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Effect of Lead and Cadmium on Soil Microbial Activities

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Abstract

This study aimed to assess the responses of microbial functional dynamics and enzymes of the soil to different concentrations of cadmium (Cd), lead (Pb) and a mixture of cadmium and lead (Cd/Pb). This study was done for 12 weeks in a greenhouse at 26±2°C and the soil moisture content was regularly rectified to water holding capacity of 60% maintained using deionized water throughout the incubation period. Soil treatments Cd_1, Pb_1 and Cd_1/Pb_1 demonstrated the highest acid phosphatase activity (780 mg p-nitrophenol/kg/h) while least activity was observed in Cd_3/Pb_3 (533 mg p-nitrophenol/kg/h). Soil treatments with the highest urease activity are Cd_3, Pb_3 and Cd_3/Pb_3 (312 mg N/kg/3h) while the least urease activity was observed in soil treatment Cd_3 (211 mg N/kg/3h). Soil treatment with the highest microbial biomass carbon was Cd_1 (125 mg /kg) while the least microbial biomass carbon was observed in Cd_3/Pb_3 (71 mg/kg). The soil treatment with the most heterotrophic bacteria, actinomycetes, and fungi counts were Cd_2/Pb_2 (72.3×10^8 CFU g^{-1}), Cd_2 (54×10^5 CFU g^{-1}) and Pb_1 (33×10^2 CFU g^{-1}) respectively while the least heterotrophic bacteria, actinomycetes, and fungi counts were Cd_3/Pb_3 (16.7×10^8 CFU g^{-1}), Cd_3/Pb_3 (33×10^5 CFU g^{-1}), Cd_3/Pb_3 (17×10^2 CFU g^{-1}) respectively. The most inhibitive effects of Pb, Cd, or a mixture of Pb/Cd on soil microbes, enzymes and properties were observed in week 2. The supplemented concentrations of the metals studied significantly altered the microbial communities, profiles and activities.

1. Introduction

Soil is an intricate environment that serves as the natural reservoir for microorganisms, plants and some animals. Soil contains a variety of microorganisms that are ubiquitous to natural ecosystems [1]. Microorganisms play vital roles in the nutritional chain that is important for the biological balance of life on earth. The conditions of the soil greatly influence crop yield, the sustainability of the environment and worldwide ecological balance [2]. The activities of soil microorganisms and their community structure effectively serve as indicators of the environmental quality of the soil [3]. The activities and diversity of soil microbes are very responsive to inorganic and organic pollutants [4].

The use of soil enzymes as a quality indicator in different systems of agriculture has been efficient [5]. Vital biochemical functions are been played by soil enzymes in the inclusive health and quality of the soil [6]. The activities of soil enzyme are negatively influenced by toxic metals [7]. The enzyme assays facilitate a comprehensive evaluation of the soil environmental conditions since they are involved in the transformations of nutrients which affect their availability to plants [8]. The assessment of soil quality using enzymes is due to their ease to measure, integrativeness and rapid response to changes in soil composition compared to other soil quality indicators [5].

Soil contamination by potentially toxic metals such as Cd, Ni, Zn, Pb, and Cu has increased severely during the last few decades [9]. Heavy metals exceptionally possess serious environmental threat as they are not biodegradable, but persist, resulting in their continuous increase in concentrations [10]. Contamination of the soil by heavy metals alters microbial diversity, microbial counts and also the activities of soil enzymes [11]. Cadmium (Cd) is toxic for living organisms and carcinogenic to human and it exhibits varying degrees of phytotoxicity [12,13]. Cadmium contaminated soil led to a remarkable decrease in the activities of urease and phosphatase [14]. Lead (Pb) is extremely toxic to microbes, animals, human and plants [15]. Across the globe, Pb has become widely distributed as a result of human activities which causes adverse effects to the environment and the health of human [16]. The toxicity of Pb depends upon its bioavailability [17]. In this study, the responses of microbial functional dynamics and enzymes of the soil to different concentrations of Cd, Pb and a mixture of cadmium and lead were assessed.

2. Methodology

2.1 Sample collection

Soil samples were collected from a cassava (*Manihot esculenta* Crantz) farm located at Adolor College Road, Benin City, Nigeria. The farm is located on Latitude 6° 23’ 17.4N and Longitude 5° 36’ 14.0E. Soil sample of 0-20 cm depth was collected in February 2018, completely air-dried and subsequently analysed.

2.2 Experimental setup

Metals used include Cd (Cadmium chloride) and Pb (Lead II chloride). Treatment and application rates (mg/g) include: Cd_1 [(1.5 mg/kg) 0.75mg = 500g of soil], Cd_2 [(3 mg/kg) 1.50mg = 500g of soil], Cd_3 [(5 mg/kg) 2.50mg = 500g of soil], Pb_1 [(150 mg/kg) 75mg = 500g of soil], Pb_2 [(300 mg/kg) 150mg = 500g of soil], Pb_3 [(500 mg/kg) 250mg = 500g of soil], Cd_1 + Pb_1 [0.75mg of Cd + 75mg of Pb = 500g of soil], Cd_2 + Pb_2 [1.50mg of Cd + 150mg of Pb = 500g of soil] and Cd_3 + Pb_3 [2.50mg of Cd + 250mg of Pb = 500g of soil]. The soil samples were incubated in a greenhouse for 12 weeks at 26±2°C. Throughout the period of incubation, the moisture content of the experimental samples were regularly examined and adjusted to 60% water holding capacity using deionized water. Samples from each experimental pot were collected at week 0 (initial week), 2, 8 and 12 for enumeration of microorganisms and enzyme assays.

2.3 Soil physicochemical properties

The pH of the soil was determined using a pH meter (FieldScout pH 110 Meter). The soil organic matter (SOM) was determined as reported by Nelson and Sommers [18]. Particle size analysis was determined as described by Ibitoye [19]. Nitrate, chloride, fluoride and sulphate were estimated with the use of atomic absorption spectrophotometer (AAS) (Perkin Elmer

Analyst 800 series Graphite Furnace AAS). AAS was also used in analyzing the digested samples for metals contaminant.

2.3 Enumeration and isolation of the bacterial, actinomycetes and fungal isolates

Stock suspension of the samples was prepared by weighing 10 g of soil from replicates of the various treatments into conical flasks containing 90 mL sterile distilled water and mixed by shaking for about 15 minutes. All stock suspensions were serially diluted (10^1 - 10^9). An aliquot of 0.1 mL from diluent 10^7 – 10^9 was inoculated onto nutrient agar (Lab M, Lancashire, UK) using the spread plate method and incubated for 18 - 24 h at 37°C. Similarly, aliquots of 0.1 mL from diluent 10^3 – 10^6 were inoculated onto a formulated glycerol asparagine agar (HiMedia, India) using spread plate method and incubated for 14 - 21 days at 25 - 30°C while aliquot of 0.1 mL from diluent 10^1 – 10^3 was inoculated onto sabourand dextrose agar (Titan Biotech LTD, India) using pour plate method and incubated for 72 h at 25-30°C. The entire number of visible colonies of heterotrophic bacteria, actinomycetes, and fungi were enumerated and expressed in colony-forming unit per gram (CFU/g).

2.4 The bioavailable fraction of metals

The bioavailable fraction of metals was determined as illustrated by Khan *et al.* [20]. Bioavailable fractions of metals were carried out by extracting 5g of the soil sample for 2 h using 8 mL of 0.5 M $CaCl_2$ (pH 7.0) and 15 mL of deionized water. After the extraction process, the separation was carried out by centrifuging at 7000 rpm for 10 min. The supernatant was filtered using a 0.45 µm filter paper and was analyzed for the concentrations of bioavailable metal using atomic absorption spectrophotometer.

2.5 Acid phosphate activity

Acid phosphate activities were determined as illustrated by Dick *et al.* [21]. The substrate used is *p*-nitrophenol phosphatase and the intensity of the filtrate's yellow colour due to *p*-nitrophenol was derived using a UV-Vis spectrophotometer (PG Instruments T80, US) at wavelength 410nm and the result was expressed as milligrams of *p*-nitrophenol per kilogram.

2.6 Urease activity

The method described by Kizilkaya and Bayraki [22] was used in measuring urease activity. One gram of the soil sample was mixed using a 0.75 mL citrate buffer (pH 6.8), 0.25 mL toluene, and 1 mL solution of 10% urea substrate. After 3 hours incubation, the soil samples were filtered at 37 °C and 10 mL of deionized water was used in diluting 1 mL of the filtrate, then 3 mL of sodium hypochloride and 4 mL of sodium phenolate solution was added. The UV-Vis spectrophotometer wavelength was adjusted to 578 nm and the formation of ammonium was determined, the result was expressed as milligrams of N per kilograms. All analysis for enzyme assays was carried out in triplicates using moist soil samples and the control was without soil. After the reaction stopped, the substrate was added to blanks and was also added before filtration of the soil suspension.

2.7 Microbial biomass carbon assay (MBC)

The microbial biomass carbon (MBC) was determined using the fumigation-extraction method [23]. For each analysis, a sufficient quantity of moist soil was taken from each sample pots. Ethanol-free chloroform was used in fumigating sub-sample of the moist soil which is equivalent to 3.0 g dry soil for 24 h at 25°C, however, another subsample of the equal weight was not fumigated. The MBC was extracted from fumigated and nonfumigated samples using a 20 mL K_2SO_4 (0.5 M) solution. The samples were agitated for 30 minutes and then centrifuged at 7000 rpm for 10 minutes. After centrifugation, the supernatant was

filtered and frozen at -20°C. A soil organic matter analyzer was used to determine the MBC contents of the extracted samples. The MBC was determined via the expression: MBC = 2.22 ($C_{fumigated} - C_{nonfumigated}$) where $C_{fumigated}$ and $C_{nonfumigated}$ are C extracted from the fumigated and nonfumigated samples, respectively.

2.8 Identification of the bacteria, fungi and actinomycetes

Selected bacteria isolates were identified using the cultural, morphological, physiological and biochemical characterization using Analytical Profile Index (API) as instructed by the manufacturer (bioMerieux, Marcy-l'Étoile, France).

2.9 Statistical analysis

Descriptive statistics and analysis of variance (ANOVA) were used in the data analysis via Statistical package (SPSS) 21.0 and Microsoft excel 2013. *P*-values less than 0.05 were considered statistically significant.

3.0 Results and Discussion

3.1 Results

3.1.1 Initial Physicochemical Properties of the Soil Sample

The initial soil samples physicochemical properties as represented in Table 1 include pH (7.8 ± 0.2), sand (40.1 ± 0.5%), clay (9.4 ± 0.2%), silt (50.5 ± 1.4%), cadmium (0.2 ± 0.06 mg kg^{-1}), lead (1.76 ± 1.03 mg kg^{-1}), soil organic matter (14.4 ± 2.1 mg kg^{-1}), nitrate (13.39 ± 1.9 mg kg^{-1}), chloride (3.01 ± 0.4 mg kg^{-1}), fluoride (0.53 ± 0.1 mg kg^{-1}) and sulphate (8.01 ± 1.1 mg kg^{-1}).

3.1.2 Bioavailable Fraction of Metals

The bioavailable fraction of the cadmium soil treatments is shown in Figure 1. The bioavailable fraction was 48.3 ± 2.1% at week 0 but it decreased to 39.6 ± 1.3% after 2 weeks. The fraction concentration decreased further to 36.4 ± 2.4% at week 8 and then to 35.1 ± 1.6% at week 12.

3.1.3 Acid Phosphatase Activities of the Soil Samples

The acid phosphatase activities of the Cd treatments is shown in Figure 2; the acid phosphatase activities of Pb treatments is depicted in Figure 3 while the acid phosphatase activities of Cd/Pb treatments is shown in Figure 4. Acid phosphatase activity was expressed in milligrams of *p*-nitrophenol per kilogram of soil per hour. The acid phosphatase activity for the treatment and incubation period range as follows: Control (779 ± 0.13 – 786 ± 0.29), Cd_1 (698 ± 0.25 – 780 ± 0.32), Cd_2 (633 ± 0.17 – 779 ± 0.14), Cd_3 (542 ± 0.14 – 778 ± 0.12), Pb_1 (728 ± 0.25 – 780 ± 0.20), Pb_2 (674 ± 0.22 – 779 ± 0.32), Pb_3 (648 ± 0.26 – 778 ± 0.41), Cd_1/Pb_1 (649 ± 0.18 – 780 ± 0.26), Cd_2/Pb_2 (620 ± 0.20 – 779 ± 0.22) and Cd_3/Pb_3 (533 ± 0.16 – 778 ± 0.18).

3.1.4 Urease Activities of the Soil Samples

The urease activity of the Cd treatments is shown in Figure 5; the urease activity of Pb treatments is shown in Figure 6 while the urease activity of Cd/Pb treatments is shown in Figure 7. Urease activity was expressed in milligrams of Nitrogen per kilogram of soil per 3 hours. The urease activity for the treatment and incubation period range as follows: control (310 ± 0.16 – 315 ± 0.20), Cd_1 (261 ± 0.25 – 310 ± 0.22), Cd_2 (242 ± 0.21 – 311 ± 0.18), Cd_3 (211 ± 0.26 – 312 ± 0.16), Pb_1 (261 ± 0.33 – 310 ± 0.23), Pb_2 (253 ± 0.29 – 311 ± 0.31), Pb_3 (247 ± 0.18 – 312 ± 0.29), Cd_1/Pb_1 (244 ± 0.31 – 310 ± 0.26), Cd_2/Pb_2 (232 ± 0.16 – 311 ± 0.22) and Cd_3/Pb_3 (221 ± 0.24 – 312 ± 0.19).

3.1.5 Microbial Biomass Carbon of the Soil Samples

The MBC of the Cd treatments is shown in Figure 8; MBC of Pb treatments is shown in Figure 9 while the MBC of Cd/Pb treatments is depicted in Figure 10. The MBC for the treatment and incubation period range as follows: control (124 ± 0.21 – 161 ± 0.20 mg kg^{-1}), Cd_1 (115 ± 0.20 – 125 ± 0.18 mg kg^{-1}), Cd_2 (109 ± 0.26 – 121 ± 0.23 mg kg^{-1}), Cd_3 (100 ± 0.24 – 123 ± 0.18 mg kg^{-1}), Pb_1 (119 ± 0.22 – 124 ± 0.24 mg kg^{-1}), Pb_2 (120 ± 0.20 – 122 ± 0.22 mg kg^{-1}), Pb_3 (109 ± 0.19 – 124 ± 0.28 mg kg^{-1}), Cd_1/Pb_1 (98 ± 0.20 – 123 ± 0.19 mg kg^{-1}), Cd_2/Pb_2 (82 ± 0.24 – 122 ± 0.25 mg kg^{-1}) and Cd_3/Pb_3 (71 ± 0.22 – 123 ± 0.21 mg kg^{-1}).

Table 1: Initial physicochemical properties of the soil sample

Properties	Values (Mean ± SD)
pH	7.8 ± 0.2
Sand	40.1 ± 0.5
Clay	9.4 ± 0.2
Silt	50.5 ± 1.4
Cd (mg kg^{-1})	0.2 ± 0.06
Pb (mg kg^{-1})	1.76 ± 1.03
SOM (g kg^{-1})	14.4 ±2.1
Nitrate (mg kg^{-1})	13.39 ± 1.9
Chloride (mg kg^{-1})	3.01 ± 0.4
Fluoride (mg kg^{-1})	0.53 ± 0.1
Sulphate (mg kg^{-1})	8.01 ± 1.1

Legend: SOM: Soil organic matter; Values are in triplicates of mean ± standard deviation of the mean

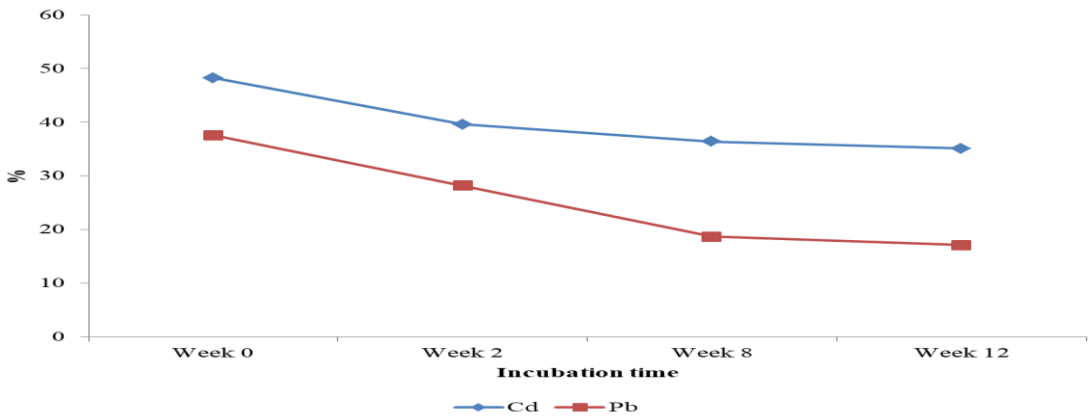

Figure 1: Bioavailable fraction of metals (%)

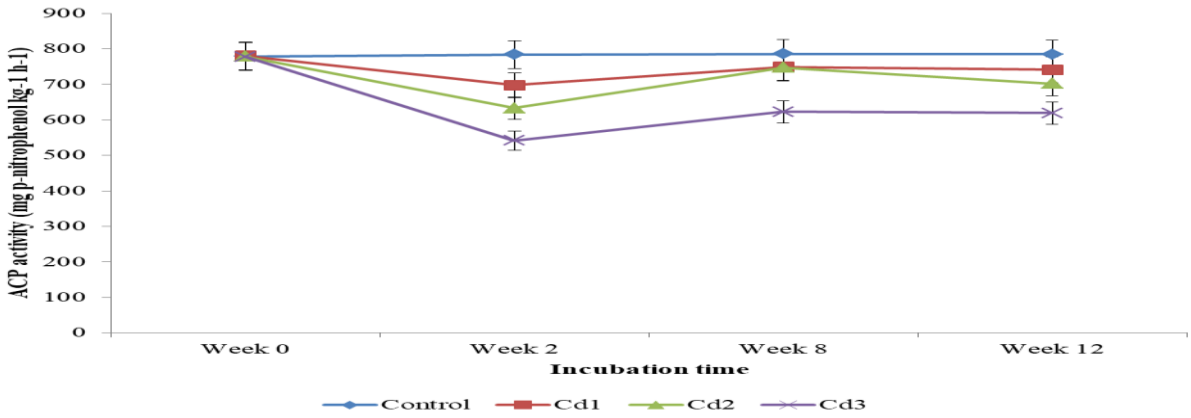

Figure 2: Acid phosphatase activities for Cd treatment
Legend: ACP activity: acid phosphatase activity; mg *p*-nitrophenol kg^{-1} h^{-1}: milligrams of *p* nitrophenol per kilogram of soil per hour.

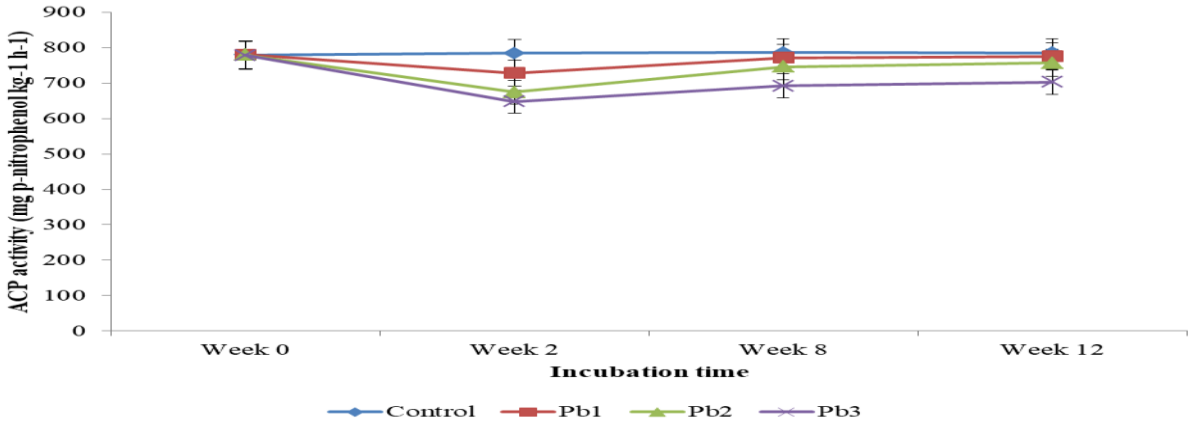

Figure 3: Acid phosphatase activities for Pb treatment
Legend: ACP activity: acid phosphatase activity; mg *p*-nitrophenol kg^{-1} h^{-1}: milligrams of *p* nitrophenol per kilogram of soil per hour.

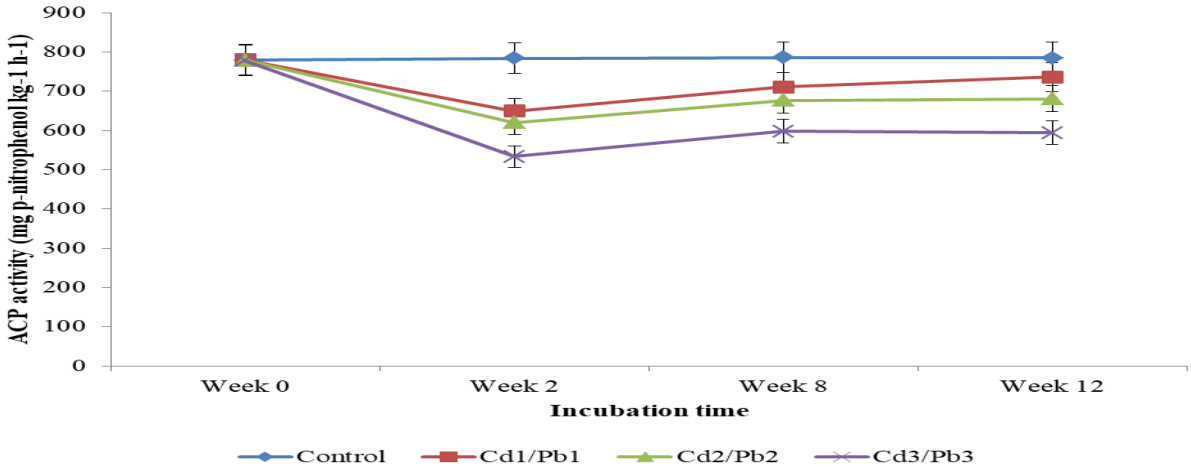

Figure 4: Acid phosphatase activities for Cd/Pb treatment
Legend: ACP activity: acid phosphatase activity; mg *p*-nitrophenol kg^{-1} h^{-1}: milligrams of *p* nitrophenol per kilogram of soil per hour.

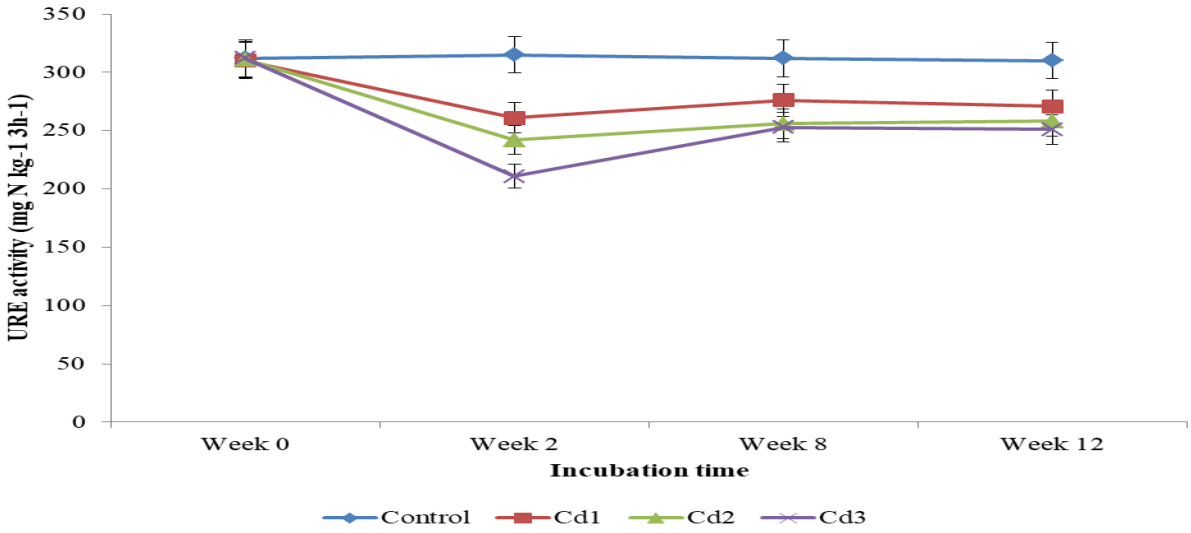

Figure 5: Urease activities for Cd treatment
Legend: URE activity: urease activity; mg N kg^{-1} $3h^{-1}$: milligrams of Nitrogen per kilogram of soil per 3 hours.

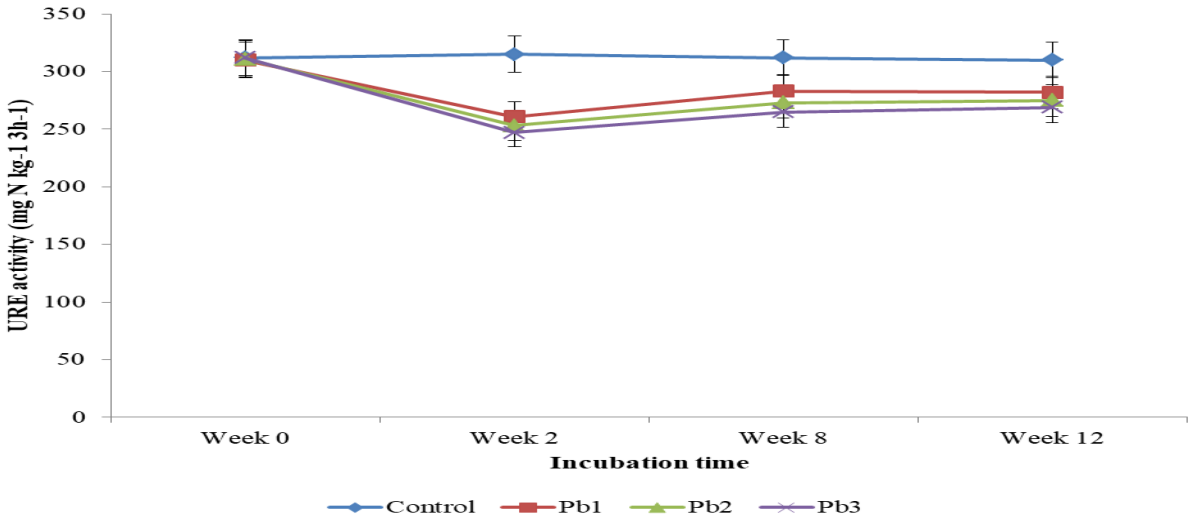

Figure 6: Urease activities for Pb treatment
Legend: URE activity: urease activity; mg N kg^{-1} $3h^{-1}$: milligrams of Nitrogen per kilogram of soil per 3 hours.

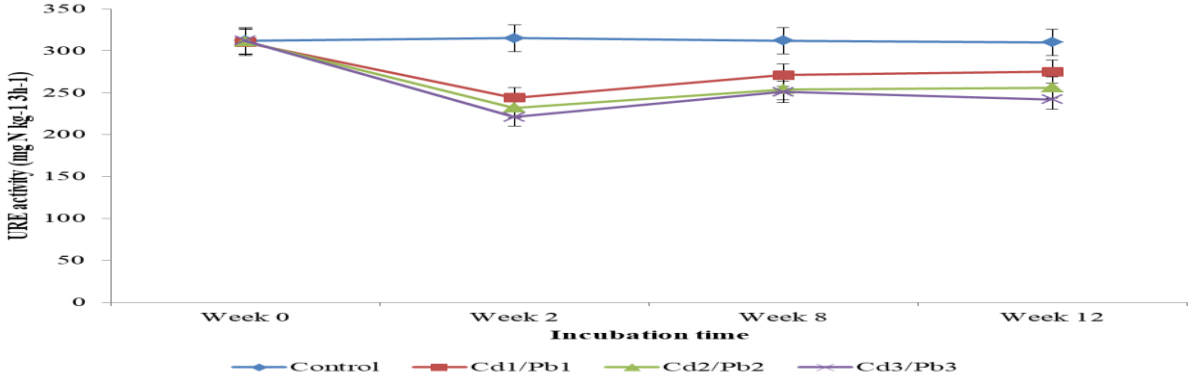

Figure 7: Urease activities for Cd/Pb treatment
Legend: URE activity: urease activity; mg N kg^{-1} $3h^{-1}$: milligrams of Nitrogen per kilogram of soil per 3 hours

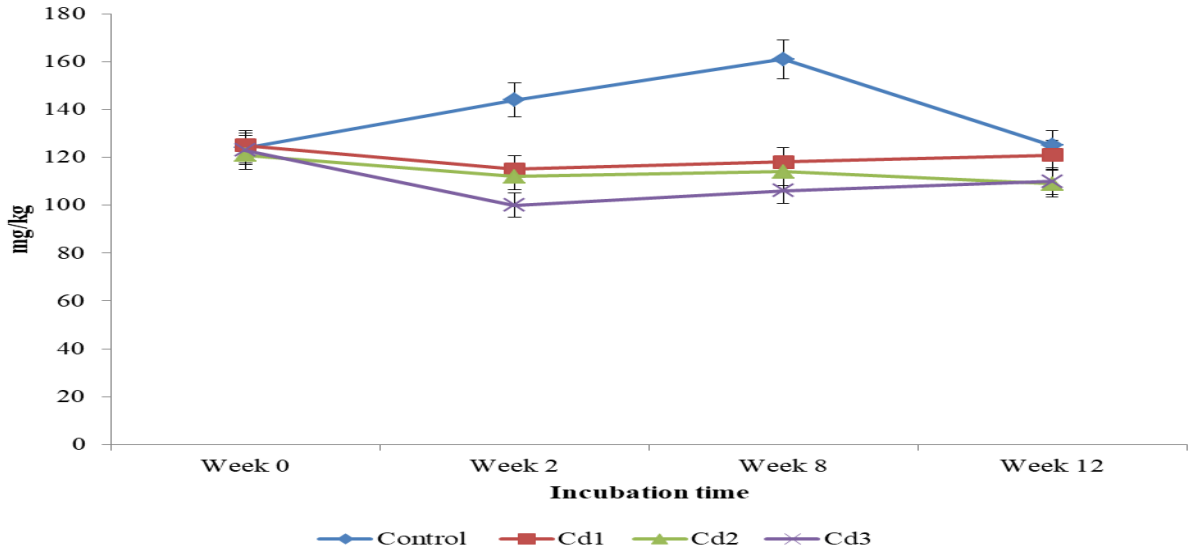

Figure 8: Microbial biomass carbon for Cd treatment

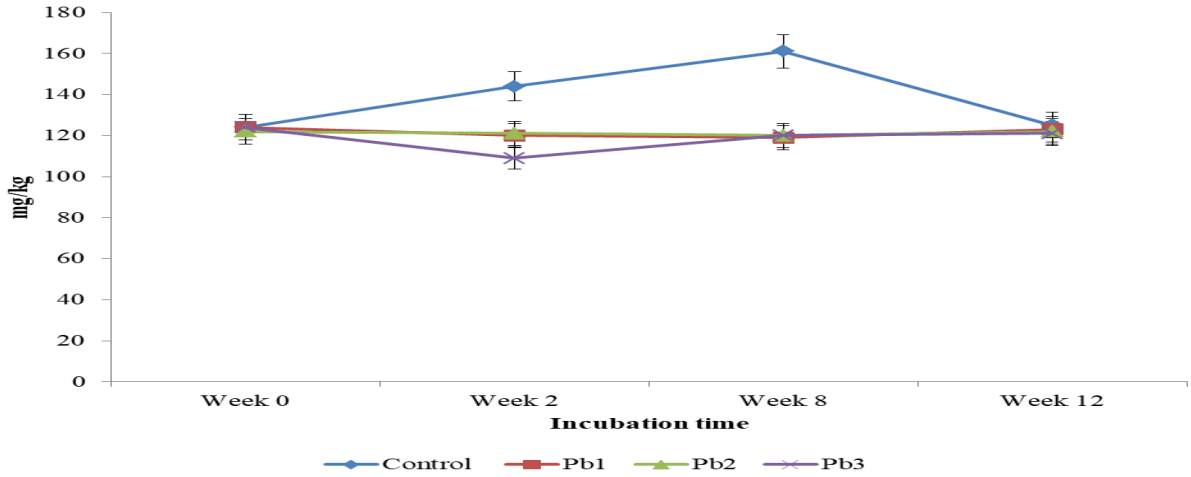

Figure 9: Microbial biomass carbon for Pb treatment

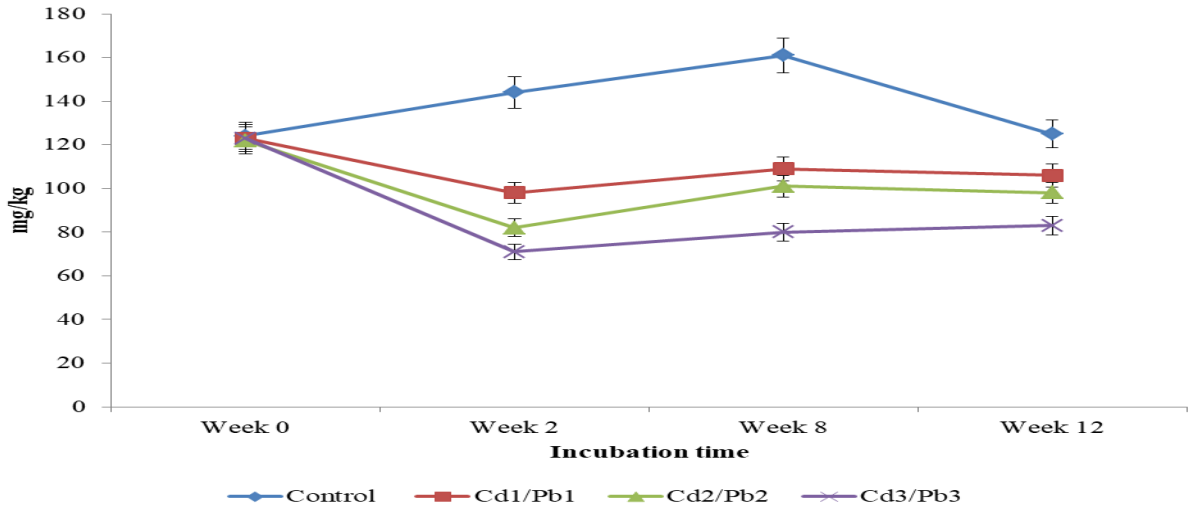

Figure 10: Microbial biomass carbon for Cd/Pb treatment

3.1.6 Heterotrophic Bacteria Count of the Soil Samples

The heterotrophic bacteria count for the treatment and incubation period in Table 2 range as follows: control ($49.8 \pm 0.37\times10^8$ - $72.7 \pm 0.70\times10^8$ CFU/g), Cd_1 ($39.7 \pm 0.32\times10^8$ - $69.3 \pm 0.65\times10^8$ CFU/g), Cd_2 ($32.7 \pm 0.24\times10^8$ - $64.6 \pm 0.50\times10^8$ CFU/g), Cd_3 ($26.2 \pm 0.18\times10^8$ - $56.0 \pm 0.44\times10^8$ CFU/g), Pb_1 ($36.4 \pm 0.31\times10^8$ - $66.8 \pm 0.59\times10^8$ CFU/g), Pb_2 ($34.5 \pm 0.25\times10^8$ - $64.3 \pm 0.55\times10^8$ CFU/g), Pb_3 ($29.9 \pm 0.22\times10^8$ - $56.2 \pm 0.45\times10^8$ CFU/g), Cd_1/Pb_1 ($28.2 \pm 0.22\times10^8$ - $63.0 \pm 0.54\times10^8$ CFU/g), Cd_2/Pb_2 ($21.9 \pm 0.15\times10^8$ - $72.3 \pm 0.60\times10^8$ CFU/g) and Cd_3/Pb_3 ($16.7 \pm 0.16\times10^8$ - $57.9 \pm 0.55\times10^8$ CFU/g).

3.1.7 Actinomycetes Count of the Soil Samples

The actinomycetes count for the treatment and incubation period in Table 3 range as follows: control ($41 \pm 0.51\times10^5$ – $53 \pm 0.67\times10^5$ CFU/g), Cd_1 ($40 \pm 0.51\times10^5$ – $50 \pm 0.61\times10^5$ CFU/g), Cd_2 ($42 \pm 0.50\times10^5$ – $54 \pm 0.66\times10^5$ CFU/g), Cd_3 ($39 \pm 0.44\times10^5$ – $44 \pm 0.49\times10^5$ CFU/g), Pb_1 ($36 \pm 0.46\times10^5$ – $50 \pm 0.69\times10^5$ CFU/g), Pb_2 ($39 \pm 0.53\times10^5$ – $49 \pm 0.62\times10^5$ CFU/g), Pb_3 ($43 \pm 0.57\times10^5$ – $51 \pm 0.68\times10^5$ CFU/g), Cd_1/Pb_1 ($42 \pm 0.58\times10^5$ – $53 \pm 0.67\times10^5$ CFU/g), Cd_2/Pb_2 ($40 \pm 0.52\times10^5$ – $49 \pm 0.62\times10^5$ CFU/g) and Cd_3/Pb_3 ($33 \pm 0.41\times10^5$ – $49 \pm 0.62\times10^5$ CFU/g).

3.1.8 Fungi Count of the Soil Samples

The fungi count for the treatment and incubation period in Table 4 range as follows: Control ($21 \pm 0.25\times10^2$ – $33 \pm 0.34\times10^2$ CFU/g), Cd_1 ($22 \pm 0.24\times10^2$ – $25 \pm 0.30\times10^2$ CFU/g), Cd_2 ($23 \pm 0.24\times10^2$ – $27 \pm 0.29\times10^2$ CFU/g), Cd_3 ($21 \pm 0.22\times10^2$ – $24 \pm 0.24\times10^2$ CFU/g), Pb_1 ($27 \pm 0.86\times10^2$ – $33 \pm 0.41\times10^2$ CFU/g), Pb_2 ($26 \pm 0.30\times10^2$ – $31 \pm 0.33\times10^2$ CFU/g), Pb_3 ($19 \pm 0.19\times10^2$ – $23 \pm 0.24\times10^2$ CFU/g), Cd_1/Pb_1 ($24 \pm 0.31\times10^2$ – $29 \pm 0.35\times10^2$ CFU/g), Cd_2/Pb_2 ($19 \pm 0.19\times10^2$ – $21 \pm 0.25\times10^2$ CFU/g) and Cd_3/Pb_3 ($17 \pm 0.19\times10^2$ – $25 \pm 0.30\times10^2$ CFU/g). The identified bacteria isolates were conducted using biochemical characterization and Analytical Profile Index (API) system were shown in Table 5a and b.

Table 3: Actinomycetes population in different incubation time and treatments

	Actinomycetes count (10^5 CFU/g)				
Treatment	**Week 0**	**Week 2**	**Week 8**	**Week 12**	***p*-value**
Control	$48 \pm 0.54^{\frac{B}{ab}}$	$53 \pm 0.67^{\frac{C}{c}}$	$46 \pm 0.56^{\frac{B}{b}}$	$41 \pm 0.51^{\frac{A}{a}}$	0.000
Cd_1	$50 \pm 0.61^{\frac{C}{ab}}$	$45 \pm 0.56^{\frac{B}{bc}}$	$41 \pm 0.51^{\frac{A}{ab}}$	$40 \pm 0.51^{\frac{A}{a}}$	0.000
Cd_2	$47 \pm 0.55^{\frac{B}{ab}}$	$42 \pm 0.50^{\frac{A}{b}}$	$43 \pm 0.50^{\frac{A}{b}}$	$54 \pm 0.66^{\frac{C}{b}}$	0.000
Cd_3	$44 \pm 0.49^{\frac{B}{a}}$	$39 \pm 0.44^{\frac{A}{b}}$	$40 \pm 0.44^{\frac{A}{ab}}$	$43 \pm 0.50^{\frac{B}{a}}$	0.001
Pb_1	$42 \pm 0.50^{\frac{B}{a}}$	$41 \pm 0.51^{\frac{B}{b}}$	$36 \pm 0.46^{\frac{A}{a}}$	$50 \pm 0.69^{\frac{C}{b}}$	0.000
Pb_2	$45 \pm 0.56^{\frac{B}{a}}$	$49 \pm 0.62^{\frac{C}{c}}$	$39 \pm 0.53^{\frac{A}{ab}}$	$46 \pm 0.64^{\frac{B}{ab}}$	0.000
Pb_3	$51 \pm 0.68^{\frac{C}{b}}$	$43 \pm 0.57^{\frac{A}{b}}$	$46 \pm 0.64^{\frac{AB}{b}}$	$44 \pm 0.57^{\frac{A}{a}}$	0.001
Cd_1/Pb_1	$53 \pm 0.67^{\frac{C}{b}}$	$42 \pm 0.58^{\frac{A}{b}}$	$44 \pm 0.56^{\frac{A}{b}}$	$50 \pm 0.68^{\frac{BC}{b}}$	0.001
Cd_2/Pb_2	$49 \pm 0.62^{\frac{C}{ab}}$	$40 \pm 0.52^{\frac{A}{b}}$	$43 \pm 0.58^{\frac{B}{b}}$	$47 \pm 0.63^{\frac{C}{ab}}$	0.000
Cd_3/Pb_3	$49 \pm 0.62^{\frac{C}{ab}}$	$33 \pm 0.41^{\frac{A}{a}}$	$34 \pm 0.40^{\frac{A}{a}}$	$38 \pm 0.57^{\frac{B}{a}}$	0.000
p-value	0.01	0.001	0.01	0.01	

Legend: Values are in mean triplicate ± standard deviation of the mean. Values which carry different uppercase superscript alphabets across rows show significant difference; while values which carry different subscript lowercase alphabet across column show significant difference.

Table 4: Fungi population in different incubation time and treatments

	Fungi count (10^2 CFU/g)				
Treatment	**Week 0**	**Week 2**	**Week 8**	**Week 12**	***p*-value**
Control	$21 \pm 0.25^{\frac{A}{a}}$	$26 \pm 0.30^{\frac{AB}{c}}$	$30 \pm 0.35^{\frac{B}{d}}$	$33 \pm 0.34^{\frac{B}{d}}$	0.001
Cd_1	$24 \pm 0.31^{\frac{B}{b}}$	$22 \pm 0.25^{\frac{A}{b}}$	$25 \pm 0.30^{\frac{B}{bc}}$	$22 \pm 0.24^{\frac{A}{b}}$	0.001
Cd_2	$25 \pm 0.30^{\frac{A}{b}}$	$24 \pm 0.31^{\frac{A}{bc}}$	$23 \pm 0.24^{\frac{A}{b}}$	$27 \pm 0.29^{\frac{B}{c}}$	0.001
Cd_3	$22 \pm 0.24^{\frac{B}{a}}$	$21 \pm 0.22^{\frac{A}{b}}$	$23 \pm 0.24^{\frac{C}{b}}$	$24 \pm 0.42^{\frac{D}{b}}$	0.000
Pb_1	$33 \pm 0.41^{\frac{B}{d}}$	$29 \pm 0.35^{\frac{A}{d}}$	$27 \pm 0.86^{\frac{A}{c}}$	$31 \pm 0.35^{\frac{B}{d}}$	0.001
Pb_2	$29 \pm 0.31^{\frac{B}{c}}$	$26 \pm 0.30^{\frac{A}{c}}$	$31 \pm 0.33^{\frac{C}{d}}$	$28 \pm 0.29^{\frac{B}{c}}$	0.000
Pb_3	$21 \pm 0.25^{\frac{B}{a}}$	$20 \pm 0.81^{\frac{B}{b}}$	$19 \pm 0.19^{\frac{A}{a}}$	$23 \pm 0.24^{\frac{C}{b}}$	0.000
Cd_1/Pb_1	$24 \pm 0.31^{\frac{A}{b}}$	$29 \pm 0.26^{\frac{B}{d}}$	$25 \pm 0.30^{\frac{A}{bc}}$	$29 \pm 0.35^{\frac{B}{c}}$	0.001
Cd_2/Pb_2	$21 \pm 0.25^{\frac{B}{a}}$	$21 \pm 0.25^{\frac{B}{b}}$	$19 \pm 0.19^{\frac{A}{a}}$	$20 \pm 0.18^{\frac{AB}{a}}$	0.001
Cd_3/Pb_3	$25 \pm 0.30^{\frac{C}{b}}$	$17 \pm 0.19^{\frac{A}{a}}$	$21 \pm 0.22^{\frac{B}{ab}}$	$20 \pm 0.26^{\frac{B}{a}}$	0.000
p-values	0.000	0.000	0.000	0.000	

Legend: Values are in mean triplicate ± standard deviation of the mean. Values which carry different uppercase superscript alphabets across rows show significant difference; while values which carry different subscript lowercase alphabet across column show significant difference.

Table 5a: Trend of bacterial succession from the heavy metal treated soil

Treatment	Week 0	Week 2	Week 8	Week 12
Bacterial isolates				
Control	*Pseudomonas putida* *Klebsiella pneumoniae* *Staphylococcus aureus*	*Pseudomonas putida* *Xanthomonas* sp. *Enterococcus durans*	*Pseudomonas aeruginosa* *Acinetobacter* sp. *Alcaligenes* sp.	*Corynebacterium* sp. *Arthrobacter* sp. *Xanthomonas* sp.
Cd_1	*Escherichia coli* *Pseudomonas putida* *Staphylococcus aureus*	*Proteus mirabilis* *Xanthomonas* sp. *Corynebacterium* sp.	*Zoogloea ramigera* *Citrobacter* sp. *Pseudomonas aeruginosa*	*Alcaligenes eutrophus* *Pseudomonas aeruginosa* *Micrococcus* sp.
Cd_2	*Enterococcus faecium* *Corynebacterium* sp. *Acinetobacter* sp.	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Micrococcus* sp.	*Arthrobacter* sp. *Alcaligenes eutrophus* *Pseudomonas aeruginosa*	*Pseudomonas aeruginosa* *Micrococcus* sp. *Alcaligenes eutrophus*
Cd_3	*Xanthomonas* sp. *Arthrobacter* sp. *Enterococcus durans*	*Micrococcus* sp. *Alcaligenes eutrophus* *Zoogloea ramigera*	*Zoogloea ramigera* *Alcaligenes eutrophus* *Micrococcus* sp.	*Alcaligenes eutrophus* *Zoogloea ramigera* *Gemella* sp.
Pb_1	*Klebsiella pneumoniae* *Enterococcus durans* *Escherichia coli*	*Xanthomonas maltophilia* *Brevibacterium frigoritolerans* *Staphylococcus saprophyticus*	*Trichococcus* sp. *Acidiphillum multivorum* *Staphylococcus saprophyticus*	*Pseudomonas ficuserectae* *Brevibacterium frigoritolerans* *Acidiphillum multivorum*
Pb_2	*Staphylococcus aureus* *Alcaligenes* sp. *Proteus mirabilis*	*Staphylococcus saprophyticus* *Hafnia* sp. *Pseudomonas ficuserectae*	*Brevibacterium frigoritolerans* *Acidiphillum multivorum* *Hafnia* sp.	*Xanthomonas maltophilia* *Pseudomonas ficuserectae* *Pseudomonas Alcaligenes*

Table 5b: Trend of bacterial succession from the heavy metal treated soil

Treatment	Week 0	Week 2	Week 8	Week 12
		Bacterial isolates		
Pb_3	*Enterococcus faecalis* *Proteus mirabilis* *Staphylococcus saprophyticus*	*Acidiphillum multivorum* *Hafnia* sp. *Pseudomonas aeruginosa*	*Acidiphillum multivorum* *Pseudomonas aeruginosa* *Trichococcus* sp.	*Hafnia* sp. *Acidiphillum multivorum* *Pseudomonas putida*
Cd_1/Pb_1	*Escherichia coli* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Pseudomonas ficuserectae* *Micrococcus* sp. *Zoogloea ramigera*	*Rastonia metallidurans* *Pseudomonas alcaligenes* *Staphylococcus saprophyticus*	*Gemella* sp. *Rastonia metallidurans* *Zoogloea ramigera*
Cd_2/Pb_2	*Enterococcus faecalis* *Arthrobacter* sp. *Corynebacterium* sp.	*Rastonia metallidurans* *Zoogloea ramigera* *Staphylococcus saprophyticus*	*Micrococcus* sp. *Gemella* sp. *Rastonia metallidurans*	*Rastonia metallidurans* *Micrococcus* sp. *Gemella* sp.
Cd_3/Pb_3	*Arthrobacter* sp. *Proteus mirabilis* *Pseudomonas aeruginosa*	*Micrococcus* sp. *Rastonia metallidurans* *Gemella* sp.	*Pseudomonas putida* *Rastonia metallidurans* *Trichococcus* sp.	*Gemella* sp. *Rastonia metallidurans* *Pseudomonas ficuserectae*

3.2 Discussion

The biological characteristics of soil are dynamic and sensitive to soil quality. Soil enzymatic activities have been severally used as a bioindicator to determine the toxicity influence of various pollutants on the quality of the microbial environment [24]. The urease and acid phosphatase activities of the soil in this study varied widely and were significantly influenced by the concentration of the metals and the period of incubation. The rate of inhibition of urease and acid phosphatase in the soil samples modified with heavy metal was also significantly higher than the control and there was an increment in the rate of inhibition as concentrations of the heavy metals increases. This could be due to the toxic effect exerted on these soil enzymes by the metal supplements. It was observed that an increase in metal concentration resulted in a corresponding increase in the inhibitory effect and increased exposure time (incubation period). This agrees with the report of Blonska and Lasota [25] that urease activity has been an effective bioindicator used in monitoring soil quality. As reported by Adetunji *et al*. [5], phosphatase activity is known to be a good indicator of soil quality and fertility. The association of heavy metals with enzyme-substrate complexes usually inhibits enzymes activities by getting the enzyme protein denatured and subsequently interact with their active sites [26]. The report of Tejada *et al*. [14] also demonstrated that soil contaminated with Cd consequently resulted in a significant decrease in urease and acid phosphatase activities.

Previous research has demonstrated that the inhibitory potency of metal pollutants on soil microbial biomass and soil enzymatic activities depends on the concentration of the metals, nature of metals pollutant and incubation period. Sardar *et al*. [27] reported that the degree of inhibition of soil enzymes increased extensively with increment in heavy metals concentrations and varied with incubation periods. This study agrees with the above-stated findings because it was observed that the degree of inhibition of the soils enzymatic activities varies and the highest degree of inhibition occur in (Cd/Pb) soil samples which happen to be the samples modified with the highest metal concentration. It was observed that cadmium poses a greater adverse effect when compared with lead. A similar report by Kabata-Pendias and Pendias [28] noted that the toxicity of cadmium is 2 to 20 times greater than any other heavy metals. Cadmium exerts a more toxic effect on enzymes than lead as a result of its lower affinity for soil colloids and greater mobility [29]. Additionally, a previous study by Chanda [30] agrees that the order of urease activity inhibition conventionally decreased according to the progression Cr > Cd > Zn > Mn > Pb. Furthermore, the higher degree of inhibition observed in this study could be attributed to the higher bioavailable fraction of metal demonstrated by cadmium when compared to lead.

Soil enzymes diminish as the concentrations of bioavailable metals increases [31]. In this study, it was noted that the values of the microbial biomass carbon in the Cd and Pb modified soil samples were decreased with the increase in the concentration of metals which varies notably in different periods of incubation. This could be due to an increase in the toxic effect exerted on the activity of the organisms resulting from an increase in the metal concentration supplements and exposure time. Metals negatively affect the biomass of soil microorganisms and deplete their activities in the soil [32]. The microbial population of the treated soil samples significantly decreased when compared to the control experiment in this study. Soil microbial counts serve as an indirect marker of soil's biological activity [33]. This validates the fact that the application of heavy metals in agricultural soils poses a toxic effect on the activities and proliferation of soil microorganisms [32]. It was also noted that the degree of microbial inhibition varies in different metal concentrations. The highest significant decrease was observed in the (Cd/Pb) samples which could be because the samples were modified with the highest metal concentration.

Furthermore, it was also observed in this study that the decrease in bacteria population was more significant than actinomycetes and fungi. This finding agrees with earlier reports of Liu *et al.* [35] and Khan *et al.* [20] that bacteria react more sensitively to heavy metals than actinomycetes or fungi. Some fungi notably thrive in these uneasy conditions, as they can tolerate extreme pH, temperature and metal concentrations [36]. The report by Gadd [37] attributed the high level of metal tolerance demonstrated by fungi to its cell walls which act as a barrier restricting the incorporation of solutes. Actinomycetes although not as tolerant as the fungi, were observed to have exhibited a higher degree of tolerance than bacteria. Actinomycetes can penetrate and solubilize polymers to persist and tolerate unfavourable environmental changes [38].

In this study aside from the elevated degree of tolerance exhibited by fungi and actinomycetes, it was also observed that a greater percentage of the bacteria isolates identified were Gram-negative organisms. At higher metal concentration Gram-negative bacteria was predominant. This could be due to their elevated level of intrinsic metal tolerance than the majority of the Gram-positive bacteria. Babich and Stotzky [39] attributed this higher level of metal tolerance demonstrated by Gram-negative bacteria when compared to Gram-positive bacteria to the distinctness in the chemical constituent of their cell wall. Gram-positive bacteria are less tolerant to heavy metals than Gram-negative bacteria and the degree of toxicity of heavy metals towards microorganisms could be expressed as Cr > Pb > As > Co > Zn > Cd > Cu [40].

The period of incubation also showed to have a pivotal influence on the microbial population, enzymatic activities and the microbial biomass carbon. The highest degree of decrease occurs in week 2. The severe inhibition in microbial activities recorded in week 2 of incubation which could be due to the modification of the soils samples with heavy metals which exposed the microorganisms' habitat to a sudden aberrant toxic condition. The effects of heavy metals which include Cd, Zn and Pb on the activities of soil urease vastly depend on the incubation time [24]. As this study progresses there was a slight increase in microbial activities at week 8 which maintain an almost steady state of activity till week 12 which could be due to the microorganisms developing tolerance to the toxic metals (Cd and Pb). The tolerance could be due to increased adaptation of the microorganisms to the metal concentration and could have initiated mechanisms to either metabolize the heavy metals or reduce their toxicity effects. However, in a previous study by Wakelin *et al*. [41], it was discovered that when metal pollution does not diminish the counts of microorganisms, they could still dwindle their diversity.Several other studies have described the qualitative and quantitative alteration in bacterial communities, in response to elevated concentration of diverse toxic metals in the soil [42]. The slight increase in microbial population exhibited in week 8 and week 12 could not be attributed to an increase in the microbial activities of the pioneer microorganism but rather the steady growth of metal tolerant microorganisms that survived the initial inhibition effect after less tolerant organisms got diminished. This is in agreement with the studies by Ruyters *et al*. [43] and Azarbad *et al*. [44] that heavy metals affect microbial diversity with the tolerant species adapting by developing genetic modifications which result in their abundance and the replacement of the more sensitive species. The less significant changes observed in the microbial enzymatic activities between week 8 and week 12 could be due to tolerance developed by the microorganisms and adaptivity to the environment; as well as decreased proliferation due to depletion of nutrients as no nutrient supplements were introduced throughout the study.

4. Conclusion

The increase in concentrations of heavy metals alters the autochthonous microbial community and their activities. The maximal inhibitory effects of the metals on soil microbial activities were observed in week 2. The rate and extent of inhibition of microbial activities in the soil are likely to

be correlative to the degree of tolerance and adaption of the microbial community, pollutant's concentration and the mechanisms of action. The sensitive and less tolerant species were diminished after exposure to the high concentration of toxic metals while tolerant species survived and proliferate.

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