© JAN 2026 | IRE Journals | Volume 9 Issue 7 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV9I7-1713736

Amended Treatment of Pb-Polluted Soil using Animal Dung Composites and *Micrococcus* sp.: Evaluating Their Effects on Maize Seedlings Growth

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Abstract-* Here, amended bioremediation of Pb-polluted soil with poultry waste, cow dung and their consortium, in addition to Micrococcus sp., used as plant growth-promoting rhizobacteria (PGPR), was carried out, and their effects on maize seedlings growth evaluated. After 25 days period of treatment of Pb-polluted soil and germination of corn seeds planted on them, growth parameters including height of shoot, and number of leaves were recorded on day 4, weeks 2, 4 and 7. Results revealed an increase in the pH, organic carbon content and soil organic matter content of the samples soil after treatment. Treatment with cow dung alone produced significantly highest alkalinity of 12.18% at 33.75 mg/kg, 14.18% at 44.02 mg/kg, and 10.05% at 52.55 mg/kg Pb pollution. The N and P contents of all treated soil samples were significantly higher than those of control samples. There was increment in the dehydrogenase activity in all treated Pb-polluted soil samples. The chlorophyll contents of seedlings grown on treated samples were more than those of seedlings on control samples. The seedlings on soil treated with cow dung only, produced the highest total chlorophyll contents of 1.06±0.07 mg/g at 32.75 mg/kg, 0.71±0.02 mg/g at 42.02 mg/kg and 0.68±0.09 mg/g total chlorophyll at 52.55 mg/kg levels of Pb pollution. The range of Pb bioaccumulation in maize leaf was 1.26±0.07 mg/kg to 3.8±0.05 mg/kg; 0.78±0.04 mg/kg to 1.84±0.1 mg/kg in the stem and 4.34±0.07 mg/kg to 8.7±0.15 mg/kg in root samples. These results prove that the amendments used in the study enhanced the seedlings growth.

***Keywords:* Micrococcus Sp., Bioremediation, Toxic Metals, Pollution, Plant Growth**

I. INTRODUCTION

The heavy metal pollution of soil has become a major problem due to the growing number of affected places worldwide, and the negative consequences of metals on the ecosystem and human health (Alves et al., 2022). The growing industrialization and urbanization have resulted in increased ore extraction and processing for a variety of purposes. A heavy metal is defined as one with a specific weight more than 5 g.cm^{-3} (Anuforo et al., 2020a). As a result, the concentrations of metal(loids) in soils increase year after year, posing a significant risk to human health and the environment, due to the high toxicity of certain of these elements. The anthropogenic causes include the use of agrochemicals such as herbicides, fertilizers, and insecticides. Other are sewage sludge, mine spoil runoff, domestic and industrial wastewater, and polluted water for agricultural irrigation. Similarly, atmospheric deposition of metal(loids) from dusts and aerosols released by mining and smelting, cement production, e-waste processing, fossil-fuel power plants, waste incineration, and vehicle use is a major soil metal pollution pathway (Anuforo et al., 2019; Korie et al., 2024a). Due to their high toxicity and nonbiodegradability, it is vital to avoid future metal pollution and restore the hundreds of damaged sites worldwide.

Plants survival in substantially metal-polluted soils is sometimes limited to a small group of plants called metallophytes, putting overall plant health at risk (Alves et al., 2022). On entry of heavy metals (HMs) and trace element like aluminum (Al), arsenic (As), cadmium (Cd), beryllium, mercury, chromium, lead, iron, zinc, copper, nickel, and thallium in the environment, they reduce food quality, disrupt the food chain, and endanger human health. They interfere with the functioning of critical biological components. This contamination affects production and food quality while also reducing cultivable and fertile farming lands (Riseh et al., 2023).

Conventional reclamation using physical and chemical approaches is costly, time-consuming, affects soil characteristics, and disrupts the soil microbiome. Furthermore, chemical treatment may cause negative side effects (Raffa et al., 2021). As a result, microbe-assisted phytoremediation has gained substantial traction due to its environmental friendliness, cost-effectiveness, and recent breakthroughs. Microorganisms are fundamental components of natural ecosystems and play an important role in their restoration. Indeed, plant-microbe interactions in metal-polluted soils are critical for plants to survive metal toxicity and grow in these severe conditions (Alves et al., 2022). Plant-growth-promoting rhizobacteria (PGPR) are microorganisms that live in the rhizosphere that can help plants develop and produce more crops. PGPRs have the potential to alter heavy metal bioavailability in the rhizosphere microenvironment, increase uptake of heavy metal by phytoremediation plants, and improve phytoremediation efficacy in heavy metal-contaminated soils (Qin et al., 2024). *Azotobacter, Arthrobacter, Azospirillum, Bacillus, Burkholderia, Klebsiella, Pseudomonas, Enterobacter, Xanthomonas, Streptomyces* and *Serratia* are among the implicated PGPR genera. Metal removal and detoxification by microbes involve processes such as adsorption, transformation, chelation, precipitation, and so on (Qin et al., 2024).

Micrococcus belongs to a genus of bacteria from the *Micrococcaceae* family. They live in a variety of habitats, including water, dust, and soil. *Micrococci* have gram-positive spherical cells that range in diameter from 0.5 to 3 μm and are often clustered together. Some *Micrococcus* strains can degrade hydrocarbons and waxes, and can flourish in settings with high salt concentrations and minimal water. *Micrococcus* can use a wide variety of uncommon substrates, including herbicides, pyridine, chlorinated biphenyls, and oil. They are most likely engaged in the detoxification or biodegradation of numerous additional environmental contaminants (Santhini et al., 2009).

According to researches, organic wastes are effective at changing the physical and chemical properties of soil, releasing nutrients for a longer period of time, and aiding in the rehabilitation of contaminated soils (Adebiyi and Salami, 2023). Consequently, understanding these plant-microbe relationships in the presence of organic matter is crucial not only for the reclamation of metal-contaminated soils, but also for improving plant development on heavy metal-polluted soils. Thus, the goal of this work is to improve the bioremediation of Pb-polluted soil by combining cow dung and poultry wastes with *Micrococcus* sp. as PGPR and evaluating their impacts on maize seedling growth.

II. MATERIAL AND METHODS

Collection and Processing of Soil and Animal Waste Samples

Using well cleaned soil auger, samples were taken from at depth of 0-30 cm of fertile soil at the Federal University of Technology, Owerri (FUTO), Nigeria, and packaged in surface-sterilized plastic bags. The obtained soil was dried, ground and sieved through 0.25 mm mesh sieve before conducting some selected physicochemical analyses. Additionally, cow dung (A1) and poultry wastes (A2) were collected from the Poultry Unit of FUTO Farms, dried, pulverized and stored in surface-sterilized nylon container.

Isolation, Identification and Standardization of Isolate

Micrococcus sp, used as PGPR, was isolated by inoculating 0.1 mL of 10^{-8} serially diluted 1 g of soil sample collected from FUTO farms, on Bushnell-

Hass agar media. The inoculated plates were incubated at 37 °C for 24 h before examination. Suspected colonies with shades of yellow and red pigments were Gram stained. Gram positive cocci arranged in tetrads or irregular clusters and without spores were streaked on freshly prepared nutrient agar slants for additional identification tests. Selected biochemical characterization, such as catalase test, motility test and oxidase test, were carried as described by Santhini et al. (2009). This procedure was also followed to identify bacterial isolates in the soil samples before and after treatments.

Pure culture of identified *Micrococcus* sp. was incubated on a freshly prepared sterile nutrient broth in cotton wool stoppered bottles, and incubated at 37 ºC, for 24 h. This was done to produce sufficient culture of isolate for soil amendment studies. The cells were harvested by centrifugation at 4000 rpm for 10 minutes, washed two times in phosphate buffered saline at pH of 7.25, and re-suspended in MS medium. Standardization of the culture was done by comparing its turbidity with 0.5 McFarland Standard against a Wickerham card.

Pot Experiment

The determination of effects of amended bioremediation of samples of Pb-polluted soil on growth of maize seedlings was studied. Maize seeds were surface-sterilized using 1% sodium hypochlorite, then with 70% ethanol, and rinsed three times in distilled water. Then 50 kg of the processed soil was weighed out in triplicates and each was separately polluted with 100 mL of 0.079M, 0.101M and 0.127M lead acetate. The polluted soil was homogenized for uniformity and then allowed for 2 days. The pot experiment was organized according to the following amendments;

(1) Cow dung only (A1),
(2) Poultry waste only (A2),
(3) Composite of poultry waste and cow dung (A1A2), and
(4) Control (absence of amendment) (C1).

Each pot contained a total of 5 kg of Pb-polluted soil sample and 10 g of organic amendments or their composite. Then the PGPR, *Micrococcus* sp. was applied at rate of 50 mL of standardized culture, per pot. All the experimental units were irrigated with distilled water, with their water contents kept at 70% water holding capacity. Amended bioremediation was allowed to take place for 25 days, before four viable maize seeds were planted in each pot. Each treatment had two replicates. In the greenhouse, there were about 9 h of sunshine on average. Also, the mean temperature (light/dark cycle) and the relative humidity were 32.1 °C and 40%, respectively. Immediately seeds germinated, the seedlings were thinned to 3 per pot. Then selected parameters of growth, including height of shoot, number of leaves were monitored on day 4, weeks 2, 4 and 7. After 7 weeks, the corn plants were harvested and analysis of both plant and soil samples were performed.

Soil Analysis

Physicochemical, minerals and toxic metal analyses

Soil samples were analysed before and after treatments. Soil pH was measured using pH meter (Mettler Toledo Delta 320), while the wet oxidation technique was adopted to determine the organic carbon and soil organic matter contents of the soil samples. Similarly, the concentrations of soil bioavailable calcium, nitrogen, magnesium, phosphorous and potassium were estimated following the method described by Lu *et al.* (2000). Digestion of soil samples for heavy metals analysis was done according to the method reported by Anuforo *et al.,* (2020a). After extraction and filtration, bioavailable Pb content of supernatant from each potted soil sample, was estimated via atomic absorption spectroscopy (AAS).

Dehydrogenase activity

The dehydrogenase activity (DHA) of the soil samples was assayed by incubating the samples with 2,3,5-triphenyltetrazolium chloride following the Thalmann procedures described by Wojewódzki et al. (2022). The triphenylformazane absorbance was measured at 546 nm, and the outcomes were presented in mg TPF kg^{-1} 24 h^{-1}.

Moisture content analysis

The method outlined by Korie et al. (2024b) was used to determine the moisture content of soil samples. After washing, the crucibles were dried. Initial weight of each crucible was recorded as W1. Subsequently, two surface-sterilized crucibles were filled with 5 g of each soil sample and the weight obtained as W2. After drying in oven at 105 ºC to constant weight, it was allowed to cool and weight recorded as W3. The percentage change in moisture content which was represented by the percentage of the difference between the starting weight and the constant weight was calculated using the equation;

$$\text{Moisture content (\%)} = \frac{W3 - W1}{W2 - W1} \times 100$$

Where W1 = initial weight of empty crucible,
W2 = weight of crucible + weight of soil before drying,
W3= final weight of crucible + weight of soil after drying

Plant Analysis

Chlorophyll and Proteins Contents

Leaves, roots and shoots of the seedlings were collected from each treatment, and decontaminated by washing with tap water. Excess water on the surface of the leaves was removed by pressing between the folds of blotting paper, and the leaves were dried in an oven at 48 ºC for 2 h. After complete drying, the samples were ground and used for analysis. The chlorophyll contents of the maize leaves were measured using the SPAD meter after 7 weeks of growth on amended treatment of samples of Pb-polluted soil.

In order to produce the standard curve that was utilized to estimate the unknown protein content, BSA was used as the standard. The extracts of leaf, stem, and root were treated with 4.5 mL of reagent 1 (48 ml of 2% sodium carbonate in 0.1N sodium hydroxide + 1 ml of 1% sodium potassium tartrate + 1 ml of 0.5% copper sulphate) and incubated for 15 minutes, as described by Sarkar et al. (2020). Subsequently, each sample was reacted with 0.5 ml of freshly made reagent 2 (1 part Folin-Ciocalteau: 1 part water) and incubated in the dark for 30 minutes. Then the protein content was calculated by measuring the absorbance at 660 nm and represented as mg BSAE/g of fresh weight.

Toxic metal contents

Digestion of the prepared samples of shoots, roots and leaves of maize seedlings was initially done using di-acid HNO_3:$HClO_4$ mixture for the estimation of Pb content, as described by Anuforo *et al.* (2020b). Then Pb contents of each supernatant were estimated using AAS.

Statistical Computations

For each parameter studied, the mean and standard deviations of the results of duplicate treatments were analysed using Microsoft Excel version 10. The One-way ANOVA, together with the LSD test were applied at ($P < 0.05$) to assess the differences among the datasets using Minitab® 17 application.

III. RESULTS

Effects of Treatments on Physicochemical Properties of Soil

Table 1 shows the effects of amendments with organic supplements and *Micrococcus* sp. as PGPR on the physicochemical properties of Pb-polluted soil samples. It was found that there was increase in the pH, organic carbon (OC) content and soil organic matter (SOM) content of the samples. For pH, treatment with cow dung only, produced significantly highest alkalinity (increase in pH) of 12.18% at 33.75 mg/kg, 14.18% at 42.02 mg/kg, and 10.05% at 52.55 mg/kg Pb pollution. Though not significantly different from other samples except controls, the same sample also yielded the highest increase in OC, with 54.31% at 33.75 mg/kg, 53.88% at 42.02 mg/kg and 66.92% at 52.55 mg/kg Pb pollution. Samples treated with mixtures of cow dung, poultry wastes and *Micrococcus* sp produced highest significant increase in SOM with 124.83% at 33.75 mg/kg, 125.51% at 42.02 mg/kg and 124.63% increase at 52.55 mg/kg Pb pollution.

Though the N and P contents of all treated soil samples were not significantly different from each other, they were significantly higher than those of control samples. While there was increase in percentage Ca contents of all samples, there was reduction in Mg and Pb contents of all samples and they did not significantly differ from each other. Samples treated with poultry wastes and *Micrococcus* sp recorded 16.70% at 33.75 mg/kg, 16.79% at 42.02 mg/kg and 15.85% of K contents at 52.55 mg/kg Pb pollution, which are significantly higher compared to other treated samples and control.

Effects on Dehydrogenase Activity

Figure 1 shows the differences in dehydrogenase activities in samples of Pb-polluted soil following a period of amended bioremediation. From the results, there was increment in the DHA in all treated Pb-polluted soil samples. DHA gradient was highest in samples treated with a composite of poultry wastes and cow dung. Here, DHA was 2.617±0.035 $\mu gTPFg^{-1}h^{-1}$ at 33.75 mg/kg Pb pollution, and 2.033±0.021 $\mu gTPFg^{-1}h^{-1}$ at 52.55 mg/kg Pb pollution. In the control samples, DHA decreased by 0.135±0.012 $\mu gTPFg^{-1}h^{-1}$ at 33.75 mg/kg and 1.104±0.012 $\mu gTPFg^{-1}h^{-1}$ at 52.55 mg/kg Pb pollution. Statistical analysis revealed that DHA produced in samples treated with poultry wastes significantly as well as combination of the organic supplements and *Micrococcus* sp were not significantly different. But they differed from those of other treated samples, as well as control.

Effects on Bacterial Diversity

Table 2 is the bacterial compositions of samples of Pb-polluted soil, bioremediated with a composite of poultry wastes and cow dung, coupled to *Micrococcus* sp. Results showed that there was more diverse bacterial isolates in treated samples than in the initial sample of soil. Also, it was observed that *Salmonella typhi, Staphylococcus aureus* and *Achromobacter* spp which were absent in the original sample, were found in treated samples. On the other hand, the diversity of bacteria found in control samples reduced after the period of the study. Results also revealed that bacterial diversities reduced as the concentrations of Pb pollution increased. Results revealed that samples treated with cow dung produced the most diverse bacterial compositions.

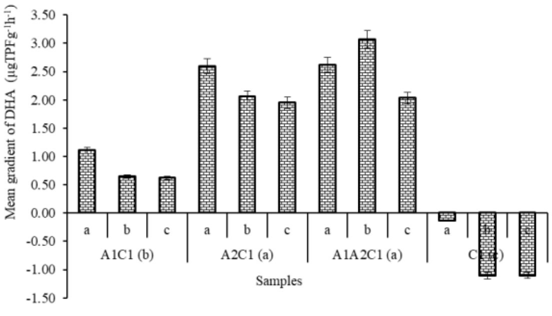

Figure 1: Changes in DHA of Pb-polluted soil samples after treatment. Legends: A1 refers to cow dung, A2 refers to poultry wastes, and C1 refers to Pb. Letters a, b and c represented 32.75 mg/kg, 42.02 mg/kg and 52.55 mg/kg levels of Pb pollution. Means of samples on horizontal bar that do not share a letter (in parenthesis) are significantly different.

Effects on Moisture Content

Figure 2 presents the moisture contents as well as their percentage variations in samples of Pb-polluted soil, at the end of amended bioremediation using composites of poultry wastes, cow dung and *Micrococcus* sp. Results showed that treatment done with poultry wastes in isolation performed better than all other treatment mixtures. It produced 8.22±0.51 (62.35%) at 32.75 mg/kg, 8.58±0.25 (68.32%) at 42.02 mg/kg 42.02 mg/kg and 14.02±0.66 (120.55%) increment in the moisture content at 52.55 mg/kg treated Pb-polluted soil samples. Moisture contents of the samples for control declined after the period of treatment. From the results of analysis, percentage change in moisture content recorded in samples treated with poultry wastes and *Micrococcus* sp only, significantly differed from that of control samples but not for other treated samples. There was no significant difference in percentage change in moisture contents recorded across the concentrations of Pb pollution.

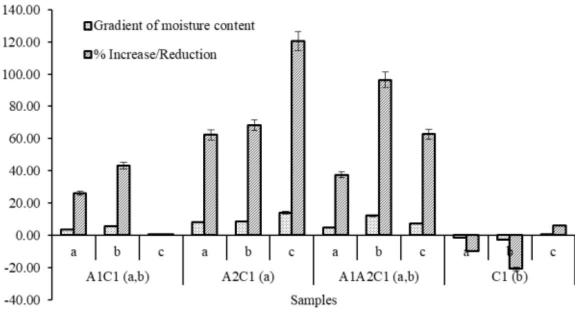

Figure 2: Moisture contents with their percentage changes in samples of Pb-polluted soil after amended bioremediation. Legends: Letters a, b and c represented 32.75 mg/kg, 42.02 mg/kg and 52.55 mg/kg levels of Pb pollution. Means of samples on horizontal bar that do not share a letter (in parenthesis) are significantly different. A1 refers to cow dung, A2 refers to poultry waste, and C1 refers to Pb.

Effects on Plant Growth Rate

On day 4 after germination of maize seedlings on treated samples of Pb-polluted soil, the results showing the number of leaves of corn seedling was between 0.0±0.0 and 2.0±0.0. The results indicated that seedlings grown on soil sample amended with cow dung alone produced more leaves than others, recording 2.0±0.0 at 32.75 mg/kg, 2.0±0.0 at 42.02 mg/kg and 2.0±0.0 leaves at 52.55 mg/kg level of

Pb pollution. The statistical analysis revealed no significant difference (α=0.05) in the numbers of leaves of seedlings grown on treated samples and control samples, except for those on samples treated with poultry dung alone. Considering the heights of seedlings, the average heights of the seedlings ranged from 1.9±0.3 cm to 4.3±0.4 cm, indicating variations among the seedlings studied. The best performed seedlings in heights were those planted on soil samples treated with a combination of cow dung and poultry wastes, recording heights of 4.3±0.4 cm at 32.75 mg/kg, 2.9±0.6 cm at 42.02 mg/kg and 3.7±0.6 cm at 52.55 mg/kg level of Pb pollution. This compared to the least performance recorded in treatment done with poultry wastes, which included 2.7±0.4 cm at 32.75 mg/kg, 1.9±0.4 cm at 42.02

Table 1: Effects of organic supplements with *Micrococcus* sp. on physicochemical properties of Pb polluted soil samples

Heavy metal type	Samples	Heavy metal conc	%Change in pH	%Change in OC	%Change in SOM	%Change in N	%Change in Ca	%Change in K	%Change in Mg	%Change in P	%Change in Pb
Pb (C1)	A_1C_1	A	12.18^{a}	$54.31^{a,1}$	68.10^{b}	68.44^{a}	$6.08^{a,1}$	6.46^{c}	$-18.17^{a,1}$	42.90^{a}	$-45.63^{a,1}$
		B	14.18^{a}	$53.88^{a,1,2}$	68.65^{b}	71.16^{a}	$3.98^{a,1,2}$	5.60^{c}	$-7.21^{a,1,2}$	50.38^{a}	$-40.75^{a,2}$
		C	10.05^{a}	$66.92^{a,2}$	68.10^{b}	61.88^{a}	$-1.49^{a,2}$	6.79^{c}	$-11.57^{a,2}$	48.75^{a}	$-35.08^{a,3}$
	A_2C_1	A	2.13^{b}	$57.26^{a,1}$	70.90^{b}	78.38^{a}	$16.45^{a,1}$	16.70^{a}	$-10.46^{a,1}$	47.25^{a}	$-43.02^{a,1}$
		B	6.16^{b}	$51.17^{a,1,2}$	69.92^{b}	71.16^{a}	$7.95^{a,1,2}$	16.79^{a}	$5.05^{a,1,2}$	55.32^{a}	$-39.54^{a,2}$
		C	8.00^{b}	$75.85^{a,2}$	68.47^{b}	63.84^{a}	$-1.86^{a,2}$	15.85^{a}	$-5.40^{a,2}$	53.98^{a}	$-33.08^{a,3}$
	$A_1A_2C_1$	A	$9.34^{a,b}$	$55.92^{a,1}$	124.83^{a}	66.45^{a}	$8.22^{a,1}$	15.62^{b}	$-15.96^{a,1}$	50.67^{a}	$-48.53^{a,1}$
		B	$12.06^{a,b}$	$55.17^{a,1,2}$	125.51^{a}	63.07^{a}	$3.61^{a,1,2}$	12.31^{b}	$-0.72^{a,1,2}$	55.98^{a}	$-40.65^{a,2}$
		C	$9.11^{a,b}$	$76.18^{a,2}$	124.63^{a}	54.05^{a}	$0.37^{a,2}$	11.32^{b}	$1.54^{a,2}$	57.58^{a}	$-35.70^{a,3}$
	C_1	A	-4.71^{c}	$-27.63^{b,1}$	-44.29^{c}	11.94^{b}	$0.00^{a,1}$	7.00^{c}	$-15.96^{a,1}$	-45.70^{b}	$-40.63^{a,1}$
		B	-5.99^{c}	$-34.87^{b,1,2}$	-44.44^{c}	19.41^{b}	$-1.08^{a,1,2}$	5.04^{c}	$-12.26^{a,1,2}$	-51.44^{b}	$-37.38^{a,2}$
		C	-9.19^{c}	$-33.13^{b,2}$	-44.77^{c}	25.07^{b}	$-2.61^{a,2}$	3.96^{c}	$-20.05^{a,2}$	-54.31^{b}	$-36.33^{a,3}$

Legends: (a) A1 refers to cow dung, C1 refers to Pb, and A2 refers to poultry dung

(b) Letters a, b and c represented 32.75 mg/kg, 42.02 mg/kg and 52.55 mg/kg levels of Pb pollution.

(c) Means in each column of samples that do not share a letter (in superscript) are significantly different.

(d) Means in each column of concentrations (for each sample) that do not share a figure (in superscript) are significantly different

Table 2: Bacterial compositions in samples of Pb-polluted soil before and after amended bioremediations.

Heavy metal type	Samples	Heavy metal conc	*Klebsella* sp	*S. typhi*	*S. aureus*	*Bacillus subtilis*	*Micrococcus* spp	*Pseudomonas aeruginosa*	*Achromobacter* spp.	*E. coli*
Pb (C1)	A_1C_1	A	+	+	+	+	+	+	+	+
		B	+	+	+	+	+	+	+	+
		C	+	+	-	-	-	-	+	-
	A_2C_1	A	+	+	+	-	+	+	+	+
		B	+	-	+	+	+	-	+	+
		C	+	+	-	-	+	+	-	-
	$A_1A_2C_1$	A	+	+	+	-	+	+	+	+
		B	+	-	+	+	+	-	+	+
		C	+	+	-	-	-	+	-	-
	C_1	a	+	-	-	+	+	+	+	+
		b	+	+	+	+	+	-	-	+
		c	+	-	-	-	-	-	-	-
	Original sample		+	-	-	+	+	+	-	+

Legends: A1 refers to cow dung, A2 refers to poultry wastes, and C1 refers to Pb. Letters a, b and c represented 32.75 mg/kg, 42.02 mg/kg and 52.55 mg/kg levels of Pb pollution.

mg/kg and 1.9±0.3 cm at 52.55 mg/kg levels of Pb pollution. Analysis indicated that there is no significant difference (α=0.05) between the heights of seedlings grown on various treated soil samples, as well between the concentrations studied.

On week 2 of growth on treated Pb-polluted soil samples, the number of leaves of seedling recorded was between 1.7±0.5 and 6.3±1.2. This implies that there are variations in the effects of treatments on the number of leaves. Generally, the number of leaves reduced with increasing concentration of Pb pollution. Basically, seedlings grown on soil samples treated with cow dung and poultry wastes composite produced the highest number of leaves, recording 6.3±1.2 at 32.75 mg/kg, 4.7±0.5 at 42.02 mg/kg and 5.3±0.5 leaves at 52.55 mg/kg level of Pb pollution. The control samples recorded 2.7±0.5 at 32.75 mg/kg, 3.7±0.5 at 42.02 mg/kg and 1.7±0.5 leaves at 52.55 mg/kg level of Pb pollution. Only the number of leaves recorded in samples treated with combination of cow dung and poultry wastes is significantly different (α=0.05) from those of other treatments, which are similar to each other. In terms of height of seedlings grown on treated Pb-polluted soil samples, it was between 11.8±0.6 cm and 22.2± 0.6 cm. Again, results indicated that growth in heights of shoots increased with reducing concentration of Pb pollution. Comparing the treated soil samples, heights of seedlings grown on samples amended with poultry wastes and cow dung composites yielded the highest. It recorded heights of 22.2±0.6 cm at 32.75 mg/kg, 18.5±0.7 cm at 42.02 mg/kg and 19.9±0.4 cm at 52.55 mg/kg levels of Pb pollution. In control samples, which recorded the slowest growth among all the samples, the heights were 14.1±0.6 cm at 32.75 mg/kg, 16.3±0.4 cm at 42.02 mg/kg and 11.8±0.6 cm at 52.55 mg/kg levels of Pb pollution. Analysis showed that heights of seedlings on amended with poultry wastes and cow dung composite, as well as isolated use of cow dung were similar, but significantly different (α=0.05) from those of control samples and samples amended with poultry wastes in isolation, which are similar, on the other hand.

On week 4 of growth on treated Pb-polluted soil samples, the average number of leaves recorded from each treatment indicated an average of number of 3.3±0.5 to 9.3±0.5 leaves, hence existence of wide variations among the seedlings. The highest numbers of leaves were recorded in seedlings planted on soil samples amended with poultry wastes and cow dung composites. These produced 9.3±0.5 leaves at 32.75 mg/kg, 7.3±0.5 at 42.02 mg/kg and 9.0±0.8 leaves at 52.55 mg/kg levels of Pb pollution. They compared to the lowest numbers of leaves found in control samples, which are 3.3±0.5 leaves at 32.75 mg/kg, 5.0±0.8 leaves at 42.02 mg/kg and 4.0±0.8 leaves at 52.55 mg/kg levels of Pb pollution. From analysis, only numbers of leaves recorded from samples treated with cow dung only, and in combination with poultry wastes are similar. But they are significantly different (α=0.05) from those of control samples, and samples treated with poultry wastes alone, two of which are similar on the other hand. With respect to the height of plants from treated Pb-polluted soils on week 4, the results obtained ranged from 41.4±3.8 cm to 91.6±4.6 cm. Wide variations of were observed among the number of leaves, indicating varied effects by different treatments. Among the number of leaves from different treated samples, those obtained from samples treated with cow dung were slightly better than those obtained from its combination with poultry wastes. These were far better than results of other samples studied. This best result recorded 68.5±2.2 cm height at 32.75 mg/kg, 91.6±4.6 cm at 42.02 mg/kg and 80.5±2.9 cm at 52.55 mg/kg levels of Pb pollution. It compares to heights of 41.4±3.8 cm height at 32.75 mg/kg, 46.8±3.8 cm at 42.02 mg/kg and 37.0±4.5 cm recorded from control samples, at 52.55 mg/kg levels of Pb pollution. Similarly, only the heights of seedlings recorded from samples treated with cow dung only, and in composite with poultry wastes are similar. But they are significantly different (α=0.05) from those of control samples, and samples treated with poultry wastes alone, two of which are similar on the other hand.

On week 7 of growth on treated Pb-polluted soil samples, the average number of leaves recorded varied between 6.0±0.0 and 17.0±0.8. Considering all the samples, it can be seen that the numbers of leaves obtained from seedling planted on samples amended with cow dung alone were higher than others across the concentrations. It recorded 11.3±0.5 leaves at 32.75 mg/kg, 13.3±0.5 at 42.02 mg/kg and 17.0±0.8 leaves at 52.55 mg/kg levels of Pb pollution. This is comparable to 6.0±0.0 leaves at 32.75 mg/kg, 7.3±0.9 at 42.02 mg/kg and 5.3±0.5 leaves obtained from control samples at 52.55 mg/kg levels of Pb pollution. Again, only the numbers of leaves recorded from samples treated with cow dung alone, and in combination with poultry wastes are similar (α=0.05). But they are significantly different (α=0.05) from those of control samples, and samples treated with poultry wastes only, two of which are similar on the other hand. With respect to heights of plants, the results obtained from measurement of seedling planted on samples of amended Pb-polluted soil bioremediation on week 7 was between 69.2±6.8 cm and 168.4±13.0 cm, indicating the existence of variations in the heights obtained. Among all the heights, those recorded from seedlings planted on samples amended with cow dung only were tallest, with heights of 115.2±2.0 cm at 32.75 mg/kg, 144.6±4.5 cm at 42.02 mg/kg and 168.4±13.9 cm at 52.55 mg/kg levels of Pb pollution. In the control samples, which were the shortest, heights were 69.8±7.2 cm at 32.75 mg/kg, 69.2±6.8 cm at 42.02 mg/kg and 71.5±9.5 cm at 52.55 mg/kg levels of Pb pollution. However, heights of seedling in samples treated with cow dung alone, and in combination with poultry wastes were the same, but are significantly different (α=0.05) from heights obtained from other treatment.

Effects on Protein Content of Leaf

Figure 3 is the concentrations of protein in maize seedlings planted on amended bioremediated samples of Pb-polluted soil. The observed range of protein contents was 26.27±2.92 mg/g to 84.50±3.71 mg/g, recording a marked difference in protein contents of the seedlings. It was also observed that the protein contents of seedlings grown on control samples are the least. The highest concentrations of proteins were recorded in seedlings treated with cow dung, with 84.5±3.71 mg/g at 32.75 mg/kg, 59.9±3.48 mg/g at 42.02 mg/kg and 45.69±2.45 mg/g of proteins at 52.55 mg/kg levels of Pb pollution. The seedlings planted on control samples, which recorded the least proteins contents, had 47.83±2/52 mg/g at 32.75 mg/kg, 33.18±2.64 mg/g at 42.02 mg/kg and 26.27±2.92 mg/g of proteins at 52.55 mg/kg levels of Pb pollution. Analysis of results at α=0.05, indicated that protein contents of seedlings treated with cow dung only, and its combinations with poultry wastes are similar. Similarly, those of samples treated with poultry wastes only, are similar to those of its combination with cow dung. Furthermore, proteins contents of seedlings on all samples are significantly different from those of control, except for those of seedlings on treatment with poultry wastes only. Across the concentrations, protein contents of seedlings on each sample are significantly different from those of others.

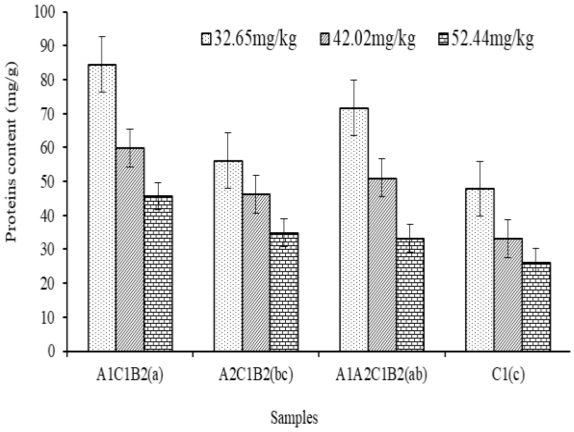

Figure 3: Concentrations of protein in maize seedlings planted on amended samples of bioremediated Pb-polluted soil. Legends: A1 refers to cow dung, A2 refers to poultry wastes, B2 refers to *Micrococcus* sp. and C1 refers to Pb. Samples with similar lowercase letters (in bracket) are not significantly different at α=0.05).

Effects on Chlorophyll Content of Leaf

Figure 4 presents the concentrations of chlorophyll in maize seedlings planted on amended samples of bioremediated Pb-polluted soil. The total chlorophyll contents recorded from treated Pb-polluted soil samples ranged from 0.34±0.02 mg/g to 1.06±0.07 mg/g, indicating a degree of variation among the samples. Again, the chlorophyll contents of seedlings grown on treated samples were higher than those of seedlings on control samples. Meanwhile, seedlings in samples treated with cow dung only, contained the highest total chlorophyll contents, and recorded 1.06±0.07 mg/g at 32.75 mg/kg, 0.71±0.02 mg/g at 42.02 mg/kg and 0.68±0.09 mg/g total chlorophyll at 52.55 mg/kg levels of Pb pollution. Conversely, 0.49±0.06 mg/g total chlorophyll content at 32.75 mg/kg, 0.43±0.05 mg/g at 42.02 mg/kg and 0.34±0.02 mg/g at 52.55 mg/kg levels of Pb pollution were recorded in seedlings planted on control samples. These represented the lowest concentrations recorded. Statistical analysis at α=0.05, showed that total chlorophyll contents of all the treated samples are similar, but are significantly different from those on control samples.

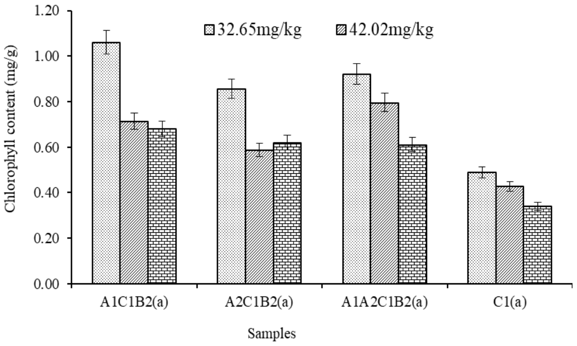

Figure 4: Total chlorophyll concentrations in maize seedlings planted on amended samples of bioremediated Pb-polluted soil. Legends: A1 refers to cow dung, A2 refers to poultry wastes, B2 refers to *Micrococcus* sp and C1 refers to Pb. Samples with similar lowercase letters (in bracket) are not significantly different at α=0.05).

Effects on Pb Bioaccumulation in Maize Organs

From the results, the range of bioaccumulation in leaf was 1.26±0.07 mg/kg to 3.8±0.05 mg/kg. It was 0.78±0.04 mg/kg to 1.84±0.1 mg/kg in the stem samples, but 4.34±0.07 mg/kg to 8.7±0.15 mg/kg in root samples. This indicates that roots bioaccumulated Pb more than other parts of corn studied. As usual, wide gap in these ranges is an indication of large variations in the capabilities of different parts of corn to bioaccumulate Pb.
In corn leaf samples, it was observed that the lowest bioaccumulation of Pb occurred in soil samples treated with *Micrococcus* sp and a combination of cow dung and poultry wastes. This recorded 1.26±0.07 mg/kg at 32.75 mg/kg, 1.56±0.09 mg/kg at 42.02 mg/kg and 1.97±0.06 mg/kg at 52.55 mg/kg levels of Pb pollution. However, at α=0.05, these were not significantly different from those recorded in soil samples treated with poultry wastes and cow dungs in isolation. Bioaccumulation obtained in control samples was significantly higher than those in all the treated samples. It recorded 2.73±0.08 mg/kg at 32.75 mg/kg, 3.14±0.16 mg/kg at 42.02 mg/kg and 3.8±0.05 mg/kg at 52.55 mg/kg levels of Pb pollution. Though bioaccumulation increased with increasing concentrations of Pb pollution, on comparison, results showed that bioaccumulation at 32.75 mg/kg pollution was significantly lower than that at 52.55 mg/kg.

In the stem, samples from control soil samples maintained the highest bioaccumulation of Pb, which was significantly higher than those of treated soil samples. They recorded 1.47±0.37 mg/kg at 32.75 mg/kg, 1.57±0.1 mg/kg at 42.02 mg/kg and 1.84±0.1 mg/kg at 52.55 mg/kg level of Pb pollution. Among samples from treated soil samples, bioaccumulation of Pb in stems of corn from soil treated with *Micrococcus* sp and cow dung only was the least. It had 0.86±0.04 mg/kg at 32.75 mg/kg, 0.82±0.03 mg/kg at 42.02 mg/kg and 1.1±0.04 mg/kg at 52.55 mg/kg levels of Pb pollution. At α=0.05, statistical analysis revealed that bioaccumulation in all the treated samples did not significantly differ. Across the concentrations, it was found that while bioaccumulation at 32.75 mg/kg and 42.02 mg/kg did not significantly differ, they significantly differed from that at 52.55 mg/kg level of Pb pollution.

In the case of roots, the least level of bioaccumulation was found in corns planted on soil samples treated with *Micrococcus* sp. and cow dung only. These recorded 4.38±0.08 mg/kg of Pb at 32.75 mg/kg, 4.35±0.05 mg/kg at 42.02 mg/kg, and 4.50±0.03 mg/kg at 52.55 mg/kg levels of Pb pollution. On the other hand, control samples recorded 7.72±0.05 mg/kg of Pb at 32.75 mg/kg, 8.25±0.1 mg/kg at 42.02 mg/kg and 8.7±0.15 mg/kg at 52.55 mg/kg levels of Pb pollution. These were significantly higher than those of treated samples, at α=0.05. Comparison of samples from treated soil samples indicated that they did not significantly differ from each other. Across concentrations of Pb pollution, only the bioaccumulation at 32.75 mg/kg significantly differed from that recorded at 52.55 mg/kg.

IV. DISCUSSION

A prior study demonstrated that soil pH varied with amendment treatment, with compost application resulting in a considerable reduction in soil pH when compared to the control. However, the pH of the soil increased following the application of vermicompost. They also stated that the effect of amendment on soil pH is determined by the initial pH of the amendment material, therefore the increase in pH might be attributed to the higher pH value of the vermicompost (pH of 7.5) compared to the soil (pH of 6.5) (Angelova, Akova, Artinova, & Ivanov, 2013). Furthermore, Romdhane et al. (2021) reported that Zn, Cu, Co, Cd, and Pb metal pollution of soil as sulfates reduced soil pH and enhanced electrical conductivity. The rise in alkalinity in soil samples following the treatment could be ascribed to products and byproducts resulting from the remediation approach. The soils treated with compost and vermicompost had a higher organic content than the control soil. Vermicompost at rates of 5 gkg^{-1} and 10 gkg^{-1} raised organic content to 3.99 and 9.36%, respectively, whereas compost increased organic content to 4.95 and 8.64 $g.kg^{-1}$, respectively (Angelova et al., 2013).

The rhizosphere is a very complex microenvironment in which soil, plant roots, and microbes interact. This nutrient-rich zone is thought to contain 1011 microbial cells per gram soil, which include invertebrates, bacteria, protists, fungus, and nematodes. This is possible as result of the overall interaction of plants and microbes, as well as a variety of elements such as soil temperature, pH, moisture, root exudates and inorganic nutrients (Alves et al., 2022). In a related study, *Enterobacter, Azotobacter, Arthrobacter, Azospirillum, Xanthomonas, Bacillus, Burkholderia, Streptomyces, Klebsiella, Pseudomonas,* and *Serratia,* were found to be the dominant genera of rhizospheric bacteria known as plant growth-promoting rhizobacteria (PGPR), which affect plant growth and yield of commercially important crops (Souza et al., 2021). Some of these bacteria were also identified in the current study. Many of these bacteria can tolerate extreme metal stress in metal-rich soils (Hou et al., 2020). Bacteria contain resistance genes to a variety of harmful elements such as Ag^{+}, Co^{2+}, Cu^{2+}, Cd^{2+}, Sb^{3+}, Hg^{2+}, Zn^{2+} and Ni^{2+}. Bacteria can tolerate heavy metal toxicity through a variety of methods, including increasing metal solubility for removal and decreasing metal solubility for immobilization (Alves, Yin, Oliveira, Silva, & Novo, 2022).

Another similar study found that the number and quantity of nutrients provided had a direct correlation with treated soil's dehydrogenase activity and microbial biomass (Adak et al., 2014). The application of a mixture of sulfur-oxidizing bacteria, elemental sulfur, cow dung, molasses (P4) resulted in a significant improvement in all the growth attributes— root fresh (96%), shoot fresh (72%) plant height (26%), dry weights (2.05 fold), shoot dry weights (1.38 fold), and root length (1.1 fold)— when compared to the corresponding control treatment as reported in another study involving ryegrass from a Pb-contaminated soil. The next was the treatment which combined elemental sulfur, cow dung, and molasses (P3) which enhanced growth parameters, such as root length (91%), root fresh (80%), plant height (20%), shoot fresh (51%) and dry weights (1.16 fold), and dry weights (1.26 fold) (Ashraf et al., 2022).

It has been shown that adding organic amendments improved plant development under metal stresses. Additionally, both *Bacillus licheniformis* and *Micrococcus luteus* produced siderophores, and the latter also demonstrated phosphorus solubilization and nitrogen fixation under challenging environments. *M. luteus* reduced the harmful effects of $NaAsO_2$ in grapevines, and the inoculation of these PGPR boosted plant biomass yields (Pinter et al., 2017). Furthermore, it was found that adding more biochar and compost organic amendments enhanced gas exchange characteristics and chlorophyll concentrations (Irfan et al., 2021). The reduction in chlorophyll content during metal stress could be due to chloroplast deformation. Similar findings from a different study showed that plant physiology, including SPAD value, chlorophyll "a," chlorophyll "b," and carotenoid levels, were negatively correlated with heavy metal contamination. The results showed that applying acidified cow dung slurry both by itself and in conjunction with molasses and SOB considerably reduced the harmful effects of heavy metals on ryegrass physiology. The SPAD value (79%), chlorophyll "a" (19%), chlorophyll "b" (35%), and carotenoid levels (75%) all significantly increased under Pb stress, indicating that the administration of P4 enhanced the plant physiology. This is in contrast to each of their individual control treatments. Treatment P3, which raised the SPAD value (55%), chlorophyll "b" (22%), chlorophyll "a" (11%), and carotenoid levels (52%), compared to control, was the next most effective. In terms of enhancing plant physiology under heavy metal stress, it was determined that treatment P4 produced the most notable results (Ashraf et al., 2022).

Furthermore, the total chlorophyll, chlorophyll 'a', and chlorophyll 'b', contents were found to be affected by the application of important plant nutrients, including sulfur, nitrogen, and iron (Jawale et al., 2017). According to some study, when plants are stressed by abiotic causes, PGP bacteria trigger the production of antioxidant enzymes. According to this study, *P. aeruginosa* under Zn stress absorbed N and P and increased wheat biomass, total soluble protein, and leaf chlorophyll (Islam et al., 2014).

The fact that Pb was found in maize suggested that it may bioaccumulate in its organs. This conclusion was supported by a prior study that found that increased levels of heavy metals in soils contaminated by wastewater irrigation cause Cr, Ni, Pb, and Zn to bioaccumulate in maize (Lu et al., 2015). Additionally, the majority of heavy metals, including Pb, have been reported to be considerably bioaccumulated by *Panicum maximum* from the soil (Anuforo et al., 2020b). According to another study, all of the treatments and soils under investigation showed a consistent drop in heavy metal concentrations as plant age increased. The "dilution effect" or the difference between the rate at which heavy metals are absorbed by plant and the rate of growth of the plant may be the reason for this decline in heavy metal concentrations with increasing plant age. However, there is a significant advantage when the shoots are fed to animals. Therefore, it is better to cut the plant after 60 days rather than after 30 days for animal nutrition. As a result, the quantities of heavy metals in maize plants decrease with age. Furthermore, applying more Ni, Pb, Cd, and Cr raised the amount of heavy metals in corn (Taalab & Shahin, 2018). In the several organs of maize, each heavy metal showed a unique pattern. The highest amounts of Ni, Cr, and Pb were found in the roots of maize, with mean values of 6.56, 105.21, and 5.12 mg/kg, respectively. Among the four maize bodies, the fruit exhibited the lowest mean values for Zn, Cr, and Pb, with 0.18, 0.23, and 0.49 mg/kg, respectively. These findings demonstrated that substantial levels of Cr, Pb, and Ni were retained in the maize roots despite the relatively low concentrations of these metals in the soil, especially Cr (Lu et al., 2015). According to a related study, applying P4 significantly improved heavy metals solubility and the uptake of Cd (56%) and Pb (52%) by ryegrass shoots. The next treatment was P3, which raised the Cd (43%) and Pb (35%) concentrations in ryegrass shoots, compared to the control treatment (Ashraf et al., 2022).

VI. CONCLUSION

The study was conducted to evaluate the effects of amendment of bioremediation of Pb-polluted soil with composites of poultry wastes and cow dung, as well as *Micrococcus* sp. on growth parameters of maize seedlings. Results obtained indicated that increase in the pH, organic carbon (OC) content and soil organic matter (SOM) content of the samples. treatment with cow dung only yielded significantly highest alkalinity of 12.18%, 14.18%, and 10.05% at 33.75 mg/kg, 44.67 mg/kg and 52.55 mg/kg Pb pollution respectively. The observed range of protein contents was 26.27±2.92 mg/g to 84.50±3.71 mg/g, recording a marked difference in protein contents of the seedlings. It was also observed that the protein concentrations in the maize seedlings planted on control samples are the least. The highest concentrations of proteins were recorded in seedlings treated with cow dung, with 84.5±3.71 mg/g at 32.75 mg/kg, 59.9±3.48 mg/g at 42.02 mg/kg and 45.69±2.45 mg/g of proteins at 52.44 mg/kg levels of Pb pollution. The chlorophyll contents of seedlings grown on treated samples were higher than those of seedlings on control samples. The range of bioaccumulation in leaf was 1.26±0.07 mg/kg to 3.8±0.05 mg/kg. It was 0.78±0.04 mg/kg to 1.84±0.1 mg/kg in the stem samples, but 4.34±0.07 mg/kg to 8.7±0.15 mg/kg in root samples. This indicates that roots bioaccumulated Pb more than other parts of corn studied. From the results, the amendments improved the growth of maize seedlings grown on treated Pb-polluted soils.

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