Occurrence of Heavy Metal and Antibiotic Resistant Bacteria in Soils from Selected Mechanic Workshops in Ibadan Metropolis, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aim: This study investigated the occurrence of antibiotic and heavy metal co - resistance in bacteria indigenous to mechanic workshops.
Study Design: The study is an experimental study.
Place: Samples were collected at mechanic workshop.
Methodology: Soil samples were collected from three mechanic workshops and tested for the presence of heavy metals. Hydrocarbon-utilizing bacteria was also isolated from the soils using Bushnell Haas medium supplemented with 1% engine oil or petrol. Isolates obtained were subjected to antibiotic resistance test and those that showed extensive drug resistance to the tested antibiotics were further tested for heavy metal resistance. Selected isolates were then identified using 16S rRNA gene sequence.
Results: The soil samples contained excessive amounts of Lead, Iron, Copper and Zinc, while the concentrations of Nickel, Cobalt, Cadmium and Chromium were within WHO permissible limit. A total of 10 hydrocarbon-degrading isolates were obtained from the soil samples, seven of which were gram negative and three were gram positive. Three of the isolates showed extensive drug resistance to 14 of the tested antibiotics. Three isolates were then subjected to heavy metal

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resistance test and all of them showed resistance to the tested heavy metals. They were identified and given ascension numbers as *Stenotrophomonas maltophilia* MW392903, *Achromobacter xylosoxidans* MW392904 and *Pseudomonas aeruginosa* MW392905.

Conclusion: It was observed that heavy metal resistance and antibiotic resistance can be selected for simultaneously as organisms adapt ways to cope with man's activities in the environment, and while traits like hydrocarbon utilization and heavy metal resistance make the organisms promising in bioremediation, the inadvertent possession of antibiotic resistance by these environmental isolates poses a challenge to the health sector.

1. INTRODUCTION

Heavy metals are chemical elements found in nature and components of the earth's crust that have a specific gravity at least five times that of water [1]. Arsenic (5.7), cadmium (8.65), iron (7.9), lead (11.34), and mercury (13.546) are some well-known metallic metals with a specific gravity greater than or equal to 5 [2]. They are regularly recycled from one earth compartment to the next due to natural processes such as weathering, erosion, and biological activity since they are non-degradable in the environment. Heavy metals have been known to accumulate to harmful or toxic amounts as a result of substantial industrialization and urbanization, as well as the rise of indiscriminate dumping of untreated industrial wastewater and municipal sewage into the environment [3-5].

Heavy metal levels in soil have been linked to poor plant development, lower water and nutrient uptake, and a variety of enzymatic disruptions [6]. Heavy metal contamination puts food safety in jeopardy. Because of the high level of heavy metal contamination in China, the food safety conference in 2009 showed that one sixth of the cultivated land was contaminated by heavy metals, and an area of more than 20 million hectares was threatened with being barred from agricultural practices [7,8]. Heavy metal uptake and accumulation have also been found in edible vegetables, grains, and seafood, according to several studies [9]. Heavy metal accumulation in human tissues and organs has resulted in the cardiovascular, kidney, and neurological system disorders, as well as bone diseases [10,11]. To address the problem of heavy metal pollution in the environment, various strategies have been developed. Chemical precipitation and solvent extraction have been used as physicochemical methods [12].These approaches, on the other hand, have shown to be difficult to apply on big surfaces, expensive, and ecologically unfriendly,

as they may necessitate the use of harmful reagents in the restoration process [12,13].

Although large levels of heavy metals have a deleterious influence on microbial populations in the impacted environment, some bacteria may tolerate or even thrive in the presence of specific metals [14]. Bacterial tolerance to heavy metals is defined as the bacteria's ability to cope with metal toxicity through intrinsic properties, whereas bacteria resistance is defined as the bacteria's ability to survive in higher concentrations of toxic metals through detoxifying mechanisms activated by the presence of the specific metals [4]. Heavy metal resistant bacteria are a superior option for heavy metal decontamination, and they have already been used successfully in the developed world [10,15]. However, there are little research in Nigeria looking into the possible use of metal-resistant bacteria for bioremediation [16].

The rise in the number of bacteria resistant to antimicrobial agents puts the control of infectious diseases at jeopardy. This is due to the fact that infections produced by resistant germs frequently fail to react to standard treatment, resulting in extended sickness and an increased risk of mortality. Antibiotic resistance is a type of drug resistance in which a microorganism can survive antibiotic exposure. Antibiotic resistance is primarily caused by bacterial genetic mutation [17]. Antimicrobial drugs are used inappropriately and irrationally, creating favorable conditions for resistant microbes to arise, spread, and persist. Regardless matter the severity of the need for the antibiotic, the longer it is exposed to the environment, the greater the danger of resistance developing. As antibiotic resistance grows increasingly frequent, there is a larger need for alternative treatments. Despite the search for new antibiotic medicines, the number of newly approved antibiotics has continued to

decline [18,19]. As a result, antibiotic resistance is a major issue.

In a heavy metal contaminated ecosystem, heavy metal resistance and antibiotic resistance can be selected at the same time, and this co-selection has also been recorded in agriculture, animal husbandry, waste water treatment systems, and sediments [10]. Organisms possessing this dual feature could be useful in the bioremediation of metal-polluted ecosystems, as well as in overcoming the inhibitory effect heavy metals have on the biodegradation of organic contaminants. Furthermore, in a contaminated environment, such dual resistance organisms might be able to compete effectively with antibiotic-producing flora.

1.1 Objective of the Study

The broad objective of this study was to investigate the occurrence of antibiotic and heavy metal co - resistance in bacteria indigenous to mechanic workshops.

2. MATERIALS AND METHODS

2.1 Soil Samples Collection

Soil samples were collected from three different mechanic workshops at sites that showed heavy oil activity. A total of three samples were collected from each workshop. Sample 1 was collected at Sango,7° 25' 47.1" N, 3° 53' 31.2" E, Sample 2 at Ologuneru situated between 7° 25' 04.0" N, 3° 51' 23.1" E and Sample 3 at Orogun situated between 7° 27' 08.0" N, 3° 54' 44.1" E. Each of these areas was located within the Ibadan metropolis, Oyo State, Nigeria. The soil samples were collected in clean polythene bags and transported to the laboratory. The control sample was collected from the Botanical Garden, University of Ibadan, where there are no anthropogenic activities such as car repairs, commercial activities, and drainage influence.

2.2 Equipment

Beakers (Pyrex), Conical flasks (Pyrex), Autoclave, Petri-dishes, Forceps, Cotton wool, Spirit lamps, McCartney bottles, Mortal and Pestle, Filter Paper, Measuring Cylinders, Refrigerator, Spatula, Test tubes, Weighing Balance SUN-5202, Incubator, UV Spectrophotometer V-730, Petrol, Engine Oil, Inoculating Loop, Antibiotic discs, Cock borer, Hand gloves, Nose masks, Compound Microscope, Slides, Standard Erlenmeyer flask, 0.5mm Sieve, Bulk Scientific 210/211 VGP Atomic absorption spectrophotometer.

2.3 Chemicals/ Reagents/ Media Used

Crystal Violet, Lugol's Iodine, Immersion Oil, Safranin, Hydrogen peroxide, Nitric Acid, Chloric Acid, Ethanol, Polyacrylamide Gel, Zinc chloride, Lead sulphate, Copper sulphate, Iron sulphate, De-ionized distilled water, Bushnell-Haas medium, Nutrient Agar, Bacteriological Agar, Nutrient Broth, Mueller-Hinton Agar.

2.4 Analysis of Heavy Metals in Soil

Soil samples were homogenized and gently crushed multiple times with a mortar and pestle before being passed through a 0.5mm mesh for analysis. 15mL of freshly made aqua regia (HNO3: HCl, 2:1) was added to a homogenized sample (0.5g) in a standard Erlenmeyer flask. The contents of the beaker were heated on a digestion block while it was covered. In a desiccators, the mixture was cooled before being filtered through a Whatman No. 42 filter paper into a 25mL standard volumetric flask. The filtrate was diluted to 25mL with de-ionized distilled water [20]. Blank solutions were also prepared using aqua regia and de-ionized water. The digested sample solutions were analyzed using the atomic absorption spectrometer {Buck Scientific 210/211 VGP Atomic absorption spectrophotometer (AAS)}. Standard solutions of the various heavy metals were analyzed. All soil samples were analyzed in triplicate to minimize error.

2.5 Enrichment and Isolation of Hydrocarbon Degrading Bacteria

One gram of soil sample was taken from each of the soil samples and serial dilution was carried out to the tenth dilution factor. Bushnell-Haas medium (BH) [21] was used for the isolation of hydrocarbon-degrading bacteria from the soil. The BH medium consist of the following composition in g/L; 0.2 $MgSO_4$, 0.02 $CaCl_2$, 1.0 K_2HPO_4, 1.0 KH_2PO_4, 1.0 NH_4NO_3, 0.05 $FeCl_3$, pH 7.0. BH was supplemented with 1% (v/v) engine oil and/or petrol as the sole carbon source. Three milliliters of dilution factors 10^{-3}, 10^{-7} and 10^{-9} were added to 250 mL Erlenmeyer flasks containing 100 mL sterile BH medium supplemented with engine oil or petrol and the

flasks were incubated at 30°C for 7 days on a rotary shaker (150 rpm). After incubation, 0.1 mL of culture was plated on solid BH medium supplemented with 1% (v/v) engine oil and/or petrol as the sole carbon source using the spread plate method and incubated for 48 hours. Colonies that grew were sub-cultured till pure cultures were obtained, which were then maintained on Nutrient Agar prepared according to Manufacturer's instruction.

2.6 Characterization of Isolated Bacteria

Isolates were characterized using conventional morphological methods.

2.6.1 Gram's staining

Twenty four-hour old culture was prepared. A smear was made by placing a drop of water on the slide and then transferring the microbial inoculum to the drop of water with a sterile cooled loop, it was then mixed and spread by using of a circular motion of the inoculating loop. The smear was air dried and heat fixed and was gently flooded with crystal violet for 1 minute and washed under running water. The smear was gently treated with three drops of Lugol's iodine and left for one minute and gently washed with running water again. The smear was decolorized with 95% concentration ethyl alcohol reagent and was gently washed under running water. Counterstaining with safranin was carried out after which the slide was dried using a filter paper and examined under the microscope using the x100 objective lens with oil immersion.

2.7 Antibiotic Susceptibility Testing of Isolated Bacteria

Single disc diffusion method [22] was used to examine isolates susceptibility to antimicrobial agents. Mueller-Hinton Agar (MHA) was used for this test and microbial sample preparations were carried out using 18-24 hour old culture which was adjusted to 0.5 Mc Farland Standard corresponding to 1.5 x 10 [8] CFU/ML. A sterile swab stick was used to spread the culture standard on the whole surface of the MHA plate after which the antibiotic discs were placed on the MHA plate with the aid of a sterile forceps. Incubation was subsequently done for 24 hours. The antibiotic sensitivity discs utilized were; streptomycin (30µg), septrin (15µg), erythromycin (15µg), zinnacef (10µg), amoxicillin (30µg), rocephin (10µg), ciprofloxacin (30µg), pefloxacin (10µg), gentamicin (30µg), ofloxacin (10µg), chloramphenicol (10µg), sparfloxacin (30µg), augmentin (10µg), ampliclox (10µg). The diameter of the inhibitory zones around the respective antibiotic discs was then measured with the aid of a meter rule [22].

2.8 Heavy Metal Resistance (HMR) test on Selected Isolates

Mueller-Hinton Agar (MHA) was the culture medium used and 18-24 hour old culture was streaked on the plate with the aid of a sterile inoculating loop. A cock borer was then used to bore wells in the MHA streaked plate. The metals Pb^{2+}, Cu^{2+}, Fe^{2+}, and Zn^{2+} were used as $PbCl_2$, $CuSO_4$, $FeCl_2$ and $ZnCl_2$ salts, respectively. Stock solutions of the metals were prepared in six varying concentrations (1g/ml, 0.1g/ml, 0.01g/ml, 0.001g/ml, 0.0001g/ml and 0.00001g/ml) and 1mL each of the solution were placed in the bored holes. The control experiment was carried out by inoculating the pure isolates on basal media without the heavy metals. The results were read after 24hours [23].

2.9 Molecular Identification

The 16S ribosomal RNA (rRNA) gene was analyzed to identify the selected bacteria strains. After the cells were cultured overnight in Nutrient Broth, the genomic DNA was extracted using ZR Bacterial DNA Miniprep. The 16S rRNA gene fragment of strains was then amplified by PCR amplification using forward primer >16S-27F(5'-AGAGTTTGATCMTGGCTAG-3') and reverse primer >16S-1492R (5'-CGGTTACCTTG TTACGATT-3'). The 16S rRNA PCR product was sequenced by 3130XL genetic analyser for Applied Biosystems. The Isolate sequences gotten after the polymerase chain reaction were opened with Bio-Edit and the sequences obtained were compared with data available in GenBank database using the Mega 6.0 Blast network service of the National Centre for Biotechnology Information (NCBI) [24]. A phylogenetic tree was constructed in MEGA 6.0 software by carrying out the multiple alignments using Jukes-Cantor method. Bootstrap values were analyzed based on 1000 replications.

3. RESULTS

3.1 Analysis of Heavy Metals Present in the Soil Samples

The soil samples were analyzed for a total of 8 heavy metals namely Iron (Fe), Copper (Cu),

Zinc (Zn), Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni) and Cobalt (Co). Iron and Cadmium had the highest and lowest concentrations respectively for each of the soil samples. The soil sample obtained from the mechanic workshop in Orogun (Sample 3) had the highest Fe concentration while that of Sango (Sample 1) had the least Fe concentration as seen in Table 1.

3.2 Characterization of Isolated Bacteria

### 3.2.1 Gram staining result and pigments of isolated bacteria

Ten (10) bacteria were isolated from this study of which 7 were gram negative and 3 were gram positive while out of the ten (10) isolates, nine (9) were cream in colour and one (1) was green in colour.

3.3 Antibiotic Susceptibility Test of Isolated Bacteria

Most of the isolates showed multiple drug resistance. However, three (3) of them $27^{B}A^{2}$, $23^{B}A^{2}$ and $17^{B}A^{1}$ showed extensive drug resistance as seen in Tables 2 and 3.

## 3.4 Heavy Metal Resistance (HMR) Test on Selected Isolates

The result showed that the organisms were resistant to the heavy metals. At higher concentrations of 1g/ml and 0.1g/ml, there were visible precipitates of the heavy metals around the cultures but none at lower concentrations as seen in Plates 1 to 3.
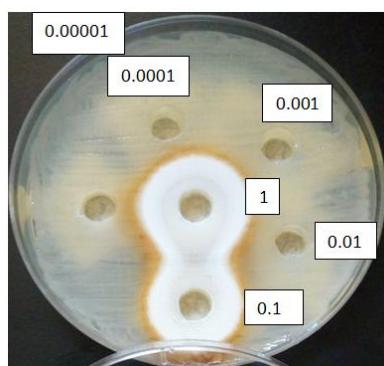

Plate 1. Plate HMR-1 showing the visible precipitates of Lead (all in g/ml) around the culture

## 3.5 Molecular Identification of Bacteria with Desired Traits

Isolates coded $17^{B}A^{1}$, $23^{B}A^{2}$ and $27^{B}A^{2}$ were identified as *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Pseudomonas aeruginosa*, respectively after subjecting their isolated DNA to 16S ribosomal RNA gene amplification and subsequent sequencing of the amplified genes. *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Pseudomonas aeruginosa* has a 98.67%, 97.99% and 97.17% identity respectively using BLAST 2.10.0N+. The 16S ribosomal RNA gene of each of the isolates was submitted to GenBank and each was assigned the following accession numbers: *Stenotrophomonas maltophilia*: MW392903, *Achromobacter xylosoxidans*: MW392904 and *Pseudomonas aeruginosa*: MW392905. The sizes of the amplicons were about 1,428bp for isolate $17^{B}A^{1}$, 1,442bp for isolate $23^{B}A^{2}$ and 1,413bp for isolate $27^{B}A^{2}$ and the DNA ladder used was Hyper ladder 1.The gel image obtained after running the amplified 16S rRNA gene of the three isolates with the molecular marker is shown in Fig. 1.
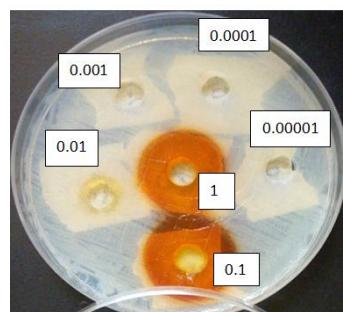
Plate 2. Plate HMR-2 showing the visible precipitates of Iron (all in g/ml) around the culture
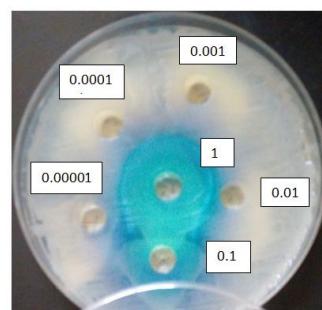
Plate 3. Plate HMR-3 showing the visible precipitates of Copper (all in g/ml) around the culture
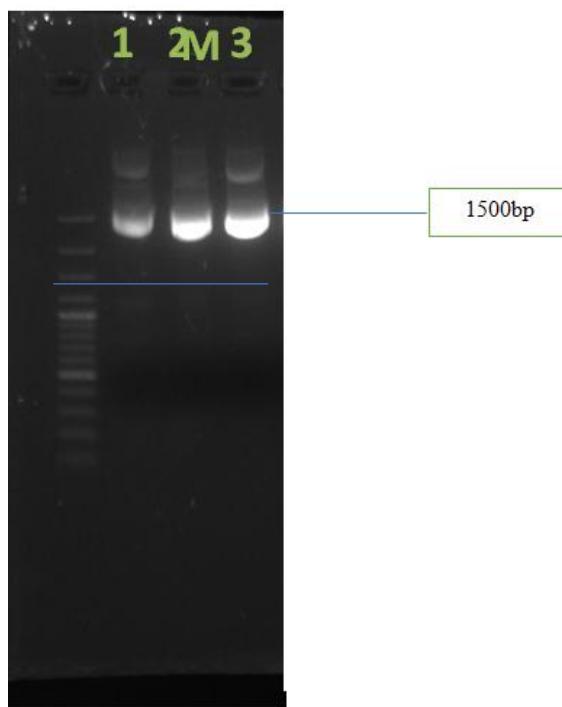
Fig. 1. Gel image for PCR of the three isolates with molecular marker/ladder

Source: Laboratory Analysis, 2020
KEY: M= Molecular marker/ladder
1= Stenotrophomonas maltophilia,
2= Achromobacter xylosoxidans
3= Pseudomonas aeruginosa

3.6 Phylogenetic Analysis of Bacteria with Desired Traits
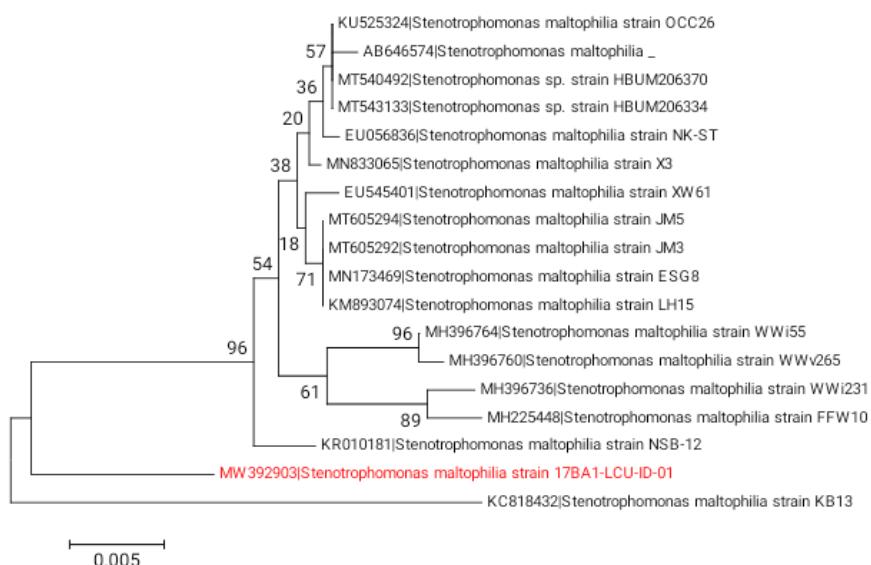
Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing *Stenotrophomonas maltophilia* **found in the GenBank Database. The bar indicates 0.005 substitutions per nucleotide position and values at nod represent percentage of 1000 bootstrap replicates**
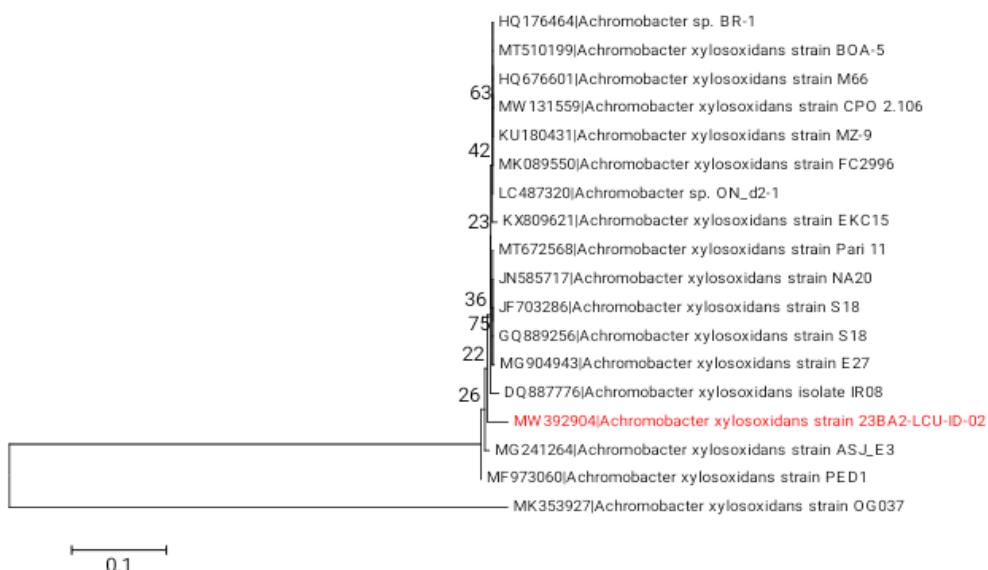
Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing *Achromobacter xylosoxidans* found in the GenBank Database. The bar indicates 0.1 substitutions per nucleotide position and values at nod represent percentage of 1000 bootstrap replicates
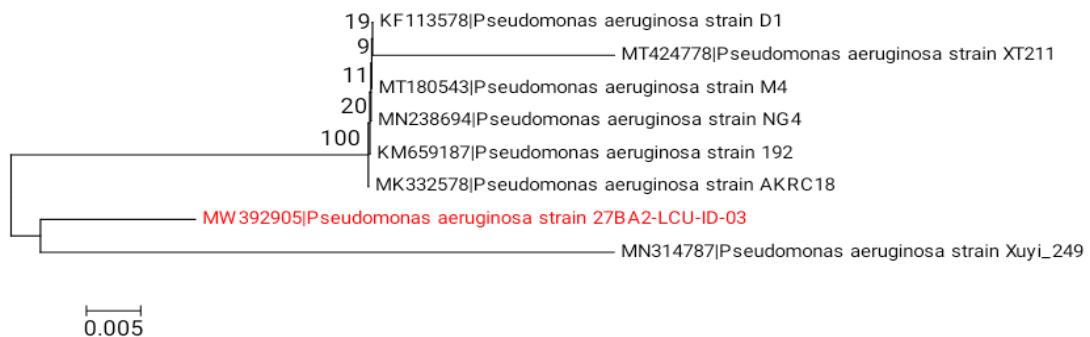

Fig. 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing *Pseudomonas aeruginosa* found in the GenBank Database. The bar indicates 0.005 substitutions per nucleotide position and values at nod represent percentage of 1000 bootstrap replicates

4. DISCUSSION

Using one-way Anova, the concentrations of heavy metals present in the three soil samples were compared relative to one another. The mean values of Fe (Iron), Cu (Copper) and Zn (Zinc) were significant when compared to one another while that of Co (Cobalt), Cr (Chromium), Cd (Cadmium), Pb (Lead) and Ni (Nickel) were insignificant.

Iron (Fe) had the greatest mean concentration of all the metals studied, ranging from 20.5 to 32.3 grams per kilogram. Several studies have found high quantities of iron in soils when compared to other metals, confirming that natural soils contain considerable amounts of iron, though not as much as soils where anthropogenic activities may have played a role [25,26,27,28]. The mean iron value found in this study is higher than that found by other researchers [28,29,30,31,32]. The soil samples from the mechanic workshop in Orogun had the highest quantity of iron. The high values are likely due to the rusting of ancient car bodywork, as well as welding and panel beating activities on the job sites. In humans, acute Fe exposure can cause vomiting, cardiac depression, and metabolic acidosis [26].

Table 1. Concentrations of the various heavy metals found in the soil samples

Sample No	g/kg Fe	g/kg Cu	g/kg Zn	g/kg Co	g/kg Cr	g/kg Cd	g/kg Pb	g/kg Ni
1	20.51±1.50	0.27±0.01	0.51±0.01	0.01±0.00	0.06±0.00	0.000	0.20±0.00	0.03±0.00
2	21.34±0.50	0.05±0.00[a]	0.34±0.03[a]	0.01±0.00	0.04±0.00	0.000	0.03±0.00	0.01±0.00
3	32.27±0.70[a,b]	0.11±0.00[a,b]	1.42±0.03[a,b]	0.02±0.00	0.05±0.00	0.000	0.22±0.00	0.03±0.00

Values are presented as mean ± standard deviation. [a]value is significant when compared with sample 1 [b]value is significant when compared with sample 2

Table 2. Antibiotic susceptibility test of Isolated bacteria

Name of Antibiotics	33[B]T	29[B]A[1]	17[B]A[2]	33[A]	23[B]A[1]	27[B]A[2]	29[B]A[2]	17[A]	23[B]A[2]	17[B]A[1]
CN	-ve	-ve	-ve	16	-ve	-ve	-ve	-ve	-ve	-ve
PEF	15	15	15	17	15	-ve	13	16	14	12
OFX	11	11	13	17	12	-ve	-ve	20	12	11
S	-ve	-ve	-ve	16	-ve	-ve	17	15	-ve	-ve
SXT	11	-ve	10	15	10	-ve	17	15	-ve	-ve
CH	12	-ve	-ve	13	-ve	-ve	-ve	-ve	-ve	-ve
SP	-ve	-ve	-ve	17	-ve	-ve	17	-ve	-ve	-ve
CPX	24	22	21	17	22	-ve	22	20	-ve	-ve
AM	-ve	-ve	-ve	17	-ve	-ve	-ve	-ve	-ve	-ve
AU	-ve	-ve	-ve	13	-ve	-ve	-ve	-ve	-ve	-ve

AU- augmentin CN- gentamycin PEF- perfloxacinOFX- tarividS- streptomycin SXT-septrin
CH- chloramphenicol SP- sparfloxacin CPX- ciprofloxacin
The diameters of susceptibility are measured in millimeter (mm). -ve means Negative

Table 3. Antibiotic susceptibility test of isolated bacteria

Name of Antibiotics	33[B]T	29[B]A[1]	17[B]A[2]	33[A]	23[B]A[1]	27[B]A[2]	29[B]A[2]	17[A]	23[B]A[2]	17[B]A[1]
CN	15	-ve	-ve	15	-ve	-ve	-ve	-ve	-ve	-ve
PEF	16	-ve	-ve	17	16	-ve	12	-ve	15	-ve
E	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
S	15	-ve	-ve	17	21	-ve	-ve	15	-ve	-ve
SXT	-ve	-ve	-ve	12	-ve	-ve	-ve	18	-ve	-ve
Z	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
R	-ve	-ve	-ve	20	-ve	-ve	-ve	-ve	-ve	-ve
CPX	20	16	22	18	20	-ve	21	20	-ve	-ve
AM	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
APX	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

R- rocephin E- erythromycin CPX- ciprofloxacin S- streptomycin SXT- septrinPEF- perfloxacin CN- gentamycin APX-ampiclox Z- zinacef AM- amoxacillin
The diameters of susceptibility are measured in millimeter (mm). -ve means Negative

Zinc contents range from 0.51 to 1.42 g/kg in soil samples. The range of results found in this study is higher than prior studies' findings [28,29, 33,34]. The highest zinc concentrations were found in Orogun. The high zinc content of this sample can be attributed to the site's antiquity as well as its position along the roadside. The wear and tear of car bodywork with galvanized steel surfaces could be to blame for high zinc levels in roadside soil [35,36]. Zinc is also utilized in vehicle brake linings because of its heat conducting qualities, and it can be released via mechanical abrasion, engine oil combustion, and vehicle tyres [37,38]. The acceptable limit of zinc in soil, according to the DPR standard, is 0.14g/kg, and the maximum zinc content in this study is 1.42g/kg. This result is more than the maximum amount of zinc that can be found in the soil. According to the WHO, the acceptable limit is 0.050 g/kg.

Chromium (Cr) concentrations ranged from 0.04 to 0.06 g/kg, and were found to be highest in Sango. Values obtained are slightly higher than those reported by some authors [34,35,36]. According to one of them, the presence of chromium in roadside soil is linked to the usage of chromic plating on various automobile parts to prevent corrosion. Chromium is carcinogenic, and people exposed to chromium-containing dust develop cancer of the respiratory organs [36]. The acceptable limit of Chromium in soil is 0.1g/kg, according to WHO and DPR standards, and the maximum chromium concentration in this study is 0.06g/kg. This number is lower than the chromium allowed limit in soil.

Lead (Pb) concentrations in all the sample sites were in the range of 0.03 to 0.22 g/kg. Thisrange of values for lead is higher in some studies and also lower than those of some other studies [32,38,39]. The concentration of lead in the soil is likely to have been derived from vehicle exhaust fumes containing some lead-rich aerosols [36]. Tetraethyl lead is used as an anti-knocking compound in gasoline, according to studies, and it is released during automotive emissions and fossil fuel combustion [40]. According to one study, petrol engine exhaust emissions account for roughly 80% of all lead in the air [38]. Furthermore, lead levels in the roadside might be linked to the deterioration of car brake linings and tyres [41]. Lead poisoning poses a major threat to one's health [41].

Nickel (Ni) values in soils from all sites varied from 0.01 to 0.03 grams per kilogram. This study's highest nickel value is higher than values published by several authors28,33,42. Airborne particles produced by brakes and tyre wear can include significant amounts of nickel, making this a likely source of nickel in the soils studied. The diesel used in automobiles35 could also be a source of anthropogenic nickel input in the studied locations. Sango and Orogun had the highest Ni levels (0.03 g/kg). Exposure to high levels of nickel from plants grown in nickel-rich soil increases the risk of lung, nose, laryngeal, and prostrate cancers, as well as respiratory failures, birth defects, and heart problems43. According to WHO standards, the maximum nickel concentration in soil is 0.035g/kg, while the maximum nickel concentration in this study is 0.03g/kg. This value is somewhat lower than the maximum amount of nickel that can be found in soil.

Copper (Cu) concentrations ranged from 0.05 to 0.27 (g/kg). This value is higher than the value reported by a previous researcher [31] Copper, lead, and antimony are all present in high concentrations in used oils that leach into the earth as leachates. Copper can also be obtained via metal bearing wear. Although, copper is required for plant growth, but only in minute levels. An excess of copper ions causes headaches, nausea, vomiting, and diarrhoea, and excessive quantities induce anemia, gastrointestinal disorders, and in extreme cases, liver and kidney failure. 44. The acceptable limit of copper in soil is 0.036g/kg, according to WHO and DPR standards, and the maximum copper concentration in this study is 0.27g/kg. This value is higher than the permissible limit for copper in the soil.

Cobalt (Co) was equally detected in all the soil samples. The sample concentration ranges from 0.01 to 0.02 g/Kg. The highest concentration value was obtained at Orogun. The values are higher than the DPR permissible limit of Cobalt in soil.

In mechanic workshops, there is a constant change in the soil micro-organisms as a result of deliberate spillage of used engine oil and other petrochemical products. This alters the biomass and ecology of the soil such that microbial communities undergo a dynamic change in species composition and grasses can no longer grow on the soil. The color and texture of the soil are also affected [22]. Some studies have shown that, oil-polluted soils are dominated by Gram negative bacteria as was seen also in this

research project [42-47]. The result of the present study revealed that soils of mechanic workshops may harbor hydrocarbon degraders that came about as a result of exposure to hydrocarbons due to indiscriminate disposal of the spent engine oil collected from the crankcase of motor vehicles, motor bikes and other machinery.

The distribution pattern of the hydrocarbon utilizing isolates gotten from this study showed that gram negative rods had the highest occurrence at 70%. This is close to a study in which isolate occurrence was 80% [48]. In that same study, gram positive cocci also had the least (10%) which correlates to the value gotten in this research where gram positive cocci had the lowest percentage.

The antibiotic susceptibility profile of the test bacterial cultures revealed that all the bacterial isolates were also multi drug resistant. Heavy metal resistance and antibiotic resistance can be selected simultaneously in a heavy metal contaminated ecosystem. This co-selection has also been reported in agriculture, animal husbandry, waste water treatment system and sediments [49]. According to some authors resistance genes to both antibiotics and heavy metals could be closely located on the same plasmid in bacteria and are thus more likely to be transferred together in the environment [50,51]. None of the bacterial isolates in this study have plasmids, thus it could be that the microorganisms while developing other mechanisms to cope with excess heavy metals such as efflux channels to pump out excess heavy metals may have adapted the same process for resisting antibiotics [52].

At 0.00001g/ml, 0.0001g/ml, 0.001g/ml, and 0.01g/ml, of Iron, zinc, copper and lead, all the bacteria isolated from the hydrocarbon polluted soil samples showed resistance to the heavy metals tested. However, at higher concentrations of 1g/ml and 0.1g/ml, there were visible precipitates of all the heavy metals around the culture. It has been observed that certain microorganisms are able to utilize heavy metals as electron donors during metabolism and are also able to precipitate heavy metals by reducing them to their insoluble forms such as chlorides or sulphates forms that are less bioactive [52]. At concentrations of 0.01g/ml, 0.001g/ml, 0.0001g/ml and 0.00001g/ml, the microorganisms could be able to utilize the heavy metals, as sources of electrons during metabolism. However, at higher concentrations

of 1g/ml and 0.1g/ml, visible precipitates were seen around cultures which most likely means that the microorganisms were precipitating the heavy metals by reducing them to their insoluble forms. The exit of the metals from the cells could be via efflux pumps [53].

The isolates exhibiting extensive drug resistance were identified as *Stenotrophomona smaltophilia*, *Achromobacterxylosoxidans* and *Pseudomonas aeruginosa* on the basis of their cultural, morphological and molecular characteristics. In polluted soil samples, similar findings of heavy metal resistance bacteria have been reported [54,55]. From a lagoon area, a group of researchers discovered Pseudomonas and Streptococcus sp. that were resistant to Cr and Pb54. From soil samples gathered from a mining site, another group discovered Pseudomonas sp. resistant to Pb [55].

The selected heavy metal bacterial isolates in this investigation were shown to be resistant to a variety of antibiotics. Stenotrophomonas maltophilia had 92.9 percent resistance to the 14 antibiotics tested, Achromobacterxylosoxidans had 85.7 percent, and Pseudomonas aeruginosa had 100 percent resistance. Pseudomonas aeruginosa has also been found to have the highest resistance pattern [56]. Heavy metal and antibiotic resistance in bacteria have been linked in a number of studies [56]. Five heavy metal resistant bacteria strains, Micrococcus sp., Nocardia sp., Acinetobacterjunni, Actinomycesturicensis, and Pseudomonas aeruginosa, were found to be resistant to all 18 antibiotics tested in a study by some researchers, which is similar to the findings of this study, in which Pseudomonas aeruginosa was resistant to all 14 antibiotics tested [35]. Metal and antibiotic resistance in bacteria most likely helps them adapt faster in the presence of high amounts of heavy metals in their environment by spreading resistant components. The organisms with antibiotic and heavy-metal resistance isolated and identified in this work could be useful in bioremediation of metal-polluted environments, as well as helping to overcome heavy metal inhibition of organic pollutant biodegradation. Furthermore, in a contaminated environment, such dual resistance organisms might be able to compete effectively with antibiotic-producing flora.

5. CONCLUSION

The environment is subject to anthropogenic pressures. Places like mechanic villages exert a

significant amount of such pressure on the environment. Microbial communities are highly sensitive to such environmental changes and the excessive heavy metals as well as hydrocarbon pollution can impose selection pressures on soil microbes. Microbes that survive usually evolves a system that could either be based on biochemical or genetically encoded mechanisms or both in order to be metabolically active and grow. While these could help select for organisms that are useful for bioremediation, the downside is the inadvertent conferment of traits like antibiotic resistance which is a major source of concern to the health sector.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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