



## Research Article

# Influence of *Lysinibacillus fusiformis* on the phytoremediation of Pb, Cu and Cd by *Helianthus annuus* in contaminated soils from New Panteka, Kaduna State, Nigeria

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## ABSTRACT

Heavy metal contamination of soils around automobile workshops poses serious environmental and public health risks due to the persistence and toxicity of metals such as cadmium (Cd), lead (Pb) and copper (Cu). This study investigated the influence of *Lysinibacillus fusiformis* on the phytoremediation performance of *Helianthus annuus* grown in contaminated soils from New Panteka, Kaduna State, Nigeria. Baseline soil metal concentrations were determined from single composite samples at three contaminated points (A–C), while post-phytoremediation concentrations were analysed in triplicate after a 30-day growth period. Metal concentrations in soil and plant tissues were quantified using atomic absorption spectrophotometry. Following phytoremediation, substantial reductions were observed for Pb and Cu, particularly at Point C. Soil Pb decreased from 392.50 mg/kg to  $49.42 \pm 15.04$  mg/kg, while Cu decreased from 55.50 mg/kg to  $12.22 \pm 8.57$  mg/kg, both falling below World Health Organization guideline values. In contrast, Cd remediation was inconsistent, with soil Cd concentrations remaining above the permissible limit (0.8 mg/kg) at all points after treatment and exhibiting negative or marginal metal removal efficiencies at some sites. High standard deviations observed for several metals indicate considerable spatial and experimental variability. Plant tissue analysis showed higher accumulation of Pb and Cd in inoculated plants compared to non-inoculated controls, suggesting increased metal bioavailability associated with microbial activity. The results indicate that *L. fusiformis* influenced Pb and Cu phytoremediation trends in *H. annuus*, while Cd remained poorly remediated under the experimental conditions. The findings highlight the potential and limitations of microbial-assisted phytoremediation in heterogeneous, metal-contaminated urban soils.

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## INTRODUCTION

Heavy metal pollution is becoming a serious issue in developing countries. It hinders sustainable growth; hence puts human health, ecosystems, and the local environment at risk. Due to their toxicity and recalcitrant nature, heavy metals have recently been found in higher amounts in the biota. This increase may also be as a result of increase in the rate of urbanization, industrialization, and other human activities (Kosta *et al.*, 2023).

Heavy metals such as cadmium (Cd), lead (Pb), and copper (Cu) extensively contaminate the environment due to activities like mining, smelting, oil exploration, waste disposal, and fertilizer production. These metals are hazardous because they are non-biodegradable and capable of causing cancer and other health problems. Lead is commonly released from mining, batteries, and electronic waste, while cadmium originates from industrial processes and natural events such as geological activities and forest fires. Cadmium is particularly toxic, contributing to cardiovascular issues, nervous system disorders, insomnia, and carcinogenic effects. Copper, though essential in trace amounts, becomes harmful at elevated concentrations (Dakasku and Galo, 2024). Most of these elements originating either from natural or artificial sources constitute serious hazards to living organisms and their environment hence necessitating measures for their remediation from the environment polluted due to their accumulation.

Phytoremediation is the plant-based method towards environmental decontamination. It mainly involves the use of plant to absorb the metals from soil and ground water. These plants are either naturally or artificially endowed with the ability to accumulate heavy metals. Different processes of phytoremediation so far reported include: phytoextraction, phytostimulation, phytovolatilization, phytodegradation, phytofiltration, phytostabilization, and rhizofiltration. In the application of any of these methods, Various plants have been used (Yan *et al.*, 2020). One of the most common phytoremediator that has been reported for its high phytoremediation potential is *Helianthus annuus* (Sunflower) which is metal-tolerant and known for its rapid growth, substantial biomass production, and ease of harvest (Niu *et al.*, 2023). The genus *Lysinibacillus* comprises Gram-positive, spore-forming,

motile bacteria that were formerly classified within the genus *Bacillus* but were later reclassified based on distinct peptidoglycan cell wall composition (Lys–Asp type), physiological characteristics, and 16S rRNA gene phylogeny. Key species such as *Lysinibacillus fusiformis* and *Lysinibacillus sphaericus* were among the original taxa reassigned to this genus. Members of *Lysinibacillus* have been isolated from diverse environments, including saline soils, compost, crop rhizospheres and anthropogenic surfaces, reflecting their broad ecological adaptability.

In recent years, *Lysinibacillus* species have attracted considerable attention due to their biotechnological potential, particularly in plant growth promotion and environmental remediation. It has been reported that *Lysinibacillus fusiformis* is recognized as a plant growth-promoting rhizobacterium (PGPR) with multiple beneficial traits, including phosphate solubilization, siderophore production, and phytohormone synthesis, which collectively enhance nutrient availability and plant performance. In addition, its tolerance to contaminated environments enables participation in the mobilisation, transformation, or immobilisation of heavy metals such as cadmium, lead, and copper. These attributes support the growing interest in *L. fusiformis* as a promising microbial agent for microbial-assisted phytoremediation and sustainable soil management strategies (Ahsan and Shimizu, 2021; Mohammad and Jamal, 2022).

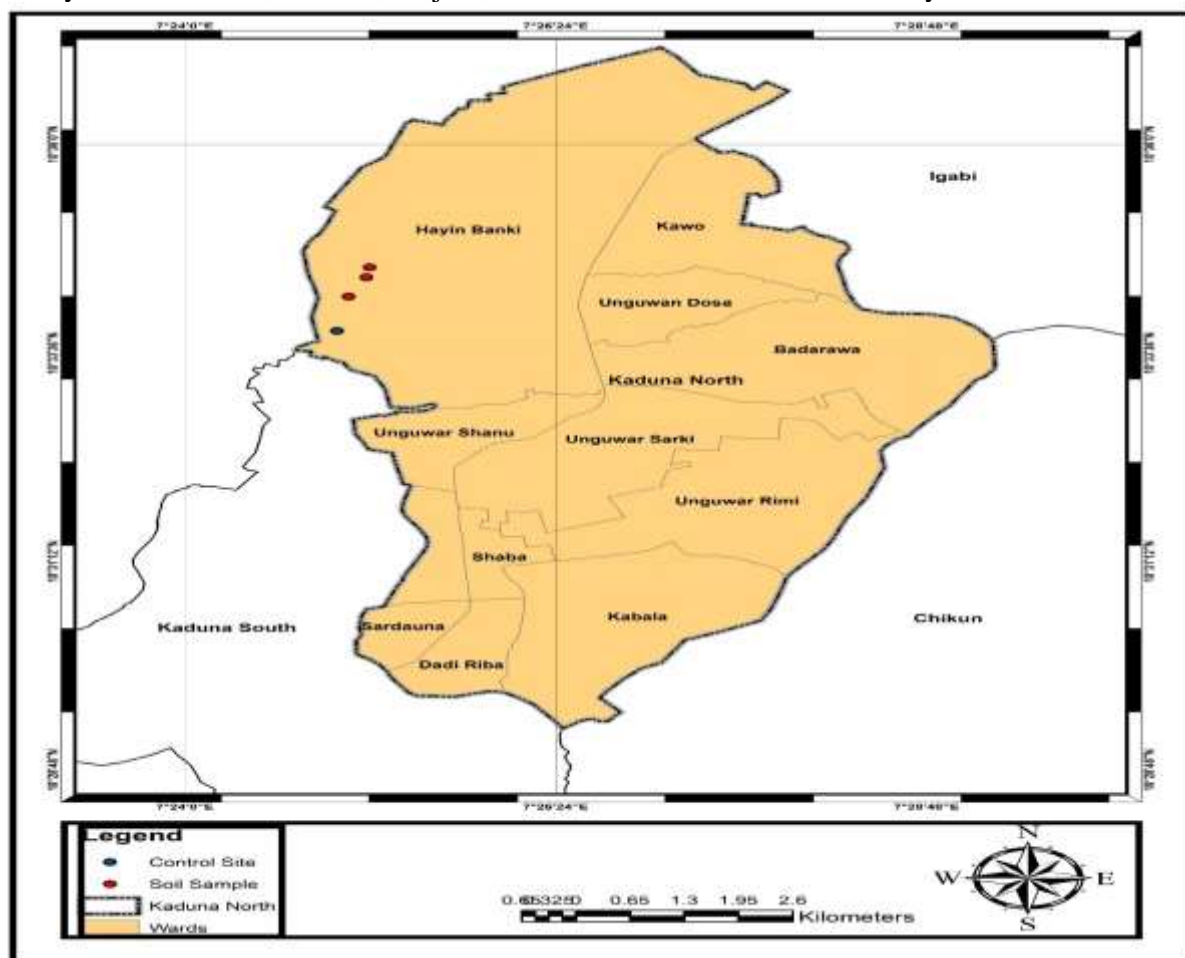
Various Studies reported the possibility of enhancing this capability in the Sunflower through its optimization with plant growth promoting rhizobacteria with the view to achieving maximum efficiency and uptake capacity in the plant (Soares *et al.*, 2021). Use of rhizospheric microorganisms for optimization of phytoremediators is a safe, cost-effective, and reliable method for eliminating heavy metals from environment. The microorganisms can survive in heavy metal-contaminated sites, increase plant growth, and enhance metal tolerance and removal. Studies have shown that Rhizobacteria can promote plant growth by producing siderophore, indole acetic acid, phosphate solubilisation, and hydrogen cyanide, which can protect plants against heavy metal toxicity in heavy metal-

contaminated soils (Kumar *et al.*, 2015; Zhu *et al.*, 2023).

## MATERIALS AND METHODS

Kaduna North Local Government Area (L.G.A.) is one of the administrative areas in Kaduna State, Nigeria, and it is regarded as the oldest Local Government in the State. The study area lies approximately between 10.56423°N, 7.41640° E and 10.57645° N, 7.41992° E, corresponding to the four sampling points (A–D). It is bordered by Igabi Local Government Area to the north and west, and by Kaduna South, Chikun, Kajuru, and

Kaura Local Government Areas to the south and east. Kaduna North L.G.A. covers an estimated area of about 72.2 km<sup>2</sup> with a population of approximately 423,580 people (NPC, 2006) and a population density of about 5,883 persons per km<sup>2</sup>. The soil composition is primarily derived from the Basement Complex formation, consisting mainly of *biotite gneisses* and *older granites* as the dominant geological materials. The region experiences two distinct seasonal patterns typical of a tropical savanna climate a cool-to-hot dry season and a humid rainy season.



**Figure 1:** Study area map showing sampling points (A–D) at New Panteka, Kaduna state, Nigeria. Source: GIS Lab, Department of Geography, NDA.

## SOIL SAMPLE COLLECTION AND PREPARATION

The fresh *Helianthus annuus* (Sunflower) seeds were purchased from National Animal Production Research Institute (NAPRI) Zaria, Kaduna State and validated at the Nigerian Defence Academy herbarium with Reference number (NDA/BIOH/202595).

Soil samples were collected randomly from 4 different points at distance of 20m in Automobile workshop in Kaduna State at New Panteka

Automechanic work shop and a control site at a depth of 0.1 meters by using Soil Auger. The soil samples were mixed properly and packed into well-labelled polythene bags and transferred to laboratory for preparation and analysis. Exactly 4 samples were collected, 3 samples were from study sites and 1 from control site and the samples were in triplicate for each Point (Ilaro *et al.*, 2021).

**PGPR** (Plant growth promoting rhizobacteria)

## BACTERIA IN IN VITRO EXPERIMENTATION

A strain of *Lysinibacillus fusiformis* was isolated from the soil sample to obtain a pure colony on TSA (Tryptic Soy Agar) which was incubated at  $26 \pm 1$  °C in the dark for at least 24–48 h. A day before the experiment, a well isolated colony was transferred to a cell culture flask containing 200 ml of TSB (Sigma-Aldrich) and was incubated overnight at  $26 \pm 1$  °C (Rosatto *et al.*, 2021). **Experimental Design and Seed Inoculation**

The experiment was designed to evaluate the effect of *Lysinibacillus fusiformis* inoculation on the phytoremediation potential of *Helianthus annuus* grown in heavy metal-contaminated soils. Sunflower seeds were divided into inoculated and non-inoculated (control) groups prior to sowing.

For the inoculated treatment, twelve viable sunflower seeds were immersed in a broth culture of *L. fusiformis* prepared in Tryptic Soy Broth (TSB) and incubated for six hours to facilitate bacterial adhesion and colonization. Following inoculation, the seeds were immediately sown into the designated contaminated soil samples. The non-inoculated control consisted of four untreated seeds sown directly into the soil without prior exposure to the bacterial suspension.

To maintain bacterial activity in the rhizosphere, a secondary inoculation was applied fourteen days after sowing to the inoculated treatment only. This consisted of 2.0 mL of washed *L. fusiformis* cell suspension applied directly to the root zone of each plant. After the second inoculation, the experiment was maintained for an additional fourteen days, resulting in a total experimental duration of four weeks (Inokulasi *et al.*, 2023).

All data interpretation was based on heavy metal concentrations normalized to soil mass and plant tissue dry weight rather than seed number, ensuring that differences in seed counts did not bias the assessment of phytoremediation performance. Baseline soil metal concentrations prior to planting were determined from single composite samples collected at each sampling point and are therefore presented as single values. In contrast, post-phytoremediation soil samples and all plant tissue samples were analyzed in triplicate, and the results are reported as mean  $\pm$  standard deviation. This approach reflects the experimental design and ensures appropriate representation of analytical variability following treatment.

## Soil and Plant Sample Preparation, Digestion and Heavy Metal Analysis

At the end of the four-week experimental period, soil and plant samples were collected for heavy metal analysis. Soil samples were air-dried at room temperature, gently crushed using a mortar and pestle, and passed through a 2 mm sieve to obtain homogeneous fine fractions.

Sunflower (*Helianthus annuus*) plants were harvested by careful uprooting and separated into roots and stems. Plant samples were thoroughly washed with distilled water to remove adhering soil particles and oven-dried at 70 °C to constant weight. The dried plant tissues were ground into fine powder using a mortar and pestle and sieved through a 2 mm mesh.

Soil and plant samples were digested using a mixed acid system of concentrated hydrochloric acid (HCl) and concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) to extract the acid-soluble fractions of cadmium (Cd), lead (Pb), and copper (Cu). This digestion approach was applied consistently to all samples and is suitable for assessing environmentally available metal fractions relevant to phytoremediation studies (Ilaro *et al.*, 2021; Abdullahi *et al.*, 2021). The digested samples were filtered and diluted with deionized water to appropriate volumes. Concentrations of cadmium (Cd), lead (Pb), and copper (Cu) in both soil and plant digests were quantified using an atomic absorption spectrophotometer (PerkinElmer PinAAcle 900H). Metal concentrations in soil were expressed in mg kg<sup>-1</sup> dry weight, while metal accumulation in plant tissues was determined separately for roots and stems.

The efficiency of phytoremediation was evaluated using metal removal efficiency (MRE), calculated as:

$$\text{MRE (\%)} = (C_1 - C_2) / C_1 \times 100$$

where  $C_1$  represents the concentration of heavy metals in the soil before planting and  $C_2$  represents the concentration of heavy metals in the soil after harvesting (Kosta *et al.*, 2023). Negative MRE values indicate an increase in soil metal concentration after treatment.

## Quality Control and Assurance for AAS Analysis

Quality assurance and quality control (QA/QC) procedures were employed during atomic absorption spectrophotometric (AAS) analysis to ensure data reliability. All glassware was thoroughly acid-washed and rinsed with deionized water prior to use. Reagent blanks were analyzed to correct for background contamination. Calibration curves for Cd, Pb, and Cu were prepared using analytical-grade

standard solutions, and instrument calibration was verified periodically during analysis.

The method detection limits (MDLs) for Cd, Pb, and Cu were 0.01 mg/kg, 0.05 mg/kg, and 0.02 mg/kg, respectively, consistent with the operational specifications of the PerkinElmer PinAAcle 900H AAS. Recovery efficiency was assessed through standard addition and spike

recovery procedures, with recoveries ranging between 90–105% for all metals analyzed. Each sample digest was analyzed in triplicate, and mean values were reported.

#### Statistical Analysis

The results of the data were subjected to analysis of variance (ANOVA) using SPSS version 20 (IBM USA) statistical package at  $p \leq 0.05$ .

## RESULTS

**Table 1:** Cadmium (Cd), Lead (Pb), and Copper (Cu) accumulation in soil sample before and after sowing respectively (mg/kg)

Sample Area	Point A (Cd)	Point B (Cd)	Point C (Cd)	Point A (Pb)	Point B (Pb)	Point C (Pb)	Point A (Cu)	Point B (Cu)	Point C (Cu)
New Panteka (Before)	0.65	1.75	1.15	19.05	12.5	392.5	40.65	25.45	55.5
New Panteka (After)	1.15±0.09	1.12±0.08	1.12±0.08	18.57±1.46	15±2.41	49.42±15.04	28.13±2.87	20.12±1.91	12.22±8.57

Key: Cd= Cadmium, Pb= Lead, Cu= Copper, Single values represent baseline composite samples, while mean ± SD values represent triplicate analyses

**Table 2:** Concentration of the heavy metals (Cd, Pb and Cu) in soil sample before and after sowing respectively in Soil and plant parts of control site

Treatment	mg/kg		
	Cd	Pb	Cu
Initial Soil Sample (concentration)	0.6	29.6	7.05
Positive Control (With <i>L. fusiformis</i> )	0.5±0.65	11.7±0.77	4.7±3.2
Stem	0.3±0.14	7.3±5.93	3.9±0.98
Root	0.4±0.2	12.8±1.02	2.8±2.48
Positive Control (Without <i>L. fusiformis</i> )	0.55	9.4	4.85
Stem	0.3	4.8	4.85
Root	0.2	6.75	3.6

Key: Cd= Cadmium, Pb= Lead, Cu= Copper, Single values represent baseline composite samples, while mean ± SD values represent triplicate analyses

**Table 3:** Determination of the Efficiency of *H. annuus* (Sunflower) to Remediate Contaminated Soil with Heavy Metals

Point	Metal	C <sub>1</sub> (Before)(mg/kg)	C <sub>2</sub> (After)(mg/kg)	MRE (%)
A	Cd	0.65	1.15	-76.92
B	Cd	1.75	1.12	36.00
C	Cd	1.15	1.12	2.61
A	Pb	19.05	18.57	2.52
B	Pb	12.50	15.00	-20.00
C	Pb	392.50	49.42	87.41
A	Cu	40.65	28.13	30.80
B	Cu	25.45	20.12	20.94
C	Cu	55.50	12.22	77.98

Key: C<sub>1</sub> is the concentration of heavy metals in the soil before planting, C<sub>2</sub> is the concentration of heavy metals in the soil after harvesting. Cd= Cadmium, Pb= Lead, Cu= Copper

**Table 4:** Cadmium (Cd) accumulation in the plant parts in New Panteka.

Treatment	Plant Part	mg/kg		
		Point A	Point B	Point C
<b>Seed without</b> <i>Lysinibacillus spp</i>	Root	0.95	1.05	1.00
	Stem	1.10	1.05	0.65
<b>Seed with</b> <i>Lysinibacillus spp</i>	Root	1.05 ± 0.09	1.05 ± 0.2	1.17 ± 0.15
	Stem	1.13 ± 0.14	1.17 ± 0.1	1.10 ± 0.12

Single values represent baseline composite samples, while mean ± SD values represent triplicate analyses

**Table 5:** Lead (Pb) accumulation in the plant parts in New Panteka.

Treatment	Plant Part	mg/kg		
		Point A	Point B	Point C
<b>Seed without</b> <i>Lysinibacillus spp</i>	Root	12.3	7.3	13
	Stem	9.6	14.15	16.5
<b>Seed with</b> <i>Lysinibacillus spp</i>	Root	10.12±1.14	13.68±2.89	11.35±0.93
	Stem	9.23±3.26	13.8±4.62	13.67±1.17

Single values represent baseline composite samples, while mean ± SD values represent triplicate analyses

**Table 6:** Copper (Cu) accumulation in the plant parts in New Panteka.

Treatment	Plant Part	mg/kg		
		Point A	Point B	Point C
<b>Seed without</b> <i>Lysinibacillus spp</i>	Root	3.45	9.45	3.1
	Stem	2.6	2.55	40.65
<b>Seed with</b> <i>Lysinibacillus spp</i>	Root	5.87±0.137	4.68±1.44	4.83±2.92
	Stem	3.46±1.05	7.13±2.59	4.5±2.33

Single values represent baseline composite samples, while mean ± SD values represent triplicate analyses

## DISCUSSION

The cadmium (Cd) concentrations obtained (0.65–1.75 mg/kg) are above the WHO/FEPA target value of 0.8 mg/kg for initial soil samples at New panteka (Iyama and Edori, 2020). Point B recorded the highest Cd level, Followed by Point C and Point A also exceeded the permissible limit compared to the control soil (0.60 mg/kg). These findings are similar to those reported by Emolade *et al.* (2025), who observed elevated cadmium levels in soils impacted by industrial activities, and agrees with earlier studies that described the persistence and non-biodegradable nature of cadmium in contaminated environments. The present result is also in line with Iyama and Edori (2020), who highlighted the frequent exceedance of FEPA standards in Nigerian

soils near industrial and urban areas may indicate a localized and limited initial effect of rhizobacterial inoculation on cadmium bioavailability, which is consistent with previous reports that plant growth promoting rhizobacteria such as *Lysinibacillus fusiformis* can enhance phytoremediation efficiency and reduce heavy metal bioavailability in soils.

The changes in heavy metal concentrations in the soil before and after phytoremediation without microbial inoculation showed both expected and anomalous trends. For cadmium (Cd), the concentrations before treatment were 0.65 mg/kg, 1.75 mg/kg and 1.15 mg/kg at points A, B and C, respectively. After phytoremediation, the Cd content at points B and C decreased, while point A has substantial

increased. The unexpected increase at point A indicates a possible mobilisation or desorption of previously adsorbed Cd from the soil particles, possibly due to pH or redox changes during plant growth, a phenomenon also described by Alloway (2013). Statistical analysis confirmed these patterns that Point B: Cd reduction was highly significant ( $t(4) = 14.36$ ,  $p < 0.001$ ), Point C: Reduction was not statistically significant ( $t(4) = 0.756$ ,  $p = 0.492$ ), Point A: Increase after treatment was statistically significant, supporting the Alloway, (2013) mobilization explanation. Baseline soil metal concentrations prior to planting were obtained from single composite samples at each sampling point, while post-phytoremediation soil and plant tissue concentrations were analysed in triplicate.

Following phytoremediation, cadmium concentrations decreased at Points B and C but increased at Point A. This trend is further supported by the metal removal efficiency (MRE) values, where Point B recorded a positive Cd removal efficiency of 36.00%, indicating moderate uptake by *Helianthus annuus*. In contrast, Point C showed a very low Cd MRE of 2.61%, reflecting minimal net removal, while Point A exhibited a negative MRE of -76.92%, confirming an increase in soil Cd concentration after treatment. The negative MRE at Point A supports the explanation of cadmium mobilisation rather than extraction, likely due to changes in soil pH or redox conditions induced by root activity, as described by Alloway (2013).

Compared to the WHO target value of 0.8 mg/kg for cadmium in uncontaminated soil (Iyama and Etori, 2020), all Cd concentrations in this study exceeded the permissible limit after treatment. This indicates that although some uptake occurred, remediation was only partially effective, with inconsistent outcomes across sampling points especially in treatments without microbial support (Emolade *et al.*, 2025). Similar high levels of cadmium were also found in the soils of mechanical workshops in Delta State, highlighting the persistent nature of Cd contamination in environments with low remediation capacity and emphasising the need for improved strategies such as bioaugmentation to improve removal efficiency.

The accumulation of cadmium observed in *Helianthus annuus* root and stem tissues indicates a potential influence of microbial

inoculation on metal uptake dynamics, which did not consistently translate into soil Cd reduction. Plants grown without *Lysinibacillus fusiformis* showed moderate Cd concentrations in both roots and stems at all sampling sites. However, Cd accumulation in the aboveground tissues was generally lower, especially at point C, where metal retention appeared to be limited without microbial enhancement. After inoculation with *Lysinibacillus fusiformis*, Cd uptake increased in all parts of the plant (Table 4). The ability of *L. fusiformis* to enhance metal uptake is consistent with the findings of Pradhan *et al.* (2014), who reported that the bacterium produces biosurfactants and exhibits catalase and citrate utilisation activities that contribute to metal mobilization, resistance to oxidative stress and enhancement of plant microbe interactions in contaminated environments. These observations are also consistent with the study by (Mohammadzadeh *et al.*, 2016), which demonstrated that plant growth promoting bacteria (PGPB) improved biomass production and Cd uptake in *Helianthus annuus* grown in soil contaminated with heavy metals. Their research supports the concept that microbe assisted phytoremediation can significantly increase the efficiency of metal extraction from polluted environments, as shown by the improved uptake in inoculated sunflower plants in the present study.

In positive control samples, the initial soil Cd concentration (0.60 mg/kg) was below the WHO target value of 0.8 mg/kg, indicating that cadmium levels in the soil did not exceed international safety limits. After inoculation with *L. fusiformis*, Cd in soil has substantially decreased, with additional uptake observed in roots and stems. Similarly, in the non-inoculated positive control, soil Cd was slightly higher and root uptake was lower (Table 2). These results suggest that although Cd was already within acceptable limits, *L. fusiformis* enhanced Cd uptake and distribution in plant tissues. The findings corroborate Abbaszadeh-Dahaji *et al.* (2021), who demonstrated improved Cd bioavailability and translocation in PGPR assisted sunflower remediation. This analysis was statistically confirmed by the ANOVA, which showed a significant plant part effect ( $p < 0.05$ ), indicating that Cd accumulation was consistently higher in stems than in roots, regardless of inoculation.

Lead (Pb) concentrations showed the most striking variation. Point C is above the WHO/FEPA permissible limit of 85 mg/kg. In contrast, points A and B were below the target value, and the control soil corresponded to the background values (Table 2). The extremely high Pb concentration at Point C suggests localized contamination, associated with automobile activities such as battery disposal and emissions from exhaust and lubricants. Similar Pb contamination has been reported in auto-mechanic workshops in Minna and Delta State (Nwakife *et al.*, 2022; Emolade *et al.*, 2025). Such elevated Pb levels present severe ecological and health risks, including neurological impairment and soil quality degradation. After phytoremediation Point A: Pb decreased slightly which was not significant from 19.05 mg/kg to  $18.57 \pm 1.46$  mg/kg ( $p > 0.1$ ), Point B: Increased from 12.50 mg/kg to  $15 \pm 2.41$  mg/kg ( $p > 0.1$ ) which was not significant, Point C: Reduced drastically from 392.50 mg/kg to  $49.42 \pm 15.04$  mg/kg ( $t(4) = 39.52$ ,  $p < 0.001$ ) which was highly significant. After phytoremediation, Pb concentrations declined slightly at Point A, increased at Point B, and decreased drastically at Point C. These changes are clearly reflected in the MRE values. Point C recorded a very high Pb removal efficiency of 87.41%, confirming substantial lead extraction despite the initial extreme contamination. At Point A, Pb showed a low positive MRE of 2.52%, corresponding to only marginal removal, while Point B exhibited a negative MRE of -20.00%, indicating Pb mobilisation rather than uptake. The negative efficiency at Point B highlights the influence of site-specific soil conditions on Pb behaviour during phytoremediation. In the case of lead (Pb), for which the WHO has set a target value of 85 mg/kg for soil, the results showed a mixed trend after treatment. At point A, Pb decreased slightly. In contrast, points B showed an increase after treatment and point C showed a substantial reduction (Table 1). *Helianthus annuus* has been shown to have strong Pb removal potential, which is also confirmed by Ngabra *et al.*, (2024), who reported efficient lead uptake in similar soil systems. Importantly, all post-treatment values in this study were well below the WHO soil limit of 85 mg/kg, indicating progress in remediating the soil to safer levels.

Furthermore, inoculation with *L. fusiformis* influenced the dynamics of Pb uptake. Without the bacterium, Pb concentrations in the stem and in the root peaked at point C. With inoculation, Pb concentrations in the stem and root showed decrease, indicating a possible immobilization effect in the rhizosphere. At point B, however, the Pb content in the roots increased, indicating that the effect of microbial inoculation could be site dependent (Table 5). Compared to the Pb-tolerant PGPR strains used by Saleem *et al.* (2018), such as *Pseudomonas gessardii* and *P. fluorescens*, *L. fusiformis* showed similar abilities to improve root uptake while limiting translocation to the aerial parts, potentially reducing toxicity risks in harvestable tissues. Lead levels in the initial soil sample (29.6 mg/kg) were also below the WHO target limit of 85 mg/kg. Following inoculation with *L. fusiformis*, soil Pb reduced, while root and stem showed appreciable accumulation. In comparison, the non-inoculated control exhibited slightly higher soil Pb but lower uptake in both roots and stems (Table 5). Although Pb values across all treatments were within WHO permissible limits, inoculation significantly improved Pb uptake and sequestration in plant tissues. This highlights the role of *L. fusiformis* in mobilizing Pb, consistent Ahsan and Shimizu (2021), who emphasized and reported that PGPR mediated siderophore production and organic acid release as mechanisms enhancing Pb solubility and plant availability as been reported in the literature.

The positive control soil sample further support the role of inoculation in improving Pb sequestration. Lead levels in the initial positive control soil were below the WHO target of 85 mg/kg. Following inoculation with *Lysinibacillus fusiformis*, soil Pb in the positive control decreased. By comparison, the non-inoculated control showed slightly higher soil Pb but lower tissue uptake (Table 2). These control data indicate that inoculation enhanced Pb uptake and sequestration in plant tissues, highlighting the capacity of *L. fusiformis* to mobilize Pb consistent with Iyama and Etori, (2020). Additionally, site-dependent responses were observed: at point b the root Pb increased after inoculation, showing that microbial effects can vary with local soil conditions. Compared to Pb tolerant PGPR strains (Saleem *et al.*, 2018), *L. fusiformis* performed similarly by improving



root uptake while limiting translocation to aerial parts, potentially lowering toxicity in harvestable tissues.

The results of this study showed that copper (Cu) concentrations ranged from 25.45 mg/kg at Point B to 55.50 mg/kg at Point C. These results are similar to the WHO/FEPA target value of 36 mg/kg reported by Iyama and Edori, (2020). The Cu levels at Point A and Point C exceeded the permissible limit, whereas Point B remained slightly below the threshold (Table 1). The findings of this study are also similar to those of Abbaszadeh-Dahaji *et al.* (2021) that copper is essential in trace amounts for plant growth, the results of this study align with previous reports that high concentrations beyond safe limits become phytotoxic, causing oxidative stress and reducing microbial diversity.

The effectiveness of *Lysinibacillus fusiformis* in supporting the phytoremediation of copper (Cu) by *Helianthus annuus* at three polluted sites in New Panteka was evident. After treatment, a significant reduction in soil Cu concentrations was observed at point A, point B and point C. All values were below the WHO guideline value of 36 mg/kg. This decrease was statistically significant ( $t(4) = 8.73$ ,  $p = 0.001$ ). Significant Cu reduction was also observed at Points A ( $t(4) = 7.51$ ,  $p = 0.002$ ) and B ( $t(4) = 4.85$ ,  $p = 0.008$ ), confirming that Cu was consistently remediated across all inoculated sites.

The effectiveness of Cu removal is strongly supported by the MRE values. Point C recorded a high Cu removal efficiency of 77.98%, reflecting substantial extraction from Cu-rich soil. Point A and Point B showed positive MRE values of 30.80% and 20.94%, respectively, confirming consistent Cu removal across all sites. These efficiencies correspond with the statistically significant reductions observed after treatment and indicate that copper was the most efficiently remediated metal in this study. These results are consistent with, Emolade *et al.*, (2025) who observed higher Cu reduction than for Cd or Pb in similar environments, and with Ahsan and Shimizu, (2021)., who has been reported that metabolic versatility of *Lysinibacillus fusiformis* including its production of siderophores and organic acids as key to improving metal solubility and uptake in phytoremediation. While the EDTA enhanced PGPRs (*Pseudomonas cedrina* and

*Stenotrophomonas* sp.) used in Abbaszadeh-dahaji *et al.*, (2019) achieved higher Cu accumulation in the plant shoots, the use of *L. fusiformis* without synthetic chelators in this study still resulted in effective soil remediation.

The positive control values for Cu further illustrate inoculation effects, the initial positive control soil Cu was well below the WHO threshold (Table 2). After inoculation, soil Cu in the positive control substantially decreased, with root and stem respectively. Without inoculation, soil Cu remained slightly higher and uptake favored the stem over the root (Table 6). These control data suggest that *L. fusiformis* modulates Cu distribution between root and stem, which can reduce translocation stress and improve plant tolerance, supporting findings by Pradhan *et al.*, (2014) and Ahsan and Shimizu, (2021). The combined field and control data indicate effective Cu remediation where microbial inoculation was applied.

The relatively large standard deviations observed for some metals, particularly copper at Point C, reflect inherent spatial heterogeneity and dynamic metal redistribution in field-contaminated soils rather than poor experimental control. Automobile workshop soils are characteristically heterogeneous due to uneven deposition of metal-containing wastes, lubricants, and particulate matter. In addition, phytoremediation and microbial activity can enhance metal mobilisation, resulting in variable metal concentrations within the rhizosphere. All analytical measurements were conducted under controlled laboratory conditions with appropriate quality assurance procedures, indicating that the observed variability primarily represents environmental heterogeneity rather than analytical uncertainty. Similar levels of variability have been reported in field-based phytoremediation studies conducted in mechanically impacted soils (Emolade *et al.*, 2025; Alloway, 2013).

## CONCLUSION

This study showed that inoculation of *Helianthus annuus* with *Lysinibacillus fusiformis* significantly improved the remediation of soils contaminated with heavy metals in New Panteka. Copper showed the most effective reduction, dropping from 55.5 mg/kg to  $12.22 \pm 8.57$  mg/kg, well below the WHO/FEPA limit of 36 mg/kg, with the roots accumulating more Cu than the stems. Lead

also decreased significantly, especially at point C from 392.5 mg/kg to  $49.42 \pm 15.04$  mg/kg, bringing soil Pb levels below the WHO guideline value of 85 mg/kg, although statistical analyses suggest that this is largely due to the high initial contamination. Cadmium remediation was limited and site-dependent, with post-treatment concentrations remaining above regulatory thresholds at all sampling points and significant reduction observed at only one location. This indicates that, unlike Pb and Cu, Cd exhibited greater persistence and mobility, and was not effectively remediated by the treatment applied. Positive control experiments confirmed that *L. fusiformis* enhanced metal uptake and distribution within plant tissues; however, improved uptake did not consistently translate into soil Cd reduction. Integration of *L. fusiformis* with *H. annuus* significantly increased Cu and Pb removal and improved plant metal interactions, supporting its potential as an affordable and sustainable bioaugmentation strategy for urban heavy metal polluted soils, although additional measures are required to address persistent Cd accumulation.

#### Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research work. All authors contributed to the study purely for academic and scientific purposes without any financial, personal, or professional conflicts influencing the outcomes of the research.

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