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Isolation and Molecular Characterization of Heavy Metal Tolerant Bacteria from Kofar Ruwa Scrap Metal Dump Site in Kano Metropolis

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Abstract

This study aimed at molecular detection of heavy metal tolerant bacteria from Kano, Kofar Ruwa scrap metal dump site, as heavy metals are one of the major setbacks to many forms of lives and their presence in the ecosystem is rapidly increasing due to anthropogenic activities, rampant scrap metal waste disposal and other industrial wastes. Bacteria were found to be among many microorganisms that are able to tolerate many heavy metals and can reduce their toxicity or even convert them to useful resources. The heavy metal content of some soil samples analyzed using Atomic Absorption Spectrophotometry (AAS). Standard methods of enrichment culture and colony count were used to isolate a total of 12 bacterial species. Using 16S rRNA gene sequence based on molecular system, the 12 isolates were identified and grouped into one genus (Bacillus). It was also observed and reported from the results that the concentration of heavy metals (Pb, Zn, Cu, Cd, and Cr) found to be high above the WHO permissible limits (Copper-2.0, Zinc-3.0, Lead-0.4, Chromium-0.05 and Cadmium-0.03). Therefore, the bacterial isolates capable of surviving at such levels of heavy metals could have potential application in the bioremediation, bioleaching, human carcinogens substances and soil infertility of heavy metal contaminants . It is therefore, recommended that further research on these bacterial species should be carried out to address their significance for the public health interest and humanity in general.

Keywords: Anthropogenic, Bioremediation Heavy metals, Molecular detection, Public health

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INTRODUCTION

Heavy metals is a combined name of metallic elements with density greater than 5g/cm^3 and atomic weights usually greater than 50g/mol (Samuel *et al.,* 2019).Among the 90 naturally occurring elements, 21 are non-metals, 16 are light metals and the remaining 53 are heavy metals (Abidin *et al.,* 2020). They are also metals that are non-biodegradable and environmental contaminants which relate to potential toxicity. Some heavy metals such as Nickel, Iron, Copper and Zinc are essential to metabolic reactions and are required as trace elements by the organisms (Srivastava & Balomajumder, 2008). Other heavy metals such as Mercury, Silver and Cadmium have no biological role in organisms, and are detrimental even at very low concentrations (Nwagwu *et al.,* 2017). Heavy-metal pollution of the environment has dramatically increased in recent years due to various human activities, such as agriculture, mining, and various other industrial processes (Fashola *et al.,* 2016). Urban soils have been affected by elevated levels of heavy metals since the beginning of industrialization which pose a considerable threat to the environment (Mustapha & Halimoon, 2015). Extremely high levels of pollutants in soil can be found in many industrial sites and waste disposable dumps, which results in accidental spillage of highly concentrated pollutants like heavy metals, and thus, metals are deposited into the soil matrix and once incorporated into the soil, they remain for very long period of time (Aliyu, 2014). The total concentration of heavy metals in soil is widely used to assess soil pollution and constitute a major hazard for the human health and ecosystem (Aliyu, 2014). Heavy metals are recognized to be powerful inhibitors of biodegradation activities.

Therefore, heavy metals are natural components of the earth's crust. Many metal ions are crucial for normal functioning of the living organisms. For example, Copper functions as a cofactor in many biochemical reactions (Mihdhir & Assaeedi, 2016); copper-containing proteins participate in such essential processes as respiration, iron transport, and protection against free radicals (Technol, 2016). Nitrogenase (Mo/Fe or V/Fe), bacteriochlorophyll (Mg), superoxide dismutase (Fe, Mn, Cu or Zn), cytochromes ,(Fe) and many other metalloproteinase could serve as examples of metal-containing enzymes (Zolgharnein *et al.,* 2010). Many bacteria are capable of using ions, some metals and metalloids as electron donors or acceptors in metabolic reactions (Technol, 2016). However, even essential for cell survival microelements would become toxic at high concentrations. Also, the toxicity of metal ions could be due to the generation of radical compounds in the cell, complexation of thiol-containing cellular components by metal ions (Copper, Cadmium, and Lead), competition between structurally similar essential elements and metal ions (Vanadate/Phosphate) (Solomon J., 2019). Studies on heavy metals affected areas were found to have a high diversity of microorganisms (Mihdhir & Assaeedi, 2016). Such microbes are autochthonous ones that have not only familiar to the new environments but have also thrived under them. Microorganisms have evolved mechanisms to tolerate metals either by presence of heavy metal through efflux, compellation or reduction of metal ions or to use them as terminal electron acceptors in respiration. Heavy metal tolerant bacteria play a significant role in reducing the environmental pollution (Addis & Abebaw, 2017). Recently, Nigeria came up with a new technique to overcome the water pollution produced by heavy metals from scrap metal dumpsite runs (Laniyan & Morakinyo, 2021). Heavy metal tolerant microorganisms include various bacteria such as *Bacillus subtilis*, *Bacillus cereus*, *Salmonella spp*., *Enterobacter* spp., *E. coli, Coliforms* and *Streptococci*. These bacterial species demonstrated to have resistance against one or more heavy metals. Microorganisms play a very vital role in the bio-geochemical cycling of toxic heavy metals and also in cleaning up or remediating metal-contaminated environments. Bacteria occupying the metal contaminated sites might have developed intrinsic tolerance to the metal present. Microbes have been exposed to different

concentrations of heavy metals presumably since the beginning of life (Kabir *et al.,* 2012a), and have sustained by maintaining a homeostasis between the available metal concentration and microbial physiology (Keesstra *et al.,* 2012). On the other hand, in a contaminated environment, the increased high levels of heavy metal result in the conformational changes in the nucleic acids and proteins, and frequently form complexes with molecules, which make them inactive (Mihdhir & Assaeedi, 2016). For example, a milli-molar concentration of Zn ions binds with the cell membrane of bacteria and interferes with cell division (Ianieva, 2018) despite the fact of being a micronutrient. Some species of microorganisms have shown to adsorb wonderfully high quantities of heavy metals. The heavy metals of great concern to human health are Mercury, Cadmium, Lead, Arsenic, Chromium, Copper, (Samuel *et al.,* 2019), and Zinc. Cleaning of heavy metal contaminants remains a major challenge in environmental Biotechnology and Microbiology. Some industrial processes occur in the release of heavy metals into aquatic systems then acting as environmental pollutants. Heavy metals such as Copper, Cadmium, Lead, Zinc, Nickel, Mercury and Chromium when deposited in soils, and water bodies, their concentrations may be harmful to plants, animals, humans and aquatic life (Gurave *et al.,* 2015). As such, this work is able to work on certain bacteria that a able to combat the effect of these toxic metals on all forms of lives.

Materials and Methods

Kofar ruwa is located in western part of Kano metropolis and it is in Dala Local Government area of Kano State. It has geographic coordinates of latitude 12^{o} 01 '19.1 "N (12.0219600^{o}) and longitude 8^{o}29'35.0 "E (8.4930600^{o}).

Collection of samples

Soil samples were collected from Kofar Ruwa scrap metal dump site in Kano metropolis. Three (3) sampling sites were selected (A, B and C), samples were carried out at 20cm from the locations each, using auger and the temperature of each site were recorded. The samples were sieved and homogenized thoroughly, then transported immediately to the laboratory for experimentation.

One gram (1g) of each of the soil samples (A, B and C) were serially diluted up to 10^{-6} dilutions and plated on nutrient agar plates. About 0.1ml of diluted samples were transferred into Petri dish and 20ml of nutrient agar containing different concentrations (0.5 to 5 mg/ml) of Copper, Zinc, Lead, Cadmium and chromium in their salt form ($CuSO_4$, $ZnSO_4$, $CdNO_3$, $PbNO_3$ and $K_2Cr_2O_7$), were poured onto the surface of the plate and incubated at 37^{o}C for 24 hours. Colonies developed were counted using colony counter and discrete distinctive colonies were sub cultured in nutrient broth and cultured on nutrient agar plates for pure isolates. Cultural characteristics of the colonies developed were examined and recorded, according to (Yaro R.S., *et al.,* 2023) that the 24 h old cultured of the isolates were aseptically smeared on sterile, cleaned, microscopic glass slides, fixed using flame for 5 S each and allowed to cool and full steps of Gram's staining technique were bloated and air dried and examined microscopically using oil immersion objective lenses (x100 power) for the Gram's staining reaction results, that is Gram's positive and negative respectively. The samples were analyzed for the heavy metals presence using Atomic Absorption Spectrophotometry (AAS), (Chen *et al.,* 2019).

Biochemical Characterization After Gram's staining technique, various biochemical tests such as Indole, citrate utilization, motility, oxidase, MR – VP amongst other tests, were performed for confirmation.

Molecular identification of the isolates DNA Extraction The extraction of the bacterial DNA was conducted in accordance with Bioneer (Accu Prep@ Genomic DNA Extraction Kit [k-3032]) Protocol. Also, the 16S rRNA was amplified with bacterial universal primers specific for eubacterial 16S rRNA genes, Forward primer: 5′-GGACTACAGGGTATCTAAT-3′ and Reverse primer: 5′-AGAGTTTGATCCTGG-3′ and 16S rRNA sequencing of the isolated strain was carried out by Dye terminator cycle sequencing (Quick start kit) (Piotrowska *et al.,* 2016).

Results

Heavy Metals (Cu, Zn, Pb, Cd & Cr) Content Analyse from the Atomic Absorption Spectroscopy (AAS) analysis result which was carried out at Central Laboratory Bayero University Kano. The pH and mean concentrations of heavy metals tested and the statistical results are shown Table1, (Akpoveta *et al.,* 2011).

Table 1: Summary of the mean concentrations of heavy metals in scrap metals dump sites from the study area.

Samples/Sites	Chromium (mg/kg)	Zinc (mg/kg)	Copper (mg/kg)	Lead (mg/kg)	Cadmium (mg/kg)
A	32.35±0.58	21.08±1.0	2.461±0.07	2.461±0.07	0.032±0.16
B	32.62±0.06	24.30± 1.5	10.72±0.14	2.372±0.04	0.065±0.004
C	33.20±0.58	15.99±0.29	5.728±0.39	4.702±0.25	0.058±0.001

Detection of Heavy Metal Tolerant Bacteria from Scrap Metal Dump Site

However, these isolates only showed tolerance to both copper and chromium even at high concentrations 5 mg/mL each. In this study, the test was conducted on nutrient agar supplemented with different Cu, Zn, Pb, Cr and Cd concentrations range from 0.5 mg/mL to 5 mg/mL. Also the growth of all the bacterial isolates was found out to be decreased with increasing concentration of copper and chromium (fig 2 and 3). Among the isolates, only three (3), (A1, B1 and C1) were able to grow in the presence of all five tested heavy metals; Cu, Zn, Pb, Cd and Cr. Thus, they were selected for further biochemical and molecular identification. On biochemical characterization the isolates were identified as *Bacillus thuringensis, Bacillus cereus* and *Bacillus* sp.

Table 2: Heavy metal ($K_2Cr_2O_7$) tolerance profile of selected bacterial isolates.

Isolate No.	GA on NA	GTVC mg/mL			
		0.5	1	3	5
A1	+++++	++++	+++	++	+
B1	+++++	++++	+++	++	+
C1	+++++	++++	+++	++	+

Table 3: Heavy metal ($CuCl_2$) tolerance profile of selected bacterial isolates.

Isolate No.	GA on NA	GTVC mg/mL			
		0.5	1	3	5
A1	+++++	++++	+++	++	+
B1	+++++	++++	+++	++	+
C1	+++++	++++	+++	++	+

GA= Growth Activity; NA= Nutrient Agar; GTVC= Growth tolerance at various concentration; +++++= Exuberant growth; ++++= Very good growth; +++= Good growth; ++= Fair growth; += Low growth.

Molecular Identification of Bacteria Tolerant for Heavy Metals

Table 4 shows the primer sequence of the 16S rRNA gene. A band of expected size of 789 bp on agarose gel electrophoresis was shown (Plate. 1). The primer amplified the 16S rRNA gene successfully from all heavy metal tolerant bacteria selected, although no clear variations in the size of rRNA gene products between the 3 isolated bacteria were observable. However, the size of the PCR amplified 16S rRNA gene product of all isolated bacteria investigated in this study was approximately 1kb to the relative DNA size ladder. The amplified 16S rRNA gene products were purified using a commercial gel purification kit. The resultant 16S rRNA sequence was edited and aligned using Chromas and Bioedit program. The additional sequence data were obtained from the Gene Bank. Comparison of the partial 16S rRNA gene sequence from the 3 bacterial isolates with sequences from the database showed that they belong to the same taxonomic lineages. The 16S rRNA analysis revealed that all isolates belonged to the genera *Bacillus*. Sequences from two isolates (C1 and B1) had a similarity equal or higher than 97% with other 16S rRNA sequences from the database, while one isolate (A1) has less than 90% similarity. The phylogenetic analysis based on the partial 16S rRNA sequences was able to discriminate the main taxonomic lineages using DNA neighbor phylogenetic tree program. Within the main lineages, the sequences obtained from the bacterial strains associated with *Bacillus* species were often formed branches separated from the sequences of other bacteria isolated from scrap metal dump site Kofar Ruwa. Neighbor-joining analysis revealed the presence of three well-resolved lineages according to 16S rRNA sequence analysis of the three isolates.

Table 4: Primer sequence of 16S rRNA gene

Primer	Sequence	Product size	Reference
RIBOSE-1F	5′-GGACTACAGGGTATCTAAT-3′	789	(Piotrowska *et al.,* 2016).
RIBOSE-2R	5′-AGAGTTTGATCCTGG-3′	789	

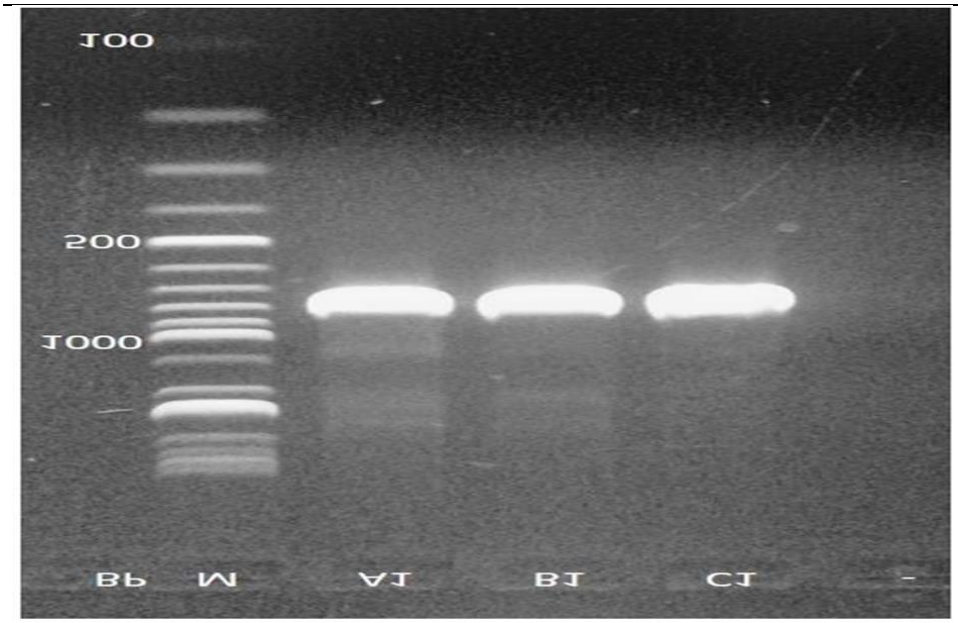

Plate. 1: Agarose gel electrophoresis shows *16S rRNA* amplicons of the three (3) isolated bacteria from scrap metal dumpsite Kofar Ruwa.
Lane 1: A1, Lane 2: B1 and Lane 3: C1 and Lane M: DNA Ladder.

DISCUSSION

In the past, many studies have demonstrated that heavy metals can significantly alter the microbial populations and diversity (Tsai *et al*., 2005). Therefore, the comparison of the bacterial isolates showed that many shared a similar *16S rRNA* genes indicating a degree of genetic relatedness. The dominant Gram positive populations belonged to the family *Bacillaceae* which accounted for more than 97% of the total bacterial populations on all the three sites A, B and C. The most represented genera were *Bacillus* and *Lysinibacillus* (Madigan *et al*., 2003). This study showed that most of the bacteria isolated from the scrap metal dump sites belonged to Gram positive *bacilli*, which agreed with the finding of AF, E. (2019), who reported that most of the heavy metal tolerant bacteria belonged to Gram positive genera. In this study, an attempt was made to identify certain bacteria from the sites around Kofar ruwa in Kano city. The sites consist various metals waste from different sources like the car garage, industries, mechanics, hospitals, agricultural factories and house wastes. The identification of the bacterial species was done on the basis of colony characteristics; microscopic examination of the Gram's stained preparations and biochemical characterization. Additionally, the amplification of *16S rRNA* of selected isolates *Bacillus thuringensis, Bacillus cereus* and *Lysinibacillus macroides* on nucleotide sequencing revealed the homology to the published sequences and thus, were identified on this basis. In this study, *Bacillus* and *Lysinibacillus sp*. were the predominant bacterial species that possessed tolerance to heavy metals. The variation in the bacterial species recovered from different dumping sites in the present study might be due to the occurrence of a particular heavy metal at a particular contaminated site as according to (Abas and Wee, 2014).

CONCLUSION

This research found the presence of bacteria that are able to tolerate heavy metals from the scrap metal dump sites. Bacteria that tolerate high concentrations of heavy metals were isolated in pure cultures. The results shown that isolate strains characterized with remarkable tolerance against heavy metals, could be potential agents for the development of soil inoculants applicable in bioaugmentation of heavy metals polluted sites. Hence, they can be used for soil amendments. The concentrations of heavy metals (Cr > Cu > Zn > Pb > Cd) in the sites were all beyond the established threshold limits set by WHO, their presence could be attributed to increasing anthropogenic activities arising from scrap metal waste disposal, burning of electronics wastes and high volume of traffic.

There is need for farmers to thrive as to control food economy, as such proper toxic metals discharge should be controlled to provide more fertile land. More enlightned campaigns and further studies on the harmful effect of heavy metals and economic advantage of uncontaminated soils should be created, so as to have a better environment.

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