DETERMINATION OF TOLERANCE POTENTIALS OF SOME BACTERIA SPECIES TO HEAVYMETAL ISOLATED FROM CONTAMINATED GOLD MINING SOIL IN ABARE, ZAMFARA STATE.

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Abstract

This aim of this research was to determine the tolerance ability of Bacillus lentus, Bacillus firmus,and Pseudomonas aeruginosa.. The soil sample was analysed for it heavy metal content lead was found to be in abundance beyond tolerable limit followed by cupper. Several bacterial specie were also isolated and identified from the sample and some selected species were tested for their tolerance ability in different heavy metal concentration, It was recorded that pseudomonas aeruginosa was tolerant to lead(Pb) at 800mgl^{-1}Bacillus lentus to cupper at 860mgl $^{-1}$ and Bacillus firmus to chromium at 1000mgl^{-1}. It was concluded that despite the toxicity of some heavy metals some bacterial specie were still able to withstand the environment.

Key words: Bacteria, Heavy metal, Tolerance, mining.

INTRODUCTION

In order to propagate in soils and for successful symbiotic interaction, the bacterium needs to sequester transition metals like iron and manganese from the environment. A number of these micronutrients are known to be essential for bacterial metabolism (Johnston *et al.*, 2007). The metal uptake in contaminated soils has to be tightly regulated to avoid toxic effects (Derek and Sharon, 2002). However, the mechanisms of metal import and resistance to toxic metal concentrations, used in common cell processes in rhizobial species, are largely unknown. Microorganisms have developed, for each metal, a specific or a set of resistance mechanisms. The efficiency of metal resistant

mechanisms depends on the metal itself, the microbial species, time, temperature, and pH the plants communities. Isolation and identification of some Rhizobiales in highly contaminated soils, presume that these soil organisms are likely to bear systems to survive with toxic metals in their habitats (Canovas *et al.,* 2003).

Elevated levels of heavy metals can adversely affect soil microbial ecology due to population loss, changes in population structure, physiological activity and shifts or changes in the composition of the microbial communities (Knight *et al.,* 1997; Kozdroj and Van-Elsas, 2000). The natural concentrations of most heavy metals in soils vary widely and are mainly related to the soil parent materials; however, anthropogenic sources such as smelters, power stations, industries and the application of metal-containing pesticides, fertilizers, composts and sludges may contribute to and at times exceed those from natural sources (McGrath *et al*., 1995). Lead (Pb) concentration in normal field soil is in the range of 10 to 100 mg/kg. (Soon and Abboud, 1993), but in contaminated soils especially near mines or by sewage sludge applications, its concentration as high as 1000 mg/kg has been reported (Peters and Shem, 1992; Pichtel *et al*., 2000). Soil microbial biomass, basal respiration and enzymes activity (Campbell *et al*., 1995; Trasar-Cepeda *et al.,* 2000; Yao *et al*., 2003) have been suggested as possible indicators in monitoring soil environmental quality.

Materials and method

Sample Collection

Soil samples was collected at the top soil from the contaminated mining sites, auger was used in sample collection, and was properly stored in polyethlene bags. It was taken

straight to Ahmadu Bello university microbiology laboratory. Due diligence was taken to prepare the sample for the specified test.

Determination Of Heavy Metals Present In The Soil Sample

The heavy metal content of the soil sample was determined by weighing one gram of the sample into a 50 ml crucible, and then 10 ml of concentrated HNO_3 was be added. The sample was heated on a hot plate until the solution becomes semi-dry. This was followed by the addition of 10ml of concentrated HNO_3.The solution was kept on a hot plate for 1hr to allow the formation of a clear suspension, which was then cooled and subsequently filtered through Whatman No. 2 filter paper. It was then transferred to a 50 ml volumetric flask and deionized distilled water will be added to the mark and it will be analyzed using AA6300 ASC (Varian) spectrophotometer.

Isolation and Charactarization of *Pseudomonas and Bacillus Species*.

The soil samples were homogeneously mixed and then sieved with the use of 2.0mm sieve to remove unwanted soil debris. One (1) gram of the soil was weighed into a test tube containing 9ml of sterile distilled water, and agitated for a minute. Serial dilution of the soil was made up to10^{-5} dilutions. Aliquot of 0.1ml of the prepared dilution was aseptically transferred onto the surface of solidified Nutrient and Centrimide agar for the isolation of *Bacillus and Pseudomonas* species respectively. It was spread well with the use of a sterile bent glass rod. Plates were prepared in duplicates and incubated at 37^{o}C for 18-48 h and were observed for bacterial growth. Different colonies observed were then purified by repeated streaking for each distinct colony on nutrient agar until a pure colony was obtained. The purified bacterial isolates were transferred on sterile nutrient agar slants and stored for further identification. Isolates were identified using the

identification scheme provided in Bergy's manual of determinative Bacteriology (1997), based on staining and biochemical reactions, such as Gram staining, motility, oxidase, catalase, coagulase, MR-VP etc. Microgen biochemical tests kit was used according to manufacturer's instruction to identify the isolates to specie level.

BIOASSAY PROCEDURE FOR HEAVY METAL TOLERANCE

Bacterial isolates were assayed for their capacity to tolerate and grow in the presence of 10, 15, 20µg/ml of the test heavy metals ion in vitro. Using the formula;-

$C_1 V_1 = C_2 V_2$

$V1 = \frac{C2V2}{C1}$

The yields of biomass in liquid shake cultures were used as an index of tolerance and growth in the presence of different concentration of heavy metals (Bennet *et al.*,2002) each test isolate was inoculated in triplicate conical flask containing 50ml of freshly prepared nutrient broth supplemented with 10,15,20µg/ ml each of lead chromium, and cupper. A control without heavy metal was used for comparative evaluation; the inoculated flask was incubated at room temperature on a rotary shaker for 24hrs.The bacterial cells produced were harvested by filtering the cultures through pre-weighed whatman filter paper (No1). The filter paper bearing the cells were dried in an oven at 70^{O}C for 24hrs and re-weighed. The yield of the dry cells was obtained by subtracting the weight of the filter paper alone from the weight of the filter paper and the cell biomass (Malik and Jaiswal2000; Bennet *et al*., 2002).

RESULTS

Table 1: Heavy metal analysis of the soil

Isolate ID	**Cr**	**Au**	**Pb**	**Cd**	**Zn**	**Cu**
A	0.302	0.359	42.474	0.000	0.318	5.498
Normal soil	40mg/kg	5ppb	35mg/kg	0.35mg/kg	90mg/kg	30mg/kg
Tolerable Level	1000mg/kg	NIL	100mg/kg	3mg/kg	300mg/kg	100mg/kg

Table 2: Identified bacteria on nutrient agar

Isolate ID	Gra Rxn	Spor	Catal	Coagul	Motilit	Oxidas	MR	VP	IND	Citras	Urea	Glucos	Lactos	Sucro	H_2S	Gas	
A1	+	+	+	+	-	+	+	-	+	-	-	+	-	-	+	-	***Firmus***
A2	+	+	+	+	+	+	-	+	+	-	-	-	+	-	+	-	***B.coagulance***
A3	+	+	+	+	+	+		-	+	+	-	+	-	+	+	+	***B.lentis***
B1	+	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	***B.lentis***
B2	-	-	+	-	+	+	-	+	+	+	-	+	-	-	-	-	***Firmus***
B3	+	+	+	+	-	+	-	+	-	+	-	+	-	+	+	+	***Firmus***
C1	+	+	+	+	+	-	-	+	-	+	-	-	-	+	+	+	***B.Cereus***
C2	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	***Firmus***
C3	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	***Firmus***
D1	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	-	***Firmus***
D2	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	-	***Firmus***
D3	+	+	-	-	-	-	+	-	-	-	-	+	+	-	-	-	***Firmus***
D4	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	***Proteus bulgaris***
E1	-	-	+	-	+	+	+	-	+	-	-	+	-	-	-	-	***Proteus mirabilis***
E2	+	+	+	+	-	+	-	+	-	-	-	-	-	+	+	-	***Brevis***
F1	+	+	+	+	+	-	+	-	-	-	+	-	-	-	-	-	***Firmus***
F2	+	+	+	+	+	-	+	-	-	-	+	-	-	-	-	-	***Firmus***
CS1	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-	-	***B.coagulance***
CS2	+	+	+	+	+	+	-	+	+	+	-	+	-	+	-	-	***Firmus***
CS3	-	-	+	+	+	+	+	-	+	-	+	+	-	+	+	-	***Proteus vulgaris***

Table 3: Identified bacteria on Centrimide ager

Isolat ID	Gra Rxn	Spo re	Catal	Coagul	Motil	Oxida		MR	VP	IND	Citr	Urea	Gluco	Lacto	Sucro		H_2S	Gas
A	-	-	+	+	-	+	+	-	-	+	+	+	-	+	+	+	***P.aeriginosa***	
B	-	+	+	-	-	+	+	-	-	+	+	+	+	-	+	+	***B.Cepacia***	
C	-	+	+	-	-	+	-	+	-	+	+	+	+	+	+	+	***Fluorescence***	
D	-	+	+	+	-	+	+	-	-	+	+	+	-	-	+	+	***P.fluorescence***	

Table 4: Maximum tolerance concentration of some isolate to Lead, cupper and Chromium

Isolate ID	Lead (mgl^{-1})	Cupper (mgl^{-1})	Chromium (mgl^{-1})
B. lentis	730	860	830
B. brevis	760	660	650
B. coagulance	800	830	830
B. cepacia	800	730	600
P. aeruginosa	800	730	600
B. firmus	730	760	1000

MTC = $\frac{Growth\ in\ the\ presence\ of\ heavy\ metals}{Growth\ in\ the\ absence\ of\ heavy\ metals}\ X1000$

Discussion

Table 1: shows the concentration of heavy metals. Generally the concentration varies with depth and distance. The level of chromium contamination at the surface soil ranges from 0.302 mg/kg which are below the permissible limit set by WHO. this result is contrary to the report of Ogundele, *et al*., 2015 who reported a high concentration of chromium in soil and plants. Gold was found in very little concentration 0.359mg/kg distribution of metals are affected by a number of factors such as accelerated weathering, mobilization, rates of slurry and the addition of reagents in the extraction of gold, these report disagrees with the report of Antonio,. 2017 who reported concentration of 7.37mg/kg at the surface and 7.38mg/kg at 1m deep from Gongo Gold mine in the Philippines. Significant findings revealed that high Gold concentration in tailings with an average of 7.38AU/t between the surface and 1m deep. Another assay revealed 0.56-0.96g of gold at Balatic mine sites in the Philippines. Furthermore, in Tasmania, Australia the Hellyer mine site contains above 2.6gAu/t was viewed as substantially valuable resources. Zinc and Cupper were also present 0.318mg/kg and 5.498mg/kg , this agrees with the report of Ezeh and Chukwu, (2011)who reported a high level of zinc at the Ishiagu mining soil in south eastern Nigeria. However, it disagreed with the result of Kakulu Samuel *et al.,* (2012), who reported a value of 30.63% zinc at the Itakpe mine in Kogi State Nigeria, and contrary with the report of Majiya *et al.,* 2014 with 0.47mg/kg in soil from Bagega ore processing site of Anka Zamfara State Nigeria. Zinc which is very mobile in weathering environment, the distribution of zinc in this area is below toxic level though it’s an important metal for the health of plants and animals, this result contradict the report of Majiya, *et al.,* 2015 which reported a value of cupper at

197.07mg/kg at the Bagega ore processing site. But contrary with another report of Majiya, *et al.,* 2015, who reported a value of 46.09mg/kg at the 10-20m deep of the ore processing site of Bagega in Anka Zamfara State, Nigeria. Cadmium was not available at the surface soil, these disagrees with the report of Majiya, *et al*., 2015, who reported 0. 035 mg/kg, and 0.03mg/kg in 10-15cm and 20-30cm at top soil of the mining site respectively the unavailability may be due to its mobility than most metals and it is weakly sorbed and is not retain in soil through cation exchange (Sparks 2005; Ferguson, 1990; Adriano 1986.). It also does not form stable methyl compounds. Lead (Pb) was the most abundant metal in the region, with a very high concentration across depth and distance of the study area, as shown in table 1. 42.474mg/kg , this is not similar to the result of Majiya, *et al.*, 2015, who reported a high level concentration of lead at the top soil (346mg/kg), and also 477.19mg/kg at the soil from the Dam sediment of the ore processing site of Anka in Nigeria. Abdu and Yusuf, 2013, reported lead concentration in the plant to be over 3500-fold higher than the recommended threshold of 50mg/kg in edible crops by FAOWHO (2001). The extremely high levels of lead in the site could have been due to atmospheric deposition, this shows that lead is relatively stable after deposition in soils, they also reported that lead concentration in plant material at this mining site must have come from dust deposition coupled with human input, result in phytotoxicity at high concentration and the transfer of this element to human and livestock diets from plant uptake. Such reports have been given by Jian-Min *et al*. (2007) and Adie and Osibanjo (2009). Report by Ezeh and Chukwu, 2011 shows high a lead concentration of 1573-1367mg kg in the analysis of agricultural soil around the Ishiagu Mining district in south eastern Nigeria. This result is beyond the standard level of lead

concentration which was reported to be 0.01mg/kg and 0.05mg/l respectively (Ezejiofor *et al*., 2013). Table 2 and 3: shows the result of biochemical characterization of bacterial isolates obtained from the sample. Twenty four bacterial isolates were obtained and were both Gram positive and Gram negative rods. Nine of the isolates were identified as *Bacillus firmus,* three *B. Cereus,* Two each of *B Lentis, B. Coagulance, P Fluorescence, P. Vulgaris* and one each of *P. aeruginosa, B. Cepacia, B. brevis, P. mirabilis,* respectively. The test organisms were identified using both conventional methods and Microgen Kit. The most predominant bacterial genera among the isolates were *Bacillus* species, which constituted 64% of the total isolates revealed. 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 to withstand environmental harshness. This agrees with Lugauskas *et al.* (2005), who found *Bacillus* species as the most abundant bacteria in the soils contaminated with lead. The presence and abundance of various species of *Bacillus* identified in this study may not be surprising because apart from their ability to produce spores they are also indigenous to soil environment and are known to persist in such environments (Atlas and Bartha, 2007). This finding is also in agreement with Kafilzadeh *et al.,* (2012) who reported *Bacillus* species among the organisms that resist lead in their findings. Next to the *Bacillus* species, the genera of *Pseudomonas (16%), Burkholderia Cepacia (4%), and Proteus (16%),* are found to have the least distribution among the bacteria isolated in the samples of the heavy metal contaminated areas of Abare Anka Local Government Area. Table: 4 showed the tolerance ability of the Bacterial species, they showed an impressive display of responses to metal ions and ability to cope with these toxic elements in a different

concentration of heavy metals, metal tolerance reflects the ability of an organism to survive in an environment with a high concentration of metal without dying. Anyanwu, C.U. *et al.*, 2011 reported that most bacteria isolated from soil were resistant to very a high concentration of heavy metals regardless of whether or not the soils were contaminated with metals. Metals tolerant species are either inherently less sensitive to metal contamination, due to either decreased functioning of nonspecific transport systems or have developed resistance to metal toxicity. The tolerance ability of the species in this study agrees with reports of researchers who state that metal exposure leads to the establishments of tolerant microbial populations, which are often represented by several Gram positives belonging to *Bacillus, Arthrobacter, pseudomonas, and Corynebacterium Burkholderia* (Ajaz *et al*., 2010 ; Ellis *et al*., 2003; Kozdro and Van Elsas, 2001).*Bacillus coagulance, Burkholderia cepacia, and Pseudomonas aeruginosa had the highest tolerance to lead* with 800mg/l each for lead while *Bacillus brevis* had 760mg/l and the lowest tolerance concentration for lead is 730mg/l by *Bacillus lentus.* The highest tolerance concentration for cupper was recorded by *B.lentis* (860mg/l) and the lowest recorded is by *Bacillus brevis* with 660mg/l. For chromium the highest tolerance concentration was recorded by *Bacillus Firmus* with 1000mg/l, the lowest recorded for chromium was from *B. Coagulance and B.cepacia* which had the same value of 600mg/l. This varying response of the species might be due to the difference in their cell wall composition or due to the variations in resistance mechanism (*Abou Zeid et al.,* 2009*)*. The tolerance of these bacteria to heavy metals indicates that they will be useful for biosorption of these metals. This is in agreement with the study of Devika *et al*., (2013) who reported that the resistance of marine bacteria to several heavy metals enthuses to

affirmatively recommend their potential to be exploited in bioremediation of heavy metals; in this study the bacterial species isolated from the contaminated soil were used for biosorption because of their maximum tolerance concentration. These results agree with the report of screening for multi-metal resistant bacteria performed in previous studies by Vullo *et al*., 2008 and Brunno *et al*., 1991).

Conclusion

The present study evaluate to determine the tolerance ability of *Bacillus lentus, Bacillus firmus,*and *Pseudomonas aeruginosa*. The most predominant bacterial genera among the isolates were Bacillus species, which constituted 64% of the total isolates revealed. 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 to withstand environmental harshness. Metals tolerant species are either inherently less sensitive to metal contamination, due to either decreased functioning of nonspecific transport systems or have developed resistance to metal toxicity. In this study, the bacterial species isolated from the contaminated soil were used for biosorption because of their maximum tolerance concentration.

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