar to reports on some plant species by several researchers. For example, zinc was found to have a negative effect on the shoot growth of *Typha latifolia* (Ye Baker, Wong, and Willis, 1998) and *Acacia auriculaeformis* (Zhang, Wong, Nie, and Lan, 1998). Also, Manivasagaperumal, Balamurugan, Thiyagarajan, and Sekar (2011) found that zinc in excess reduces chlorophyll, carotenoid, sugar, amino acid and growth of cluster beans (*Cyamopsis tetragonoloba*). In another research, early seedling growth was inhibited by Pb in soya bean and rice (Huang *et al.,* 1974), maize (Miller *et. al.* 1975), barley, tomato and some legumes (Sudhakar, Syamalabai, and Veeranjaneyulu, 1992). The variation that occurred in growth reduction might

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not be limited to the type of metal and concentrations alone, other factors might have contributed to it. Similarly, in contaminated soil, the degree to which root elongation was inhibited was suggested to be depended upon the concentration of lead and ionic composition and pH of the medium (Goldbold and Hutterman, 1986). Concentration dependent inhibition of root growth has also been observed in *Sesamum indicum* (Kumar and Singh,1993). High levels of Zn in soil was found to inhibit many plant metabolic functions which resulted in retarded growth and caused senescence, limited growth of both root and shoot in French marigold (Choi *et al.,* 1996). Zinc toxicity can cause chlorosis in the younger leaves *Brassica* species, which can extend to older leaves after prolonged exposure to high levels (Ebbs and Kochian, 1997). The chlorosis may arise partly from an induced iron (Fe) deficiency as hydrated Zn^{+2} and Fe^{+2} have similar radii (Marschner, 1986). Excess Zn can also give rise to a hindered transfer of micronutrients from root to shoot leading to the appearance of a purplish red colour in leaves, which is ascribed to phosphorus (P) deficiency (Ren, Liu, Liu, and Hu, 1993; Fontes and Cox, 1995).

Many of the growth parameters measured in this study were significantly reduced even at a low concentration of 20 mg/kg of the metals. This is similar to the discovery that very low concentrations of heavy metals in the growth medium are capable of negative effect on plant growth (Kibra, 2008). Kibra (2008) noticed a significant reduction in the height of rice plants growing on soil contaminated with 1 mg Hg/kg with the reduction in tiller and panicle formation. Likewise, Cd toxicity reduced the shoot and root growth in wheat plants at a concentration as low as 5 mg/L in the soil (Ahmad *et al.,* 2012). Borkert *et al*. (1998) recorded growth reduction in *Arachis hypogaea,* while Pandey and Sharma (1999) obtained a similar result in *Carthamus tinctorius* (safflower) due to soil heavy metal contamination. Most of the reduction in growth parameters of plants growing on heavy metal polluted soils can be attributed to reduced photosynthetic activities, plant mineral nutrition, and reduced activity of some enzymes (Kabata-Pendias and Pendias, 2001).

The negative influence of heavy metals on the growth and activities of soil microorganisms might also indirectly affect the growth of plants according to Huang *et al.* (2009). Reduction in the number of beneficial soil microorganisms due to high metal concentration may lead to decrease in organic matter decomposition resulting in a less soil fertility (Pogrzeba, Rybka, Krzyżak, and Prokopiuk, 2013). Enzyme activities that are useful for plant metabolism might have been hampered due to heavy metal interference with activities of soil microorganisms (Zurek *et al.,* 2013). These toxic effects (both direct and indirect) result in a decrease in plant growth which can finally cause the death of plant (Schaller and Diez, 1991).

This study revealed that the plant species could remove Pb and Zn from the soil by accumulating them in plant tissues. In the same vein, Ayesa *et al*. (2018) reported that *Tithonia diversifolia* and *Chromolaena odorata* reduced heavy metals in polluted soils. Al-Jobori, *et al*. (2019) deduced that sunflower plant can absorb and accumulate quantities of lead without affecting the production of biomass, indicating the possibility of using it as hyper accumulator successfully. Aransiola *et al*. (2013) demonstrated the potential of *Glycine max* L. to remediate lead (Pb) contaminated soil.

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This plant generally had the highest accumulation of lead (Pb) in its seeds after 12 weeks of remediation and therefore recommended for phytoremediation purposes. The position of the researchers above is a pointer to the fact that *T. diversifolia* is capable of removing Pb and Zn from contaminated soil, accumulate them in the tissues and when harvested, the soil will be free from excess metals.

Though plants are grown in soil with mycorrhiza accumulated higher quantity of heavy metals in their tissues than the control, inoculation of soil with mycorrhizal fungus further enhanced heavy metal accumulation in the plant. This might be because arbuscular mycorrhizas are characterized by the formation of unique structures, arbuscules and vesicles by fungi of the phylum Glomeromycota which help plants to capture nutrients such as phosphorus, sulfur, nitrogen and micronutrients from the soil (Bücking and Shachar-Hill (2005). It is believed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonization of land by plants and in the evolution of vascular plants (Li *et al.,* 2017). Bücking and Shachar-Hill (2005) observed that new approach to restoring land is to inoculate the soil with arbuscular mycorrhiza fungi when reintroducing vegetation in ecological restoration projects (phytoremediation); this has enabled host plants to establish themselves on degraded soil and improve soil quality and health. However, the negative effect of heavy metals on the plant as recorded when AM was introduced to the soil could be as a result of a high level of metals accumulation in plant tissues. This is because when heavy metals are accumulated in the living cells, it causes reduction of cell activities, inhibition of growth or various deficiencies in plants (Farooqi *et al*., 2009; Kabir *et al*., 2008). Inoculation of mycorrhizal fungus (*Glomus clarum)* in this experiment led to the suppression of negative toxicity effect of heavy metals on the growth of the plant as compared to the treatments that did not receive the mycorrhizal inoculation. This can be linked to the inferences made by Bücking and Shachar-Hill (2005) that plant growth increases due to high nodulation in the presence of arbuscular mycorrhiza fungus. The high nodulation can enhance the population of nitrogen-fixing bacteria which help to fix atmospheric nitrogen into the soil thereby improving plant growth better than when mycorrhizal is absent.

Table 1: Growth parameters of Tithonia diversifolia grown in lead (Pb) contaminated soil with/without mycorrhizal bio-augmentation

Soil status	Growth parameter	Quantity of heavy metal applied (mg/kg of soil)					
		0	**20**	**40**	**60**	**80**	**100**
With mycorrhizal bio-augmentation	**Plant height (cm)**	126.40±8.32^{b}	111.80±10.65^{b}	80.04±4.10^{ab}	93.96±4.26^{ab}	83.08±5.06^{ab}	97.00±12.54^{ab}
	Number of leaves	108.80±18.77^{b}	76.20±11.85^{ab}	41.00±1.00^{a}	51.60±3.72^{a}	48.60±10.37^{a}	38.20±3.53^{a}
	Number of branches	11.00±2.78^{b}	2.00±1.00^{a}	1.00±0.01^{a}	2.60±0.24^{a}	3.00±1.00^{a}	2.20±1.77^{a}
	Stem girth (cm)	21.00±1.84^{a}	16.40±1.17^{a}	15.40±0.51^{a}	16.20±0.86^{a}	19.40±1.91^{a}	18.75±1.31^{a}
	Leaf area (cm^{2})	130.00±13.87^{c}	75.00±22.17^{b}	39.00±2.45^{ab}	41.00±7.48^{ab}	55.00±8.94^{ab}	25.00±2.74^{a}
	Root length (cm)	46.33±17.32^{a}	35.80±3.48^{a}	33.20±5.74^{a}	36.60±2.66^{a}	41.20±5.71^{a}	31.50±3.62^{a}
	Number of roots	5.33±0.33^{a}	5.20±0.37^{a}	4.20±0.37^{a}	5.30±0.93^{a}	5.00±0.68^{a}	5.00±0.82^{a}
	Plant height (cm)	106.20±5.51^{c}	69.40±1.89^{a}	79.40±4.82^{ab}	80.60±7.78^{ab}	95.20±2.13^{ab}	84.50±6.06^{ab}

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	Number of leaves	72.20±15.90^{b}	25.60±2.71^{a}	25.60±0.93^{a}	29.20±4.59^{a}	56.80±18.65^{a}	40.00±15.75^{a}
Without	**Number of branches**	6.20±3.72^{a}	1.80±0.37^{b}	2.60±0.40^{b}	2.40±0.40^{b}	4.00±2.49^{b}	3.50±1.50^{b}
mycorrhizal	**Stem girth (cm)**	20.40±1.03^{a}	14.75±0.48^{a}	15.33±0.67^{a}	17.80±0.66^{a}	18.00±2.05^{a}	16.20±1.46^{a}
bio-	**Leaf area (cm^2)**	118.00±14.63^{b}	98.00±12.00^{ab}	69.00±5.57^{ab}	63.00±16.40^{ab}	65.60±22.23^{ab}	44.20±3.48^{a}
augmentation							
	Root length (cm)	40.60±2.98^{a}	38.25±11.25^{a}	36.67±4.70^{a}	37.20±5.46^{a}	39.80±2.85^{a}	39.60±12.31^{a}
	Number of roots	5.20±0.66^{a}	4.50±0.64^{a}	5.03±0.88^{a}	4.80±0.73^{a}	4.60±0.97^{a}	4.60±0.68^{a}

Values are mean ± standard error of 5 replicates. Means with a similar letter(s) in superscript along the row are not significantly different from each other (Tukey HSD test at p = 0.05).

Table 2: Growth parameters of Tithonia diversifolia grown in zinc (Zn) contaminated soil with/without mycorrhizal bio-augmentation

Soil status	**Growth parameter**	**Quantity of heavy metal applied (mg/kg of soil)**					
		0	**20**	**40**	**60**	**80**	**100**
	Plant height (cm)	137.60±5.44^{b}	111.00±3.44^{a}	111.80±5.68^{a}	118.00±3.74^{a}	108.60±6.41^{a}	102.20±9.98^{a}
	Number of leaves	117.60±5.44^{b}	114.00±3.34^{a}	112.80±5.68^{a}	108.00±3.78^{ab}	106.60±6.41^{a}	99.38±9.95^{a}
With	**Number of branches**	12.40±3.17^{b}	11.60±2.11^{b}	7.40±1.78^{ab}	5.80±0.58^{ab}	2.20±0.20^{a}	3.60±0.40^{a}
Mycorrhizal	**Stem girth (cm)**	18.40±0.81^{b}	19.20±2.13^{b}	19.20±1.39^{b}	13.80±0.49^{a}	12.48±0.91^{a}	12.88±0.39^{a}
bio-	**Leaf area (cm^2)**	58.40±1.50^{b}	51.80±4.52^{b}	52.60±3.56^{b}	51.80±4.12^{b}	41.00±1.70^{ab}	40.00±2.74^{ab}
augmentation							
	Root length (cm)	43.20±6.83^{a}	43.00±8.50^{a}	40.80±8.84^{a}	36.25±6.29^{a}	40.00±4.97^{a}	40.20±5.67^{a}
	Number of roots	8.20±1.24^{a}	4.80±0.37^{a}	5.20±0.97^{a}	7.00±1.96^{a}	5.25±0.25^{a}	5.20±0.50^{a}
	Plant height (cm)	95.10±6.42^{a}	93.56±8.60^{a}	90.92±9.48^{a}	82.66±5.39^{b}	77.55±2.86^{b}	81.66±4.59^{b}
	Number of leaves	44.80±2.58^{b}	30.80±3.02^{a}	29.00±1.41^{a}	29.00±1.52^{a}	23.80±1.11^{a}	22.60±2.84^{a}
Without	**Number of branches**	2.80±0.37^{b}	1.20±0.45^{ab}	1.00±0.01^{ab}	1.00±0.01^{ab}	1.00±0.01^{ab}	1.60±0.60^{ab}
Mycorrhizal	**Stem girth (cm)**	17.80±1.02^{b}	18.20±0.86^{b}	12.60±0.40^{a}	14.50±0.29^{a}	13.60±0.53^{a}	13.52±0.88^{a}
bio-	**Leaf area (cm^2)**	44.00±3.67^{b}	41.40±8.72^{b}	35.00±1.58^{ab}	31.00±3.31^{ab}	21.00±2.45^{a}	24.00±4.84^{a}
augmentation							
	Root length (cm)	40.80±4.50^{a}	40.60±5.15^{a}	35.40±4.48^{a}	40.40±2.42^{a}	39.40±8.60^{a}	38.20±4.33^{a}
	Number of roots	7.40±1.50^{a}	5.00±0.63^{a}	6.20±0.58^{a}	6.60±0.87^{a}	5.40±0.51^{a}	7.00±0.93^{a}

Values are mean ± standard error of 5 replicates. Means with a similar letter(s) in superscript along the row are not significantly different from each other (Tukey HSD test at p = 0.05).

Table 3: Fresh and dry mass of Tithonia diversifolia grown in lead (Pb) contaminated soil with/without mycorrhizal bio-augmentation

	Parameter	**Plant part**	**Quantity of heavy metal applied (mg/kg of soil)**					
			0	**20**	**40**	**60**	**80**	**100**
		Root	48.64±2.66^{a}	41.95±2.14^{a}	22.27±2.87^{b}	20.68±1.07^{b}	18.92±1.32^{b}	16.64±1.04^{b}
	Fresh mass (g)	**Stem**	66.82±3.72^{a}	50.92±2.04^{a}	60.23±2.05^{a}	44.82±2.74^{ab}	52.34±1.22^{ab}	33.30±1.20^{ab}
With		**Leaf**	43.46±1.6^{a}	13.03±1.28^{b}	13.97±0.73^{b}	15.44±1.50^{b}	16.80±1.52^{b}	18.44±0.76^{b}
Mycorrhizal								
bio-augmentation		**Root**	18.92±1.11^{a}	13.08±0.11^{a}	8.70±0.14^{b}	7.56±0.03^{b}	6.44±0.08^{b}	4.76±0.03^{b}
	Dry mass (g)	**Stem**	29.56±2.32^{a}	26.70±1.27^{a}	24.87±2.97^{a}	17.50±1.09^{ab}	18.80±0.45^{ab}	12.94±0.63^{ab}
		Leaf	8.82±0.98^{a}	4.18±0.07^{b}	4.87±0.02^{b}	4.40±0.10^{b}	4.38±0.05^{b}	5.88±0.92^{b}
		Biomass (g)	57.49±3.23^{a}	43.22±3.0^{ab}	37.94±2.29^{b}	29.54±2.79^{b}	29.00±2.97^{b}	24.08±2.22^{b}
		Root	48.67±2.67^{a}	29.68±1.24^{b}	28.92±1.37^{b}	23.50±1.62^{b}	23.52±1.08^{b}	21.07±1.61^{b}
	Fresh mass (g)	**Stem**	61.10±3.32^{a}	57.92±2.40^{a}	54.14±2.10^{a}	48.58±2.8^{a}	44.42±1.59^{a}	40.95±1.10^{ab}
		Leaf	47.93±3.43^{a}	28.50±2.79^{b}	20.22±1.43^{b}	23.38±1.88^{b}	27.62±1.85^{b}	22.43±1.15^{b}
Without								
Mycorrhizal		**Root**	17.73±1.75^{a}	9.68±0.70^{b}	8.32±0.62^{b}	8.08±0.47^{b}	7.74±0.88^{b}	5.20±0.05^{bc}
bio-augmentation	**Dry mass (g)**	**Stem**	26.03±0.61^{a}	24.76±0.36^{a}	24.82±1.92^{a}	22.98±1.31^{a}	22.08±1.49^{a}	17.70±0.54^{a}
		Leaf	7.00±0.06^{a}	4.46±0.02^{b}	3.74±0.08^{b}	3.94±0.03^{b}	4.18±0.05^{b}	4.35±0.07^{b}
		Biomass (g)	50.74±3.34^{a}	38.23±3.04^{b}	37.00±2.91^{b}	35.76±2.33^{b}	34.04±2.10^{b}	27.77±2.06^{b}

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Values are mean ± standard error of 5 replicates. Means with a similar letter(s) in superscript along the row are not significantly different from each other (Tukey HSD test at p = 0.05).

Table 4: Fresh and dry mass of Tithonia diversifolia grown in zinc (Zn) contaminated soil with/without mycorrhizal bio-augmentation

Soil status	Parameter	Plant part	Quantity of heavy metal applied (mg/kg of soil)					
			0	20	40	60	80	100
With Mycorrhizal bio-augmentation	Fresh mass (g)	Root	49.77±3.64^{a}	49.70±2.38^{a}	42.68±3.91^{a}	17.33±1.71^{b}	11.00±1.65^{b}	16.89±1.55^{b}
		Stem	66.88±4.34^{a}	45.97±2.25^{ab}	33.46±2.78^{b}	12.48±1.16^{c}	7.21±0.4^{cd}	3.90±0.12^{cd}
		Leaf	43.97±2.19^{a}	37.47±2.58^{a}	43.28±2.83^{a}	18.56±2.82^{b}	8.41±1.99^{c}	6.61±0.30^{c}
	Dry mass (g)	Root	19.56±2.19^{a}	18.64±1.53^{a}	21.29±1.30^{a}	13.16±0.12^{b}	5.47±0.09^{c}	3.18±0.09^{c}
		Stem	30.27±1.84^{a}	28.83±1.20^{a}	26.47±1.46^{a}	12.26±1.91^{b}	3.52±0.03^{c}	2.90±0.01^{c}
		Leaf	8.97±0.19^{a}	13.39±0.41^{a}	10.80±0.52^{a}	7.15±0.82^{b}	5.29±0.11^{b}	1.81±0.01^{c}
		Biomass (g)	59.98±3.17^{a}	59.65±3.16^{a}	57.43±3.34^{a}	32.33±3.22^{b}	14.84±1.11^{b}	7.86±0.16^{b}
Without Mycorrhizal bio-augmentation	Fresh mass (g)	Root	47.89±2.92^{a}	50.96±2.36^{a}	27.98±2.14^{b}	34.32±0.79^{b}	29.80±1.09^{b}	27.21±1.63^{b}
		Stem	60.98±1.98^{a}	59.96±1.89^{a}	20.68±0.50^{b}	32.22±1.50^{b}	26.47±2.50^{b}	29.2±8.11^{b}
		Leaf	47.78±2.92^{a}	24.91±1.58^{b}	19.49±1.70^{b}	16.30±0.20^{b}	11.63±0.31^{bc}	15.40±0.27^{b}
	Dry mass (g)	Root	18.94±0.45^{a}	13.52±0.24^{ab}	8.79±0.87^{b}	9.61±0.48^{b}	9.85±0.64^{b}	7.92±0.58^{b}
		Stem	26.47±1.91^{a}	17.53±0.21^{b}	9.37±0.45^{b}	11.14±0.16^{b}	6.36±0.02^{b}	8.66±0.07^{b}
		Leaf	8.02±0.40^{b}	3.56±0.05^{a}	2.37±0.74^{a}	3.15±0.07^{a}	1.56±0.38^{a}	3.88±0.31^{a}
		Biomass (g)	55.01±1.27^{a}	31.55±1.07^{ab}	20.63±1.23^{b}	24.07±1.2^{b}	21.74±1.19^{b}	20.15±1.07^{b}

Values are mean ± standard error of 5 replicates. Means with a similar letter(s) in superscript along the row are not significantly different from each other (Tukey HSD test at p = 0.05).

Table 5: Concentration of Pb (%) in the root and shoot of Tithonia diversifolia grown in Pb contaminated soil with/without mycorrhizal bio-augmentation

Soil status	Plant part	Quantity of heavy metal applied (mg/kg of soil)					
		0	20	40	60	80	100
With mycorrhizal bio-augmentation	Root	0.38±0.01^{a}	1.45±0.01^{b}	1.67±0.01^{b}	1.75±0.01^{b}	2.56±0.01^{b}	2.56±-0.01^{c}
	Shoot	0.37±0.01^{a}	1.44±0.01^{b}	1.46±0.01^{b}	1.75±0.01^{b}	2.51±0.01^{b}	2.42±0.06^{c}
Without mycorrhizal augmentation	Root	0.27±0.01^{a}	1.32±0.01^{b}	1.37±0.0 1^{b}	1.46±0.01^{b}	1.51±0.01b	2.55±0.01c
	Shoot	0.22±0.01^{a}	1.27±0.01^{b}	1.35±0.01^{b}	1.42±0.01^{b}	1.47±0.01^{b}	2.51±0.01c

Values are mean ± standard error of 3 replicates. Means with a similar letter(s) in superscript along the row are not significantly different from each other (Tukey HSD test at p = 0.05).

Table 6: Concentration of Zn (%) in the root and shoot of Tithonia diversifolia grown in Zn contaminated soil with/without mycorrhizal bio-augmentation

Soil status	Plant part	Quantity of heavy metal applied (mg/kg of soil)					
		0	20	40	60	80	100
With mycorrhizal bio-augmentation	Root	0.72±0.01^{a}	2.49±0.01^{b}	2.65±0.01^{b}	3.04±0.01^{bc}	3.46±0.01^{c}	3.71±0.01^{cd}
	Shoot	0.73±0.01^{a}	2.88±0.01^{b}	3.96±0.01^{b}	3.26±0.01^{c}	3.64±0.01^{c}	4.70±0.01^{c}
Without mycorrhizal bio-augmentation	Root	0.77±0.0^{a}	2.15±0.01^{b}	2.40±0.01^{b}	2.74±0.01^{b}	3.23±0.02c	3.35±0.01^{c}
	Shoot	0.55±0.08^{a}	1.72±0.02^{b}	2.12±0.02^{c}	2.42±0.01c	2.62±0.02c	3.14±0.01^{c}

Values are mean ± standard error of 3 replicates. Means with a similar letter(s) in superscript along the row are not significantly different from each other (Tukey HSD test at p = 0.05).

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CONCLUSION

Growth and biomass production were reduced by soil contamination with Pb and Zn with variations depending on metal type, concentration applied, parameter determined and soil status (presence or absence of AM). Plants seemed to perform batter when arbuscular mycorrhiza was added to the soil than without it. Lead and zinc contamination resulted in accumulation of the metals in plant tissues which was further elevated by soil inoculation with AM fungus. *Tithonia diversifolia* is a potential phytoremediation agent for Pb and Zn polluted soils, and bio-augmentation with *Glomus clarum* will further enhance its potential.

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