A COMPARATIVE STUDY ON THE BIOREMEDIATION EFFECT OF PHOSPHATE *PSEUDOMONAS SPECIE* AND HYDROXYAPATITE ON HEAVY METAL CONTENT IN A MECHANIC WORKSHOP SOIL IN OSASOGIE, BENIN CITY, NIGERIA.

BY:

OSARETIN BINTA IGBINIJESU (MISS)

MATRICULATION NUMBER:

LSC2006931

AN UNDERGRADUATE DISSERTATION SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY

DATE:

NOVEMBER, 2025

CERTIFICATION

This is to certify that this research titled **"A COMPARATIVE STUDY ON THE BIOREMEDIATION EFFECT OF PHOSPHATE *PSEUDOMONAS SPECIE* AND HYDROXYAPATITE ON HEAVY METAL CONTENT IN A MECHANIC WORKSHOP SOIL IN OSASOGIE, BENIN CITY, NIGERIA"** was carried out by **"OSARETIN BINTA IGBINIJESU (MISS)"** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfilment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of a Bachelor of Science degree in Environmental Management and Toxicology.

________________________ **DR. (MRS) G. E. OGBEIDE** **(PROJECT SUPERVISOR)**	________________________ **DATE**
________________________ **PROF. (MRS) E. T. AISIEN** **(HEAD OF DEPARTMENT)**	________________________ **DATE**

DECLARATION

I **"OSARETIN BINTA IGBINIJESU (MISS)"** declare that **"A COMPARATIVE STUDY ON THE BIOREMEDIATION EFFECT OF PHOSPHATE *PSEUDOMONAS SPECIE* AND HYDROXYAPATITE ON HEAVY METAL CONTENT IN A MECHANIC WORKSHOP SOIL IN OSASOGIE, BENIN CITY, NIGERIA"** is my work and that all sources that I have used or quoted have been acknowledged using complete references and that this work has not been submitted before for any other degree at any other University.

OSARETIN BINTA IGBINIJESU **DATE**

DEDICATION

This report is dedicated to God Almighty, for his guidance and protection during this project. I also want to dedicate this report to my beloved parents Mr and Mrs Igbinijesu for their unwavering support, prayers, love and financial assistance throughout my academic journey.

ACKNOWLEDGEMENT

First and foremost, I will like to thank God Almighty for His divine blessing that made me complete the project successfully.

My gratitude goes to Dr. Mrs G. E. Ogbeide; my project supervisor who made my project work a reality.

Great thanks to my parents Mr and Mrs Igbinijesu who have been my financial and emotional backbone throughout my academic race and also for the advice, words of encouragement and prayers.

I also recognize the impact of my family members, siblings and all my friends Uyi, Elect, Ayomide and all those I can’t remember now for their continuous support for the entirety of the project.

Thank you all for your encouragement.

TABLE OF CONTENTS

CONTENT **PAGE**

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

ABSTRACT

Environmental pollution from heavy metals in mechanic workshop soils poses serious threat to soil fertility, microbial health and groundwater quality. This study evaluated the bioremediation potential of phosphate solubilizing bacteria (Pseudomonas sp.) and hydroxyapatite in reducing heavy metal concentrations in contaminated soil. The study was carried out in a mechanic workshop in Osasogie, Ugbowo, Benin city, Edo state, Nigeria with four treatments: soil only (O1), soil + phosphate solubilizing bacteria (O2), soil + hydroxyapatite (O3) and soil + phosphate solubilizing bacteria + hydroxyapatite (O4). Microbial count and heavy metal concentrations (iron, copper and arsenic) were tested after 14 days. According to results hydroxyapatite was most effective in increasing the growth of *Pseudomonas sp*. and in the remediation of soil contaminated with copper and iron while phosphate solubilizing bacteria was the least effective due to the short period of remediation. In conclusion, *Pseudomonas sp*. and hydroxyapatite showed great promise in cleaning up heavy metal contaminated soil from a mechanic workshop.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

The ever-growing human population has led to a rise in intensive farming and worldwide industrialization, which has contaminated the ecosystem, especially the soil (Alori and Fawole, 2017). According to Sales da Silva *et al*. (2020), soil contaminants alter the physical, chemical, and microbiological properties of soil and have immunotoxin, carcinogenic, and mutagenic consequences. Lead, copper, iron, cadmium, arsenic, and zinc are among the potentially hazardous metals that are introduced into soils by anthropogenic activities such mining, industrial discharges, pesticide application, and waste disposal. According to Alori and Babalola (2018), these soil contaminants have the potential to enter human food and water supplies and cause major problems for soil, human health, and the ecosystem. As a result, recovering contaminated soils becomes more practical. To clean up polluted soils, a variety of techniques have been used, including burning, excavation, and chemical use; however, these procedures are too costly and do not offer a complete solution, as some only move the contamination from one location to another (Alori, 2015). They frequently also lead to the creation of secondary pollutants, which have an adverse effect on the environment (Divya *et al*., 2015). Consequently, a safer and more economical alternative approach is required.

By employing biological processes to detoxify, break down, or change contaminants into a harmless state, bioremediation provides an alternate option. By eliminating pollutants from the environment, bioremediation uses microorganisms to return the ecosystem to its initial state

(Ayangbenro and Babalola, 2017). One easy, affordable and successful method of getting rid of contaminants is bioremediation. Biological remedies have more social acceptance and eco-friendly qualities (Aparicio *et al*., 2022). It doesn't unintentionally harm the local plants, animals, or natural resources. Because of their capacity to stable pollutants, lower mobility and restore soil health, immobilization strategies utilizing soil additives in conjunction with advantageous microorganisms are becoming more and more popular among the different bioremediation approaches.

The efficacy of treating polluted soil using hydroxyapatite (HAP) and phosphate solubilizing bacteria (PSB) separately as well as the combined impact of both is the main focus of this project. PSB in the soil enable the transformation of the soil's insoluble phosphorus into phosphorus that the plant can absorb and use directly, increasing the plant's phosphorus content. By encouraging plant growth and the management of metal uptake, PSB improves soil enzymatic activity, supports microbial diversity, and can indirectly lower metal toxicity (Sharma *et al*., 2013). By binding to heavy metals via a variety of processes, including surface adsorption, complexation, and ion exchange, PSB can efficiently immobilize and remove the metals from the soil solution. The total toxicity of the soil can be decreased by some PSB's ability to metabolize heavy metals, changing them into less harmful forms or even volatilizing them. Because of its excellent adsorption and fixation capabilities, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), a naturally occurring calcium phosphate mineral is helpful in soil remediation, especially for immobilizing heavy metals. Through processes like ion exchange, surface adsorption, and precipitation of stable metal-phosphate complexes, it can lower the availability of metals like lead (Pb), cadmium (Cd), arsenic (As), iron (Fe), zinc (Zn), and copper (Cu) in soil (Chen *et al*., 2007; Misra and Chaturvedi, 2007). In addition to decreasing the bioavailability of harmful metals, hydroxyapatite (HAP) treatment increases the amount of phosphorus available in soils, which may promote plant development. According to research, PSB and HAP may work in concert to improve remediation effectiveness and soil fertility. HAP supplies phosphate that can be released gradually, while PSB speeds up its solubilization.

1.2 AIM

In order to improve the in-situ immobilization of heavy metal pollutants in contaminated soil and support sustainable soil remediation techniques, the project's overall goal is to explore and optimize the individual and synergistic use of HAP and PSB.

1.3 OBJECTIVES

1. To evaluate the contaminated soil's physicochemical characteristics both before and after HAP and PSB treatment.

2. To assess how well heavy metals in polluted soil are immobilized by HAP.

3. To assess PSB's ability to immobilize heavy metals in polluted soil.

4. To look at how HAP and PSB work together to improve the bioavailability and immobilization of heavy metals in polluted soil.

5. To evaluate the treated soils' potential for long-term stability and a decrease in ecotoxicity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. INTRODUCTION TO SOIL CONTAMINATION

Numerous pollutants that are toxic to living things have been released into the environment as a result of urbanization and industrialization. Pollutants from various industrial processes are major sources of soil contamination, and the presence of heavy metals in soil has caused significant public concern both domestically and internationally (Zhao *et al*., 2019). Various types and quantities of heavy metals are released during industrial production processes and as effluents after further industrial production (Methneni *et al*., 2021). For example, fertilizers, pesticides, and herbicides in the agricultural sector generate pollutants that include aluminum, copper, zinc, nickel, lead, and arsenic (Ayilara *et al*., 2020; Prabagar *et al*., 2021). Additionally, crude oil contributes significantly to environmental pollution, especially through pipeline vandalism, transportation leaks, and/or unintentional spills (Ogunlaja *et al*., 2019). According to earlier research, heavy metals can be transferred into the soil through processes including mining and smelting (Obiora *et al*., 2016). Certain compounds that are harmful to the near environment, like lead, arsenic, cadmium, and copper, are discharged during mining. During the mining process, other environmentally hazardous chemicals are utilized, such as sulfuric acid and cyanide, among others (Ayangbenro *et al*., 2018). According to Jafari *et al*. (2019), other industrial wastes, like those generated in the cement industry, also release copper, zinc, and cadmium into the top soils. Furthermore, pollutants from the coal industry include copper, arsenic, mercury, chromium, lead, nickel, cadmium, and zinc (Sun *et al*., 2019). In the meantime, an overabundance of heavy metals in soils endangers human health and ecological security in addition to lowering agricultural productivity, microbial activity and soil quality (Meng *et al*., 2018). Terrestrial environments and their inhabitants are highly poisoned by these heavy metals. Lead causes liver and kidney dysfunction, cardiovascular disorders, and immune and reproductive system malfunctions, whereas mercury, cadmium and lead all affect the human central nervous system, particularly in newborns. Neurotoxic and nephrotoxic complications, skeletal diseases, malignancies and reproductive system malfunction are all brought on by cadmium (Zwolak *et al*., 2019; Fashola *et al*., 2020). Heavy metal-containing wastes are frequently dumped into the earth incorrectly. As a result, these contaminants must be cleaned up using physical, chemical or biological techniques. More economical and ecologically friendly methods, such as bioremediation and integrated remediation, have been developed in recent decades to lessen the negative effects of heavy metal contamination in soil on people and the environment. Because of their in-situ and economic properties, soil amendments have been extensively utilized in the in-situ treatment of heavy metal contaminated soils (Lwin *et al*., 2018).

2.2 INTRODUCTION TO BIOREMEDIATION

Cleaning up, lowering, eliminating or neutralizing pollutants or toxins from the environment in order to return it to a state that is safe for ecosystems, human health or future use is known as remediation. In order to make contaminated areas safe, intentional steps are taken to clean them up. The physical, chemical and biological methods are the three categories of remediation. Booms, sorbent materials and skimmers are used in the physical cleanup process. Before a subsequent cleanup process is conducted, a boom which is a physical barrier composed of materials, collects oil pollutants and stops them from spreading (Vocciante *et al*., 2019). After booms, contaminants are further absorbed and adsorbable using sorbents and skimmers (Kumari *et al*., 2020). Because the bloom remediation technique depends on buoyancy and roll reaction, it presents a significant problem. The boom floats and stays on the water's surface for a longer period of time when it is buoyant. The torque needed to rotate the bloom from its upright position is known as the roll response. In other words, a greater remediation process is the outcome of a higher roll reaction (Dhaka and Chattopadhyay, 2021). To stabilize and eliminate heavy metals from the environment, chemical remediation involves the addition of chemicals like sulfide, phosphate, charcoal, aluminum salts, clay minerals and silicocalcium compounds. Adsorption, reduction, oxidation, complexation, precipitation and ion exchange are the mechanisms underlying the utilization of these compounds (Xu *et al.,* 2022). Although chemical treatment is a quick, easy and straightforward method, the chemicals utilized may also pollute the environment (Xu *et al*., 2022). Although both chemical and physical bioremediation techniques have been employed for many years, they have disadvantages. For example, the chemical bioremediation process requires specialized equipment and a specialist, while the physical bioremediation process is costly. Because of this, a better option, biological remediation, or bioremediation, has become necessary.

One of the most successful, economical and environmentally beneficial technologies for transforming pollutants is bioremediation (Sonune, 2021). Although both plants and microorganisms can be used in biological restoration, microbes are preferred because plants are more difficult to manipulate and require longer to develop (Hussain *et al.,* 2022). The kind, location, and degree of pollution determine whether this approach is feasible (Patel A. K. *et al*., 2022). One of the most advanced methods for dealing with environmental heavy metal remediation is bioremediation technology (Tayang and Songachan, 2021). This method provides a sustainable solution to the issue by using microorganisms, plants or animals to degrade or neutralize toxins. The conversion of heavy metal cations from active to inactive forms, reduction of heavy metal toxicity and ease of removal from contaminated areas are all made possible by these metabolites.

2.2.1 Mechanisms of microbial bioremediation

Through a variety of methods, microbes can eliminate contaminants from the environment. (Ndeddy-Aka and Babalola 2016; Verma and Kuila 2019) divided these mechanisms into two main groups: immobility and mobilization.

2.2.1.1 Mobilization

Enzymatic oxidation, bioleaching, biostimulation, bioaugmentation and enzymatic reduction are all steps in the mobilization process.

2.2.1.1.1 Enzymatic oxidation

By oxidizing harmful molecules from a higher oxidation state to a lower one, a process known as enzymatic oxidation, heavy metals lose an electron and become less hazardous. The bacteria involved release an enzyme called oxidoreductase, which is used in this process. This technique works very well for cleaning up contaminants that are difficult for microorganisms to break down, such as dyes and phenols (Unuofin *et al*., 2019). According to Unuofin *et al*. (2019), oxidative enzymes generate radicals that can be separated into various fractions and ultimately produce molecules with a high molecular weight. Laccase is an example of an oxidoreductase enzyme that catalyzes the aromatic amines.

2.2.1.1.2 Enzymatic reduction

In contrast to enzymatic oxidation, this mechanism transforms the contaminants into a reduced oxidized state that renders them insoluble. The procedure is carried out by facultative and obligatory anaerobes; it works well for bioremediation of substances like dibenzofurans and polychlorinated dibenzo-p-dioxins (Zacharia, 2019). Similarly, azoreductase cleaves the azo bonds to reduce the azo compounds, while chrome reductase catalyzes the reduction of hexavalent chromium to trivalent chromium (Saxena *et al*., 2020). To identify other creatures that can bioremediate environmental contaminants, much more research is required.

2.2.1.1.3 Bioaugmentation

In a method known as bioaugmentation, microorganisms are specifically introduced to contaminated areas so they can consume harmful contaminants. It is an extremely efficient, quick, and economical bioremediation technique (Mahmoud, 2021). To supplement the native microorganisms in contaminated areas, external bacteria are introduced. In some situations, it can also entail separating and genetically altering microorganisms from the polluted area before reintroducing them there for cleanup. The purpose of genetically modifying the bacteria that live in contaminated areas is to improve their capacity to degrade pollutants, as these organisms may not be able to do so naturally. In other instances, non-resident microorganisms are introduced to contaminated sites in order to encourage the breakdown of contaminants. The ability to compete with the resident bacteria and adapt to the new environment are two criteria that determine how effective these new strains are (Fashola *et al*., 2016; Goswami *et al*., 2018). When applied to a polluted site, *Burkholderia sp*. FDS-1 has been shown to break down nitrophenolic compounds found in pesticide-polluted soil to a less harmful form at a temperature of roughly 30° C and a slightly acidic pH (Goswami *et al*., 2018).

2.2.1.1.4 Biostimulation

To boost the activity of the resident microbes and speed up the remediation process, biostimulation involves adding nutrients (like nitrogen, potassium, and phosphorus), metabolites, electron donors, enzymes, electron acceptors, biosurfactants, etc. to the soil (Ojuederie and Babalola, 2017). The procedure is economical, eco-friendly, and effective (Goswami *et al*., 2018). Because native bacteria are more competitive than introduced ones (Sayed *et al*., 2021), the biostimulation approach is better than the bioaugmentation method and contributes to preserving the environment's natural microbial diversity balance. *Bacillus, Rhodococcus, Staphylococcus sp., Klebsiella, Pseudomonas sp.,* and *Citrobacter sp.* were found to be successful in the bioremediation of heavy metals using the biostimulation technique by Nivetha *et al*. 2022. Sadly, despite the potential benefits of this bioremediation technique, the extra nutrients in the ecosystem may cause eutrophication and other environmental problems. Additionally, if the nutrients come from synthetic sources, they may pollute the environment, negating the original goal of bioremediation.

2.2.1.1.5 Bioleaching

The method of using acidophilic microorganisms to encourage the solubilization of solid heavy metals from the sediment matrix is known as bioleaching. For iron or sulfur pollution, the method works very well (Sun *et al*., 2021). Thus, bacteria that oxidize iron or sulfur are mostly sought for this process; *A. thiooxidans, Aspergillus sp., Mucor sp., Penicillium sp., Pseudomonas sp., Cladosporium sp.,* and *Rhizopus sp*. are examples of such organisms (Medfu-Tarekegn *et al.,* 2020). According to Medfu-Tarekegn *et al.* (2020), these bacteria produce an acidic environment that dissolves heavy metals in an aqueous solution when they are immobilized.

2.2.1.2 Immobilization

Bioaccumulation, complexation, biosorption and precipitation (solidification) are all processes that contribute to immobilization (Tak *et al.*, 2012). Microbes aid in the conversion of contaminants into final products like carbon dioxide, water, or other intermediate metabolic chemicals during the mineralization process. The transformation of chemicals into a form that makes them unavailable in the environment is known as immobilization. As an example, nitrate nitrogen is transformed into organic nitrogen (Pratush *et al.*, 2018). The technique is typically applied to heavy metal bioremediation, particularly in extremely polluted areas. Both in-situ and ex-situ techniques can be used to immobilize metals (Pratush *et al.*, 2018). In order to immobilize the metal ions causing the contamination, the ex-situ method entails moving contaminated soils from the pollution site to a different area where they will go through a microbiological process (Ayangbenro and Babalola, 2017). However, the contamination is treated on-site in the in-situ technique. Heavy metals that damage the environment have been shown to be immobilized by microbes including *B. cereus and E. asburiae* (Fashola *et al.,* 2020). Microbes create a hydrophobic or solvent efflux pump to shield the cell's outer membrane from harmful substances during microbial bioremediation (Verma and Kuila, 2019).

2.2.1.2.1 Biosorption

Proton and ion displacement, complexation, chelation and physical contact with electrostatic forces are the methods used to adsorb heavy metals from pollution (Mahmoud, 2021). It entails the elimination of impurities from solutions due to the outer cell shield of bioremediation agents such as bacteria, fungus, and algae. The active groups of the chemicals that are present at the cell surface layer are typically what connect metals. This causes an ion transfer between the negatively charged active group potentials at the exterior of the microorganism's structure and metal cations. It has been shown that *Bacillus anthracis, Pseudomonas sp., Streptomyces sp. K11,* and *Rhodococcus erythropolis* can bioremediate using the biosorption method (Sedlakova-Kadukova *et al.,* 2019). Heavy metal pollution, such as copper, zinc, and gold, frequently have some economic value and are highly beneficial in industrial processes. Therefore, it is a good method since the compounds can be retrieved by a reversible step in biosorption termed desorption (using a weak mineral solution or chelating substances) (Medfu-Tarekegn *et al*., 2020).

2.2.1.2.2 Complexation

According to Ayangbenro and Babalola (2017), complexation is the process of employing a ligand to create a complex with inorganic metals, which are environmental contaminants, particularly solid waste. High molecular weight ligands, siderophores, toxic metal-binding chemicals, and low molecular weight organic acids (alcohols, tricarboxylic acids and citric acids) are the primary agents used in complexation (Pratush *et al*., 2018). Complexation happens when heavy metals that damage the environment interact with extracellular polymeric materials present on microbial surfaces. According to Xiao *et al*. (2019), biochar may remove copper (II) oxide and hexavalent chromium from wastewater through a complexation-based mechanism. According to Wang *et al*., (2019), *B.lichenformisis* and *Rhodobacter blasticus* are among the species implicated in complexation. Microbes create siderophores, which are iron chelators when they are exposed to a contaminated environment with low iron levels. Siderophores contain binding groups including hydroxamate, catecholate and phenolates that combine with heavy metals to form complexes and make them more soluble. The ability of siderophores to generate reactive oxygen species improves their ability to act as organic pollutants' bioremediation agents (Albelda-Berenguer *et al*., 2019). Because they produce siderophores, cyanobacteria have been shown to be effective bioremediation agents. For instance, they can bioremediate complex compounds like polythene and produce a variety of siderophores, such as schizokinen, synechobactin, and anachelin (Arstol and Hohmann-Marriott, 2019; Sarmah and Rout, 2020).

2.2.1.2.3 Bioaccumulation

When a compound's rate of absorption exceeds its rate of loss, this phenomenon is known as bioaccumulation. The intracellular portion of the microorganisms accumulates (toxically) as a result of this process (Sharma *et al*., 2022). Various methods, including ion pumps, protein channels and carrier-mediated transport are used by heavy metals to pass across bacterial membranes. A large number of organisms are highly active in the bioaccumulation of heavy metals, for instance, *Pseudomonas putida* bioremediates cadmium, *Aspergillus niger* bioremediates thorium, *Rhizopus arrhizus* bioremediates mercury, and so on (Sharma *et al.,* 2022).

2.2.1.2.4 Precipitation

This process lowers the toxicity level of heavy metals or contaminants by converting them into precipitates or crystals. Due to enzyme activity and the galactosis of secondary metabolites, this process can take place during the biogeochemical cycling to produce the deposition of metals (iron and manganese), mineralized manganese and silver, and microfossils (Sharma *et al.,* 2022). For example, when the pH is alkaline sulfate-reducing bacteria can change organo-phosphate into ortho-phosphate (Pratush *et al.,* 2018). In a similar vein, *Bacillus subtilis* and

Oceanobacillus indicireducens have also been linked to environmental heavy metal precipitation (Maity *et al*., 2019).

2.3. HYDROXYAPATITE IN SOIL REMEDIATION

The main mineral component of bone and teeth, hydroxyapatite (HAP), has the general formula $Ca_{10}(PO_4)_6(OH)_2$ and is primarily responsible for their strength and hardness. This bio-inspired material's exceptional structure and intrinsic qualities make it highly desirable in a variety of industries. According to a few, hydroxyapatite is highly valued for medical applications, where it can be applied as a biocompatible coating for bioimplant materials that degrade quickly or as a material for bone restoration (Mondal *et al.,* 2016). Additionally, HAP is used as a medication release agent (Son *et al.,* 2011) and protein delivery medium (Fu *et al*., 2013) in the pharmaceutical industry. HAP is also widely utilized in the chemical industry where hydroxyapatite column chromatography provides a potent method for protein and nucleic acid separation. On the other hand, with regard to the field of pollution control, the use of HAP can prove to be very advantageous for the removal of contaminants present in gas, liquid, and solid phases. This is because there are currently increasing efforts being made towards the environmental remediation industry (Condit *et al.,* 2017), with the focus being shifted towards the development of new and improved ways of eliminating air, soil, and water pollution (Fihri *et al*., 2017). Phosphorus containing minerals, such as apatite, potassium dihydrogen phosphate, superphosphate and hydroxyapatite, have been demonstrated in numerous studies to successfully lower the activities of heavy metals, including Pb, Cd, Fe and Cu, in soil and wastewater (Valipour *et al*., 2016). Because of its potent adsorption and fixation capabilities for heavy metals, hydroxyapatite has been utilized extensively to clean up heavy metal-contaminated soil and sediments [Guo *et al.,* 2018]. In addition to being a safe, clean and sustainable way to remove pollutants from contaminated sites, using hydroxyapatite (HAP) in air, water, and soil cleanup will also serve as a valuable resource recovery route because HAP can be successfully extracted from biological sources like mineral rocks, plants and most importantly waste, which primarily consists of animal bones (fish, chicken, cattle, etc.) and biogenic products (eggshells, mussel shells). Hydroxyapatite can combine with heavy metals or change their active state to an inactive state however, it cannot completely eliminate the amount of heavy metal pollution.

2.3.1. Structure of Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$)

A compact collection of tetrahedral PO_4 groups, each of which is shared by a single column and delineates two different kinds of disconnected channels, can be used to characterize the structure of hydroxyapatite. Ca^{2+} ions (referred to as Ca (I)) encircle the first channel, which has a diameter of 2.5 Å. With a diameter of about 3.5 Å, the second type is surrounded by triangular Ca^{2+} ions (called Ca (II)) and contains OH groups along the c-axis to balance the matrix's positive charge (Prakasam *et al*., 2015).

2.3.2 Mechanism by which HAP immobilizes heavy metals

Because of their toxicity even at low concentrations, non-biodegradable nature and ability to bioaccumulate, heavy metals are known to pose a major risk to humans, animals and plants. As a result, eliminating heavy metal ions from polluted grounds and aqueous solutions has become essential for the environment, necessitating the installation of decontamination procedures and treatment technologies. Of all the methods that are accessible, sorption turns out to be the most effective, cost-effective and straightforward. Given that hydroxyapatite is a mineral with the following exceptional qualities it is an ideal sorbent for long-term pollution containment, non-toxic, affordable and easily accessible, it also has a high adsorption capacity, low water solubility and high stability under reducing and oxidizing conditions. In fact, HAP proved to be highly effective at immobilizing a wide range of metals, including Cr, Pb, Cd, Ni, Zn, Al, Cu, Fe, Co, Mn, and Fe (Ferri *et al*., 2019; Mobasherpour *et al*., 2012). However, it is important to note that hydroxyapatite showed preferential adsorption of particular cations over others in the work of Mobasherpour *et al*., 2012 because of its higher affinity for those cations. Actually, $Pb^{2+} > Cd^{2+} > Ni^{2+}$ was the sequence in which the elimination capacities of Pb^{2+}, Cd^{2+} and Ni^{2+} rose. This cation preference trend was explained by a number of theories. One was the variation in these ions' acidity levels. Cd^{2+} and Ni^{2+} are categorized as soft Lewis's acids, but Pb^{2+} is a borderline hard Lewis acid. The increased affinity of hydroxyapatite for lead cations can be explained by the fact that its phosphate and hydroxyl groups are hard Lewis bases. Pb^{2+}'s stronger electronegativity than that of Cd and Ni, which would promote surface complexation processes is another explanation for its preferential adsorption on HAP. Furthermore, studies have shown that cations with ionic radii larger than Ca^{2+} (0.099 nm) have a higher chance of being included in HAP's structure than cations with ionic radii that are comparable to or smaller. As Pb^{2+}, Cd^{2+} and Ni^{2+} have respective ionic radii of 0.118 nm, 0.097 nm, and 0.072 nm, it makes perfect sense to derive the above-mentioned order of preferential adsorption. Furthermore, HAP's degree of crystallinity is a significant component that could affect its sorption capacities. In fact, a study by Stötzel *et al*. (2009) discovered a relationship between the ion adsorption behavior of hydroxyapatite and its crystallinity. These authors stated that a decrease of HAP's crystallinity leading to a growth of its specific surface area is beneficial to this material's sorption properties and its ability to remove heavy metal ions. Additionally, this study showed that nanocrystalline hydroxyapatite powder performs almost an order of magnitude better than activated carbon in removing the two divalent metals they studied (Pb^{2+} and Zn^{2+}), highlighting the allure of this apatitic sorbent as a heavy metal immobilizer. A change in the Ca/P molar ratio of hydroxyapatite can also impact its adsorbent property performance by altering its surface characteristics, including the location and kind of carbonate species found in HAP. In fact, the effectiveness of the HAP solid's metal removal process may be determined by the type of contaminating metal (Campisi *et al*., 2018). Furthermore, HAP outperformed the other phosphate-containing materials (phosphate rock, triplesuperphosphate, and diammonium phosphate) in its ability to reduce the bioavailability of the metals under study (cadmium, copper, iron, lead, and zinc) in a soil that had been artificially contaminated with metals. We should note that hydroxyapatite was found to be an affordable and effective sorbent for heavy metals, even when made from waste materials like phosphogypsum waste (Mousa *et al*., 2016) and discarded oyster shells.

2.3.3 Mechanisms of metal adsorption

2.3.3.1. Dissolution-precipitation

The hydroxyapatite dissolves in this kind of mechanism releasing phosphate ions into the environment that can precipitate other nearby metal cations (Me) and form a new metal phosphate crystal with an apatitic structure. These two equations can be used to illustrate this process:

$Ca_{10}(PO_4)_6(OH)_2 + 14H^+ \rightarrow 10Ca^{2+} + 6H_2PO_4^- + 2H_2O$

$10Me^{2+} + 6H_2PO_4^- + 2H_2O \rightarrow Me_{10}(PO_4)_6(OH)_2 + 14H^+$

Lead is the one heavy metal that stands out among the others whose sorption mechanisms were investigated in the presence of a hydroxyapatite solid as being mostly immobilized through a dissolution precipitation mechanism. Indeed, a number of investigations have demonstrated that the primary mechanism for the elimination of Pb ions is the dissolution of hydroxyapatite and the creation of a lead-phosphate substance. Although the type of new crystal that forms can change according on the anions in the solution this has no bearing on the HAP's capacity to remove lead. According to studies, hydroxyapatite did in fact immobilize Pb^{2+} cations in the presence of NO_3, Cl, F, SO_4, and CO_3 anions, proving its enormous potential for use in Pb^{2+} removal from contaminated wastes where these anions are unavoidably present. The type of lead precipitate that was produced was the sole change seen when these anions were added to the media. Chloropyromorphite [$Pb_5(PO_4)_3Cl$] and fluoropyromorphite [$Pb_5(PO_4)_3F$] were created in the presence of Cl and F, respectively, whereas hydroxypyromorphite [$Pb_5(PO_4)_3OH$] was formed in the presence of NO_3, SO_4 and CO_3.

2.3.3.2. Ion exchange

Ion exchange is a distinct but equally prevalent sorption mechanism in which calcium ions in the HAP lattice are replaced by divalent metal ions (Me) by a process that is represented by the following equation:

$Ca_{10}(PO_4)_6(OH)_2 + xMe^{2+} \rightarrow Ca_{10-x}Me_x(PO_4)_6(OH)_2 + xCa^{2+}$

A dissolution-precipitation process is slower and less advantageous than an ion exchange with HAP for some metal ions such as nickel or cadmium (Mobasherpour *et al.*, 2011). Surprisingly, a study of how the pH of the solution affects the HAP absorption of the heavy metals in the solution may provide a proxy for the sorption process involved for each cation under consideration because an ion-exchange process is made possible under alkaline conditions whereas a dissolution-precipitation process is enhanced in an acidic environment. In fact, Vila *et al*. (2011) discovered that the effectiveness of HAP's lead removal process which uses a dissolution-precipitation mechanism was stronger at low pH and declined as alkalinity increased. This can be simply explained by the fact that hydroxyapatite solubility changes with pH (it lowers as pH rises) which when reduced, restricts the amount of phosphate ions that can dissolve and consequently, the amount that is available for the Pb precipitation process. Although the sorption of these cations on HAP was primarily carried out through a mechanism different from the dissolution-precipitation one, such as ion-exchange, research that looked at the effects of pH on heavy metal removal by mineral apatite found that a low pH could be harmful for the removal of cadmium and zinc from the solution. They clarified that by raising the pH to a level higher than the pHPZC (point of zero charge) of HAP, the latter's surface becomes negatively charged, increasing the electrostatic forces that attract the surface to the cations in metalized water and improving metal immobilization at higher pH values.

2.4 PHOSPHATE SOLUBILIZING BACTERIA

The second most important macronutrient for plants after nitrogen is phosphorus which plays a direct role in the synthesis of nucleic acids, cell division and the development of new tissues. It is also required for many cellular functions including photosynthesis, energy production, carbohydrate metabolism, redox homeostasis and signaling in plants (Elhaissoufi *et al*., 2022).

With levels up to 0.2% of the dry weight of plants, phosphorus is also necessary for plant growth and development (Alori *et al*., 2017). However, the concentration of plant-available phosphorus rarely surpasses 10 μM, and arable land has a higher total phosphorus content and a lower available phosphorus content globally. This has a significant impact on agricultural development and plant growth.

Thirty to sixty-five percent of the phosphorus in soil is organic and the remaining thirty to seventy percent is inorganic. Inorganic phosphorus readily reacts with soil ions like Fe^{3+}, Al^{3+} and Ca^{2+} to produce insoluble phosphate while organic phosphorus typically exists in the soil in an inert form and is fixed to form insoluble phosphorus forms that are difficult for plants to directly absorb and use. As a result, long-term phosphorus fertilizer application overwhelmingly results in phosphate that is difficult for plants to directly absorb and cannot fully address phosphorus deficiencies in soils. In addition to harming the food chain, over use of phosphate fertilizers can cause eutrophication, imbalances in soil nutrients and devastation of the soil microbial ecosystem. Finding alternate, economically feasible and environmentally acceptable methods to raise the phosphorus levels in low-phosphorus or phosphorus-deficient soils for plant growth is necessary due to the negative effects of phosphorus fertilizer application and the decline in global phosphorus stocks.

The issue of effective phosphorus deficiency in soils can now be resolved thanks to the discovery of phosphate solubilizing bacteria (PSB). By mineralizing organic phosphorus through acid secretion and hydrolyzing inorganic phosphorus minerals through enzyme activity, PSB contribute significantly to the soil phosphorus cycle. This process solubilizes insoluble phosphorus and raises the amount of available phosphorus in soils (Liang *et al*., 2020).

Numerous recent investigations have demonstrated that PSB can change the insoluble forms of phosphorus found in soils into forms that plants can directly absorb and use through a variety of methods. Examples include the production of dissolved phosphate by chelation (siderophores, extracellular polysaccharides), enzyme secretions (phytase and phosphatase) and organic and inorganic acids (Liang *et al*., 2020). Numerous PSB types can be divided into two primary groups based on the various substrates they interact with: inorganic PSB, which can change insoluble inorganic phosphorus into soluble organic phosphorus, and organic PSB, which can mineralize organic phosphorus. Many PSB can also dissolve inorganic phosphorus and mineralize organic phosphorus simultaneously (Timofeeva *et al*., 2022). Numerous other factors also have a significant impact on PSB distribution like the distribution of PSB which varies depending on habitat types, soil characteristics and rhizosphere effects; these variations might have a direct impact on PSB species and abundance. The abundance of PSB communities, the condition of their dispersion and the variety and composition of PSB communities in soil are currently being assessed using high-throughput sequencing technologies. Additionally, the effects of various conditions on PSB dispersion vary. In all soil types, *Bacillus species, Burkholderia species,* and *Pseudomonas* species are currently widely distributed and abundant. *Bacillus, Pseudocystis,* and *Burkholderia* species have been found in a variety of soil types, including tea gardens, saline soil, heavy metal-containing soils, and forest soil, as well as in crop rhizosphere soils, these species are known to have a high relative abundance in the bacterial community and a potent ability to solubilize phosphorus (Tang *et al*., 2020). The distribution of PSB is also influenced by soil characteristics (varying soil characteristics result in variations in the distribution of PSB with various functions). Two PSB, *Pseudomonas carboxylans* and *Pseudomonas malodorans,* were identified from soils that were highly contaminated with heavy metals these bacteria showed remarkable tolerance to lead. Bacteria isolated from saline soils were Bacillus species, including *Bacillus amyloliquefaciens*, and these PSB were more effective at improving saline soils (Zhang *et al*., 2023). These distributional properties make PSB suitable for remediating particular soil types.

2.4.1 Mechanisms of Phosphorus Solubilization by PSB

In addition to being associated with bacterial species, phospholysis-related genes also regulate the three key elements of the soil phosphorus cycle; dissolution–precipitation, mineralization–fixation and adsorption–desorption that are the focus of phosphorus solubilization mechanisms. In addition to chelating or complexing with metal cations (Ca^{2+}, Fe^{2+} and Al^{3+}) in the soil to liberate phosphate ions, the majority of phosphate-solubilizing bacteria have the ability to mineralize or hydrolyze the insoluble phosphate in the soil by secreting acids and enzymes. In order to solubilize insoluble phosphate, a few phosphate-solubilizing bacteria can indirectly alter the pH of the surrounding environment by releasing gas molecules (CO_2 through respiration and hydrogen sulfide from PSB). By changing the soil's microbial community, PSB can also raise the amount of phosphorus in soils thereby improving crop development and production.

2.4.1.1. Solubilizing Action of Acids

Both organic and inorganic acids are involved in acidolysis. Majority of PSB release organic acids while very few release inorganic ones. Low molecular weight organic acids, including butyric acid, lactic acid, 2-ketogluconic acid, fumaric acid, oxalic acid, glutamic acid, propionic acid, fumaric acid, acetic acid, tartaric acid, malonic acid, glutamic acid and succinic acid can be secreted by PSB bacteria as they grow. Under low pH conditions, these low molecular weight organic acids can chelate with metal ions in the soil (Fe^{3+}, Al^{3+} and Ca^{2+}) through hydroxyl and carboxyl groups. Their ability to chelate Ca^{2+} is crucial and they compete with phosphates for phosphorus adsorption sites in the soil this increases the solubilizing capacity of inorganic phosphorus and improves soil phosphate uptake, which in turn increases the solubility and availability of mineral phosphates (Billah *et al*., 2019). The type of the organic acids also affects solubilization efficacy; aliphatic acids are more successful than phenolic, citric and fumaric acids in solubilizing phosphates, while tricarboxylic and dicarboxylic acids are often more effective than mono and aromatic acids. However, different PSB produce different kinds of organic acids, and the adsorption capabilities of these organic acids for phosphate vary greatly (Wei *et al*., 2018). Some PSB also release inorganic acids such as carbonic, nitric, sulfuric and hydrochloric acids which dissolve inorganic phosphorus and lower soil pH but are less efficient than organic acids at the same pH.

2.4.1.2. Mineralization Action of Enzymes

One of the primary dephosphorylation processes is the mineralization of organophosphorus by enzymes released by PSB. Phosphates, phytases and C-P cleaving enzymes are the primary enzymes that have been shown to have a dephosphorylating effect additionally, numerous experiments have clearly shown that PSB increases soil enzyme activity, which raises the soil's available phosphorus content. However, different hydrolytic enzymes play distinct roles in the mineralization of organic phosphorus (Khourchi *et al*., 2022). Acidic, alkaline and neutral phosphatases are the three forms of phosphophosphatase sometimes referred to as phosphomonolipase. In acidic soils with a pH below 7, acidic phosphatases (ACP) is more successful in mineralizing organic phosphorus. In soils with a pH greater than 7, alkaline phosphatases (ALPs) primarily catalyze the breakdown of phospholipids (phosphoglucose-6 and ATP) and liberate inorganic phosphorus. Conversely, neutral phosphatases have less of an impact on phosphorus mineralization and hydrolysis than do acid and alkaline phosphatases. Another kind of phosphatase is called phytotase and it mostly mineralizes organic phosphorus in phytate, because the ester bond in phytate is so persistent, phosphatases cannot fully hydrolyze it instead phytase must transform it into a form that phosphatases can break down ultimately releasing inorganic phosphorus. Like many of the enzymes listed above, C-P lyase contributes to the mineralization of organophosphorus; however, it is distinct in that it hydrolyzes organophosphorus which eventually liberates phosphorus in its free form (Elhaissoufi *et al*., 2022).

2.4.1.3. Chelation and Complexation

Based on the idea that a functional group binds to metal cations in the soil to release phosphate for phosphorus solubilization, chelation and complexation are two significant phosphorus solubilization mechanisms. Extracellular polysaccharides, siderophores (made by phosphorus-solubilizing bacteria) and the breakdown of plant and animal waste are the primary sources of chelation and complexation. Phosphate-dissolving bacteria generate siderophores, which are tiny molecules with low molecular weights to chelate Fe^{3+}, Al^{3+} and Ca^{2+} in the soil and release phosphate ions for phosphate dissolution when there is less iron stress. In order to increase iron uptake by plants and stimulate plant growth, the generated metal-iron carrier complexes can attach to iron carrier receptor proteins on cell membranes and enter cells (Li *et al*., 2021). PSB secretes extracellular polysaccharides, which are high molecular weight sugar polymers that are affixed to the surfaces of bacteria. These polymers have a lot of -OH and -COOH acid groups on their surface, as well as unique anionic functional groups (phosphate, carboxyl, and succinate groups) that can complex with the cations of heavy metal elements in the soil. Humic acid-like compounds are created when PSB breaks down plant and animal waste, these compounds can also chelate Fe^{3+}, Al^{3+} and Ca^{2+} in the soil releasing phosphate and raising the amount of phosphorus that is available in the soil.

2.5 INTERACTION OF HYDROXYAPATITE AND PSB IN BIOREMEDIATION

Because each agent enhances the other's limitations, the combined use of HAP and PSB presents a promising remedial approach. PSB speeds up the solubilization of HAP-bound phosphorus so that plants can use it, whereas HAP immobilizes heavy metals and raises the amount of phosphorus in the soil.

2.5.1 Synergistic mechanisms between HAP and PSB

2.5.1.1 Enhanced phosphate release from HAP

Organic acids secreted by PSB have the ability to breakdown hydroxyapatite and liberate soluble phosphate. As a result, metal-phosphate complexes precipitate more effectively since the interaction between phosphate and heavy metals is accelerated (Li *et al*., 2015).

2.5.1.2 Sustained metal immobilization

A sustained immobilization system for heavy metals is created by PSB, which guarantees continual solubilization while HAP offers a long-term source of phosphate.

2.5.1.3 Microbial colonization on HAP surfaces

By creating biofilms on the surface of HAP particles, PSB can improve dissolving by causing localized acidity. Hot areas for metal sequestration are produced by this biomineral interaction.

2.5.1.4 Improved soil health and plant growth

In addition to immobilizing heavy metals, the combination of HAP and PSB increases plant phosphorus availability, promoting vegetative development in contaminated soils. In addition to stabilizing the soil, vegetation also inhibits erosion and increases microbial activity in the rhizosphere.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

The study was carried out in a mechanic workshop in Osasogie with latitude 6°23'18"N 5°37'3"E in Ugbowo, Benin city, Edo state.

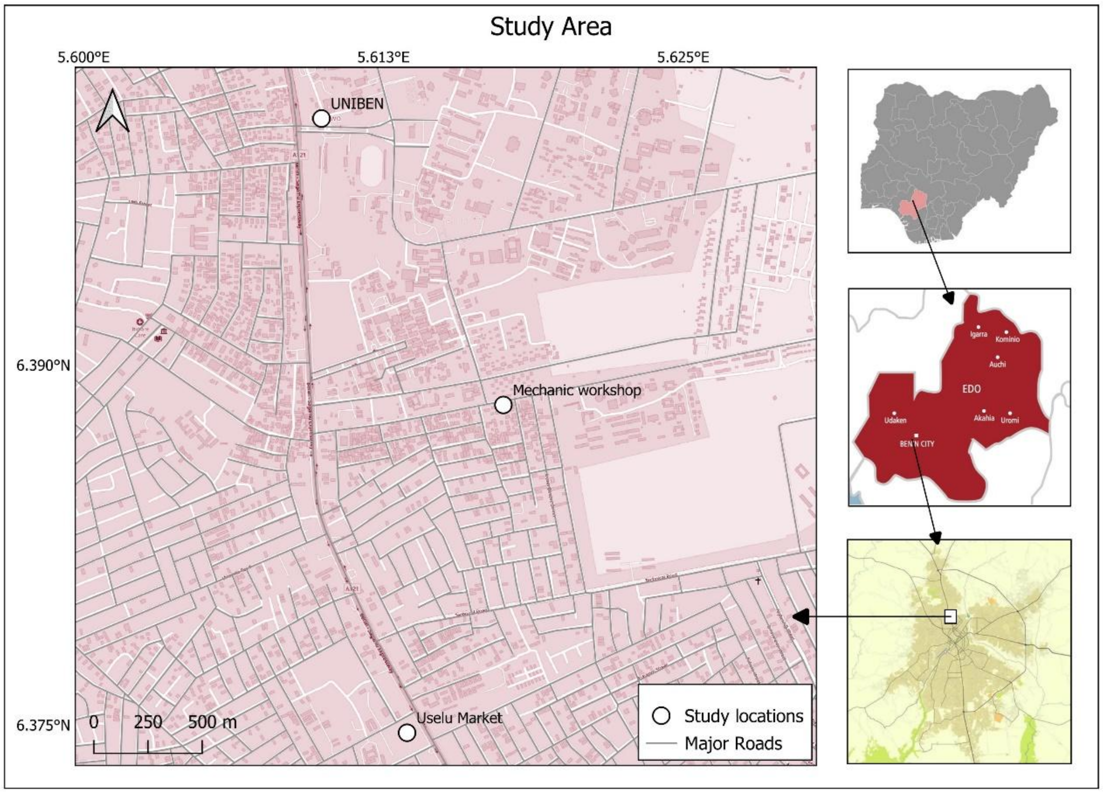

Figure 3.1: A map of the area where soil sample was collected

3.2 SAMPLE COLLECTION AND PREPARATION

The soil samples were collected by dividing the site into 4 and samples were collected from the 4 different points in the site in clean polyethylene bags. The samples were then air dried and sieved with a 2.8mm sieve to remove large particles and debris.

3.3 EXPERIMENTAL DESIGN

This bioremediation setup consists of soil sample from Osasogie. 100g was weighed from each point and mixed thoroughly in a clean bowl to form a composite sample. This was repeated for three bowls having 400g of composite soil sample in four bowls each. The bowls are labelled Soil only (O1), Soil + PSB (O2), Soil + HAP (O3) and Soil + PSB + HAP (O4) and perforated in other to allow air into the soil samples. The 20ml of isolated PSB was then diluted with 80ml of distilled water to make 100ml, 50ml from the 100ml is then added to O2 (Soil + PSB) and the remaining 50ml added to O4 (Soil + PSB + HAP). 40g of HAP was added to O3 (Soil + HAP) and another 40g was added to O4 (Soil + PSB + HAP). Bowls O2, O3 and O4 are then mixed thoroughly and soil moisture was maintained by replenishing evaporative losses with sterile distilled water.

After 14 days from the introduction of PSB and HAP into the soil, heavy metals will be tested (copper, iron and arsenic) and microbial count will be taken.

3.4 PROCEDURE OF ANALYSIS

3.4.1 Phosphate Solubilizing Bacteria Isolation

Samples of soil and rhizosphere were taken from specific agricultural fields and plant root zones. Using sterile spatulas, about 10 grams of soil were extracted from a depth of 5 to 15 cm for bulk soil sample. In order to gather rhizosphere samples, healthy plants were carefully uprooted and the soil that stuck to their root surfaces was brushed off and placed in sterile containers. After being labeled with the date, location, and plant species (for rhizosphere samples), each sample was brought to the lab in a refrigerator. To maintain microbial viability, samples were processed within 24 to 48 hours and kept at 4 °C. In 250 mL Erlenmeyer flasks, 10 grams of each soil sample were suspended in 90 mL of sterile distilled water. To separate microbial cells from soil particles, the suspension was shaken for 30 minutes at 150 rpm on a rotary shaker. Next, sterile distilled water was used to prepare serial dilutions up to 10^{-2}.

1 ml portions of each dilution were aseptically moved into sterile Petri dishes. Each dish received 15–20 mL of molten nutritional agar that had been cooled to 45 °C. To guarantee that the inoculum was distributed evenly, the plates were gently swirled before being left to harden. The incubation period was 24 to 48 hours at 28 ± 2 °C. To obtain pure isolates, colonies with diverse morphologies were chosen and subcultured onto new nutrient agar plates (Ihoeghian *et al*., 2023; Isagba *et al*., 2023).

Tricalcium phosphate (TCP) is the only insoluble phosphorus source in Pikovskaya's agar medium, which was used to screen purified bacterial isolates for phosphate-solubilizing ability. A loopful of each isolate was applied to the agar surface in order to accomplish spot inoculation. For three to seven days, the plates were incubated at 28 ± 2 °C. The creation of transparent halo zones surrounding the colonies as a result of TCP breakdown demonstrated phosphate solubilization (Ihoeghian *et al*., 2023; Isagba *et al*., 2023). A digital calliper was used to measure the diameter of the bacterial colony and the diameter of the clear zone which included the colony following three days of incubation. The formula was used to determine the solubilization index:

SI = Halo zone diameter + Colony diameter / Colony diameter

Higher SI isolates were chosen for additional characterization because they were thought to be more effective phosphate solubilizers (Ihoeghian *et al*., 2023; Isagba *et al*., 2023).

To ascertain their phenotypic characteristics, a few phosphate-solubilizing isolates were put through Gram staining and simple biochemical assays like oxidase and catalase activity. The bacterial isolates were initially identified and categorized by these assays. Gram staining was used to examine the isolates' cellular and colony morphology (shape, elevation, border, color, and opacity). Gram staining was carried out, and stained smears were analyzed for cell shape and Gram response under oil immersion (100×).

3.4.1.1 Biochemical Assays

Using a variety of staining methods and biochemical tests, the recovered bacterial isolates were microbiologically characterized to ascertain their taxonomic identity, metabolic capacities and gram reaction. In order to supplement molecular and genomic investigations, these assays produced fundamental phenotypic data.

3.4.1.1.1 Staining with Gram

Each bacterial isolate was prepared as a thin smear on a clean glass slide, heat-fixed and then sequentially stained with crystal violet (primary stain) for three minutes, rinsed with water and then stained with Gram's iodine (mordant) for three minutes before another round of rinsing. The decolorization step involved using 95% ethanol (decolorizer) for ten to fifteen seconds, counterstained, and safranin for one to three minutes. After air drying, the slides were inspected using an oil immersion light microscope at a magnification of 1000×. Bacteria are distinguished by gram staining according to the structural variations in their cell walls. Because of their thick peptidoglycan coating, which appears purple under a microscope, gram-positive bacteria are able to preserve the crystal violet-iodine combination. Gram-negative bacteria have an outer membrane and a weaker peptidoglycan layer, which allows the dye to be washed out and replaced by the counterstain (safranin), giving them a pink or red appearance. However, the primary stain's color is retained by the Gram-positive bacteria.

3.4.1.1.2 String Test with KOH

On a spotless glass slide, a drop of 3% KOH solution was combined with a loopful of new bacterial culture. After 30 seconds of stirring, the fluid was progressively raised with the loop to see if strings formed. A quick way to verify the Gram response is the KOH string test. In 3% potassium hydroxide, gram-negative bacteria lyse, releasing DNA that, when lifted with a loop, creates a viscous string. Gram-positive bacteria do not form a string and are resistant to lysis. Gram-negative bacteria are therefore indicated by a positive string test result, and vice versa.

3.4.1.1.3 Catalase Test

A sterile loop was used to introduce a colony of the test organism to a clean slide that had a drop of 3% hydrogen peroxide on it. A good reaction is indicated by the cells in the loop bubbling right away. The catalase test finds the catalase enzyme, which converts hydrogen peroxide into oxygen and water. A positive catalase result is indicated by the visible bubbling that results from the release of oxygen, whereas a negative catalase result is indicated by the lack of gas bubbles or bubbling.

The bacterial isolates were kept on nutrient agar slants at 4°C to guarantee their long-term availability. Glycerol stocks (20% v/v) were also made and kept for later use at -80°C.

3.4.2 Heavy Metals Test

For heavy metal test an Atomic Absorption Spectrometer (AAS) is used. In order to use AAS the soil samples need to be digested by taking 1g of finely grinded sample into a conical flask, 10ml of nitric-perchloric acid mixture was added and allowed to soak overnight. A small glass funnel was then inserted to act as a reflux condenser and it was heated for 1 hour at 150°C, gradually the temperature was raised to 235°C. When dense white fume occurred, heating was continued until a colourless solution was obtained. The colourless solution was then poured into a 100ml flask and the previous flask was rinsed 5 times adding distilled water used for rinsing into the 100ml flask and the volume was made up with distilled water. Blank samples were prepared using the same procedure. The digested sample was then put into a calibrated AAS and the digested sample is first aspirated into the flame of the AAS whose high temperature converts the analyte ions into atoms in vapour state, absorption occurred when a ground state atom absorbed energy in form of a light as a specific wavelength and is elevated to an excited state. The relationship between the amount of light absorb and the concentration of the analyte present in known standard can be used to determine unknown concentration by measuring the amount of light absorbed. The digested samples and blank were then run on the AAS to obtain the absorbance values. Concentrations of the metals in the sample were calculated from the equation of the calibration curve.

CHAPTER FOUR

4.0 RESULTS

4.1 MICROBIAL COUNT

Table 4.1 shows microbial count after 14 days of introduction of *Pseudomonas sp*.

2.5×10^6 CFU/g was introduced into the soil and at day 14 (2 weeks) the number of *Pseudomonas sp*. in O2 (soil + PSB) reduced to $1.50 \pm 0.00 \times 10^5$ CFU/g which can be due to the fact that some of the bacteria could not survive because of the unfavourable environment, it can also be due to nutrient depletion and competition for available nutrients in the soil.

The number of *Pseudomonas sp*. in O4 (soil + PSB + HAP) also reduced to $13.75 \pm 0.71 \times 10^5$ CFU/g this can also be due to unfavourable environmental conditions and nutrient depletion but the number of *Pseudomonas sp*. here is higher than in O2 this can be because of the addition of HAp which can help reduce heavy metal concentration thereby making the soil more favourable to live in. Also, *Pseudomonas sp*. which is a phosphate solubilizing bacteria can use the phosphorus and calcium in hydroxyapatite as food source thereby having more food source than *Pseudomonas sp* in O2.

Table 4.1: Showing microbial count after 14 days of introduction of *Pseudomonas sp*.

S/N	SOIL SAMPLES	TOTAL HETEROTROPHIC BACTERIA COUNT (×10^5 CFU/g)
1	SOIL + PSB (O2)	1.50 ± 0.00
2	SOIL + PSB + HAP (O4)	13.75 ± 0.71

4.2 HEAVY METAL RESULT

Table 4.2 shows iron (Fe), copper (Cu) and arsenic (As) concentration after 14 days of bioremediation.

After 14 days, there was some reduction in iron (Fe) and copper (Cu) concentrations while arsenic (As) concentration was too minute to be detected.

Table 4.2 shows that soil + HAP (O3) was the most effective at reducing Fe and Cu because of its strong sorption and precipitation for metals and also ion exchange while soil + PSB (O2) recorded some minor reductions this may be because biological processes are slow and 14 days is a short period of time for the *Pseudomonas sp* to remediate the soil. Soil + PSB + HAP (O4) recorded intermediate reduction, it was higher than O2 but lower than O3 this shows that the synergistic effect of PSB and HAP is effective at reducing Fe and Cu although the result for O4 Cu is closer to O3 than the result for Fe. Arsenic showed no detection because it was in very minute concentration that could not be detected.

Figure 4.1 shows a graph of the concentration of iron, copper and arsenic.

This is a graphical representation of table 4.2 which shows that Fe was present in the highest concentration followed by Cu while Arsenic was below detectable limit in all samples. The high concentration of Fe and Cu in O1 shows how contaminated the soil is which is typical of mechanic workshop soils.

Table 4.2: Showing heavy metals (iron, copper and arsenic) concentration after 14 days of bioremediation

S/N	SOIL SAMPLES	IRON (mg/kg)	COPPER (mg/kg)	ARSENIC (mg/kg)
1	SOIL ONLY (O1)	6619.72	193.7	NO DETECTION
2	SOIL + PSB (O2)	6549.30	193.18	NO DETECTION
3	SOIL + HAP (O3)	5000.00	143.6	NO DETECTION
4	SOIL + PSB + HAP (O4)	5915.49	151.54	NO DETECTION

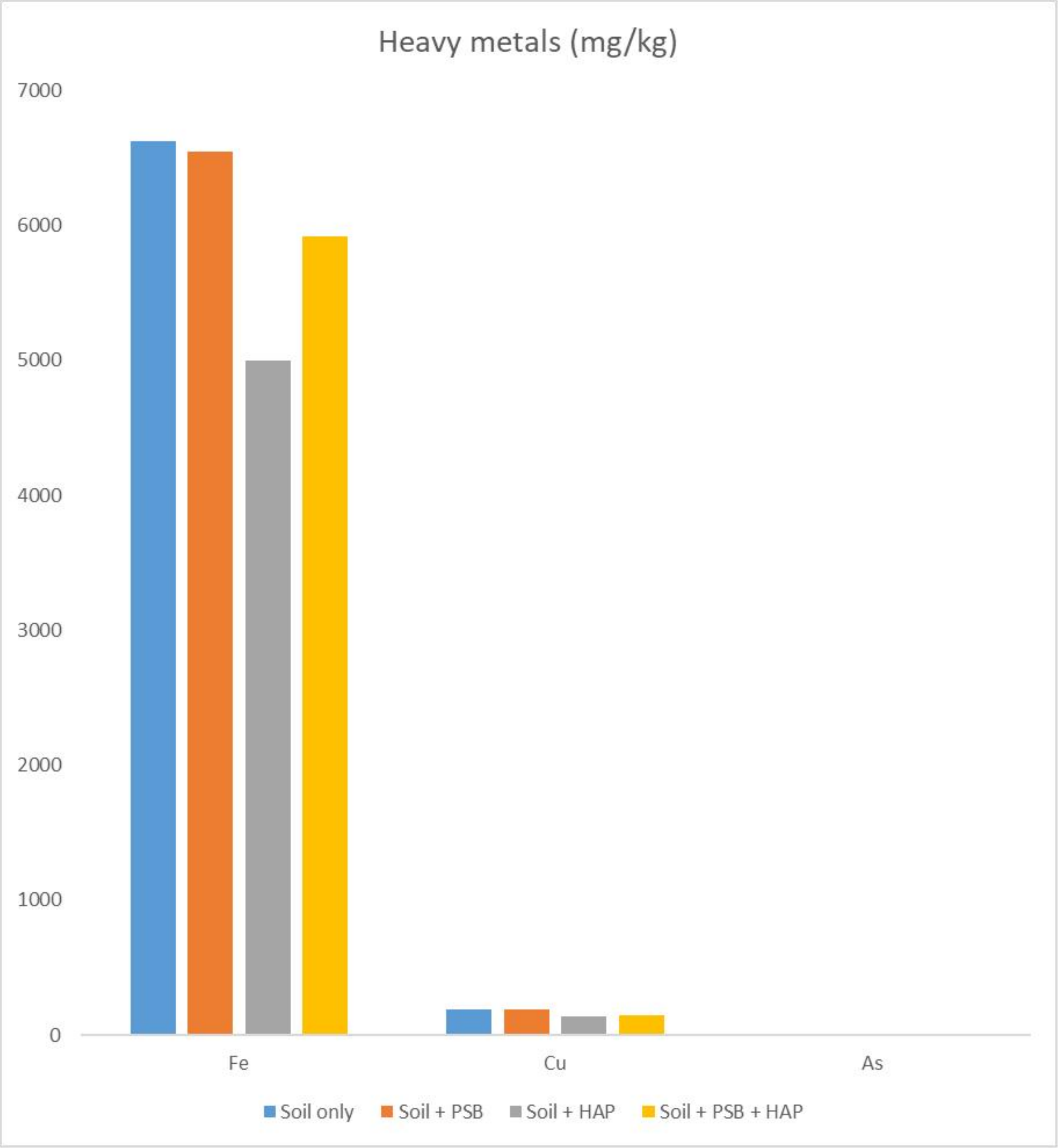

Figure 4.1: Showing a graph of iron, copper and arsenic concentration after 14 days of bioremediation

4.3 PERCENTAGE REMOVAL OF IRON, COPPER AND ARSENIC

Table 4.3 shows the percentage removal of iron (Fe), copper (Cu) and arsenic (As) after 14 days.

The table shows that the percentage removal of Fe ranged from 1.063791% in soil + PSB (O2), 24.4681% in soil + HAP (O3) and 10.63837% in soil + PSB + HAP (O4) which suggests that O3 was the most effective at reducing Fe concentration for the short period of time (14 days).

The table also shows that the percentage removal of Cu ranged from 0.268456% in O2, 25.86474% in O3 and 21.76562% in O4 which suggests that O3 was more effective. Also, the percentage removal in O4 was also high demonstrating that the microbial activity of *Pseudomonas sp.* supported Cu stabilization.

The table also shows no percentage removal for arsenic which is because there was no detection of arsenic in the soil samples.

Overall, the percentage removal data shows that O3 and O4 were more effective than O2 because using bacteria alone is a slower process which requires more time to be effective.

Table 4.3: Showing the percentage removal of iron, copper and arsenic after 14 days

S/N	HEAVY METALS	PERCENTAGE REMOVAL OF SOIL + PSB (O2)	PERCENTAGE REMOVAL OF SOIL + HAP (O3)	PERCENTAGE REMOVAL OF SOIL + PSB + HAP (O4)
1	Iron (Fe)	1.063791	24.4681	10.63837
2	Copper (Cu)	0.268456	25.86474	21.76562
3	Arsenic (As)	No detection	No detection	No detection

CHAPTER FIVE

5.0 DISCUSSION

5.1 DISCUSSION

This work investigated the bioremediation potential of *Pseudomonas sp*. and hydroxyapatite in heavy metal polluted soil collected from a mechanic workshop in Osasogie, Benin city, Nigeria.

The bioremediation potential of hydroxyapatite and *Pseudomonas sp*. in heavy metal polluted soil taken from a mechanic workshop in Osasogie, Benin city, Nigeria was examined in this work. High concentrations of Fe and Cu which are indications of pollution from mechanic workshops were found in the soil although arsenic was not found. Bioremediation was assessed by tracking changes in the microbial population and metal concentration after 14 days.

5.1.1 Microbial Analysis

About 2.5×10^6 CFU/g of *Pseudomonas sp*. were injected into the contaminated soil. Due to adverse environmental factors like nutrient depletion, heavy metal toxicity and competition with local bacteria, the number of *Pseudomonas sp.* decreased after 14 days in O2 and O4 where the bacteria were introduced.

The *Pseudomonas sp*. count dropped dramatically to 1.5×10^5 CFU/g in soil + PSB (O2). This decrease implies that microbial growth was hindered by heavy metal toxicity. Choudhury and Das (2012) showed similar reductions in bacteria populations under metal stress pointing out that metal ions like Cu^{2+} and Fe^{2+} can damage enzyme systems and cell membranes.

The *Pseudomonas sp*. population was significantly greater in soil+ PSB + HAP (O4) with the value of $13.75 \pm 0.71 \times 10^5$ CFU/g. This greater population suggests that by adsorbing and precipitating metals, hydroxyapatite reduced metal stress thereby creating a more favourable environment for *Pseudomonas sp*. survival. Through surface complexation and ion exchange, hydroxyapatite can trap metals and create stable metal-phosphate minerals (Chen *et al.*, 2017). Also, *Pseudomonas sp*. are phosphate solubilizing bacteria that can use hydroxyapatite's calcium and phosphorus as sources of nutrients. This explains why bacteria survival is higher in O4 than in O2. Similar interactions between hydroxyapatite and phosphate solubilizing bacteria have shown to enhance microbial activity and phosphorus bioavailability.

Therefore, table 4.2 shows that the combination of hydroxyapatite and PSB (*Pseudomonas sp.)* offers a synergistic advantage.

5.1.2 Heavy Metals

After 14 days of bioremediation, the concentrations of Fe and Cu showed different effects among the various treatments. Fe and Cu were prevalent contaminants in the soil samples because of the constant use of lubricants and its use in automobile parts (Diagi *et al*., 2023). Arsenic was not detected because of its minute level in the soil sample leading to no detection.

The remarkable ability of hydroxyapatite alone (O3) to immobilize metals through adsorption and precipitation mechanisms was confirmed by the highest metal reduction of 24.4681% for Fe and 25.86474% for Cu (Wei *et al*., 2016). The decrease in Fe and Cu indicates the formation of insoluble metal-phosphate complexes ($Cu_3(PO_4)_2$ and $FePO_4$) that restrict bioavailability.

Significant reduction was obtained from the combination of PSB and hydroxyapatite (O4) with Fe being 10.63837% and Cu being 21.76562% which was marginally less effective for Fe removal than HAP alone but almost equal for Cu. This suggests that hydroxyapatite and

Pseudomonas sp. might compliment one another. The organic acids that the bacteria create improve phosphate solubilization and change the pH of the soil which affects metal mobility (Ahemad, 2014). Meanwhile, hydroxyapatite maintains a balance between solubilization and stability by buffering excessive acidity and adsorbing released metal ions.

Because of the short incubation period and metal stress, microbial activity alone was insufficient within 14 days, as demonstrated by the low metal reduction in O2 (Soil + PSB). However, *Pseudomonas sp*. may encourage prolonged immobilization through metal sequestration and increased enzyme production with longer remediation period (Zhu *et al*., 2017).

While *Pseudomonas sp*. improves the overall restoration process by enhancing nutrient cycling and soil biological quality, Fe and Cu reduction patterns suggest that hydroxyapatite is the major immobilizing agent. Pseudomonas sp. makes a biological contribution by improving nutrient cycling and long term stabilization potential while hydroxyapatite quickly immobilizes metals through chemical reactions. Thus, the combined treatment provides a sustainable and well rounded remediation approach that incorporates both biological and rapid mechanisms.

5.2 CONCLUSION

Pseudomonas sp. and hydroxyapatite showed great promise in cleaning up heavy metal contaminated soil from a mechanic workshop. *Pseudomonas sp*, probably led to better nutrient turnover and long term remediation stability while hydroxyapatite was crucial in immobilizing Fe and Cu. According to findings, combining mineral and biological amendments presents a viable approach to long term soil restoration.

REFERENCES

Ahemad, M. (2014). Phosphate-solubilizing bacteria assisted phytoremediation of metalliferous soils: a review. *3 Biotech,* **5**(1): 111-121.

Albelda-Berenguer, M., Monachon, M., and Joseph, E. (2019). Siderophores: from natural roles to potential applications. *Advances in. Appllied Microbiology,* **106**(1): 193–225.

Alori, E., Glick, B., and Babalola, O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology*, **8**(1): 971-982.

Alori, E.T. (2015). Phytoremediation using microbial community. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L, (eds). Phytoremediation: Management of Environmental Contaminants, vol. 1&2 Springer International Publishing. Switzerland p. 190.

Alori, E.T., and Babalola, O.O. (2018). Microbial inoculant for improving crop quality and human health in Africa. *Frontiers in Microbiology,* **9**(1): 1–12.

Alori, E.T., and Fawole, O.B. (2017). Microbial inoculants-assisted phytoremediation for sustainable soil management. In: Ansari AA, Gill R, Gill SS, Lanza GR, Newman L (ed)

Phytoremediation: Management of Environmental Contaminants vol. 5 Springer International Publishing, Switzerland, p17.

Aparicio, J.D., Raimondo, E.E., Saez, M.J., Gutierrez, S.B., Alvarez, A., Benimeli, C.S., and Polti, M. (2022). The current approach to soil remediation: a review of physicochemical and biological technologies, and the potential of their strategic combination. *Journal of Environmental Chemical Engineering,* **10**(1): 107-141.

Arstol, E., and Hohmann-Marriott, M. F. (2019). Cyanobacterial siderophores– physiology, structure, biosynthesis, and applications. *Marine Drugs*, **17**(5): 1-281.

Ayangbenro, A. S., and Babalola, O. O. (2017). A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *International Journal of Environmental Research and Public Health,* **14**(1): 1-94.

Ayangbenro, A.S., and Babalola, O.O. (2018). Metal(loid) bioremediation: strategies employed by microbial polymers. *Sustainability,* **10**(9), 28-40.

Ayangbenro, S.A., and Babalola (2017). A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *International Journal of Environmental Research and Public Health,* **14**(1): 94-101

Ayilara, M.S., Olanrewaju, O.S., Babalola, O.O., and Odeyemi, O. (2020). Waste management through composting: challenges and potentials. *Sustainability*, **12**(11): 1-23.

Billah, M., Khan, M., Bano, A., Hassan, T., Munir, A., and Gurmani, A. (2019). Phosphorus and phosphate solubilizing bacteria: keys for sustainable agriculture. *Geomicrobiology Journal,* **36**(10): 904–916.

Bridget, D.E., Okorondu, J.N., Ajiere, S.I., Ekweogu, C.V., Edokpa, D.O., Acholonu, C., and Edeh, S. (2023). Assessment of heavy metal contamination of soil in mechanic workshops at Nekede and Orji, Owerri zone, Imo state, Nigeria. *Journal of Scientific Research and Reports,* **29**(7): 8-16.

Campisi, S., Castellano, C., and Gervasini, A. (2018). Tailoring the structural and morphological properties of hydroxyapatite materials to enhance the capture efficiency towards copper(II) and lead(II) ions. *New Journal of Chemistry*, **42**(6): 4520–4530.

Chen, S.B., Xu, M.G., Ma, Y.B., and Yang, J. (2007). Evaluation of different phosphate amendments on availability of metals in contaminated soil. *Ecotoxicology and Environmental Safety*, **67**(2): 278–285.

Chen, Z., Ma, Y., and Wang, H. (2017). Hydroxyapatite as an efficient immobilization agent for heavy metal contaminated soil. *Environmental Science and Pollution Research,* **24**(22): 930-940.

Choudhury, R., and Das, P. (2012). Effect of heavy metals on soil microbial community and enzyme activity. *Environmental Monitoring and Assessment,* **185**(3): 2191-2201.

Condit, W., Hawley, E., Rectanus, H., and Deeb, R. (2017). Global trends in the environmental remediation industry, *Journal of Environmental Management*, **204**(1): 705- 708.

Dhaka, A., and Chattopadhyay, P. (2021). A review on physical remediation techniques for treatment of marine oil spills. *Journal of Environmental Management*, **288**(1): 112-128.

Divya, M., Aanand, S., Srinivasan, A., and Ahilan, B. (2015). Bioremediation an eco-friendly tool for effluent treatment: a review. *International Journal of Applied Research*, **1**(12): 530–537.

Elhaissoufi, W., Ghoulam, C., Barakat, A., Zeroual, Y., and Bargaz, A. (2022). Phosphate bacterial solubilization: a key rhizosphere driving force enabling higher P use efficiency and crop productivity. *Journal of Advanced Research***, 38**(9): 13–28.

Fashola, M. O., Ngole-Jeme, V. M., and Babalola, O. O. (2016). Heavy metal pollution from gold mines: environmental effects and bacterial strategies for resistance. *International Journal of Environmental Research and Public Health*, **13**(12): 10-47.

Fashola, M.O., Ngole-Jeme, V.M., and Babalola, O.O. (2020). Heavy metal immobilization potential of indigenous bacteria isolated from gold mine tailings. *International Journal of Environmental Research***, 14**(1), 71–86.

Ferri, M., Campisi, S., Scavini, M., Evangelisti, C., Carniti, P., and Gervasini, A. (2019). In-depth study of the mechanism of heavy metal trapping on the surface of hydroxyapatite. *Applied surface science*, **475**(1): 397–409.

Fihri, A., Len, C., Varma, R.S., and Solhy, A. (2017). Hydroxyapatite: a review of syntheses, structure and applications in heterogeneous catalysis. *Coordination Chemistry Review*, **347**(1): 48–76.

Fu, H., Rahaman, M.N., Brown, R.F., and Day, D.E. (2013). Evaluation of BSA protein release from hollow hydroxyapatite microspheres into PEG hydrogel. *Materials Science and Engineering C,* **33**(4): 2245–2250.

Goswami, M., Chakraborty, P., Mukherjee, K., Mitra, G., Bhattacharyya, P., Dey, S., Mitra, G., and Tribedi, P. (2018). Bioaugmentation and biostimulation: a potential strategy for environmental remediation. *Journal of Microbiology and Experimentation*, **6**(5): 223–231.

Guo, F., Ding, C., Zhou, Z., Huang, G., and Wang, X. (2018). Stability of immobilization remediation of several amendments on cadmium contaminated soils as affected by simulated soil acidification. *Ecotoxicology and Environmental Safety*, **161**(1): 164–172.

Hussain, A., Rehman, F., Rafeeq, H., Waqas, M., Asghar, A., Afsheen, N., et al. (2022). In-situ, ex-situ, and nano-remediation strategies to treat polluted soil, water, and air–a review. *Chemosphere,* **289**(3): 133-252.

Ihoeghian, N. A., Amenaghawon, A. N., Ogofure, A., Oshoma, C. E., Ajieh, M. U., Erhunmwunse, N. O., Obuekwe, I. S., Edosa, V. I. O., Tongo, I., Emokaro, C., Ezemonye, L. I. N., Semple, K. T., & Martin, A. D. (2023). Biochar-facilitated batch co-digestion of food waste and cattle rumen content: An assessment of process stability, kinetic studies, and pathogen fate. *Green Technologies and Sustainability*, **1**(3): 1-17.

Isagba, E. S., Ajieh, M. U., Oshoma, C. E., Amenaghawon, A., Ogofure, A. G., Obatusin, V., Obuekwe, I. S., Tongo, I., Ihoeghian, N., Edosa, V. I. O., Erhunmwunse, N., Lag-Brotons, A. J., Emokaro, C., Ezemonye, L. I. N., & Semple, K. T. (2023). Assessment of Anaerobic Digestate Amended with Wood Ash and Green Vegetable Matter and Impacts on Microbial Growth. *Waste and Biomass Valorization*, **14**(9), 3013-3025.

Jafari, A., Ghaderpoori, M., Kamarehi, B., and Abdipour, H. (2019). Soil pollution evaluation and health risk assessment of heavy metals around douroud cement factory, Iran. *Environmental Earth Sciences*, **78**(8), 1–9.

Khourchi, S., Elhaissoufi, W., Loum, M., Ibnyasser, A., Haddine, M., Ghani, R., Barakat, A., Zeroual, Y., and Rchiad, Z. (2022). Delaplace, P. Phosphate solubilizing bacteria can significantly contribute to enhance P availability from polyphosphates and their use efficiency in wheat. *Microbiological. Research*, **262**(1): 12-70.

Kumari, V., and Tripathi, A. (2020). Remediation of heavy metals in pharmaceutical effluent with the help of bacillus cereus-based green-synthesized silver nanoparticles supported on alumina. *Applied Nanoscience***, 10**(6): 1709–1719.

Li, K., Zeghbroeck, J.V., Liu, Q., and Zhang, S. (2021). Isolating and characterizing phosphorus solubilizing bacteria from rhizospheres of native plants grown in calcareous soils. *Frontiers in Environmental Science,* **9**(1): 24-56.

Liang, J., Liu, J., Jia, P., Yang, T., Zeng, Q., Zhang, S., Liao, B., Shu, W., and Li, T. (2020). Novel phosphate-solubilizing bacteria enhance soil phosphorus cycling following ecological restoration of land degraded by mining. *International Society for Microbial Ecology Journal*, **14**(6): 1-14.

Lwin, C.S., Seo, B.H., Kim, H.U., Owens, G., and Kim, K.R. (2018). Application of soil amendments to contaminated soils for heavy metal immobilization and improved soil quality a critical review. *Soil Science and Plant Nutrition*, **64**(2): 156–167.

Mahmoud, G.A.E. (2021). Microbial scavenging of heavy metals using bioremediation strategies, in Rhizobiont in bioremediation of hazardous waste, Springer, Singapore, pp289.

Maity, J. P., Chen, G.-S., Huang, Y.H., Sun, A.C., and Chen, C.Y. (2019). Ecofriendly heavy metal stabilization: microbial induced mineral precipitation (MIMP) and biomineralization for heavy metals within the contaminated soil by indigenous bacteria. *Geomicrobiology*, **36**(7): 612–623.

Medfu-Tarekegn, M., Zewdu Salilih, F., and Ishetu, A. I. (2020). Microbes used as a tool for bioremediation of heavy metal from the environment. *Cogent Food & Agriculture*, **6**(1): 1-15.

Meng, J., Tao, M., Wang, L., Liu, X., and Xu, J. (2018). Changes in heavy metal bioavailability and speciation from a Pb-Zn mining soil amended with biochars from co-pyrolysis of rice straw and swine manure. *The Science of the Total Environment*, **633**(1): 300–307.

Methneni, N., Morales-Gonzalez, J.A., Jaziri, A., Mansour, H.B., and FernandezSerrano, M. (2021). Persistent organic and inorganic pollutants in the effluents from the textile dyeing industries: ecotoxicology appraisal via a battery of biotests. *Environmental Research*, **196**(1): 110-156.

Misra, V., and Chaturvedi, P. K. (2007). Plant uptake/bioavailability of heavy metals from the contaminated soil after treatment with humus soil and hydroxyapatite. *Environmental Monitoring and Assessment*, **133**(3): 169–176.

Mobasherpour, I., Salahi, E., and Pazouki, M. (2011). Removal of nickel (II) from aqueous solutions by using nano-crystalline calcium hydroxyapatite. *Journal of Saudi Chemical Society*, **15**(2): 105-112.

Mobasherpour., I., Salahi, E., and Pazouki, M. (2012). Comparative of the removal of Pb2+, Cd2+ and Ni2+ by nano crystallite hydroxyapatite from aqueous solutions: adsorption isotherm study. *Arabian Journal of Chemistry*, **5**(4): 439–446.

Mondal, S., Pal, U., and Dey, A. (2016). Natural origin hydroxyapatite scaffold as potential bone tissue engineering substitute. *Ceramics International*, **42**(16): 18338–18346.

Mousa, S.M., Ammar, N.S., and Ibrahim, H.A. (2016). Removal of lead ions using hydroxyapatite nanomaterial prepared from phosphogypsum waste. *Journal of Saudi Chemical Society,* **20**(3): 357–365.

Ndeddy-Aka, R. J., and Babalola, O. O. (2016). Effect of bacterial inoculation of strains of pseudomonas aeruginosa, alcaligenes feacalis and bacillus subtilis on germination, growth and heavy metal (Cd, cr, and Ni) uptake of brassica juncea. *International Journal of Phytoremediation,* **18**(2): 200–209.

Nivetha, N., Srivarshine, B., Sowmya, B., Rajendiran, M., Saravanan, P., Rajeshkannan, R., Rajasimman, M., Pham, T.H.T., Shanmugam, V., and Dragoi, E.N. (2022). A comprehensive review on bio-stimulation and bioenhancement towards remediation of heavy metals degeneration. *Chemosphere*, **312**(1):137-199.

Obiora, S.C., Chukwu, A., and Davies, T.C. (2016). Heavy metals and health risk assessment of arable soils and food crops around Pb–Zn mining localities in Enyigba, Southeastern Nigeria. *Journal of African Earth Sciences*, **116**(1): 182–189.

Ogunlaja, A., Ogunlaja, O.O., Okewole, D.M., and Morenikeji, O.A. (2019). Risk assessment and source identification of heavy metal contamination by multivariate and hazard index analyses of a pipeline vandalised area in Lagos state, Nigeria. *The Science of The Total Environment*, **651**(1): 2943–2952.

Ojuederie, O. B., and Babalola, O. O. (2017). Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. *International Journal of Environmental Research and Public Health*, **14**(12): 15-24.

Patel, A. K., Singhania, R. R., Albarico, F. P. J. B., Pandey, A., Chen, C.W., and Dong, C.D. (2022). Organic wastes bioremediation and its changing prospects. *The Science of The Total Environment,* **824**(2):153-192.

Prabagar, S., Dharmadasa, R.M., Lintha, A., Thuraisingam, S., and Prabagar, J. (2021). Accumulation of heavy metals in grape fruit, leaves, soil and water: a study of influential factors and evaluating ecological risks in jaffna, Sri Lanka. *Environmental and Sustainability Indicators,* **12**(9): 100-147.

Prakasam, M., Locs, J., Salma-Ancane, K., Loca, D., Largeteau, A., and Berzina-Cimdina, L. (2015). Fabrication, properties and applications of dense hydroxyapatite: a review. *Journal of Functional Biomaterials,* **6**(4): 1099–1140.

Pratush, A., Kumar, A., and Hu, Z. (2018). Adverse effect of heavy metals (As, Pb, Hg, and cr) on health and their bioremediation strategies: a review. *Microbiology,* **21** (3): 97–106.

Sales-da-Silva, I.G., Gomes-de-Almeida, F.C., Padilha-da-Rocha-Silva, N.M., Casazza, A.A., Converti, A., and Sarubbo, L.A. (2020). Soil bioremediation: overview of technologies and trends. *Energies,* **13**(1): 46-64.

Sarmah, P., and Rout, J. (2020). Role of algae and cyanobacteria in bioremediation: prospects in polyethylene biodegradation, in advances in cyanobacterial biology, Elsevier, Amsterdam, pp349.

Saxena, G., Kishor, R., and Bharagava, R. N. (2020). Application of microbial enzymes in degradation and detoxification of organic and inorganic pollutants, in bioremediation of industrial waste for environmental safety, Springer, New York, pp51.

Sayed, K., Baloo, L., and Sharma, N. K. (2021). Bioremediation of total petroleum hydrocarbons (TPH) by bioaugmentation and biostimulation in water with floating oil spill containment booms as bioreactor basin. *International Journal of Environmental Research and Public Health*, **18**(5): 22-26.

Sedlakova-Kadukova, J., Kopcakova, A., Gresakova, L., Godany, A., and Pristas, P. (2019). Bioaccumulation and biosorption of zinc by a novel streptomyces K11 strain isolated from highly alkaline aluminium brown mud disposal site. *Ecotoxicology and Environmental Safety,* **167**(1): 204–211.

Sharma, P., Dutta, D., Udayan, A., Nadda, A. K., Lam, S. S., and Kumar, S. (2022). Role of microbes in bioaccumulation of heavy metals in municipal solid waste: impacts on plant and human being. *Environmental Pollution*, **305**(4): 119-248.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. and Gobi, T.A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus for managing phosphorus deficiency in agricultural soils. *SpringerPlus,* **2**(1): 587-598.

Son, J.S., Appleford, M., Ong, J.L., Wenke, J.C., Kim, J.M., Choi, S.H., and Oh, D.S. (2011). Porous hydroxyapatite scaffold with three-dimensional localized drug delivery system using biodegradable microspheres. *Journal of Controlled Release*, **153**(2): 133–140.

Sonune, N. (2021). Microbes: a potential tool for bioremediation, in Rhizobiont in bioremediation of hazardous waste. In: Vivek, K, Ram, P and Manoj, K. (eds) Phytoremediation: Management of Environmental Contaminants Vol 3 Springer, Singapore, pp407.

Stötzel, C., Müller, F.A., Reinert, F., Niederdraenk, F., Barralet, J.E., and Gbureck, U. (2009). Ion adsorption behaviour of hydroxyapatite with different crystallinities. *Colloids and Surfaces B: Biointerfaces*, **74**(1): 91–95.

Sun, L., Guo, D., Liu, K., Meng, H., Zheng, Y., Yuan, F., and Zhu, G. (2019). Levels, sources, and spatial distribution of heavy metals in soils from a typical coal industrial city of Tangshan, China. *Catena,* **175**(1): 101–109.

Sun, W., Cheng, K., Sun, K. Y., and Ma, X. (2021). Microbially mediated remediation of contaminated sediments by heavy metals: a critical review. *Current Pollution Reports*, **7**(4): 201–212.

Tak, H. I., Ahmad, F., and Babalola, O. O. (2012). Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. *Reviews of Environmental Contamination and Toxicology,* **223**(1): 33–52.

Tang, A., Haruna, A., Majid, N., and Jalloh, M. (2020). Potential PGPR properties of cellulolytic, nitrogen-fixing, phosphate-solubilizing bacteria in rehabilitated tropical forest soil. *Microorganisms,* **8**(3): 44-52.

Tayang, A., and Songachan, L.S. (2021). Microbial bioremediation of heavy metals. *Current science*, **120**(6): 1013–1025.

Timofeeva, A., Galyamova, M., and Sedykh, S. (2022). Prospects for using phosphate-solubilizing microorganisms as natural fertilizers in agriculture. *Plants,* **11**(16): 21-29.

Unuofin, J. O., Okoh, A. I., and Nwodo, U. U. (2019). Aptitude of oxidative enzymes for treatment of wastewater pollutants: a laccase perspective. *Molecules,* **24**(11): 20-64.

Valipour, M., Shahbazi, K., and Khanmirzaei, A. (2016). Chemical immobilization of lead, cadmium, copper, and nickel in contaminated soils by phosphate amendments. *Clean-Soil, Air, Water*, **44**(5): 572–578.

Verma, S., and Kuila, A. (2019). Bioremediation of heavy metals by microbial process. *Environmental Technology & Innovation,* **14**(1): 100-369.

Vila, M., Sánchez-Salcedo, S., Cicuéndez, M., Izquierdo-Barba, I., and Vallet-Regí, M. (2011). Novel biopolymer-coated hydroxyapatite foams for removing heavy-metals from polluted water. *Journal of Hazardous Materials*, **192**(1): 71–77.

Vocciante, M., Finocchi, A., De Folly D′ Auris, A., Conte, A., Tonziello, J., Pola, A., and Reverberi, A.P. (2019). Enhanced oil spill remediation by adsorption with interlinked multilayered graphene. *Materials*, **12**(14), 22-31.

Wang, L., Liu, Y., Shu, X., Lu, S., Xie, X., and Shi, Q. (2019). Complexation and conformation of lead ion with poly-g-glutamic acid in soluble state. *Public library of Science One*, **14**(9): 1-22.

Wei, L., Wang, S., Zuo, Q., Liang, S., Shen, S., and Zhao, C. (2016). Nano-hydroxyapatite alleviates the detrimental effects of heavy metals on plant growth and soil microbes in e-waste contaminated soil. *Environmental Science: Processes and Impacts,* **18**(6): 760-767.

Wei, Y., Zhao, Y., Shi, M., Cao, Z., Lu, Q., Yang, T., Fang, Y., and Wei, Z. (2018). Effect of organic acids production and bacterial community on the possible mechanism of phosphorus solubilization during composting with enriched phosphate-solubilizing bacteria inoculation. *Bioresource Technology*, **247**(10): 190–199.

Xiao, F., Cheng, J., Cao, W., Yang, C., Chen, J., and Luo, Z. (2019). Removal of heavy metals from aqueous solution using chitosan-combined magnetic biochars. *Journal of Colloid for Interface Science*. **540**(1): 579–584.

Xu, Q., Wu, B., and Chai, X. (2022). In situ remediation technology for heavy metal contaminated sediment: a review. *International Journal of Environmental Research and Public Health,* **19**(24): 1-19.

Zacharia, J. T. (2019). Degradation pathways of persistent organic pollutants (POPs) in the environment. *Persistent Organic Pollutants*, 17–30.

Zhang, C., Chen, H., Dai, Y., Chen, Y., Tian, Y., and Huo, Z. (2023). Isolation and screening of phosphorus solubilizing bacteria from saline alkali soil and their potential for Pb pollution remediation. *Frontiers in Bioengineering and Biotechnology,* **11**(1): 11-34.

Zhu, Y., Xing, Y., Li, Y., Jia, J., Ying, Y., and Shi, W. (2024). The role of phosphate solubilizing microbial interactions in phosphorus activation and utilization in plant-soil systems: a review. *Plants,* **13**(19): 2686-2690.

Zhuo, H., Fu, S., Liu, H., Song, H., and Ren, L. (2019). Soil heavy metal contamination and health risk assessment associated with development zones in Shandong, China. *Environmental Science and Pollution Research*, **26**(29): 30016–30028.

Zwolak, A., Sarzyńska, M., Szpyrka, E., and Stawarczyk, K. (2019). Sources of soil pollution by heavy metals and their accumulation in vegetables: a review. *Water Air and Soil pollution*, **230**(7), 1–9.