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Isolation and Characterization of Lead Tolerance Bacteria Collected from Different Mining Site in Minna, Niger State

Hassan Mohammed*

Biochemistry Research,
Bayero University, Kano
Kano, Nigeria
*Corresponding author: E-mail: hassanalamin329@gmail.com
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ABSTRACT

Lead released from mining site into the environment has caused serious environmental pollution thereby threatening human and animal lives. Hence this study focus on characterization of lead tolerance bacteria isolated from lead contaminated soil of different mining site in Minna, Niger State. Isolation was carried out using Nutrient agar and MacConkey agar. Tolerance test was done using Nutrient Agar containing lead acetate at different concentrations ranging from 0.1-1.0mg/ml. The ability of the isolate to utilize lead was tested using agar-agar at different concentration ranging from 0.2-0.5mg/ml of lead acetate. Degradation potential of the isolates was determined using peptone water containing 0.06mg/ml lead acetate; absorbance was taken using Jenway 6320D spectrophotometer at 600nm for seven days at interval 24hhrs. The isolates were identified by morphological and biochemical tests. Few bacteria colonies were observed on both nutrient agar and MacConkey agar. The bacteria isolates sub cultured; HM1, HM2, HM3, and HM4 were identified as Staphylococcus aureus, Bacillus cereus, and Micrococcus letus. From the tolerance result, all the isolates were able to tolerate lead at 0.1mg/ml. For utilization test, all the bacteria isolates zone of inhibition increases as the concentration increases with Staphylococcus aureus having the highest zone of inhibition of 26.22. The cumulative percentage of degradation on the 7th day for Staphylococcus aureus, Bacillus cereus, and micrococcus letus are 32.81%, 29.15%, 26.0% and 27.84% respectively. These bacteria can be utilized as potential bioremediation agent to reduce lead pollutant in environment.

Keywords: Bacteria isolates, Lead, Bioremediation, Tolerance

INTRODUCTION

Lead is a heavy, pliable, inelastic metal element, having a bright, bluish colour, ubiquitous, most abundant of transition metal and is detectable in all phases of the inert environment such as air, water and soil as well as in most biological systems. It belongs to the elemental group 14 and period 6. Due to its unique features, lead is one of the most commonly use heavy metal in the world unlike other heavy metals. It is rarely found in elemental form, rather in variety of compounds and complexes. It had been used in large amount for 2500 years and recently as a fuel additive, although its toxicity to human and animal had been known for long. The release of lead into aquatic ecosystems of many nations is of great concern to several individuals. Lead finds its way into aquatic environment as a result of several activities such as mining, energy and fuel producing, fertilizers and pesticides. Lead released into the environment has caused serious environmental pollution thereby threatening human and animal lives. The removal of lead from aqueous solution and from other substance has become an important issue that many are faced; thousands of tons of lead are released from electric battery manufacturing, lead smelting internal combustion, engines fuelled with petroleum that has contents of lead in it and mining activities. Human exposure to lead at low level is toxic and has acute and chronic effects on the human health. Lead is a multi-organ toxicant (i.e it affects different organs or has wide organ system effect) that can produce ROS (reactive oxygen species) such as peroxide, superoxide and hydroxide that causes degenerative diseases like neurological, cardiovascular, renal, gastrointestinal, hematological and reproductive effects [2].

MATERIALS AND METHODS

The degree and type of effect depends on the duration, level of exposure. It is mainly accumulated in the bone and may serve as a source of exposure in life. According to UNEP (2010) organo-lead compounds, such as tri-alkyl-lead and tetra-alkyl-lead compounds, are more toxic than inorganic forms of lead. Quite a number of techniques and processes are currently employed in order to remove lead from the environment. The most commonly used techniques are precipitation, ion exchange, membrane processes, evaporation and filtration. The difficulties with these techniques includes the following; technological management of waste generated, incomplete metal removal, high reagent and energy requirement among others (Table 1).

Table 1: Lead-Related Symptoms of earliest and chronic exposure.

Earliest symptoms of lead poison	**Symptoms of chronic exposure**
Head ache	Impotence
Insomnia	Constipation
Irritability	Inability to concentrate
Diminished libido	In coordination
Weight loss of 10 IBS or more without known cause	Numbness and tingling in extremities
Tremulousness	Short time memory loss
Personality changes	Depression

Diffuse muscle weakness	Nausea / vomiting
Dyagia	Abdominal pain/ cramping
Joint pain/ arthritis	Frank paralysis
General fatigue / lethargy	Abdominal colic
Loss of appetites	Somnolence / severe lethargy
Unusual test in mouth/ change in test of food	

Particularly, these microorganisms are well adapted to environments contaminated with high level of toxic pollutants such as lead, mercury, nickel pesticide etc, and are useful for bioremediation applications [2]. The existence of microorganisms in polluted soil depends on intrinsic biochemical and structural properties, genetic and physiological adaptation including morphological changes of cells, as well as ecological modifications of metal speciation (Table 2).

Table 2: Signs of lead poison in general and in children.

In general	**In children**
Tremors	Abdominal pain
Hypertension	Language delay
Hyper-reflexia	Growth failure
Blood level over 10µg/Dl	Aminoaciduria (reversible)
Decreased nerve conduction velocity	Behavioral change/ hyperactivity
Upper extremity weakness	
Papilledema	
Increased in cranial pressure	
Buccal lead staining	
Muscular gray stain	
Forearm extensor weakness (wrist drop)	

Bioremediation is a technique that involves the use of living organisms to remove contaminant or to transform hazardous materials to harmless compounds (Datta J. et al. 2012). According to Shams S. (2012) that microorganisms are environmental friendly and does not destroy the ecosystem and are very useful to remove pollutants. Thus, there is no doubt that bioremediation has great potential for dealing with certain types of site contamination. Soil contains variety of microorganisms such as bacteria and fungi that can be found in any natural ecosystem [3].

Collection of sample

Soil sample for this study was collected from three different mining sites in Minna, Niger State. Minna is a city in Niger, Nigeria. It is located 9.62 latitude and 6.55 longitudes and it is situated at elevation 243 meters above sea level. Minna has a population of 291,905 making it the biggest city in Niger. It operates on the WAT time zone [4].

Collection of substrate

Lead acetate used for this work to test the tolerance ability of the organisms isolated was collected from department of biochemistry, Ibrahim Badamasi Babangida university lapai, Niger State.

Preparation of culture media (Culturing)

The culture media used were Nutrient Agar and MacConkey agar. The media were weighed and prepared according to the Manufacturers specification. Soil samples collected from three different location were mixed together to obtain a homogenous mixture. A serial dilution of the mixture of the soil sample was performed and serial dilution of test tube 10-4 and 10-5 was placed on nutrient agar and MacConkey agar each and then incubated. After incubation for 24hrs at 37℃ the bacteria growths were subculture on a freshly prepared nutrient agar to give a pure culture. The pure cultures were there after incubated for 24hrs at 37℃ after which a slant of the pure culture was prepared and kept in an incubator to moderate the growth.

Testing the bacterial isolate for ability to tolerate lead

4g of lead acetate was weighed and suspended into 46±10ml of distilled. 14g of nutrient agar was also weighed and dissolved in 500ml of distilled water. Both nutrient agar and lead acetate (substrate) were sterilize by autoclaving at 15psi (121℃) for 15minutes and allowed to cool at room temperature. Substrate (lead acetate) with the following concentration 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml were discharged into Petri dish before pouring prepared nutrient agar and allowed to solidify at room temperature. Four bacteria isolate were spread by streaking on each of the Petri dish containing the substrate concentration and incubated for 24hrs at 37℃. Bacterial growth was observed.

Testing the bacterial isolate for ability to utilize lead

14g of agar-agar was weighed and dissolved into 500ml of distilled water. 9ml of distilled water was laced into 9 test tubes. Agar-agar solution and the diluted test tubes were sterilize by autoclaving at 15psi (121℃) for 15 minutes and allowed to cool at room temperature. Agar-agar was pour plated and allowed to solidify. After solidification an agar well was bored. Bacterial isolate were spread by streaking on the solidified agar-agar media. 0.1ml of substrate (lead acetate) with different concentration was inoculated into agar well and for the control 0.1ml of the first substrate concentration was spread by streaking. Isolates were inoculated into the agar well. Both the samples and control were incubated for 24hrs at 37℃. A ring was observed on the edge of the agar well which determines the zone of inhibition [5].

Testing the bacterial isolate for ability to degrade lead

28g of peptone water sample was suspended into 1000ml of distilled water.9ml of distilled water was poured into two set of test tube.40ml of prepared peptone water was discharged into MacCantney bottles (i.e 10 bottles for set A and B). Diluted test tube and the 10 set of bottles containing peptone water were sterilize by autoclaving at 15psi (121℃) for 15 minutes and allowed to cool at room

temperature. 1ml of lead acetate (substrate) was discharged into set A bottles and 2ml of same substrate discharge into set B bottles containing peptone water. Serial dilution of the isolate was made. 1ml was taken from the serial dilution and discharge into each of the set A and set B bottles. It was incubated at room temperature. Absorbance was taking using Jenway 6320 spectrophotometer at wavelength of 550, 600, and 650nm for seven days [2].

Enumeration of Total microbial populations

The counting of the microbial population was done depending on the nature of their growth. The counting for bacterial growth was done after 24hours of incubating. Colonies were counted on plates that showed good distribution.

Morphological/Biochemical characterization (using Bergy Manual)

To characterize the isolates biochemically, the following tests: Citrate, Catalase, Gram staining Coagulase, Methyl red, Mannitol and Urease test were performed using Bergey's manual.

RESULT

Bacteria isolation and population on study soil

Four bacteria growth were observed; Bacteria HM1 and HM2 on MacConkey agar, HM3 and HM4 on Nutrient agar. This shows the total counts of bacterial populations of soil used in the present study. These results seem to indicate a relatively low capacity of the soils to sustain higher above 105 CFU/g of soil. The isolates were subculture to obtain pure colony (Table 3).

Table 3: Bacteria colonies count on media.

S/N	Bacteria	Colonies count
1	HM1	$3.5x\ 10^5$
2	HM2	5.0×10^5
3	HM3	4.0×10^5
4	HM4	5.5×10^5

The Bacteria characterization

Isolates were identified to a specie level based on morphological features and biochemical characteristics. Bacteria HM1, HM2, HM3 and HM4 were identified as Bacillus cereus. Staphylococcus aureus., micrococcus sp (Table 4).

Table 4: Bacteria isolated from some selected mining sites in Minna.

S/N	Samples	Bacteria species isolated
1	HM1	Staphylococcus aureus
2	HM2	Bacillus cereus
3	HM3	Micrococcus sp.
4	HM4	Micrococcus sp.

Determination of tolerance Bacteria

Bacteria growth was observed on Petri plate containing 0.1mg/ml for the four bacterial isolates. In which bacteria HM1 carries the highest growth; moderate growth in HM2, HM3and HM4 has low growth while there was no bacteria growth on the Petri plate containing the following concentrations 0.2mg/ml, 0.4mg/ml, 0.8mg/ml and 1.0mg/ml. This shows that the bacteria can survive in conditions lower than 0.1mg/ml and cannot survive in any conditions above that (Table 5).

Table 5: Determination of tolerance bacterial from study soil.

SN	Probable bacteria	0.1mg/ml	0.2mg/ml	0.4mg/ml	0.8mg/ml	1.0mg/ml
1	Staphylococcus aureus	+++	-	-	-	-
2	Bacillus cereus	++	-	-	-	-
3	Micrococcus sp.	+	-	-	-	-
4	Micrococcus sp.	+	-	-	-	-

This shows the bacteria index of the tolerance determination. Since the bacterial isolates were able to grow on 0.1mg/ml the number of growth of each bacteria isolate was counted and the bacteria percentage index was calculated (Figure 1).

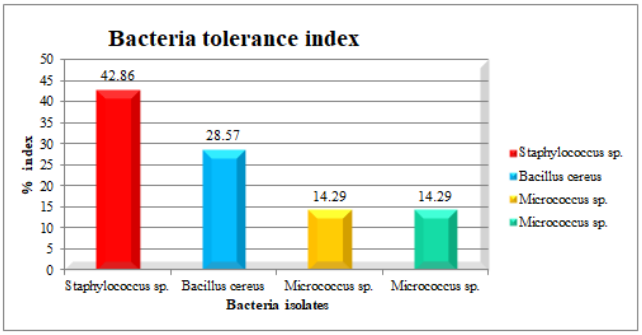

Figure 1: Graphical representation of bacteria index showing minimum inhibitory growth of bacteria isolated from lead contaminated soil collected from mining site.

Bacterial utilization Test

This shows the measured length of clear zone of inhibitions. For staphylococcus aureus as the concentration increases from 0.20mg/ml-0.5mg/l, the zone of the bacteria increases from 13mm,

15mm, 20mm, to 22mm respectively for the different concentrations. The same pattern was observed for both bacillus and micrococcus sp (Table 6).

Table 6: Bacterial Utilization Test from study soil.

Probable Bacterial	0.20mg/ml	0.25mg/ml	0.33mg/ml	0.50mg/ml
Staphylococcus aureus	13mm	15mm	20mm	22mm
Bacillus cereus	12mm	13mm	22mm	22mm
Micrococcus sp.	13mm	14mm	16mm	18mm
Micrococcus sp.	10mm	16mm	20mm	21mm

This shows the Bacteria zone of inhibition and was obtained, by summing up the measured length of clear zone of inhibition of each of the bacteria (Figures 2 and 3).

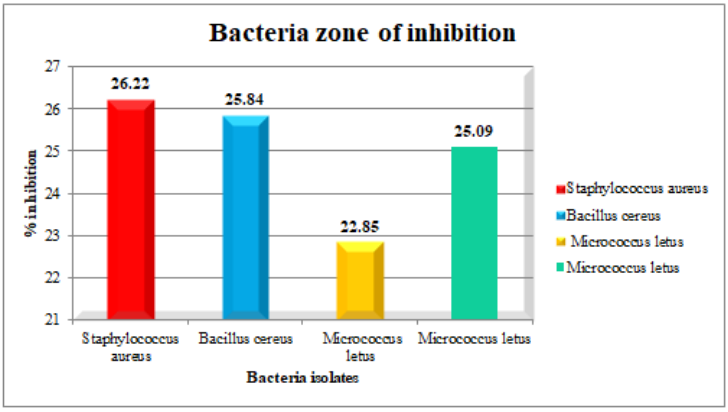

Figure 2: Graphical representation of Bacteria zone of inhibition.

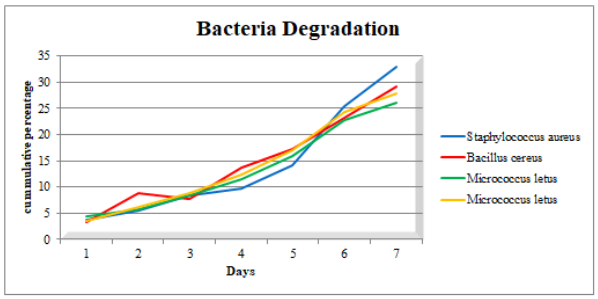

Figure 3: Comparative ability of different bacteria on degrading lead.

DISCUSSION

Contamination of heavy metals in the environment is a major global problem because they are toxic and create many health problems to human and animal and aquatic life especially Pb contamination. This study was carried out basically to check the tolerance ability of some isolated bacteria from

different mining site in Minna, Niger State. Biochemical tests were carried out to determine and characterize the isolated organisms. A combination of factors including poor moisture conditions linked to the coarse textured soil and poor substrate availability induced by very low values of percentage organic carbon and percentage nitrogen cause most microorganisms may fail to survive under such conditions. This is similar to the observations made by Carney and Matson (2005) that fine textured soils support more microbial population than coarse textured soils. The distribution of microorganisms in these various soil textural classes might be related to soil moisture and nutrient contents as explained by Heritage et al. (2003), that sandy soils cannot retain water very well and drain very quickly and fail to hold nutrient for longer periods of time. Four bacteria growth were observed; Bacteria HM1 and HM2 on MacConkey agar, HM3 and HM4 on Nutrient agar. This shows the total counts of bacterial populations of soil used in the present study. These results seem to indicate a relatively low capacity of the soils to sustain higher above 105 CFU/g of soil. The isolates were subculture to obtain pure colony. Isolates were identified to a specie level based on morphological features and biochemical characteristics. Bacteria HM1, HM2, HM3 and HM4 were identified as Bacillus cereus. Staphylococcus aureus., micrococcus sp. Respectively. This result is similar to the report of Kolawole and Obueh (2015) that staphylococcus spp., E.coli, Bacillus Cereus, Pseudomonas spp., Salmonella and klebsiella as heavy metal tolerant bacteria. This study also agrees with in part with the study of Ugoh and Monke (2011) as regards to the isolated bacteria, He isolated Serratia Sp. In addition to those isolated in this study from mining site. This shows the bacteria index of the tolerance determination. Since the bacterial isolates were able to grow on 0.1mg/ml, the number of growth of each bacteria isolate was counted and the bacteria percentage index was calculated. This shows the tolerance and effect of different concentration (0.1mg/ml, 0.2mg/ml, 0.4mg/ml, 0.6mg/ml,0.8mg/ml and 1.0mg/ml) of lead. All the isolates were able to tolerate lead at 0.1mg/ml. the bacteria isolates showed similar pattern of lead tolerance and this could be attributed to the fact that isolates are closely related genetically as the gram staining results showed that, the isolates were gram positive. It can be said that the concentration of lead has direct effect on the growth or microbial level of tolerance depends on the concentration. Microbial tolerance to lead concentration is dependent, the higher the concentration the lower the tolerance and the lower the concentration the higher the tolerance. The microbial level of the tested bacteria decreases with increase in the concentration of lead, the microbial level reduces and more zones of inihibition was observed. This is in accordance with the work of Hookoom and Puchooa (2013). For Staphylococcus aureus as the concentration increases from 0.20mg/ml-0.50mg/ml, the zone of inhibition of the bacteria increases from 13mm, 15mm, 20mm, to 22mm respectively for the different concentrations. The same pattern was observed for both Bacillus and Micrococcus sp. This shows the Bacteria zone of inhibition and was obtained, by summing up the measured length of clear zone of inhibition of each of the bacteria. Graphical representation of Bacteria percentage for staphylococcus aureus, Bacillus Cereus, and micrococcus letus with percentage inhibition of 26.22%, 25.84%, 22.85% and 25.09% respectively. In general, results presented in fig 5 shows the involvement of different species of bacteria including Staphylococcus aureus, Micrococcus Sp., Bacillus cereus exhibibt the ability to

degrade lead. The cumulative percentage of degradation on the 7th day for staphylococcus aureus, Bacillus cereus, and Micrococcus letus are 32.81%, 29.15%, 26.0%, and 27.84% respectively.

CONCLUSION

In conclusion, this study demonstrates that bacteria confer positives result in bioremediation process. This also suggests the possibility of Micrococcus sp., Staphylococcus aureus, and Bacillus cereus which exists in the mining soil within the vicinity of Minna has the ability in the bioremediation processes. Therefore, these bacteria can be utilized as potential bioremediation agent to eliminate or decrease lead pollutant in future. However, more study on this matter should be executed in order to reconfirm the bioremediation activity by the bacteria, the presence of the bacteria, as well as bacteria identification genotypic ally and the concentration of lead polluted in the soil.

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