Study on the Effect of Lead on Bacterial Flora of Soil Sample

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***Abstract*: The effects of lead on bacteria flora of soil samples were studied. Soil samples collected from Agricultural Technology farm of Akanu Ibiam Federal Polytechnic Unwana, were distributed into five buckets at 1kg each. The total bacteria load of the soil sample were determined before the introduction of different grams of lead, the result of initial bacteria load revealed 6.28×10^5cfu/g using serial dilution method. Different grams of lead were used to pollute the soil samples and allow to stay for three weeks. The microbial loads of the samples were determined weekly. The result revealed the first week of pollution with different grams of lead at 20g, 15g, 10g, 5g and control are 1.0×10^5cfu/g, 1.8×10^5cfu/g, 4.5×10^5cfu/g, 5.0×10^5cfu/g and control 5.21×10^5cfu/g and second week had 0.9×10^5cfu/g, 1.5×10^5cfu/g, 4.0×10^5cfu/g,4.3×10^5cfu/g and control 5.26×10^5cfu/g and the third week had 0.7×10^5cfu/g, 1.3×10^5cfu/g, 3.0×10^5cfu/g, 4.0×10^5cfu/g and control 6.23×10^5cfu/g respectively. The isolates were identified as *pseudomonas aeruginosa, Bacillus subtils, Micrococus letus, Corynebacterium speices* and *Staphylococcus aureus* through morphological characteristics and biochemical tests. The study showed that various bacteria genera were associated with lead polluted soil samples. The growth of bacteria observed showed delayed growth in polluted samples whereas growth was rapid in control sample. The bacteria counts were generally high in the control when compared to those of the polluted sample. This revealed that lead had depressing effect on the growth and activity of soil bacteria,**

***Key words:* lead, flora, serial dilution, depressing genera and polluted.**

I. INTRODUCTION

Lead is a metallic element belonging to group iv A of the periodic table and classified as a heavy metal elements with metallic properties and an atomic number >20 (1). Pure lead is a silvery-white metal that oxides and turns blue-grey when exposed to air with metallic luster and a particularly high density compared with other metals such as copper and aluminum, it is a poor conductor of electricity. On the other hand in comparison to most metals it has a very low melting point of 32.7℃ and is therefore readily fusible, lead is soft, highly malleable, ductile and characterized by low strength and high resistance to corrosion in most common environments (2, 1, 3).

Environmental biotechnology strategies must address and solve in a long term perspective the formidable environment problems now facing the world, such as soil contamination with metals, pesticides or hydrocarbons, disposal of animal manures, treatment of waste water or recovery of reusable products and energy from waste. Thus, bioremediation is becoming increasingly used mostly in cases of removal of heavy metal from contaminated environment, as a cheaper alternative to chemical technology. On the other hand microorganisms capable of biodegrading or detoxify heavy metals usually are already present in contaminated soils and ground water. There is therefore, a need to isolate, identify and characterize the microorganisms that exist and interact in contaminated environment and to isolate the genetic determinants of resistance frequently located on plasmids and transposing.

Studies have describes the isolation, screening and genetic characterization of the lead resistance bacteria strains isolated from the battery manufactured polluted soil. Global industrialization has been attributed to the pollution crisis all round the world. One of the major environment problems today is heavy metals contamination resulting from industrial activities such as battery industry (4). The examples of industrial waste are lead, oil spills, dyes and petroleum products such as diesel, gasoline and other energy sources. One of the currently most important environmental problems is global contamination by toxic substances. The aim of this work is to pollute the soil sample with different concentrations of lead and to determine the effect of lead on bacteria flora of the soil sample.

II. MATERIALS AND METHODS

Collection of Soil Samples

Soil samples were collected from the agricultural technology farm in Akanu Ibiam Federal Polytechnic Unwana, with the aid of soil trowel at depths of 10-15cm into sterile bucket and were taken to the laboratory to measure and distribute into other buckets. The study conducted between the months of August and December

Experimental Setup

The experimental design consisted of five perforated plastic buckets containing 1kg of each soil. One of the plastic buckets served as a control (lead free soil, LFS) while the other four buckets were contaminated with lead of 20g, 15g, 10g, and 5g (lead polluted soil, LPS). The five experimental setup as shown in table 1, were incubated in the laboratory at a minimum aseptic condition for a period of three weeks and homogenized before analyzed. These buckets were perforated to increase aeration and covered with net to avoid external penetration.

Experimental Protocol

Table 1: Experimental design.

Total sand	Total lead used
1st Bucket	20g
2nd Bucket	15g
3rd Bucket	10g
4th Bucket	5g
5th Bucket	Control

From the table shown above different grams of lead at 20g, 15g, 10g and 5g respectively were applied to 1kg of soil sample each.

Bacteriological Analysis

Serial Dilution

Serial dilution was carried out to determine the number of cells in a bacterial culture. Since bacteria cell number are usually very high in agricultural soil sample plating out this sample in an undiluted fashion would just lead to the creation of bacteria lawn (a smear of many individual bacteria colonies that are all growing next to or on top of one another).

Identification of Bacterial Isolates

Bacterial isolates used in this study were identified based on: Morphological characteristics of colony.Staining and microscopic visualization. Gram staining and biochemical tests were performed on the bacterial isolates following the guidelines outlined by Prescott and Harley [5].

III. RESULTS

After the serial dilution and plating for the period of three weeks the following results were obtained.

Table 2: Bacterial counts after first week of contamination

Different grams of lead	Total bacteria count (cfu/g)
20g	1.0×10^5
15g	1.8×10^5
10g	4.5×10^5
5g	5.0×10^5
Control	5.21×10^5

Table 3: Bacterial counts after second week of contamination

Different grams of lead	Total bacteria count (cfu/g)
20g	0.9×10^5
15g	1.5×10^5
10g	4.0×10^5
5g	4.3×10^5
Control	5.26×10^5

Table 4: Bacterial counts after third week of contamination

Different grams of lead	Total bacteria count (cfu/g)
20g	0.7×10^5
15g	1.3×10^5
10g	3.0×10^5
5g	4.0×10^5
Control	6.23×10^5

Table 5: Preliminary biochemical tests for isolated strains

Suspected isolated	Morphology	Gram rxn	Catalase	Citrate	Motility	Coagulase
Pseudomonas spp	Rod	-	+	+	+	-
Bacillus subtilis	Rod	+	+	-	+	+
Corynebacterium spp	Rod	+	+	+	-	-
Micrococcus luteus	Cocci	+	+	-	-	-
Staphylococcus aureus	Cocci	+	+	+	+	+

Key: (+) Positive, (-) Negative, (rnx) Reaction

IV. DISCUSSION

Soil contains a variety of microorganisms including bacteria that can be found in any natural ecosystem. Microorganisms play an important role on nutritional chains that are an important part of the biological balance in the life in our planet. Where, bacteria are essential for the closing of nutrient and geochemical cycles such as the carbon, nitrogen, sulfur and phosphorous cycle. Without bacteria, soil would not be fertile and organic matter such as straw or leaves would accumulate within a short time [6]The five buckets used for this experiment was filled with 1kg of soil sample with different quantity of lead applied to it. 1st bucket contain 20g, 2nd bucket contain 15g, 3rd bucket contain 10g, 4th bucket contain 5g of lead while the 5th bucket contain no lead which is the control as shown in table 1.

Table 2. shows the bacteria count after first week of contamination of the soil sample with different contraction of lead of 20g, 15g 10g, 5g and control had the total bacterial count of 1.0×10^5cfu/g, 1.8×10^5cfu/g, 4.5×10^5cfu/g, 5.0×10^5cfu/g, and control 5.21×10^5cfu/g. Table 3. Had 0.9×10^5cfu/g, 1.5×10^5cfu/g, 4.0×10^5cfu/g, 4.3×10^5cfu/g1 and 5.26×10^5cfu/g for second week of

contamination. Table 4. Had 0.7×10^5cfu/g, 1.3×10^5cfu/g, 3.0×10^5cfu/g, 4.0×10^5cfu/g1 and 6.23×10^5cfu/g for the third week of contaminating the soil with lead respectively. Even though the results obtained by plate enumeration underestimate the actual soil microbial populations, the evaluation of the cultivable bacteria fraction in a contaminated soil can provide useful information about the impact of the contamination on the autochthonous micro flora. Enumeration of total heterotrophic aerobic bacteria was performed by using nutrient agar thus unique populations of microorganism can be cultured (7),Lead have been known to disrupt ecosystem structure and functioning for a long time. In the multicellular organisms, heavy metal target specific organs and pathways. Resulting in disruption of definitive metabolic functions. By the same token, one can expect that some pathways in microbes would be more sensitive to metal than others, resulting in selective inhibition as metal concentration increases this first datum seems to suggest a good adaptation and resistance to the contamination by the established bacterial population. The immediate reported effect of lead stress is a decrease in microbial biomass as metal sensitive microbes are inhibited over a longer period. There can be a gradual change in the microbial composition in which natural selection, if tolerant community develops in soil as this seems to be the case then the decrease in biomass and activity may be temporary (8). It was observed that the microbial load decreased with increases on the number of weeks .Studies have shown that long-term lead contamination of soils has harmful effects on soil microbial activity, especially microbial respiration [9]. *Conynebacterium specie, Staphylococcus aureus,* and *Pseudomonas aeruginosa Bacillus subtilis and Micrococcus luteus* were isolated

V. CONCLUSION

Nowadays, lead is worldwide one of the pollution of major concern due to its widespread diffusion, persistence and toxicity. Indeed lead (Pb) has been widely used since ancient time and its spreading in the environment is connected to industrial, agricultural and urban activities such as manufacturing and smelting of batteries, land application of sewage sludge (1,8,9). Indeed organic lead has been used for almost a century as anti-knocking additive for petrol, leading to an ubiquitous pollution of both organic and inorganic lead (Pb) in low concentration and much more severe contaminations at manufacturing and distributing level, implying the need for remediation (10).

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