



Soil bacterial flora and enzymatic activities in zinc and lead contaminated soil

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Abstract

Soil bacterial flora and enzymatic activities in lead and zinc contaminated soil of Ishiagu, Nigeria were investigated. The physicochemical properties measured showed that the mining pit had acidic pH (5.6) which gradually increased till 7.5 in the control. Organic matter was only 2.57mg/g in the pit but gradually reached 7.41mg/g in control. Pb concentration was higher at pit 360.52mg/g, 305.46mg/g at 5m away and lowest at control 36.16mg/g. Zn was 217.47mg/g at the pit, 176.32mg/g at 5m, 106.18mg/g at 10m and only 40.67mg/g at control. This showed a gradual fall away from the pit. Major organisms at the pit were *Pseudomonas* and *Bacillus* species (30% each) and *Mocrococcus* and *Chromobacter* species (20% each) *E. coli*, *Salmonella* and *Lactobacillus* species, which occurred in the control soil, were absent in the pit soil but occurred at various rates in other soil samples. Bacterial prevalence, diversity, and bioload were all high in the control, followed by 100m away while values decreased significantly towards the pit. Soil enzymatic activities correlated negatively with heavy metal concentration. This showed that the higher the heavy metal concentration the lower the enzymatic activities. Urease, dehydrogenase activity, hydrogen peroxidase and polyphenol oxidase were adversely affected but alkaline phosphatase did not show any significant effect.

Keywords: Soil, Zinc, Lead, Bacteria, Bioload, gradient

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INTRODUCTION

Microbial flora in relation to soil bioload and contamination is a significant component of the quality of the soil. The type of activities prevalent in any given environment determines the type of contamination in that area¹⁻³. Soil and water bodies have been sinks for many hazardous wastes, organic wastes sewage and several other waste types generated from different human activities. Many of these wastes contain heavy metals which contaminate the receiving sink.

The soil serves several human needs and several other natural functions. Nature too, has placed several minerals (metallic and non metallic) in the soil. To reach and obtain them requires extensive drilling or excavation⁴⁻⁸. Most industrial wastes are often not well treated before disposal⁹⁻¹¹.

The most commonly encountered heavy metals include Pb, Cd, Zn, Hg and As^{12,13}. Heavy metal toxicity represents an uncommon but clinically significant medical hazard often unrecognized and inappropriately treated. In nature, Pb and Zn are often found together^{13,14}. Both metals have found extensive use in man's activities. Waste resulting from their mining and use litter many places.

In most cases, large ponds and heaps of wastes are left in the trail of excavations for these elements. While rains wash the waste heaps into the surrounding water bodies and farmlands, the ponds overflow their banks resulting in pollution even outside the area of production¹⁵. The above scenario is typical of the Pb or Zn mining fields of Ishiagu, Ebonyi State in Nigeria. Several reports have it that such pollution inhibits soil microbial activities but information from the study area is lacking in spite of the extensive mining activities there. This work then aims at establishing the level of pollution, soil bacterial flora and enzymatic activities in Ishiagu in relation to the soil health or quality.

MATERIALS AND METHODS

Ishiagu, the study area lies in the northern part of Abia State, South East Nigeria where rock blasting and heavy metal (Pb or Zn) mining activities are common. Two major heavy metal

mining companies in the area excavate for Pb and Zn and abandon the wastes generated in heaps leaving large ponds in the typical Guinea Savannah climate farmland. Indigenes also scavenge for the same minerals in the abandoned heaps.

Soil properties and heavy metal extraction

The climatic condition of the area is Guinea savannah and has typical uniform sandy, loamy soil. The soil pH and temperature were determined directly at the site using multipurpose tester (Jenway HANNA 1910 model). Soil Organic matter content was determined using loss of ignition method as described by Lee *et al.*² involving the use of furnace (MAC 2000). The soil moisture was also determined by the drying to constant weight method as in the publication of the American Public Health Association (APHA)¹⁶.

Soil heavy metals concentrations were estimated using the Atomic Absorption Spectrophotometric (AAS) method after acid digestion as described in APHA¹⁶ with HACH/D2/2010 spectrophotometer.

Soil enzymatic activities

The enzymes analysed in this work include dehydrogenase activity (EC 1.1.1.1) which is the reduction of tetrazolium chloride (TTC) to triphenyl formazon (TPF), urease, hydrogen peroxidase, poly- phenol oxidase, acid and alkaline phosphatases activities. The dehydrogenase activity was determined as described by Cassida *et al.*¹⁷ and modified by Li *et al.*¹⁸. 5.0g of soil was mixed in 10ml 0.25% aqueous triphenytetrazolium chloride (TTS). This was incubated in sealed tubes at 30°C for 6 hours. The absorbance at 485nm of the methanol extracts of the triphenylformazon (TDF) formed was measured using methanol as blank. The result was expressed as TPF⁻¹ dry soil 6h

The urease activity was estimated using the colourimetric method based on NH₃-N formation in the Urea-amended soil sample. The soil was incubated at 37°C for 24 hours and the result expressed as mg NH₃-N g⁻¹ dry soil 24h^{19,20}.

Soil hydrogen peroxidase activity was determined by the KMnO₄ titration method.

The result was expressed as The result was expressed as mL g⁻¹ dry soil soil²¹. The polyphenol oxidase activity was estimated by the colorimetric method as described in Ma *et al*²² and Li *et al*¹⁸ modified from Tabatabai and Bremearr²³. This was based on the purpurogallin formation in the pyrogalllic acid-supplemented soil samples. The amended soil was incubated at 30°C for 3 hours and the result expressed as mg purpuragallin g⁻¹ dry soil 3h¹.

The activities of both acid and alkaline phosphatases were determined using the methods of Tabatabai (24 which involved the use of Nitrophenyl/phosphate with CaCl₂ and NaOH added to stop the reactions and reading the result at 410nm.

Microbiological analysis

Prevalence of soil bacterial species was determined using the culture technique. Ten soil samples from each sampling point were collected at one-week intervals and cultured on Tryptone soil Agar, McConkey Agar, and Mineral salt Agar. The frequency of each organism was taken as occurrence or prevalence. Different bacterial groups were also investigated using various culture media including Pb and Zn amended ones at concentration of 3mg/ml of the Pb and Zn¹⁰. The groups were Total Heterotrophic Bacteria (THB), Coliform Bacteria (CB), Nitrifying Bacteria (NB), Pb-resistant bacteria (PRB) and Zn-resistant Bacteria (ZRB). The bioloads of these organisms were determined after ten-fold serial dilution of 1.0g of fresh soil sample as described by Chessbrough²⁵.

RESULTS

The results obtained in the physicochemical parameters and the heavy metal concentration assessments are shown in Table 1. There was a general gradient in all the parameters-either increasing or decreasing with distance away

from the excavated pit. The pH of the pit soil was weakly acidic (5.6) but gradually changed to neural in 100m and control soil (7.0 and 7.5) respectively. The organic matter that was only 2.57 in pit soil rose to 7.41 in control. However soil moisture gradually decreased away from the water filed pit but no significant change was observed in temperature (Table 1).

Concentrations of Pb and Zn followed the same pattern and were quite significant ($p = 0.05$) highest at the pit lowest and further away. Pb had 360.52 at the pit, 305.46 at 5m away while 100m and control had 112.53 and 36.16 respectively. Zn had 217.47 at the pit, 176.32 at 5m away and 40.67 at control (Table 1) with 51.77 and 41.67 for 100m and control soil respectively. Pb and Zn values correlated negatively with pH and organic matter content but positively with moisture content.

The frequency (occurrence) of each bacterial species observed in the work is shown in Table 2. All the organisms had their lowest prevalence in the pit soil but increased gradually till 100m which had no significant statistical difference with control ($p = 0.05$). *Bacillus* and *Pseudomonas* species were the most prevalent in the heavy metal source (pit) while *E-coli*, *Staphylococcus* and *Lactobacillus* were found in 100m and control samples (Table 2).

Table 3 shows the bioload of each of the groups of bacteria estimated in relation to the effects of the heavy metals in soil. The bacterial groups had their lowest bioload in pit, followed by the 5m away soil while the highest bioloads were in the 100m and control soil samples.

The most affected was the nitrifying bacteria with only 1.7×10^1 of the pit and 2.8×10^4 and 3.1×10^4 in 100m and control respectively. The least affected bacterial group was the total heterotrophic bacteria

TABLE 1: Concentrations of lead and Zinc and some other parameters of the various soils samples analyzed

Soil sample distance from pit	Pb mg/g	Zn mg/g	pH	Temp. °C	Organic matter mg/g	Soil moisture %
Pit soil	360.52	217.47	5.6	30.2	2.57	37.00
5m	305.46	176.32	6.2	29.6	3.67	35.00
10m	216.24	106.81	6.8	29.7	5.14	34.00
100m	105.31	81.77	7.2	29.6	7.12	32.00
Control	36.16	40.67	7.5	29.5	7.41	30.00

*Values are the mean values of three times sampling.

TABLE 2: Prevalence of bacterial species isolated from the various samples

Organisms	Pit	5m	10m	100m	Control
<i>Pseudomonas</i> species	30.00	30.00	40.00	60.00	30.00
<i>Micrococcus</i> species	20.00	30.00	40.00	60.00	60.00
<i>Xanthomonas</i> speccies	-	-	20.00	30.00	20.00
<i>Azotobacter</i> species	20.00	20.00	40.00	60.00	60.00
<i>E. coli</i>	-	20.00	60.00	80.00	90.00
<i>Salmonella</i> species	-	-	-	20.00	10.00
<i>Chromobacter</i> species	20.00	30.00	40.00	60.00	40.00
<i>Bacillus</i> species	30.00	40.00	80.00	100.00	100.00
<i>Staphylococcus</i> species	20.00	20.00	30.00	60.00	70.00
<i>Lactobacillus</i> species	-	-	20.00	40.00	50.00

*Values are mean percentages of isolates obtained from ten times sampling; - Not observed

TABLE 3: Bioloads of various groups of bacterial species from the various soil samples

Bacterial group	Pit	5m	10m	100m	Control
THBC	2.3×10^4	6.1×10^4	4.3×10^5	5.7×10^6	6.4×10^6
NBC	1.2×10^1	2.9×10^2	4.1×10^3	2.8×10^4	3.6×10^4
CBC	3.1×10^2	4.1×10^3	2.9×10^4	2.1×10^4	3.2×10^6
PRBC	1.6×10^2	1.8×10^2	2.7×10^3	2.7×10^3	2.1×10^3
ZRBC	2.1×10^2	2.5×10^2	3.1×10^3	2.3×10^3	2.7×10^3

*Bioload values are mean values of five times estimation; THBC: Total Heterotrophic Bacterial Count; NBC: Nitrifying Bacterial Count; CBC: Coliform Bacterial Count; PRBC: Lead (Pb) Resistant Bacteria Count; ZRBC: Zinc (Zn) Resistant Bacteria Count

TABLE 4: Soil enzymatic activities in the various soil samples examined

Enzymes	Pit	5m	10m	100m	Control
Dehydrogenase $\text{mg g}^{-1} \text{6h}^{-1}$	0.72	0.99	1.31	4.11	4.42
Urease $\text{mg g}^{-1} \text{24h}^{-1}$	0.61	0.92	2.2	3.10	3.32
Polyphenol oxidase $\text{mg g}^{-1} \text{3h}^{-1}$	0.91	1.51	1.74	2.1	2.61
Hydrogen oxidase $\text{mL g}^{-1} \text{1h}^{-1}$	0.93	1.32	1.82	2.4	2.52
Acid phosphatase ($\mu\text{mol-p-nitrophenol}$)	0.54	0.72	1.01	1.53	1.62
Alkaline phosphoatase ($\mu\text{mol-p-nitrophenol}$)	0.97	1.12	12a	1.4b	1.61

Soil enzymatic activities were significantly lower in the soil from the pit compared to other soil sampling points. However, the activities increased with distance away from the excavation pit correlating negatively with the heavy metal concentrations in soil. The most sensitive enzyme activity was the

dehydrogenase, which had 6.13 times activities less in the pit compared to the control and 4.3 times less in the 100m away. Urease also showed high sensitivity to Pb and Zn poisoning with 5.44 times less than the value obtained in the control (3.32). The phenol oxidase was 2.86 times less in pit than

control. Acid phosphatase and hydrogen peroxidase, also showed similar gradient in activities (Table 4). The least sensitive i.e more resistant enzyme was the alkaline phosphatase which had only 1.66 time less activities in the most heavy metal contaminated soil pit.

DISCUSSION

Results obtained in this work showed that soil pH and organic matter content were adversely affected by high Pb and Zn concentrations observed nearest the pit. The values obtained at the pit and 5m away were above the acceptable levels²⁶ hence affected the pH and organic matter content. Oliveira and Pampulha¹, Babich and Stotzky²⁷ and Christensen²⁸ agree that low pH (acidic) reduces solubility and speciation of metals in soil and soil solution which directly rubs off in soil organic matter. This was the case in this study where pH and organic matter in the pit soil were lower than others. This could have caused the high adverse effect observed in the soil at the pit and 5m away. This assertion tally well with Nwaugo *et al*²⁹ and Chinyere³⁰ that pollutants have highest concentrations at the discharge point or sources.

Though ten bacterial species were observed in this work, only five were seen in the immediate vicinity of the pollution pit. The bacteria increased in prevalence and diversity away from the pit indicating a negative correlation with Pb & Zn concentrations. Fagade and Adetutu¹⁰, Nwuba³¹ and Abdou *et al*¹⁵ stated that heavy metal suppressed microbial growth but allows resistance ones to grow slowly which was observed in this work. However, away from the high Pb and Zn concentration, microbial prevalence and diversity increased. This observation was further buttressed by results obtained in the bioload analysis as bioload of most bacterial groups were higher distances away from the pit. Lee *et al*², Kuperman and Carreiro³², and Fagade and Adetutu¹⁰ reported that most heavy metals are toxic to soil micro-organisms at high concentrations and even inhibit the enzymes resulting in low microbial occurrence in such polluted soils.

All the various groups of bacteria examined were not affected at the same rate. This work

agrees with Nwaugo *et al*³³, Martensson³⁴ and Oliveira and Pampulha¹ that nitrifying bacteria which is a complex group of phylogenetically and physiologically diverse bacteria are very sensitive to pollution. This gives a very positive indication that the nitrifying bacteria can be used as good indicators of anthropogenic pollution of the soil. There was less effect on THB, which could be understood as the group is the sum total of the heterotrophic (all variable and culturable) bacteria present in the soil at that point. The suppression of even the Pb and Zn resistant bacteria groups of the pit and 5m away show that no group of bacteria is spared by adverse environmental conditions, though the extent varies.

The significance of soil enzymatic activities assessment is enormous as even the activities of the unculturable bacterial types are equally assessed with the culturable ones. This is because the enzymatic activities correlate well with their parent organisms^{1,2,35-37}. Analysis of this work show that the oxidoreductases used in the work (dehydrogenase, urease, phenol oxidase, peroxidase and the phosphatases- (alkaline and acid) were sensitive to Pb and Zn contamination. However dehydrogenase, urease, phenol oxidase and acid phosphatase were more sensitive to the heavy metals pollution. The work agrees with Quilchairo and Maronon³⁸, Leiros *et al*³⁹, Konapka *et al*³ and a host of others that soil enzymatic activities could easily be applied in assessing soil quality, especially the intracellular ones which are directly attached to the organisms.

The soil fertility could also be assessed by these enzymatic activities along with the nitrifying bacterial bioload. These determine the level of Nitrogen in the soil in question which agrees with Mantellin and Touraine⁴⁰. Dehydrogenase activity, urease and acid phosphatase were very sensitive to the heavy metal contamination. However, the work disagrees with Wyzkowska and Kuchariski⁴¹ who stated that acid phosphatase was less sensitive in the soil. This difference could be due to the type of soil contaminant examined as Wyzkowska and Kuchariski⁴¹ had worked on petrol-contaminated soil.

Lee *et al*², Wyzkowska and Kuchurski⁴¹, and Mantellin and Touraine⁴⁰ reported that plants

assimilate and accumulate these heavy metals in their tissues which could be dangerous to man if such plants are consumed. In conclusion, this work therefore suggests that the excavation for Pb and Zn with the consequent inappropriate disposal of the resultant wastes, cause high concentration of these elements in the soil. The concentration of the contaminants gradually decreased with distance away from the source of pollution, which agrees with Nwaugo *et al*⁴². The Pb and Zn contamination adversely affected the soil microbial quality in both prevalence and diversity and calls for remediation if the soil must be used for agricultural purposes.

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