International Journal of Agriculture, Environment and Bioresearch

Vol. 4, No. 05; 2019

ISSN: 2456-8643

ISOLATION AND IDENTIFICATION OF HEAVY METAL TOLERANT BACTERIA FROM ARAPAJA DUMPSITE, IBADAN,OYO STATE

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http://doi.org/10.35410/IJAEB.2019.4453

ABSTRACT

Dumpsites constitute nuisance and lots of environmental pollution because of different components that make up the dump which are mostly household and industrial wastes. Decomposition of these wastes introduce some toxic materials into the soil that harbour microorganisms and possibly have negative impact on the soil microflora. Therefore this research investigated the level of heavy metal contamination in the soil from Arapaja dumpsite and also the presence of bacteria tolerant to the high level of contamination. Relatively high concentration of all metals investigated was recorded which are in no way compared to WHO standard for permissible limit for metals in soil. Presence of some bacteria were observed which indicates that those isolates can tolerate the level of heavy metal contamination and present them as good candidates for restoration of heavy metal contaminated soils.

Keywords: Contamination, Toxic, Microflora, Wastes.

1. INTRODUCTION

In Nigeria waste are dumped recklessly with no regards to the environmental implications, while in some dumpsites, waste is burnt in the open and ashes abandoned at the sites. The burning of waste gets rid of the organic materials and oxidized the metals, leaving the ash richer in metal contents. After the process of oxidation and corrosion, these metals will dissolved in rain water and leached into the soil from where they are picked up by growing plants, thereby entering the food chain. Improper wastes management methods also pilot the contamination of underground water, while most of the metals are being washed away by runoff into streams and rivers thus contaminating the marine environment. Consequently, these metals accumulate in fish and other organisms (aquatic), hence posing a health threat to the consumers (Njojju & Ayoka, 2006).

Recent studies have also reviewed that waste dumpsite can transfer significant levels of these toxic and persistent metals into the soil environment. And eventually these metals are taken up by plant part and transfer same into the food chain. Consequently, higher soil heavy metals concentration can result in higher levels of uptake by plants. Although, the rate of metal uptake by crop plants could be influenced by factors such as metal species, plants species, plant age and plant part. Heavy metals are metals with a density at least five times that of water. Heavy metal

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ISSN: 2456-8643

is the term commonly adopted as a group name for the metals which are associated with pollution and toxicity. They may also include some elements which are very essential for living organisms at low concentrations. Among these heavy metals some have been found to be of serious hazard to plants and animals and have listed by the European Commission to include; As, Cd, Cr, Cu, Pb, Hg, Ni, Al and Zn. Heavy metals are stable elements that cannot be metabolized by the body, as such they are passed up in the food chain to human beings (Bioaccumulation). The most common and harmful heavy metals are Al, As, Cd, Cu, Pb, Hg and Ni etc. Heavy metals in general have no basic function in the body and can be highly toxic. They are present in drinking water, food and countless human-made chemicals and products. In the last 50 years, human infection by heavy metals had risen dramatically; this is as a result of an exponential increase in the use of heavy metals in industrial processes and other human activities. Pollution of the environment by heavy metals results mainly from these activities. For example, refuse dumping, agricultural activities, industrial activities among others. The presence of heavy metals in the environment especially in food materials poses a serious threat both health and socio-economic well being of man and his environment. With the knowledge that waste dumps are potential sources of heavy metals contamination, it is believed that there would be heavy metals contamination in residential and commercial areas in urban areas (Jekin, 1989)

Heavy Metal Tolerant Bacteria.

Reports have shown that indigenous microbes and plant–microbe symbionts tolerate high heavy metal concentrations in different ways and may play a significant role in the restoration of contaminated soil (Carrasco *et al*., 2005; Ge *et al*., 2009). It is important to study the indigenous microorganisms in heavy metal polluted sites. It may provide new insight into bacterial diversity under unfavourable conditions, new isolates and probably new genetic information on heavy metal resistance, which could be exploited in revegetation in future (Fabienne *et al*., 2003). This research seeks to investigate level of heavy metal contamination and heavy metal tolerant bacteria diversity in the site.

2. MATERIALS AND METHODS

Sample Collection

Soil samples were collected from Arapaja dumpsite at Oluyole Local Government of Oyo State. Samplings was carried out from soil surface and at a depth of approximately 20cm were taken in sterilized polyethylene bags and stored at 4 ^{o}C until examination.

Heavy Metal Analysis of soil Samples.

Soil samples were digested using the EPA 3050B method involving a sequential addition of nitric acid, hydrogen peroxide and hydrochloric acid. The sample digest obtained was filtered into 100ml volumetric flask and made up to mark (USEPA, 1996), the sample extracts analysed for Cd, Ni ,Pb , Zn and Fe Using Atomic Absorption Spectrophotometry.

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Isolation and Identification of Bacteria

The soil sample was serially diluted up to 10-6 dilutions and plated on Nutrient Agar and Minimal Agar plates. 1ml of diluted sample was spread onto the surface of Nutrient Agar medium and minimal agar medium and incubated at 37ºC for 24 h. Single colony was picked spread on the Nutrient Agar plates and subcultured in nutrient broth and again cultured on plates in order to obtain pure cultures. Pure bacterial strain were obtained after successive transfer of a single colony on Nutrient Agar plates and incubated for 24hrs at 37ºC temperature. Morphological characters were determined by Gram's Staining and motility was determined by hanging drop method.

Biochemical Characterization

Biochemical screening was done according to Bergey's Manual of Systematic Bacteriology. By performing tests such as Sugar utilization, H_2S production, Urease production, Catalase detection, Starch Hydrolysis test, Antibiotic sensitivity tests etc.

Physiological Characterization

Physiological characteristics were screened by supplying wide range of growth temperature10°C-50°C and 4-12 pH range for growth, tolerance up to 5% NaCl concentrations were tested. To examine the ability of isolate to tolerate heavy metals, Spot inoculation was done on nutrient agar plate provided with different concentrations (0.5, 1.0, 3.0 and 5.0 mg) of heavy metals (Cadmium, Nickel, Chromium, Lead, Copper and Iron and incubated at 37°C for 24 hours and cell growth were observed.

3. RESULT AND DISCUSSION

Table1. Showing the P_H and Heavy Metal Analysis of the Site.

S/Code	pH	Cd mg/kg	Ni mg/kg	Cr mg/kg	Pb mg/kg	Cu mg/kg	Fe mg/kg
PointA	8.52	13.45	6.89	23.32	429.56	363.59	18360.11
PointB	8.26	10.46	3.78	20.17	371.55	512.33	17695.44
Control	7.93	ND	0.21	0.34	ND	23.65	26.21

Table 2. Bacterial Isolates from the sites.

Sites	**Bacterial Isolates**

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A	*Pseudomonas aeruginosa, Bacillus subtilis, , Pseudomonas fluorescens, Escherichia coli, Staphylococcus aureus,*
B	*Pseudomonas aeruginosa, Klebsiella pneumonia Bacillus subtilis, Pseudomonas fluorescens, Escherichia coli, Staphylococcus aureus,*
C	*Pseudomonas aeruginosa, Bacillus subtilis, Pseudomonas fluorescens, Escherichia coli, Staphylococcus aureus, Pseudomonas putida, Klebsiella pneumonia, Proteus sp.*

Results of Heavy Metals and bacterial isolates in Soil

Table 1 presents the results of the pH of the soil as well as the soil heavy metal concentration. The pH of the soil from all the sites ranges from 7.93 - 8.52 indicating alkalinity in soil. The concentration of heavy metals in the soil from the sampled sites is as follows: Pb (ND - 429.56 mg/kg), Cd (10.46 - 13.45 mg/kg), Cr (0.34 - 23.32 mg/kg), Fe (26.21 – 18360.11 mg/kg). The background concentration of heavy metal analysed for in the control site were within the WHO permissible limit for soil heavy metal thus, no observable contamination. This however, is in sharp contrast with the levels observed in the other sites which show relatively high contamination with cadmium, Chromium, Lead, Nickel, copper and Iron in points A and B, exceeding the permissible limit in the soil. This high level of contamination observed in this location may be attributed to the activities going on the site.

Table 2 shows the bacteria isolates from all the sampled sites and it reveals that there are common bacteria in all the locations analyzed except for few more found in the control site. The availability of these bacteria in this contaminated soil indicates their ability to withstand the relatively high concentration of the heavy metals which implies that they have resistance mechanism to this harsh condition.

4. CONCLUSION

The presence of bacteria capable of tolerating heavy metals from soil samples from heavy metal contaminated sites was investigated. Bacteria that resist high levels of heavy metals were isolated and in pure cultures. The results show that isolate strains characterized with remarkable tolerance against heavy metals, could be potential agents for the development of a soil inoculants applicable in bio augmentation of heavy metals polluted sites. Hence they show traits that present them as biofertilizers that can be used for soil amendments. There is also need to look into the health implication this high level of contamination at this site pose on the people living in and around because of possible contamination of underground water due to seepages of the toxic compounds.

REFERENCES

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ISSN: 2456-8643

De La Iglesia R, Castro D, Ginocchio R, Van Der Lelie D, Gonzalez B,b(2006). Factors influencing the composition of bacterial communities found at abandoned copper-tailings dumps. J. Appl. Microbiol. 100: 537-544.

Diels L, Mergeay M (1990). DNA probe-mediated detection of resistant bacteria from soils highly polluted by heavy metals. Appl. Environ. Microbiol 56: 1485-1491.

Duan XJ, Min H (2004). Isolation, identification and preliminary studies on the resistance gene detection of a Cd2+ resisting bacterium. Acta Sci. Circums, 1: 154-158.

Fabienne G, Antonis C, Hauke H (2003). Comparative 16S rDNA and 16S rRNA sequence analysis indicates that Actinobacteria might be a dominant part of the metabolically active bacteria in heavy metal contaminated bulk and rhizosphere soil. Environ. Microbiol. 10: 896-907.

Ge HW, Lian MF, Wen FZ, Yun YF, Jian FY, Ming T (2009). Isolation and characterization of the heavy metal resistant bacteria CCNWRS33-2 isolated from root nodule of Lespedeza cuneata in gold mine tailings in China. J. Hazard Mater. 162: 50-56.

Giller K, Witter E, McGrath SP (1998). Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol. Biochem. 30: 1389-1414.

Gilotra U, Srivastava S (1997). Plasmid-encoded sequetration of copper by *Pseudomonas pickettii* strain US321. Curr. Microbiol. 34: 378-381.

Goris J, De VP, Coenye T, Hoste B, Janssens D, Brim H, Diels L, Mergeay M, Kersters K, Vandamme P (2001). Classification of metal resistant bacteria from industrial biotopes as *Ralstonia campinensis* sp. nov., *Ralstonia metallidurans* sp. nov. and *Ralstonia basilensis*

Steinle et al. 1998 emend. Int. J. Syst. Evol. Microbiol. 51: 1773-
1782.
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