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Research Article

Distribution of Bacteria in Lead Contaminated Soil in Anka Local Government Area, Zamfara State, Nigeria

Mustapha Bashir Kazaure*

Department of Science Laboratory Technology, College of Science and Technology, Jigawa State Polytechnic Dutse, Nigeria

***Corresponding Author:** Mustapha Bashir Kazaure, Department of Science Laboratory Technology, College of Science and Technology, Jigawa State Polytechnic Dutse, Nigeria.

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Abstract

This study was to determine the distribution of bacteria in lead contaminated soil of Anka Local Government Area of Zamfara State, Nigeria. Soil samples were collected from five different places of the local government which include Abare, Bagega (BGG), Dan Kamfani (DNK), Dareta (DRT) and Anka town (ANT) where illegal gold mining activities is taking place. The samples were collected for the period of three months (November, December and January). The physicochemical and bacteriological parameters of the soil samples were analyzed. The soil samples were serially diluted and 0.1ml of 10^6 dilution factors was inoculated into nutrient agar and incubated for 24 hours at 37^0C. The highest concentrations recorded for lead was 57 × 101mg/kg from the DNK. Soil from ANT had the highest total bacterial counts of 6.9×10^6cfu/g, 5.5×10^6 cfu/g and 7.2×10^6 cfu/g for November, December and January respectively. The predominant bacterial isolates were identified as species of *Bacillus, Proteus, Achromobacter, Citrobacter, Corynebacerium, Alcaligenes, Pseudomonas, Staphylococcus, Klebsiella, Escherichia, Agrobacterium, Enterobacter* and *Diplococcus. Bacillus* species had the highest frequency of occurrence (45.67%). *Achromobacter, Agrobacterium, Enterobacter* and *Diplococcus* species had a very low frequency of occurrence (0.79%).

Keywords: Bacteria; Lead; Contaminated; Soil

Introduction

Anthropogenic activities have resulted in localized ecosystem contamination with heavy metals that serve no known biological purpose such as mercury, arsenic, cadmium, and lead [1]. Anthropogenic sources of heavy metal pollution include but not limited to mining, iron smelting, fossil burning and municipal and industrial waste disposal. Illegal mining activities in Zamfara, Northern Nigeria were reported to have killed hundreds of people, mostly children in 2010 [2]. Heavy metal contamination is a major environmental threat worldwide due to their adverse effects (toxicity) on natural biota and humans which is manifested as DNA damage, lipid peroxidation, binding to -SH groups of essential proteins and enzymes and generation of reactive oxygen species [3]. Heavy metals present in soils pose serious threat to human and animal health. Neither are they neutral to plants microorganisms [4]. They can have inhibitory effect on the development of bacteria, fungi and actinomycetes [5,6]. Heavy metals reduce the biomass of microorganisms and lower their soil activity [5,7] and even if they do not reduce their number, they depress their biodiversity [8].

Lead, a ubiquitous and versatile metal, has been used since prehistoric times. It has become widely distributed and mobilized in the environment and human exposure to and uptakes of this non-essential element have consequently increased [9]. At high levels of human exposure there is damage to almost all organs and organ systems, most importantly the central nervous system, kidneys and blood, culminating in death at excessive levels. At low levels, haeme synthesis and other biochemical processes are affected, psychological and neuro behavioral functions are impaired, and there is a range of other effects [10,11], collectively known as lead poisoning [12]. There is a long history of public exposure to lead in food and drink. Lead poisoning was common in Roman times because of the use of lead in water pipes and earthenware containers, and in wine storage. Lead poisoning associated with occupational exposure was first reported in 370 BC [13]. It became common among industrial workers in the 19th and early 20th centuries, when workers were exposed to lead in smelting, painting, plumbing, printing and many other industrial activities. In 1767, Franklin obtained a list of patients in La Charite´ Hospital in Paris who had been admitted because of symptoms, which, although not recognized then, were evidently those of lead poisoning. All the patients were engaged in occupations that exposed them to lead [9,13].

Exposure of human populations to environmental lead was relatively low before the industrial revolution but has increased with industrialization and large-scale mining. Lead contamination of the environment is high relative to that of other nonessential elements [14]. Globally, the extensive processing of lead ores is estimated to have released about 300 million tonnes of lead into the environment over the past five millennia, mostly within the past 500 years. The concentrations of heavy metals in soils are associated with biological and geochemical cycles and are influenced by anthropogenic activities, such as agricultural practices, transport, industrial activities and waste disposal [14].

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 [15]. The establishment of the strong binding between soil particulates and bacteria is probably a gradual process, involving a variety of binding mechanisms [16]. Soil bacteria and fungi play pivotal roles in various biogeochemical cycles (BGC) and are responsible for the cycling of organic compounds. Soil microorganisms also influence above-ground ecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility [17]. Soils normally contain low background levels of heavy metals. However, in areas where agricultural, industrial or municipal wastes are land-applied as fertilizer, concentrations may be much higher. Excessive levels of heavy metals can be hazardous to man, animals and plants. Although most organisms have detoxification abilities (i.e. mineralization, transformation and/or immobilization of pollutants), particularly, bacteria play a crucial role in biogeochemical cycles and in sustainable development of the biosphere [18].

Materials and Methods

Experimental Design and Sample Collection

The study area includes four different villages of Anka Local Government Area of Zamfara State, Nigeria. These are Abare (ABR), Bagega (BGG), Dan Kamfani (DNK), Dareta (DRT) and Anka town (ANT). The soil from Anka town where no mining activity is taking place was used as control. Purposive and random sampling method was also applied to each mining site. In each of the locations, ten soil samples were randomly collected in triplicates from the top 0 - 20 cm of the soil with the aid of soil auger and bulked into polythene bags. The soil samples were transported to the Usmanu Danfodiyo University, Sokoto Postgraduate Microbiology Research Laboratory for analysis. The soil samples were collected at after one-month interval for the months of November, December [2013] and January [2014] respectively.

Characterization and Identification of Isolates

Pure isolates of bacteria were identified based on colonial, morphological Gram staining properties and biochemical characteristics following the guidelines outlined by Barrow and Feltham [19] as well as Prescott and Harley [20]. Some of the biochemical tests employed are Triple sugar iron test, Urease production test, Methyl red reaction test, Voges-Proskauer test, Indole production test, Citrate utilization test, Catalase, Oxidase and Starch hydrolysis.

The data generated from the experiments were analyzed by analysis of variance (ANOVA) using INSTAT statistical package at 95% statistical significance.

Results

Physicochemical Analysis of Soil

Results obtained from the study of the physicochemical parameters were shown on table 1. Soil pH was observed to be slightly acidic, ranging from 5.6 to 6.3. Highest pH (6.3) was recorded in Abare (ABR) soil and least pH (5.6) was also recorded in DNK. DRT soil had the highest organic carbon (OC) (1.12%) and organic matter (OM) (1.93%) content. BGG samples were poorest in both the organic carbon (OC) and organic matter (OM) 0.14 and 0.24 respectively. The ANT had moderate amount of both the parameters 0.34% and 0.59% respectively. Nitrogen and phosphorus contents of the soils were shown to differ although without statistical significance ($p \geq 0.05$). Soil from DRT, ABR and DNK had higher nitrogen content 0.109%, 0.095% and 0.067% respectively. Similarly, ABR, DRT and BGG soils recorded 0.95 ppm, 0.86 ppm and 0.82 ppm of phosphorus respectively. Moreover, DRT soil was shown to contain more calcium (1.75 cmol/kg), potassium (1.62 cmol/kg), sodium (0.70 cmol/kg) and cation exchange capacity (9.64 cmol/kg) than the other soils except for magnesium where ABR soil had higher content (1.06 cmol/kg).

The lowest quantities of Potassium K, Sodium Na, and Cation Exchange Capacity CEC) were observed in the Soil of ANT with potassium (K), 0.82 cmol/kg, sodium (Na), 0.39 cmol/kg and Cation Exchange Capacity (CEC), 7.74 cmol/kg). DNK had the lowest magnesium (Mg) content of 0.65 cmol/kg.

Site	Parameter (mean ± SD)									
	Ph	OC (%)	OM (%)	N (%)	P (ppm)	Ca (cmol/kg)	Mg (cmol/kg)	K (cmol/kg)	Na (cmol/kg)	CEC (cmol/kg)
ABR	6.3	0.40	0.69	0.095	0.95	1.30	1.15	1.23	0.57	8.50
BGG	5.7	0.14	0.24	0.032	0.82	1.65	0.90	1.44	0.65	9.36
DNC	5.6	0.46	0.79	0.067	0.79	0.80	0.65	1.15	0.48	8.66
DRT	5.9	1.12	1.93	0.109	0.86	1.75	1.05	1.62	0.70	9.64
SAT	6.0	0.34	0.59	0.056	0.77	0.70	0.90	0.82	0.39	7.74

Table 1: Monthly Physicochemical Parameters of the Soil Sample.

SD: Standard Deviation; ABR: Abare; BGG: Bagega; DNC: Dan Company; DRT: Dareta; SAT: Soil from Anka Town; OC: Organic Carbon; OM: Organic Matter; N: Nitrogen; P: Phosphorus; Ca: Calcium; Mg: Magnesium; K: Potassium; Na: Sodium; CEC: Cation Exchange Capacity.

Enumeration of Bacteria in Soil Samples

The soil samples from Anka Town (ANT) which was used as control had the highest number of the bacterial counts in all the three months. The mean counts were recorded as 6.9×10^6 cfu/g, 5.5×10^6 cfu/g and 7.2×10^6 cfu/g for the months of November, December and January respectively. The mean counts of ABR followed that of ANT in the months of November and January as 5.6×10^6 cfu/g and 4.3×10^6 cfu/g respectively, however the mean value recorded for the month of December was 1.3×10^6 cfu/g. Dareta (DRT) is having next to the least mean of bacterial count in November and January as 2.0×10^6 cfu/g and 1.7×10^6 cfu/g respectively, while in December it had the least mean bacterial count of 1.0×10^6 cfu/g. Bagega (BGG) followed ABR mean of the total of bacterial count in November and January as 4.7×10^6 cfu/g and 1.7×10^6 cfu/g respectively, while in December the mean total of bacterial count was recorded as 3.9×10^6 cfu/g followed that of Soil from Anka Town (SAT). Dan Company (DNC) had the least mean of the total of bacterial count in November and January as 1.1×10^6 cfu/g and 1.1×10^6 cfu/g respectively.

Sampling site	Mean Count (cfu/g) × 10⁶*		
	November	December	January
SAT	6.9^a	5.5^a	7.2^a
ABR	5.6	1.3^{abcd}	4.3
DRT	2.0	1.0^b	1.7^a
BGG	4.7	3.87^c	1.7^a
DNC	1.1^a	1.7^d	1.1^a

Table 2: Total Aerobic Heterotrophic Bacterial Count.

*Values with the same superscript in a column are significantly different $p \leq 0.05$.

While in December the mean total of bacterial count of DNC was recorded as 1.7×10^6 cfu/g followed that of BGG. Table 3 showed the bacterial isolates in the soil samples. *Bacillus* species had the highest frequency of occurrence than the rest of the isolates. *Achromobacter, Agrobacterium, Enterobacter* and *Diplococcus* species are having a least frequency of occurrence with (0.79%). Other isolates have their frequencies of occurrence ranging from 7.87% to 1.57%.

Concentration of Lead in Soil Sample Collected from Lead Contaminated Sites

Results obtained from the study of the lead concentration in the soil were shown on Table 4. Soil samples from DNK had the highest level of lead (0.57 ppm) whereas that from Bagega (BGG) had the least (0.16 ppm) in the month November. Soil sample from Dan Company (DNC) also had the highest level of lead (0.51 ppm) and the soil sample from Bagega (BGG) had the least (0.13 ppm) in the month of December. In the month of January also the soil sample from Dan Company had the highest level of lead and soil from Bagega BGG still have least lead level that were recorded as 0.63 ppm and 0.10 ppm respectively. Abare (ABR) soil lead level followed that of soil from Anka town with 0.35 ppm, 0.39 ppm and 0.30 ppm for the month of November, December and January respectively.

Discussion

The pH of the soils was weakly acidic (Table 1). The acidity may be attributed to microbial activities as found by Hamza [21], who recognized microbial activities, root respiration and exudation as important causes of soil acidity. This is also in accordance with the findings of Stephen and Ijah [22], where acidic soil pH was observed in phytoremediation studies. Some pH parameters of the soil in rainy season slightly changed to basic that is closed to neutral and this agrees with the finding of Asraf and Adam [23] who worked on the effect of heavy metals on the soil microbial community.

Bacteria species	Occurrence (%)					Total
	SAT	DRT	BGG	DCN	ABR	
Bacillus species	8 (27.59)	12 (42.86)	21 (80.80)	9 (50.00)	8 (30.77)	58 (45.67)
Proteus species	4 (13.79)	3 (10.71)	2 (7.69)	3 (16.67)	3 (11.54)	15 (11.81)
Achromobacter species	1 (3.45)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.79)
Citrobacter freundii	0 (0)	2 (7.14)	0 (0)	0 (0)	1 (3.85)	3 (2.36)
Corynebacterium species	5 (17.24)	1 (3.57)	0 (0)	0 (0)	1 (3.85)	7 (5.51)
Alcaligenes species	1 (3.45)	0 (0)	0 (0)	0 (0)	1 (3.85)	2 (1.57)
Pseudomonas species	4 (13.79)	4 (14.29)	0 (0)	1 (5.56)	5 (19.23)	14 (11.02)
Staphylococcus species	1 (3.45)	3 (10.71)	0 (0)	2 (11.11)	4 (15.38)	10 (7.87)
Klebsiella species	3 (10.34)	2 (7.14)	1 (3.85)	0 (0)	3 (11.54)	9 (7.09)
Escherichia species	2 (6.90)	1 (3.57)	2 (7.69)	0 (0)	0 (0)	5 (3.94)
Agrobacterium species	0 (0)	0 (0)	0 (0)	1 (5.56)	0 (0)	1 (0.79)
Enerobacter species	0 (0)	0 (0)	0 (0)	1 (5.56)	0 (0)	1 (0.79)
Diplococcus species	0 (0)	0 (0)	0 (0)	1 (5.56)	0 (0)	1 (0.79)
Total	29 (22.83)	28 (22.05)	26 (20.47)	18 (14.17)	26 (20.47)	127 (100)

Table 3: Frequency of Occurrence of the Distribution of Aerobic Heterotrophic Bacteria in Lead Contaminated Soil.

KEY: SAT: Soil Sample from Anka Town; DRT: Dareta; BGG: Bagega; ABR: Abare; DNC: Dan Company.

Site	Lead concentration (ppm)			Mean ± SD*
	November	December	January	
SAT	0.19	0.25	0.22	0.22 ± 0.03^{ab}
ABR	0.36	0.39	0.30	0.35 ± 0.05^{acd}
DRT	0.28	0.26	0.24	0.26 ± 0.02^{ef}
BGG	0.16	0.13	0.10	0.13 ± 0.03^{ceg}
DNC	0.57	0.51	0.63	0.57 ± 0.06^{bdfg}

Table 4: Levels of Lead in soils collected from lead contaminated site.

KEY: *Values with the same superscript are statistically significant p ≤ 0.05.

Soil SAT: Sample from Anka Town; DRT: Dareta; BGG: Bagega; ABR: Abare; DNC: Dan Company.

The mean bacterial counts of the two seasons show that Soil from Anka Town (SAT) had the highest mean total in both seasons. It had 6.9×10^6cfu/g, 5.5×10^6 cfu/g and 7.2×10^6 cfu/g of November, December and January respectively for the dry season. It also had 8.1×10^6 cfu/g, 6.1×10^6 cfu/g and 5.5×10^6 cfu/g of July, August and September respectively for raining season. The highest number of the bacterial count has to do with less concentration of the lead in the soil sample when compared to many of the soil sample used. The standard level of lead concentration in sediment and water was reported to be 0.01mg/l and 0.05 mg/l respectively by Ezejiofor., *et al.* [24] and the levels of lead concentration from the soil sample SAT which was used as control was found to exceed the above standards with little less than one figures. This may be due to the fact that the Anka town soil is surrounded by villages where the local gold mining activities is taking place and the possibility of the Anka soil to have lead content is revealed. The mean levels of lead concentration in soil of SAT were recorded as 0.19 ppm, 0.25 ppm and 0.22 ppm for November, December and January respectively. Also, the mean levels of lead in soil of SAT were recorded as 0.25 ppm, 0.19 ppm and 0.20 ppm for July, August and September respectively. The more the lead concentration in a soil samples the less the number of bacterial count and vice versa. This finding agrees with work of Wyszkowska., *et al.* [5] where he reported that "In a soil contaminated by higher doses of heavy metals regardless of the soil use, cadmium, copper and lead significantly reduced the count of heterotrophic bacteria".

The soil from DNC was found to have the highest lead concentration and lowest bacterial counts when compared to all the five places in both the dry and rainy seasons. The least bacterial count of DNC soil was probably due to the high concentration of the lead as it agrees with the findings of McGrath., *et al* [25]. This low bacterial count in relation to high heavy metal concentration was also reported by Wyszkowska., *et al.* [5], where their work revealed that heavy metal contamination of soil adversely affects the abundance and activity of microorganisms involved in organic decomposition and nutrient recycling.

The most predominant bacteria among the isolates ware *Bacillus* species which occupied 45.67% of the isolates. This may be attributed to their ability to resist harsh environmental conditions and heavy metal contamination in soils. *Bacillus* species are known to produce spores that enable them with stand environmental harshness. This agrees with Laugauskas., *et al.* [6], where he found *Bacillus* species as the most bacteria in the soils contaminated with lead. This finding is also in agreement with Ezejiofor., *et al.* [24], where he reported Bacillus species among the organisms that resist lead highest in his findings. Next to the *Bacillus* species, the species of *Pseudomonas, Proteus, Staphylococcus, Klebsiella, Corynebacterium* and *Escherichia* are found to have moderate distribution among the bacteria isolated in the five places of the lead contaminated areas of Anka Local Government Area [26-92].

Conclusion

The result of this study revealed the distribution of bacteria in lead contaminated soils of Anka local Government Area Zamfara State which provided the baseline information for the bacteria present in lead contaminated soils of five places, the bacteria resisting lead and the possible candidates for bioremediation of the affected polluted places. The *Bacillus* species were found to be best survivours of the lead contaminated soils. The result of this work also shows high lead level in all the soil samples collected.

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Volume 1 Issue 7 July 2018