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Heavy Metals Tolerant Bacteria Detection from Selected Scrap Metal Dump Site: A Review

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Abstract

This study aimed to detect heavy-tolerant bacteria from selected scrap metal dump sites. Heavy metals are the major setbacks to many forms of life and their presence in the ecosystem rapid increase of heavy metal contamination is due to anthropogenic activities, rampant scrap metal waste disposal, and other industrial wastes. Bacteria were found to be among the many microorganisms that can tolerate many heavy metals and can as well reduce their toxicity or even convert them to useful resources. This study aims to detect the heavy metal concentration and the bacterial species capable of tolerating the identified heavy metals from selected metal dump sites. The heavy metals content of the soil samples was analyzed using Atomic Absorption Spectrophotometry (AAS). Standard methods of enrichment culture and colony count were used to isolate a total of 12 bacterial species. Using 16S rRNA gene sequence-based molecular systematics, the 12 isolates were identified and grouped into one genus (*Bacillus*). It was observed from the results that the heavy metals (Pb, Zn, Cu, Cd, and Cr) concentrations found to be high above the WHO permissible limits (Copper-2.0, Zinc-3.0, Lead-0.4, Chromium-0.05, and Cadmium-0.03). Therefore, the bacterial isolates capable of surviving at such levels of heavy metals could have a potential application in the bioremediation and bioleaching of heavy metal contaminants.

Keywords: Heavy Metals, Bacteria, Anthropogenic, Scrap Metal Dumpsite

Introduction

Heavy metals are non-biodegradable and are, therefore, considered as a key to an environmental contaminant related to potential toxicity. Some heavy metals such as Nickel, Iron, Copper, and Zinc are essential to metabolic reactions and are required as trace elements by the organisms. Other heavy metals such as Mercury, Silver, and Cadmium have no biological role in organisms, and they are detrimental even at very low concentrations (Nwagwu *et al.,* 2017). Heavy-metal pollution of the environment has dramatically increased in recent years due to various human activities, such as agriculture, mining, and various other industrial processes (Fashola *et al.,* 2016). Urban soils have been affected by elevated levels of heavy metals since the beginning of industrialization, these heavy metals pose a considerable threat to the environment (Mustapha & Halimoon, 2015). Contamination due to heavy metals is a major environmental problem because of their toxicity. Extremely high levels of pollutants in the soil can be found in many industrial sites and waste disposable dumps, which results in accidental spillage of highly concentrated pollutants like heavy metals, thus, metals are deposited into the soil matrix and once incorporated into the soil, they remain for a very long period (Aliyu Haliru, 2014). The total concentration of heavy metals in soil is widely used to assess soil pollution and constitutes a major hazard to human health and the ecosystem. Heavy metals are recognized to be powerful inhibitors of biodegradation activities.

Generally, scrap metal dumping has greatly affected the microbial biota, vegetation, and human well-being due to the toxic effect of some heavy metals in the scraps dumped. Scrap metals are the most valuable substances among informal urban street collectors. Huge amounts of electrical tools and e-waste appliances rich in metals can be managed by people who sell these scrap materials to recycling companies. A scrap metal dump site is a site used to dispose of recyclable materials left over from product manufacturing and consumption, such as parts of vehicles, building supplies, and surplus materials. Unlike other solid waste, scrap metal has monetary value especially recovered metals, and non-metallic materials are also recovered for recycling processes (Haladu M. *et al.,* 2024).

Heavy Metals

The presence of heavy metals is a great environmental concern, and this is due to their high adverse effect on all forms of life. However, some of these elements are essential for living organisms at low concentrations (Akpoveta *et al.,* 2011). These elements are usually transition metals. They have high densities (>5 g cm^{-3}) when compared with other materials (Baker & Banfield, 2003).

Heavy metals harm human physiology and other biological systems (Bailey *et al.,* 1999). They show a great affinity for other elements such as sulfur, disrupting enzyme functions in living cells by forming bonds with this group. Cadmium, lead, and mercury ions can bind to cell membranes, interfering with the cell transport processes (Manahan, 2017). Heavy metals may also cause the formation of free radicals and reactive oxygen species, which may lead cells into oxidative stress (Kehrer *et al.,* 2015). They are non-biodegradable and tend to accumulate in the tissues of living organisms, and this is a process called bioconcentration (Kobya *et al.,* 2005).

Sources and Toxicity of Heavy Metals

The origins of heavy metals fluctuate amongst nations according to how their products are used, both now and in the past. Numerous investigations revealed that industrial, agricultural, and human activities, as well as historical use, are the main sources of heavy metals (Genchi *et al.,* 2020).

Chromium: Chromium (Cr) is popularly known as a metal with two roles, it can either be beneficial or harmful to humans and animals depending on its oxidation state and concentration. Chromium, which is produced by different industries, occurs in various oxidation states but Cr (III) and Cr (VI) are the most important (Tiago *et al.,* 2005). Cr (III) is deemed to be a trace element essential for the well-functioning of living organisms (Aguilar-López *et al.,* 2013). It is widely used in industries such as electroplating, paint and pigment manufacturing, textile, fertilizer, and leather tanning (Sultana *et al.,* 2020). These industries discharge trivalent and hexavalent Chromium with waste effluent to the soil and surface water. Chromium (hexavalent) is highly toxic, mutagenic, and carcinogenic (Lee *et al.,* 2001). However, Cr (VI) is highly mobile in many environments, solely due to its soluble nature. In spite of the fact that heavy metals are acutely toxic to most microbes, there are metal-tolerant bacteria. In terms of a balanced diet, at lower concentrations, Cr (III) is an essential component of a balanced human and animal diet for preventing adverse effects in the metabolism of glucose and lipids, e.g., impaired glucose tolerance, increased fasting insulin, increased cholesterol and triglycerides, and hypoglycemic symptoms (Zayed & Terry, 2003). Cr (III) at high concentrations can interfere with many metabolic processes because of its high ability to coordinate different organic compounds resulting in inhibition of some metal-enzyme systems. Hexavalent chromium is reported to possess mutagenic and carcinogenic properties that have deleterious effects on mammals including human beings (Sadeesh *et al.,* 2021). Chromium causes bladder cancer, allergic dermatitis, and nasopharyngeal cancer.

Lead: Lead (Pb) is non-essential biologically. This metal is a great pollutant that is found in soil, water, and air as a waste of great hazard. Its toxicity affects humans, animals, plants, and microbes highly (Wani *et al.,* 2015). The main sources of Lead are petroleum, scrap metals dump sites, electronic industries, batteries, paint, stained glass, biocide preparation, etc. The use of leaded gasoline in cars is one of the major sources of Pb pollution in cities around the globe (Ekong *et al.,* 2007). According to an estimate made, road traffic is highly responsible for over a thousand tons of Pb each year, due to lead additives in petrol (M. Kumar *et al.,* 2021). Soils, plants, and food are major receptacles for these atmospheric emissions. Only 3% of Pb in soil is translocated via the root to the shoot of plants while the remaining are through foliage (Sharma *et al.,* 2000). Research on foods exposed to highways like smoked fish shown a relationship between the vehicular traffic volume and the fish Pb levels. This is a potential threat to the health, security, and safety of such food items (Masindi *et al.,* 2021). Lead is one of the deleterious heavy metals found in the environment. Lead affects the systems of the human body (Tiwari *et al.,* 2013). On low doses, Lead can cause developmental disorders in fetuses, infants, and young children, brain damage, respiratory problems, intoxication of the central nervous system, and male infertility WHO, 1996, (M. Kumar *et al.,* 2021).

Cadmium: Cadmium (Cd) is a member of group II-B of the periodic table and is a comparatively rare metal and this makes it uncommon in most "natural" soils and waters (Aguilar-López *et al.,* 2013). The average content of Cd in soil is less than 1 ppm, with the normal range of 0.005-0.02 ppm in plants. It is very similar to Zinc, undergoing joint geochemical processes, and its oxidation state is (like Zinc) +2 (Manahan, 2017). Cd is rendered as a by-product of the mining and smelting of Lead and Zinc (Sultana *et al.,* 2020).

The production of this element has grown rapidly in the last few decades from 11000t in 1960 to 19000 t in 1985 (Hussaini *et al.,* 2021). This heavy metal is used in semiconductors, nickel-cadmium batteries, electroplating, PVC manufacturing, various alloys, pigments, and control rods for nuclear reactors. Soil and water contamination by Cd is initiated by the mining and smelting industries, atmospheric pollution, and sewage sludge application, rampant scrap metals dumping, and burning of fossil fuels. Cd has no essential biological function and is thus highly toxic to living organisms. Chronic exposure to cadmium in humans has many toxic effects, such as high blood pressure, and kidney, lung, liver, and testes damage. Cd is also associated with a disease called Itai-Itai, meaning "it hurts" in Japanese, and it is characterized by bone pain, pathological fractures, and signs of renal impairment (Sinha & Paul, 2014).

Effects of Heavy Metals on Bacteria

Microorganisms are normally the first biota to be affected by heavy metal contamination. Bacterial habitats are the most affected by high heavy metal concentrations as compared to fungal communities (Naguib *et al.,* 2019). The significant or harmful effect of heavy metal on the cell microorganisms is a function of its concentration and the state in which it exists in the environment. The essential metals help in building the structure of an organism or assist in metabolic functions as a component of enzymes. The Presence of heavy metals like Zn, Cu, Pb, Ni, and Fe at low concentrations is important for several microbial activities, they help in the metabolism and redox processes. Exposure to high heavy metal concentrations results in selection pressure on the microbial community leading to the establishment of heavy metal-resistant microbial populations with low diversity when related to a clean environment. The community profile is affected by reducing the number, biomass, alteration of morphological structure, and loss of activity in microbial-assisted soil processes such as nitrification, denitrification, and decomposition of organic matter. The decrease in diversity can also occur in soil erosion as a result of reduced soil aggregation and bad soil structure. Heavy metals are also coupled with the microbe's life cycle and cause a decrease in the pigmentation of microbial cells (Piotrowska-Długosz, 2016). (Fashola *et al.,* 2016), studied the effect of three heavy metals (Zn, Cd, and Pb) on colony forming unit (CFU), enzymatic activities, and microbial biomass carbon: oxidizable carbon content (C-biomass: Cox ratio) of a soil's microorganisms. They found out that all the measured parameters were significantly affected by the heavy metal concentrations. Reasonable reduction was observed on CFU which was most significant in the sporeforming and oligotrophic bacteria. Major inhibition of C-biomass was observed in these soils and the C-biomass: ox ratio decreased with increasing soil pollution.

Bacteria

Structures of Bacterial surfaces are highly vital to understanding their interactions with the nearby environment, particularly with metals. Bacteria can be Gram-positive or Gram-negative depending on the constituent of the cell wall membrane. Gram-negative cell walls are structurally multilayered with an outer membrane comprising of lipopolysaccharide (e.g. lipopolysaccharide layer [LPS]), phospholipids, and a little peptidoglycan layer. Likewise, Gram-positive cells have as much as 90 % of the cell wall comprising of peptidoglycan in several layers, with little quantity of teichoic acid usually present (Rohde, 2019). These negatively charged structures can interact with metal ions.

Microorganisms have obtained different types of mechanisms for adaptation to the presence of poisonous heavy metals. Traditional methods for the removal of heavy metals are electrochemical treatment, chemical precipitation, reverse osmosis, ion exchange, evaporation, and sorption, but each method has been found to have technical and economic constraints. Removal of metals and their recovery is one of the great concerns in sewage and industrial effluent treatment, which is both in municipal and industrial interest. Heavy metal pollution of soil and wastewater is an important environmental problem. Wastewater from industries and sewage sludge applications have constant toxic effects on humans and the environment (Angaye *et al.,* 2016).

Microbial Diversity

The depth of educational background about prokaryotes remains incomplete and controversial in spite of all the modern technological advances. Due to their abundance and diversity, they obviously play a vital role in biochemical processes such as primary production, organic matter and nutrient cycling in soil and marine environments, nitrogen fixation, and microbial interaction with plants (Berlanga, 2010). The estimated complete number of prokaryotes species on earth is reported to be 10^{30} (Dykhuizen, 1998); (Whitman *et al.,* 1998). Specifically, average cellular densities of 10^{10} cells per gram of soil and 10^{6} cells per milliliter in seawater have been reported. After analyzing these numbers, the conclusion can be made that prokaryotic biomass represents more than half of the total biomass on the earth (Whitman *et al.,* 1998).

Soil Microbial Diversity

From phylogenetic diversity in soil it is reported that one gram of soil contains approximately 6000 species (Øvreås & Torsvik, 1998); (Curtis *et al.,* 2002). Molecular-based studies on soil samples have supported this idea (Lichtfouse *et al.,* 2003). To add to the large reported values of microbial diversity, the diverse metabolic activities of microorganisms give them the ability to inhabit all types of habitats. Bacteria can be found in environments that offer good conditions for life such as soils, rivers, estuaries, oceans, and other organisms (symbiosis, parasitism, mutualism), but also in extreme environments like hydrothermal vents, sea ice, acid mine drainage, saline basins and soda lakes and deserts. (Nealson & Popa, 2005).

When understanding the microbial diversity in a particular microbial community several aspects such as microbial phylogenetic diversity and microbial functional diversity are key points to properly describe a microbial community (Schloss *et al.,* 2016). Culture-dependent techniques only identify a small proportion of the total microorganisms sampled, as these grow at best under the enriched conditions provided (Chien *et al.,* 2008). Since the emergence of modern microbiology microbiologists have based their studies on tools such as microscopy, staining methods, and pure cultures (Brehm-Stecher & Johnson, 2004). However, once bacteria have similar shapes like rods or spheres, it is almost impossible to differentiate microorganisms based on morphology. Other conventional tools such as biochemical properties and metabolic activities are also highly undependable, changing according to environment adaptation, and not providing a sound frame for the classification of microorganisms. Due to the above evidence, molecular-based methods are now considered crucial to characterize microorganisms and microbial communities (Øvreås & Torsvik, 1998).

The developmental speediness of novel molecular techniques has given new ways of studying microorganisms in diverse environments. The first proposed cultivation-independent approaches to study microbial populations were proposed by (Quevedo *et al.,* 2011). This comprised the analysis of the 5S or 16S rRNA gene sequences directly in nucleic acids extracted from environmental samples. The arrival of the Polymerase Chain Reaction (PCR) technology eases the experimental performance of this analysis. More recently, PCR amplification of the 16S rDNA sequences from environmental bacterial DNA, cloning of amplicons, and comparison of the obtained sequences with already known sequences allow their assignment to a given phylogenetic group within a phylogenetic tree. This method is now commonly utilized to determine the phylogenetic diversity in microbial communities. As a consequence of using these new techniques, the number of recognized phyla has largely increased, from 11 phyla in 1987 (Soo *et al.,* 2014) to 53 phyla, 25 of which do not include culturable members (Webster *et al.,* 2020).

Bacteria in Heavy Metal Contaminated Soil and Sediments

In a general sense, heavy metal-contaminated environments constitute a common environmental problem constituting a major hazard for ecosystems and the health of all forms of life with expensive cleanup costs. The rampant dumping of heavy metals and their input by industries and agriculture has led to the release and improper disposal of enormous amounts of heavy metals (Bazrafshan *et al.,* 2015). Heavy metals can be found in soils as free cations, as complexes (e.g. $CdCl^{3-}$, $ZnCl^{+}$) with organic and inorganic ligands, and associated with soil colloids (R.Cole's *et al.,* 2005), they can accumulate in biological systems finding their way into the food web via different mechanisms.

The environmental pressure caused by heavy metals generally reduces the diversity and activity of soil bacterial populations leading to a reduction of the total microbial biomass, a decrease in numbers of specific populations such as rhizobia, and a shift in microbial community structure (Sandaa *et al.,* 2001). Soil microbial population responses to heavy metal contamination give a relevant model for ecological studies to assess the influence of environmental characteristics (Guo *et al.,* 2009). Many studies have shown that metals influence microorganisms by affecting their growth, morphology, and biochemical activity (Peréz-de-Mora *et al.,* 2006) and diversity (Dell'Amico *et al.,* 2005).

Interactions of Microorganisms with Heavy Metals

Low concentrations of some metals such as Zinc, Copper, Cobalt, and Nickel are crucial for the metabolic activity of bacterial cells. Other metals like Pb, Cd, Hg, and Cr have no known effects on cellular activity and are cytotoxic (Tsai *et al.,* 2005); (Abou-shanab *et al.,* 2003). It is obvious that microbial activity plays a greater role in metal speciation and transport in the environment (Spain & Alm, 2003). When present in abundance, heavy metal ions become toxic to cells. Due to the fact that some heavy metals are necessary for enzymatic functions (e.g. Zn) and growth, the cell has various processes for metal uptake, this can be achieved by bioaccumulation or biosorption.

Bioaccumulation is a specific substrate process, driven by ATP (Errasquín & Vázquez, 2003) and is an energetic process of heavy metal uptake. There are three known metal transport mechanisms in the bacterial cell: passive diffusion, facilitated diffusion, and active transport. Some of these systems are metal-selective. On the other hand, there are some exceptions, Cd can be transported by the same transporters as Zn (Poonam *et al.,* 2021). A drawback of bioaccumulation is the recovery of the accumulated metal which has to be done by destructive means leading to damage to the biosorbent structural integrity (Bazrafshan *et al.,* 2015).

Table 1: Heavy metals concentrations from selected scrap metals dump site

		Heavy metals concentration (mg/kg)			References
S/N	pH	Cr	Pb	Cd	
A	6.12	32.62	24.30	10.72	Haladu M. *et al.,* 2024
B	7.39	27.77	36.90	24.83	Eghomwanre *et al.,* 2019
C	7.89	18.72	14.47	1.65	Akpoveta *et al.,* 2010
D	8.52	23.32	429.56	13.45	Bankole *et al.,* 2019

Discussion

Table 1 presents the results of the pH of the soil as well as the soil's heavy metal concentration. The pH of the soil from all the sites ranges from 6.12 to 8.52 indicating both acidity and alkalinity in the soil. The concentration of heavy metals in the soil from the sampled sites is as follows: Cr (18.72 -32.62 mg/kg), Pb (14.47- 429.56mg/kg), Cd (10.72 - 24.83mg/kg). However, these concentrations are not within the WHO permissible limit, and the relatively high contamination of Cadmium, Chromium, and Lead in these locations is due to the activities going on at the sites.

Conclusion

In conclusion, the study shows that many bacterial species were able to tolerate high concentration levels of some heavy metals, and their ability to be good bioremediation agents. However, these metals (Chromium, Cadmium, and Lead) were found to have high concentrations above the WHO permissible limit thus, they have a potential effect on the soil ecosystem.

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