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ORIGINAL RESEARCH

Heavy Metal Tolerant *Bacillus* species Isolated from hydrocarbons Polluted Soil.

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Abstract:

Introduction: Indiscriminate dumping of spent oils enriched with heavy metals has led to increase in heavy metals load in the soil. Heavy metals exert toxic effects on biodegradation of organic pollutant in co-contaminated soil and there is need to find suitable strategies for their removal.

Aim: The aim of this study was to assess the heavy metals resistance capability of indigenous *Bacillus* species in hydrocarbon polluted soil to nickel (Ni), Cadmium (Cd), Lead (Pb) and Chromium (Cr).

Materials and Methods:

Heavy metal tolerant bacteria were isolated from hydrocarbon polluted soil using Luria-Berthani agar supplemented with the respective metals and spread plate techniques. The isolates were putatively identified on the basis of their colonial morphology and biochemical characteristics and their antibiotics susceptibility pattern were evaluated using disc diffusion method.

Results: The maximum tolerable concentration (MTC) of the four heavy metals to the selected isolates was 2 mM. Four bacteria isolates able to withstand the MTC were putatively identified as *Bacillus subtilis, Bacillus megaterium, Bacillus laterosporus* and *Bacillus polymyxa*. Out of the four *Bacillus* species, only *B. laterosporus* did not show multiple tolerance to the tested antibiotics which show that there is correlation between heavy metal tolerance and antibiotics resistance by the isolates.

Conclusion: Multiple heavy metal tolerance *Bacillus* spp. was isolated from hydrocarbon polluted soil. These bacteria could be suitable agents for bioaugmentation of hydrocarbon polluted soil co-contaminated with heavy metals.

Key words: Antibiotics resistance, *Bacillus* spp., Hydrocarbons, Multiple tolerances, Heavy metals.

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1. INTRODUCTION

The continuous growing demands for oil and other oil-related products have led to increasing severity of petroleum hydrocarbons pollution. In Nigeria, one of the foremost producers of crude oil, soil and water pollution with petroleum hydrocarbons has been worsened by the nonchalant and uncontrolled disposal of hydrocarbons into the environment [1]. Hydrocarbon polluted sites are usually co-contaminated with heavy metals such as nickel (Ni), cadmium (Cd), lead (Pb) and chromium (Cr) among other metals in trace concentrations. With reference to Chevron (Nig.) Ltd., every litre of crude oil extracted from Nigeria oil wells contain not less than 4.0 mg of nickel. Heavy metals are naturally occurring metals having an atomic number greater than 20 with a density greater than 6 g cm^{-3} and a molecular weight greater than 53 [2,3].

Heavy metals and hydrocarbons are two of the most dominant and potentially harmful pollutants in various countries [4]. Anthropogenic activities emanating from the use of petroleum products has led to introduction of the metal components into the environment. This raises the toxicity limits of the metals permissive for faunas, floras, microbiota, and consequently imparting the public health [5]. Exposure of human to heavy metals has been reported to cause growth inhibition, cancer, nervous system and organ damage as well as death. Heavy metals exert their toxic effects on bacterial species by reducing their population, diversity, alteration in morphological structure, change in conformation of nucleic acids and proteins. They also cause inhibition of enzyme activity, disruption of membrane functions and oxidative phosphorylation as well as alteration of the osmotic balance of the bacterial cells [6, 7].

Petroleum hydrocarbons and heavy metals produce synergistic effect in soils that exacerbate the toxicity and damage to the environment, making treatment more difficult [8]. Unlike organics, heavy metals are stable and persistent contaminant of the environment since they cannot be degraded and they also frustrate bioremediation strategies of hydrocarbon polluted soil and water [9, 10]. Hence, the accumulation of metals in soil presents a health risk for people and other living things in soil and water near the polluted sites [11].

Despite the toxic effects of high metals concentration on microbes, Bacteria have however been able to tolerate the presence of metals and use them for their growth. Bacteria inhabiting metal polluted soils have evolved various mechanisms to tolerate metal stress. These mechanisms include the efflux of metal ions outside the cell, intracellular accumulation of the metal and reduction of the metal ions to a less toxic state (12,14). Various genera of heavy metal tolerant bacteria such as *Bacillus*, *Athrobacte*r, *Pseudomonas*, *Micrococcus* and *Enterobacter*, have been previously reported (13,17,42).The relationship between microbes and metals forms the basis for bioremediation of metals using microbes. Bacteria that are resistant to heavy metals also play an important role in biogeochemical cycling of metal ions [12]. The use of microorganisms for decontamination of soil and water in polluted sites has gained more attention. Most biological heavy metal remediation approaches rely on the detoxification and immobilization of the metal to reduce the biological toxicity and to retard metal transport [13, 14]. Biological transformation of metals is a significant detoxification mechanism that is carried out by different bacterial species. The action of bacteria on heavy metal may also result in changes in valency and/or conversion of HM into organometallic compounds that are volatile or less toxic [15]. Although there are many reports of tolerance of *Bacillus* spp to high metals concentration in different polluted sites, only few reports [18] exist in literature on isolation of metal tolerance *Bacillus* spp.from hydrocarbon polluted sites.

This study therefore aimed to isolate and identify *Bacillus* spp tolerance to Ni, Cd, Pb and Cr in hydrocarbon polluted soil which can be use as inoculants in immobilization of heavy metals from such environment.

2. MATERIALS AND METHODS

2.1. Collection of Soil Samples

Composite surface soil samples were collected from Lagos State University (LASU), Faculty of Science power generating plant soil that lies between longitude 3ºE12'02.9'' and latitude 6º27'55.9''N. This site is known to be chronically contaminated with diesel, engine oil, exhaust soot and other hydrocarbons for more than 15 years. The samples were collected at a depth of 15 cm with sterile trowel. Samples for physicochemical and heavy metals analysis were collected in polyethylene bags, while those for microbiological studies were kept in sterile sample bottles. Samples were transported to the laboratory and treated within 4 h.

2.2. Physicochemical properties and heavy metal contents of the soil sample

The pH of the soil samples was determined with a pH meter (Jenway 3051) in 1:1 soil solution in distilled water. The moisture content of the sample was determined using the gravimetric method previously described by Odu et al [19]. The total hydrocarbon was extracted from the soil using n-hexane: dichloromethane solvent systems (1:1) and the extract was analyzed using GC-FID. Conventional aqua regia (a mixture of 32% HCl and 55% HNO_3 in a ratio of 3:1) was used to extract the heavy metals (Pb, Ni, Cr and Cd) in the sample as described by [20, 21]. One gram of the soil sample was weighed into a beaker and 10 ml of aqua regia was added and the resulting mixture was evaporated to near dryness in a water bath maintained at 110°C. Another 15 ml of aqua regia was added to the mixture after which it was then evaporated to near dryness. One molar HNO_3 was added to the mixture and the extract was filtered using

acid washed Whatmann no. 42 filter paper. The filtrate obtained was used to determine the concentrations of the four heavy metals in the sample using atomic absorption spectrometry (PerkinElmer, Canada).

2.3. Microbiological analysis of the soil sample

The total heterotrophic bacterial and fungal counts were enumerated by plating aliquots (0.1 ml) of appropriate diluted soil samples on nutrient agar and acidified potato dextrose agar containing streptomycin (1 mg/100 ml), respectively. Likewise, the population of hydrocarbon-utilizers was estimated on mineral salts medium (MSM) formulated by Katsner et al. [22]. The medium contained (in g/l) Na_2HPO_4, 2.13 g; KH_2PO_4, 1.30 g; NH_4Cl, 0.50 g and $MgSO4.7H_2O$, 0.20 g. Sterile trace element solution (1.0 ml/l) of Bauchop and Elsden [23] was aseptically added to the medium after sterilization. The pH of the medium was adjusted to 7.2 and 5.6 respectively for bacterial and fungal estimations. The MSM was also fortified with nystatin (50 µg/ml) for bacteria and streptomycin (10 µg/ml) for fungi. Sterile crude petroleum served as the sole carbon and energy source and was made available to the cultures through vapour-phase transfer [24]. Plates were counted after incubation at room temperature (27 ± 2.0°C) for 5 to 7 days.

2.4. Chemicals and stock solution of heavy metals

The heavy metal salts ($CdCl_2.H_2O$, $Pb(NO_3)_2$, $CrCl_2$ and $NiCl.6H_2O$) used were purchased from Sigma-Aldrich Corp (St. Louis, MO, USA). All other chemicals were of analytical reagent grade.

One molar stock solutions of Cd, Ni, Pb and Cr were prepared by dissolving adequate amounts of $CdCl_2.H_2O$, $Pb(NO_3)_2$, $CrCl_2$ and $NiCl.6H_2O$ separately in deionized water and sterilized using 0.22 μm pore-size sterile filters (Millipore Corporation, Bedford,MA, USA) and the solutions were stored at 4°C. All plastic and glassware used were acid-washed in 2 N HNO_3 and thoroughly rinsed several times with deionized water before use to avoid metal contamination.

2,5. Isolation and selection of Heavy Metal Tolerant Bacteria

The metal tolerant bacteria were isolated using agar dilution method as described by Kannan and Krishnamoorthy [25]. Morphologically distinct bacterial isolates were selected and inoculated on Luria Berthani agar plates supplemented with 0.5 mM concentration of each metal solution and the plates were incubated at (27 ± 2.0°C) for 72 hours and observed for growth. Bacteria able to grow in the presence of the 0.5 mM metal solutions were noted and further inoculated into 1 mM concentration of the respective metals and incubated for growth at the same conditions. Distinctive bacteria colonies observed on the cultured plates were picked and subcultured on the same medium for further experiment.

2.6. Determination of Maximum tolerable concentration (MTC) of metals

The bacterial strains able to tolerate the 1 mM concentration of the metals were further streaked on increasing concentrations (1.5 mM, 2.0, 2.5 and 3 mM) of the four metals. The MTC was determined when the isolate failed to show growth on the plates after three days of incubation. All experimental set-ups were prepared in duplicate.

2.7. Maintenance and Identification of Isolates

Pure colonies of the selected isolates growing on LB plates were harvested with sterile inoculating loop, pooled and transferred to glycerol nutrient broth (1:1 v/v) medium. The resulting mixture was shaken well to homogenize and preserved at -20°C for further studies. The isolates were identified on the basis of their colonial morphology, cellular and biochemical characteristics as described by Cowan and Steel's Manual [26].

2.8. Determination of Antibiotic Resistance

The bacterial isolates were tested for susceptibility to 10 different antibiotics (Abtek Biologicals Limited, United Kingdom) using disc diffusion method. The antibiotics tested were Septrin SXT (30 µg), Chloramphenicol CH (30 µg), Sparfloxacin SP (10 µg), Ciprofloxacin CPX (10 µg), Amoxacillin AN (30 µg), Gentamycin CN (10 µg), Augmentin AU (30 µg), Perfloxacin PEF (30 µg), Tarivid OFX (10 µg), Streptomycin S (30 µg).

The antibiotics discs were placed on Mueller Hinton agar plates previously seeded with cell suspension with a turbidity of 0.5 McFarland standards. The plates were incubated at room temperature (27 ± 2.0°C) for 24 h and observed for zones of inhibition. The zones of inhibition were measured and scored with reference to the National Committee for Clinical Laboratory Standards [27].

3. RESULTS

3.1. Physicochemical and Microbiological analysis of Soil Samples

The physicochemical and microbiological properties of the polluted soil are shown in Table 1. The total petroleum hydrocarbon content of the polluted soil is 474.6 mg/kg. As shown in Fig. 1, aromatic and middle range aliphatics hydrocarbon fractions such as acenaphthylene, anthracene, anthraquinone, nC14-tetradecane had high peak value as compared with lighter fractions such as hexane, benzene, pentane and heptane fractions. The pH was slightly acidic (6.09) with moisture content of 10%. Heavy metal analysis showed that the soil was also contaminated with Pb, Ni, Cd and Cr.

The microbial load of the polluted soil shows a total heterotrophic bacterial (THB) and fungal counts (THF) of $6.6x10^8$cfu/g and $1.2x10^4$cfu/g respectively. Additionally, the total hydrocarbon utilizing bacteria (THUB) and fungi (THUF) are $7.9x10^4$cfu/g and $2.0x10^2$ cfu/g respectively.

Table 1. Physicochemical and microbiological analysis of soil sample.

Parameters	Value
pH	6.09
Moisture (%)	10.3
TPH (mg/kg)	474.64
Cadmium (mg/kg)	1.58
Lead (mg/kg)	76.72
Chromium (mg/kg)	15.41
Nickel (mg/kg)	4.28
THB (cfu/g)	6.6×10^8
THF (cfu/g)	1.2×10^4
THUB (cfu/g)	7.9×10^4
THUF (cfu/g)	2.0×10^2

TPH: Total petroleum hydrocarbon, THB (Total heterotrophic bacteria), THF (Total heterotrophic fungi), THUB (Total hydrocarbon utilizing bacteria), THUF (Total hydrocarbon utilizing fungi)

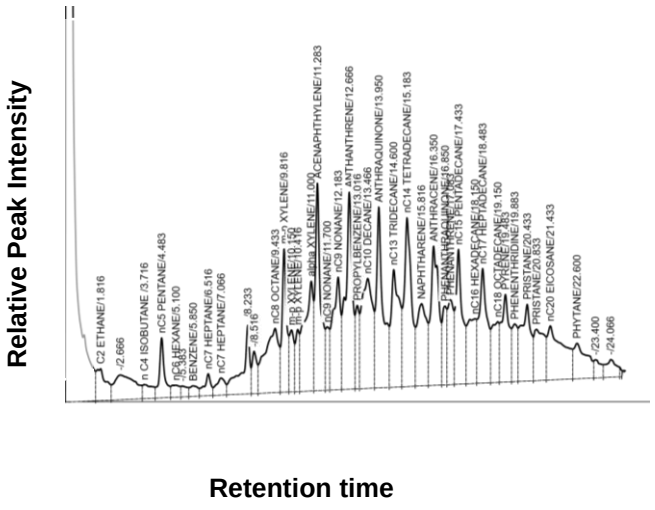

Fig 1. Gas chromatographic traces of n-hexane extract of hydrocarbon contents of the polluted soil. The hydrocarbon components were separated on 30 m long HP-5 column (internal diameter, 0.25 mm; film thickness, 0.25 µm) in a Hewlett Packard 5890 Series II gas chromatograph equipped with flame ionization detector (FID).

3.2. Isolation and determination of MTC of the bacterial isolates

A total of 28 culturable bacteria showing distinct colony characteristics like size, pigmentation, opacity, texture, form, elevation and margin surface were isolated from the composite soil sample. Out of these, twelve isolates able to withstand 0.5 mM concentrations of the four metals were selected and screened further on higher concentration of the metals.

At the end of the screening process, the highest concentration of the four metals that some of the isolates could tolerate was 2 mM. Two (16.7%) of the isolates were able to grow at 2 mM concentration of Ni, while 3 (25%) isolates was recorded for Cd and 6 (50%) was noted for Cr. Lead recorded the highest tolerance of 8(66.7%). Thus, the pattern of tolerance showed by the bacteria isolates to the four heavy metals was Pb> Cr > Cd > Ni as shown in Table 2.

Table 2: Concentrations of heavy metals at which the different bacterial isolates were able to grow.

Highest concentration of heavy metals at which bacteria were able to grow in (mM)				
Isolate	Cd	Cr	Ni	Pb
HD-1	2.0	2.0	1.5	2.0
HD-2	1.0	1.0	1.0	2.0
HD-3	0.5	2.0	0.5	1.0
HD-4	1.5	2.0	2.0	2.0
HD-5	2.0	2.0	2.0	2.0
HD-6	0.5	0.5	0.5	0.5
HD-7	2.0	2.0	1.5	2.0
HD-8	0.5	1.0	1.0	1.0
HD-9	1.0	1.0	1.0	2.0
HD-10	0.5	0.5	0.5	0.5
HD-11	0.5	1.0	1.0	2.0
HD-12	0.5	2.0	1.0	2.0

At the end of the screening process, four out of the twelve isolates HD-1, HD-4, HD-5 and HD-7 with highest tolerance to the tested metals were selected for further study. The morphological and biochemical characteristics of the four isolates selected are shown in Table 3. All the isolates were creamish in color, Gram positive rod and motile spore formers. They were all positive for catalase, oxidase, nitrate, casein and gelatin hydrolysis but negative for coagulase, indole and methyl red test. They all ferment glucose, lactose and sucrose and unable to ferment galactose, raffinose and maltose and were putatively identified as *Bacillus subtilis, B.megaterium, B.laterosporus and B.polymyxa.*

Table 3: Biochemical characteristics of Isolates

Test	HD-1	HD-4	HD-5	HD-7
Catalase	+	+	+	+
Oxidase	+	+	+	+
Indole	-	-	-	-
Motility	+	+	+	+
Methyl red	-	-	-	-
Voges Proskauer	+	-	+	+
Citrate utilization	+	-	-	+
Urease activity	-	+	-	-
Starch hydrolysis	+	+	-	+
Casein	+	+	+	+
Gelatin hydrolysis	+	+	+	+
Spore	+	+	+	+
Nitrate reduction	+	+	+	+
Coagulase	-	-	-	-
Glucose	+	+	+	+
Sucrose	+	+	+	+
Lactose	+	+	+	+
Fructose	+	+	-	-
Mannitol	+	+	+	+
Maltose	-	-	-	-
Raffinose	-	-	-	-
Xylose	+	+	-	-
Galactose	-	-	-	-
Putative identity	BS	BM	BL	BP

BS: *Bacillus subtilis***,** BM **:** *Bacillus megaterium,* BL*: Bacillus laterosporus,* BP: *Bacillus polymyxa*
+: Positive response; - : negative response

3.3. Antibiotic susceptibility patterns of isolates

The susceptibility patterns of the four *Bacillus* isolates to various antibiotics tested are shown in Table 4. All the isolates were susceptible to ciprofloxacin, tarivid but resistance to chloramphenicol. Out of the four *Bacillus* spp. only *B. laterosporus* did not show multi drug resistance. *B. subtillis* was resistance to 7 out of the 10 antibiotics tested while *B. polymyxa* recorded resistance to 6 and *B. megaterium* to 3 antibiotics as shown in Table 4.

4. DISCUSSION

Contamination with oil spills and other hydrocarbons is a persistent and widespread pollution problem ravaging almost all compartments of the environment and imposing serious health implication and ecological disturbances [28,29]. Crude oil is a complex mixture of hydrocarbons in different proportions depending on the source. Heavy metals such as Pb, Cr, Cd and Ni, among other metals in trace concentrations, have been reported to be associated with crude oil. Heavy metals may inhibit biodegradation of organic pollutants in co-contaminated soil and water environments; hence there is urgent need for suitable bioremediation approach for removal of heavy metals for environmental and human health preservation.

Table 4: Antibiotic susceptibility patterns of bacteria

Antibiotics	HD-1	HD-4	HD-5	HD-7
Septrin	R	S	R	R
Chloramphenicol	R	R	R	R
Sparfloxacin	R	S	S	S
Ciprofloxacin	S	S	S	S
Amoxicillin	R	S	S	R
Gentamycin	R	S	S	R
Augmentin	R	S	S	R
Perfloxacin	S	R	S	S
Tarivid	S	S	S	S
Streptomycin	R	R	S	R

R: resistant; S: susceptible

Recent advances in bioremediation of co-contaminated environments have focused on the use of metal-resistant bacteria to reduce bioavailable heavy metal concentrations [10].
The present study determined the soil pH, moisture level, total petroleum hydrocarbon, concentration of Pb, Cd, Cr, and Ni present in petroleum hydrocarbons polluted soil and the MTC of the metals by selected heavy metals tolerant bacteria strains. The concentration of TPH observed in this study is higher than the regulatory limits of 50 mg/Kg recommended limit set by Department of petroleum resources (DPR) (30) in Nigeria which signifies high level of pollution. The preponderance of aromatics fractions of the TPH compared to their aliphatic counterparts could be as a result of weathering of the pollutant due to the combined effect of chemical and physical factors such as volatilization, photo-oxidation, chemical-oxidation, thus leaving behind the more recalcitrant aromatic fractions. The soil pH (6.09) recorded shows that the soil is slightly acidic which implies that the heavy metals present in the sample will be labile as heavy metal mobility increases under acidic conditions [16].

The metal analysis of the samples showed that the polluted soil was evidently co-contaminated with heavy metals. The metal levels recorded were below the target and intervention values stipulated for Nigerian soils except Cd that recorded 1.58 kg that is higher than the target value of 0.8 kg but still fall within the intervention value of 17 kg recommended by DPR [30]. This is of great concern as cadmium is one of the

metals that are toxic at very low concentration. It exerts its toxic effects on microbes by interacting with calcium and zinc metabolism. It also cause various effects on the functional activities of the microbes [31,32].

The tolerance ability of the four *Bacillus* strains: *Bacillus subtilis, Bacillus megaterium, Bacillus laterosporus* and *Bacillus polymyxa* observed in this study is not surprising. Previous studies have indicated the extensive distribution and tolerance ability of *Bacillus* spp in various heavy metals contaminated environments. Hookom and Poochoa [33] reported isolation of *Bacillus* spp from agricultural soil and Industrial water in Mauritius and the isolates were able to grow in the presence of high concentration of Pb. Oyetibo *et al* [34] also established the heavy metal tolerance ability of *Bacillus* spp. from sediments of creek polluted with petroleum cuts and industrial waste waters from Ikeja Industrial Estate, Lagos, Nigeria. The isolates showed tolerance to the high concentrations of Co and Ni encountered at the polluted sites. Cadmium tolerant *Bacillus* species were also reported from contaminated soil samples [35, 36]. In contrast to this study, Mustapha *et al.* [37] reported the dominance of heavy metal tolerant *Microbacterium* spp. in hydrocarbon polluted soil. The ability of *Bacillus* spp. to form spores in nutrients limiting environments makes this genus self-sustainable bioremediation agent. The four *Bacillus* species selected in this study showed multiple tolerance to the four metals tested. Multiple tolerances are common phenomena among heavy metal tolerant bacteria. The metal tolerance ability of the bacteria could be as a result of metal binding metallothioneins (MTs) which form complexes with metals or due to an efflux P-type ATPases [31]. Bacteria with multiple tolerances to metal ions have also been reported by several researchers, including multiple metal resistance by *Bacillus* spp. isolated from platinum and gold mine tailings in South Africa [16; 38]. Bacteria usually display numerous genetic and physiological mechanisms to withstand the toxic effects of metal ions. A number of processes such as efflux systems, bioaccumulation and biosorption by cell biomass, biotransformation, alteration in cell morphology and oxidation reactions have been attributed to bacteria tolerance to heavy metals [6].These mechanisms could be utilized for detoxification and removal of metal from the environments [39]. Efflux pumps has been identified to be the best-known mechanisms of nickel resistance in microorganisms [40]. Chromium tolerance by the isolates could be due to biotransformation processes that involve conversion of toxic form of a metal to a nontoxic form. For example, bacteria tolerance to Cr^{6+} toxicity has been reported to be by conversion of Cr^{6+} to the less toxic form Cr^{3+} through enzymatic reduction [13].

This study also recorded multi-drug resistance in all three out of the four isolates which shows co-occurrence of heavy metal resistance with antibiotic resistance. This finding is in conformity with previous reports [41, 42, 43]. Exposure of bacteria to heavy metals may result in the acquisition of resistance to different types of antibiotics due to the increased selective pressure in the environment. This is because genes responsible for bacteria tolerance to heavy metals are often located together with antibiotic resistance genes on mobile genetic elements. Under conditions of metal pressure, metal and antibiotic resistance in microorganisms possibly help them to adapt faster by the spread of resistant factors than by mutation and natural selection [44]. The multi-drug resistance recorded by the isolates is a serious concern. This is because the various antibiotics resistance genes of the isolates can be spread to non-resistant bacteria populations in the soil through horizontal gene transfer which can subsequently be transferred to humans.

5. CONCLUSION

This study showed co-existence of heavy metal in petroleum products polluted generator house soil and the presence of bacteria that can tolerate the stress imposed by these two important pollutants in the polluted soil. Relationship between heavy metal tolerance and antibiotics resistance were also seen in the four selected *Bacillus* spp. isolated from the sample. These isolates are potential soil inoculants in overcoming the inhibitory effects of heavy metals on biodegradation of hydrocarbon in co-contaminated environments. Further research is needed to determine the mechanisms of the *Bacillus* spp tolerance to the metals which can be exploited for the treatment of heavy metals in the environments.

COMPETING INTERESTS

There is no conflict of interest.

AUTHORS' CONTRIBUTIONS

MOF designed the study, collated and interpreted the data, prepared the manuscript and see to execution of the experiments. AKO managed the literature search and wrote the first draft of the manuscript, AA; participated in sample collection and experimentation, HAJ and ADO participated in sample collection, experimentation and data collation. All authors read and approved the final manuscript.

ETHICAL APPROVAL/ CONSENT

The sample and procedures used in this study by the authors do not make use of animal or human in part or whole.

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REFERENCES

1. Odjegba VJ, Sadiq A. Effects of spent engine oil on the growth parameters, chlorophyll and protein level of *Amaranthus hybridus L*. Nigerian J Appl Sci. 2000; 7: 1–46.
2. Howlett NG, Avery SV. Induction of lipid peroxidation during heavy metal stress in *Saccharomyces cerevisiae* and influence of

plasma membrane fatty acid unsaturation. Appl Env Microbiol. 1997;63: 2971–2976.
3. Ali H, Khan, E. What are heavy metals? long-standing controversy over the scientific use of the term 'heavy metals'—proposal of a comprehensive definition, Toxicol Environ Chem. 2017; DOI: 10.1080/02772248.
4. Schwartz C, Gerard E, Perronnet K, Morel JL: Measurement of in situ phytoextraction of zinc by spontaneous metallophytes growing on a former smelter site. Sci Total Environ. 2001; 279:215–221.
5. ATSDR. Public Health Statement: Toxic Substances and Health. Agency for Toxic Substances and Disease Registry. Division of toxicology and environmental medicine, Atlanta, GA (September bulletin). 2008
6. Fashola MO, Ngole-Jeme VM, Babalola OO. Heavy metal pollution from gold mines: environmental effects and bacterial strategies for resistance. Int J Environ Res Public Health. 2016; **13**: 1047
7. Xie Y, Fan J, Zhu W, Amombo E, Lou Y, Chen L, Fu J. Effect of Heavy Metals Pollution on Soil Microbial Diversity and Bermudagrass Genetic Variation Front Plant Sci. 2016; 7:755.
8. Chibuike GU, Obiora SC, Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods. Appl Environ Soil Sci. 2014: 1-12.
9. Bamforth SM, Singleton I. Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. J Chem Technol Biotechnol. 2005; 80: 723–736.
10. Olaniran AO, Balgobind A, Pillay B. Bioavailability of Heavy Metals in Soil: Impact on Microbial Biodegradation of Organic Compounds and Possible Improvement Strategies. Int J Mol Sci. 2013; 14: 10197-10228.
11. Fashola MO, Ngole-Jeme VM, Babalola OO. Heavy metal immobilization potential of indigenous bacteria isolated from gold mine tailings. Int J Environ Res. 2020; **14**: 71-86.
12. Issazadeh K, Nadiya J, Pourghorbanali F, Raeisi G, Faekhondeh, J. Heavy metals resistance by bacterial strains. Ann Biol Res. 2013; 4 (2):60-63.
13. Shekhar S, Sundaramanickam A, Vijayansiva G. Detoxification hexavalent chromium by potential chromate reducing bacteria isolated from turnery effluent. American J Res Comm. 2014; 2(2): 205-216.
14. Oves MI, Saghir KM, Huda A, Nadeen FM, Almeelbi T. Heavy Metals: Biological Importance and Detoxification Strategies. J Biorem Biodegrad. 2016; 7(2): 1-15.
15. Gadd, GM. Metals Minerals and Microbes: Geomicrobiology and Bioremediation. Microbiol. 2010; 156(3):609-43.
16. Fashola MO, Ngole-Jeme VM, Babalola OO. Physicochemical properties, heavy metals, and metal-tolerant bacteria profiles of abandoned gold mine tailings in Krugersdorp, South Africa. Canadian J Soil Sci. 2020;100(3):217-233.
17. Govarthanan M, Lee KJ, Cho M, Kim JS, Kamala-Kannan S, Oh BT. Significance of autochthonous *Bacillus* sp. KK1 on biomineralization of lead in mine tailings. Chemosphere. 2013; 2013(90):2267-2272
18. Godheja J, Sudhir K, Shekhar, GNV, Satyanarayan S, Pratap S, Dinesh RM. Antibiotic and heavy metal tolerance of some indigenous bacteria isolated from petroleum contaminated soil sediments with a study of their aromatic hydrocarbon degradation potential. Int J Curr Microbiol App Sci. 2017; 6(3):194-211
19. Odu CT. Babalola IO, Udo EJ, Ogunkunle AO, Bakare TA, Adeoye GO. Laboratory manual for agronomic studies in soil plant and Microbiology. Department of Agronomy, University of Ibadan, Nigeria. 1986; 83
20. Chen M, Ma LQ. Comparison of three aqua regia digestion methods for twenty Florida soils. Soil Sci Soc Am J. 2001; 65: 491-499
21. Ngole VM, Ekosse GI. Characterizing associations between geochemical lead fractions and selected properties of a sewage sludge-amended montmorillonitic vertisol. Fresenius Environ Bull. 2012; 21: 171-180.
22. Kästner M, Breuer-Jammali M, Mahro B. Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil sites able to mineralize polycylic aromatic hydrocarbons. Appl Microbiol Biotechnol. 1994; 41:267-273.
23. Bauchop T, Elsden SR. The growth of microorganisms in relation to their energy. J Gen Microbiol. 1960; 23:457-459
24. Raymond RL, Hudson JO, Jamison VW. Oil degradation in soil. Appl Environ Microbiol. 1976; 31:522–53.
25. Kannan SK, Krishnamoorthy R. Isolation of mercury resistant bacteria and influence of abiotic factors on bioavailability of mercury — a case study in Pulicat Lake North of Chennai, South East India. Sci Total Environ. 2006; 367: 341-353
26. Barrow GI, Feltham, RKA. Cowan and Steel's manual for identification of medical bacteria. 3rd ed., Cambridge University, Cambridge
27. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing: Ninth informational supplement, v. 19. NCCLS, Document No. M-100-S9. NCCLS Wayne, PA; 1999; 1-112.
28. Bundy JG, Paton GI, Campbell CD. Microbial communities in different soil types do not converge after diesel contamination. J Appl Microbiol. 2002; 92: 276–288.

29. Okoh AI, Trejo-Hernandez MR. Remediation of petroleum hydrocarbon polluted systems: Exploiting the Bioremediation Strategies. Afri J Biotechnol. 2006; 5: 2520-2525
30. DPR-EGASPIN. Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN), Department of Petroleum Resources, Lagos, Nigeria. 2002.
31. Nies DH. Microbial heavy metal resistance. Appl. Microbiol. Biotechnol. 1999; 51:730–750.
32. Ahemad M. Implications of bacterial resistance against heavy metals in bioremediation: A review, IIOAB J. 2012; 3: 39-46.
33. Hookoom M, Puchooa D. Isolation and identification of heavy metals tolerant bacteria from industrial and agricultural areas in Mauritius. Curr Res Microbiol Biotechnol. 2013; 1: 119-123
34. Oyetibo GO, Chien M, Ikeda-Ohtsubo W, Suzuki H, Obayori, OS, Adebusoye, SA, Ilori,MO, Amund OO, Endo G. Biodegradation of crude oil and phenanthrene by heavy metal resistant *Bacillus subtilis* isolated from a multi-polluted industrial wastewater creek. Int Biodeterior Biodegr. 2017; 614-625
35. Chauhan M, Solanki, M. Isolation and molecular characterization of three *Bacillus* Strains for their tolerance against various heavy metals. J Microbiol Biotech Food Sci. 2017; 6 (4) 1065-1069.
36. Gomez-Ramirez M, Rojas-Avelizapa NG, Hemandez-Gama R, Tenorio-Sanchez SA, Lopez-Villegas EO. Potential use of *Bacillus* genera for metals removal from spent catalysts. J Environ Sci Health. 2019; 54(8): 701-710.
37. Mustafa S, Al-Douseri A, Majik K, Al-Saleh E. Potential of crude oil-degrading bacteria to co-resist heavy metals in soil. Trans Ecol Environ. 2013; 173: 697-705
38. Ndeddy-Aka RJ, Babalola OO. Identification and characterization of Cr-, Cd-, and Ni-tolerant bacteria isolated from mine tailings. Biorem J. 2017; 21:1-9
39. Malik A. Metal bioremediation through growing cells. Environ Int. 2004; 30: 261-278.
40. Abou-Shanab RA, van JS. Berkum IP, Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria. Chemosphere. 2007; 90: 2267-2272.
41. Oyetibo GO, Ilori MO, Adebusoye SA, Obayori OS, and Amund OO. Bacteria with dual resistance to elevated concentrations of heavy metals and antibiotics in Nigerian contaminated systems. Environ Monit Assess. 168:305–314.
42. Owolabi JB, Hekeu MM. Isolation and characterization of zinc resistant bacteria from a coil coating industrial wastewater treatment plant. Intl J Environ Sci. 2015; 5(5): 1030-1042.
43. Jafarzade M, Mohamad S, Usup G, Ahmad A. Heavy-metal tolerance and antibiotic susceptibility of red pigmented bacteria isolated from marine environment. Nat Resour J. 2012; 3: 171-174.
44. Silver S, Misra TK. Plasmid mediated metals resistance. Annu Rev Mcrobiol. 1988; 42: 717–743.