KWARA STATE UNIVERSITY, MALETE, NIGERIA

SCHOOL OF POSTGRADUATE STUDIES (SPGS)

ANTIBIOTICS AND METAL SENSITIVITY OF BACTERIA ISOLATED FROM SOME METAL SCRAP DUMPING SITE SOIL SAMPLES IN ILORIN METROPOLIS

BY

RUKAYYA, BUSHOLA SHITTU

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August, 2021

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A M.Sc. THESIS SUBMITTED

By

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DEPARTMENT OF BIOSCIENCE AND BIOTECHNOLOGY

FACULTY OF PURE AND APPLIED SCIENCES,

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August, 2021

DECLARATION PAGE

I hereby declare that this thesis titled (Antibiotics and Metal Sensitivity of Bacteria Isolated from Soil Samples from Metal Scrap Dumping Site within Ilorin) is a record of my research. It has neither been presented nor accepted in any previous application for higher degree.

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RUKAYYA BUSHOLA SHITTU **Signature / Date**

APPROVAL PAGE

This is to certify that this thesis by (RUKAYYA, BUSHOLA SHITTU) has been read and approved as meeting the requirements of the Department of Microbiology for the award of the degree of Masters of Science (M.Sc.) in Microbiology.

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DEDICATION

I dedicate this work to Almighty Allah the most beneficent, most merciful for his guard throughout my life and for giving me privilege to complete this work. I also dedicated it to my late father, Mal. Azeez Olagunju. May his soul rest in peace and also to my husband for his support financially, morally and spiritually.

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Abstract

Soil contaminated by heavy metals is one of the major environmental problems worldwide. Heavy metals can move to food chains from contaminated soil, water, and air, causing food contamination that threatens human and animal health. Although some of these metals have a vital role for functioning of cellular enzymes, but their existence in high dose can be a source of death for many biota including soil microorganisms and plants. The aim of this study was to determine antibiotics and metal sensitivity pattern of bacteria isolated from soil samples from metal scrap dumping sites within Ilorin metropolis, Nigeria. Samples were collected from metal dumping site at Ita-elepa and Sango within Ilorin metropolis, Nigeria. Microbiological and physicochemical analysis of the samples were carried out. Bacteria isolated were subjected to biochemical, antibiotic sensitivity test and molecular techniques for identification. The metals detected were carried out by absorption spectrophotometer analysis. Samples from Ita-elepa had a higher total viable counts than that of Sango in Cfu/ml. Gram negative bacteria were more than the Gram positive bacteria. Ofloxacin and gentamicin were the most active antibiotics and *E. coli* was the most susceptible bacterium. The physicochemical parameters of the soil samples revealed temperature of 27.9 to 28.1^{o}C at Ita-elepa and Sango at 35.0 to 35.5^{o}C, pH of 6.0 to 6.3 at Ita-elepa and that of Sango ranged from 6.4 to 6.5. Also, the moisture content of Ita-elepa ranged from 6.1 to 6.3 and that of Sango ranged from 10.0 to 10.4; organic carbon (%) of Ita-elepa was 2.9 to 3.0, while that of Sango ranged from 2.4 to 2.6; organic matter (%) of Ita-elepa ranged from 5.3 to 5.9 and that of Sango ranged from 6.2 to 6.4. The metal detected were zinc, lead, manganese, cadmium and copper. It could be concluded from the study that the isolated organisms were tolerant to some of the metal at higher concentration and could therefore, be used for bioremediation of metal contaminated soils.

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CHAPTER ONE

1.0 Introduction

1.1 Background of the Study

Soil contaminated by heavy metals is one of the major environmental problems worldwide. Heavy metal polluted soil and the environment has been accelerated in modern and developing society due to industrialization, intensified agriculture, increase in pesticides manipulation, and poor controlling system to manage these wastes (Su *et al*., 2014; Abdel-lateif, 2017). Although some of these metals have a vital role for functioning of cellular enzymes, but their existence in high dose can be a source of death for many biota including soil microorganisms and plants (Selvin *et al*., 2009; Gollop *et al*., 2011; Ghosh *et al*., 2013; Abdel-lateif, 2017). Moreover, heavy metals can move to food chains from polluted natural resources, particularly soil, water, and air, causing food contamination that threatens human and animal health. Accumulation of heavy metals, due to their biological property, influences negatively on the soil microbiota as their concentrations increased.

The impact of heavy metal on the soil microbial community play a fundamental role in nutrient recycling and could lead to distsurbance in microbial balance, decrease their diversity and metabolic activity and death (Chaudri *et al*., 2008; Stan *et al*., 2011). Therefore, toxic effect of heavy metal could be a disaster for microbial community especially those that live in symbiotic relation with higher plants that able to fix atmospheric nitrogen. It has been reported that with the elevated concentrations of heavy metals such as copper, zinc, and or lead, the numbers of nitrogen fixing organisms like *Rhizobium* sp. cells are decreases and also their nod genes expression are interrupted (Chaudri *et al*., 2008; Stan *et al*., 2011).

Soil microbial biomass and their activity are used as good indicators for the level of heavy metals-contaminated soil (Aceves *et al*., 1999). Heavy metals constitute one of the major pollutant

groups that are kept under surveillance in leachate from landfills for municipal solid waste. According to Lovleen and Swati (2014), municipal solid waste landfill is not a safe method of disposal, which are biochemically active and toxic substances are gradually leached and released into the surrounding environment. Due to migration process of leachate, soils have been contaminated with heavy metals such as lead, copper, zinc, manganese, chromium and cadmium and these heavy metals in soils lead to serious problems as they cannot be biodegraded (Hong *et al*., 2002). The main sources of heavy metals in the municipal solid waste are pharmaceuticals, certain detergents, personal care products, fluorescent tubes, garden pesticides, photographic chemicals, waste oil, batteries, wood treated with dangerous substances, electronic waste, electrical equipment and paint etc. generated at the household (Slack *et al.*, 2005).

Concentrations of metals in the leachate varies from microgram to the milligram per liter concentration (Christensen *et al*., 2001) and significant variation of metals in leachate is due to seasonal and environmental condition (Malyuba *et al*., 2013). Metals are transported to aerosol by two ways; one is the transport of the fine material enriched with metals from municipal solid waste dumpsite. The second is the emission of heavy metals from the uncontrolled self-ignition and the incineration residue including metals suspected to be aerosol and transported by winds (Mohamed and Elsayed, 2007). Kumar *et al*. (2010) reported that metals play an intrinsic role in the living organism. Some metals like Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni and Zn are essential for metabolism and regulation of osmotic pressure (Kumar *et al*. 2010). Trace metals serve as micronutrients, while many other metals like Ag, Al, Cd, Au, Pb, and Hg have no biological role and they are nonessential (Kumar *et al*. 2010).

They enter the body via food, drinking water, air and can lead to poisoning. Achiba *et al*. (2009) and Mohamed *et al.* (2011) concluded in their report that there was an increased and

accumulation of heavy metals in A horizon of soil. To reduce hazardous pollution from the environment, bioremediation is the best choice. Bioremediation is the process by which microorganisms are used to reduce or remove the pollutant from the contaminated site. According to Garima and singh (2014) two methods of bioremediation technologies is known. One is the intrinsic which makes use of microorganisms occurring naturally to degrade contaminants without engineered interventions at the site. Another method involves engineered interventions which involve alteration in environmental conditions for enhancing microorganism's activity to remove heavy metals.

1.2 Statement of Research Problem

Metals is one of the major sources of pollution, heavy metals are threat to human life and environment. They enter the body via food, drinking water and air we breathe in. When it exceeds the permissible level, it can lead to poisoning. Some microorganisms are resistance to heavy metal and therefore, can grow in the environment having rich heavy metal concentration**.** As a result, heavy metal resistant bacteria might be used as organic monitors of environmental contamination and these heavy metal resistance bacteria can be isolated and used for the remediation of the heavy metal contaminated environment.

1.3 Justification of the Study

Many researchers have carried out studies on isolation and identification of heavy metal resistant bacteria from petroleum soil, isolation of metal resistant bacteria from municipal solid waste dumpsites, physicochemical parameters and heavy metals content of soil samples. But only little work has been reported on antibiotics and metal sensitivity of bacteria isolated from soil samples from metal scrap dumping site within Ilorin metropolis, Nigeria

1.4 Aim

The aim of this study is to determine antibiotics and metal sensitivity pattern of bacteria isolated from soil samples from metal scrap dumping site within Ilorin metropolis.

1.5 Specific Objectives

The specific objectives of this research are to:

i. isolate and identify heavy metal resistant bacteria from metal scrap dumping site
ii. carried out antibiotics sensitivity of the bacteria isolated from metal scrap dumping site.
iii. biochemical and molecular characterization of the bacteria isolated from metal scrap dumping site.

CHAPTER TWO

2.0 Literature Review

2.1. Multidrug Resistance Bacteria

Increase in mortality rate is related to antimicrobial resistance. This makes antibiotic therapy more restrictive, and it is difficult to treat infections caused by multi resistant microorganisms. In the 21st century, infections with carbapenem-resistant gram-negative bacilli, mainly Enterobacteria, become public health problem (Tacconelli *et al*., 2014). Multidrug resistance (MDR) Gram-negative bacteria such as *Acinetobacter baumannii*, *Pseudomonas aerugionosa*, extended-spectrum beta-lactamase (ESBL) producing Enterobacteria, and carbapenem-resistant Enterobacteria (CRE) are main causative agents of nosocomial infections (Teerawattanapong *et al*., 2017). Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococcus (VRE) have been said to be the most common bacterial pathogens, and have been isolated from foods of animal origins, water, and animals. Multidrug resistance *Pseudomonas aeruginosa*, Carbapenem-resistant Enterobacteriaceae, and *A. baumannii* are mainly associated with clinical samples, but some strains have been isolated from foods, animals and water (Teerawattanapong *et al*., 2017).

2.1.1 Methicillin-Resistant *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive ubiquitous strain known to produce several virulence factors that facilitate disease causation and help rapidly develop antimicrobial resistance against antimicrobial agents used for its control, a feature that increases the importance of this microorganism as a pathogen (Chang *et al*., 2003). Initially, infections caused by *S. aureus* were

easily controlled by penicillin. However, *S. aureus* acquired a plasmid-encoded beta-lactamase that conferred resistance to penicillin already in the 1940s, shortly after its introduction for clinical use. To solve this problem, a new, semisynthetic, narrow spectrum, beta-lactamase-resistant antibiotic named methicillin was developed and introduced to combat penicillin-resistant strains in 1959. Unfortunately, methicillin-resistant strains arose quickly (Hiramatsu *et al*., 2001). Since 1961, MRSA has spread worldwide, and infections caused by this microorganism are regarded as one of three major infectious diseases threatening human health. This bacterium apart from causing infections in cutaneous lesions can result in severe cases of pneumonia, meningitis, endocarditis, septicemia, and even systemic infections, with risk of death (Togneri *et al*., 2017). Infections caused by *S. aureus* strains sensitive to methicillin, those caused by MRSA usually have more severe clinical manifestations and are the most difficult to treat. Methicillin resistance affects other virulence factors and enhances the pathogenesis of the bacterium (Schlievert *et al*., 2010). According to the data published by the Center for Disease Control and Prevention (CDC) of the United States, more than 80,000 illnesses and 11,000 deaths in the hospital setting were caused by MRSA during 2011 (CDC, 2011). The European Centre for Disease Prevention and Control also published in 2013 that *S.* aureus is among the most frequently isolated pathogen from health care-associated infections (Togneri *et al*., 2017).

2.1.2. Vancomycin-Resistant Enterococcus (VRE)

Enterococci are indigenous flora of the gastrointestinal tracts of animals and humans, and the species *Enterococcus faecium* and *Enterococcus faecalis* have the ability to cause serious infections and their intrinsic resistance to antimicrobials, including Vancomycin (Silva *et al*., 2011). Vancomycin resistance has been reported considerably less frequently in *E. faecalis* globally (Raven *et al*., 2016). In Europe, Vancomycin resistance related to community infections,

transmitted by animal based food products to humans, and associated with the use of Avorpacina (an antimicrobial of the group of glycopeptides) as a growth promoter in livestock (Raven *et al*., 2016). A similar situation occurred in Europe, where the spread of hospital-related VRE occurred in the 2000 (O'Driscoll and Crank, 2015). The occurrence of outbreaks has been documented in hospitals from Brazilian cities (d'Azevedo *et al*., 2008).VRE was described as ''serious'' (the second highest threat level among organisms causing severe human infections) by CDC. Among hospitalized patients, over 20,000 experienced an infection due to VRE each year and it was estimated that 1,300 deaths occur directly due to these resistant infections in the United States (CDC, 2011).

VRE is a common cause of nosocomial infections and has also associated with urinary tract infections, hospital-acquired bloodstream infections, endocarditis, abdominal and pelvic abscesses, and chronic periodontitis (Chang *et al*., 2010). Resistance to vancomycin is determined by one of nine resistance determinants (vanA, B, C, D, E, G, L, M, and N), but the vanA and vanB genotypes predominate worldwide. These genetic determinants could be carried in the mobile genetic element, such as Tn1546, mostly located on conjugative plasmids (variants of the vanA and vanB-type) or located on the chromosome (vanC) (Iweriebor *et al*., 2015). Linezolid is the first-line drug for treatment of VRE infections, but the first reports of Linezolide-resistant VRE have appeared, reducing treatment options (O'Driscoll1 and Crank, 2015). Daptomycin have been suggested as an effective agent in the treatment of VRE infections (Kohinke and Pakyz, 2017).

2.1.3. Carbapenem-Resistant Enterobacteria

Carbapenem-Resistant Enterobacteria (CRE) is one of the major public health problems in the world. Carbapenems are important for the treatment of critically ill patients at the risk of multi resistant bacterial infection (Braun *et al*., 2014). They have been used as a drug of choice for the

treatment of infections caused by extended spectrum β-lactamases (ESBLs) producing enterobacteriaceae for years, which is also one of the main factors responsible for the emergence of CREs through selective pressure (Braun *et al*., 2014). Among the Enterobacteriacea, *Klebsiella pneumoniae* is the most common bacterium exhibiting carbapenem resistance followed by Enterobacter species. Others such as *E. coli* have been reported less frequently. CREs have emerged in recent decades, but have become one of the major concerns of hospital infection control services. High prevalence of infections by these bacteria is present in several countries on all continents, leading to an important restriction in treatment options (Lerner *et al*., 2015). *Klebsiella pneumonia* is an important cause of nosocomial infections in some countries (Gonc¸alves *et al*., 2016). Early reports of CREs were related to overexpression of ampC, and ESBL associated with loss or modifications of porins. However, later they were confirmed to produce a new type of enzymes (carbapenemases) with the capacity to inactivate any type of beta-lactam, including the carbapenems (Nordmann *et al*., 2012).). *Klebsiella pneumonia* Carbapenemase has been also detected in many other strains, including *Klebsiella oxytoca, Enterobacter* spp., *E. coli, Salmonella* spp., *Serratia* spp., *Citrobacter freundii*, *Proteus mirabilis*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Pseudomonas putida* (Queenan and Bush, 2007). Beta-lactamases exhibit activity against carbapenems but do not degrade cephalosporins (Nordmann *et al*., 2012). Cases of infections caused by *K. pneumoniae*-producing carbapenemases, the mortality rate may be as high as 75%, but the associated factors are age, comorbidities, functional status, and underlying disease may influence this rate (Daikos *et al*., 2014).

2.1.4. Carbapenem-Resistant *Acinetobacter baumannii*

The genus Acinetobacter consists of gram-negative, aerobic cocobacillus that are ubiquitous, immobile, non-fermenting, catalase positive, and oxidase negative. *The A. calcoaceticus-A.*

baumannii complex is responsible for most of the community or hospital-acquired infections (Chusri *et al*., 2014). The different species of Acinetobacter present in diverse natural habitats can be isolated from the soil, water, vegetables, animal and human hosts. They are part of the commensal flora of human skin and mucous membranes. *A. baumannii* can survive in a variety of settings in the hospital environment: in dialysis machines, mechanical ventilation systems, water sources, skin and mucous membranes of health professionals and patients, medicinal preparations, and disinfectants (Vahdani *et al*., 2011). Presence of several virulence factors allow it to survive hospital environment and enhance its capacity to cause disease resulting in one of the main causes of nosocomial infections in many countries (Smani *et al*., 2014). World Health Organization announced this microorganism as the priority pathogen, for which research and development for new antibiotics are seriously needed (WHO, 2017*).* This bacterium can also be highly resistant to antimicrobials, especially those isolated from the patients in the intensive care units. Infections caused by MDR *A. baumannii* strains may worsen patient outcomes due to inadequate initial therapy, limited treatment options, and high toxicity of available therapies. This microorganism has progressively accumulated resistance to penicillins, cephalosporins, quinolones, and aminoglycosides. Mechanisms of resistance of *A. baumannii* can be intrinsic or acquired and are mediated by loss of membrane permeability, production of betalactamases, enzymes that degrade beta lactam antibiotics (Gusatti *et al*., 2009).

2.1.5. Multidrug-resistant Pseudomonas

Pseudomonas aeruginosa is a non-fermenting gram-negative bacillus, widely distributed in nature and in hospital environment. Responsible for nosocomial infections, it is one of the most important opportunistic pathogen causing bloodstream infection, urinary tract infection, and ventilator-associated pneumonia, especially in critically ill patients receiving intensive care (Biswal *et al*.,

2014). It is highly resistant to antibiotics makes it a major public health concern (Utchariyakiat *et al*., 2016). Aeruginosa is intrinsically resistant to several antimicrobials and has great versatility to acquire new genes that confer resistance to many drugs. The antibiotic resistance of this bacterium is due to the low cell wall permeability of this organism, which restricts the uptake of antibiotics, associated with wide resistance mechanisms, such as efflux pumps and enzymes, which modify or degrade antibiotics and drug targets (Lambert, 2002). Carbapenems are usually part of the first line of therapeutic choice for treatment of Multidrug-resistant Pseudomonas (MDR). *P. aeruginosa* resistance to carbapenems occurs mainly due to the impermeability to the drug, loss of porin, and action of efflux pumps, but the production carbapenemases is the most important mechanism (Castanheira *et al*., 2014). The main carbapenemases expressed by *P. aeruginosa* are from class B of Ambler, called metallo-ß-lactamases (IMP, VIM, SPM, GIM, NDM, and SIM families). These enzymes confer resistance to carbapenems and are encoded in plasmids and integrons of class 1, which are responsible for their rapid global spread by horizontal transfer (Araujo *et al*., 2016). *Aeruginosa* resistant to carbapenems (PARC) has become one of the major problems for hospitals. Outbreaks of infection caused by PARC have been reported by several countries, including Brazil (Araujo *et al*., 2016).

2.1.6. Multidrug-resistant *Stenotrophomonas maltophilia*

Stenotrophomonas maltophilia is a motile, aerobic, non-fermentative Gram-negative bacillus with the ability to survive on any humid surface, form biofilm and colonize humid surfaces. It is an opportunistic pathogen with multidrug resistance, which particularly affects immunocompromised patients (with malignances and post-organ transplantation) (Brooke, 2012 and Baker and Satlin, 2016). *Stenotrophomonas maltophilia* is intrinsically resistant to carbapenems and frequently carries genetic elements that provide resistance to other β-lactams, fluoroquinolones,

aminoglycosides and tetracyclines. *Stenotrophomonas maltophilia* has the ability to acquire genes involved in antibiotic resistance from other bacterial species. The most relevant mechanisms of antibiotic resistance include β-lactamase (L1 and L2) production, multidrug efflux pumps (which are resistance to macrolides, quinolones, aminoglycosides, polymyxins), antibiotic-modifying enzymes, mutations of bacterial topoisomerase, gyrase genes and reduction in outer membrane permeability (Brooke, 2012). Although *Stenotrophomonas maltophilia* has been reported in patients with cystic fibrosis, it can affect healthy individuals through contaminated wounds or infected catheters (Cha *et al*., 2016; Gelle *et al*., 2018). Transmission of *S. maltophilia* may occur through direct contact with contaminated source such as contaminated water or medical devices (Brooke, 2012). Community-acquired pneumonia (CAP) caused by *S. maltophilia* is associated with high mortality rates. Hemorrhagic pneumonia is one of the most severe forms of *S. maltophilia* infection (Mori *et al.*, 2014).

2.2. Mechanisms of Resistance among Organisms

Antibiotic producing bacteria contain a variety of sophisticated mechanisms for self-defense against their own antibiotics. Very often they contain multiple mechanisms simultaneously to ensure complete protection from the biologically active molecules produced by them. Interestingly, the genetic determinants for self-resistance are clustered together with the antibiotic biosynthesis genes, and their expression is co-regulated (Mak *et al*., 2014).

2.2.1. Antibiotic Modification or Degradation Mechanism

Antibiotic modification is a commonly used strategy for rendering an antibiotic ineffective, especially in the case of aminoglycoside antibiotics (for example, kanamycin, gentamycin, and streptomycin), chloramphenicol, and β-lactams. A large number of aminoglycoside modification

enzymes (AMEs), including N-acetyl transferases (AAC), O-phosphotransferases (APH), and O-adenyltransferases (ANT) that acetylate, phosphorylate, or adenylylate the aminoglycoside antibiotic, respectively, are known to exist in producer bacteria. Synthesis of aminoglycosides and the presence of modification enzymes in producer organisms leads to direct correlation. Streptomycin resistance in the producer *S. griseus* involves the function of the modification enzyme streptomycin 6-phosphotransferase that converts streptomycin to an inactive precursor streptomycin 6-phosphate. Streptomycin 6-phosphotransferase is the last enzyme in the biosynthetic pathway, and the expression of the gene encoding this enzyme is co-regulated with biosynthesis genes (Mak *et al*., 2014). It has been speculated that these enzymes may not be directly involved in resistance in producers, but instead may perform other metabolic functions (Martinez, 2018).

Modification of the antibiotic as a mechanism for self-defense is also seen for other classes of antibiotics. For example, the bleomycin (BLM) family members (bleomycin (BLM), tallysomycin (TLM), phleomycin (PLM) and zorbamycin (ZBM)) are subject to acetylation. BLMs and TLMs are produced by *Streptomyces verticillus* and *Streptoalloteichus hindustanus*, respectively, and their biosynthesis gene clusters contain genes for N-acetyltransferases, BlmB and TlmB. These enzymes carry out acetylation of the metal-free forms of BLMs and TLMs, thus preventing correct formation of the metal-binding domain of these antibiotics (Coughlin *et al*., 2014). Chloramphenicol is another antibiotic that can be acetylated by a large group of enzymes known as chloramphenicol acetyl transferases (CATs) (Schwarz *et al*., 2004). Resistance to β-lactam antibiotics is normally conferred by antibiotic-hydrolyzing enzymes known as β-lactamases (Sattler *et al*., 2015 and Ogawara, 2016).

2.2.2. Antibiotic Efflux Mechanism

Efflux of antibiotics is another commonly used mechanism for self-resistance, it occurs in conjunction with other mechanisms, such as modification of the antibiotic or the target. Example of antibiotic efflux among producers of antibiotics organisms is found in *Streptomyces peucetius*, which produces two closely related anticancer antibiotics, daunorubicin (Dnr) and doxorubicin (Dox). These two antibiotics intercalate with DNA preventing further rounds of replication. Efflux of these antibiotics in *S. peucetius* occurs by an ABC (ATP Binding Cassette) family transporter DrrAB coded by the drrAB genes embedded within the gene cluster responsible for biosynthesis of these antibiotic (Li *et al*., 2014). The DrrA protein functions as the catalytic nucleotide binding domain (NBD). Carrier protein such as DrrB functions as the and forms the trans membrane domain (TMD) (Li *et al*., 2014). Location of the drrAB genes in the Dox biosynthesis gene cluster, this system is considered to be a dedicated transporter of Dnr and Dox in *S. peucetius*. The DrrAB system is similar to the mammalian ABC multidrug transporter P-glycoprotein (Pgp), which is overexpressed in human cancer cells and is one of the major causes for failure of chemotherapy (Chufan *et al*., 2015). Oxytetracycline have OtrC found in producer *Streptomyces rimosus* as another example of a self-resistance efflux system that exhibits multidrug specificity. Self-resistance in *S. rimosus* is conferred by two efflux proteins: OtrB (previously known as TetB) located in the biosynthesis cluster, and OtrC located outside of the cluster (Mak *et al*., 2014). Protein such as OtrC is ABC family protein, like DrrAB, it also confers resistance to multiple antibiotics and MDR substrates, including ampicillin, oxytetracycline, doxorubicin, ethidium bromide, ofloxacin and vancomycin (Yu *et al*., 2012 and Mak *et al*., 2014).

2.2.3. Antibiotic Sequestration Mechanism

Sequestration involves the function of drug-binding proteins, which prevent the antibiotic from reaching its target. In producers of the bleomycin family of antibiotics, the primary mechanism of resistance involves sequestration of the metal bound or the metal-free antibiotic (Sugiyama and Kumagai, 2002) by binding proteins TlmA, BlmA, and ZbmA in *S. hindustanus* ATCC 31158 (Rudolf *et al*., 2015). Each bleomycin-family producer member has one or more genes related to ABC transporters in their biosynthesis clusters (Du *et al*., 2000; Tao *et al*., 2007; Galm *et al*., 2009), which may be used to remove the antibiotics bound to binding proteins. (Pozzi *et al*., 2016).

2.2.4. Target Modification Mechanism

Target modification acts as a self-resistance mechanism against several classes of antibiotics, including β-lactams, glycopeptides, macrolides, lincosamides, and streptogramins (MLS), and aminoglycosides. The β-lactam antibiotic has a similar structure to PBP substrates (peptidoglycan precursors), thus allowing the antibiotic to associate and cause acylation of the active site serine resulting in its inhibition (Yeats *et al*., 2002). The producer Streptomyces species, despite being Gram-positive, are highly resistant to penicillin's, which is due to either overproduction of PBPs or synthesis of low-affinity PBPs (Ogawara, 2015). Three classes of PBPs (A, B, and C) are found in bacteria (Ogawara, 2015). Analysis of the biosynthesis clusters of β-lactam producing bacteria showed that they often contain genes for PBPs, suggesting their role in self-resistance (Liras and Martin, 2006; Ogawara, 2015). Some of these PBPs indeed have low affinity for β-lactams most likely due to the absence of a serine/threonine protein kinase domain (STPK) that binds β-lactams (Marcone *et al*., 2014; Frasch *et al*., 2015).

Target modification is also seen for MLS antibiotics, which bind to the 50S ribosomal subunit. This mechanism involves methylation of 23S rRNA at residue A-2058 by 23S rRNA methyltransferases (Douthwaite *et al*., 2004). Monomethylation (MLS type I) typically provides moderate level of resistance, while dimethylation (MLS type II) provides strong resistance (Fyfe *et al*., 2016). Finally, resistance against aminoglycosides by target modification uses 16S rRNA methyltransferases, which methylate at residue A1408 or G1405 (Shakil *et al*., 2008). This mechanism for self-resistance may work in conjunction with the AMEs. Other resistance mechanisms bypass the original target by producing additional low affinity targets. Examples include synthesis of additional B subunit of DNA gyrase for novobiocin resistance, alternate resistant RNA polymerase for rifamycin resistance, or an alternate fatty acid synthase for resistance to platensimycin (Schmutz *et al*., 2003; Sanchez-Hidalgo *et al*., 2010; Peterson *et al*., 2014).

2.3. Environmental Contamination

An environment can be polluted or contaminated. Pollution differs from contamination; however, contaminants can be pollutants, and pose detrimental impact on the environment. Pollution is defined as the introduction by man, directly or indirectly, of substances or energy into the environment resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to environmental activities and impairment of quality for use of the environment and reduction of amenities (Wong, 2012).

Contamination on the other hand is the presence of elevated concentrations of substances in the environment above the natural background level for the area and for the organism. Environmental pollution can be referred to undesirable and unwanted change in physical, chemical and biological characteristics of air, water and soil which is harmful for living organisms both

animal and plants. Pollution can take the form of chemical substances or energy, such as noise, heat or light (Wong, 2012). Pollutants, the elements of pollution, can either be foreign substances/energies or naturally occurring contaminants. Environmental pollutants continue to be a world concern and one of the great challenges faced by the global society. Pollutants can be naturally occurring compounds or foreign matter which when in contact with the environment cause adverse changes. There are different types of pollutants, namely inorganic, organic and biological.

2.3.1. Inorganic Pollutants

Industrial, agricultural and domestic wastes contribute to environmental pollution, which cause adverse harm to human and animal health. From such sources, inorganic pollutants are released. Inorganic pollutants are usually substances of mineral origin, with metals, salts and minerals (Wong, 2012). Inorganic pollutants as material found naturally but have been altered by human production to increase their number in the environment. Inorganic substances enter the environment through different anthropogenic activities such as mine drainage, smelting, metallurgical and chemical processes, as well as natural processes. These pollutants are toxic due to the accumulation in the food chains.

2.3.2. Organic Pollutants

Organic pollution can be defined as biodegradable contaminants in an environment. These sources of pollution are naturally found and caused by the environment, but anthropogenic activity has also been contributing to their intensive production to meet the human needs. Some of the common organic pollutants which have been noted to be of special concern are human waste, food waste, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic

hydrocarbons (PAHs), pesticides, petroleum and organochlorine pesticides (OCPs) (EI-Shahawi *et al*., 2010). Organic pollutants have become one of the major problem in the environment because of its high lipid solubility, stability, lipophilicity and hydrophobicity. These properties give organic pollutants the ability to easily bioaccumulate in the different spheres of the environment, thus causing toxicological effects (Lepp, 2012; Van-Ael *et al*., 2012)

2.3.3. Biological Pollutants

Biological pollutants are described as pollutants which exist as a result of humanity's actions and impact on the quality of aquatic and terrestrial environment. This type of pollutants include bacteria, viruses, moulds, mildew, animal dander and cat saliva, house dust, mites, cockroaches and pollen. Different sources of these pollutants are pollens originating from plants, viruses transmitted by people and animals, bacteria carried by people, animals, and soil and plant debris (Elliott, 2003).

2.4. Heavy Metals

Heavy metal is a naturally occurring element having a high atomic weight and high density which is greater than that of water (Banfalvi, 2011). Among all the pollutants, heavy metals have received more attentions to environmentalist due to their toxic nature. Heavy metals are present in trace amounts in natural waters but many of them are toxic even at very low concentrations (Herawati *et al*., 2000). Metals such as arsenic, lead, cadmium, nickel, mercury, chromium, cobalt, zinc and selenium are highly toxic even in minute quantity. Increasing quantity of heavy metals in the environment is a greater concern, since a large number of industries are discharging their metal containing effluents into the environment without any adequate treatment. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. They can enter

the human body through food, water, air or absorption through the skin when they come in contact with humans in agriculture, manufacturing, pharmaceutical, industrial or residential settings. Ingestion is the most common route of exposure in children. Natural and human activities are contaminating the environment because they are discharging more than what the environment can handle (Herawati *et al*., 2000; He *et al*., 2005).

2.4.1. Sources of Heavy Metals

Heavy metals in the environment can be from both natural and anthropogenic processes and can be found in different environmental compartments (soil, water, air and their interface).

2.4.1.1. Natural Processes

Natural emissions of heavy metals occur in different ways such emissions include volcanic eruptions, sea-salt sprays, forest fires, rock weathering, biogenic sources and wind-borne soil particles. Natural weathering processes can lead to the release of metals from their endemic spheres to different environment compartments. Heavy metals can be found in the form of hydroxides, oxides, sulphides, sulphates, phosphates, silicates and organic compounds. The most common heavy metals are lead (Pb), nickel (Ni), chromium (Cr), cadmium (Cd), arsenic (As), mercury (Hg), zinc (Zn) and copper (Cu). Although some heavy metals can be found in traces, they still cause serious health problems to human and other mammals (Herawati *et al*., 2000).

2.4.1.2. Anthropogenic Processes

Industries, agriculture, wastewater, mining and metallurgical processes, and runoffs also lead to the release of pollutants to different environmental compartments. Anthropogenic processes of heavy metals have been noted to go beyond the natural fluxes for some metals. Metals naturally emitted in wind-blown dusts are mostly from industrial areas. Some important anthropogenic sources which significantly contribute to the heavy metal contamination in the environment

include automobile exhaust which releases lead; smelting which releases arsenic, copper and zinc; insecticides which release arsenic and burning of fossil fuels which release nickel, vanadium, mercury, selenium and tin. Human activities have been contributed to environmental pollution due to everyday activities such as manufacturing of goods to meet population needs (He *et al*., 2005).

2.5. Sources of Soil Heavy Metal Pollution

Chemical compounds, accumulate in soil and water due to different human activities. Therefore, soil may be regarded as a long-term reservoir of pollutants, from which these compounds may be introduced to food chains or groundwater (Pečiulyté and Dirginčiuté-Volodkiené, 2009). Inappropriate and careless disposal of industrial waste often results in environmental pollution. The pollution includes point sources such as emission, effluents and solid discharge from industry, vehicle exhaustion and metal smelting or mining, as well as nonpoint sources such as the use of pesticides or excessive use of fertilizers (McGrath *et al*., 2001). Each of the sources have their own damaging effects on plant, animal and human health, but those that add heavy metals to soils are of serious concern due to the persistence of these elements in the environment. They cannot be destroyed, but are only transformed from one state to another (Gisbert *et al*., 2003).

Pollution of soil including heavy metals, may be of natural origin, like volcanic eruptions, animal excrements or ore leaching. Human activity such as chemical industry, mining and metallurgy, as well as municipal management and traffic emissions are the main source of environmental pollution. Some authors also mention that waste disposal, waste incineration, fertilizer application and long-term application of wastewater in agricultural lands may result in heavy metal pollution of soils (Bilos *et al*., 2001). Heavy metals occur naturally in soils due to weathering of parent materials, however concentrations of these metals are regarded as trace and rarely toxic (Pierzyński *et al*., 2000). Heavy metals occurring in soils from anthropogenic sources

tend to be more mobile, therefore more bioavailable than pedogenic or lithogenic ones (Kaasalainen and Yli-Halla, 2003). Communication routes, such as roads, railways etc., are an important source of soil pollution, especially in the case of lead and zinc. Despite restricted use of leaded gasoline adopted in most countries, lead remains one of the most serious automotive-originating metal pollutant. Apart from lead and zinc, chromium, cadmium, nickel and platinum are among the pollutants emitted by combustion engine-powered vehicles. Heavy metals enter the environment as a result of tire wear and damage of vehicle parts. Moreover, grease used in vehicles may also be the source of cadmium pollution along roads (Antonkiewicz and Macuda, 2005). Nickel emission results from this metal being added in gasoline and atmospheric abrasion of nickel-containing parts of automobiles (Voegborlo and Chirgawi, 2007). The changes in the concentrations of lead, nickel, cadmium, copper and zinc in roadside soils are attributed to traffic density (Arslan and Gizir, 2006).

Standard agricultural practices are also a significant source of heavy metals in soils, as application of fertilizers and pesticides has contributed to a continuous accumulation of these elements. Heavy metals can accumulate in soils due to the application of liquid and solid manure, as well as inorganic fertilizers (Atafar *et al*., 2010). The application of livestock manures, composts and municipal sewage sludge on agricultural soils could leads to the accumulation of various heavy metals, such as, Cd, Cr, Cu, Hg, Mo, Ni, and Zn (Basta *et al*., 2005). Copper oxychloride is annually applied on vineyards as a fungicide to control a significant number of plant diseases. This Cu ends up in the agricultural soil and affect natural vegetation (Du-Plessis *et al*., 2005). High fertilizer applications and acid atmospheric deposition, with insufficient liming, may cause a decrease in pH and increase heavy metal bioavailability, elevating the problem of deteriorating food quality, metal leaching and impact on soil organisms (De Vries *et al*., 2002).

The application of municipal wastewater or industrial waste as fertilizers requires constant monitoring of the amount and proportion of harmful factors. Airborne sources of heavy metals include stack emissions or fugitive emissions such as dust from storage areas or waste heaps. All solid particles in smoke from fires and other emissions from factory chimneys are deposited on land or sea. Another source of soil pollution is the emission of lead from combustion of petrol containing tetraethyl lead; this contributes to the content of Pb in soils mostly in urban areas (Wuana and Okieimen, 2011). Metal containing compounds are deposited onto the landscape in form of dust emissions from smelters: pentlandite (Ni,Fe) 9S8, pyrrotite Fe7Sg(Nix), chalcopyrite CuFeS2, chalcosite Cu_2S, covellite CuS, cuprite Cu_2, tenorite CuO, and metal copper and nickel (Barcan, 2002). Chemical and biological oxidation of mineral pyrite (FeS_2) occurs due to the unearthing of pyrite-containing rock formations and this results in an acidification of the dump material. Under acidic conditions, the majority of heavy metals is leached from the waste dump and they are transported in stream waters (Hafeburg and Kothe, 2007).

2.6. Environmental Impacts of Heavy Metals

The presence of heavy metals in the environment leads to a number of adverse impacts. Such impacts affect all spheres of the environment, that is, hydrosphere, lithosphere, biosphere and atmosphere. Until the impacts are dealt with, health and mortality problems break out, as well as the disturbance of food chains.

2.6.1. Effect on Soil

Emissions from activities and sources such as industrial activities, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilisers, animal manures, sewage sludge, pesticides, wastewater irrigation, coal combustion residues and spillage of petrochemicals

lead to soil contamination by heavy metals. Soils have been the major sinks for heavy metals released into the environment by many anthropogenic activities. Most heavy metals do not undergo microbial or chemical degradation because they are non-degradable, and their concentrations last for a long time after being released to the environment (Athar and Vohora, 2001; Lepp, 2012). The presence of heavy metals in soils is a serious issue due to its presence in food chains, thus destroying the entire ecosystem. Organic pollutants can be biodegradable, their degradation rate is decreased by the presence of heavy metals in the environment, and this in turn doubles the environmental pollution, that is, organic pollutants and heavy metals thus present. There are various ways through which heavy metals present risks to humans, animals, plants and ecosystems as a whole. Such ways include direct ingestion, absorption by plants, food chains, consumption of contaminated water and alteration of soil pH, porosity, colour and its natural chemistry that have impact on the soil quality (Musilova *et al*., 2016).

2.6.2. Effects on Water

There are many sources of water contamination, industrialisation and urbanisation are the major causes for the increased level of heavy metal water contamination. Heavy metals are carried by runoff from industries, municipalities and urban areas. Most of these metals end up accumulating in the soil and sediments of water bodies (Musilova *et al*., 2016). Heavy metals can be found in traces in water sources and can still be very toxic and impose serious health problems to humans and other ecosystems. Toxicity level of metal depends on various factors such as the organisms which are exposed to it, its nature, its biological role and the period at which the organisms are exposed to the metal. Food chains and food webs symbolize the relationships among organisms. Therefore, the contamination of water by heavy metals actually affects all organisms. Humans, as

an example of organisms feeding at the highest level, are more prone to serious health problems because the concentrations of heavy metals increase in the food chain (Lee *et al*., 2002).

2.6.3. Effects on Air

Rapid growth of industrialization and urbanization, have made air pollution as a major environmental problem around the world. The air pollution was reported to have been accelerated by dust and particulate matters (PMs) particularly fine particles which are released through natural and anthropogenic processes. Natural processes which release particulate matters into air include dust storms, soil erosion, volcanic eruptions and rock weathering, while anthropogenic activities are more industrial and transportation related (Soleimani *et al*., 2018). Particulate matters are important and require special attention as they can lead to serious health problems such as skin and eyes irritation, respiratory infections, premature mortality and cardiovascular diseases. These pollutants can also cause deterioration of infrastructure, corrosion, formation of acid rain, eutrophication and haze (Herawati *et al*., 2000). Among others, heavy metals such as group 1 metals (Cu, Cd, Pb), group 2 metals (Cr, Mn, Ni, V and Zn) and group 3 metals (Na, K, Ca, Ti, Al, Mg, Fe) originate from industrial areas, traffic and natural sources, respectively (Ventura *et al*., 2017 ; Soleimani *et al*., 2018).

2.6.4. Effects of Heavy Metals on Soil Microorganisms

Metals without biological functions are tolerated only in minute concentrations, whereas essential metals with biological functions, are tolerated in higher concentrations (Hafeburg and Kothe, 2007). They have either metabolic functions as constituents of enzymes or meet structural demands by supporting the cell envelope. Concentration of metal determine whether it is useful or harmful to microbial cells (Hafeburg and Kothe, 2007). Microorganisms are the first biota that

undergoes direct and indirect impacts of heavy metals. Some metals (e.g. Fe, Zn, Cu, Ni, Co) are of vital importance for many microbial activities when presence at low concentrations. These metals are often involved in the metabolism and redox processes. Metals facilitate secondary metabolism in bacteria, actinomycetes and fungi (Hafeburg and Kothe, 2007). For example chromium is known to have stimulatory effect on both actinorhod in production and growth yield of the model actinomycete *S. coelicolor*. High concentrations of heavy metals may have inhibitory or even toxic effects on living organisms (Bruins *et al*., 2000).

Adverse effects of metals on soil microbes result in decreased decomposition of organic matter, reduced soil respiration, decreased diversity and declined activity of several soil enzymes. Metals can cause general changes in morphology, disruption of the life cycle and the increase or decrease of pigmentation of the soil microorganisms (Hafeburg and Kothe, 2007). Rajapaksha *et al*. (2004) compared the reactions of bacteria and fungi to toxic metals in soils (Zn and Cu). The authors concluded, that bacterial community is more sensitive to increased concentrations of heavy metals in soils than the fungal community. The relative fungal/bacterial ratio increased with increasing metal levels. They also reported the varying effect of soil pH on the microbial reaction to soil pollution. Lower pH in contaminated soils enhanced the negative effect on bacteria, but not on fungi. The toxic concentration of heavy metals may cause enzyme damage and their inactivation, as the enzymes-associated metals can be displaced by toxic metals with similar structure (Bruins *et al*., 2000). Heavy metals also alter the conformational structures of nucleic acids and proteins, and consequently form complexes with protein molecules that render them inactive. Those effects result in disruption of microbial cell membrane integrity or destruction of entire cell (Bong *et al*., 2010). Heavy metals also form precipitates or chelates with essential

metabolites (Sobolev and Begonia, 2008). Juwarkar *et al*. (2007) indicate that long-term contamination of soils with heavy metals has adverse effects on soil microbial activity.

Lenart and Wolny-Koładka (2013) reported that except for fungi, the soil-dwelling microorganisms were much less abundant in heavy metal polluted soils than in uncontaminated soils. Heavy metal contamination results in reduction of microbial biomass and even if they do not cause the reduction in their number, they reduce biodiversity or disturb their community structure. However, one of the reasons of decreasing biodiversity of microorganisms in heavy metal polluted soils is the selection for tolerant species or strains. Metal exposure may lead to the establishment of tolerant to the microbial populations of Gram positive genera such as *Bacillus* spp, *Arthrobacter* spp and *Corynebacterium* spp or Gram-negatives such as *Pseudomonas spp*, *Alcaligenes spp*, *Ralstonia* spp or *Burkholderia* spp (Piotrowska-Seget *et al*., 2005). The impact of heavy metals on the bacterial metabolism depends on the growth form. The resistance towards metals seems higher in consortia than in pure cultures (Sprocati *et al*., 2006). A great number of heavy metal-resistant bacteria, such as *Cupriavidus metallidurans* possess efflux transporters that excrete toxic or over concentrated metals outside the cell (Nies, 2003). Efflux transporters have high substrate affinity and can maintain low cytosolic concentration of metals (Hafeburg and Kothe, 2007).

Microbial cells may prevent intoxication by the release of metal-binding compounds into the extracellular surroundings. So, metals are chelated outside the cell and blocked from entering the cell through the membrane transporters that facilitate the influx (Hafeburg and Koth, 2007). Some fungal and bacterial species are able to keep metals outside their cells by the extracellular active melanin. It is a secondary metabolite that has strong cation chelating properties through the anionic function such as carboxyl and deprotonated hydroxyl groups (Hafeburg and Kothe, 2007). Number of soil microorganisms, such as *Aspergillus niger* solubilize metals by the release of

organic acids or by the immobilization of metals through excretion of different compounds, such as oxalates. Some microorganisms possess the abilities to protect their cells by a cytosolic sequestration mechanisms. These mechanisms are activated once the metal enters the cell and cannot be excreted. In this case internal inclusion bodies, e.g. polyphosphate granules (volutin) bind large amounts of metal cations.

2.7. Permissible Level of Heavy Metal in Soil and Toxic Effect of its High Concentration

The mean concentration of mercury limit is 2.00 mg/kg for soils, permissible limit for lead (Pb) is 50.00 mg/kg for soils and the mean concentrations of copper permissible limit is 100 mg/kg for soils (WHO/FAO, 2001). The mean concentration of zinc by WHO/FAO (2001) permissible limit is 300.00 mg/kg for soils. The presence of zinc in soil at the various sites could be attributed to the occurrence of dry cells in the municipal waste (Thorpe and Harrison, 2008) and the burning of e-waste materials. Zinc is an essential microelement which plays a very essential catalytic role in enzyme reactions but its content varies with the type of soil (Knezevic *et al*., 2009).

High concentration of Zn can pose health threats to humans. The mean concentrations of cadmium by WHO/FAO (2001) permissible limit is 3 mg/kg for soils. Cadmium is very much connected with non-residual fractions of heavy metals and thus makes them mobile and potentially bio-available for uptake by plants (Zhuang *et al*., 2009). The mean concentration of nickel by WHO/FAO (2001) permissible limit is 50 mg/kg for soils. The mean concentrations of arsenic (As) by WHO/FAO (2001) permissible limit is 20.00 mg/kg for agricultural soils. Ashtray (2008) reported that lead causes both acute and chronic poisoning and thus, poses adverse effects on kidney, liver, vascular and immune system. Cadmium mean concentrations by WHO/ FAO (2007) permissible limit is 0.20 mg/kg. Jabeen *et al*. (2010) and Maobe *et al*. (2012) reported that

cadmium causes both acute and chronic poisoning. It also causes adverse effect on kidney, liver, vascular and the immune system.

Mean copper values by FAO/WHO (2001) permissible limit is 3.0 mg/kg. According to Maobe *et al*. (2012) high levels of copper can cause metal fumes fever with flu-like symptoms, hair and skin decolouration, dermatitis, irritation of the upper respiratory tract, metallic taste in the mouth and nausea. The concentration of nickel by WHO/FAO (2001) permissible limit is 1.63 mg/kg in edible plants. Nickel in plants could be attributed to cadmium–nickel batteries in the electrical gadgets and some paints used to polish the surfaces of the gadgets which might have spread to adjoining sites. High concentration of nickel can lead to health risks. According to Khan *et al*. (2008) Ni deficiency results in liver disorder. The concentration of Sn recommended by FAO/WHO (2001) permissible limit is 200.00 mg/kg in edible plants. The presence of arsenic in the vegetation could be attributed to the recycling activities in the area. According to Luo *et al*. (2011) atmospheric deposition is a major factor for high metal accumulation in plant samples. The uptake of heavy metals by plants occurs during the vegetative period (Krstic *et al*., 2007; Knezevic *et al*., 2009). The presence of toxic heavy metals in the soil results in the bio-accumulation and bio-magnification of toxins in plant tissues (Suciu *et al*., 2008; Fagbote and Olanipekun, 2010).

CHAPTER THREE

3.0 Materials and Methods

3.1. Collection of Samples

Soil samples were collected with auger from two metal scrap dumping sites at three point on each contaminated site, at 10 cm depth in a clean polythene bag and were conveyed to the laboratory for further analysis.

3.2. Preparation of the Enriched Media

Nutrient agar was prepared according to manufacturer's instruction supplemented with individual heavy metals Cd, Cu, Zn, Pb, Mn and Ni in the form of their salts $CdCl_2$, $CuCl_2$, $ZnCl_2$, $MnSO_4$ and $NiSO_4$ respectively at concentration of 50 ppm sterilized at 121^oC for 15 min. and use for the isolation of bacteria. The plates were incubated at 37^oC for 24-48 hours.

3.3. Serial Dilution and Isolation of Microorganisms

Eight test tubes each containing 9 ml of distilled water were set up, and 1 gram of soil sample was introduced into the first test tube containing 9 ml of distilled water. From the first test tube, 1ml was pipetted into the second test tube containing 9 ml of distilled water and continuously until the last test tube of 10^{-8} dilution was made for each soil sample. Exactly 0.1 ml of the serial dilution from test tube10^{-4}, 10^{-5} and 10^{-8} was inoculated into the Petri dishes of enriched nutrient, molten agar was pour into the plate. The plate were swirled round for proper mixing before the content was allowed to solidify (Oyeleke and Manga, 2008). The isolates were subcultured repeatedly to obtain pure culture on nutrient agar.

3.4. Gram's staining

Gram staining was carried out according to Fawole and Osho, (2004) and Oyeleke and Manga, (2008). A drop of water was placed on a microscopic slide (at the center of the slide). A colony of

24 hours old was picked using a sterilized wire loop and then emulsified with the drop of water on the slide to obtain a thin smear. The smear was air dried and then heat fixed by passing over flame. The slide was flooded with crystal violet for one minute and wash with water. Secondly, Lugol's iodine was also flooded for one minute and washed off with water. The slide was decolorized with 95 % ethanol and allowed to run out of the slide for 3 seconds and washed with water. The slide was finally flooded with safranin for 1 minutes before it was washed with water and allowed to air dry. Immersion oil was added and the slide was viewed under the microscope using x100 objective. Gram positive cells stained purple, while Gram negative cells stained pink (Oyeleke and Manga, 2008).

3.5 Motility

Sulphide indole motility medium (SIM), is a semi-solid media and was prepared to see how bacteria swarm. Different test organism was inoculated into the test tube by stabbing the medium. The test tubes were incubated for 35-37^{o}C and examined for a diffuse zone of growth flared out from the line of inoculation. Hazy growths that spread throughout the medium rendering it slightly opaque indicates a positive motility while growth confined to the stab-line, with sharply defined margins leaving the surrounding clearly transparent indicates negative motility (Cappuccino and Sherman, 2008 and Tille and Forbes, 2014).

3.6. Biochemical Tests

3.6.1. Catalase Test

Catalase test was carried out as described by Oyeleke and Manga, (2008). A drop of hydrogen peroxide (H_2O_2) was placed on a slide and bacteria culture was emulsified with a drop of hydrogen peroxide on the slide. The presence of catalase enzyme was indicated by bubbles gas (oxygen) that shows positive, while no bubbles indicate absence of catalase.

3.6.2. Coagulase Test

Coagulase is an enzyme capable of coagulating certain blood plasma. This test is used to differentiate pathogenic staphylococcus from non-pathogenic staphylococcus species. A drop of water was placed on a slide and a pure culture was emulsified with a drop of water on the slide to obtained suspension. A drop of blood plasma was then mixed with the suspension on the slide and it was observed for agglutination. Coagulase positive was indicated by clumping of colonies, while coagulase negative was indicated by absence of clumping of colonies (Oyeleke and Manga, 2008).

3.6.3. Indole Test

Indole test is used to determine ability of the organism to convert tryptophan to indole. Each isolate was inoculated into sterile peptone water enriched with 1 % tryptophan in the test tube and incubated. Kovac's reagent was added and gently shaken. A red violet colour at the top surface of the tube indicated a positive result, while yellow coloration indicate a negative result (Cheesbrough, 2006).

3.6.4. Citrate Test

This test is used to test ability of an organism to use citrate as a sole source of carbon and energy. Each isolate was inoculated into a Simmon citrate agar in a slant test tube and was incubated. A positive citrate test was confirmed by the formation of blue colour, while the initial green color denotes negative test (Cheesbrough, 2006).

3.6.5. Oxidase Test

A piece of filter paper was soaked with few drops of oxidase reagent (Tetramethyl-p-phenylenediamine dihydrochloride). Sterile inoculating loop was used to pick a colony of the test organisms and smeared on the filter paper. If the organism produced oxidase, the

phenylenediamine in the reagent was oxidized to a deep purple colour indicated a positive result, while absence of purple colour indicated a negative result (Cheesbrough, 2006).

3.6.6. Lactose Fermentation Test

This test was carried out to determine the ability of organisms to ferment sugars with production of acid and gas. Sugar indicator broth was prepared using peptone water medium containing 1% lactose and 0.01% phenol red. About 10 milimeters of sugar broth was dispensed into each of the test tubes, Durham tube which would trap the gas if produced was inverted carefully. The test tubes were autoclaved and inoculated with a loopful of 24 hour old culture of the test organisms after then incubated for 2-7 days at 36^{o}C and observed for acid and gas production. Yellow colouration indicated acid production and red colouration indicated negative result, while gas production was indicated by displacements of the medium in the Durham tube (Fawole and Oso, 2004).

3.6.7. Methyl-Red (MR) Test

A test organism was inoculated in the glucose phosphate water medium and incubated at 37^{o}C for 48 hours. Few drop of methyl-red was added to the culture and positive result was indicated with red colour formation, while no change in colour denote negative result (Fawole and Oso, 2004).

3.6.8. Hydrogen Sulphate (H_2S) Production Test

Triple sugar iron agar medium was prepared to detect the production of H_2S from different test organism. Each test organism was inoculated into test tube by stabbing the medium. The test tubes were then incubated for 24 hours at 37^{o} C. A black colour along the line of stabbing indicates a positive result, while absence of black colouration along the line of stabbing indicated a negative result (Oyeleke and Manga, 2008).

3.6.9. Nitrate Reduction Test

Each test organisms was inoculated into a test tubes containing nitrate agar and incubated at 37^{o} C for 24 hours to determine ability of an organism to reduce nitrate to nitrite. Nitrite reagents (sulfanilic acid and α-naphthylamine) six drops each was added and the change in color was observed red colouration signify positive result, absent of red colour signifies negative result (Tille and Forbes, 2014).

3.7. Antibiotics Sensitivity Test

Medium was inoculated with tested isolate, it was seeded throughout the plate after it has been diluted at a standard concentration (approximately 1.5 x 10^{8} colony forming units per ml) (according to Mcfarlan standard). Commercially prepared disks, each of which are pre-impregnated with a standard concentration of a particular antibiotic, were then evenly dispensed and lightly pressed onto the agar surface. The test antibiotic immediately begins to diffuse outward from the disks, creating a gradient of antibiotic concentration in the agar such that the highest concentration was found close to the disk with decreasing concentrations further away from the disk. After an overnight incubation, the bacterial growth around each disc was observed. If the test isolate is susceptible to a particular antibiotic, a clear area of "no growth" was observed around that particular disk. The zone around an antibiotic disk that has no growth was referred to as the zone of inhibition since this approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. This zone was then measured in mm and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant (Giguère *et al*., 2006).

3.8. Minimum Inhibitory Concentration of Metals on Some of the Identified Organisms

The minimum inhibitory concentration (MIC) is identified as the lowest concentration of metal that inhibits the visible growth of the test microorganisms. The MIC of metal detected by physicochemical analysis of the contaminated soil were determined. Resistant bacterial were determined by the nutrient agar dilution method (Aleem *et al*., 2003). The metals were used to prepare 1000 mg ml-1stock solutions in sterile deionized water. Preparation of various concentration (100, 300, 500 μg/ml) of individual metal from this stock solution used for the inoculation of isolated bacteria individually into plates. The bacteria were incubated at 30 ^{O}C for 72 h. The lowest concentration of metals that completely prevented the growth of each bacterium were considered as MIC.

3.8.1. Minimum Bactericidal Concentration Metals on Some of the Identified Organisms

Minimum bactericidal concentration (MBC) assay is performed to determine the bactericidal activity of the metal against specific bacterial isolate (CLSI, 2018). From minimum inhibitory concentration, test tubes with least metal concentration that showed no growth or turbidity after 24 h incubation was subculture into freshly prepared nutrient agar with different metal concentration and incubated for 24 h. Any plates that showed no growth after incubation were regarded as minimum bactericidal concentration (MBC).

3.9. Determination of the Physicochemical Properties of Soil from Metal Scrap Dumping Site

3.9.1. Soil pH

The pH of soil samples was determined. Ten grams of the air dried sample were weighed into 100 ml beaker and 100 ml of distilled water was added. The mixture was allowed to stand for 30 minutes with occasional stirring with glass rod. The electrode probe of calibrated pH meter (Horiba

pH meter D-51) was inserted into the partially settled suspension and the pH of the soil was measured (AOAC, 2000).

3.9.2. Temperature

Temperature was measured using thermometer (0 – 100 ^{o}C). The temperature of each point where soil samples was collected was taken on-site and recorded (AOAC, 2000).

3.9.3. Moisture Content

The moisture content of the soil is an indication of the amount of water present in the soil. It was determined according to the method described by George and Martin (2020). One gram of a representative sample of the soil was placed in a clean dry crucible of known mass. The mass of the container and soil were determined (W2) using an analytical balance (OHAUS Advance AR 3130 Model). The crucible was placed in an oven maintained at 110± 5^{o}C for 4 hours to obtain a constant weight (W1). The measurement was done in duplicate. % Moisture was calculated as follows:

% Moisture = (W2 – W1)/Sample weight; Where W2 = weight of crucible + weight of sample before oven drying; W1 = weight of crucible + weight of sample after oven drying.

3.9.4. Determination of Organic Carbon

The organic carbon content of soils was determined by the Walkey- Black and digestion method. About 1 g of soil sample was placed into a block digester tube (sample weight) and added 5 ml of potassium dichromate solution and 7.5 ml of concentrated H_2SO_4. The tube was placed in a pre-heated block at 145-155 ^{o}C for 30 minutes, then removed and allowed to cool. The digest was quantitatively transferred into a 100 ml conical flask and then added 0.3 ml of ophenanthrene-ferrous complex (ferroin) indicator solution, then stirred and mixed properly using magnetic

stirrer. The digest was titrated with ferrous ammonium sulphate solution with end point indicating a change from greenish to brown colouration. The organic carbon content expressed in percentage as follows was based on 77% recovery factor (Griffin *et al*., 2013)

% Organic C = N (T – B) /W× 390.0

Where N = Normality of $KMnO_4$; T = Volume of $KMnO_4$ used in titration of soil; B = Volume of $KMnO_4$ used in titration of blank; and W = Weight of soil in gram

3.9.5. Determination Organic Matter

Organic matter was determined using acid extraction method by weighing 100 g of the sample into a beaker and 50 cm^3 of 30 % H_2O_2 was added. The solution was heated at 450 ^oC for 1 hr. The calculation of the amount of OM was done by the difference in mass of dry sample before and after extraction (Amacher *et al*., 2003). OM % = Initial mass of dry sample before extraction–Final mass of dry sample after extraction (Hoyle, 2013).

3.9.6. Metal Analysis by Atomic Absorption Spectroscopy

Zinc, lead, cadmium, manganese, Nickel, copper were analyzed by atomic absorption spectroscopy (AAS). For each soil sample, three different extractions conditions were used to determine the total, available (fraction presenting long-term mobility) and soluble concentrations (Lienard *et al*., 2014). Prior to the analyses, the soil samples were ground and sieved at 2 mm. For the extraction of the soluble fraction. Five grams of dried soil were extracted by 50 ml of 0.01 M $CaCl_2$ solution for two hours under agitation. The solutions were then filtered (Whatman Grade 595 ½) and analyzed after less than 24 h. The available fraction was determined by extracting 10 g of dried soil by 50 ml of an extractive solution containing EDTA (0.002 M), ammonium acetate (0.5 N), and acetic acid (0.05 N). It is considered that EDTA can be used to estimate the long-term

mobility of the metallic elements (Labanowski *et al*., 2008). The solution was then agitated for 30 min. filtered and analyzed after less than 24 h. the determination of psedo-total MTE (digestion with aqua regia) concentrations was made as follows : three grams of soil sample were treated by a mixture of 22.2 ml HCl 37 % and 7.5 ml HNO_3 65 % (aqua regia) in a Gerhardt asher. The resulting solution and the nitric acid 0.5 M from the vapor trap were then pooled in a 100-ml vial, completed with distilled water. The solution was filtered with Whatman Grade 602 H1/2 and analysed after less than 24 h. The quantification of Zn, Cd, Pb, Mn, Cu, and Ni was then performed using a SpectrAA 110/220, VARIAN ASS (flame atomic absorption spectrometry).

3.10. Molecular Characterization

3.10.1. Deoxyribonucleic acid (DNA) Extraction

Bacterial Genomic DNA was isolated using the Insta GeneTM Matrix Genomic DNA isolation kit. An isolated bacterial colony was picked and suspended in 1 ml of sterile water in a micro centrifuge tube. It was centrifuged for 1 minute at 10,000–12,000 rpm to remove the supernatant. Two hundred microliter (200 µl) of Insta Gene matrix was added to the pellet and incubated at 56 °C for 15 minutes. Vortexed at high speed for 10 seconds and placed the tube in a 100 °C in heat block or boiling water bath for 8 minutes. Finally, vortexed the content at high speed for 10 seconds and spin at 10,000–12,000 rpm for 2 minutes. Twenty µl of the supernatant was used per 50 µl PCR reaction.

Polymerase chain reaction (PCR) Protocol: 16S rRNA universal primers gene fragment was amplified using MJ Research Peltier Thermal Cycler, primer was used depend on the bacteria genomic. One µL of template DNA was added to 20 µL of PCR reaction solution. Primers was used for bacteria, and then PCR reaction was performed accordingly. Initial Denaturation was done at 94℃ for 2 mins and amplified at 94℃ for 45 secs and 72℃ for 60 secs. Final extension

was done at 72℃ for 10 min. Deoxyribonucleic acid (DNA) fragments are amplified at about 1,400 bp in case of bacteria. Include a positive control (*E.coli* genomic DNA) and a negative control in the PCR.

3.10.2. Purification of Polymerase Chain Reaction (PCR) Products

Polymerase Chain Reaction **(**PCR) primers and dNTPs from PCR products using Montage PCR Clean up kit (Millipore). The PCR product was sequenced using primers. Sequencing reactions were performed using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme).

3.10.3. Sequencing Protocol

Single-pass sequencing was performed on each template using 16s rRNA universal primers. The fluorescent labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer.

3.10.4. Sequencing Primer

Sequence data was aligned and analyzed for Identifying the Sample.

3.10.5. Bioinformatics Protocol

The 16s r RNA sequence was BLAST using NCBI BLAST similarity search tool. The phylogeny analysis of sequence with the closely related sequence of BLAST results was performed followed by multiple sequence alignment. The program MUSCLE 3.7 was used for multiple alignments of sequences (Edgar, 2004). The resulting aligned sequences were cured using the program Gblocks 0.91b. This Gblocks eliminates poorly aligned positions and divergent regions (removes alignment noise) (Talavera and Castresana, 2007). Finally, the program PhyML 3.0 aLRT was used for phylogeny analysis and HKY85 as Substitution model. PhyML was shown to be at least as accurate

as other existing phylogeny programs using simulated data, while being one order of magnitude faster. The program Tree Dyn 198.3 was used for tree rendering.

3.11. Statistical Analysis

Analysis of variance (ANOVA) is a statistical technique used to check if the means of two or more groups are significantly different from each other. Analysis of variance (ANOVA) checks the impact of one or more factors by comparing the means of different samples. https://www.analyticsvidhya.com/blog/2018/01/anova-analysis-of-variance/.

In this research, a one-way ANOVA was used. One way ANOVA is used for three or more groups of data, to gain information about the relationship between the dependent and independent variables. Tukey post-hoc analysis was used. Tukey's test compares means of all treatments to the mean of every other treatment and is considered the best available method in cases when confidence intervals are desired or if sample sizes are unequal https://en.wikipedia.org/wiki/Tukey's_range_test#Advantages_and_disadvantages.

CHAPTER FOUR

4.0 Results

4.1 Total Viable Counts

The mean ± standard deviation of total viable counts of isolates from the two soil samples in cfu/ml are shown in Table 1. Ita-elepa has higher viable bacterial counts of cfu/ml than Sango

Table 1: Total Viable Bacterial Count

SN	Samples	Bacterial Count (Cfu/ml × 10^4)
1	E1	1.3 ± 0.07
2	E2	9.4 ± 0.21
3	E3	9.2 ± 0.21
4	S1	9.7 ± 0.07
5	S2	2.1 ± 0.14
6	S3	1.1 ± 0.21

Key: S1-S3 are point from Sango site, E1-E3 are point from Ita-elepa site, Data are means of two replicates each ± Standard deviation. The mean difference is significant at the 0.05 level.

4.2 Growth of Isolates on Enriched Media

The growth of the organisms on metal enriched media is presented in Table 2. Zinc and manganese supported the growth of bacteria, while lead, copper, cadmium and mixtures of all the metal in a medium does not support the growth of the bacteria.

Table 2: Growth of Bacterial Isolate on Enriched Media

SN	Isolated organisms	Lead	Manganese	Cadmium	Nickel	Copper	Zinc	Mixtures
1	*Pseudomonas* spp.	-	+	-	-	-	+	-
2	*Bacillus* spp	-	+	-	-	-	+	-
3	*Micrococcus* spp	-	+	-	-	-	+	-
4	*Escherichia* spp	-	+	-	-	-	+	-
5	*Enterobacter* spp	-	+	-	-	-	+	-
6	Total	00	5	00	00	00	5	00

Key: + means growth, - means no growth

4.3. Colonial Morphology, Motility and Gram's Reaction of Bacteria Isolates from Soil Samples

Table 3: Colonial Morphology, Motility and Gram's Reaction of Bacteria Isolates from Soil Samples

S/N	Isolates	Cell form	Colony Margin	Elevation	Opacity	Texture	Pigmentation	Motility	Gram's reaction
1	*Pseudomonas* spp	Circular	Entire	Raised	Opaque	Mucoid	Green	-	- Rod
2	*Bacillus* spp	Irregular	Lobate	Flat	Opaque	Smooth	Cream	+	+ Rod
3	*Micrococcus* spp	Rhizoid	Lobate	Flat	Opaque	Smooth	Cream	-	+ Cocci
4	*Escherichia* spp	Circular	Entire	Raised	Opaque	Mucoid	Pink	+	- Rod
5	*Enterobacter* spp	Circular	Undulate	Flat	Opaque	Smooth	Yellow	+	- Rod
6	*Pseudomonas* spp	Circular	Entire	Flat	Opaque	Mucoid	Green	+	- Rod

4.4 Biochemical Characteristics of Isolates

This was carried out to identify the probable organisms isolated from the two soil samples

Table 4: Biochemical Characteristics of Isolates

Isolates	Citrate	Nitrate reduction	Methyl red	Catalase	Coagulase	Indole	Lactose fermentation	Hydrogen sulphide production	Oxidase	Probable Organisms
1	+	+	+	+	-	+	-	-	+	***Pseudomonas* spp.**
2	-	-	-	+	-	-	+	+	+	***Miccrococcus* spp.**
3	+	-	-	+	+	-	+	-	-	***Enterobacter* spp.**
4	+	+	-	+	-	-	+	+	+	***Bacillus* spp.**
5	+	+	+	+	-	+	-	-	+	***Pseudomonas* spp.**
6	-	+	+	+	-	+	-	-	+	***Escherichia* spp**

Key: - means negative, + means positive

4.5 Molecular Identification

The bacterial isolates identity were confirmed through gene sequencing. The phylogenetic trees were also constructed Figures 1-5. The organisms confirmed were *Pseudomonas* spp*, Lysinibacillus sphaericus*, *Lecleria* spp, *Enterobacter mori*, *Morganella morganii*

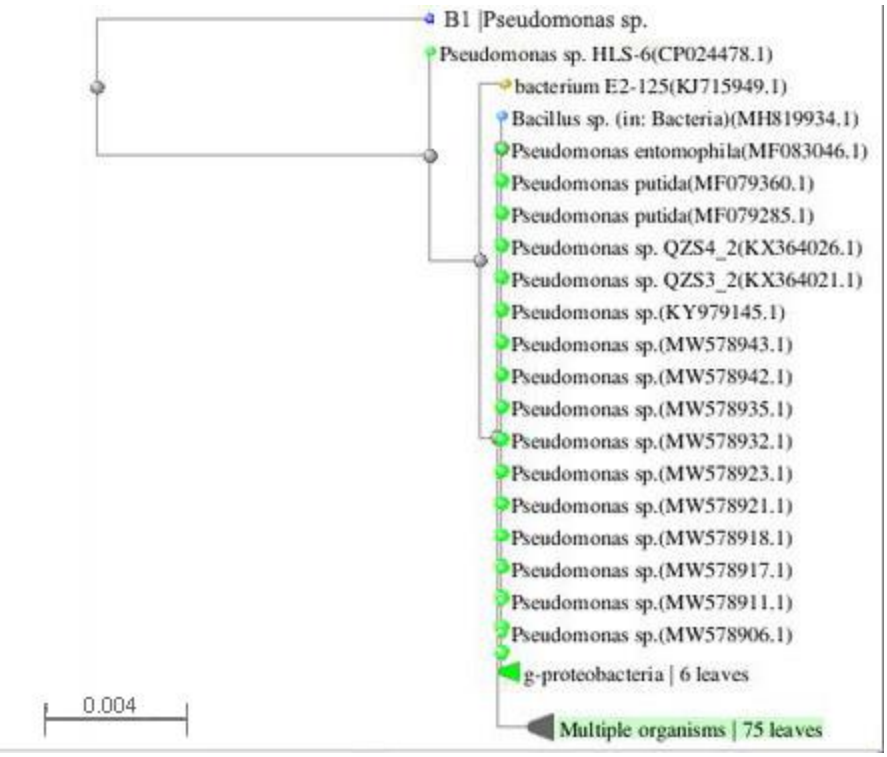

Figure 1: Phylogenetic tree of B1 (*Pseudomonas* spp.)

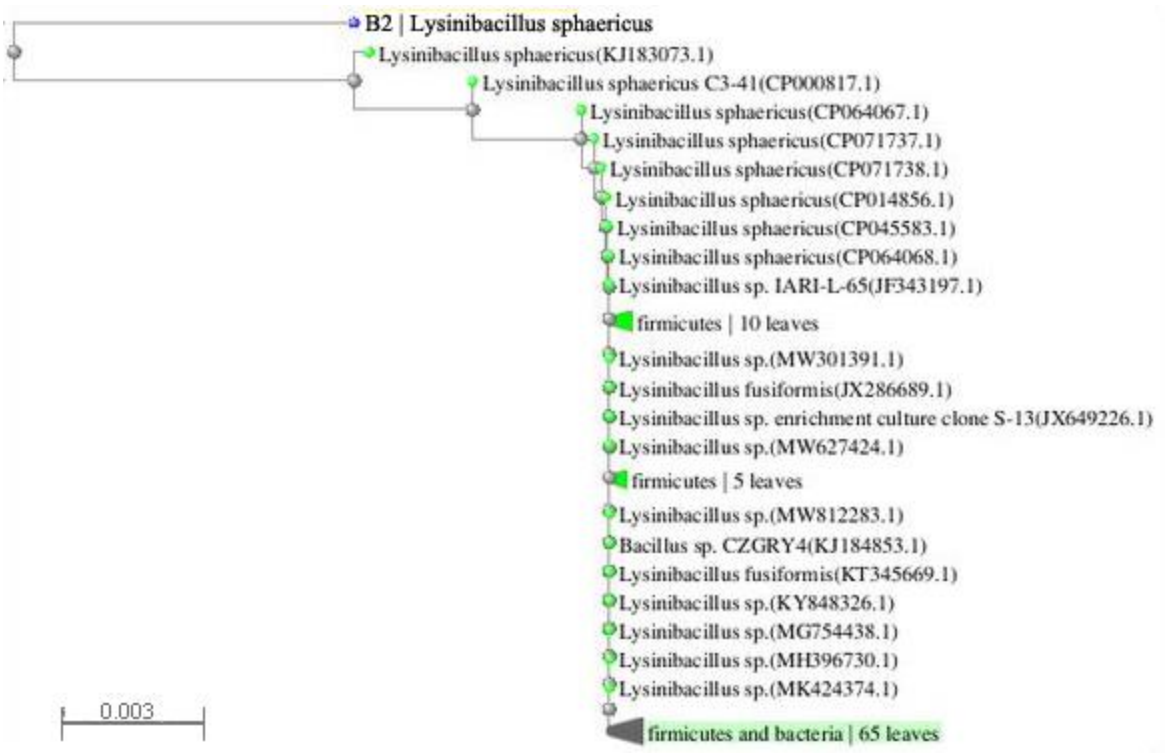

Figure 2: Phylogenetic tree of B2 (*Lysinibacillus sphaericus*)

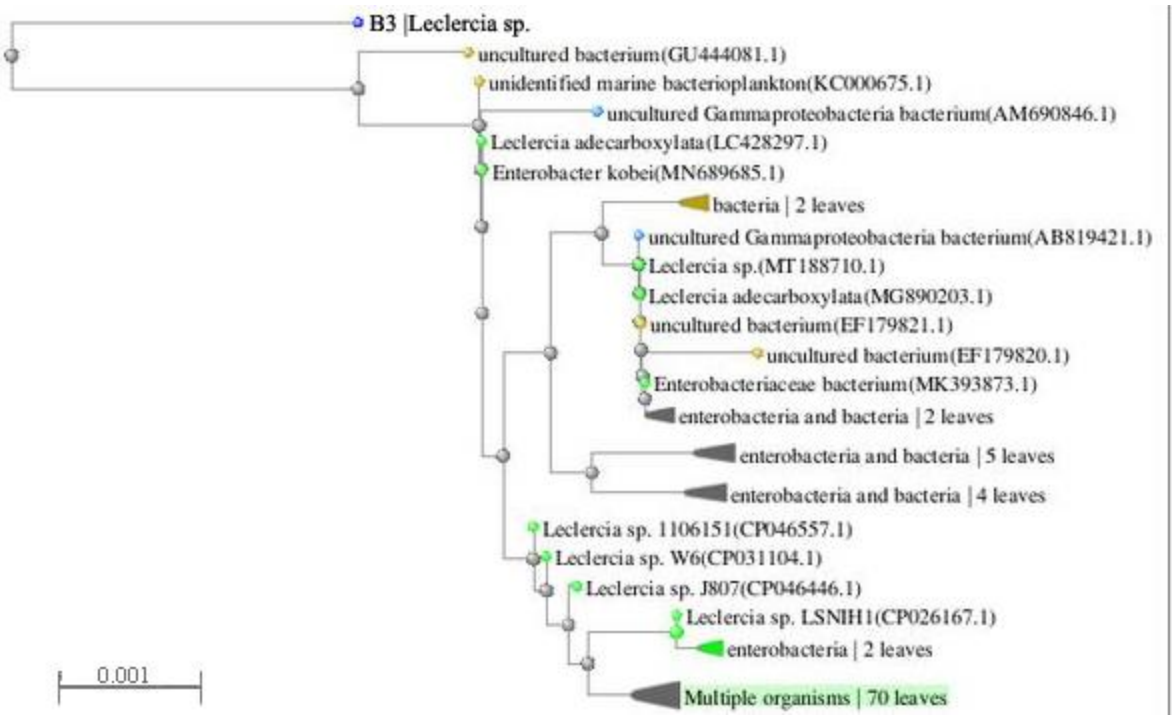

Figure 3: Phylogenetic tree of B3 (*Lecleria* spp.)

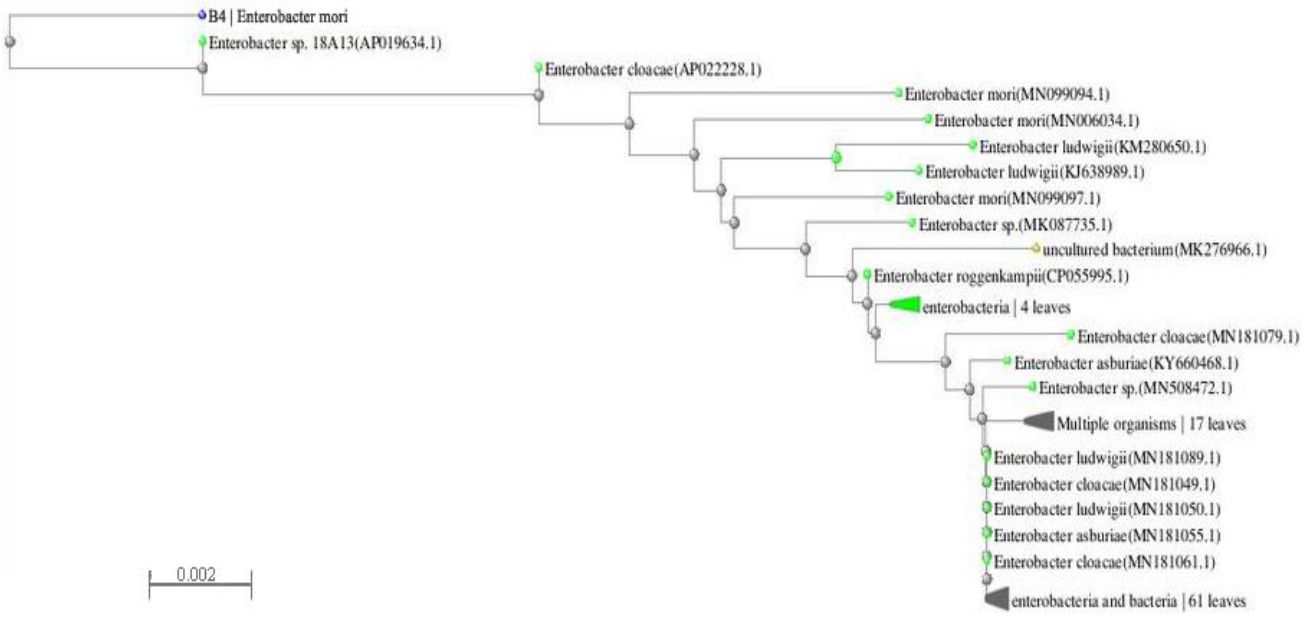

Figure 4: Phylogenetic tree of B4 (*Enterobacter mori)*

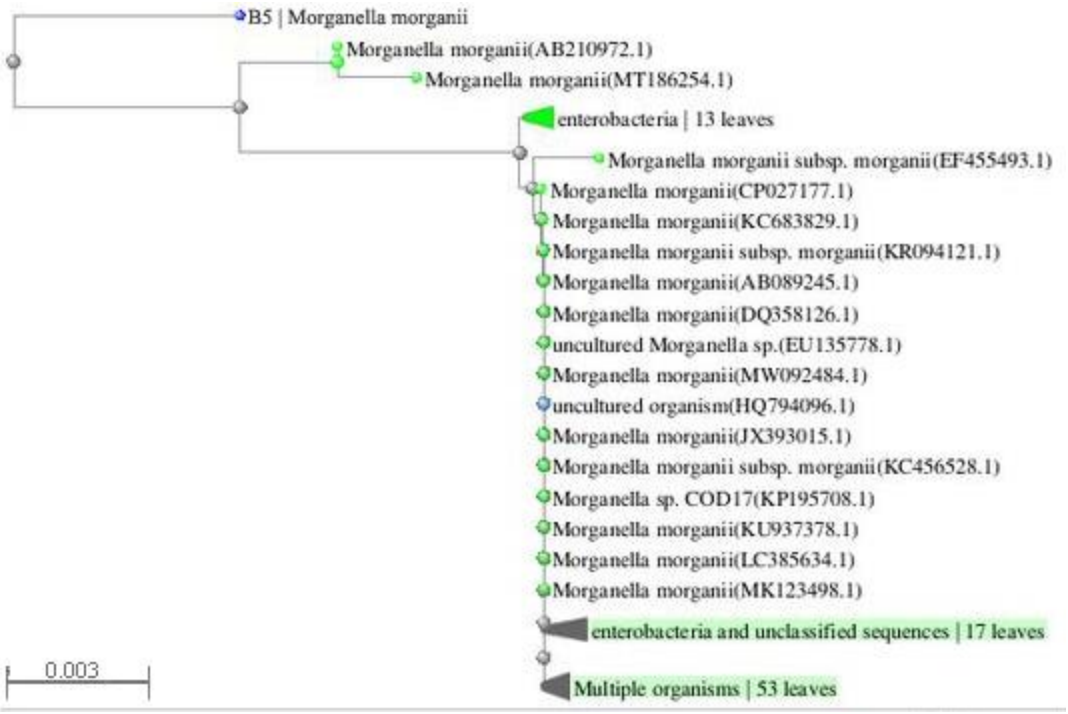

Figure 5: Phylogenetic tree of B5 (*Morganella morganii)*

4.6 Antibiotic Susceptibility of Isolates

The isolates were mostly susceptibility to ofloxacin and gentamicin. They are broad spectrum antibiotics. *E. coli* was the most sensitive organism among the isolates tested with the antibiotics.

Table 5: Antibiotic Susceptibility of Isolates

	Antibiotics										
	Zones of Inhibition (mm)										
	OFL	**GEN**	**ERY**	**CPR**	**CRX**	**CAZ**	**NIT**	**AMP**	**AUG**	**CXC**	**CTR**
Pseudomonas spp	**25.0**	**14.0**	**NA**	**0.0**	**0.0**	**0.0**	**0.0**	**16.0**	**0.0**	**NA**	**NA**
Bacillus spp.	**26.0**	**17.0**	**0.0**	**NA**	**0.0**	**0.0**	**NA**	**NA**	**0.0**	**0.0**	**0.0**
Micrococcus spp.	**24.0**	**16.0**	**0.0**	**NA**	**0.0**	**0.0**	**NA**	**NA**	**0.0**	**0.0**	**0.0**
Escherichia spp	**26.0**	**17.0**	**NA**	**13.0**	**0.0**	**0.0**	**22.0**	**20.0**	**0.0**	**NA**	**NA**
Enterobacter spp.	**25.0**	**20.0**	**NA**	**18.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**NA**	**NA**
Pseudomonas spp.	**23.0**	**19.0**	**NA**	**0.0**	**0.0**	**0.0**	**20.0**	**16.0**	**0.0**	**NA**	**NA**

Key: ^{CTR}: Ceftriaxone 30 µg, ^{NIT}: Nitrofurantoin 300 µg, ^{CRX}: Cefuroxime 30 µg, ^{AMP}: Ampicillin 5 µg, ^{OFL}: Ofloxacin 5 µg, ^{CPR}: Ciprofloxacin 5 µg, ^{GEN}: Gentamicin 10 µg, ^{CAZ}: Ceftaxidime 30 µg, ^{AUG}: Amoxycillin/Clavulanate 30 µg, ^{CXC}: Cloxacillin 5 µg, ^{ERY}: Erythromycin 30 µg. ^{NA}: Not Applicable to the organisms

4.6 Physicochemical Characteristics of Soil Samples

The physicochemical characteristics of the soil samples are Temperature, pH, moisture content, organic carbon and organic matter of both samples.

Table 6: Physicochemical Characteristics of Soil Samples

SN	Samples	Temp (^{O}C)	pH	Moisture content	Organic carbon (%)	Organic matter (%)
1	E1	28.1 ± 0.4	6.0 ± 0.07	6.3 ± 0.07	2.9 ± 0.07	5.3 ± 0.00
2	E2	28.0 ± 0.7	6.3 ± 0.07	6.1 ± 0.07	3.0 ± 0.07	5.9 ± 0.07
3	E3	27.9 ± 0.7	6.3 ± 0.00	6.1 ± 0.07	2.9 ± 0.07	5.8 ± 0.07
4	S1	35.0 ± 0.7	6.4 ± 0.07	10.0 ± 0.70	2.4 ± 0.07	6.4 ± 0.00
5	S2	35.2 ± 0.5	6.5 ± 0.07	10.1 ± 0.07	2.6 ± 0.07	6.2 ± 0.07
6	S3	35.5 ± 0.0	6.5 ± 0.07	10.4 ± 0.70	2.5 ± 0.07	6.2 ± 0.07

Key: S1-S3 are point from Sango site, E1-E3 are point from Ita-elepa site, Data are means of replicates ± Standard deviation. The mean difference is significant at the 0.05 level.

4.7 Metals Detected in the Soil Samples

Metals detected from the two soil samples were Zinc, lead, cadmium, manganese and copper ($mgkg^{-1}$)

Table 7: Metals Detected in the Soil Samples

	Soil Samples	Metals ($mgkg^{-1}$)					
SN		Zn	Pb	Mn	Cd	Ni	Cu
1	E1	521 ± 0.00	116 ± 0.70	431 ± 0.70	1.1± 0.00	0.00	59.9 ± 0.70
2	E2	523 ± 0.07	113 ± 0.00	436 ± 0.70	1.0 ± 0.07	0.00	58.5 ± 0.70
3	E3	521 ± 0.70	113 ± 0.70	430 ± 0.70	1.2 ± 0.00	0.00	56.0 ± 0.07
4	S1	640 ± 0.70	112 ± 0.70	443 ± 0.70	1.6 ± 0.07	0.00	73.5 ± 0.70
5	S2	640 ± 0.70	111 ± 0.70	441 ± 0.70	1.4 ± 0.07	0.00	74.5 ± 0.70
6	S3	638 ± 0.00	112 ± 0.00	440 ± 0.70	1.5 ± 0.07	0.00	74.0 ± 0.00

Key: S1-S3 are point from Sango site, E1-E3 are point from Ita-elepa site, Data are means of replicates ± Standard deviation. The mean difference is significant at the 0.05 level.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of metals on Bacteria Isolates

No MIC at the highest concentration used.

CHAPTER FIVE

5.0 Discussion, Conclusion and Recommendation

5.1 Discussion

Soil is an important component of terrestrial ecosystem. It is essential because, it is the top layer of the earth where plants grow. This makes it play a vital role in food chain. Heavy metal pollution in soil and the environment generally have been accelerated in modern and developing society due to industrialization, intensified agriculture, increase in pesticides manipulation, and poor controlling system to manage these wastes (Su *et al*., 2014; Abdel-lateif, 2017).

The two sampling sites were observed to have heavy microbial load but, samples from Ita-Elepa (E1-E3) recorded higher microbial counts than samples from Sango (S1-S3). This could probably be due to the fact that Sango dump site has been in use for a longer period of time as discovered during this research, and high concentrations of heavy metals may have inhibitory or even toxic effects on living organisms (Bruins *et al*., 2000).

Bacteria isolated based on biochemical characterization were *Pseudomonas* spp, *Micrococcus* spp, *Bacillus* spp, *Escherichia* spp and *Enterobacter* spp. Agostinho *et al*. (2012) in their work isolated similar bacteria from sewage. Also, Ramya and Boominathan (2017) reported the isolation of *Bacillus* and *Pseudomona*s spp. from industrial effluent. Similarly, Rajbanshi (2008) isolated *Escherichia coli, Bacillus and Pseudomonas* spp. from oxidation ditch of wastewater treatment plant. The occurrence of *Bacillus* and *Pseudomonas* spp. at this dumping sites could due to the fact that both organisms are heavy metals tolerant ((Piotrowska-Seget *et al*., 2005). *Pseudomonas aeruginosa* could metabolize heavy metal complex due to resistant genes, the genes which could be chromosomal or plasmid borne (Salah *et al*., 2019). *Micrococcus* spp is

another organism reported to possess the ability to transform heavy metals using a plasmid gene (Lolo *et al*. 2017). Moreover, *Bacillus, Micrococcus* and *Pseudomonas* spp have been reported to be able to transform heavy metals through bioaccumulation (Spain and Alm, 2003; Umrania, 2006; Rani and Goel, 2009; Ahemad and Malik, 2011). *Pseudomonas* spp. was found to occur mostly at these sites. This finding negates the findings of Ellis *et al*. (2003) who concluded that *Bacillus* spp. had a higher relative abundance in the most heavy metal–contaminated soil.

The findings in this research also observed that there are more Gram negative bacteria than Gram positive bacteria. This observation contradicted the work of Piotrowksa-Seget *et al*. (2005) and Robinson and Olubukola (2017) who concluded that most metal-tolerant strains were Gram-positive. However, the authors also submitted that Gram-negative organisms could also display tolerance to heavy metals. Several authors have also observed that Gram negative bacteria could be more tolerant to heavy metals than Gram positive bacteria (Madigan *et al*. 2003; Bennisse *et al*. 2004). The cell wall in Gram-negative bacteria is much more effective barrier against toxic metals (Benyehuda *et al*., 2003).

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, **1993**). The tree with the highest log likelihood (-4577.05) was showed. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 6 nucleotide sequences. Codon positions included were $1^{st} + 2^{nd} + 3^{rd}$ + Noncoding. There were a total of 1553 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018).

The findings showed susceptibility of the bacterial isolated to different antibiotics. Isolated organisms were resistance to most of the tested antibiotics. The present research showed that *Micrococcus* spp. were resistance to most of the tested antibiotics, but was only sensitive to gentamicin and ofloxacin. This differ from the findings of Sulaimon *et al*. (2015) who reported that a strain of *Micrococcus luteus* had lowest resistance ability to drugs. In like manner, it was observed that *Bacillus* spp. was sensitive to gentamicin and ofloxacin and resistance to other antibiotics tested. This observation was similar to the finding of Fazlani *et al*. (2011). *Pseudomonas* spp. isolated was found to be sensitive to four of the antibiotics (ofloxacin, gentamicin, nitrofurantoin and ampicillin). Similar observation has also been reported (Rind and Shaikh, 2001; Fazlani *et al*. 2011). *Escherichia* spp isolated display the highest sensitivity to tested antibiotics (ofloxacin, gentamicin, ciprofloxacin, nitrofurantoin and ampicillin) and it the most sensitive organisms to the tested antibiotics, which was similar to the observation of Fazlani *et al*. (2011).

The findings this study showed the physicochemical parameters and metal detected from the two sites. According to WHO/FAO (2001), permissible limit of Zn is 300.00 mg/kg for soils. But the recorded zinc level in this study exceeded the permissible limit. The presence of zinc in soil at the various sites could be attributed to the occurrence of dry cells in the municipal waste (Thorpe and Harrison, 2008) and the burning of e-waste materials. High concentration of Zn can pose health threats to humans. Also, the permissible limits of lead is 50.00 mg/kg for soils. The mean lead concentrations of the two sites exceeded the permissible limits. Ashtray (2008) reported that lead causes both acute and chronic poisoning and thus, poses adverse effects on kidney, liver, vascular and immune system. The mean concentration of manganese in the present study exceeded

that reported by Smith *et al*. (1995) and Eze *et al*. (2018). It also exceeded the concentration reported by Gryoschko *et al*. (2004) for soils within abattoirs farm sites in Rivers State, Nigeria. The Mn concentration however, exceeded the allowable limits recommended for agricultural soils (200 mg/kg) (European Union, 2006; Mohammed and Folorunsho, 2015) but it does not exceed the 500 mg/kg (SEPA, 2005). Manganese is an essential metal for both plants and animals but, higher accumulation can have adverse effects on the central nervous system of humans (Farooq and Ishretullah, 2000).

The mean permissible limits of cadmium by WHO/FAO (2001) was 3 mg/kg for soils. Cadmium of both sites does not exceed the permissible limits of 2001 but, exceeded the limit allowed in 2007. Neclam *et al*. (2014) investigated heavy metal concentrations of soils from irrigated farmland in Makera Area of Kaduna State, Nigeria and reported relatively higher concentrations of Cd (3.05 to 8.02 mg/kg). Levels of Cd in this study were higher than the 0.2 mg/kg (SEPA, 2005; Deepak *et al*., 2013) and lower than 3 mg/kg allowed in agricultural soil (EU, 2006). Cadmium is very much connected with non-residual fractions of heavy metals and thus, makes them mobile and potentially bio-available for uptake by plants (Zhuang *et al*., 2009). Jabeen *et al*. (2010) and Maobe *et al*. (2012) reported that cadmium causes both acute and chronic poisoning, has adverse effect on kidney, liver, vascular and the immune system. Cadmium has no essential biological function and is highly toxic to plants and animals (Farooq and Ishretullah, 2000). Mean concentration of Cu was higher than the levels reported by Umar and Ebong (2013) for soil from farms along Jabi Lake, FCT, Abuja, Nigeria. FAO/WHO (2001) permissible limit of copper is 3.0 mg/kg. Both sites exceeded the permissible limits. According to Maobe *et al*. (2012) high levels of copper can cause metal fumes fever with flu-like symptoms, hair and skin decolouration, dermatitis, irritation of the upper respiratory tract, metallic taste in the mouth and

nausea. Copper (Cu) is one of the most essential elements for plants and animals. From this research all the metal analysed exceeded the permissible limits of WHO/FAO (2001). Only cadmium was less than the permissible limits of WHO/FAO (2001) but, exceeded the permissible limits of WHO/FAO (2007).

Bacteria were able to metabolize zinc and manganese at different concentrations because zinc and manganese are essential for metabolism and regulation of osmotic pressure and both are trace metals, as trace metals serve as micronutrients (Kumar *et al*. 2010). The isolated bacteria however, could not utilize the other heavy metals for growth. This could lead to accumulation of this metals in soil. The metals could invariably leach into underground water and rivers. Such water when used for irrigation could have adverse effects on man, animals and plants. Some of the metals may possess carcinogenic properties (Musilova *et al*., 2016). Heavy metal accumulation in soil is also dangerous to indigenous microbial fauna, some of the organisms that could not metabolize the heavy metals go into extinct (D'Amore *et al*., 2005), while some ended up accumulating the metals in their cell. The fact that most of the isolates were resistant to more than two of the tested antibiotics is of important health concern. The consumption of such organisms in food or water could lead to multidrug resistant infections. Such in the community could lead to community acquired resistance to antibiotics commonly used. Thus, leading to difficulty in and increase in cost of treatment (Lerner *et al*., 2015).

On the other hand, the ability of isolates to tolerate zinc and manganese up till 500 mg/ml suggested that they could metabolize metal and therefore, could be used in breaking down zinc and manganese complexes in the soil. The organisms may also be effective in remediating zinc and manganese contaminated soil.

5.2. Conclusion

Soils have been the major sinks for heavy metals released into the environment by many anthropogenic activities. Most heavy metals do not undergo microbial or chemical degradation because they are non-degradable, and their concentrations last for a long time after being released to the environment. Bacteria isolated from this study have high resistance ability to zinc and manganese, so this organisms could be used for bioremediation of zinc and manganese contaminated environment.

5.3. Recommendation

State and Local government should make law against the discharge of metals into the environment and they should encourage the use of microorganisms for the remediation of contaminated environment rather than using chemicals that can pose another problems to the environment.

5.4 Contribution of the Study to Knowledge

Isolated organisms should be tested on the concentration of zinc and manganese higher than 500mg/mL used in this research for the MIC.

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