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Evaluation of effects of animal dung composites and *Bacillus* sp in amended bioremediation of Zn-polluted soil on maize seedlings growth

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Abstract

Heavy metal pollution of arable soils reduces their productivity, hence the need for ecofriendly techniques to ameliorate this increasing menace. In this study, cow dung, poultry wastes and their composites were applied as organic supplements, together with Bacillus sp as plant growth promoting rhizobacteria to amend bioremediation of zinc-polluted soil sample. Effects of amendments on physicochemical, mineral contents, and dehydrogenase activity of soil, as well as on corn seedlings growth rate, protein and chlorophyll contents and bioaccumulation of Zn were analyzed. Results demonstrated an increase in pH and nutrient contents including N, organic carbon, soil organic matter, K etc., of amended soil samples. Amendment with composite of two organic supplements produced highest increase in dehydrogenase activity, recording 2.35±2.04 µgTPFg-1h-1 at 33.75 mg/kg, and 2.19±0.04 µgTPFg-1h-1 at 52.55 mg/kg level of Zn pollution. Number of leaves recorded in most samples reduced with increasing Zn pollution, with seedlings grown on soil samples amended with cow dung alone outperforming others and recorded 10.3±0.5, 11.3±0.5 and 12.3±0.5 leaves at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. Heights of seedlings in most of amendments also decreased with increasing Zn pollution. Also, seedlings grown on samples treated with cow dung alone were tallest, and recorded 109.0±3.4 cm, 115.8±4.3 cm and 127.3±2.6 cm at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. Zn bioaccumulation different parts of corn seedlings was generally in the order; roots>leaves>stems, while it was; control samples>composite of poultry wastes and cow dung samples>only poultry wastes samples>only cow dung samples, for various treatments. This buttresses the usefulness of organic amendments, especially cow dung, in bioremediation of Zn-polluted soil used for agricultural activities.

Keywords: Organic amendments; Bioremediation; Soil pollution; Growth rate; Chlorophyll content

1. Introduction

All elements (metals and metalloids) with atomic density higher than 6gcm^{-3} are generally referred to as "heavy metal(loid)s", with the exception of arsenic (As), boron (B), and selenium (Se) [1]. Both physiologically essential elements (such as copper (Cu), manganese (Mn), cobalt (Co), chromium (Cr), and zinc (Zn) and non-essential elements (such as lead (Pb), cadmium (Cd), and mercury (Hg)) are included in this group. Some essential elements, also referred to as "trace elements" or "micro nutrients," are needed in small amounts, while some known as macro nutrients are required in larger quantities for the nourishment of plants, animals, and humans. However, at excessive quantities, both groups are poisonous to humans, animals, and/or plants. The non-essential metal(loid)s are referred to as "toxic elements" because they are zootoxic and/or phytotoxic [2].

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Through both anthropogenic and pedogenic (or geogenic) activities, heavy metal(loid)s are introduced into the soil environment. In soil parent materials, the majority of metal(loid)s are found naturally, primarily in forms that are inaccessible to plants. The metal(loid)s in the parent materials are generally not soluble enough to be absorbed by plants, which results in minimal effects on soil organisms. According to Romdhane et al. [3], origin and nature of the parent materials play a major role in the concentrations of metal(loid)s released into the soil system by natural pedogenic (or weathering) processes. Anuforo et al. [2] included the following human activities, such as vehicle emissions, smelting, manufacturing, municipal trash, pesticide and fertilizer use in agriculture, and industrial effluents, as contributors of increased release of heavy metals into the environment.

There are increasing concerns among health authorities worldwide, over the impact of heavy metal(loid)s on human and environmental health, as well as global trade [1]. Pump and treat systems, incineration, soil vapour extraction, and containment are examples of conventional techniques used to eliminate, minimize, or lessen harmful compounds released into soil or ground water through anthropogenic activities and processes. According to Roy et al. [4], there are several obvious disadvantages to using any of these traditional methods for treating contaminated soil, water, or both. These include the fact that they frequently harm the surrounding ecology, are costly, and need treating vast amounts of hazardous wastes. Another way to clean up contaminated environments is by the use of bioremediation; a developing field of research and technology [1].

The term "cow dung" refers to the partially digested leftovers of meals that herbivorous bovine animals defecate. It is a mixture of urine and faeces in the ratio 3:1, and primarily composed of cellulose, lignin, and hemicelluloses, along with trace amounts of iron, copper, magnesium, manganese, cobalt, and sulphur. It also includes 24 distinct minerals, including potassium and nitrogen [5]. Numerous bacterial genera, including *Enterobacter aerogenes, Citrobacter koseri, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Morgarella morganii, Kluyvera spp., Providencia alcaligenes, Pasteurella spp., Providencia stuartii,* and *Pseudomonas* spp., were isolated from cow dung by Sawant et al. [6]. Cow dung is utilized in agriculture as a co-product for manure, biopesticides, pest repellent, biofertilizer, and energy production [5].

One of the most common bacterial genera in soil is the genus *Bacillus*, of which numerous species have been identified from a variety of biological settings. With their immense genetic and metabolic diversity, *Bacillus* species play a variety of ecological roles in the soil environment, ranging from nutrient cycling to providing plants with stress tolerance. Numerous advantageous characteristics are known to exist in members of the genus *Bacillus*, which benefit plants either directly or indirectly through nutrient uptake, defense against infections and other abiotic stresses and general growth enhancement through the synthesis of phytohormones [7]. Numerous *Bacillus* species, including *B. circulans, B. megaterium, B. velezensis, B. subtilis, B. coagulans, B. macerans, B. azotofixans,* etc., have been identified as rhizobacteria that promote plant growth (PGPR) [8]. PGPR refers to a group of free-living beneficial bacteria that provide agricultural plants with health benefits [9].

Therefore, there is a pressing need to investigate cost-effective and environmentally friendly ways to lower the bioavailability of heavy metals and guarantee food security [10]. This study was focused on applying a composite of cow dung, as an organic amendment, together with *Bacillus* sp, as PGPR, to remediate Zn-polluted soil and evaluate their effects on the growth characteristics of maize seedlings. This is targeted at furthering studies on developing more effective bioremediation mechanisms.

2. Material and methods

2.1. Soil and animal waste samples collection and processing

Soil sample used in this study were collected from 0–30 cm depth of farmland at the SAAT Farms, Federal University of Technology, Owerri (FUTO), Nigeria, using well cleaned soil auger. It was packaged in sterilized plastic pouches and transported to the laboratory where it 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. Furthermore, cow dung (A1) and poultry wastes (A2) samples were obtained from the Animal husbandry Unit of FUTO Farms. They were ground after drying and preserved in clean, sterilized plastic bags.

2.2. Bacterial analysis

Isolation of *Bacillus* sp which served as used as plant growth promoting rhizobacteria (PGPR), was carried out following the inoculation of aliquot of serially diluted soil sample, obtained from FUTO farms, on nutrient agar medium. First, 50 g of soil sample was added to 150 mL of sterilized distilled water and heat treated for 15 minutes at 30 °C. Inoculation was done by spreading 0.1 ml of the soil suspension on already prepared nutrient agar plates. Afterwards, incubation of plates was done at 30 °C for 24-48 h. This was followed by examination of plates for presence of rough and abundant colonies with irregular spreading edge and waxy growth (1-4 mm) diameter. Observed colonies were further Gram stained, and all Gram positive bacilli were sub-cultured on nutrient agar slants for more tests [11]. Then biochemical tests, such as including motility test, crystal formation, penicillin susceptibility test were done following the method of Shambhavi et al. [12]. Isolates with conforming characteristics were then sub-cultured on polymixin egg yolk mannitol bromothymol blue agar (PEMBA) and after incubation, their morphologies were confirmed. After identification, *Bacillus* sp sufficient quantity of cells needed for amendment was obtained by sub-culturing its pure culture on a freshly prepared sterile nutrient broth in cotton wool stoppered Balch tubes. This was incubated for 120 h, at 30 ^{o}C. They were harvested by centrifuging at 3500 rpm for 20 minutes, before washing twice in phosphate buffered saline at pH of 7.25. They were subsequently transferred into sterile Eppendorf tubes, and re-suspended in MS medium. Other autochthonous soil bacterial isolates were isolated and characterized according to the method of Cheesbrough [13] as presented in Anuforo et al. [14].

2.3. Setting up of pot studies

Study of effects of amendment on parameters of growth of corn cultivated on Zn-polluted soil samples, and its bioaccumulation potential in plant organs was carried out. Corn seeds were surface-sterilized by mild washing in turns in 5% sodium hypochlorite, 96% ethanol, and thrice with distilled water. Pollution of soil sample was done by introducing 10 mL of 0.2M zinc nitrate to 50 kg of soil, followed by thorough mixing to give rise to uniform concentrations. Amendment of bioremediation was set up according to the treatments below;

- Cow dung only (A1),
- Poultry waste only (A2),
- Combination of cow dung and poultry waste (A1A2), and
- No amendment (control).

In each case, 1000 g of organic supplement or their combination and 5 kg of Zn-polluted soil were prepared. Introduction of *Bacillus* sp as plant growth promoting rhizobacteria, was done at rate of 50 mg (w/w) per pot. Sterilized distilled water was used for irrigation of the pots, and water content was maintained at about 70% water holding capacity. These were allowed for 25 days to undergo amended bioremediation, before four corn seeds were planted in each of the pots. Treatments were prepared using duplicates. The conditions of greenhouse were 6.86±3.7 h of average sunshine, 30.14±3.8 °C temperature and 40% relative humidity. Upon seeds germination, 3 seedlings in each pot were maintained, and their growth parameters, such as number of leaves and height of shoot were recorded on day 4, weeks 2, 4 and 7. At week 7, corn seedlings and treated soil samples were collected and analysed.

2.4. Determination of soil physicochemical, minerals and Zn compositions

Analysis was done to determine the physicochemical, selected mineral salts and Zn contents of soil samples before and after amended treatments. Soil organic matter and organic carbon contents were obtained by the wet oxidation method. Soil available nitrogen, phosphorous, magnesium, calcium, and potassium contents were determined according to the method of Lu et al. [15]. The pH of soil samples was obtained using pH meter (Mettler Toledo Delta 320). Digestion of soil samples for determination of Zn contents was done as reported reported by Anuforo et al. [2]. Following extraction, bioavailable Zn content of each soil sample was measured using atomic absorption spectroscopy (AAS) (AA-240FS Varian, USA). Moisture content of soil samples was measured following the procedure of Nwinyi and Akinmulewo [16], in which crucibles were dried after washing. Their weights were measured and recorded as W1. This was followed by adding a quantity of each soil sample into sterile weighed crucible dish and new weight recorded as W2. They were dried in the oven at 105° C until constant weight was achieved following repeated weighing after cooling. These new weights were recorded as W3. Differences in weights between the initial and constant final weights which indicated the moisture content were computed using the relationship below;

$$\text{Percentage moisture content } = \frac{W3 - W1}{W2 - W1} \times 100$$

2.5. Determination of dehydrogenase activity of soil

Assessment of dehydrogenase (DEH) activity in soil samples was done according to Thalmann protocol presented by Wojewódzki et al. [17]. This involved incubation of samples using 2,3,5-triphenyltetrazolium chloride, measuring the absorbance of triphenylformazane (TPF) at 546 nm and recording the results in mg TPF kg^{-1} 24 h^{-1}.

2.6. Analysis of chlorophyll and proteins contents

After 7 weeks of growing on amended bioremediated Zn-polluted soil samples, corn leaves, shoots and roots were harvested from each treatment. They were washed under tap water, followed by 0.1N HCl solution for proper decontamination, before rinsing with distilled water and allowed to dry. Leaf samples were then dried in an oven for 72 h at 48 ^{o}C, and ground for analysis. Chlorophyll compositions of corn leaves were determined using the SPAD meter.

In order to establish a standard curve for the extrapolation of protein concentrations from unknown samples, BSA was initially utilized as the standard reagent. The root, leaf, and stem sample extracts were each reacted with 4.5 mL of reagent 1 (1 ml of 1% sodium potassium tartrate + 48 ml of 2% sodium carbonate in 0.1N sodium hydroxide + 1 ml of 0.5% copper sulphate) before being incubated for 15 minutes. Following that, 0.5 ml of freshly made reagent 2 (one part water to one part Folin-Ciocalteau) was added to each sample, and it was incubated for 30 minutes in the dark. The protein content was then measured at 660 nm using absorbance, and it was expressed as mg BSAE/g of fresh weight [18].

2.7. Determination of toxic metal contents of plant

Ground samples of leaves, shoots, and roots of harvested corn seedlings were first digested with di-acid $HClO_4$:HNO_3 mixture for measurement of Zn concentration of plant samples [2]. Then concentration of Zn in each supernatant was measured using atomic absorption spectroscopy (AA-240FS Varian, USA).

2.8. Statistical analysis of data

Mean and standard deviations (SD) of the set of duplicates of each treatment 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 determine variations among each set of data using Minitab® 17 software.

3. Results and discussion

3.1. Effects of amendments on physicochemical properties of Zn-polluted soil

From the results in Figure 1, it was observed that pH of all samples increased after the period of treatment, except for the control in which it decreased. Also, alkalinity increased with increase in concentration of Zn pollution. Among the treated samples, combination of poultry waste and cow dung with *Bacillus* sp produced highest increase in pH of 10.31% at 33.75 mg/kg Zn content.

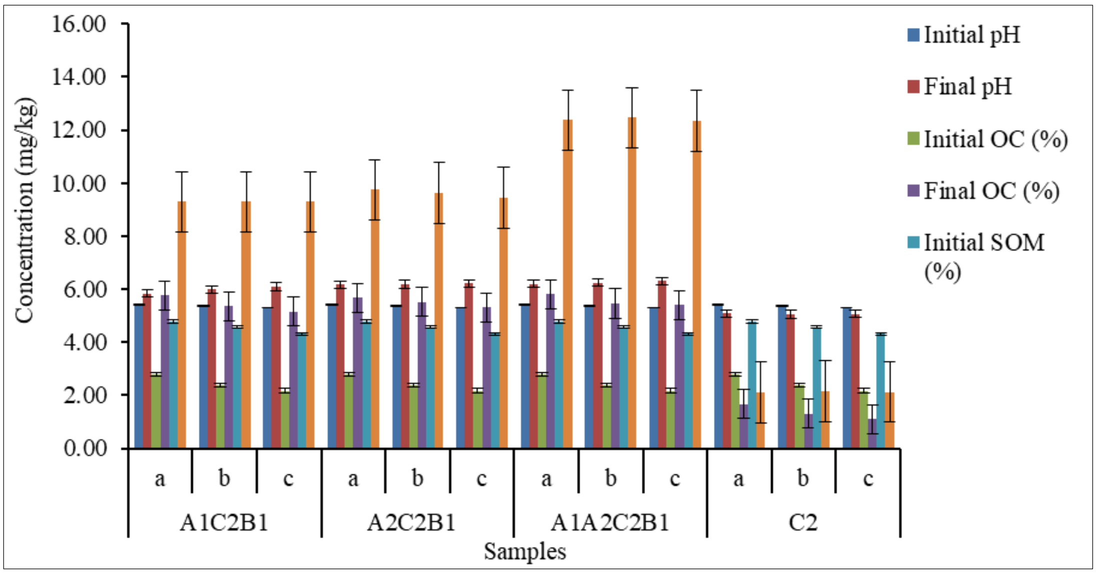

Figure 1 pH, OC and SOM contents in Zn-polluted soil samples before and after treatment. Legends: A1 is cow dung, C2 is Zn, A2 is poultry dung, and B1 is *Bacillus* sp

In the same way, both OC and SOM of all treated Zn-polluted soil samples increased after the period of treatment.

The percentage increases in concentration of OC and SOM increased with increasing concentration Zn-pollution, and were highest (59.82% and 186.50% for OC and SOM respectively) at 52.55 mg/kg Zn-pollution, in treatment carried out with combination of cow dung and poultry wastes. OC and SOM, which decreased in control samples, recorded their maximum reduction of 98% for OC, and 56% for SOM, at 52.55 mg/kg and 33.75 mg/kg Zn-pollution respectively.

The N, Ca and K contents increased after the period of treatment of Zn-polluted soil samples. Generally, among the three nutrients, N recorded highest percentage increase, with poultry waste and *Bacillus* sp outperforming other treatment options, recording 68%, 18% and 12% increase at 33.75 mg/kg Zn pollution, as well as 68%, 9.57% and 21.73% at 52.55 mg/kg Zn pollution. Unlike in Ca, it was observed that concentrations of N and K in sample after treatment increased with increasing concentration of Zn-pollution. Except for K, where there was slight reduction in concentration, after treatment, other control samples recorded increase in concentrations of nutrients, as shown in Figure 2.

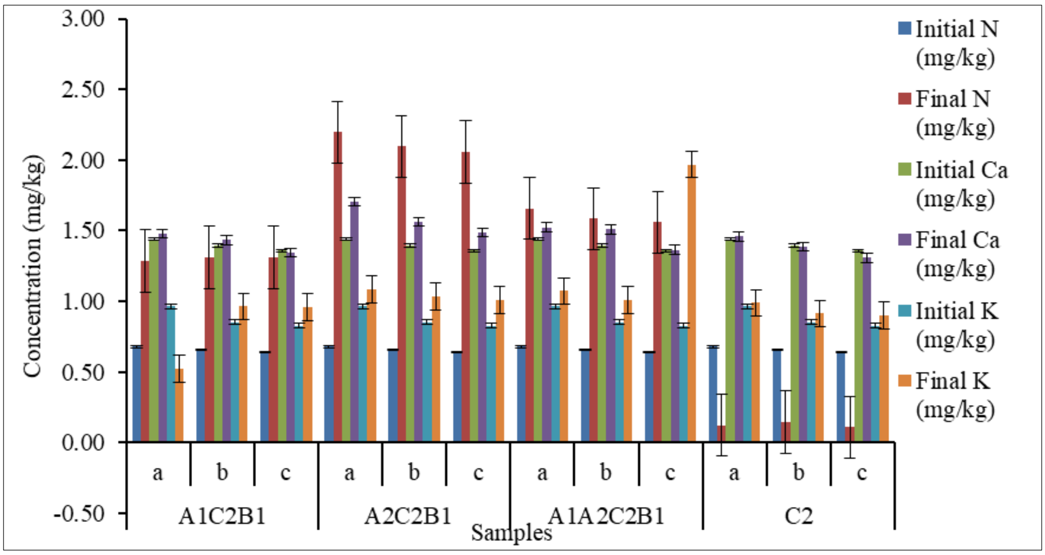

Figure 2 N, Ca and K concentrations in Zn-polluted soil samples before and after treatment. Legends: A1 is cow dung, C2 is Zn, A2 is poultry dung, and B1 is *Bacillus* sp.

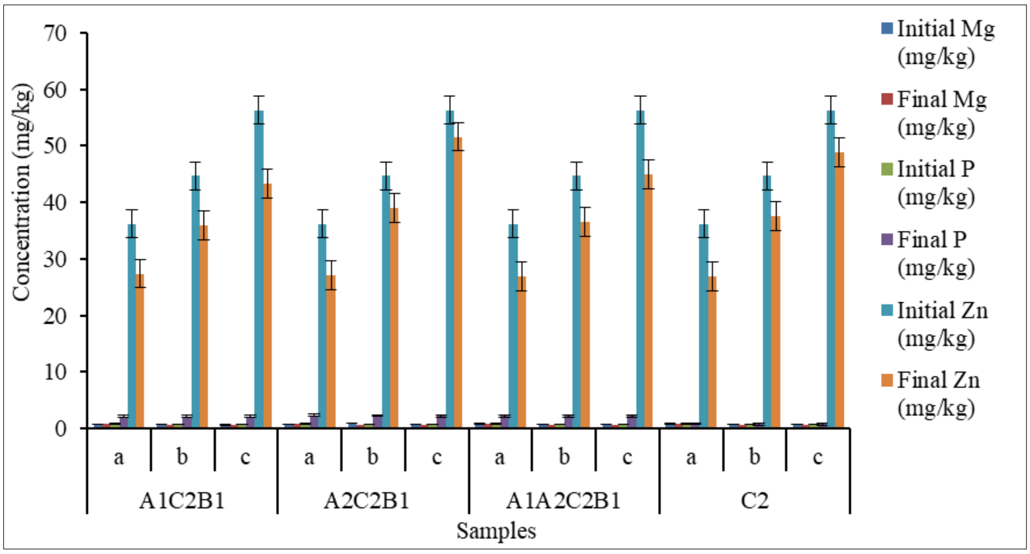

Figure 3 Mg, P and Zn concentrations in Zn-polluted soil samples before and after treatment. Legends: A1 is cow dung, C2 is Zn, A2 is poultry dung, and B1 is *Bacillus* sp.

Conversely, the concentrations of Mg and Zn in the polluted samples reduced after treatment, unlike concentration of P which increased. Reduction was least in samples treated with cow dung and *Bacillus* sp, which recorded 8.13% and 24.26% reduction at 33.75 mg/kg for Mg and Zn respectively; and 7.52% and 19.43% reduction at 52.55 mg/kg Zn pollution. Treatment with poultry waste produced the highest increase in concentration of Mg, with 173.72% increase at 33.75 mg/kg, and 18.87% increase at 52.55 mg/kg, as shown in Figure 3.

The natural process of bioremediation, which can be improved by adding organic amendments to soils, modifies the bioavailability of metal(loid) through the action of soil bacteria and higher plants [1]. Due to their greater biodegradability, lower cost, and improved soil qualities, organic amendments have advantages over inorganic ones when added to polluted soil [19]. When organic amendments are added to acidic and nutrient-poor soils, they have been especially helpful for microbial productivity and plant growth [1]. According to Santiago et al. [20], the pH of rhizosphere soils is frequently lowered by the application of organic amendments such as manures and biosolids. A number of mechanisms are responsible for the acidity of the rhizosphere, including the cation–anion exchange balance, the release of organic acids (such as citric, lactic, malaeic, propanic, oxalic, and butyric acids); the exudation and respiration of roots; and redox-coupled activities involving the consumption or production of H^+ and modifications in the oxidation state of Mn, Fe, and N [1]. However, this is at variance with the findings of the present study in which pH of Zn-polluted soil increased on amendment with organic supplements. Studies have also reported that the incorporation of organic amendments promotes the growth of microorganisms. Treatment of bauxite processing residual sand with a variety of organic additions, including biosolids, green waste compost, wasted mushroom compost, and green waste-derived biochar increased its water retention, mesoporosity, and available water holding capacity, while decreasing bulk density [1]. According to Jones et al. [21], the inclusion of these amendments increased basal respiration, microbial biomass carbon, soluble organic carbon, and the activity of the enzymes, such as l-asparaginase, β-glucosidase, and alkali phosphatase. The soil's microbial activity is enhanced by the increased porosity, which may also boost the oxygen concentration and diffusion.

In Zn-polluted soil samples (C2), moisture content of soil samples slightly increased with increasing concentration of Zn pollution, among all the treated samples. Here, treatment carried out with cow dung alone produced the highest increase in moisture contents, with 8.03±0.86 (63.57%), 12.19±1.58 (96%) and 11.34±1.30 (85%.74%) at 32.75mg/kg, 42.02mg/kg and 52.44mg/kg Zn pollution respectively. In the control sample, there was a decline in moisture content at higher concentrations, with 6.87±0.67 (54.21%) increase, -0.92±0.02 (-7.30%) and 0.88±0.02 (-6.68%) reduction in moisture content. These can be seen in Figure 4.

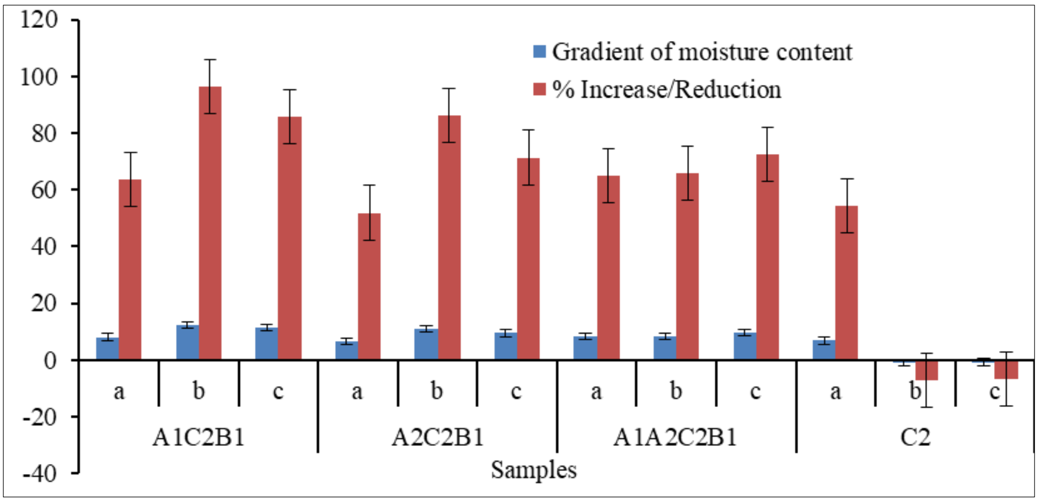

Figure 4 Moisture content and their percentage increase/reduction in Zn-polluted soil samples, treated with mixtures of cow dung, poultry wastes and *Bacillus* sp.

3.2. Effects of amendments on dehydrogenase activities in Zn-polluted soil

After the period of treatment, it was observed that DHA marginally increased in all treated samples, with the gradient ranging from 1.03±0.15 μgTPFg^{-1}h^{-1} to 2.35±2.04 μgTPFg^{-1}h^{-1}. Treatment undertaken with a combination of the two organic supplements yielded the highest increase in DHA, with 2.35±2.04 μgTPFg^{-1}h^{-1} recorded at 33.75 mg/kg Zn pollution, and 2.19±0.04 μgTPFg^{-1}h^{-1} at 52.55 mg/kg Zn pollution. Conversely, DHA reduced in control samples, with 0.03±0.01 μgTPFg^{-1}h^{-1} and 0.04±0.02 μgTPFg^{-1}h^{-1} recorded at 33.75 mg/kg and 52.55 mg/kg respectively, as shown in Figure 5. Dehydrogenase activities of remediated soil groups increased significantly (P=0.05) over the course of four weeks. However, they eventually decreased when compared to those of controls values. The increase could have been caused by an initial rise in the number of microorganisms during the first four weeks, which then declined as available nutrients or carbon sources were depleted over time, resulting in a decline in microbial activity [22].

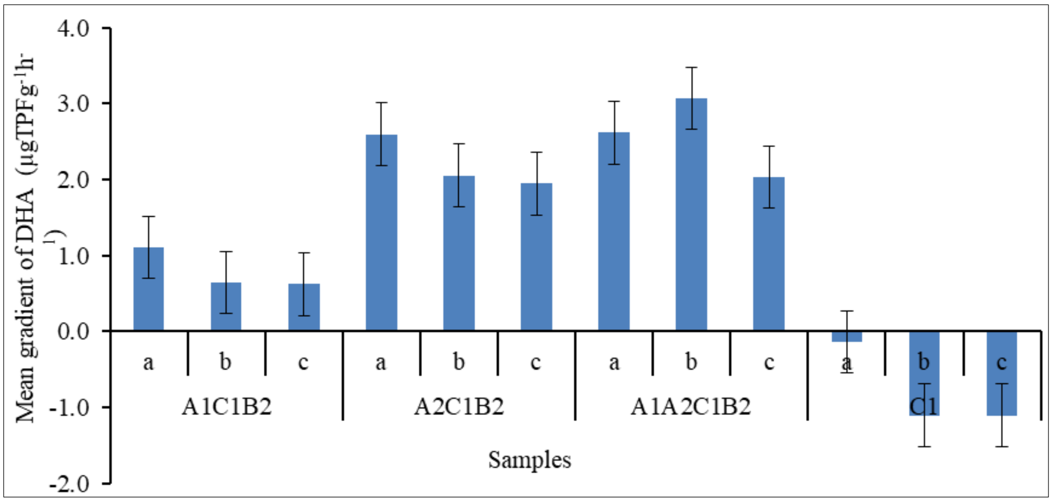

Figure 5 Gradients of DHA in Zn-polluted soil samples after period of treatment. Legends: A1 is cow dung, A2 is poultry wastes, C2 is Zn and B1 is *Bacillus* sp.

3.3. Effects of amendments on bacterial diversity of Zn-polluted soil samples

From the results obtained, there was variation in the dominance and diversities of bacteria in the samples. In the original soil sample, it is observed that bacterial isolates, such as *Salmonella typhi* and *Staphylococcus aureus* were absent, but present in all other treatments. Least diversity of bacteria was recorded in control sample, in which diversity was lower than was obtained in the original sample. Among all the treatments, Zn-polluted soil sample treated with poultry wastes and *Bacillus* sp, produced the most diversity of bacteria, as shown in Table 1. Sawant et al. [23] have isolated many different bacterial genera such as *Enterobacter aerogenes, Citrobacter koseri, Morgarella morganii, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Kluyvera* spp., *Pasteurella* spp., *Providencia stuartii, Providencia alcaligenes,* and *Pseudomonas* spp. from cow dung.

3.4. Effect of amendments on growth rate of corn grown on Zn-polluted soil

The number of leaves recorded on day 4 ranged from 0.0±0.0 to 2.0±0.0. The highest number of leaves was recorded in sample treated with cow dung and *Bacillus* sp, with 2.0±0.0 number of leaves across all the concentrations levels of Zn studied. The control sample, as well as samples treated with poultry wastes in isolation, was the least, recording 0.0±0.0 number of leaves across all the concentrations used. In terms of height of plant recorded on day 4, results ranged from 2.0±0.2 cm to 3.8±0.3 cm. Among the treated samples, results showed that plants grown in samples treated with cow dung alone grew tallest, 3.2±0.3 cm, 2.7±0.3 cm and 3.8±0.3 cm at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg respectively. Samples treated with poultry dungs were the shortest, recording only 0.8±0.2 cm, 0.8±0.3 cm and 0.9±0.2 cm at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg respectively. However, statistical analyses revealed that, on day 4, there is no significant difference (α-0.05) between the numbers of leaves produced, as well as heights of plants, across the different concentrations and samples studied.

On week 2 of treatment, the number of leaves recorded from seedlings growing on treated Zn-polluted soil samples ranged from 2.3±0.5 to 4.7±0.5. There is no much variation in the number of leaves produced by the treated samples. However, seedlings grown on treatment carried out with combination of poultry wastes and cow dung relatively had more leaves than others. It recorded 4.7±0.9, 4.7±0.9 and 4.3±0.5 leaves at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. Statistically, the control samples and sample treated with poultry wastes alone are similar in their numbers of leaves, and are significantly different (α=0.05) from other treatments used in this study. The control recorded 2.3±0.5, 3.7±0.5 and 3.7±0.5 number of leaves at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. With respect to heights of seedlings recorded on week 2 of treatment of Zn-polluted soil samples, they ranged from 9.7±0.5 cm to 18.0±0.5 cm. Among the seedlings grown on treated Zn-polluted soil samples, those on treatment with poultry wastes alone were the poorest in their heights, without much variation from the control samples. Seedlings grown on treatment conducted with cow dung alone were the tallest, recording 17.7±1.1 cm, 17.4±0.4 cm and 18.0±0.5 cm heights at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. There is no significant difference in heights of seedlings grown on various concentrations of Zn used in this study. Also, heights of seedlings grown on the control samples are statistically similar to those of treatments done with poultry wastes alone, which are significantly different (α-0.05) from other treatments considered in this study.

Metal(loid) solubility can be improved by some microbial processes, which increase their bioavailability and possible toxicity. On the other hand, immobilization can occur from other processes, which can decrease their bioavailability. One method of solubilizing metal(loid)s is chemoorganotrophic (heterotrophic) and chemolithotrophic (autotrophic), which mobilize the material primarily through the release of siderophores, inorganic and organic acids, and other complexing agents. This process speeds up methylation, demethylation, redox, and biodegradation [1]. However, immobilization of metal(loid) mediated by microbes can happen through a variety of processes, including localization, biosorption, sequestration, reduction, precipitation, intracellular deposition, and accumulation [24].

On week 4 of treatment of Zn-polluted soil samples, the number of leaves produced by seedlings ranged from 3.0±0.8 to 8.0±0.8. There were clear variations in number of leaves produced by seedlings recorded from different treatments studied. The control samples recorded the poorest number of leaves, with 3.0±0.8, 3.3±0.5 and 4.3±0.5 leaves at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. Observations indicated that seedlings on treatment done with cow dung alone possessed the highest number of leaves, recording 8.0±0.8, 8.0±0.8 and 8.3±0.5 leaves at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. Unlike in earlier weeks of treatment, statistical analysis revealed that numbers of leaves by treatments were significantly different (α=0.05) from each other, except for the control samples and those treated with poultry wastes alone, which were not. Considering the heights of seedlings cultivated on Zn-polluted soil samples, the results obtained showed a range of 24.3±2.5 cm to 72.4±2.2 cm. Overall, the heights of seedlings on the control samples were the shortest, and among treated samples, those grown on treatments done using only poultry wastes and *Bacillus* sp recorded the poorest growth in heights. From observations, seedlings grown on treatments done with cow dung alone witnessed highest growth in height, with 72.4±2.2 cm, 64.0±1.7 cm and 64.7±1.8 cm at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. Analysis showed that each of the samples, including the control, is significantly different (α-0.05) from the others.

On week 7 of treatment of Zn-polluted soil samples, the numbers of leaves recorded from seedlings ranged from 4.3±0.5 to 12.3±0.5. The number of leaves recorded in seedlings on treated samples was more than those of seedlings on control samples. Also, the number of leaves found in most samples reduced with increasing concentration of Zn pollution. Among the treated samples, seedlings grown on soil samples that were treated with cow dung alone outperformed others. It recorded 10.3±0.5, 11.3±0.5 and 12.3±0.5 leaves at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. On control samples, the number of leaves was 4.7±0.5, 5.0±0.8 and 4.3±0.5 at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. Statistically, each of the average number of leaves in each treatment is significantly different (α=0.05) from others, including the control. In terms of heights of plants, results showed that it ranged from 70.5±3.2 cm to 127.3±2.6 cm. The heights of seedling planted on treated soil samples were obviously higher than those on control samples, which recorded 74.2±1.3 cm, 71.8±2.3 cm and 64.3±3.7 cm at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. Similarly, the heights of seedlings in most of the treatments decreased with increasing concentrations of Zn pollution in the soil samples. Among the treated samples, seedlings grown on samples treated with cow dung alone were the tallest. It recorded 109.0±3.4 cm, 115.8±4.3 cm and 127.3±2.6 cm at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. Analysis of the results showed that heights of plants grown on soil samples treated with poultry wastes alone were similar to those of seedlings grown on control samples. However, their heights were significantly different (α=0.05) from those of seedlings grown cow dung alone, and combination of cow dung and poultry wastes, which were on the other hand similar to each other.

Findings in previous studies have suggested that when soil zinc concentrations increased, both the number of leaves and plant heights dropped. This might be explained by plants becoming more harmed due to toxicity. Zinc, cobalt and copper are vital micronutrients that have been demonstrated in numerous studies; yet, excess of these elements can lead to phytotoxicity [25]. The enhancement of soil fertility parameters such as N and organic matter, as well as the phytostabilization of Cd, Pb, 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 [10].

Table 1 Bacterial diversity in Zn-polluted soil samples, treated with combinations of cow dung and poultry wastes, together with *Bacillus* sp.

Heavy metal type	Samples	Heavy metal conc	K. sp	S. typhi	S. aureus	Bacillus subtilis	Micrococcus spp.	Pseudomonas aeruginosa	A. flavus	Achromoba er spp.	E. coli
Zn (C2)	$A_1C_2B_1$	a	+	+	+	+	-	-	+	+	+
		b	+	+	+	+	-	-	+	-	+
		c	+	+	-	-	-	-	-	-	+
	$A_2C_2B_1$	a	+	+	+	+	-	+	+	+	+
		b	-	+	+	+	+	-	+	+	+
		c	+	+	+	+	-	-	-	-	+
	$A_1A_2C_2B_1$	a	+	+	+	+	-	+	+	+	+
		b	+	+	-	+	+	+	+	+	-
		c	+	+	-	-	-	+	+	-	+
	C_2	a	-	-	+	-	-	+	+	-	-
		b	-	-	-	+	-	+	+	-	+
		c	+	-	+	+	-	+	+	+	+
	Original sample		+	-	-	+	+	+	+	-	+

Legends: A1 is cow dung, A2 is poultry wastes, C2 is Zn, B1 is *Bacillus* sp

3.5. Effect of amendments on protein and chlorophyll contents of corn, grown on Zn-polluted soil

Results indicated that the protein contents of all seedlings were in the range of 10.20±20 mg/g to 92.69±1.89 mg/g, which signified the existence of a wide margin in their protein contents. Moreover, seedlings on treated samples recorded higher protein contents than those on control samples. Among the treated samples, seedlings grown on samples treated with cow dung only, recorded highest protein contents, with 92.69±1.89 mg/g, 88.26 mg/g and 71.69 mg/g protein contents, at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution, respectively. On the other hand, lowest protein contents were found in seedlings grown on control samples, which recorded 21.71±2.11 mg/g, 15.14±1.86 mg/g and 10.2±2.15 mg/g protein contents, at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution, respectively. These are shown in Figure 6. Statistical analysis of results, at α=0.05, indicated that protein contents of seedlings grown on samples treated with cow dung only, were significantly different from others, which were similar to each other. According to reports of a previous study, high levels of soil zinc pollution reduce both the initial and maximum levels of chlorophyll fluorescence, which suppresses PSII activity in a variety of plants [3]. This corresponded with the finding of the present study. Moreover, a study revealed reported reduced protein contents of plants following their exposure to heavy metal pollution. *Murraya koenigii* had a total protein content (mg BSAE/g of FW) of 62.94±2.06, *Ocimum canum* had 10.59±0.77, *Ocimum gratissimum* had 52.40±1.64, *Ocimum tenuiflorum* had 23.95±1.62, *Andrographis paniculata* had 36.84±1.69, *Nyctanthes arbor-tristis* had 42.79±2.73, and *Carica papaya* had a total protein content of 73.72±1.76 [18].

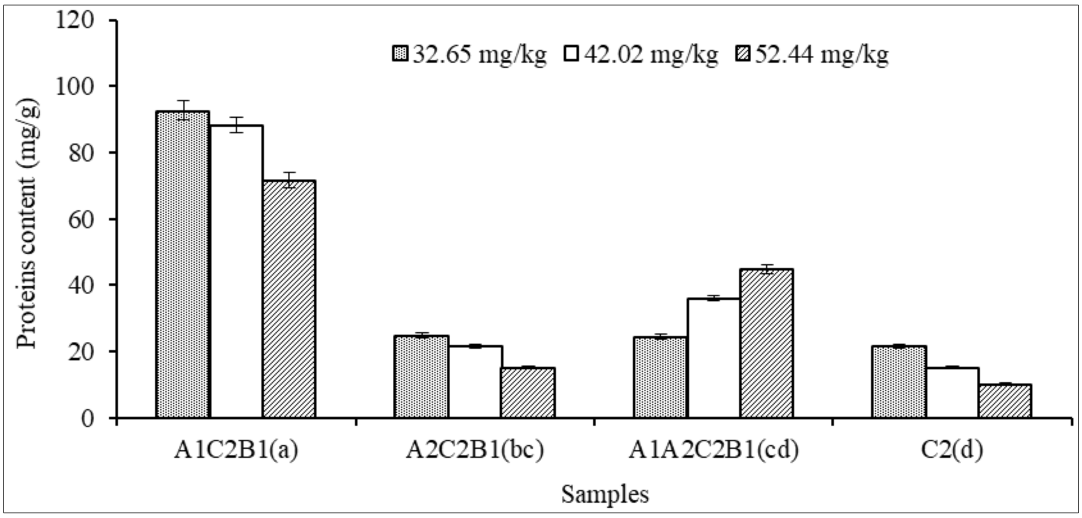

Figure 6 Protein contents of corn seedlings grown on different concentrations of Zn-polluted soil samples treated with different samples. Legends: A1 is cow dung, A2 is poultry wastes, B1 is *Bacillus* sp and C2 is Zn. Samples with similar lowercase letters (in bracket) are not significantly different at α=0.05)

Similarly, results of total chlorophyll contents of the seedlings indicated a range of 0.38±0.01 mg/g to 0.96±0.02 mg/g. It is also found that protein contents of seedlings on treated samples were slightly higher than those of control samples. Generally, seedlings on samples treated with a combination of poultry wastes and cow dung recorded the highest chlorophyll contents, with 24.49±1.88 mg/g, 36.07±2.8 mg/g and 44.91±1.72 mg/g chlorophyll contents at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution, respectively. Again, 21.71±2.11 mg/g, 15.14±1.86 mg/g and 10.20±2.15 mg/g chlorophyll contents were recorded in control samples, at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution, respectively (Figure 7). Due to modifications in mitotic activity, restriction of cell proliferation, and changes in membrane permeability and integrity, high Zn levels can result in shoot necrosis [26]. However, high rates of absorption of zinc can be attributed to its continued importance in numerous physiological and metabolic activities, including photosynthesis [3]. When biochar and compost were applied instead of control, there was a significant increase ($p < 0.05$) in the total chlorophyll contents, photosynthetic rate, transpiration rate, and stomatal conductance. The control plants had the lowest values of stomatal conductance, photosynthetic rate, transpiration rate, and total chlorophyll contents. The plants modified with 4% biochar and irrigated with the highest rate of compost application showed the highest values. When 4% biochar was applied, there was an increase in total chlorophyll levels of 92%, transpiration rate of 125%, photosynthetic rate of 138%, and stomatal conductance of 125% as compared to the control [10].

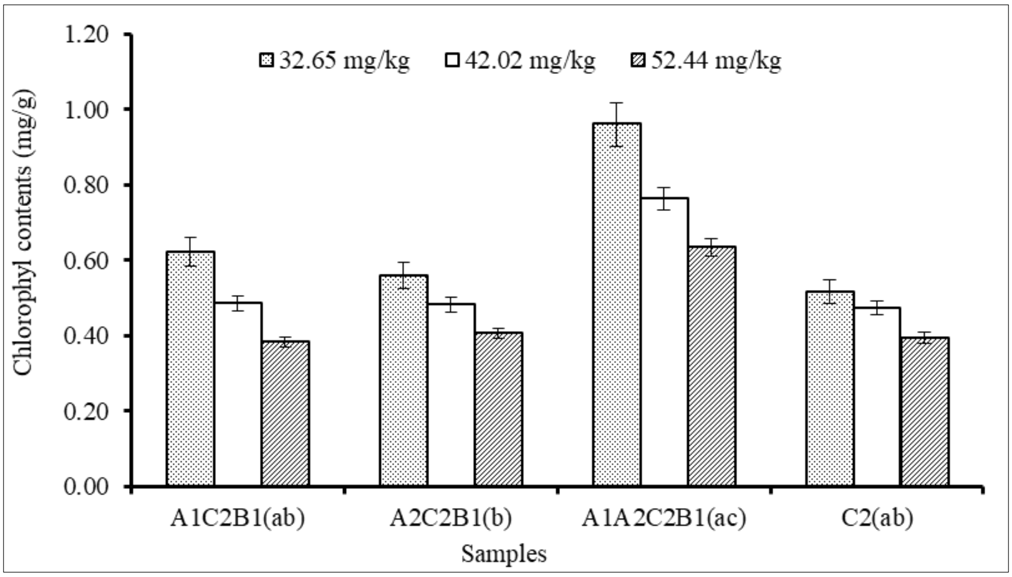

Figure 7 Chlorophyll contents of corn seedlings grown on different concentrations of Zn-polluted soil samples treated with different samples. Legends: A1 is cow dung, A2 is poultry wastes, B1 is *Bacillus* sp and C2 is Zn. Samples with similar lowercase letters (in bracket) are not significantly different at α=0.05)

3.6. Effect of amendments on bioaccumulation of toxic metals in corn, grown on Zn-polluted soil

Results showed that bioaccumulation of Zn in leaf, stem and root samples of corn, cultivated on treated Zn-polluted soil samples ranged from 23.14±1.04 mg/kg to 49.59±0.61 mg/kg, 12.97±0.57 mg/kg to 25.24±0.82 mg/kg, and 54.94±1.0 mg/kg to 156.24±1.31 mg/kg respectively. As well, differences in the ranges are indicative of large variations in Zn bioaccumulation in the samples. It was found that Zn bioaccumulation in all the samples of corn increased with increasing Zn pollution in soil samples. Also, bioaccumulation of Zn in corn samples grown on treated soil samples were significantly lowers than those on control soil samples. In leaf samples, lowest bioaccumulation of Zn was recorded in corn grown on soil samples treated with *Bacillus* spp and poultry wastes only. It was 23.14±1.04 mg/kg, 24.42±0.57 mg/kg and 27.44±0.11 mg/kg at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution, respectively. These were significantly lower than 46.94±0.25 mg/kg, 47.59±0.29 mg/kg and 49.59±0.61 mg/kg bioaccumulation of Zn at 32.75 mg/kg, 42.02 mg/kg and 52.44mg/kg levels of Zn pollution, respectively, which were recorded in control samples. Results of statistical analysis α=0.05, revealed that there was no significant difference in bioaccumulation of Zn in corn leaves from all the treated soil samples. But they were significantly different from bioaccumulation recorded in corn leaf from control soil samples. With respect to level of pollution, bioaccumulation of Zn at 32.75 mg/kg pollution was significantly different from when the pollution increased to 52.44 mg/kg.

Other results indicated that corn stems from control soil samples had highest bioaccumulation of Zn, with 16.79±0.45 mg/kg, 20.04±0.25 mg/kg and 25.24±0.82 mg/kg at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution, respectively. On the other hand, lowest bioaccumulation was obtained in stems from soil samples treated with *Bacillus* sp and poultry wastes only, with 12.97±0.57 mg/kg, 15.17±1.31 mg/kg and 17.74±0.35 mg/kg at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution, respectively. When subjected to statistical analysis at α=0.05, results showed that bioaccumulation in stems from control soil samples was significantly different from all others, except in samples from soil treated with cow dung. Moreover, bioaccumulation in stems from soil samples treated with *Bacillus* sp and poultry wastes only, was not significantly different from all others, except for that of samples from control soil samples. For concentrations, bioaccumulation in stems at 52.44 mg/kg level of Zn pollution was significantly different from others, which are similar.

In corn root samples, highest bioaccumulation of Zn was recorded in samples from control soil samples, which recorded 135.49±0.61 mg/kg, 146.69±1.19 mg/kg and 156.24±1.31 mg/kg at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution, respectively. This was significantly different from Zn bioaccumulation in samples from all other treated soil samples. Though there were variations in bioaccumulation recorded from samples all treated soil samples, they were not significantly different from each other. In terms of concentrations, bioaccumulation at 52.44mg/kg was significantly different from others, except at 42.02 mg/kg. In comparison, Zn bioaccumulation in samples of different parts of corn plants was generally in the order; roots>leaves>stems, while it was; control samples>composite of poultry wastes and cow dung samples>only poultry wastes samples>only cow dung samples, for various treatments.

Previous studies reported that different plants have demonstrated the capabilities to bioaccumulate heavy metals when grown on heavy metals contaminated sites, which include Pb, Mn, Cu and Cd by *Manihot esculenta* [27], Cu and Zn by *Brassica juncea* [28]; Pb, Cu, Mn and Cd by *Panicum maximum* [29]. In a different study, the order of heavy metal concentrations in the various plant sections was roots>leaves>stem [30]. Al-Wabel et al. [31] 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. Zn concentrations in mature plants, however, can reach 120 mg kg-1 and in kernels, 40 mg kg-1 in contaminated areas [32]. According to Hussaina et al. [33], the results of this investigation unequivocally demonstrated that cow dung had a major impact on the bioassimilation of Pb and Cd in *P. posthuma* tissues during the composting or remediation phase.

A study reported that Zn concentrations in shoots of maize grown in contaminated soil at the 3-5 leaf stage, were much higher than both the controls (76 vs. 2.8 mg kg^{-1} DW) and the average values of 66 mg kg^{-1} DW that are typically seen in normal plants [34]. Reduced plant growth and crop productivity are the results of heavy metal accumulation, which disrupts plant growth processes such as transpiration, photosynthetic rate, and respiration [35]. Significant amounts of organic amendments, such as biosolid, composted manure, and municipal solid waste, are utilized to enhance the physical characteristics and fertility of soils while also serving as a source of nutrients. Due to their effects on the adsorption, reduction, complexation, and volatilization of metal(loid)s, these organic amendments with low metal(loid) content can be employed as a sink to decrease the bioavailability of metal(loid)s in polluted soils and sediments [1].

When poultry manure compost was applied, 47.8–69.8% of the soluble/exchangeable Cd was converted to the organic-bound fraction. Consequently, plants absorbed 56.2–62.5% less Cd than in the control group [36]. Nevertheless, a significant inherent issue with using organic amendments to immobilize metal(loid)s is that, even while their bioavailability decreases, the overall concentration of these substances in soils doesn't alter. Through the natural weathering process or the dissolution of organic matter–metal(loid) complexes, the immobilized metal(loid)s may eventually become plant accessible [1]. It had been suggested that application of combination of different organic amendments would significantly decrease the amount of heavy metal bioaccumulated by plants when grown on spent-engine oil contaminated soil (Adebiyi and Salami, 2023).

4. Conclusion

Bioremediation had remained the choice alternative to remediating heavy metal polluted soil samples. In this study, addition of organic amendments including cow dung, poultry wastes and their composites, together with *Bacillus* sp as plant growth promoting rhizobacteria to bioremediation of zinc-polluted soil sample demonstrated some remarkable effects on cultivated corn seedlings. Results indicated that with addition of these amendments, there was increase in pH and nutrient contents, including N, organic carbon, soil organic matter, K etc., of treated Zn-polluted soil. Treatment undertaken with a combination of the two organic supplements yielded the highest increase in DHA, with 2.35±2.04 $\mu gTPFg^{-1}h^{-1}$ recorded at 33.75 mg/kg Zn pollution, and 2.19±0.04 $\mu gTPFg^{-1}h^{-1}$ at 52.55 mg/kg Zn pollution. The number of leaves found in most samples reduced with increasing concentration of Zn pollution. Among the treated samples, seedlings grown on soil samples that were treated with cow dung alone outperformed others. It recorded 10.3±0.5, 11.3±0.5 and 12.3±0.5 leaves at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. The heights of seedlings in most of the treatments decreased with increasing concentrations of Zn pollution in the soil samples. Among the treated samples, seedlings grown on samples treated with cow dung alone were the tallest. It recorded 109.0±3.4 cm, 115.8±4.3 cm and 127.3±2.6 cm at 32.75 mg/kg, 42.02 mg/kg and 52.44 mg/kg levels of Zn pollution respectively. Zn bioaccumulation in samples of different parts of corn plants was generally in the order; roots>leaves>stems, while it was; control samples>composite of poultry wastes and cow dung samples>only poultry wastes samples>only cow dung samples, for various treatments. Thus, application of organic amendments, especially cow dung, has shown to be useful in bioremediation of Zn-polluted soil used for agricultural activities.

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare that no conflict of interest exists.

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