The Effects of Plant Growth-promoting Bacteria on Lead Uptake by Chromolaena Odorata (Siam Weed)

Elizabeth Temitope. Alori (aloritope@yahoo.com)
Landmark University
Faith Oyerei Dimowo
Landmark University
Faridat Temilomo. Olaniyan
Landmark University
Aruna Olasekan Adekiya
Landmark University
Bisola Bolajoko Ayorinde
Landmark University
Fisayo Yemisi Daramola
Cape Peninsula University of Technology Wellington Campus
Olubukola Oluranti Babalola
North-West University

Article

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Abstract

A study was designed to access the phytoremediation potential of microbial-assisted *Chromolaena odorata* of lead (Pb). The pots experiment was carried out in a Factoria experiment in a Completely Randomized Design, with nine treatments and three replications. Viable seeds of *Chromoleana odorata* were planted in 10kg of soil placed in each plastic pot having 10ppm, 20ppm, and 30ppm of Pb, respectively. Seedlings were inoculated with either *Pseudomonas aeruginosa* or *Bacillus subtilis.* The study was carried out for 12 weeks under natural conditions. Physiochemical properties of the soil were determined using standard methods. The results revealed that the microbes (*Pseudomonas aeruginosa* and *Bacillus subtilis*) used showed the potential for remediating lead. However, *C. odorata* inoculated with *Bacillus subtilis* remediated lead (Pb) was significantly higher than that inoculated with *Pseudomonas aeruginosa* at 10 weeks after planting. The levels of Pb in the roots and shoot of *C. odorata* after 12 weeks ($B_{10,}$ 0.22) showed that a more bioavailable pool of Pb was translocated from the root to leaves and stem in the *Bacillus subtilis* assisted C. *odorata* compared with other treatments. The results suggested that *C. odorata* has phytoextraction ability and could restore soil polluted with Pb. *Bacillus subtilis* assisted *C. odorata to* extract Pb via phytoaccumulation in shoot while *Pseudomonas aeruginosa* assisted *C. odorata* in removing Pb via phytostabilization. It also suggested that plant growth-promoting bacteria helped the uptake of lead from the soil.

Introduction

Heavy metals, especially lead (Pb), are significant pollutants that endanger the environment and human and animal health. Heavy metal contamination of the soil has existed for decades. Still, its scope has grown in the last 60 years due to technological advances and increased consumer use of products containing these metals [1]. Any metallic chemical element with a relatively high density that is toxic or poisonous at low concentrations is heavy metal. Mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead are examples of heavy metals [2]. These heavy metals are natural and cannot be lost or degraded [2]. They get into our bodies in small amounts through food, drinking water, and breathing. Heavy metals (e.g., copper, selenium, and zinc) are needed as trace elements to keep the human body's metabolism running smoothly. They can, however, cause poisoning at higher concentrations [3]. Lead is a heavy metal with high toxicity and harmful effects on human health. Due to its widespread use, lead poisoning is still a public health issue of concern, particularly in developing countries [4].

Pead is an industrial metal that has become widespread in soil, air, food, and water [5]. Sources of lead poisoning in human include; consumption of lead-contaminated food and water; industrial production such as metallurgy, mining, battery manufacture, and recycling; use of; leaded gasoline, lead paint, electronic waste, and some lead contaminated traditional medicines [6]. Also as revealed by [7] some of the anthropogenic sources of lead in agricultural soil include herbicides and insecticides. These pollutants affect and alter the chemical and biological properties of soil [8].

The conventional method of remediating heavy metal pollution such as excavation will transfer the pollutant to a new location where they must be monitored; this method also spreads polluted soil and dust particles during the removal and transport of contaminated soil, and the relatively high cost is equally a disadvantage [9]. The chemical method of stabilizing heavy metals in situ is also costly [8]. Hence there is a need for an environmental and economically friendly remediation process.

Bioremediation uses living organisms such as microorganisms and green plants to treat soil contaminated with toxic materials [4]. Bioremediation is a method for extracting/converting hazardous contaminants such as heavy metals into less harmful substances and/or removing radioactive elements from polluted environments; or decaying organic substances and eventual mineralization of organic substances into carbon dioxide, water, nitrogen gas, and other gases, using dead or living biomass [4].

Phytoremediation is a technique that involves using specialized green plants and eliminating, sequester, killing, or minimizing the concentration or toxic effects of pollutants in contaminated environments, especially soil and water [10].

Microorganisms are found worldwide and play a critical role in the biogeochemical transformations of metals between soluble and insoluble species [11]. Interactions between metals and microbes may be beneficial or harmful [12]. Plant growth-promoting bacteria (PGPB) can improve plant growth and protect plants from disease and abiotic stresses through various mechanisms

[13]. Bacteria that form close relationships with plants, such as endophytes, may be more effective in promoting plant growth. In addition, bacterial inoculants can help boost agronomic productivity by lowering production costs and pollution levels[14].

Chromolaena odorata (Siam weed), was chosen for this research due to its invasiveness and survival in almost all soils, as well as its apparent non-vegetative use in Nigeria. Heavy metals have been found in higher concentrations in some parts of the derived savannah. This is due to practices like mining, vehicle emissions, and agricultural inputs like fertilizers, herbicides, and pesticides, among others [6]. The soils in these areas become unfit for agricultural use because of the poisonous nature of these heavy metals when taken up by plants and eaten by livestock [15]. In addition, some soils in Nigeria's derived savannah agro-ecological zone are low-yielding due to their low fertility status, which can be related to high heavy metals content due to industrial activities in the areas in which those soils exist.

[16] studied the Phytoremediation Potential of *Chromolaena odorata* (L.) King and Robinson (Asteraceae) and *Sida acuta* Burm. f. (Malvaceae) Grown in lead-Polluted Soils. [17], assessed the phytoremediation potential of *Chromolaena odorata*, *Impatiens patula*, and *Gynura pseudochina* grown in cadmium-polluted soils while [18], studied effect of bacterial inoculation of strains of Pseudomonas aeruginosa, Alcaligenes feacalis and Bacillus subtilis on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of Brassica juncea.. There is, however minor, or no study on the possibility of *C. odorata* assisted with microbial inoculant in the remediation of lead polluted soil in the derived savannah of Nigeria. On the other hand, *C. odorata* (Siam weed**)** helped with *Pseudomonas*, and *Bacillus* spp inoculants promise to be a cost-effective, environmentally friendly, and sustainable clean-up technology for environmental contaminants.

Therefore, this study was conducted to assess the potential of C. odorata inoculated with *Pseudomonas* and *Bacillus* spp. to remediate soils polluted with lead and assess the effect of inoculation with *Pseudomonas* and *Bacillus* spp. on the growth of *C. odorata*.

Results And Discussion

INITIAL SOIL CHARACTERISTICS

Table 1 shows the physicochemical properties of the soil before planting. The pH of the soil was strongly acidic, with a textural class of sandy loam. The exchangeable K was moderate, while the exchangeable bases, Na, Ca, Mg are all suitable.

Table 1: Soil physical and chemical properties prior planting (0-15 cm)

Parameter	Values
Sand (%)	79.56
Silt (%)	8.00
Clay (%)	12.44
Textural Class	Sandy Loam
pH (H_20)	4.86
Organic Carbon (mg/kg)	1.95
Exchangeable Bases	
K (cmol/kg)	0.12
Ca (cmol/kg)	2.55
Mg (cmol/kg)	1.60

EFFECT OF MICROBES ON PLANT GROWTH

Table 2 showed the vegetative parameters collected at Week 4, 6, 8, and 10, respectively. It also shows the biomass of the plant, that is, the length and weight of the plant parts after harvest. It revealed that the plants inoculated with *Bacillus* had higher plant height and the number of leaves than *Pseudomonas aeruginosa* inoculated plants. *C. odorata* not inoculated with microbes had less shoot and root weight. The *Bacillus subtilis* assisted *C. odorata* had higher shoot and root weight than *C. odorata* assisted by *Pseudomonas aeruginosa*. The microbial assisted C. *odorata* has a higher root length, which may be responsible for lesser lead content in the soil compared to the non-microbial assisted C. *odorata*.

Table 2: Effect of *Bacillus* and *Pseudomonas* on growth of *C.odorata*

Treatment	PH Week 4	PH Week 6	PH Week 8	PH Week 10	NOL Week 4	NOL Week 6	NOL Week 8	NOL Week 10	Root Length	Root Weight	Shoot Weight
B_{10}	29.67^{h}	36.35^{c}	49.60^{f}	58.47^{e}	18.67^{f}	27.00^{f}	69.00^{i}	145.67^{i}	47.00^{g}	17.12^{h}	47.29^{i}
B_{20}	25.67^{g}	35.47^{c}	48.16^{f}	58.33^{e}	18.67^{f}	26.00^{e}	60.00^{h}	113.00^{h}	46.83^{g}	16.59^{g}	46.83^{h}
B_{30}	25.00^{fg}	33.87^{c}	44.33^{e}	52.00^{d}	16.33^{e}	25.33^{b}	58.00^{g}	108.00^{g}	44.83^{f}	15.77^{f}	42.78^{g}
P_{10}	24.00^{ef}	32.13^{cd}	43.34^{e}	51.73^{d}	13.00^{c}	23.67^{d}	45.50^{f}	92.00^{f}	43.17^{e}	14.08^{e}	39.17^{f}
P_{20}	23.33^{e}	36.35^{c}	38.68^{d}	51.47^{d}	13.00^{c}	19.33^{c}	42.00^{e}	84.00^{e}	38.90^{d}	13.30^{d}	36.41^{e}
P_{30}	19.67^{d}	27.31^{ab}	38.28^{d}	47.50^{c}	12.00^{b}	19.00^{c}	41.00^{d}	82.00^{d}	37.67^{c}	13.21^{d}	35.90^{d}
N_{10}	17.33^{c}	26.72^{ab}	35.78^{c}	46.06^{b}	10.33^{a}	18.67^{c}	33.00^{c}	80.00^{c}	36.00^{b}	10.97^{c}	32.30^{c}
N_{20}	15.33^{b}	25.34^{ab}	30.69^{b}	45.67^{b}	10.67^{a}	15.33^{b}	32.00^{b}	66.00^{b}	35.67^{b}	9.60^{b}	28.16^{b}
N_{30}	13.33^{a}	22.88^{a}	29.01^{a}	43.00^{a}	10.00^{a}	13.33^{a}	29.00^{a}	59.00^{a}	33.93^{a}	9.14^{a}	27.81^{a}

(Means in a column under any given treatment followed by the same letter(s) do not differ significantly at 0.05

level of probability using the Duncan Multiple Range Test (DMRT).

PH stands for Plant Height.

NOL stands for Number of Leaves.

B represents C.*odorata* inoculated with *Bacillus subtilis*.

P represents C.*odorata* inoculated with *Pseudomonas aeruginosa*.

N represents the non-inoculated C.*odorata*.

LEAD CONTENT OF THE SHOOT, ROOT AND SOIL 12 WEEKS AFTER PLANTING

Table 3 indicated that the treatments with no microbes had higher levels of lead concentration in the soil. Increased soil lead concentration resulted in increased lead concentration in shoot and root. *Pseudomonas*-assisted *C. odorata* soil had a significantly higher lead concentration than *Bacillus*-assisted *C. odorata* soil. The non-assisted *C. odorata* had significantly less concentration of lead in its shoots and roots compared to the microbes assisted *Chromolaena*. The concentration of extractable lead in *Bacillus subtilis* assisted *C. odorata* decreased between 77 %-86 %, while *Pseaudomonas aeruginosa* helped *C. odorata* decreased between 60 %-77 %. *Bacillus* assisted *C. odorata* extracted more lead from the soil, followed by *Pseudomonas*-assisted *C. odorata*

Table 3: Lead content of the shoot, root and soil 12 weeks after planting

Treatment	Shoot	Root	Soil
B_{10}	0.22^{c}	0.21^{c}	0.02^{a}
B_{20}	0.23^{c}	0.22^{cd}	0.02^{a}
B_{30}	0.26^{d}	0.23^{d}	0.04^{b}
P_{10}	0.23^{c}	0.22^{cd}	0.03^{a}
P_{20}	0.23^{c}	0.22^{cd}	0.06^{c}
P_{30}	0.23^{c}	0.22^{cd}	0.08^{d}
N_{10}	0.13^{a}	0.15^{a}	0.13^{e}
N_{20}	0.14^{a}	0.17^{ab}	0.15^{f}
N_{30}	0.15^{b}	0.18^{b}	0.18^{g}

(Means in a column under any given treatment followed by the same letter (s) do not differ significantly at 0.05 level of probability using the Duncan Multiple Range Test (DMRT).

B represents *C. odorata* inoculated with *Bacillus subtilis*.

P represents *C. odorata* inoculated with *Pseudomonas aeruginosa*.

N represents the non-inoculated *C. odorata*.

Bio-concentration factor (BCF) and Translocation Factor (TF)

This study assessed the ability of *C. odorata* inoculated with microbial inoculants to accumulate metals from contaminated soil by BCF in accordance to Yadav et al. (2009) as reported in table 4. Highest bio concentration factor (11.5) for the shoots were recorded in *C. odorata* inoculated with *Bacillus subtilis*. The shoot BCF of *C. odorata* inoculated with *Pseudomonas spp* ranges from 2.9-7.6 while the un-inoculated *C. odorata* were 0.8-1.0 (Table 4). The highest BCF of root and whole plant (11 and 22.5 respectively) was also recorded in *C. odorata* inoculated with *Bacillus subtilis*. BCF of whole *C. odorata* augmented with *Bacillus subtilis* increased by 85%-91% while the BCF of whole *C. odorata* augmented with *Pseudomonas aeruginosa* increased by 68%-86%. It also revealed that the highest TF (1.12) was recorded in *C. odorata* inoculated with *Bacillus subtilis* while the least 0.8 was recorded in *C. odorata* that was not augmented by microbial inoculant.

Table 4: Bio concentration and translocation factor

Treatment	BCF Shoot	BCF Root	BCF Whole plant	TF
B10	11	10.5	21.5	1.05
B20	11.5	11	22.5	1.05
B30	6.5	5.8	12.3	1.12
P10	7.6	7.3	15	1.05
P20	3.8	3.7	7.5	1.05
P30	2.9	2.8	5.6	1.05
N10	1	1.2	2.15	0.83
N20	0.9	1.1	2.	0.83
N30	0.8	1	1.8	0.8

B represents *C. odorata* inoculated with *Bacillus subtilis*.

P represents *C. odorata* inoculated with *Pseudomonas aeruginosa*.

N represents the non-inoculated *C. odorata*.

Discussion

The uptake of metal is highly influenced by soil factors such as cation exchange capacity, organic matter content, and pH. Plant species, cultivation, and plant ageare other factors that also play important role in the uptake of heavy metal. The solubility and hydrolysis of metal hydroxides, phosphates, and carbonates are largely dependent on soil pH. Morealso soil pH controls ion-pair formation and solubility of organic matter, as well as the surface charge of Mn, Fe, and Al-oxides, clay edges, and organic matter.

For instance, soils with high; pH value, clay, and organic matter content usually have low mobility and availability of heavy metals[19].

This result gotten is in line with that of [20] whose data showed that the B. *subtilis* and B. *amyloliquefaciens* shortened emergence time, increased plant growth, produced fewer but larger tubers, and increased potato tuber yield over control treatment. [18] reported an improved growth in microbial inoculated plant compare to the un-inoculated plant. Other researchers stated that the increase in plant growth as a result of bacterial inoculation was due to production of plant growth substances such as IAA that promote plant root elongation and shoot growth and the solubilization of minerals such as phosphorus [18, 21, 22].

Also, [23] stated that plant growth-promoting bacteria are capable of enhancing the growth of plants and protecting them from diseases and abiotic stress.

The reduction in Pb concentration in the soil by *C. odorata* observed is in line with the study of [16] who stated that C. *odorata* showed accumulative potential for lead hence, reducing lead concentration in soil. Significantly lesser Pb concentration in polluted soil treated with *C. odorata* inoculated with *Pseudomonas* and *Bacillus* spp. implies that the microbial inoculants enhanced the remediation potential of *C. odorata*. This agrees with [24], which reported that *Pseudomonas aeruginosa* shows potential for bioremediation. This may be due to enhanced root length and weight as recorded in Table 2.

More also, [25] stated that plants in association with microbial inoculants, can remove or transform contaminants into harmless substances. *Bacillus subtilis* have the ability to translocate Pd from root to upper part of plant (0.22-0.26) than *Pseaudomonas aeruginosa* (0.23). The results show that *C. odorata* inoculated with *Bacillus subtilis* and *Pseaudomonas aeruginosa* removed larger amount of Pb to the shoot biomass because higher concentration was recorded in shoot compared to the root. According to [26], the phytoextraction process is determined by ability of the shoots to remove heavy metals. This implies that plant species having a higher metal concentration in its shoots than in its roots can be considered as an accumulator for phytoremediation.

This study assumed that plants with BCF value > 1 are accumulators while plants with BCF <1 are excluders [16]. The results in this study shows that remediating Pb polluted soil with *C. odorata* inoculated with *Bacillus subtilis* and *C. odorata* inoculated with *Pseudomonas aeruginosa* had BCF values > 1, indicating that this plant when inoculated with these species of *Bacillus and Pseudomonas,* have the potential to be used as accumulators of lead. The un-inoculated *C. odorata* also had

values > 1. This also implies that *C. odorata* have the potential remediate Pb in soil polluted with Pb by process of bio-accumulation. This agrees with previous research by [16] that reported that *C. odorata* accumulates significant concentration of Pb from soil polluted with Pb

The TF of un inoculated *C. odorata* in this study were < 1, which reveals that these restricted the Pb transfer from their roots to the shoots. [27] also reported TFs of less < 1 in lead accumulation by *C. odorata*.

Conclusion

This study confirmed that *C. odorata* may be used as a phytoremediator of soils polluted with lead. *C. odorata* will remediate lead polluted soil significantly higher when assisted with plant growth-promoting bacteria such as *Bacillus subitilis* and *Pseudomonas aeruginosa. C. odorata* remediates lead polluted soil via phytoextraction techniques by accumulation. *Bacillus subtilis* and *Pseudomonas aeruginosa* improve plant growth considerably. It was also recorded that *Bacillus subtilis* improves *C. odorata* growth more and *C. odorata* inoculated with *Bacillus subtilis* extracts more Pb from soil polluted with Pb than *C. odorata* inoculated with *Pseudomonas aeruginosa*

Materials And Methods

Description of the Experimental Site

This potted experiment was conducted at the Teaching and Research Farm, Landmark University, Omu-Aran Kwara State, Nigeria, located at Latitude 8° 9'N and Longitude 5° 61'E.

Soil Collection

The soil was obtained from the screen house, Teaching and Research Farm, Landmark University from the top 0 – 15 cm depth. The soil samples were air-dried for 5 days and sieved using 2 mm mesh gauze to remove debris and stones. The sieved soil was sterilized by potting soil mixed with a bit of water into the electrical soil sterilizer and then sterilized for one hour. The soil is then poured out and bagged to weigh 10 kg per pot.

Sources of Materials

Siam seeds were gotten from around the Teaching and Research farm. The Bacteria isolates (*Pseudomonas and Bacillus spp.*) were obtained from the Entomology lab, Federal Institute of Industrial Research Oshodi (FIIRO), Oshodi, Lagos State, Nigeria. Lead nitrate was obtained from the Chemistry laboratory, Landmark University Omu-Aran, Kwara State Nigeria.

Experimental design and soil treatment application

The experiment was a 3×3 factorial laid out in a Completely Randomized Design (CRD), and each treatment was replicated three times. This consist of soil was polluted at three different levels (10ppm, 20ppm, and 30ppm) and three (3) microbial inoculant (*Pseudomonas and Bacillus* spp and Non-inoculant). Pollutant was obtained by preparing a stock solution of 1M lead nitrate, and the 10ppm, 20ppm, and 30ppm concentrations were obtained from this stock solution. Each bag of soil was thoroughly mixed in the lead nitrate as expected

Microbial Inoculant Preparation

The bacterial isolates (*Pseudomonas and Bacillus subtilis*) were cultured for 48 hrs in Luria-Bertani Broth (LB) medium, and then cells were harvested by centrifugation at 12000g for 10 minutes. Each of the *Pseudomona aeruginosa* and *Bacillus subtilis* was suspended in sterile distilled water, and the concentration was adjusted to give 10^8cells/ml

Seed sowing and plant inoculation

All methods were performed in accordance with the relevant guidelines and regulations. Siam seeds were sown one week after the pollution of the soil. Planting was done at 3 seeds per bag. Twenty mls (20 mls) of the prepared microbial inoculants *(Pseudomonas and Bacillus spp.)* were then introduced into the soil at the plant root two weeks after planting.

Preparation of plant samples for analysis

Methods were performed in accordance with the relevant guidelines and regulations. Twelve (12) weeks after planting, the plant were harvested and carefully rinsed under running tap water to remove clogged soil particles. Water droplets were removed from the plant roots using blotting papers. Then, the plant samples were separated into roots and shoots.

Determination of heavy metal (Pb) in Soil

The soil samples were collected twelve (12) weeks after planting. 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 content of the soil was determined using Atomic Absorption Spectrometer (AAS) (Model number: AA320N).

Determination of Pb in plant material

All methods followed the relevant guidelines and regulations. [28] acid digestion method was employed to digest grounded plant samples (shoot and root). One g of the plant materia was weighed into a beaker of 50 ml capacity. Then 10 ml mixture of analytical grade acids: NO3;H2SO4; HClO4 in the ratio 1:1:1. Was added. 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, another 10 ml of the acid mixture was added. This mixture was 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. Lead concentrations in the samples were analyzed with AAS (Model number: AA320N)

Determination of Physical and Chemical Properties of Soil of the Study Site.

As shown in table1, the physical and chemical properties measured were; Soil pH using Kent pH meter model 7020, organic matter content using the wet oxidation method as described by [29]. The hydrometer method of [30] was employed to determine particle size. Total nitrogen was estimated by the macro Kjel-dahl method [31], available phosphorus (P) was determined by Bray-1 extraction method [32], and to determine ECEC, the method of [33] was employed.

Determination of bio concentration and translocation factor

Bio-concentration factor (BCF) and translocation factor (TF) were calculated using the formula of [34] and [35] as

$$\text{Shoot bio concentration factor (BCF)} = \frac{\text{Concentration of heavy metal in shoot (mg/kg)}}{\text{Concentration of heavy metal in soil (mg/kg)}}$$

$$\text{Root bio concentration factor (BCF)} = \frac{\text{Concentration of heavy metal in root (mg/kg)}}{\text{Concentration of heavy metal in soil (mg/kg)}}$$

$$\text{Whole plant bio-concentration factor (BCF)} = \frac{\text{Conc. of heavy metal in whole plant (mg/kg)}}{\text{Concentration of heavy metal in soil (mg/kg)}}$$

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

C aerial = Metal concentration in the aerial part of the plant (shoot).

C root = Metal concentration in the root of the plant.

Statistical analysis

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

Declarations

Authors Contributions

ETA conceptualize the research, supervised the experiment and wrote the the manuscript. FOD, FTO and BBA carried out the experiment. AOA and FYD did the statistical analysis and interpretation while OOB supervised and reviewed the manuscript

Availability of data

All data generated from this research are included in this published article

Conflicts of Interest

There are no conflicts of interest among authors.

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