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Antibiogram Profile and Heavy Metal Tolerance of Bacteria Cultured from Soils Collected from Metal Scrap Dump-Sites Located in Benin City, Nigeria

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Abstract

The study investigated the antibiogram profile and heavy metal tolerance of bacteria cultured from soils collected from metal scrap dumpsites in Benin City. Enumeration and characterization of bacterial isolates were carried out using standard procedures. The agar diffusion and Kirby-Bauer disc diffusion methods were used in determining the minimum inhibitory concentration (MIC) and the antibiotic susceptibility profile respectively. The total heterotrophic bacteria count ranged from 12.50 ± 3.44 to 22.50 ± 8.12 (×10^{3}cfu/g), while the control sample has 34.50±11.57(×10^{3}cfu/g), Six (6) bacterial isolates were identified; Bacillus subtilis, Micrococcus sp., Arthrobacter sp., Corynebacterium sp., Pseudomonas aureginosa and Escherichia coli. The Minimum inhibitory concentrations of the various isolates ranged from 5 to 25mg/l. Lead and cadmium showed the highest inhibition against bacterial isolates with MIC values between 5 and 10mg/l except Pseudomonas aeruginosa which tolerated cadmium at 15mg/l. Iron and zinc highly inhibited the isolates with MIC values ranging from 10 to 25mg/l. The bacteria isolates showed about 67% resistance to at least three test antibiotics with a multiple antibiotic resistance index of 0.3. The presence of resistant bacteria to heavy metal and antibiotics in the scrapyard soils could be critical to the health of the nearby residents as the scrapyard may act as a reserviour for the transmission of the antimicrobial agents

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1. Introduction

Much research in recent years has focused on the loss of the therapeutic effect of antibiotics due to the surge in antibiotic-resistant bacteria [1]. More recently, the natural environment as a sink or reservoir for the exchange of antimicrobial-resistant genes has generated considerable research interest [2,3].

Scrapyards are haphazardly sited in urban centres in Nigeria where all kinds of scraps from abandoned automobiles, machinery, and electrical appliances are disassembled and recycled for further usage. One of the major contaminants present in metal scrapyards soil is heavy metals. They are toxic at high levels and adversely affect the environment when not properly managed [4]. Exposure of bacteria to toxic levels of heavy metals and antibiotics, as a result of diverse anthropogenic activities and abuse of antibiotics have contributed to the emergence of bacterial

resistant genes [5,6]. The co-existence of more than one antimicrobial such as antibiotics and heavy metals in microorganisms was due to the mechanisms of co-resistance and cross-resistance [7,8]. Co-resistance involves the presence of phenotypic resistance genes on a similar transmissible genetic element such as plasmids, transposons, and integrons which serve as physical vectors for the transmission of resistance genes from one microorganism to another conjugation [7]. On the other hand, cross-resistance occurs when varying antimicrobial agents – such as heavy metals and antibiotics target similar microorganisms through the same pathway, thereby resulting in cell death [7]. Several authors have reported the adverse effect of metals such as cadmium, copper, lead and zinc on the microbiological balance of the soil, especially when present in high concentrations [9,10,11,12]. Decreasing microbial counts and diminishing microbial diversity are the common disorders associated with the increased heavy metal presence in soil [13]. However, little attention has been given to the effect of the co-occurrence of more than one antimicrobial on soil bacteria. Studies on the relationship between heavy metals and antibiotic resistance in the environmental media cannot be overemphasized, since environmental reservoirs are one of the several routes by which antimicrobial resistance genes are transmitted to humans [14]. This study, therefore, assessed the presence of heavy metal and antibiotic resistance profile of bacterial isolates from scrapyard soils around Benin City, Southern Nigeria.

2. Materials and Method

2.1 Study area

Twelve scrapyards, (four each) were randomly selected across Oredo, Egor and Ikpoba Okha Local Government areas in Benin City, Nigeria. The choice of these locations stems from the proximity of the scrapyards to residential neighbourhoods.

The scrapyards were located in Aduwawa, St. Saviour, Ibiwe, Iyaro and Uwelu areas of the city and were made up of various abandoned automobiles, pieces of machinery and electrical appliances which are brought by scavengers for sale.

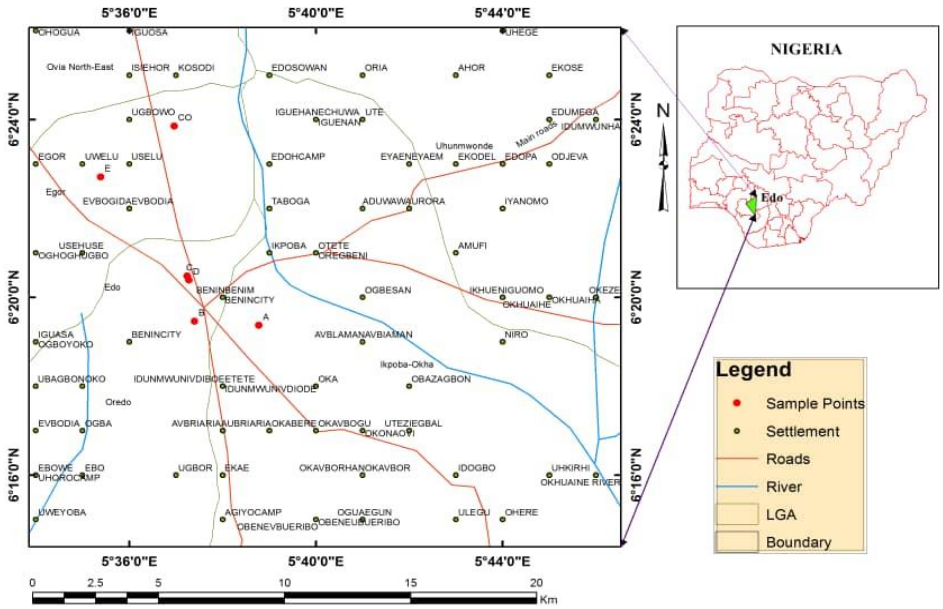


Figure1: Map of Benin City showing the sampling locations

2.2 Sample collection:

Surface soil samples were collected from 12 scrapyards between February and April 2020. About 100g of the surface soil samples were obtained in triplicates at a depth of 2 – 20 cm with the aid of

a soil auger. The surface debris on the soils was removed before sampling. The samples were dispensed into sterile containers and appropriately labelled. A control soil sample was obtained from the University of Benin botanical site Ugbowo, Benin City.

2.3 Enumeration and identification of heterotrophic soil microflora

The mean heterotrophic bacteria in the soil samples were examined with the aid of serial dilution and pour plate technique as described by [15,16]. Quantification of the discrete bacterial colonies was done and expressed in colony-forming units (CFU/g). After enumeration, sub-culturing was carried out in duplicates, the culture plates were agitated and left to solidify and incubated at 35^{o}C for 48 h. Distinct colonies of bacterial were purified and identified by the methods described by [17].

2.4 Determination of minimum inhibitory concentrations (MICs) of heavy metals

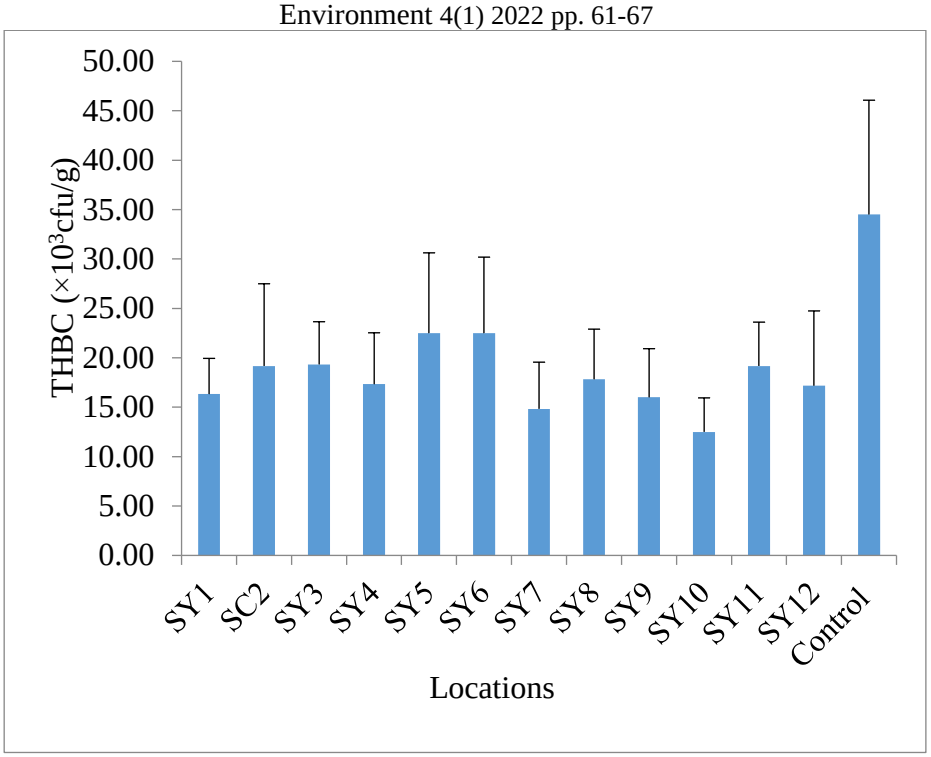
The Agar diffusion method was used in determining the MICs of the metals as described by [18]. The metals Pb^{2+}, Cd^{2+}, Zn^{2+} and Fe^{2+} were used as $PbCl_2$, $CdCl_2$, $ZnCl_2$ and $FeCl_2$ salts respectively. Stock solutions of the metals (100mg/l) were prepared and five varying concentrations (5 mg/l, 10 mg/l, 15 mg/l, 20 mg/l and 25 mg/l) of the different metal salts were tested on the bacterial isolated from the scrapyards. Zones of inhibitions (mm) were measured after 24 h of incubation using a meter rule Pure isolates were inoculated on basal media without the salts of the heavy metals to serve as the control**.**

2.5 Determination of antibiotic susceptibility profiles

The Kirby-Bauer disc diffusion method following modified [19]. was employed to examine the resistance of bacterial isolates to antimicrobial agents. A loop full of the test bacteria was inoculated into 3.5 mL of normal saline. The suspension of the test isolates was then adjusted to 0.5 McFarland turbidity standards. Each of the test suspensions was streaked on Mueller-Hinton agar plates using a sterile swab stick. Antibiotic disc obtained from Becton Dickinson; the USA was aseptically placed in the streaked agar plates. The antibiotic sensitivity discs utilized include; aminoglycosides {streptomycin (30µg) and Gentamycin (30µg)}, Septrin (15µg), macrolides {(erythromycin (15µg), Ampicillin (30µg)}, Beta-lactamase inhibitors {(Amoxicillin (30µg), Augmentin (10µg)}, quinolones {Ciprofloxacin (30µg), Pefloxacin (10µg), Sparfloxacin (30µg)}, phenicols {(Chloramphenicol (10µg). The impregnated agar plates were left to absorb for 10 min and incubated at 37°C for 24 h. The diameter of the zones of inhibition shown by the various scrapyard soil bacterial isolates against the test antibiotics was interpreted into resistance and susceptibility categories according to the [19].

2. Results and discussion

The mean concentrations of the total heterotrophic bacteria count in the sampled scrapyard soils is shown in Figure 2. The average bacteria population ranged between 12.50 ± 3.44 and 22.50 ± 8.12 ($\times 10^3$cfu/g) across the sampling sites. The highest count was recorded at SY5 and SY6 while SY10 has the least concentration of bacteria. The concentration of bacterial in the scrapyard sites was lower than the mean bacterial counts in the control soil 34.50 ± 11.57 ($\times 10^3$cfu/g) (Figure 2).



SY=Scrapyard

Figure 2: Mean concentration of total bacteria isolates

Lower bacteria counts were obtained in the scrapyard soils compared to the control soils. The reduction in the microbial population could be associated with the influence of stressors such as heavy metals present in the scrapyard soils. This may have resulted in increased utilization of energy by the microbial populations and subsequent reduction in the available substrates for their growth [20,21]. This finding was however at variance with the earlier studies by [22,23] who reported higher bacterial counts from contaminated systems in Nigeria.

The phenotypic characterization of isolates revealed six bacterial genera which include *Bacillus subtilis*, *Micrococcus* sp., *Arthrobacter* sp., *Corynebacterium* sp. *P. aeruginosa* and *Escherichia coli*. The study revealed that the bacteria isolates exhibited varying growth on the agar impregnated with heavy metal salts ranging from 0 to 25 mg/l concentration of heavy metals (Pb^{2+}, Cd^{2+} Zn^{2+} and Fe^{2+}).

Table 1: Minimum inhibitory concentration (MIC) Bacterial isolates

	Highest concentration of heavy metal at which bacterial isolates were able to grow (mg/l)			
Bacterial Isolates	Lead	Cadmium	Iron	Zinc
Bacillus subtilis	5.0	5.0	10.0	20.0
Micrococcus sp.	0.0	0.0	10.0	10.0
Arthrobacter sp.	5.0	5.0	10.0	10.0
Corynebacterium sp	5.0	5.0	15.0	15.0
Pseudomonas aeruginosa	10.0	10.0	20.0	25.0
Escherichia coli	5.0	5.0	10.0	10.0

There was abundant growth of all isolates in the control agar plates without heavy metal salts. All bacteria isolates exhibited low MIC values towards Pb and Cd except *Pseudomonas aeruginosa*, which tolerated Pb and Cd at 10mg/l.

The inhibition of bacteria isolates exhibited by Pb and Cd as revealed by the low MICs in this study is similar to the reports of [22,24] who also observed the toxic effect of Pb and Cd on microorganisms. Cadmium and lead can enter bacterial cells through transport systems for essential bivalent cations, such as Mn^{2+} [25,26]. Bacterial toxicity by Cd^{2+} and Pb^{2+} is caused by interaction with nucleic acids, binding to essential respiratory proteins, oxidative damage by the production of reactive oxygen species, and by displacing Ca^{2+} and Zn^{2+} in proteins [27] *Pseudomonas* sp. can synthesize extracellular polymers (EPS) that attaches cations of toxic metals, thereby protecting metal susceptibility and essential components of bacteria.

This study revealed that all bacterial isolates from scrapyards were highly tolerant to iron and zinc with MIC ranging from 10 to 25mg/l. *Bacillus subtilis* and *Corynebacterium* sp. exhibited the highest minimum inhibitory concentration (MIC) of 25mg/l, 20mg/l and 15mg/l respectively for Zinc. Iron showed the highest MIC of 20mg/l towards *Pseudomonas aeruginosa* and the least MIC of 5mg/l towards *Corynebacterium* sp. (Table 1). This finding agrees with the reports of [24] who noted that bacterial isolates from contaminated systems tolerated high concentrations of Zn and Fe. Unlike Cd and Pb, Zinc and Iron act as an essential trace element in the development, growth and differentiation of all living systems including bacteria [28] One of the fundamental characteristics of all living cells including bacteria is the ability to maintain the intracellular Zn^{2+} and Fe^{2+}concentration within safe limits. Zinc concentration is maintained in bacterial cells by sequestration of metal ions and Zn^{2+} export across the cytoplasmic membrane [29]. While the presence of iron-oxidizing systems in bacteria enhances metal ion transformations.

The antibiotic susceptibility test showed that the isolated gram-positive bacteria (*Bacillus subtilis*, *Micrococcus* sp., *Arthrobacter* sp., *Corynebacterium* sp. isolates were resistant to Ampiclox and Ampicillin but were susceptible to Ciprofloxacin, Penicillin and Erythromycin. *Pseudomonas aeruginosa* and *E. coli* were resistant to chloramphenicol, pefloxacin and streptomycin and Sparfloxacin (Table 2). The maximum antibiotic resistance index of the test antibiotics ranged from 0.2 to 0.3.

Table 2: Zone of inhibition (mm) for antibiotic sensitivity test

Gran positive bacteria	**PEF**	**APX**	**AMX**	**CPX**	**PEN**	**ERY**
Bacillus subtilis	13.0 (I)	0.0 (R)	0.0 (R)	22.0 (S)	24.0 (S)	22.0 (S)
Micrococcus sp.	10.0 (R)	0.0 (R)	0.0 (R)	28.0 (S)	25.0 (S)	28.0 (S)
Arthrobacter sp.	18.0 (S)	0.0 (R)	10.0 (R)	25.0 (S)	20.0 (S)	25.0 (S)
Corynebacterium sp	0.0 (R)	0.0 (R)	0.0 (R)	20.0 (S)	24.0 (S)	20.0 (S)
Gram negative bacteria	**CHL**	**SP**	**CPX**	**GEN**	**PEF**	**S**
Pseudomonas aeruginosa	0.0 (R)	14.0 (I)	18.0 (S)	15.0 (I)	0.0 (R)	08.0 (R)
Escherichia coli	0.0 (R)	10.0 (R)	26.0 (S)	18.0 (S)	0.0 (R)	12.0 (I)

KEY: SXT= CHL= Chloramphenicol, SP= Sparfloxacin, CPX= Ciprofloxacin, AMX= amoxicillin, GEN= Gentamycin, PEF= Pefloxacin, S= Streptomycin, PEN=Penicillin, APX=Ampicillin, ERY=Erythromycin

Table 3: MAR index of bacterial isolates

Bacterial isolates	**Antibiotics**	**MAR Index**
Bacillus subtilis	PEF, APX	0.2
Micrococcus sp.	PEF, APX, AMX	0.3
Arthrobacter sp.	APX, AMX	0.2
Corynebacterium sp	PEF, APX, AMX	0.3
Pseudomonas aeruginosa	PEF, PEN, ERY	0.3
Escherichia coli	PEF, APX, PEN	0.3

MAR = Maximum antibiotic resistance

The antibiogram profile showed 66.7% resistance to at least three antimicrobial agents with a multiple antibiotic resistance index of 0.3 while 33.3 % of the isolated bacteria were resistant to two

antibiotics. This finding is similar to that of [30] who also found multiple antibiotic resistance bacteria in contaminated soils across Lagos, Nigeria. Several authors have also isolated similar heavy metal resistant bacterial isolates [22,30] The presence of bacteria resistant to antibiotics and heavy metals in contaminated soil in different areas have been reported [20, 23,31]. The presence of heavy metal and antibiotic-resistant bacteria in scrapyard soils as revealed in this study could be due to co-resistance i.e., the physical linkage of antibiotic resistance and metal resistance encoded on the plasmid, which confers these resistances to the bacteria even when one of the antimicrobials is co selected [32] Co-occurrence of antimicrobial resistance agents can also be caused by cross-resistance mechanism where both antibiotic and heavy metal access similar route to year target bacteria [7].

The occurrence of dual resistance bacteria in the scrapyard soils may increase the risk of diseases related to antibiotic-resistant bacterial infections for nearby vulnerable residents thereby constituting a public health threat.

4. Conclusion

This study assessed the presence of antibiotic and heavy metal resistant bacteria from scrapyard soils. The bacteria population was higher in the scrapyard soils compared to the control soil. The bacterial isolates were more tolerant to iron and zinc heavy metals but exhibited lower MIC values against lead and cadmium. Most of the isolates also showed multiple antibiotic resistance to more than two antibiotics used in the study. The isolation of bacteria resistant to heavy metals and antibiotics in the scrapyard soil is a public threat to surrounding residents. Therefore, an urgent effort is needed in the evacuation of the scrapyard sites from the locations to protect the health of the close-by residence.

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