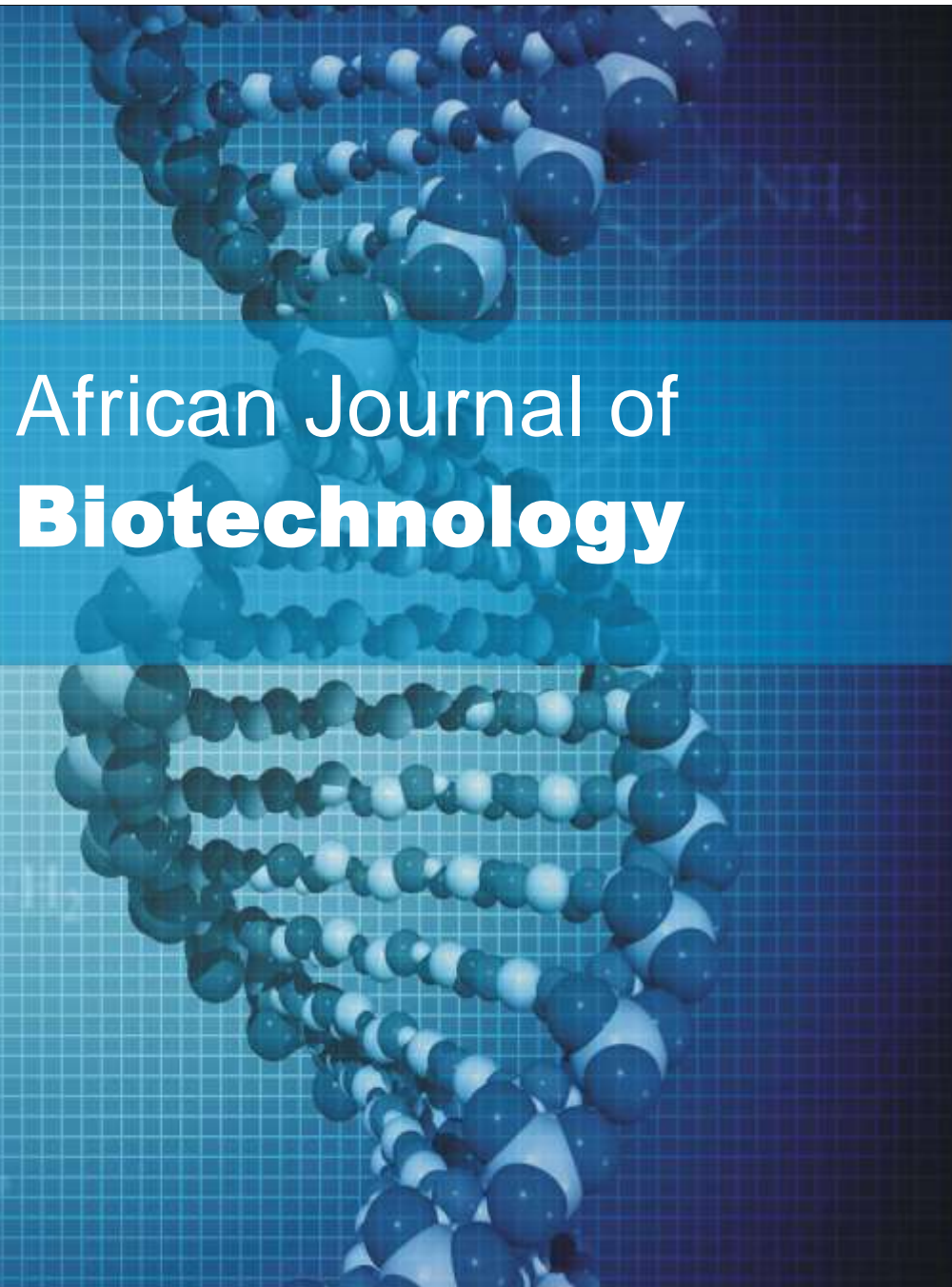


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Bioremediation potentials of sunflower and Pseudomonas species in soil contaminated with lead and zinc

Elizabeth Temitope Alori, Abiodun Joseph, Ojo Timothy Vincent Adebisi,
Ajibola Patrick Aluko and Chidera Onyekankeya

Full Length Research Paper

Bioremediation potentials of sunflower and *Pseudomonas* species in soil contaminated with lead and zinc

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This study was conducted to assess the ability of sunflower (*Tithonia diversifolia*) inoculated with *Pseudomonas* species to remediate soils contaminated with heavy metals (lead and zinc). The contaminants were added to 20 kg soil in pots as lead nitrate ($Pb(NO_3)_2$) and zinc nitrate ($Zn(NO_3)_2$); 400 mg/l metal concentrations were added to 20 kg soil in pots. The experimental design was a randomized complete block design (RCBD) with the two metal types (lead, zinc) and three species of *Pseudomonas* replicated thrice. The results reveal that all the *Pseudomonas* sp. (*P. putida* ATCC 29352, *P. cepacia* ATCC 29351 and *P. fluorescens* F113) used showed the potential for remediating zinc and lead. However, *P. fluorescens* inoculated sunflower remediate zinc (Zn) the best, followed by sunflower inoculated with *P. putida* ATCC 29352, and sunflower inoculated with *P. cepacia* ATCC 29351 at 8 Weeks After Planting (WAP). The order of remediation ability of sunflower inoculated with *Pseudomonas* spp. in soil polluted with lead (Pb) is in the order of sunflower inoculated with *P. cepacia* ATCC 29351 > sunflower inoculated with *P. putida* ATCC 29352 > sunflower inoculated with *P. fluorescens* F113. The shoot and root of the plant were analysed for Zn and Pb uptake after 8 weeks. The bio-concentration factor (BCF) and translocation factor (TF) assessed at 8WAP showed that translocation of Zn from root to shoot by sunflower with *Pseudomonas* spp., was higher than Pb. In conclusion this research suggests that sunflower inoculated with *Pseudomonas* spp., has phytoextraction ability and could be used to remediate soil contaminated with Zn and Pb.

Key words: Bioremediation, phytoremediation, *Pseudomonas* spp., heavy metals.

INTRODUCTION

Increase in the use of organic and inorganic materials as soil amendments have raised the concerns about their harmful effects on the environment. Modern agricultural practices pollute the soil to a large extent. With the

advancing agro-technology, huge quantities of fertilizers, pesticides and herbicides added to increase the crop yield are reported to cause soil pollution (Olanrewaju et al., 2017; Önder et al., 2011). Heavy metals belong to the

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types of toxic substances that have adverse effects on health. The most common heavy metals are Cd, Cr, Cu, Hg, Pb, and Zn having their atomic number greater than 20 and with metallic properties. These metals cannot be easily degraded, and the clean-up usually requires their removal (Alori, 2015).

Lead is a metal which has been associated with human activities for several decades. It is an industrial metal that has become widespread in air, water, soil, and food. These pollutants affect and alter the chemical and biological properties of soil. Dixit et al. (2015), has reported herbicides and insecticides as one of the anthropogenic sources of lead in agricultural soil. More also in Nigeria, gasoline with an average Pb content of 0.66 g/dc³ remains in use (Aransiola et al., 2013). Zinc though occurs naturally in water, air and soil, its concentrations have tremendously increased through human activities (Subhashini and Swamy, 2013).

The remediation of metal contaminated sites often involves expensive and environmentally invasive and civil engineering-based practices (Olanrewaju et al., 2017). A range of technologies such as fixation, leaching, soil excavation, and landfill of the top contaminated soil *ex situ* have been used for the removal of metals. Many of these methods have high maintenance costs and may cause secondary pollution or adverse effect on biological activities, soil structure, and fertility (Abioye et al., 2013). The high cost of these approaches necessitated the need for less expensive clean up techniques. Bioremediation plays a major role in cleaning of polluted or contaminated site.

Bioremediation is the correction of soil polluted or contaminated with hazardous materials using living organisms such as microorganisms and green plants. Phytoremediation is a technology that uses specialized green plants together with associated soil microbes to remove, destroy, sequester or reduce the concentration or toxic effects of contaminants in polluted environment especially soil and water (Alori, 2015). Some microorganisms have developed and adopted various detoxifying mechanisms such as, bioaccumulation, biosorption, biomineralization, and biotransformation and hence, their ability to survival in heavy metal-polluted habitats. These organisms can be exploited for bioremediation either *ex situ* or *in situ* (Lin and Lin, 2005; Malik, 2004). Examples are *Staurastrum* sp., *Bacillus* sp., *Paenibacillus* sp., *Synechococcus* sp., *Saccharomyces cerevisiae*, *Synechococcus* sp., *Pseudomonas* sp. and fungi such as *Aspergillus* sp., and *Corlospora* sp. (Dixit et al., 2015). *Pseudomonas* spp. have been reported to have the ability to remediate polluted soils (Stamenov et al., 2015).

The life cycle and luxuriant growth habits of sunflower, in particular, on soils with poor nutrient and on roadsides exposed to frequent Pb emission from automobiles necessitated the investigation of this plant for their metal accumulation potentials. Therefore, this study assessed

the ability of sunflower and *Pseudomonas* spp. to remediate metal impacted soils.

MATERIALS AND METHODS

The soil sample used for this study was collected from a depth of 0-15 cm within Landmark Teaching and Research Farm, Omu-aran, Kwara State, Nigeria and transported to Crop and Soil Science Laboratory of Landmark University in plastic bags. The soil sample was air dried and sieved with 2 mm diameter mesh. The soil physical and chemical properties including particle size analysis, available phosphorus, total nitrogen, organic matter content, magnesium, calcium, potassium and soil pH were examined.

The pH of the soil samples was determined with a pH meter. Organic matter content was determined using the wet oxidation method, described by Shamshuddin et al. (1994). The hydrometer method of Gee and Or (2002), was employed in the determination of particle size. Exchangeable bases (K, Mg, Na, and Ca), were determined by ammonium acetate method of Chapman (1965). The total soil Nitrogen was determined by macroKjedahl analysis, (Bremner, 1965). The Bray1 method was used in the determination of available phosphorus, (Murphy and Riley, 1962).

The experimental soil was a loamy sand texture soil that is slightly acidic. Nutrients such as phosphorus (P), Nitrogen (N), and organic matter are available moderately available (Table 1).

Heavy metal contaminants preparation

The contaminants was added as lead nitrate (Pb (NO₃)₂) and zinc nitrate (Zn (NO₃)₂); 1.599 g of Pb (NO₃)₂ and 2.897 g of Zn (NO₃)₂ and was weighed and dissolved in 1,000 ml distilled water to make stock solutions. A 40 ml amount was measured out from the stock solutions into a 100 ml measuring cylinder and made up to mark to give 400 mg/dm³ metal concentrations. This therefore, was added to 20 kg soil in pots. The 400 mg/kg represents upper critical soil concentration for both Pb and Zn (Kabata-Pendias and Pendias, 1984).

Experimental layout

The experimental design was a Randomized Complete Block Design (RCBD) with the two metal type (lead and zinc), four species of *Pseudomonas* replicated thrice to make a total of 24 experimental pots. Each of the experimental pots was contaminated with the heavy metals at a concentration of 400 mg/kg. Ten seeds each of sunflowers inoculated with *Pseudomonas* were planted in each pot. The seeds in each pot started germinating 1 week after planting (WAP) which was monitored for 8 weeks. At 8 WAP, plants were harvested, separated into two compartments, viz. shoot and root and each compartment was then oven dried at 70°C. The soil on which plant were grown was also analysed for heavy metal content.

Determination of lead and zinc in plant material

Yusuf et al. (2003) acid digestion method was employed for the digestion of grounded plant samples. From each of this 1 g was weighed into 50 ml capacity beaker, followed by addition of 10 ml mixture of analytical grade acids: HNO₃;H₂SO₄; HClO₄ in the ratio 1:1:1. The beakers containing the samples were covered with watch glasses and left overnight. The digestion was carried out at temperature of 70°C until about 4 ml was left in the beaker. Then, a further 10 ml of the mixture of acids was added. This mixture was

Table 1. Physical and chemical properties of the experimental soil.

Parameter	Value
Sand (2,000-50 μm) %	81.68
Silt (50-2 μm)%	13.00
Clay (<2 μm)%	5.32
Texture	Loamy sand
pH (H ₂ O)	6.50
Organic matter (OM) (mg/kg)	2.98
Total N (g/kg)	6.04
Available P(mg/kg)	6.04
Ca (mg/kg)	448
Mg (mg/kg)	173
Na (mg/kg)	0.92
K (mg/kg)	819
Pb (mg/kg)	9.32
Zn (mg/kg)	37.5

allowed to evaporate to a volume of about 4 ml. After cooling, the solution was filtered to remove small quantities of waxy solids and distilled water was added to make up to a final volume of 50 ml. Atomic Absorption Spectrometer (AAS) (Model number: AA320N) was used to determine the lead and zinc concentration.

Determination of Lead and Zinc in soil

Five gram (5 g) of soil was weighed into 100 ml plastic bottle. 50 ml

of 0.1 M HCl was added and shaken for 30 min. Soil suspension was filtered. Pb, and Zn was determined AAS.

Determination of bioconcentration and translocation factor

Bioconcentration factor (BCF) and translocation factor (TF) were calculated using the formula of Yadav et al. (2009) as:

$$\text{Bio concentration factor (BCF)} = \frac{\text{Average metal conc. in the whole plant (mg/kg)}}{\text{Metal conc. in soil (mg/kg)}}$$

$$\text{Translocation Factor (TF)} = C_{\text{aerial}} \times \frac{1}{C_{\text{root}}} = \frac{C_{\text{aerial}}}{C_{\text{root}}}$$

C_{aerial} = Metal concentration in the aerial part of plant (shoot).

C_{root} = Metal concentration in root of plant.

Statistical analysis

Data were subjected to analysis of variance using SPSS (version 21). Means were separated using Duncan multiple Range Tests at significant level of $P < 0.05$.

RESULTS

Effects of sunflower inoculated with *Pseudomonas* spp. on the concentration of heavy metals in polluted soil at 8 weeks after planting

Figure 1 shows the concentration of Zn and lead in polluted soil remediated with sunflower inoculated with

Pseudomonas spp. At 8 WAP, zinc concentration in the soil has been significantly reduced by sunflower. All the *Pseudomonas* sp. used showed the potential for remediation of zinc polluted soil. However, sunflower inoculated with *P. cepacia* ATTC 29351 remediates the best followed by sunflower inoculated with *P. putida* ATTC 29352, and sunflower inoculated with *P. fluorescens* F113 (Figure 1a).

Figure 1b shows the concentration of Pb in polluted soil remediated with sunflower inoculated with *Pseudomonas* spp. at 8 WAP. The concentration of Pb in the polluted soil remediated with sunflower without *Pseudomonas* was higher than the concentration of Pb in polluted soil remediated with sunflower inoculated with *Pseudomonas* sp. The degree of remediation is in the order of sunflower inoculated with *P. putida* ATTC 29352 > sunflower inoculated with *P. fluorescens* F113 > sunflower inoculated with *P. cepacia* ATTC 29351.

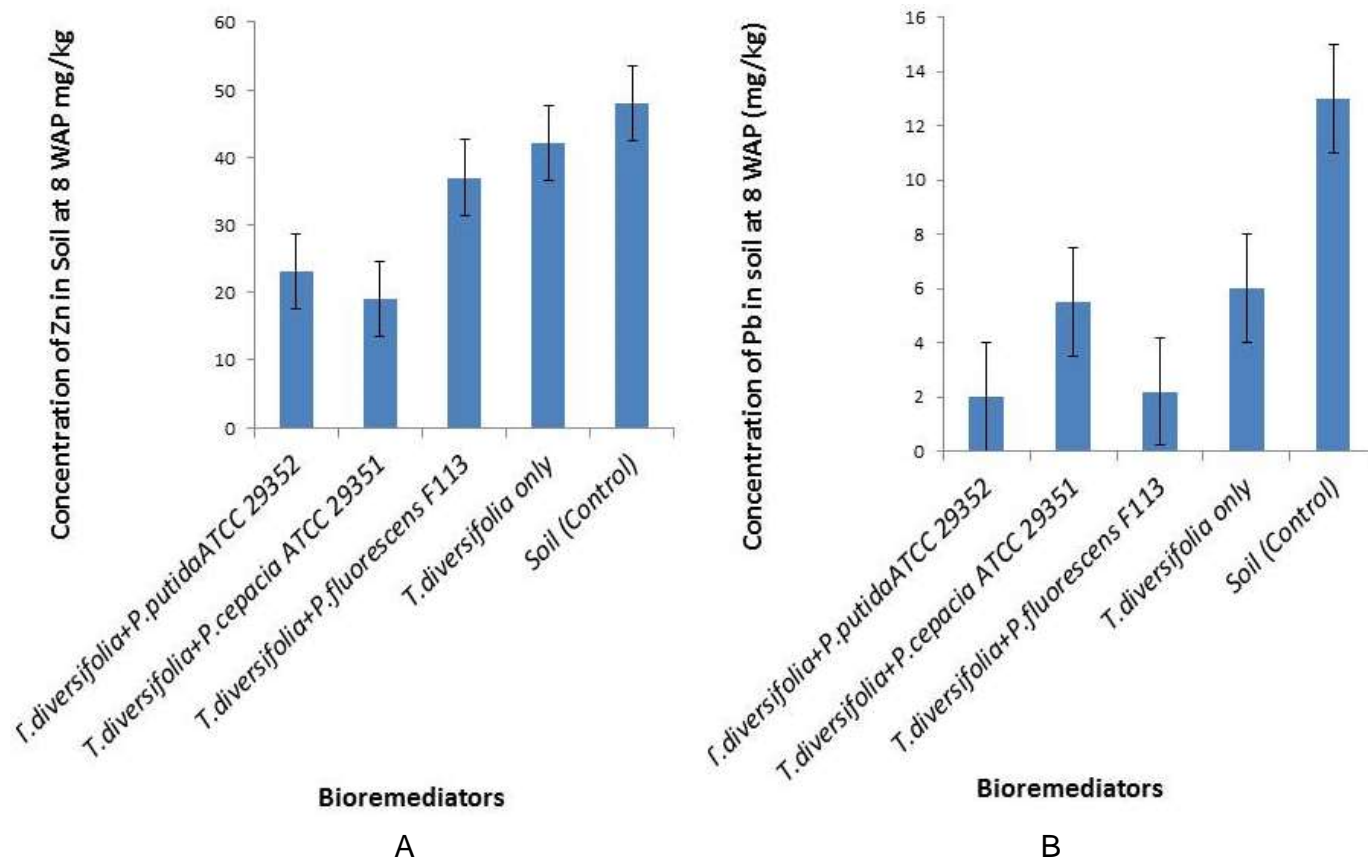


Figure 1. Concentration of metals in polluted soil remediated with sunflower and *Pseudomonas* spp. at 8 weeks after planting. (a) Concentration of Zn in polluted soil remediated with *T. diversifolia* and *Pseudomonas* spp. at 8 weeks after planting. (b) Concentration of Pb in polluted soil remediated with *T. diversifolia* and *Pseudomonas* spp. at 8 weeks after planting.

The concentration of Pb and Zn in the root of *T. diversifolia* inoculated with *Pseudomonas* spp.

The concentration of Pb and Zn in the root of sunflower inoculated with *Pseudomonas* spp. is revealed in Figure 2. The root of sunflower without *Pseudomonas* spp. accumulates more Pb compared with the root of sunflower inoculated with *Pseudomonas* spp. The concentration of Zn at the root of sunflower un-inoculated with *Pseudomonas* spp. was lower than the root of sunflower inoculated with *Pseudomonas* spp.

Bio-concentration factor (BCF) and translocation factor of metals in sunflower inoculated with *Pseudomonas* spp.

This study evaluated the ability of sunflower inoculated with *Pseudomonas* spp. to accumulate metals from contaminated soil by BCF in accordance to Yadav et al. (2009). Table 2 reveals that the highest BCF of zinc polluted soil was recorded in polluted soil remediated with sunflower inoculated with *P. cepacia*. The shoot (stem

and leaves) of sunflower inoculated with *P. fluorescens* F113 had the highest TF of zinc followed by sunflower inoculated with *P. putida* ATCC 29352 and then sunflower inoculated with *P. cepacia* ATCC 29351 while sunflower not inoculated with *Pseudomonas* spp. had the least. In the same vein, Table 2 shows the highest BCF of lead polluted soil was recorded in polluted soil remediated with sunflower inoculated with *P. putida* ATCC 29352 while the least was recorded in polluted soil remediated with sunflower not inoculated with *Pseudomonas* spp. The highest value of TF for soil polluted with lead was recorded in polluted soil remediated with sunflower inoculated with *P. putida* ATCC 29352 and then followed by sunflower inoculated with *P. cepacia* ATCC 29351 > sunflower inoculated with *P. fluorescens* F113 > sunflower not inoculated with *Pseudomonas* spp.

In soil polluted with Zn, all the treatments (sunflower inoculated with *P. putida* ATCC 29352, sunflower inoculated with *P. cepacia* ATCC 29351, sunflower inoculated with *P. fluorescens* F113 and sunflower not inoculated with any *Pseudomonas* spp) had TF > 1 (Table 2a). In Pb polluted soil, all the treatment (sunflower inoculated with *P. putida* ATCC 29352, sunflower

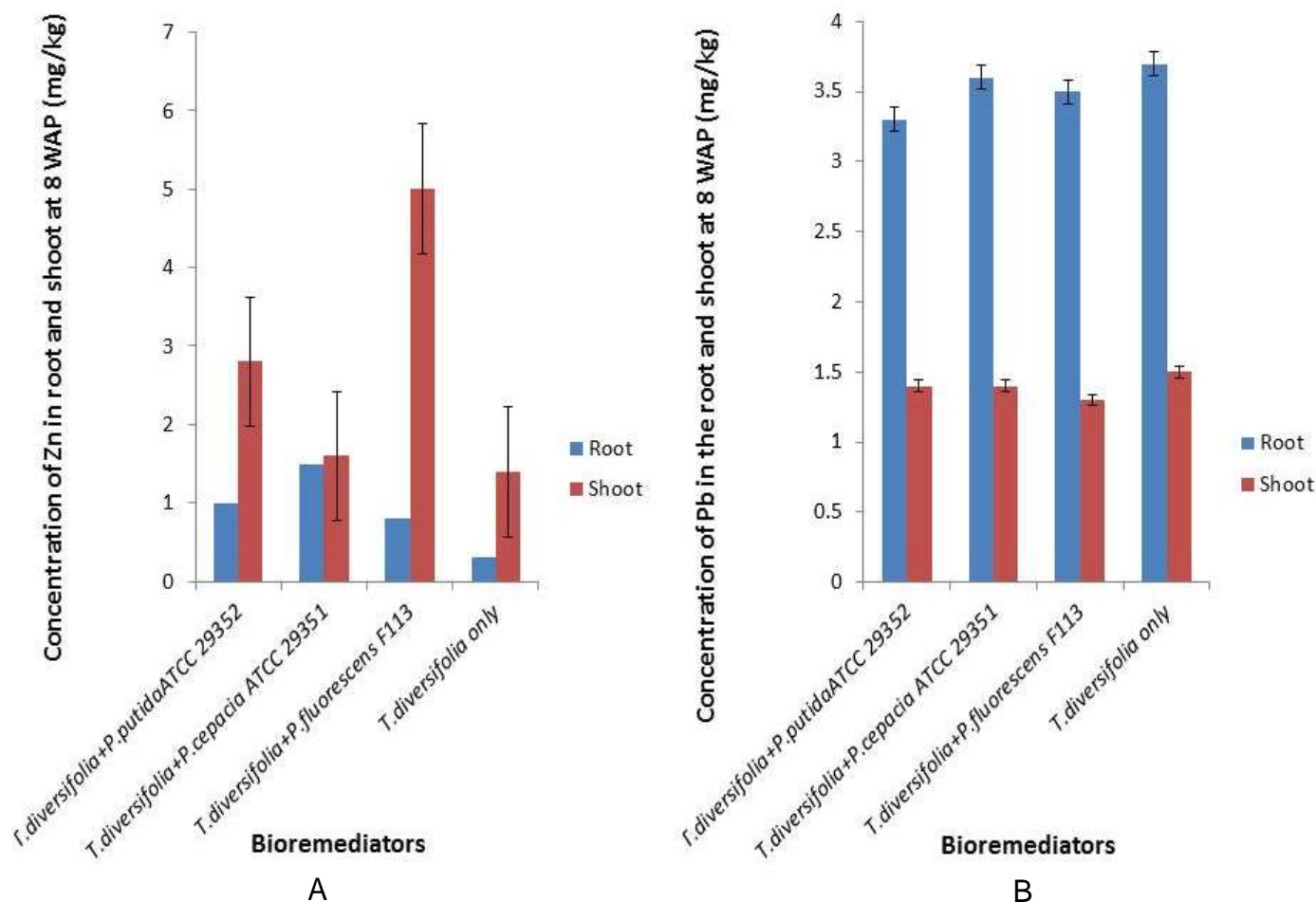


Figure 2. Concentration of metals in the root and shoot of *T. diversifolia* inoculated with *Pseudomonas* spp. at 8 weeks after planting. Bars are standard error bars. (a) Concentration of Zn in root and shoot of *T. diversifolia* inoculated with *Pseudomonas* spp. (b) Concentration of Pb in root and shoot of *T. diversifolia* inoculated with *Pseudomonas* spp.

inoculated with *P. cepacia* ATCC 29351, sunflower inoculated with *P. fluorescens* F113 and sunflower not inoculated with any *Pseudomonas* spp) had TF < 1 (Table 2b).

DISCUSSION

Effects of sunflower inoculated with *Pseudomonas* spp. on the concentration of heavy metals in polluted soil at 8 weeks after planting

The reduction in Zn concentration by sunflower observed is in line with the study of Adesodun et al. (2010), who stated that sunflower demonstrated accumulative potential for zinc. Stamenov et al. (2015), also reported that *Pseudomonas* species show potential for bioremediation. Plants, in association with microbial inoculant, can remove or transform contaminants into harmless substance (Alori et al., 2017). Lower Pb

concentration in polluted soil treated with sunflower inoculated with *Pseudomonas* spp. implies that the *Pseudomonas* spp. inoculants enhanced remediation potential of sunflower in lead polluted soil. *Physalis minima* Linn has potential to remediate soil polluted with Zinc (Subhashini and Swamy, 2013).

The concentration of Pb and Zn in the root of sunflower inoculated with *Pseudomonas* spp.

The *Pseudomonas* spp. used have the ability to translocate Zn from root to upper part of the plant while the reverse is the case with Pb. Inoculation of sunflower with *Pseudomonas* spp. aid translocation of Zn but not Pb from the root. The results indicate that sunflower inoculated with *Pseudomonas* spp. mopped up substantial concentration of Zn to above ground biomass as higher concentration was recorded in shoot compare to concentration in root.

Table 2. Bio-concentration factor (BCF) and translocation factor of zinc and lead in *T. diversifolia* inoculated with *Pseudomonas* spp

Treatment	BCF Zn	TF Zn (Shoot)	BCF Pb	TF Pb (Shoot)
<i>T. diversifolia</i> + <i>P. putida</i> ATCC 29352	0.16	2.75	2.13	0.42
<i>T. diversifolia</i> + <i>P. cepacia</i> ATCC 29351	0.17	1.04	0.84	0.38
<i>T. diversifolia</i> + <i>P. fluorescens</i> F113	0.15	8.03	1.69	0.35
<i>T. diversifolia</i> only	0.04	4.84	0.89	0.39

Bio-concentration factor (BCF) and translocation factor of metals in sunflower inoculated with *Pseudomonas* spp.

Sunflower inoculated with *P. cepacia* ATCC 29351 accumulates more zinc than the other treatments.

Translocation factor which reveals the measure of the ability of plants to transfer accumulated metals from the roots to the shoots is given by the ratio of concentration of metal in the shoot to that in the roots (Cui et al., 2004). This result agrees with those of other researcher such as Aransiola et al. (2013) which shows that plant species may effectively and selectively act as accumulators and indicators.

This study assumed that plants with BCF value > 1 are accumulators while plants with BCF <1 are excluders (Aransiola, 2013). The results in this study show that remediating zinc polluted soil with sunflower inoculated *P. putida* ATCC 29352 and sunflower inoculated with *P. fluorescens* F113 had BCF values > 1, indicating that this plant when inoculated with these species of *Pseudomonas*, have the potential to be used as accumulators of lead while sunflower inoculated with *P. cepacia* ATCC 29351 had BCF < 1 for Pb and sunflower inoculated with *P. putida* ATCC 29352, sunflower inoculated with *P. fluorescens* F113 and sunflower inoculated with *P. cepacia* ATCC 29351 had BCF < 1 for Zn. According to Usman and Mohamed (2009), the success of phytoextraction process depends on heavy metal removal by the shoots. It is therefore suggested that plant species having the higher metal concentration in its shoots than in its roots can be considered as accumulator for phyto remediation. However, since *P. cepacia* had BCF < 1 for Pb and sunflower inoculated with *P. putida* ATCC 29352, sunflower inoculated with *P. fluorescens* F113 and sunflower inoculated with *P. cepacia* ATCC 29351 had BCF < 1 for Zn, they could also be an excluder in phyto remediation processes. This is in line with the report by Aransiola et al. (2013). Ma et al. (2001) classified plants with TF value > 1 as high efficiency plants for metal translocation from the roots to the shoots. Both sunflower and sunflower inoculated with these *Pseudomonas* spp. could be classified as high efficient plant for Zn translocation from roots to the above ground parts of the plant, hence could be good phyto remediators for Zn polluted soil as suggested by Wei and Chen (2006). However, sunflower stored the

metal removed from the soil in the root. Ma et al. (2001) and Srivastava et al. 2006) explained that one of the factors that determine the efficacy of phytoextraction in metal contaminated soil is root uptake.

Conclusion

This study demonstrated that *Pseudomonas* spp. enhance bioremediation potential of sunflower and that the combination can be used as bioremediator in soil polluted with zinc and lead. Sunflower inoculated with *P. cepacia* ATCC 29351 best remediates soil polluted with Zn, whereas that inoculated with *P. putida* ATCC 29352 performed significantly better than the rest in soil polluted with Pb. It also recorded that sunflower inoculated with *Pseudomonas* spp. accumulates more zinc in the shoot and more lead in the root.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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