__Original Research Article__

Assessment of Effects of Amended Bioremediation of Toxic Metal-polluted Soil using Organic Composites and *Bacillus* sp on Plant Growth Parameters

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ABSTRACT

It has become pertinent to develop improved bioremediation techniques, to avert potential effects of heavy metal pollution on agricultural productivity, and safety of its produce. In this study, cow dung, poultry wastes and their composites used as organic amendments were collected and processed, while *Bacillus* sp used as plant growth promoting rhizosphere was isolated and identified. Then 24 kg of fertile soil was polluted by introducing 20 mL of 0.1M lead acetate solution, before bagging 3 kg of polluted soil into ten perforated nylons. Soil sample was then analyzed before and after treatment. After mixing with *Bacillus* sp suspension, two of the pot experiments were amended with each of 0.5 kg cow dung only, 0.5 kg poultry wastes only and 1:1 ratio of their composites. Control samples were set up without amendment, and they were bioremediated for 25 days. Following germination of sown corn seedlings, heights of their shoots and number of leaves were monitored on day 4, weeks 2, 4 and 7, before harvesting for analysis together with soil samples. Results obtained revealed that seedlings grown on treated samples as well as properties of treatment of soil were generally better than those of control samples. Highest percentage increase in organic matter content of soil was recorded using mixture of cow dung only and *Bacillus* sp, with 9.28% and 7.63% increase at 33.75 mg/kg and 52.55 mg/kg of Pb pollution. Seedlings grown on samples treated with poultry wastes only, recorded the highest protein content of 161.25±5.5 mg/g, 104.99±4.95 mg/g and 79.75±3.2 mg/g protein contents, at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively. The amendments also reduced bioaccumulation of Pb in corn seedlings, which was in the order; stem<leaf<root. Therefore, these amendments can be exploited for improved bioremediation of heavy metals.

Keywords: Pollution, bioremediation, phytotoxicity, plant growth, chlorophyll content

1. INTRODUCTION

The most common environmental heavy metals are nickel (Ni), copper (Cu), lead (Pb), chromium (Cr), cadmium (Cd), iron (Fe), arsenic (As) and mercury (Hg). Heavy metals are metals whose specific gravity exceeds 5 g cm^{-3} [1]. Low concentrations of some heavy metals, such as copper (Cu), iron (Fe), zinc (Zn), cobalt (Co), manganese (Mn), selenium (Se), nickel (Ni), and molybdenum (Mo) are necessary for metabolic activity, but their high concentrations are toxic. Other heavy metals including arsenic (As), mercury (Hg), silver (Ag), lead (Pb), cadmium (Cd), and chromium (Cr(VI)) have no known biological function, and may cause serious toxicity and carcinogenicity, even at low concentrations. Hence they are included in the priority metals for public health concern. These metals are toxic to microorganisms, plants, and humans, even at low concentrations. They cause metabolic abnormalities to organisms which consume foods obtained from plants cultivated on contaminated soil [2].

In general, heavy metals are naturally occurring substances found in vastly varying amounts in the earth's crust. However, the high occurrence of heavy metals in the ecosystem is a result of human-caused pollution [3]. Manufacturing, smelting, pesticide and fertilizer application in agriculture, vehicle emissions, municipal wastes, and industrial effluents are additional human activities that lead to release of heavy metals [1]. According to Manj´on et al. [4], the most frequent risks associated with

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heavy metals-contaminated soil include consumption of contaminated water or vegetables and direct inhalation of dust, aerosols, or particulate matter.

Many variables, including soil pH, organic matter content, temperature, and others, affect the bioavailability of heavy metals [5]. For normal growth, plants take up nutrients from the soil; but, due to the non-specificity of the absorption processes, when they grow in contaminated substrates, they may take up hazardous amounts of both essential and non-essential heavy metals [6]. Plant aerial tissues may potentially become contaminated if heavy metals (HMs) are dropped directly on biomass above ground. Worldwide, one of the main causes of health concerns is the environmental pollution caused by heavy metals and the resulting health impacts [3]. Over $10 billion is thought to be the annual economic cost of heavy metal pollution globally [7]. Although the price and length of soil remediation vary depending on the technology used and the particular site involved, estimates range up to $500 ton^{-1} waste and 15 years duration [8].

Microbial remediation and phytoremediation are two types of bioremediation procedures. In order to clean up contaminated soil, a process known as phytoremediation entails carefully cultivating specific plants, such Cruciferae plants [9]. The most crucial thing in this situation is to find plants that have a strong capacity to accumulate and tolerate heavy metals. These kinds of plants must have a particular hyper accumulation capacity for pollutants in the soil [3]. However, microbial remediation uses a variety of microorganisms to carry out the heavy metal absorption, deposition, oxidation, and reduction in the soil. The primary bio-remediators in this process include fungi, bacteria, and archaea [10]. The majority of metals may be treated using bioremediation procedures, which are also inexpensive and environmentally safe. Because of this, biologically based remediation strategies are gaining a lot of attention among other accessible options [11]. The purpose of this study was to evaluate the possible impacts of employing composites of cow dung and chicken wastes along with Bacillus sp. on the growth patterns of corn seedlings, and to modify bioremediation of Pb-polluted soils.

2. MATERIAL AND METHODS

2.1 Collection and processing of soil and animal waste samples

Using well cleaned soil auger, samples were taken from upper layer of soil, at 0–30 cm depth of productive land in the SAAT Farms, Federal University of Technology, Owerri (FUTO), Nigeria, and packaged in sterilized plastic pouches. The obtained soil was dried, and ground. Some portion of prepared soil about 0.5 kg was further ground and passed through 0.25-mm sieve for the basic physicochemical characteristics. Additionally, cow dung (A1) and poultry wastes (A2) were collected from the Poultry Unit of FUTO Farms, dried, ground and stored in sterilized plastic container.

2.2 Isolation and Identification of Isolate

*Bacillus*sp isolate, used as plant growth promoting rhizobacteria (PGPR), was isolated by inoculating serially diluted soil sample, collected from FUTO farms, on nutrient agar medium. Fifty gram soil sample was transferred to 150 mL sterile distilled water and heat treated at 80 °C for 15 minutes. After that 0.1 ml of soil suspension was spread over pre-sterilized nutrient agar plates. The inoculated plates were incubated at 30 °C for 24-48 h. The plates were examined after incubation period for rough and abundant colonies with waxy growth (1-4 mm diameter) and irregular spreading edge. Suspected colonies were Gram stained, and Gram positive bacilli were maintained on nutrient agar slants for additional identification tests [12]. Selected biochemical tests including motility test, crystal formation, penicillin susceptibility test were carried as described by Shambhavi*et al.* [13]. Suspected isolates were further sub-cultured on polymixin egg yolk mannitolbromothymol blue agar (PEMBA) and their morphologies were confirmed after incubation.

Pure culture of identified *Bacillus* sp was incubated on a freshly prepared sterile nutrient broth in cotton wool stoppered Balch tubes, and incubated at 30 ^{o}C, for 120 h. This was done to produce

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critical mass of cells required for soil amendment studies. The cells were harvested by centrifugation at 35 x 100 rpm for 50 minutes, washed two times in phosphate buffered saline at pH of 7.25, transferred into a sterile Eppendorf tubes, and re-suspended in MS medium.

2.3 Pot experiment

Determination of effects of amendment on maize growth was performed to examine the influence of amended bioremediation of Pb-polluted soil samples on corn seedlings growth, and their bioaccumulation in its tissues. The seeds were surface-disinfected using 5% sodium hypochlorite, 96% ethanol, and thrice with distilled water. Soil sample was polluted by adding 20 mL of 0.1M lead acetate to 50 kg of soil and homogenizing to obtain uniform concentrations. The pot experiment was organized according to the following amendments;
(1) Cow dung only (A1),
(2) Poultry waste only (A2),
(3) Composite of cow dung and poultry waste (A1A2), and
(4) Absence of amendment or control (C1).

Each pot contained 1000 mg of organic amendment or their combination, and 5 kg of Pb-polluted soil sample. Then plant growth promoting rhizobacteria (PGPR), *Bacillus* sp was applied at rate of 50 mg (wet weight) 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 seeds were planted in each pot. Each treatment had two replicates. In the greenhouse, there were 9.86 h of sunshine on average. Also, the mean temperature (light/dark cycle) and the relative humidity were 32.14 °C and 40%, respectively. Immediately seed germination began, 3 seedlings in each pot were maintained. Then growth parameters, 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.

2.4 Soil Analysis

2.4.1 Physicochemical, minerals and toxic metal analyses

Soil samples were analyzed before and after treatments. Soil pH was measured using pH meter (Mettler Toledo Delta 320), organic carbon and soil organic matter contents of the soil samples were calculated by using the wet oxidation method. Similarly, soil available nitrogen, calcium, magnesium, phosphorous and potassium contents were estimated following the method described by Lu *et al.* [14]. Digestion of soil samples for heavy metals analysis was done according to the method reported by Anuforo*et al.* [1]. After extraction and filtration, bioavailable Pb content of supernatant from each potted soil sample, was estimated using atomic absorption spectroscopy (AAS) (AA-240FS Varian, USA).

2.4.2 Dehydrogenase activity

The activity of dehydrogenases (DEH) was assayed with consideration of Thalmann protocol described by Wojewódzki*et al.* [15], with sample incubation using 2,3,5-triphenyltetrazolium chloride. Measurement of the absorbance of triphenylformazane (TPF) was done at 546 nm. The results were presented in mg TPF kg^{-1} 24 h^{-1}.

2.4.3 Moisture content analysis

The method outlined by Nwinyi and Akinmulewo (2019) 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 sterile crucible dishes were filled with 5 g of each soil sample and weight recorded as W2, before drying in oven set at 105 ^{o}C for three hours. After allowing it to cool, the weight was recorded as W3. After cooling for thirty minutes, it was put back in the oven, heated and weighed again as W3. This was repeated until constant weight was achieved. The moisture content

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which was represented by 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 + soil before drying,
W3= final weight of crucible + soil after drying

2.5 Plant Analysis

2.5.1 Chlorophyll and Proteins Contents

Leaves, roots and shoots of the seedlings were collected from each treatment, decontaminated by washing first with tap water and in 0.1N HCl solution to remove residues of chemical spray materials on the leaf surface. This was followed by washing in distilled 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 ^{o}C for 72 h. After complete drying, the samples were ground in a grinder and used for analysis. The chlorophyll contents from maize leaves were measured using the SPAD meter after 7 weeks of growth on amended bioremediated Pb-polluted soil samples.

In order to produce the standard curve that was utilized to estimate the unknown protein content, BSA was used as the standard reagent. The leaf, stem, and root extracts 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 per the protocol of Sarkar et al. [17]. 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. Following that, the protein content was calculated by measuring the absorbance at 660 nm and represented as mg BSAE/g of fresh weight.

2.5.2 Toxic metal contents

The prepared samples of shoots, leaves and roots of corn seedlings were first digested with di-acid HNO_3:$HClO_4$ mixture for the estimation of Pb content, as described by Anuforo *et al.* (2020). Then toxic metal contents of each supernatant were estimated using atomic absorption spectroscopy (AA-240FS Varian, USA).

2.6 Statistical Analysis

For each parameter studied, mean and standard deviations (SD) of the set of duplicates for all the treatments were computed using Microsoft Excel version 10. One-way analysis of variance (ANOVA), followed by the LSD test were carried at ($P < 0.05$) to assess differences among the data using Minitab® 17 software.

3. RESULTS AND DISCUSSION

3.1 Effects on physicochemical, minerals and Pb contents of soil samples

Results of effects of addition of cow dung and poultry waste, as organic supplements, and *Bacillus* spp, as plant growth promoting rhizobacteria (PGPR), on physicochemical properties of Pb-polluted soil samples, before and after seven (7) weeks of treatment, are shown in Figure 1. It was observed that addition of both organic supplements, together with *Bacillus* spp resulted in increase in pH of Pb-polluted soil samples. Percentage increase in pH of treated soil samples ranged from 6.76% in poultry waste treatment at 33.75 mg/kg Pb pollution to 11.62% recorded at its 52.55 mg/kg Pb pollution. Although there was no appreciable difference in the pH of all treated samples, the mixture of cow dung (A1) only and *Bacillus* (B1),produced the highest increase in alkalinity among the various combinations of treated soil. On the other hand, the pH of untreated Pb-polluted soil (control) sample

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decreased by 4.71% and 9.19% at 33.75 mg/kg and 52.55 mg/kg Pb pollution respectively, after the period of treatment. It was also observed that as concentration of Pb pollution increased, pH of soil also increased both before and after treatment. Generally, the percentage organic carbon (OC) content of all treated Pb-polluted soil samples increased after treatment. However, it was observed that the highest percentage increase occurred in treatment done with mixture of cow dung and *Bacillus* sp, with 9.28% and 7.63% increase at 33.75 mg/kg and 52.55 mg/kg of Pb pollution. After treatment, the OC content of control sample decreased by 37.8% at 33.75 mg/kg Pb pollution and 49.08% at 52.55 mg/kg Pb pollution. For soil organic matter (SOM) content, the highest percentage increase was recorded in treatment which was done using a mixture of cow dung, poultry waste and*Bacillus* sp. SOM decreased in control sample by 54.04% and 54.66% at 33.75 mg/kg and 52.55 mg/kg Pb pollution respectively.

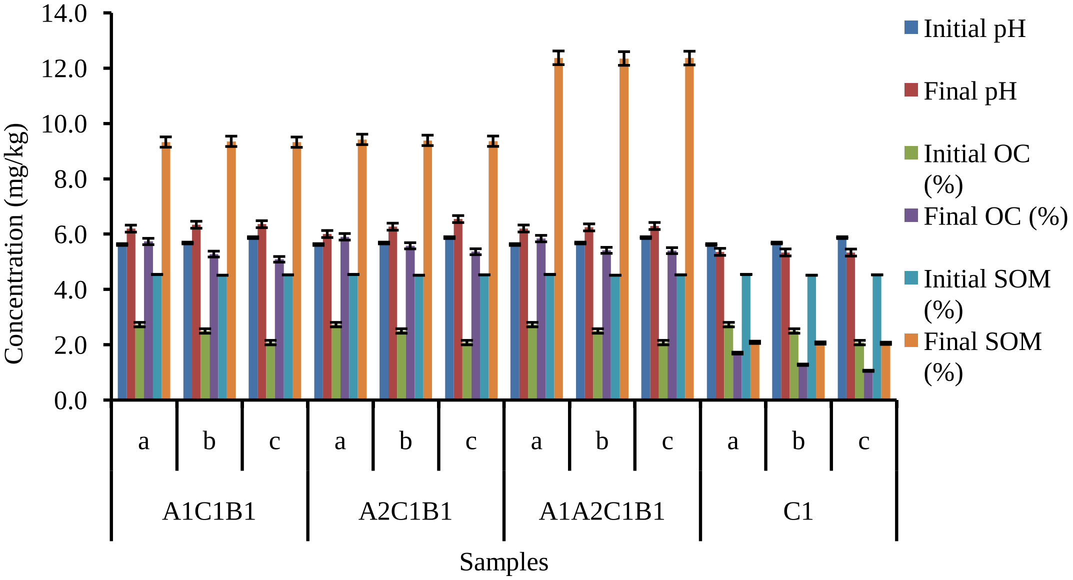

Fig. 1.pH, organic carbon and soil organic matter content of Pb-polluted soil samples, before and after treatment. A1 is cow dung, A2 is poultry waste, C1 is Pb, B1 is *Bacillus* sp.

In an earlier study, it was reported that samples of soil from areas with high and medium levels of pollution had pH values of 6.58 and 6.60, respectively, indicating that the soils were neutral to slightly acidic [18]. The pH of the soil reduced and electrical conductivity increased with addition of metals as sulfates [19]. Significantly greater quantities of metals, including Pb, Cu, and Co, were discovered in the shoot tissues of maize at the 3–4 leaf stage due to increased bioavailability in the soil pore water [19]. Another study reported that after adding compost and biochar to soil, the pH of the mixture rose noticeably ($p < 0.05$). After applying 4% biochar, 4% compost, and 2% biochar, the pH of the soil rose from 7.53 (control) to 7.96 (biochar). The pH of the soil increased somewhat as the amounts of compost and biochar increased [20]. The findings indicated that both the quantities of compost and biochar considerably ($p < 0.05$) increased the amount of organic matter content of the soil. The control group exhibited the lowest organic matter (1.09%), whereas the maximum organic matter (1.72%) was observed at 4% biochar application, which was subsequently followed by 4% compost. According to Irfan et al. [20] there was a consistent rise in organic matter when both biochar and compost levels rose.

Similarly, there was an increase in concentration of nitrogen in all bioremediated Pb-polluted soil samples, but not in control samples. Amendment of treatment with a combination of cow dung, poultry waste and *Bacillus* sp produced the highest increase in nitrogen content, recording 61.82% increase at 33.75 mg/kg and 61.22% at 52.55 mg/kg Pb pollution. For calcium, there was a slight increase in its concentration across all samples, except in control where it slightly decreased, after the period of study. Highest percentage increase of 16% at 33.75 mg/kg Pb pollution was observed in treatment with poultry waste only and *Bacillus* sp. Conversely, Ca content reduced with increasing concentration of Pb pollution in all samples, including the control. Similar to Ca content, results indicated that concentration of K in polluted soil marginally increased in all samples, including the control. However, the increase was higher in treated samples, with that of poultry waste only and *Bacillus* sp, recording the highest percentage of 30% at 33.75 mg/kg Pb concentration, after treatment. Percentage increase in the control sample was 7% at 33.75 mg/kg and 3% at 52.55 mg/kg Pb pollution, as shown in Figure 2. According to Irfan et al. [20], the application of compost increased the total N as well as the accessible P and K contents in the soil significantly ($p < 0.05$).

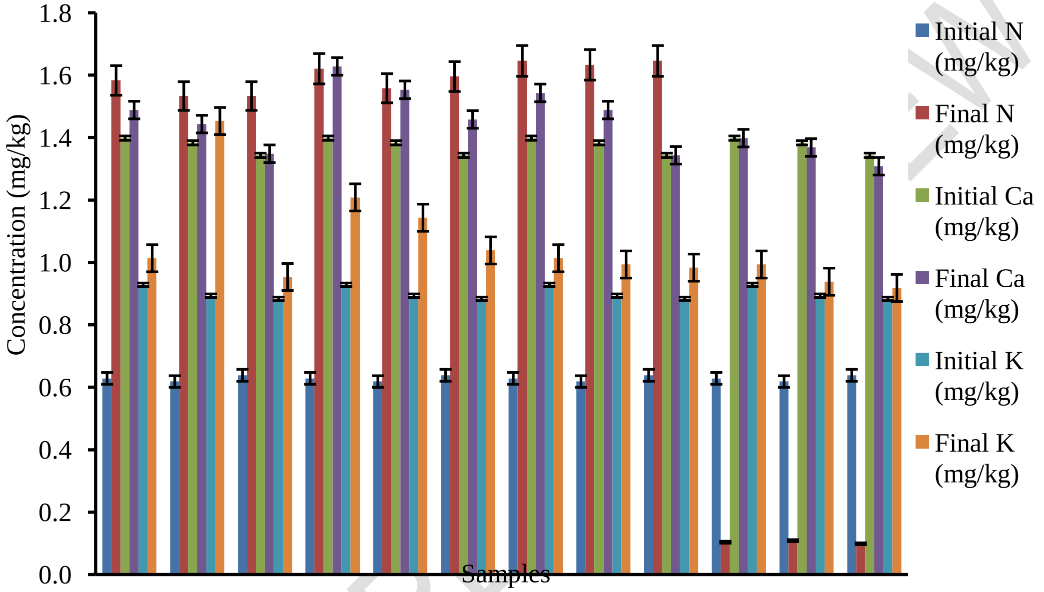

Fig. 2.Nitrogen, calcium and potassium contents of Pb-polluted soil samples before and after amended treatment. A1 is cow dung, A2 is poultry waste, C1 is Pb, B1 is *Bacillus* sp.

Furthermore, concentration of magnesium in the Pb-polluted soil samples decreased in both treated and control samples. However, the highest consistent percentage decrease in its concentration was recorded in the control sample, with 20% and 12% at 33.75 mg/kg and 52.55 mg/kg Pb pollution respectively. Among the treated samples, treatment with cow dung and *Bacillus* sp yielded the highest decrease in Mg content. The concentration of phosphorus in all the treated samples increased after the period of treatment. Sample treated with poultry waste and *Bacillus* sp recorded the highest increase of 56% content of P, at 33.75 mg/kg Pb content, while cow dung and *Bacillus* sp produced the least increase in its concentration. The concentration of P in control sample decreased by 54% at 52.55 mg/kg and 45% at 33.75 mg/kg Pb pollution as shown in Figure 3.

Reducing the bioavailability of metals in polluted soils is the first step towards reducing metal pollution [21]. Toxic components in contaminated areas have been immobilized using a variety of organic fertilizers [22]. According to Ramli et al. [23], organic amendments are a source of plant nutrients and may continue to be the main source of nutrients for maintaining the fertility and quality of the soil. Organic compounds made from plant and animal leftovers offer a number of advantageous qualities that can increase crop performance and soil quality [24]. When added to highly polluted soils, these materials can be especially helpful in their bioremediation [25]. Rich in iron (Fe) and phosphorus (P), compost can immobilize harmful metals found in contaminated soil. Depending on how much the organic matter has decomposed, different organic additions have different effects on the bioavailability of hazardous substances. The electrostatic complexation between the metal and soil

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organic matter (SOM) has a major impact on the oxidizable percentage of heavy metals that is linked to SOM. This process also influences the toxicity of metals in contaminated soils [26].

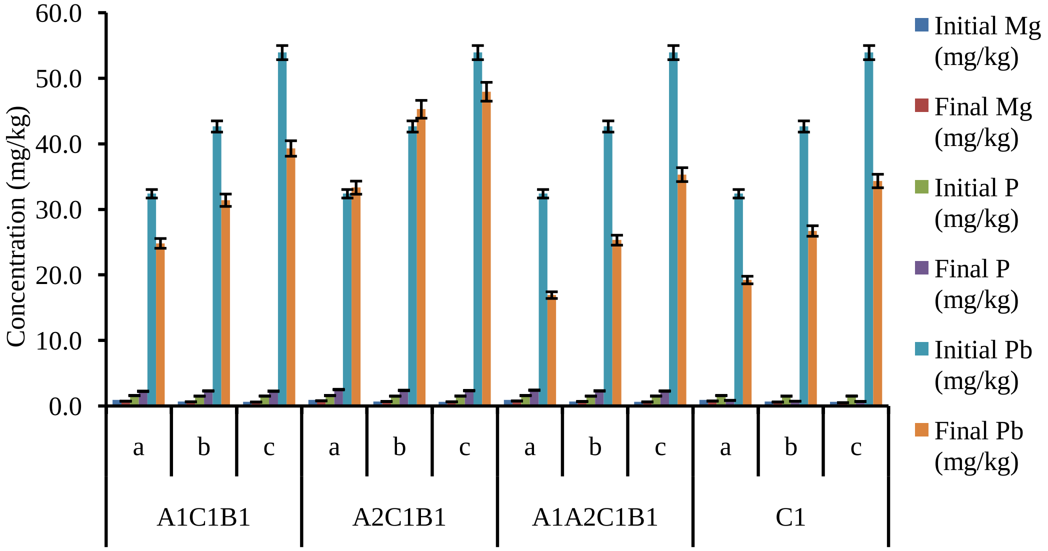

Fig. 3.Magnesium, phosphorus and lead contents of Pb-polluted soil samples before and after amended treatment. A1 is cow dung, A2 is poultry waste, C1 is Pb, B1 is *Bacillus* sp.

A study had reported that the availability of Pb, Cd, and Cr were considerably ($p < 0.05$) reduced in artificially polluted soil, when biochar and compost alone were supplied at doses of 0.5, 1, 2, and 4%. After applying 4% biochar and 4% compost, the Pb concentration in the control soil dropped from 18.26 to 3.82 mg kg^{-1} [20]. It is anticipated that the impacts of compost and manure, which comprise non-decomposing organic materials, will differ in terms of how they affect the bioavailability of heavy metals due to their different chemical compositions [22]. After growing protein maize and non-protein maize, the concentration of Pb increased from 36.44 mg/kg in pre-cropped soil to 110.73 ± 28.03 and 78.43 ± 19.72, respectively [27].

3.2 Effects on moisture content of soil samples

In Pb-polluted soil samples (C1), it was found that when cow dung and poultry wastes were used in isolation, moisture content of soil decreased with increasing concentration of Pb- pollution. However, when they were combined, there was a reversal of the declining trend of moisture content of soils from 42.02 mg/kg to 52.44 mg/kg of Pb. On the average, among the treated samples, samples treated with combination of cow dung and poultry wastes showed highest moisture content, as well as its percentage increase across the range of Pb concentrations studied. It produced 8.36±0.52 (63.4%) increase at 32.75 mg/kg Pb concentration, which declined to 7.68±0.79 (61.11%) at 42.02 mg/kg Pb concentration, but reversed to 9.88±0.68 (84.93%) at 52.44 mg/kg Pb concentration. In control samples, it was found that the moisture of soil declined at the end of experiment by 1.30±0.57 (9.87%) and 0.7±0.1 (6.06%), at 32.75 mg/kg and 52.44 mg/kg Pb pollution respectively. These can be seen in Figure 4.

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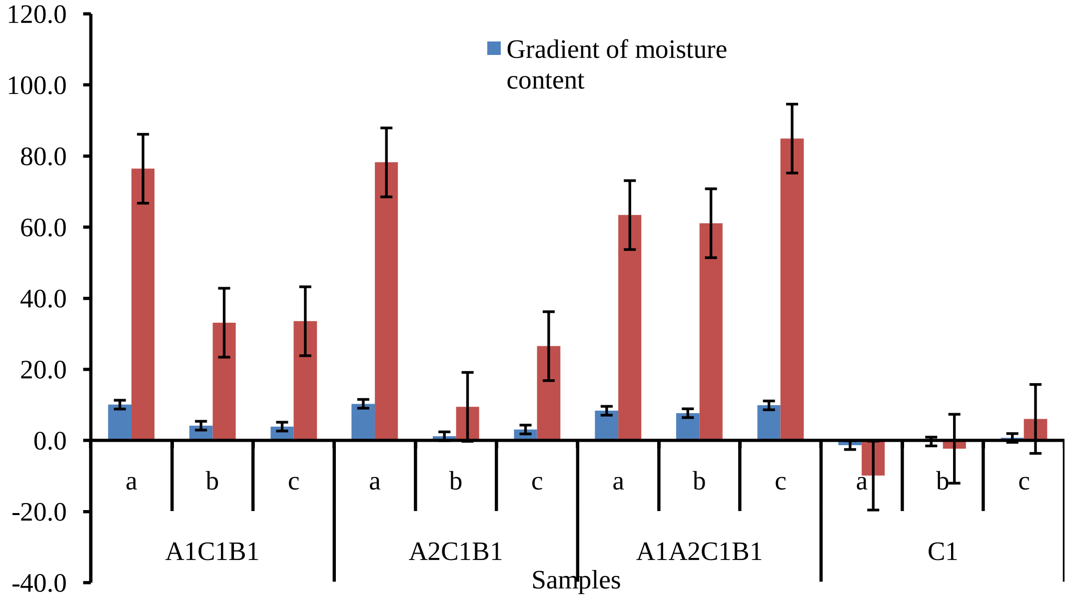

Fig. 4. Moisture content and their percentage increase/reduction in Pb-polluted soil samples, treated with mixtures of cow dung, poultry wastes and *Bacillus* sp.

3.3 Effects on dehydrogenase activity

DHA in Pb polluted soil which was treated with different combinations of cow dung, poultry wastes, together with *Bacillus* sp increased in all treated samples. The gradient of DHA recorded before and after treatment ranged from 0.32 to 2.07 $\mu gTPFg^{-1}h^{-1}$. The highest increase in DHA was found in the sample that was treated using a mixture of cow dung and poultry wastes, with 2.07 $\mu gTPFg^{-1}h^{-1}$ recorded at 33.75 mg/kg Pb pollution, which reduced to 1.26 $\mu gTPFg^{-1}h^{-1}$ at 52.55 mg/kg Pb pollution. Least DHA was recorded in samples treated with cow dung alone. Unlike in treated samples, it was observed that after treatment, the DHA in control samples reduced, with reduction of 0.14 $\mu gTPFg^{-1}h^{-1}$ and 1.10 $\mu gTPFg^{-1}h^{-1}$ impacted on the samples at 33.75 mg/kg and 52.55 mg/kg respectively. These are illustrated in Figure 5. A study by Chukwuma et al. [28] reported that after four weeks, there was a significant (P=0.05) increase in the dehydrogenase activities of bioremediated soil samples compared to their control samples, although these activities decreased at the conclusion of bioremediation. The observed rise in microbial population could potentially be attributed to an initial surge during the first four weeks of the experiment, which was subsequently followed by a decrease in microbial activity due to the depletion of available nutrients or carbon sources. This suggested that higher dehydrogenase activities recorded in this study was due to increased nutrient availability, following applications of these amendments.

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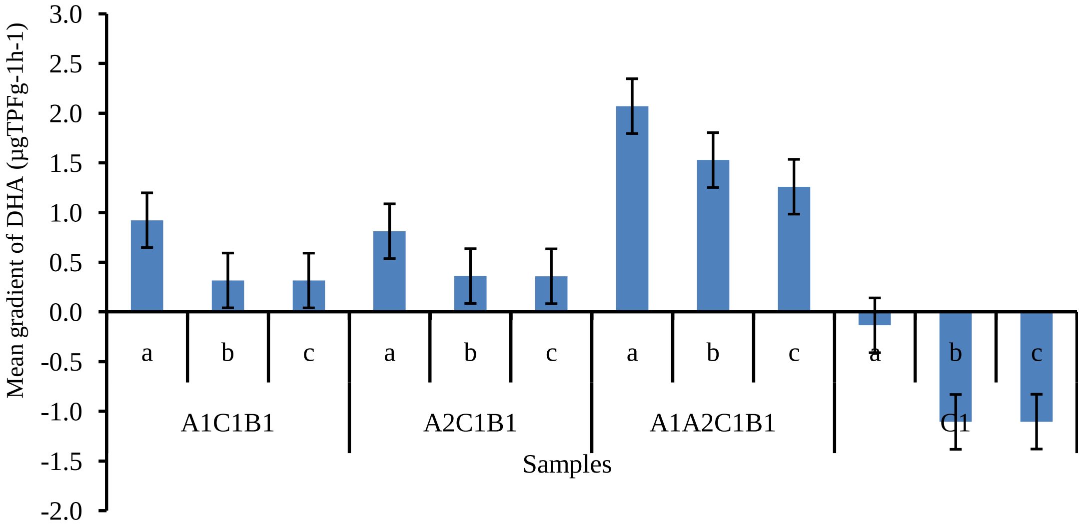

Fig. 5.Gradients of DHA in Pb-polluted soil samples after period of treatment. A1 is cow dung, A2 is poultry wastes, C1 is Pb and B1 is *Bacillus* sp.

3.4 Effects of amendments on growth patterns

Results of effects of organic and PGPR amendment of bioremediation of Pb-polluted soil on growth patterns (Figure 6) indicated that on day 4 after planting of seedlings, the number of leaves per seedling ranged from 2.00±00 to 0.00±00. However, all the treated samples performed better than the control samples, which recorded 0.0±0.0, 2.0±0.0 and 0.0±0.0 number of leaves at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution. Though there were some observable variations in the number of leaves among treated samples, they are not significantly different from each other (α=0.05).

On week 2 of treatment, there was a slight increase in the number of leaves recorded across the treatment, which ranged from 3.7±0.5 to 4.7±0.5. As seen on day 4 in Pb-polluted soil samples, all treated samples produced more numbers of leaves than the samples for control, which recorded only 3.7±0.5, 3.7±0.5 and 2.3±0.5 at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg respectively. Among all the treated samples, results indicated that samples treated with poultry wastes, in isolation, and in combination with cow dung equally performed better than others. Consequently, there was no significant difference (α=0.05) between the numbers of leaves produced by plants grown on samples treated with poultry wastes, in isolation, and in combination with cow dung. But they were significantly different (α=0.05) from numbers of leaves recorded from samples for control and using cow dung alone.

On week 4, the results recorded from the samples indicated increments in number of leaves by plants grown on the samples. The number of leaves ranges from 3.30±0.5 to 8.0±1.4. Similarly, corn seedlings grown on treated Pb-polluted soil samples produced more leaves than those on the control samples. Among all the treated Pb-polluted soil samples, results showed that seedlings grown on treatment with combination of cow dung and poultry wastes slightly produced more leaves. It recorded 8.0±0.8, 7.3±0.5 and 7.3±0.5 numbers of leaves at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg respectively. On the other hand, control samples recorded 3.3±0.5, 5.0±0.8 and 4.0±0.8 numbers of leaves at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg respectively. Statistical analysis revealed that there is no significant difference (α=0.05) between the numbers of leaves produced by seedlings at various concentrations of Pb studied. Similarly, there is no significant difference between the treated samples. However, control samples are significantly different (α=0.05) from all the treated samples.

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At week 7 of treatment, the results showed that the number of leaves produced by corn seedlings ranged from 5.3±0.5 to 13.0±1.4 in Pb-polluted soil samples. Seedlings grown on treated soil samples had more numbers of leaves than were found from different concentrations of Pb pollution. Among all the treated samples, seedlings grown on samples treated with poultry wastes alone produced more leaves than the rest. It recorded 13.0±1.4, 13.0±1.4 and 12.3±0.9 leaves at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg respectively. This compares to 6.0±0.0, 7.3±0.9 and 5.3±0.5 leaves recorded at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg respectively, for the control samples. Statistically, all the treated samples are significantly different from the control. Moreover, the number of leaves produced by seedlings that were treated with poultry dung alone was significantly different (α=0.05) from those of other treatments used.

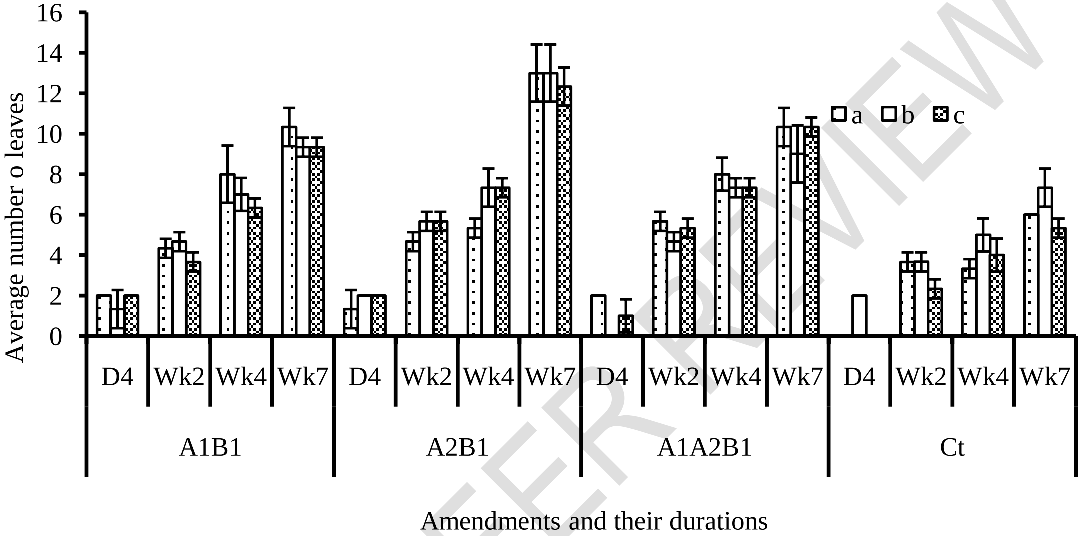

Fig. 6.Effects of different amendments on number of leaves of plants over time.Letters a, b and c represent 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively.

On day 4 of planting, range of plant height was from 2.3±0.3 cm to 4.6±0.3 cm (Figure 7). On the average, it was observed that there was better growth in the soil sample that was treated with poultry wastes in isolation. It recorded 2.9±0.3 cm at 32.75 mg/kg, 4.0±0.2 cm at 42.02 mg/kg and 4.6±0.3cm at 52.44mg/kg Pb pollution. In control samples, it was 2.6±0.3cm, 3.3±0.2 cm and 2.7±0.2 cm at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution respectively. However, there was no significant difference between all the results obtained. There is no significant difference (α=0.05) between the numbers of leaves produced as well as the heights of seedlings recorded across the various concentrations for the treatment carried out with cow dung in isolation.

With respect to plant heights, the results obtained on week 2 showed a range of 16.3±0.5 cm to 20.1±1.0 cm. Plant heights in all the treated samples were higher than those of the control, which recorded 14.1±0.6 cm, 16.3±0.4 cm and 11.8±0.6 cm at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg respectively. Comparatively, plants grown on samples treated with cow dung alone were the tallest, with 20.1±1.0 cm, 20.1±1.2 cm and 17.6±0.5 cm at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg respectively. However, there is no significant difference (α=0.05) between the heights across different samples and concentrations studied.

For the heights of seedlings, there were variations among the samples, as well as concentrations used in the study. The range of heights of all the plants is from 37.0±4.5 cm to 74.5±1.7 cm. Seedling cultivated on treated Pb-polluted soil samples grew taller than those on the control samples. Particularly, seedlings on soil samples treated with combination of poultry wastes and cow dung outperformed all other treatments, recording heights of 74.5±1.7 cm, 54.3±2.1 cm and 69.6±1.8 cm at

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32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg respectively. Though heights of seedlings reduced with increasing concentrations of Pb in the soil, in most cases, the variations observed are not significantly different from each other, including the controls. Statistical analysis showed that though there is no significant different among the treated samples, the variations between all treated samples and the control is significant (α=0.05).

In terms of height of plants on week 7 of treatments, the results showed a range of 69.2±6.8 cm to 125.1±4.1 cm. Observation showed that plants grown on control samples recorded the poorest growth in height, with 69.8±7.2 cm, 69.8±6.8 cm and 71.5±9.5 cm at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg Pb levels of pollution respectively. Among the treated samples, tallest seedlings with heights of 119.3±9.1 cm, 122.5±4.9 cm and 125.1±4.1 cm were recorded at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg Pb levels of pollution respectively, for soil samples treated with poultry wastes only. Similar to the number of levels, the height of plants in control samples were significantly different (α=0.05) from all the treated samples. Also, seedlings on poultry wastes-treated soil samples are significantly different from those cultivated on cow dung treatment alone.

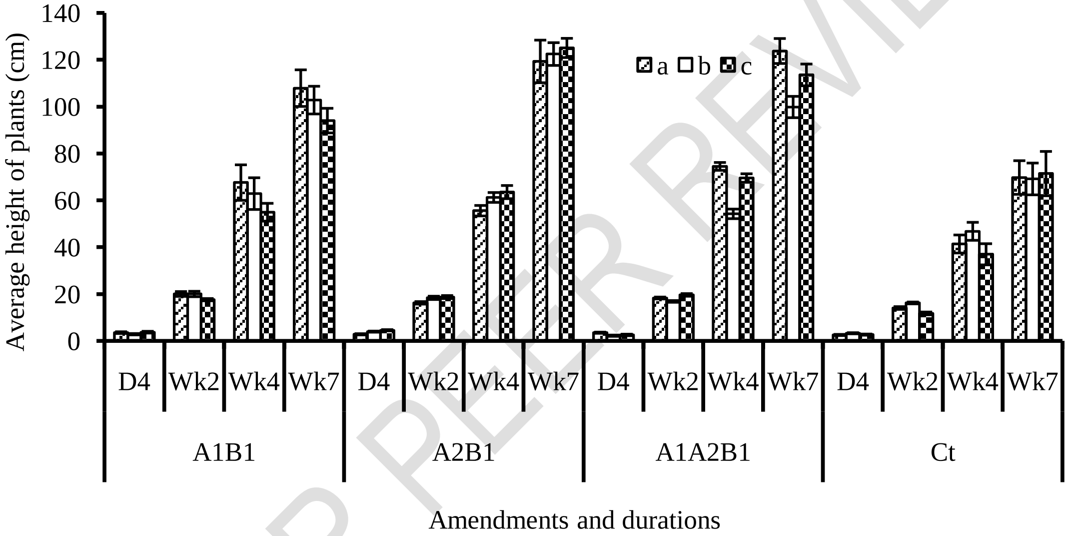

Fig. 7.Effects of different amendments on heights of plants over time. Letters a, b and c represent 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively.

According to the findings of a previous study, in terms of growth analysis, heavy metal contamination significantly decreased the fresh weights of shoot and root systems, with the impairments in the shoot system being higher than in the root system (-91% and -72%, respectively; $p < 0.05$). Similar reduction in dry biomass (-72%; $p \leq 0.05$) were noted in the roots, but there was less damage in terms of dry biomass (-88% vs. -91%; both $p \leq 0.05$) above ground, indicating altered water status/turgor in plants stressed by metal [19]. Contamination resulted in a relatively significant reduction in the fraction (%) of finer roots with respect to the distribution of total root length and surface area across root diameter intervals. Root surface area was reduced even further (-65% and -70% for the two diameter classes, respectively), and root length was reduced by 43% in the 0.25–0.50 mm diameter interval and 30% in the <0.25 mm diameter interval [19. Al-Wabel et al. [29] have shown that the application of biochar resulted in a considerable decrease in the buildup of harmful metals and an increase in maize growth and biomass output.

3.5 Effects on chlorophyll and proteins contents

After seven weeks of treatment of Pb-polluted soil samples (C1), protein and chlorophyll content were determined. Results of average protein contents ranged from 26.27±27 mg/g to 161.25±5.5 mg/g, signifying a large variation in protein contents of the samples. Among the treated samples, seedlings

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grown on samples treated with poultry wastes (A2) only, recorded the highest protein content, with 161.25±5.5 mg/g, 104.99±4.95 mg/g and 79.75±3.2 mg/g protein contents, at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively. On the other hand, the lowest protein contents were found in control samples (C1), with 47.83±2.06 mg/g, 33.18±2.16 mg/g and 26.27±2.01 mg/g protein contents, at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively (Figure 8). Analysis of results, at $\alpha=0.05$, indicated that the protein contents of corn seedlings grown on all the samples were similar, except for those on samples treated with *Bacillus* sp and poultry wastes only, which was significantly higher than all the other treated samples and control. Also, protein contents across different concentrations were similar, except at 32.75 mg/kg, which was significantly different from others. From the results, it was evident that the protein contents of treated samples were much higher than those of seedlings on control samples.

Similarly, a study had shown that when biochar and compost were applied, there was a significant increase ($p < 0.05$) in the transpiration rate, photosynthetic rate, total chlorophyll contents, and stomatal conductance, compared to control samples which had the lowest values. The plants on soil treated with 4% biochar and irrigated with the highest rate of compost application showed the highest values of each of these parameters. Comparing the application of 4% biochar to the control, there was an increase in total chlorophyll levels of 92%, photosynthetic rate of 138%, transpiration rate of 125%, and stomatal conductance of 125% [20]. The enhancement of soil fertility parameters, such as nitrogen and organic matter contents, as well as the phytostabilization of Pb, Cd, and Cr with these organic amendments, may be the cause of the improvement in the growth parameters of maize plants. It is possible that the deformation of the chloroplast is the cause of the decrease in chlorophyll content under metal stress [20].

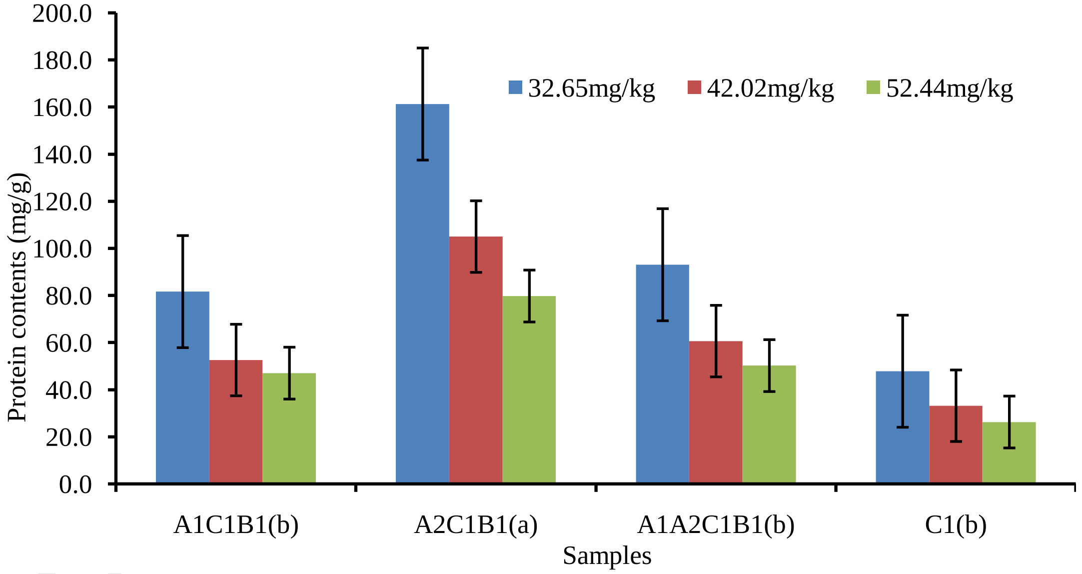

Fig. 8. Protein contents of corn seedlings grown on different concentrations of Pb-polluted soil samples treated with different samples. Legends: A1 is cow dung, A2 is poultry wastes, B1 is *Bacillus* sp and C1 is Pb. Samples with similar lowercase letters (in bracket) are not significantly different at α=0.05).

For total chlorophyll contents measured on week 7, the range among corn seedlings was 0.34±0.02 mg/g to 1.04±0.11 mg/g, which represented an appreciable difference in total chlorophyll contents of the samples. From observations, total chlorophyll contents of seedlings on treated samples were higher than those on control samples. Among them all, seedlings on samples treated with a blend of cow dung and poultry wastes yielded the highest total chlorophyll contents, recording 1.04±0.11 mg/g, 0.83±0.02 mg/g and 0.47±0.02 mg/g of total chlorophylls, at 32.75 mg/kg, 42.02 mg/kg and

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52.44 mg/kg levels of Pb pollution, respectively. Conversely, the least total chlorophyll contents were recorded in control samples, with 0.49±0.06 mg/g, 0.43±0.05 mg/g and 0.34±0.02 mg/g of total chlorophyll, at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively, as shown in Figure 9. Statistical analysis at α=0.05, indicated that chlorophyll contents of seedlings on all the treated samples were the same. However, only those on samples treated with poultry wastes only, and its combination with cow dung, were significantly different from chlorophyll contents of seedlings on control samples. In terms of concentrations of Pb pollution, chlorophyll contents of seedlings on 32.75 mg/kg Pb pollution were significantly different from those of other concentrations studied.

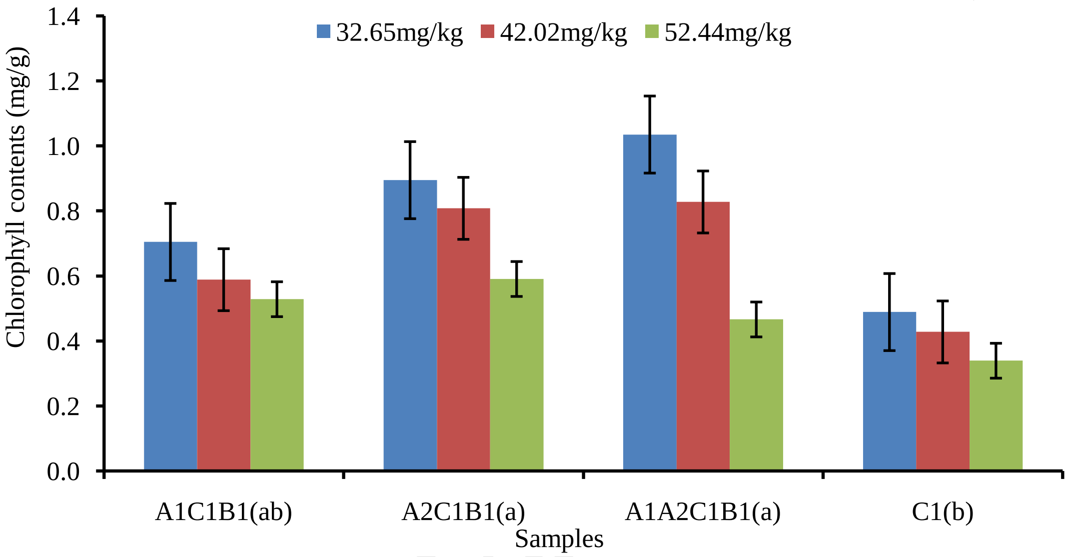

Fig. 9. Chlorophyll contents of corn seedlings grown on different concentrations of Pb-polluted soil samples treated with different samples. Legends: A1 is cow dung, A2 is poultry wastes, B1 is *Bacillus* sp and C1 is Pb. Samples with similar lowercase letters (in bracket) are not significantly different at α=0.05)

It has been reported that the application of organic fertilizers, such as manure and sewage sludge, significantly altered the chemical, physical, and biological characteristics of the rhizosphere and raised the biomass of maize and sunflower, as well as the populations and activity of microorganisms in the soil [30]. By altering the physiological and morphological properties of plants, boosting soil fertility, and enhancing the physical qualities of the soil, they increased the production of biomass and uptake of heavy metals [31]. The dry matter of maize increased with addition of minor amount of heavy metals in conjunction with fertilizer treatment. Nevertheless, the maize plant's ability to produce dry matter was reduced when more additional Cd, Ni, Pb, and Cr were added. The concentrations of heavy metals in the roots and shoots of the maize plant increased in tandem with the decrease in dry matter.

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3.6 Effects on bioaccumulation of Pb in corn seedling

For treated Pb-polluted soil samples, the concentrations of Pb found in corn leaf, stem and root samples are shown in Figure 10. These ranged from 1.55±0.04 mg/kg to 3.80±0.05 mg/kg, 0.85±0.04 mg/kg to 1.84±0.1 mg/kg, 4.14±0.07 mg/kg to 8.70±0.15 mg/kg in the leaf, stem and root samples respectively. The widened range of concentration revealed a significant variation in Pb concentration in the each set of samples. In all the samples, results indicated that corn seedlings grown on control samples significantly bioaccumulated higher concentrations of Pb than in those of the treated soil samples. Leaf samples from corn seedlings grown on soil samples treated with poultry wastes only and *Bacillus* sp recorded the lowest concentrations of Pb, with 1.55±0.04 mg/kg, 1.61±0.03 mg/kg and 1.79±0.05 mg/kg at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively. This is comparably lower than the concentrations in the control samples, where the average Pb concentrations were 2.73±0.08 mg/kg, 3.14±0.16 mg/kg, and 3.80±0.05 mg/kg at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively. Bioaccumulation of Pb in leaf samples increased as levels of Pb pollution increased in the soils. Statistical analysis at $\alpha=0.05$, shows that only the bioaccumulation recorded in leaves samples from control soil samples is significantly different from all others. On the other hand, it was not significantly different in all other samples. Bioaccumulation was shown to be Pb concentration dependent, as it increased significantly with increasing PB pollution. Thus bioaccumulation at 32.75 mg/kg Pb pollution was significantly different from that of leaf samples at 52.44 mg/kg.

In stem samples, lowest concentrations of Pb were found in seedlings grown on soil samples treated with *Bacillus* spp and cow dung only, which recorded 0.85±0.04 mg/kg, 1.02±0.04 mg/kg and 1.19±0.05 mg/kg at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively. Similarly, all samples from treated soils recorded significantly lower Pb concentrations than those on control soil samples, which were 1.47±0.37 mg/kg, 1.57±0.1 mg/kg and 1.87±0.1 mg/kg at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively. Statistically, at $\alpha=0.05$, bioaccumulation of Pb in samples on control was significantly different from all others. Also, bioaccumulation in samples from soils treated with cow dung and *Bacillus* spp only was significantly different from others, except for that of samples on poultry wastes and *Bacillus* spp only. On comparing the bioaccumulation of Pb in stem with respect to concentrations, it was found that it varied significantly across the three concentrations used in this study.

In root samples, it was found that samples obtained from soil samples treated with *Bacillus* sp and a combination of cow dung and poultry wastes recorded the lowest bioaccumulation of Pb, with 4.14±0.07 mg/kg, 4.31±0.03 mg/kg and 4.48±0.07 mg/kg at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively. This was significantly lower than those of control samples, which recorded 7.72±0.05 mg/kg, 8.25±0.1 mg/kg and 8.7±0.15 mg/kg of Pb at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Pb pollution, respectively. At $\alpha=0.05$, statistical analysis of results indicated that bioaccumulation in root samples of corns from treated soils were not significantly different. However, they were significantly different from that of samples on control. In terms of concentration, bioaccumulation at 52.44 mg/kg level of Pb pollution was significantly different from that of samples from on 32.75 mg/kg Pb pollution.

The safe limit of Pb in whole plants, as established by the FAO/WHO-Codex Alimentarious Commission in 1984, is 5 µg.g^{-1}. An earlier study had inferred that though lower concentration of Pb was recorded in test plant samples, compared to control samples, they were still over recommended limits. It showed that the levels of heavy metals in samples studied varied greatly, with Pb having a range of 0.64−5.97 µg.g^{-1}. Because there is no effective mechanism for the removal of heavy metals from the human body, it is risky to consume vegetables grown in soils contaminated with toxic metals from industrial activities. This is because they would have absorbed and accumulated heavy metals in their edible and non-edible parts in amounts that can cause clinical problems for animals and humans overtime [32]. A study had reported that the order of heavy metal concentrations in different plant components, specifically in roots, leaves, and stems, was determined to be roots>leaves>stem [32]. According to Taalab and Shahin [33], the concentration of heavy metals in roots increased significantly more than in shoots.

Soil amendments have shown significant potential in reducing Pb bioavailability [34]. The fact that, despite becoming less bioavailable, the overall concentration of metal(loid)s in soils does not change poses a significant inherent issue with the application of organic amendments for the immobilization of these substances. Through natural weathering or the dissolution of organic matter-metal(loid) complexes, the immobilized metal(loid)s may eventually become plant accessible [34]. Soils contaminated with poisonous metal(loid)s can be efficiently remedied by using organic amendments

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low in metal(loid)s. By adsorption and complexation processes, the application of organic amendments lowers the bioavailability of metal(loid)s, which in turn inhibits their transfer through plant uptake and leaching [34]. The amounts of heavy metals in these samples varied greatly; for example, the quantities of Pb were 0.64−5.97 μg g^{-1}, Cd was 0.22–1.28 μg g^{-1}, Ni was 4.02–46.5 μg g^{-1}, Co was 0.39–2.62 μg g^{-1}, and Cr was 1.71–6.99 μg g^{-1}. Growing on highly polluted land, spinach roots were shown to acquire significant levels of Cd, Ni, and Cr (1.28, 46.5, and 2.62 μg g^{-1}, respectively). Red amaranth stems in low-level polluted areas had low concentrations of Pb (0.64 μg g-1) whereas spinach leaves had greater concentrations of Pb (5.97 μg g-1) [32]. Conversely, a previous study had shown that as a result of the addition of organic waste, including poultry, pig, and cow manure, the uptake of metal concentration increased in plants [35]. According to Egene et al. [36], while the use of certain organic modifiers, such biochar, decreases the mobility of heavy metals, the addition of modifiers, like compost and nitrogen fertilizers, enhances the availability and concentration of heavy metals in plants.

4. CONCLUSION

The study was aimed at determining the effects of amendment of bioremediation of lead-polluted soil with composites of animal dungs and *Bacillus* sp on growth patterns of corn seedlings. Results obtained indicated that amended bioremediation improved the overall pattern of growth of corn seedlings, compared to the control. On the other hand, results showed that amendments decreased the Pb bioaccumulation potentials of the seedlings. However, it was observed that none one specific organic amendment was able to provide the best effects on all the growth parameters studied. Thus, in some cases application of cow dung alone or poultry waste alone yielded best results, on other cases, their composites outperformed others. Nevertheless, the percentage of best performance recorded for composites of the two organic amendments was higher than other treatments. Consequently, application of composites of cow dung and poultry wastes in bioremediation of heavy metals polluted soil is advocated.

REFERENCES

1 Anuforo HU, Akujobi CO, Umeh PK, Ejimadu PI. Pattern of distribution and concentration of selected heavy metals in farmlands near roadsides in Owerri, Nigeria.AnaleleUniversităţii din Oradea, FasciculaBiologie. 2020;22(1):32-38.

2 Bakshi S, Banik C, He Z. The impact of heavy metal contamination on soil health. In: Reicosky A, editor. Managing soil health for sustainable agriculture, 2(8): 1–36 2018.

3 Nyiramigisha P, Komariah R, Sajidan S. Harmful impacts of heavy metal contamination in the soil and crops grown around dumpsites. Reviews in Agricultural Science. 2021;9:271-282. https://dx.doi.org/10.7831/ras.9.0_271

4 Manj´on I, Ramírez-Andreotta MD, S´aez AE, Root RA, Hild J, Janes MK, et al. Ingestion and inhalation of metal(loid)s through preschool gardening: an exposure and risk assessment in legacy mining communities, Sci. Total Environ. 2020;718:134639. https://doi.org/10.1016/j.scitotenv.2019.134639.

5 S´anchez-Castro I, Molina L, Prieto-Fern´andez M, Segura A. Past, present and future trends in the remediation of heavy-metal contaminated soil-remediation techniques applied in real soil-contamination events. Heliyon. 2023;9(e16692):1-24.

6 Clemens S, Ma JF. Toxic heavy metal and metalloid accumulation in crop plants and foods.Annu.Rev. Plant Biol. 2016; 67:489-512. https://doi.org/10.1146/annurev-arplant-043015-112301.

7 He Z, Shentu J, Yang X, Baligar VC, Zhang T, Stoffella PJ. Heavy metal contamination of soils: sources, indicators, and assessment. Journal of Environmental Indicators 2015; 9:17-18.

UNDER PEER REVIEW

8 Liu L, Li W, Song W, Guo M. Remediation techniques for heavy metal-contaminated soils: principles and applicability. Sci. Total Environ. 2018;633:206-219. https://doi.org/10.1016/j.scitotenv.2018.03.161.

9 Singh P, Siddiqui H, Sami F, Arif Y, Bajguz A, Hayat S. Cadmium: A threatening agent for plants, in plant responses to soil pollution. Pollut. 2020;59–88.

10 Su C, Jiang L, Zhang W. A review on heavy metal contamination in the soil worldwide: Situation, impact and remediation techniques. Environ. Skept.Critics.2014;3(2):24-38.

11 Rongxin G. Xusheng Z, Yiwen Y, Dexun Z, Yanping L. Remediation of heavy metals contaminated soils: a bibliometric network analysis. J Soil Water Sci. 2021;5:18-29. https://doi.org/10.36959/624/446.

12 Amin M, Rakhisi Z, Ahmady AZ. Isolation and identification of *Bacillus* species from soil and evaluation of their antibacterial properties. Avicenna J ClinMicrobInfec.2015;2(1):e23233.

13 Shambhavi RK, Akash T, Purushottam JS, Shishu PS. Isolation and identification of *Bacillus* species from soil for phosphate, potassium solubilisation and amylase production. International Journal of Current Microbiology and Applied Sciences. 2020;9(5):415-426. https://doi.org/10.20546/ijcmas.2020.905.046

14 Lu KP, Yang X, Gielen G, Bolan N, Ok YS, Niazi NK, et al. Effect of bamboo and rice straw biochars on the mobility and redistribution of heavy metals (Cd, Cu, Pb and Zn) in contaminated soil. Journal of Environmental Management.2017;186:285–292.

15 Wojewódzki P, Lemanowicz J, Debska B, Haddad SA. Soil enzyme activity response under the amendment of different types of biochar.Agronomy. 2022;12:569. https://doi.org/10.3390/agronomy12030569

16 Nwinyi OC, Akinmulewo BA. Remediation of soil polluted with spent oil using cow dung. IOP Conf. Series: Earth and Environmental Science. 2019;331(012058): doi:10.1088/1755-1315/331/1/012058

17 Sarkar S, Mondal M, Ghosh P, Saha M, Chatterjee S. Quantification of total protein content from some traditionally used edible plant leaves: A comparative study. Journal of Medicinal Plants Studies. 2020;8(4):166-170. https://doi.org/10.22271/plants.2020.v8.i4c.1164

18 Naser HM, Rahman, MZ, Sultana S, Quddus, MA, Hossain, MA. Heavy metal accumulation in leafy vegetables grown in industrial areas under varying levels of pollution.Bangladesh J. Agril. Res. 2018;43(1):39-51.

19 Romdhane L, Panozzo A, Radhouane L, Dal Cortivo C, Barion G, Vamerali T. Root characteristics and metal uptake of maize (*Zea mays* L.) under extreme soil contamination. Agronomy. 2021;11(178):1-14. https://doi.org/10.3390/agronomy11010178

20 Irfan M, Mudassir M, Khan MJ, Dawar KM, Muhammad D, Mian IA, Ali W, et al. Heavy metals immobilization and improvement in maize (*Zea mays* L.) growth amended with biochar and compost. Scientific Reports. 2021;11(18416):1-9. https://doi.org/10.1038/s41598-021-97525-8.

21 Ding Z, Alharbi S, Almaroai YA, Eissa MA. Improving quality of metal-contaminated soils by some halophyte and non-halophyte forage plants. Sci. Total Environ. 2021;794:142885.

22 Li J, Chang Y, AL-Huqail AA, Ding Z, Al-Harbi MS, Ali EF et al. Effect of manure and compost on the phytostabilization potential of heavy metals by the halophytic plant wavy-leaved saltbush. Plants. 2021;10(2176):1-13. https://doi.org/10.3390/plants10102176

23 Ramli H, Azim K, Daoud S, Harouni MC. Effect of organic amendments on soil fertility and quality of organic zucchini under greenhouse condition. ActaHortic. 1286. ISHS 2020 XXX IHC – Proc. II Int. Symp. on Organic Hort. for Wellbeing of the Environ. and Population. 2020:61-72. https://doi.org/10.17660/ActaHortic.2020.1286.10

24 Aytenew M, Bore G. Effects of organic amendments on soil fertility and environmental quality: A review. J. Plant Sci. 2020;8:112–119.

25 Eissa MA. Effect of compost and biochar on heavy metals phytostabilization by the halophytic plant old man saltbush [*Atriplexnummularia*Lindl].Soil Sediment Contam. 2019;28:135-147.

UNDER PEER REVIEW

26 Xia Y, Liu H, Guo Y, Liu Z, Jiao W. Immobilization of heavy metals in contaminated soils by modified hydrochar: Efficiency, risk assessment and potential mechanisms. Sci. Total Environ. 2019;685:1201-1208.

27 Adewole MB, Oyebanji BO, Igbekele, K. Phytoremediation potential of two maize varieties cultivated on metal-particulate-contaminated soil. Ghana Jnl Agric. Sci. 2019;54(1):38-46. https://dx.doi.org/10.4314/gjas.v54i1.4

28 Chukwuma CC, Monanu MO, Ikewuchi JC, Ekeke C. Variance in protease, dehydrogenase, phosphatase and respiratory activities during phytoremediation of crude oil polluted agricultural soil *Using Schwenkiaamericana*L. and *Spermacoceocymoides*Burm. f. Annual Research & Review in Biology. 2018;28(6):1-9.

29 Al-Wabel MI. Conocarpusbiochar as a soil amendment for reducing heavy metal availability and uptake by maize plants. Saudi J. Biol. Sci. 2015;22:503-511.

30 Sedghiani M, Sepehr, E. The effect of application of sewage sludge and livestock fertilizers on nitrogen mining and rhizosphere characteristics of corn and sunflower plants. J. Water Soil. 2011;25:327-337.

31 Lin Z, Dou C, Li Y. Nitrogen fertilizer enhances zinc and cadmium uptake by hyperaccumulator*Sedum alfredii*Hance. J Soil Sediment. 2019;31-45. doi:10.1007/s11368-019-02405-4.

32 Naser HM, Rahman MZ, Sultana M, Quddus SA, Hossain, MA. Heavy metal accumulation in leafy vegetables grown in industrial areas under varying levels of pollution.Bangladesh Journal of Agricultural Resources.2018;43(1):39-51.

33 Taalab AS, Shahin RR. Effect of fertilizer application on the uptake of heavy metals by corn plant grown in different soils.Middle East Journal of Agriculture Research.2018;07(02):505-511.

34 Park JH, Lamb D, Paneerselvam P, Choppala G, Bolan N, Chung J. Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils. Journal of Hazardous Materials.2011;185:549-574. 10.1016/j.jhazmat.2010.09.082

35 Hussaina M, Liaqata I, Bukharib SM, Khanc FS, Adalatc R, Salman M, et al. The impact of cow dung augmentation on soil restoration and bio-accumulation of metals (Lead and Cadmium) in *Pheretimaposthuma* (Annelida: Clitellata). Brazilian Journal of Biology. 2023;83(e247562):1-7. https://doi.org/10.1590/1519-6984.247562

36 EgeneCE, Van-PouckeR, Ok YS, MeersE, Tack FMG. Impact of organic amendments (biochar, compost and peat) on CD and Zn mobility and solubility in contaminated soil of the campine region after three years.Sci Total Environ. 2018;626:195-202.

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