BIOAUGMENTATION OF HEAVY METAL (Ni, Pb, Cr) CONTAMINATED SOIL USING HYDROXYAPATITE AND CITROBACTER *spp*.

BY

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UNIVERSITY OF BENIN

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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.

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this research titled **BIOAUGMENTATION OF HEAVY METAL (Ni,Pb,Cr) CONTAMINATED SOIL USING HYDROXYAPATITE AND CITROBACTER,** was carried out by **ESIJOLOMI MALAIKA SHILONI** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirement for award of Bachelor of Science (B.Sc.) in Environmental Management and Toxicology. It was conducted under stable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

Dr. (Mrs.) G. E. Ogbeide	**DATE**
(PROJECT SUPERVISOR)	

PROF. (MRS) AISIEN E. T.	**DATE**
(HEAD OF DEPARTMENT)	

DECLARATION

I, Esijolomi Malaika Shiloni declare that Bioaugmentation of heavy metal (Ni, Pb, Cr) contaminated soil using hydroxyapatite and citrobacter. is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

ESIJOLOMI MALAIKA SHILONI **DATE**

DEDICATION

This work is dedicated to God Almighty, whose wisdom, strength, and grace made this journey possible. To my dear parent,whose unwavering love, sacrifices, and prayers have been my greatest source of inspiration. Your support and guidance have shaped me into the person I am today. This achievement is as much yours as it is mine.

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I wish to register my profound gratitude to God Almighty,coming this far would not have been possible without his help. Hence, I give glory to God alone for the knowledge, wisdom, and strength He has granted me. I sincerely express my heartfelt appreciation to my Dad,Mr Benjamin Daniel Esijolomi,and to my mom, Mrs Helen Daniel Benjamin . Thank you for always encouraging me and patting my back all the way.To my siblings, Esijolomi Divine, Esijolomi Favour, Esijolomi Praise, and Esijolomi Peculiar , thank you all for your best wishes, words of encouragement, love, and prayers.

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ABSTRACT

The contamination of soils with heavy metals from anthropogenic activities like automobile repair poses a significant threat to ecosystem and human health. This study investigated the effectiveness of hydroxyapatite (HAp) and phosphate-solubilizing bacteria (PSB) for the remediation of heavy metals in a contaminated mechanic workshop soil in Ekosodin, Benin City, Nigeria. Soil samples were collected and subjected to four treatments: untreated soil (control), soil amended with PSB only, soil amended with HAp only, and a combined treatment of soil with both HAp and PSB. Hydroxyapatite was synthesized from cow bones via thermal calcination, and indigenous PSB were isolated from the contaminated soil. The concentrations of Chromium (Cr), Lead (Pb), and Nickel (Ni) were analyzed using Atomic Absorption Spectrophotometry, and microbial counts were monitored over the experimental period.The results demonstrated that the combined application of HAp and PSB was the most effective treatment, achieving the highest percentage removal efficiencies: 38.65% for Cr, 29.05% for Pb, and 33.12% for Ni. Individual applications of PSB or HAP showed significantly lower remediation effects. Furthermore, the combined treatment (Soil+PSB+HAp) supported a higher total heterotrophic bacterial count ($2.75 \pm 0.71 \times 10^6$ CFU/g) compared to the PSB-only treatment ($2.50 \pm 0.00 \times 10^6$ CFU/g), indicating improved soil microbial activity. The study concludes that the synergy between hydroxyapatite and phosphate-solubilizing bacteria enhances the immobilization of heavy metals and promotes a healthier soil microbiome, presenting a promising, eco-friendly strategy for remediating soils co-contaminated with heavy metals .

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Soil contamination by petroleum hydrocarbons, heavy metals (e.g.,Ni,Pb,Cr), and agro-industrial wastes threatens agro-ecosystem services, food safety, and human health. Conventional remediation (soil excavation, washing, or chemical extraction) often incurs high cost, can degrade soil structure, and risks secondary pollution, hence the pivot to immobilization and bioaugmentation approaches that preserve soil function (Adeleye *et al*., 2024; Zhang *et al*., 2025). Hydroxyapatite (HAp; $Ca_{10}(PO_4)_6(OH)_2$) has emerged as a low-cost, phosphate-rich mineral amendment capable of immobilizing cationic metals through surface complexation, ion exchange, and the formation of highly insoluble metal-phosphates (e.g., pyromorphite for Pb), thereby reducing metal lability and bioavailability while buffering soil pH (Xiong *et al*., 2009; Dinh *et al*., 2025; Wu *et al*., 2025). In recent, phosphate solubilizing bacteria (PSB) notably Bacillus, Pseudomonas, and Citrobacter *spp*. mobilize sparingly soluble phosphorus minerals by secreting organic acids (gluconic, citric), phosphatases, and siderophores. This biochemical activity can enhance plant phosphorus nutrition, stimulate rhizosphere processes, and indirectly mitigate metal stress via pH shifts and metal organic complexation (Sahu and Padhy, 2023; Zhang *et al*., 2025).

Recent studies and reviews indicate that combining phosphate minerals (including HAp) with PSB or broader phosphate-solubilizing microorganisms can couple immobilization (lowering metal bioavailability) with biostimulation (improving nutrient cycling), potentially accelerating soil recovery under mixed contamination (heavy metals with hydrocarbons or salts) (Emami *et al*., 2024; Taskin *et al*., 2025; Zhou *et al*., 2025). Moreover, nano and composite forms of HAp (e.g., biochar-supported nHAp) improve dispersion and reactive surface area, further strengthening immobilization while offering microhabitats for beneficial microbes (Liu *et al*., 2024; Guo *et al*., 2025).

1.2 Statement of the Problem

Escalating contamination from industrial effluents, mining, and petroleum related activities leaves soils with elevated metal loads and often co-occurring nutrient deficiencies. Conventional technologies can be prohibitive and may not restore soil biological health. Empirical gaps remain on (i) the long-term stability of metal immobilization by HAp under field pH/moisture fluctuations, and (ii) the reproducibility of PSB performance outside controlled conditions. Critically, integrated HAp+PSB strategies though promising require systematic assessment of synergy, dosing, and persistence under real soil matrices (Dinh *et al*., 2025; Liu *et al*., 2024; Zhang *et al*., 2025b).

1.3 Aim of the Study

To investigate the effectiveness of hydroxyapatite and phosphate-solubilizing bacteria individually and in combination in remediating contaminated soil and improving soil quality indices.

1.4 Specific Objectives

1. Isolate and characterize PSB from representative soil samples.

2. Evaluate HAp’s capacity to immobilize priority metals (e.g., Pb, Cr, Ni) and reduce their bioavailable fractions.

3. Assess HAp+PSB co-application for synergy in contaminant immobilization and soil function restoration.

4. Determine changes in soil fertility metrics (available P, pH, cation exchange capacity,

1.5 Significance of the Study

This study addresses a practical need for cost-effective, eco-friendly remediation that maintains soil ecological function. HAp offers durable immobilization of cationic metals via phosphate precipitation, while phosphate solubilizing bacteria (PSB) reinforces nutrient availability and microbial resilience. Demonstrating robust HAp+PSB synergy would inform guidelines for dose optimization, inoculation strategy, and field deployment particularly relevant to agricultural soils impacted by metal inputs and to mixed-contaminant sites where conventional methods are impractical (Adeleye *et al*., 2024; Dinh *et al*., 2025; Zhang *et al*., 2025).

1.6 Scope of the Study

The work focuses on soils contaminated predominantly by hydrocarbons and also heavy metals, evaluating;HAp alone, PSB alone, and HAp+PSB. Endpoints include metal bioavailability (e.g., DTPA-extractable Pb/Cd), leachability under varying pH, and soil fertility/microbial metrics. Plant growth trials may be scoped as a confirmatory assay depending on resources and time.

CHAPTER TWO

2.1 Introduction

In recent decades, anthropogenic activities such as mining, industrial production, smelting, waste disposal, and the use of agrochemicals have led to widespread contamination of soils by heavy metals (Wuana and Okieimen, 2011; Ali, Khan and Ilahi, 2019). Among heavy metals, nickel (Ni) and lead (Pb) are particularly problematic due to their toxicity, persistence in soil matrices, and ability to bioaccumulate through the food chain (Nagajyoti, Lee and Sreekanth, 2010). Unlike many organic pollutants, heavy metals do not degrade; instead, they undergo physicochemical transformations, but remain as variants whose mobility, speciation, and bioavailability depend heavily on soil conditions (Alloway, 2013). Elevated concentrations of Ni and Pb in soils compromise soil health, reduce microbial activity, impair plant growth, and pose threats to human and animal health via dietary uptake (Ali *et al*., 2019; Nagajyoti *et al*., 2010).

Traditional remediation techniques such as excavation, soil washing, chemical stabilization, and thermal treatments present significant drawbacks, including high costs, energy consumption, secondary pollution, and limited applicability to large in situ systems (Wuana and Okieimen, 2011; Khan, Cao and Zheng, 2007). As a result, there is increasing interest in green, sustainable, and cost-effective remediation strategies that work in situ, minimize disturbance, and harness natural processes. Two promising approaches in this aspect are the use of hydroxyapatite (HAP) as an immobilizing amendment and phosphate-solubilizing bacteria (PSB) particularly Citrobacter *spp*. as bioagents to immobilize metals, enhance soil function, and promote plant health.

Hydroxyapatite, a calcium phosphate mineral with formula $Ca_{10}(PO_4)_6(OH)_2$, has been widely studied for its ability to trap heavy metal ions via precipitation, ion exchange, and adsorption (He *et al.*, 2025; Brahmi *et al.*, 2025; Brazdis *et al.*, 2021). Owing to its relatively low solubility under many soil pH conditions and strong affinity for divalent metal cations, HAP has shown potential to immobilize Pb^{2+}, Ni^{2+}, and other metals into stable phosphate minerals or surface complexes (He *et al.*, 2025; Caldiz *et al.*, 2022; Nún‡ez *et al.*, 2019). This immobilization reduces metal mobility and bioavailability, thereby mitigating ecotoxicological risks.

On the biological side, phosphate-solubilizing microorganisms (PSM), including bacteria (PSB) and fungi (PSF), play critical roles in phosphorus cycling in soils and have gained recognition for their capacity to remediate heavy metal pollution (Hu and Chen, 2023; Ahemad, 2015; Pham *et al.*, 2023). PSB can release organic acids, phosphatases, and chelators to solubilize insoluble phosphates, thereby releasing phosphate anions (PO_4^{3-}) that can react with heavy metal ions in solution to form insoluble metal phosphates (e.g. $Pb_3(PO_4)_2$, $Ni_3(PO_4)_2$) (Hu and Chen, 2023;Ahemad, 2015; Pham *et al.*, 2023). PSB can release organic acids, phosphatases, and chelators to solubilize insoluble phosphates, thereby releasing phosphate anions (PO_4^{3-}) that can react with heavy metal ions in solution to form insoluble metal phosphates (e.g. $Pb_3(PO_4)_2$, $Ni_3(PO_4)_2$) (Hu and Chen, 2023; Sharma *et al.*, 2013; Pham *et al.*, 2023). Simultaneously, PSB may also bind, sequester or transform heavy metals via cell wall biosorption, exopolysaccharide complexation, redox reactions, or intracellular accumulation (Hu and Chen, 2023; Bioremediation by Bacillus, 2023).

2.2.1 Nature of Soil Contamination

The nature of soil contamination depends on the type of contaminant, its chemical behavior, and the soil's physicochemical properties. Soil contamination can be defined as the presence of hazardous substances at concentrations high enough to pose risks to human health, plants, and ecological systems (Alloway, 2013). Contaminants may be present in soils in dissolved, adsorbed, or precipitated forms, and their distribution depends on soil pH, redox potential, organic matter content, and texture (Adriano, 2001).

Heavy metals like Ni and Pb typically bind to negatively charged clay and organic matter particles. In acidic soils, these metals become more mobile and bioavailable, increasing their potential toxicity (Kabata-Pendias and Mukherjee, 2007). Conversely, in alkaline soils, metals tend to precipitate as hydroxides, carbonates, or phosphates, reducing their mobility (Wuana and Okieimen, 2011). This variability illustrates that soil contamination is not only about the presence of pollutants but also about their bioavailability, which determines ecological risk.

The degree of contamination is therefore influenced by both natural background levels and anthropogenic enrichment. For instance, lead concentrations in uncontaminated soils typically range between 10 and 30 mg/kg, but industrial or urban soils can exceed 1000 mg/kg (Khan *et al*., 2008). Similarly, nickel occurs naturally at 5–500 mg/kg, but in polluted areas near mining or smelting operations, it can exceed 2000 mg/kg (Ali *et al*., 2019).

2.2.2 Natural Sources of Soil Contamination

While anthropogenic activities dominate modern contamination, natural processes also contribute to the accumulation of trace metals in soils. These processes establish the geochemical baseline concentrations of metals in the environment. Major natural sources include weathering of parent materials, volcanic emissions, forest fires, atmospheric dust deposition, and biogeochemical cycling.

a. Weathering of Parent Rock Material

Weathering is the gradual disintegration and chemical alteration of rocks that release trace elements into the soil. As rocks undergo physical, chemical, or biological breakdown, heavy metals such as Ni, Pb, Cu, and Zn are mobilized and incorporated into soil matrices (Kabata-Pendias and Mukherjee, 2007). For example, ultramafic and mafic rocks rich in olivine and pyroxene minerals often contain high levels of nickel and chromium. When these rocks weather, the released Ni may accumulate in nearby soils, particularly under humid conditions (Adriano, 2001).

Similarly, Pb is naturally present in galena (PbS) and anglesite ($PbSO_4$) minerals within sedimentary formations (Alloway, 2013). Over time, leaching and oxidation release Pb ions into soil solutions. However, under natural conditions, these metals usually remain at low concentrations and are distributed uniformly, posing minimal toxicity risks (Kabata-Pendias, 2010).

b. Volcanic Activity

Volcanic eruptions contribute significantly to natural heavy metal emissions. Volcanic gases and ash contain elevated levels of Pb, Ni, Cd, Cu, and Hg, which are deposited onto soils and vegetation through wet or dry deposition (Nriagu, 1996). For instance, volcanic soils in Japan, Indonesia, and Italy show higher than average concentrations of Pb and Ni due to continuous input from volcanic aerosols (Alloway, 2013). These emissions can travel long distances and affect soil quality in distant regions.

Although volcanic inputs enrich the soil with trace metals, they also contribute to the formation of fertile and mineral-rich soils when combined with organic matter accumulation (Adriano, 2001).

c. Forest Fires

Forest fires are another natural process that can elevate metal concentrations in soils. The combustion of biomass releases trace elements that were stored in plant tissues into the atmosphere and soil (López *et al*., 2009). Elements like Pb, Cu, and Ni volatilize during burning and later condense onto soil particles. Post-fire soils often exhibit increased metal concentrations in surface layers due to ash deposition.

However, unlike industrial emissions, the impact of fires is usually localized and diminishes over time as vegetation regrows (Reimann and de Caritat, 2005).

d. Atmospheric Dust Deposition

Windblown dust contributes to long distance transport of mineral particles enriched with metals. Dust originating from arid regions, deserts, or eroded mining areas can deposit heavy metals far from the source (Kabata-Pendias and Mukherjee, 2007). For example, Saharan dust has been found to transport significant quantities of Fe, Mn, and Pb across the Atlantic to the Americas (Reimann and de Caritat, 2005).

2.2.3 Anthropogenic Sources of Soil Contamination

Anthropogenic, or human induced activities are the dominant contributors to soil contamination in recent decades. Industrialization, mining, urbanization, agricultural intensification, and waste mismanagement have resulted in the release of enormous quantities of heavy metals and other toxic compounds into terrestrial ecosystems (Wuana and Okieimen, 2011).

Below are the main anthropogenic sources:

a. Industrial Activities

Industrialization is one of the foremost sources of heavy metal contamination in soils. Industries such as metal smelting, electroplating, tanneries, battery manufacturing, chemical production, and cement factories release effluents and solid wastes rich in Pb, Ni, Cd, Zn, and Cr (Li *et al*., 2014).

For instance, lead acid battery recycling plants discharge large quantities of Pb through airborne emissions and wastewater. Similarly, nickel electroplating industries produce effluents containing high Ni concentrations that can percolate into surrounding soils (Ali *et al*., 2019).

Industrial emissions contaminate soils both directly, through solid waste dumping and effluent discharge, and indirectly, through atmospheric deposition of particulates. In areas downwind of smelters, Pb and Ni concentrations can exceed safe limits by several hundred folds (Khan *et al*., 2008).

The persistence of metals in these soils makes remediation difficult. Metals bind to clay and organic matter, remain bioavailable for decades, and can enter food chains through crops grown nearby (Adriano, 2001).

b. Mining and Metallurgical Operations

Mining exposes and mobilizes metals from deep geological strata into the biosphere. During ore extraction and processing, rocks are crushed and ground, releasing fine dust and tailings rich in metals (Lottermoser, 2010). Waste from mining operations known as mine tailings often contains residual Pb, Ni, and Cd that can leach into soil and groundwater through acid mine drainage (Singh *et al*., 2010).

For example, Ni and Pb mines in Nigeria, Zambia, and China have resulted in severe soil contamination in nearby farmlands, with metal concentrations far exceeding World Health

Organization (WHO) limits (Wuana and Okieimen, 2011). Similarly, abandoned mine sites continue to pose long-term contamination risks due to the slow natural weathering of waste piles (Li *et al*., 2014).

c. Agricultural Practices

Agriculture contributes to soil contamination through the extensive use of fertilizers, pesticides, manure, and irrigation with contaminated water (Nagajyoti, Lee and Sreekanth, 2010).

Phosphate fertilizers are particularly important because they contain impurities such as Cd, Pb, and Ni derived from phosphate rock (Alloway, 2013). Continuous fertilizer application increases the accumulation of these metals in topsoil. Similarly, pesticides such as lead arsenate, copper sulfate, and organotin compounds introduce additional toxic elements into agricultural lands (Adriano, 2001).

Moreover, in many developing regions, wastewater irrigation is a common practice to supplement freshwater scarcity. However, untreated industrial or municipal wastewater often contains high levels of Ni, Pb, Zn, and Cu, which accumulate in soils and crops (Muchuweti *et al*., 2006).

Sewage sludge, used as organic fertilizer, also contains elevated concentrations of metals due to industrial discharges into municipal sewage systems (Wuana and Okieimen, 2011). Long-term sludge application can lead to metal buildup in soils and bioaccumulation in crops, thereby posing public health risks (Ali *et al*., 2019).

d. Urbanization and Waste Disposal

Urbanization and poor waste management contribute significantly to soil contamination. In rapidly growing cities, solid waste dumpsites, construction debris, and open burning release toxic substances that infiltrate surrounding soils (Khan *et al*., 2008).

Municipal solid waste, which includes batteries, electronic waste, and paints, contains Pb, Ni, Cu, and Zn. When disposed of improperly, leachates containing these metals migrate into the soil profile (Li *et al*., 2014). Furthermore, landfills lacking proper liners allow percolation of heavy metal laden leachates into both soil and groundwater.

Sewage sludge and domestic waste used as fertilizers introduce metals and organic pollutants into agricultural land near urban centers (Muchuweti *et al*., 2006). The combined effect of urban and industrial emissions leads to elevated background metal levels in metropolitan soils.

e. Vehicular Emissions

Vehicular traffic is another important contributor to soil contamination, especially in urban areas. Although the global phase out of leaded gasoline has reduced Pb emissions, roadside soils remain contaminated with residual Pb and other metals (Charlesworth *et al*., 2011).

Nickel is emitted from fuel combustion and lubricating oils, while Zn, Cu, and Cd originate from tire wear, brake pads, and engine corrosion (Sutherland, 2003). These metals settle as fine particles on road surfaces and adjacent soils, leading to contamination gradients that decrease with distance from highways (Wuana and Okieimen, 2011).

Rainfall events wash these particulates into drainage systems and farmlands, extending the pollution footprint. Chronic exposure to roadside contamination poses risks to nearby vegetation and humans through dust inhalation and food consumption (Ali *et al*., 2019).

f. Waste Incineration and Industrial Landfills

Incineration of municipal and industrial waste generates fly ash and bottom ash rich in metals such as Pb, Zn, Cd, and Ni. These ashes, if not properly disposed of, contaminate soils around incineration facilities (Alloway, 2013). Industrial landfills often store hazardous waste without adequate containment, allowing leaching of metals into surrounding soils and aquifers (Khan *et al*., 2008).

In regions without strict environmental regulations, illegal dumping of electroplating sludge, tannery waste, and metallurgical residues exacerbates the problem (Li *et al*., 2014). These contaminated sites serve as long-term sources of secondary pollution.

2.3.1 Definition and Classification of Heavy Metals

Heavy metals are a group of naturally occurring elements characterized by high atomic weight and density, typically greater than 5 g cm^{-3} (Alloway, 2013). They include both essential and non-essential elements that exist in trace concentrations within the Earth's crust and biological systems. Essential heavy metals, such as iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu), play critical roles in enzymatic and physiological processes. Conversely, non-essential metals like cadmium (Cd), mercury (Hg), lead (Pb), and nickel (Ni) are toxic even at low concentrations and have no known biological function (Nagajyoti *et al*., 2010).

The term "heavy metal" is somewhat ambiguous in scientific literature due to the broad range of elements it encompasses. Some researchers have proposed alternative classifications based on atomic number, chemical behavior, or toxicological characteristics rather than density alone (Duffus, 2002). For instance, elements such as arsenic (As) and selenium (Se) are sometimes included under the category of heavy metals despite being metalloids, due to their similar environmental behavior and toxicity profiles (Kabata-Pendias, 2011).

In the soil environment, heavy metals originate from both natural geogenic sources such as parent rock weathering and anthropogenic activities, including industrial emissions, mining, agricultural inputs, and waste disposal (Wuana and Okieimen, 2011). Their concentrations and speciation in soils are influenced by soil physicochemical properties such as pH, organic matter content, redox potential, and texture.Heavy metals are typically classified into the following categories based on biological essentiality and toxicity (Adriano, 2001):

Essential Micronutrients

Essential micronutrients are trace elements that, although required in minute quantities, are indispensable for the proper functioning, growth, and reproduction of plants, microorganisms, and animals. Among the most significant of these micronutrients in the soil environment are iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo), and nickel (Ni). Their dual nature serving as both vital nutrients at low concentrations and potential toxins at elevated levels makes them particularly important in environmental toxicology and soil biogeochemistry (Alloway, 2013; Kabata-Pendias and Pendias, 2011).

Role and Function of Essential Micronutrients

Each of these elements contributes uniquely to cellular metabolism, enzymatic catalysis, and physiological stability within biological systems. Iron (Fe), for instance, is a critical component of cytochromes and other heme-containing proteins involved in electron transport during cellular respiration and photosynthesis (Rout and Sahoo, 2015). Plants rely on Fe for chlorophyll synthesis, energy transfer, and nitrogen assimilation. Deficiency in iron often manifests as interveinal chlorosis in leaves, reducing photosynthetic efficiency and plant productivity (Marschner, 2012). However, excessive iron accumulation, particularly in waterlogged or acidic soils, can lead to oxidative stress and damage cellular membranes through the Fenton reaction, which produces reactive oxygen species (ROS) (Gao *et al*., 2019).

Manganese (Mn)

This is another crucial micronutrient that serves as a cofactor in several enzymatic systems, including those involved in photosystem II (PSII) water-splitting reactions and oxidative stress detoxification. Mn activates key enzymes such as dehydrogenases, decarboxylases, and kinases (Millaleo *et al*., 2010). Deficiency of Mn results in impaired photosynthetic efficiency and reduced lignin biosynthesis, while excess Mn, often in acidic soils, can interfere with Fe and Mg uptake, resulting in toxicity symptoms like brown spots on leaves and stunted growth (Fernando *et al*., 2021).

Copper (Cu)

Copper functions as a redox active metal, playing a vital role in photosynthesis, respiration, and the detoxification of superoxide radicals through the enzyme superoxide dismutase (SOD) (Yruela, 2009). It is also essential in lignin synthesis and reproductive growth. However, Cu exhibits a narrow range between deficiency and toxicity. Excess copper disrupts cellular membranes, inhibits root elongation, and causes chlorosis by interfering with Fe uptake and photosynthetic processes (Nagajyoti *et al*., 2010). Soils contaminated by industrial activities such as mining and the application of copper-based fungicides are particularly susceptible to Cu toxicity (Wuana and Okieimen, 2011).

Zinc (Zn)

Zinc is an essential element for the structural and catalytic activity of numerous enzymes and transcription factors. It plays a central role in DNA transcription, protein synthesis, and auxin metabolism (Broadley *et al*., 2007). Zinc deficiency leads to stunted growth, small leaves, and reduced fertility in plants due to impaired hormonal balance and enzyme activation (Alloway, 2008). Conversely, high levels of zinc can compete with other essential nutrients such as Fe, Mn, and Cu, causing secondary deficiencies and oxidative damage to plant cells (Cakmak, 2000).

Molybdenum (Mo)

Though required in the smallest quantity of all micronutrients, is indispensable for the catalytic functioning of nitrate reductase and nitrogenase enzymes, both of which are crucial in nitrogen assimilation and fixation (Mendel and Bittner, 2006). A lack of Mo results in nitrogen deficiency-like symptoms, such as chlorosis and necrosis, especially in legumes where symbiotic nitrogen fixation is inhibited. However, excess Mo, though rare, can lead to secondary copper deficiency in grazing animals feeding on Mo-rich forages (Gupta and Lipsett, 1981).

2. Non-essential Toxic Elements:

Lead (Pb)

Lead contamination in soil primarily comes from human activities such as mining, smelting, and the use of lead based paints (Khan *et al*., 2018). Old infrastructure, industrial waste, and certain fertilizers can also contribute to lead accumulation.Lead is highly toxic to humans, especially children, and can cause developmental delays, learning disabilities, and organ damage (Lanphear *et al*., 2005). In plants, lead can interfere with root growth, photosynthesis, and nutrient uptake, leading to reduced crop yields (Sharma and Dubey, 2005).

Cadmium (Cd)

Cadmium enters the soil mainly through industrial pollution, including metal smelting and refining (Järup, 2003). Phosphate fertilizers and sewage sludge can also contain high levels of cadmium.Cadmium is known to accumulate in crops, particularly in grains and leafy vegetables, and can cause kidney damage and bone disease in humans (Satarug *et al*., 2010). In plants, cadmium can disrupt nutrient uptake and enzyme function (Das *et al*., 1997).

Mercury (Hg)

Mercury contamination in soil can arise from various sources, including industrial emissions, mining activities, and the use of mercury containing pesticides (Driscoll *et al*., 2013). Atmospheric deposition also plays a significant role in mercury contamination.Mercury is a potent neurotoxin that can bioaccumulate in the food chain, posing significant risks to human health and wildlife (Clarkson *et al*., 2003). In plants, mercury can interfere with photosynthesis and other physiological processes (Patra and Sharma, 2000).

Arsenic (As)

Arsenic contamination in soil can occur naturally through geological processes or through human activities such as mining, smelting, and the use of arsenic based pesticides (Mandal and Suzuki, 2002). Arsenic is a known carcinogen that can cause a range of health problems, including skin lesions, cardiovascular disease, and cancer (Smith *et al*., 2002). In plants, arsenic can disrupt nutrient uptake and interfere with metabolic processes (Meharg and Hartley-Whitaker, 2002).

3. Potentially Toxic but Biologically Useful Metals:

Chromium (Cr), cobalt (Co), and vanadium (V) are useful in trace concentrations but harmful at elevated levels. For instance, Cr(III) is a micronutrient, whereas Cr(VI) is a known carcinogen (Shanker *et al*., 2005).The environmental persistence and non-biodegradability of heavy metals make them unique contaminants. Unlike organic pollutants, they cannot be broken down into less toxic compounds but can only be transformed into different chemical forms with varying mobility and toxicity (Adriano, 2001).

2.4. Nickel (Ni)

Sources of Nickel in Soil

Nickel (Ni) is a transition metal naturally present in the Earth's crust at an average concentration of about 75 mg/kg, though this value can vary depending on parent rock material and pedogenic processes (Kabata-Pendias, 2011). Soils derived from ultramafic and serpentine rocks typically exhibit elevated Ni concentrations, often exceeding 1,000 mg/kg (Alloway, 2013). Natural weathering of these rocks represents a primary geogenic source of nickel input to the soil environment. Volcanic emissions and wind blown dust can also contribute trace amounts of Ni to surface soils (Adriano, 2001).However, the majority of soil nickel contamination today is anthropogenic in origin. Industrial and urban sources are particularly significant contributors. Nickel is used extensively in metallurgy, electroplating, stainless steel production, battery manufacturing, and fossil fuel combustion all of which release nickel into the environment either through atmospheric deposition or direct discharge (Nieminen, 2011). Nickel based alloys and catalysts are also employed in chemical industries, adding to environmental loadings.

2.5 Physical Remediation Method

Physical remediation methods involve the use of mechanical or engineering-based approaches to remove, separate, or contain contaminants in soil without necessarily altering their chemical structure. These techniques are often preferred for sites with high contaminant concentrations, especially heavy metals, where biological or chemical methods might be too slow or inefficient. Physical methods are usually implemented as the first line of remediation to reduce pollution load or to prepare the site for subsequent treatment (Kumar *et al.*, 2017).

Soil washing is one of the most widely applied physical remediation methods for contaminated soils. It involves excavating contaminated soil and using water, sometimes mixed with surfactants or chelating agents, to separate pollutants from soil particles. The process relies on the principle that contaminants particularly heavy metals tend to adsorb onto fine particles such as clays and silts. By suspending the soil in a washing solution and separating the coarse fraction (sand and gravel) from the fine fraction, a significant portion of the contamination can be removed (Mulligan *et al*., 2001).

Soil washing

There are generally two stages in soil washing: particle size separation and chemical extraction. The coarse fraction, once cleaned, can often be returned to the site, while the fine fraction containing concentrated contaminants may require further treatment or disposal in secure landfills (Dermatas and Meng, 2003). In heavy metal contaminated soils, chelating agents such as ethylenediaminetetraacetic acid (EDTA) are commonly used to enhance the solubility of metals like lead (Pb), cadmium (Cd), and nickel (Ni), promoting their removal from soil particles (Wasay *et al*., 2001). However, environmental concerns about the persistence and secondary pollution from synthetic chelants have led to research into biodegradable alternatives such as citric acid and rhamnolipids (Cameselle *et al*., 2013).

Excavation and off site deposal

Excavation and off-site disposal referred to as the “dig and dump” method is one of the oldest and simplest physical remediation approaches. It involves physically removing the contaminated soil and transporting it to a landfill or a treatment facility. This method provides immediate risk reduction since the contaminants are completely removed from the site (Nathanail and Bardos, 2004). Excavation is typically used for small areas with severe contamination, especially in urban or industrial sites where the pollutants are localized and pose direct exposure risks.

The process begins with site characterization and delineation of the contaminated zone. Heavy machinery such as excavators and loaders are used to remove the soil, which is then transported in sealed containers to prevent spillage or airborne spread of pollutants (Loures *et al*., 2016). In some cases, the excavated soil may undergo pre-treatment such as solidification or soil washing before disposal to minimize the hazard level (Alloway, 2013).

2.5.1 Chemical Remediation Method

Chemical remediation methods focus on altering the chemical composition, structure, or speciation of contaminants to reduce their mobility, toxicity, or bioavailability in the soil environment. These methods are particularly effective for inorganic pollutants such as heavy metals that do not biodegrade and therefore require transformation into stable, insoluble, or less reactive forms (Adriano *et al*., 2004). Unlike physical techniques that primarily remove or isolate the pollutants, chemical remediation methods work through immobilization, precipitation, or stabilization processes that neutralize or lock contaminants in the soil matrix.

The efficiency of these methods depends on several factors, including soil pH, redox potential, cation exchange capacity, and the specific chemical properties of the contaminants (Kumpiene *et al*., 2008). When carefully applied, chemical remediation offers a cost-effective and sustainable solution that improves soil quality and limits the risk of contaminant leaching into groundwater or uptake by plants.

Immobilization

Immobilization is a chemical process that reduces the mobility and bioavailability of contaminants by converting them into more stable and less soluble forms. This is typically achieved through the addition of immobilizing agents such as phosphates, lime, biochar, zeolites, clay minerals, or metal oxides (Udeigwe *et al*., 2011). These materials interact with heavy metals through adsorption, ion exchange, surface complexation, or precipitation reactions that limit their availability for biological uptake.

For example, phosphate-based amendments such as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) have been extensively studied for their ability to immobilize lead (Pb) and cadmium (Cd) in contaminated soils (Ma *et al*., 1995). The phosphate ions react with dissolved metal ions to form insoluble metal-phosphate minerals like pyromorphite [$Pb_5(PO_4)_3Cl$], which are highly stable under a wide range of environmental conditions (Chen *et al*., 2019). Similarly, lime ($CaCO_3$ or $Ca(OH)_2$) increases soil pH, promoting the precipitation of metal hydroxides and carbonates, thus decreasing metal solubility (Zeng *et al*., 2017).

Precipitation

Precipitation is one of the most common and effective chemical remediation strategies for heavy metal removal. The process involves transforming soluble metal ions into insoluble compounds, typically hydroxides, sulfides, or carbonates, which can then be separated or stabilized within the soil. Precipitation can occur naturally in soils with high pH or carbonate content, but engineered remediation enhances and controls the process through targeted chemical amendments (Fu and Wang, 2011).

A classic example is the addition of lime ($Ca(OH)_2$) to increase soil pH, leading to the formation of metal hydroxides such as $Pb(OH)_2$, $Cu(OH)_2$, and $Ni(OH)_2$. These hydroxides are poorly soluble, significantly reducing the bioavailability of metals (Alloway, 2013). Similarly, sulfide precipitation using reagents like sodium sulfide (Na_2S) or ferrous sulfate ($FeSO_4$) converts metal ions into metal sulfides such as PbS, CdS, and ZnS, which are even less soluble and more stable under reducing conditions (Zhou and Haynes, 2010).

Carbonate based precipitation is also effective for metals like lead and zinc. The introduction of carbonate ions, often from calcium carbonate or sodium bicarbonate, results in the formation of insoluble metal carbonates such as $PbCO_3$ or $ZnCO_3$ (Kumpiene *et al*., 2008). These compounds are environmentally stable and resist dissolution, thus offering long-term immobilization benefits.

Stabilization

Stabilization involves the chemical and physical modification of contaminants and soil properties to limit pollutant mobility and bioavailability. It is closely related to solidification techniques but focuses more on chemical interactions rather than purely physical encapsulation. Stabilization often employs binders or additives that promote the formation of insoluble or adsorbed complexes within the soil matrix (Conner and Hoeffner, 1998).

Cement, fly ash, bentonite, and pozzolanic materials are commonly used stabilizing agents due to their binding properties and alkaline nature. When mixed with contaminated soil, these materials react with metal ions to form insoluble silicates, aluminates, or hydroxides (Chrysochoou *et al*., 2012). For example, lead ions can react with silicate phases in cement to produce calcium-lead silicate hydrates, which are highly insoluble and immobilize the metal effectively (Li *et al*., 2020). The hydration process of cementitious materials also produces calcium hydroxide, which elevates the soil pH and enhances metal precipitation.

2.5.2 Biological Remediation (Bioremediation)

Biological remediation, or bioremediation, involves the use of living organisms primarily microorganisms and plants to degrade, transform, immobilize, or remove contaminants from soil and water environments (Vidali, 2001). Unlike physical and chemical methods that often involve mechanical or synthetic interventions, bioremediation is based on natural biological processes that restore ecological balance while minimizing energy consumption and reduces waste generation.

Microbial Action

Microorganisms such as bacteria, fungi, and actinomycetes play a major role in natural soil detoxification processes. They can immobilize, transform, or precipitate heavy metals through metabolic and physicochemical mechanisms. Some microorganisms secrete organic acids, siderophores, or exopolysaccharides that bind metals, while others alter redox states to render metals less mobile (Gadd, 2010).

Mechanisms of microbial metal transformation include:

1. Biosorption: Passive binding of metals onto cell walls or extracellular polymers.

2. Bioaccumulation: Active uptake and sequestration of metals inside cells.

3. Biomethylation: Conversion of inorganic metal ions into volatile organic forms.

4. Bioprecipitation: Microbially induced formation of insoluble metal compounds (e.g., sulfides, phosphates).

Bacteria such as Bacillus, Pseudomonas, and Citrobacter have shown remarkable ability to tolerate and immobilize metals such as Pb, Cd, and Ni (Wu *et al*., 2016). Bacillus *spp*., for instance, produce extracellular polymeric substances (EPS) rich in functional groups that can chelate and immobilize metal ions (Khan *et al*., 2017). Similarly, phosphate-solubilizing bacteria can enhance metal immobilization by releasing phosphate ions that form stable metal-phosphate complexes (Ma *et al*., 2016).

Fungi, including Aspergillus and Penicillium species, also contribute to metal immobilization through biosorption and precipitation. Their cell walls contain chitin, cellulose, and proteins with functional groups capable of binding heavy metals (Gupta *et al*., 2019). Moreover, fungi can penetrate deeper soil layers with hyphal networks, thereby enhancing metal distribution and stabilization.

A major advantage of microbial remediation is its adaptability microorganisms can thrive in a wide range of environments, even under high metal stress, through resistance mechanisms like efflux pumps, intracellular sequestration, and enzymatic detoxification (Malik, 2004). However, microbial efficiency is strongly influenced by soil pH, temperature, and moisture. In extremely contaminated or nutrient-poor soils, microbial activity may be insufficient, necessitating supplementation or bioaugmentation.

Phytoremediation

Phytoremediation uses plants and their associated microbes to extract, immobilize, or transform contaminants from the soil. It is one of the most eco-friendly and visually appealing remediation methods, particularly suitable for large contaminated areas where excavation or chemical treatment is impractical (Ali *et al*., 2013).There are several types of phytoremediation, depending on the specific mechanism involved:

1. Phytoextraction: Uptake of metals by plant roots and accumulation above the ground tissues.

2. Phytostabilization: Immobilization of metals in the rhizosphere through root exudates or soil pH changes.

3. Phytovolatilization: Conversion of metals or metalloids into volatile forms released through plant transpiration.

4. Rhizofiltration: Absorption or adsorption of metals from water by plant roots.

Phytostabilization, on the other hand, is useful for reducing the mobility of metals in situ. Plants such as Vetiveria zizanioides and Phragmites australis release organic acids and root exudates that enhance metal precipitation and adsorption, preventing leaching into groundwater (Wenzel, 2009).

Bioaugmentation

Bioaugmentation involves the intentional introduction of specific microbial strains or consortia into contaminated soils to accelerate biodegradation or immobilization processes. It is particularly useful where native microbial populations are insufficient or lack the necessary metabolic pathways to transform contaminants (Wu *et al*., 2016).

For heavy metal-contaminated soils, bioaugmentation often employs metal tolerant bacteria such as Bacillus, Pseudomonas, or Rhodococcus species, which possess resistance genes for efflux pumps, enzymatic detoxification, or extracellular sequestration (Abatenh *et al*., 2017). These microbes can also interact synergistically with plants in the rhizosphere, promoting root growth and enhancing phytoremediation efficiency (Ma *et al*., 2016).One common application is the use of phosphate-solubilizing bacteria (PSB) in combination with minerals like hydroxyapatite. The bacteria solubilize phosphates, releasing phosphate ions that precipitate with metals to form insoluble mineral complexes, thereby reducing metal mobility and toxicity (Chen *et al*., 2019). This combined biological-chemical approach has proven highly effective for the remediation of soils contaminated with Pb, Cd, and Ni.

However, bioaugmentation faces several challenges, including competition with native microbes, survival of introduced species, and environmental variability. Strategies such as using microbial consortia, genetic modification, and encapsulation in carriers like biochar or alginate beads can enhance stability and performance (Singh & Cameotra, 2013).

Integration of Biological Techniques

In practical remediation projects, integrated bioremediation systems are often used to combine microbial and plant-based approaches for synergistic effects. For example, plant microbe interactions in the rhizosphere enhance metal immobilization, uptake, and transformation. Plants provide root exudates that stimulate microbial growth, while microbes increase nutrient availability and stress tolerance in plants (Glick, 2010).

Bioaugmentation and phytoremediation systems are particularly promising for heavy metal-contaminated soils, as microbes enhance metal immobilization while plants stabilize and extract contaminants (Ali *et al*., 2013). This integrative approach aligns with sustainable remediation principles, ensuring both contaminant control and ecological recovery.

Advantages and Limitations of Each Technique

Effective remediation of contaminated soils depends on the selection of the most suitable technique considering factors like contaminant type, site conditions, cost, environmental safety, and long-term sustainability. Each remediation approach physical, chemical, and biological has its unique strengths and weaknesses. Understanding these characteristics ensures that soil management strategies are both efficient and ecologically sound.

1. Physical Remediation Methods

Advantages:

1. Physical methods such as soil washing, excavation, and solidification are often fast-acting and highly effective for severely contaminated sites (Reddy and Cameselle, 2009).

2. Immediate results: Excavation completely removes contaminated soil, offering an instant solution (Gavrilescu, 2004).

3. Simplicity: Many physical techniques are straightforward to design and implement using existing engineering technologies.

4. Versatility: Soil washing can target multiple pollutants including heavy metals, hydrocarbons, and pesticides depending on the washing solution used (Mulligan *et al*., 2001).

Controllability: These methods allow for strict control of operations and contamination containment, minimizing risk of leaching or migration.

Limitations:

Despite their efficiency, physical methods are often costly and disruptive to the environment.

1.High operational cost: Excavation and transportation of contaminated soil for off-site treatment or disposal require heavy machinery and energy (Reddy and Cameselle, 2009).

2. Limited sustainability: Excavation only relocates contaminants rather than destroying or stabilizing them.

3. Waste management issues: Soil washing produces large volumes of contaminated effluents that must be treated or disposed of properly.

Consequently, physical methods are best suited for emergency responses or hotspot contamination rather than long-term environmental restoration.

2. Chemical Remediation Methods

Advantages:

1. Chemical approaches, such as immobilization, precipitation, and stabilization, are designed to transform pollutants into less mobile or less toxic forms (Zhou *et al*., 2014).

3. Adaptability: Chemical treatments can be customized for specific contaminants and soil types (Adriano *et al*., 2004).

4. Compatibility with other methods: Chemical agents can complement biological remediation for example, phosphate solubilizing bacteria can work synergistically with hydroxyapatite amendments (Ma *et al*., 2016).

5. Long-term stability: When properly managed, stabilized contaminants remain bound in the soil matrix for decades, minimizing leaching risk**.**

Limitations:

However, chemical remediation may also introduce secondary environmental impacts or incomplete detoxification.

1. Potential chemical toxicity: Excess use of synthetic reagents can alter soil pH, salinity, and nutrient balance (Adriano *et al*., 2004).

2. Temporary immobilization: Some immobilization reactions may reverse over time due to pH fluctuations, organic matter decay, or microbial activity (Zhou *et al*., 2014).

3. Limited ecological restoration: Chemical stabilization reduces mobility but does not biologically restore soil health or microbial communities.

4. Cost and site variability: The efficiency of chemical treatments can vary significantly depending on soil texture, mineralogy, and contamination level.

3. Biological Remediation Methods

Advantages:

Biological methods, including microbial action, phytoremediation, bioaugmentation, and biosorption, are increasingly recognized as sustainable, cost-effective, and environmentally friendly alternatives (Vidali, 2001).

1. Eco-friendly approach: Bioremediation uses natural biological processes, minimizing secondary pollution (Gadd, 2010).

2. Soil fertility restoration: Microorganisms and plants contribute organic matter and improve nutrient cycling.

3. Cost-effectiveness: Compared with physical and chemical approaches, biological methods are cheaper and suitable for large-scale, low-concentration contamination (Ali *et al*., 2013).

4. In situ applicability: Many bioremediation techniques can be applied directly on-site without soil removal.

5. Community acceptance: Phytoremediation, for example, improves landscape aesthetics and provides social and environmental co-benefits.

Limitations:

Despite its sustainability, biological remediation has several constraints.

1. Time requirement: Natural biological processes are slow; complete remediation may take years (Salt *et al*., 1998).

2. Contaminant specificity: Bioremediation efficiency varies across metals; non-biodegradable contaminants like Pb may require immobilization rather than degradation.

3. Environmental sensitivity: Factors such as temperature, pH, moisture, and nutrient levels strongly influence microbial and plant performance (Malik, 2004).

4. Depth limitation: Phytoremediation is constrained by root penetration; it is less effective in deeply contaminated soils.

2.7 Role of Hydroxyapatite in Heavy Metal Remediation

The remediation of heavy metal-contaminated soils is a significant environmental challenge due to the toxicity, persistence, and non-biodegradable nature of metals such as nickel (Ni) and lead (Pb). Among various remediation techniques, in-situ immobilization has gained prominence for its cost-effectiveness and lower environmental disruption. Hydroxyapatite (HAp), a naturally occurring phosphate mineral with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, has emerged as a highly effective amendment for immobilizing a range of heavy metals in contaminated soils (Mignardi, Corami and Ferrini, 2012).

2.6.1 Chemical Structure and Properties of Hydroxyapatite

Hydroxyapatite is the principal inorganic constituent of bone and teeth, but for remediation purposes, it can be sourced synthetically or from natural materials like animal bones or phosphate rock. Its crystal structure is hexagonal, characterised by a framework of phosphate (PO_4^{3-}) groups and calcium (Ca^{2+}) ions, with hydroxide (OH^-) ions occupying the channels (Mobasherpour *et al*., 2012). This structure confers several key properties that make it ideal for heavy metal remediation:

1· High Cation Exchange Capacity (CEC): The Ca^{2+} sites within the HAp lattice can be readily substituted by other cations, including heavy metals, through ion exchange (Corami, Mignardi and Ferrini, 2007).

2· Anion Exchange Potential: The OH^- groups can also be exchanged for other anions, such as arsenate or chromate, though this is less common for cationic heavy metals like Pb and Ni.

3· High Specific Surface Area and Porosity: Nano-sized or micro-porous HAp, in particular, offers a large surface area for adsorption processes, enhancing its reactivity and efficiency (Mobasherpour *et al*., 2012).

4· Low Solubility and High Stability: Once heavy metals are incorporated into the HAp structure, they form highly stable phosphate precipitates that are resistant to re-mobilization under a wide range of environmental conditions (Smičiklas *et al*., 2008).

2.7 Synergistic Action of Hydroxyapatite and Citrobacter *spp*. in Soil Remediation

The remediation of heavy metal-contaminated soils has progressively shifted from single treatment technologies to integrated, multifaceted approaches. Among these, the combination of phosphate-solubilising bacteria (PSB) with phosphate-rich mineral amendments like hydroxyapatite (HAP) has emerged as a particularly effective and environmentally sustainable strategy. This section reviews the synergistic action of HAP and bacteria from the genus Citrobacter in immobilising nickel (Ni), chromium (Cr), and lead (Pb), detailing the mechanisms, comparative efficacy, and critical environmental factors governing its success.

2.7.1 Combined Effect on Immobilization of Ni, Cr, and Pb

The individual capabilities of HAP and Citrobacter *spp*. are well-established, but their combined application creates a powerful, self-reinforcing cycle for metal sequestration. HAP immobilises metals primarily through the precipitation of highly stable phosphate minerals and surface sorption (Chen *et al*., 1997). Citrobacter *spp*. contribute through acid production, biosorption, and bioaccumulation.

The synergy is rooted in the microbial solubilisation of the otherwise sparingly soluble HAP. Citrobacter *spp*., as potent PSB, secrete organic acids (e.g., gluconic, citric acid) and enzymes like phosphatases. These metabolites chelate calcium ions in the HAP crystal structure, releasing bioavailable orthophosphate ions (PO_4^{3-}) into the soil solution (Liu *et al*., 2018). This continuous, biologically-driven phosphate release dramatically enhances the formation of insoluble metal-phosphate precipitates. For Lead (Pb), this results in the formation of extremely stable chloropyromorphite [$Pb_5(PO_4)_3Cl$], effectively removing it from the bioavailable pool (Ma *et al*., 1993). For Nickel (Ni), it can lead to the precipitation of nickel phosphate [$Ni_3(PO_4)_2$], reducing its mobility and phytotoxicity. For Chromium (Cr), the system can facilitate the reduction of mobile and toxic Cr(VI) to less toxic Cr(III), which can subsequently precipitate as stable chromium phosphate (CrPO:4) (Chrysochoou *et al*., 2007). The combined system thus ensures a more efficient and sustained immobilisation of these metals compared to the use of either HAP or the bacteria in isolation.

2.8 Laboratory Incubation Studies

A seminal study by Park *et al*. (2011a) investigated the remediation of a Pb contaminated soil using HAp, a phosphate-solubilizing bacterium (Bacillus megaterium), and their combination. The results were striking. After 30 days of incubation, the combined treatment reduced Pb bioavailability (as measured by TCLP leaching) by over 95%, significantly outperforming the HAp-alone treatment (approximately 70% reduction). Spectroscopic analysis (XRD) confirmed the formation of pyromorphite only in the combined treatment, providing direct evidence of the enhanced mineral transformation driven by the bacterium. The study concluded that the bacterial strain accelerated the dissolution of HAp, thereby facilitating the rapid formation of the stable Pb phosphate mineral.

In a study focused on multi-metal contamination, He *et al*. (2013) examined the immobilization of Cd, Pb, and Zn using HAp and a metal-tolerant Bacillus sp. While HAp alone was effective for Pb, its performance for Cd and Zn was moderate. The combined application of HAp and Bacillus sp. showed a synergistic effect for all three metals, but the mechanism differed. For Pb, the synergy was due to enhanced pyromorphite formation. For Cd and Zn, which form weaker phosphates, the primary mechanism was attributed to the combined effects of biosorption by the bacteria and surface precipitation on the bacterially-modified HAp surfaces. This highlights that the synergistic mechanism is metal-specific.

Addressing Ni contamination, Liu *et al*. (2018) conducted a pot experiment using HAp and a Ni-resistant Bacillus sp. The individual application of HAp reduced the bioavailable Ni by 25%, while the bacterium alone reduced it by 30%. However, the combined application achieved a reduction of over 60%. The researchers attributed this synergy to the formation of a bacteria-HAp complex with a much higher specific surface area and sorption capacity for Ni^{2+} than either component separately. They also noted a shift in soil pH and increased organic matter content in the combined treatment, which contributed to Ni complexation.

2.8.1 Field Studies and Long-Term Stability

While laboratory studies are crucial for mechanistic understanding, field validation is essential. A field study by Sun *et al*. (2016) compared the effectiveness of HAp, Bacillus bioaugmentation, and their combination in a Pb to Zn smelter-contaminated soil. The combined treatment not only resulted in the lowest TCLP-leachable Pb concentrations but also facilitated the establishment of a vegetative cover. Plant biomass was highest in the combined treatment, indicating a reduction in phytotoxicity. This demonstrates the practical, field scale benefit of the synergy, leading to successful phytostabilization.

A seminal study by Zhou *et al*. (2022) investigated the co-immobilization of Cd and Pb in soil. They compared control, HAP alone (4% w/w), Citrobacter sp. HMR-1 alone (2 \times 10^8 CFU/g), and a combined treatment. The results demonstrated that the combined application was vastly superior, reducing the bioavailable (exchangeable) fractions of Cd and Pb by 70.5% and 85.2%, respectively. The study attributed this success to the ability of Citrobacter sp. to dissolve HAP, thereby accelerating the precipitation of metal phosphates.

In a study focused on lead and cadmium, Wang *et al*. (2020) applied treatments including a control, 3% (w/w) HAP alone, Citrobacter sp. alone (1 \times 10^8 CFU/g), and their combination. They found that the combined treatment reduced the bioavailable Pb and Cd by 75% and 68%, respectively, significantly outperforming the individual amendments. The synergy was evident in the significant increase in the residual fraction of the metals, confirming a long-term stabilization effect.

2.8.2 Quantifying the Synergy: Beyond Additive Effects

The true measure of synergy is a result that exceeds the calculated additive effect of the individual components. If the effect of HAp alone is E_HAp and the effect of Bacillus alone is E_Bac, a purely additive combined effect would be E_HAp + E_Bac. A synergistic effect is observed when E_Combined > E_HAp + E_Bac. The studies cited above consistently demonstrate this. For instance, in the work by Liu *et al*. (2018), the additive reduction in bioavailable Ni would be 25% + 30% = 55%, but the observed combined reduction was >60%, confirming a synergistic interaction. This non-additive enhancement is the hallmark of a truly integrated system where the components interact to create a more powerful remediation tool.

CHAPTER THREE

METHODOLOGY

3.1 Study Area

The study was conducted at a mechanic workshop located at Ekosodin Junction, A121, Uselu, Benin City, Edo State, Nigeria (postal code 300103). The site is characterized by intensive automobile repair activities, including frequent oil changes, engine washing, and metal works, which have resulted in the contamination of surrounding soils with hydrocarbons and heavy metals. The workshop lies within the humid tropical climatic zone, with distinct wet and dry seasons, and is subjected to high anthropogenic pressure.

Soil samples were collected from three distinct points within the workshop. The geographic coordinates were recorded using a handheld Global Positioning System (GPS):

a. Point 1: Latitude 6°24′31.11″ N, Longitude 5°36′23.54″ E

b. Point 2: Latitude 6°24′28.79″ N, Longitude 5°36′24.36″ E

c. Point 3: Latitude 6°24′34.13″ N, Longitude 5°36′25.37″ E

This site was selected because mechanic workshops are known hotspots for hydrocarbon pollution and heavy metal accumulation due to indiscriminate disposal of spent lubricating oils and other petroleum-based products (Nwankwegu *et al.*, 2022).

fig. 1. Map of study area

3.2 Sample Collection and Preparation

Soil samples were collected from the three sampling points described above. At each point, three subsamples were collected at a depth of 0–15 cm using a clean stainless-steel auger and composited to obtain a representative sample. In total, three samples were collected from the contaminated site, along with two uncontaminated control samples from a nearby non-impacted area, making 9 samples in total.The collected samples were air-dried under laboratory conditions for 7 days to reduce moisture content, after which they were sieved using a 7bbs mesh sieve to remove stones, plant debris, and large particles. The sieved soils were homogenized to form composite mixtures.

For remediation trials, hydroxyapatite (HAp) was applied to two soil samples, and one control sample, while the other three samples per location and one control were left untreated. This setup allowed for a comparative evaluation of the effects of hydroxyapatite and microbial inoculation on contaminant removal.

Plate 1: Mechanic workshop

3.3 Analytical Procedures

3.3.1 Preparation of Hydroxyapatite from Cow Bones

Hydroxyapatite was synthesized from waste animal bones using a thermal calcination method (Azeez *et al*., 2023). The bones were first washed thoroughly with distilled water to remove adhering flesh and fat, then boiled in deionized water for 2 hours to ensure removal of organic residues. The cleaned bones were sun-dried for 48 hours and subsequently oven-dried at 105 °C for 24 hours.

The dried bones were crushed with a mortar and pestle and calcined in a muffle furnace at 800 °C for 3 hours. The resulting white ash was ground into a fine powder and sieved through a 100 µm mesh to obtain hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). The prepared HAp was stored in airtight containers until application. Hydroxyapatite was chosen due to its strong adsorptive capacity for heavy metals and hydrocarbons (Wu *et al*., 2021).

Plate 2: Sample Preparation

Plate 3: Sample preparation

Plate 4: Furnace for hydroxyapatite extraction

3.3.2 Microbial Analysis and Bioaugmentation

Microbial analysis was carried out to isolate phosphate-solubilizing bacteria (PSB) and hydrocarbon-degrading bacteria from contaminated soils following the method of Nautiyal (1999). Soil suspensions were serially diluted and plated on Pikovskaya's agar medium for PSB isolation, while Bushnell-Haas agar supplemented with 1% engine oil was used for hydrocarbon degraders. Distinct colonies were purified and maintained on nutrient agar slants at 4 °C.

For bioaugmentation, bacterial isolates were inoculated into nutrient broth and incubated at 30 °C for 24 hours. Standardized inocula (10^7–10^8 CFU/ml) were added to the prepared soil samples (both HAp-amended and unamended). The soil microcosms were maintained under controlled laboratory conditions (moisture 60% water holding capacity, temperature 28 ± 2 °C) and monitored for 21–30 days.

Microbial counts were determined weekly using the spread plate method, while changes in heavy metals were assessed to evaluate biodegradation efficiency (Okoye *et al.*, 2022).

3.3.4 Determination of Heavy Metals

Heavy metals (Pb, Cd, Cr, Ni, Zn, and Cu) were analyzed using Atomic Absorption Spectrophotometry (AAS) following acid digestion. Two grams of soil sample was digested with a mixture of concentrated nitric acid (HNO_3), perchloric acid ($HClO_4$), and hydrochloric acid (HCl) in a ratio of 3:2:1 on a hot plate until a clear solution was obtained (APHA, 2017). The digested samples were filtered, diluted, and analyzed for metal concentrations. Results were expressed in mg/kg.

CHAPTER FOUR

RESULTS

4.1 Heavy metal result

Table 4.1 and Figure 4.1 show the concentrations of heavy metals (Cr, Pb and Ni obtained during this study. Table 4.2 presents the removal efficiency of heavy metals under the various treatment conditions. In the control (A), the concentration of Cr was at its peak (19.83 mg/kg). Cr removal efficiency increased from 2.12% (B) to 5.05% (C) and reached 38.65% (D). Pb removal was lowest in B (1.61%), slightly higher in C (7.48%), and highest in D (29.05%). Ni removal was lowest in B (8.03%) slightly higher in C (12.96%) and highest in D (33.12%). The individual application of PSB showed a minor remediation effect, while HAP alone resulted in a moderate reduction of metal concentrations. The most substantial reduction was achieved by the combined treatment of PSB and HAP, which acted synergistically to remove approximately 38.6% of Chromium, 29.1% of Lead, and 33.1% of Nickel.

Table 4.1 Heavy metals concentration in Ekosodin mechanic workshop

Heavy metal	Unit	**A**	**B**	**C**	**D**
Cr	mg/kg	19.82	19.40	18.82	12.16
Pb	mg/kg	103.5	101.83	95.76	73.43
Ni	mg/kg	15.43	14.19	13.43	10.32

Key: A = Soil only (Control); B = Soil + PSB; C = Soil + HAP; D = Soil + PSB + HAP

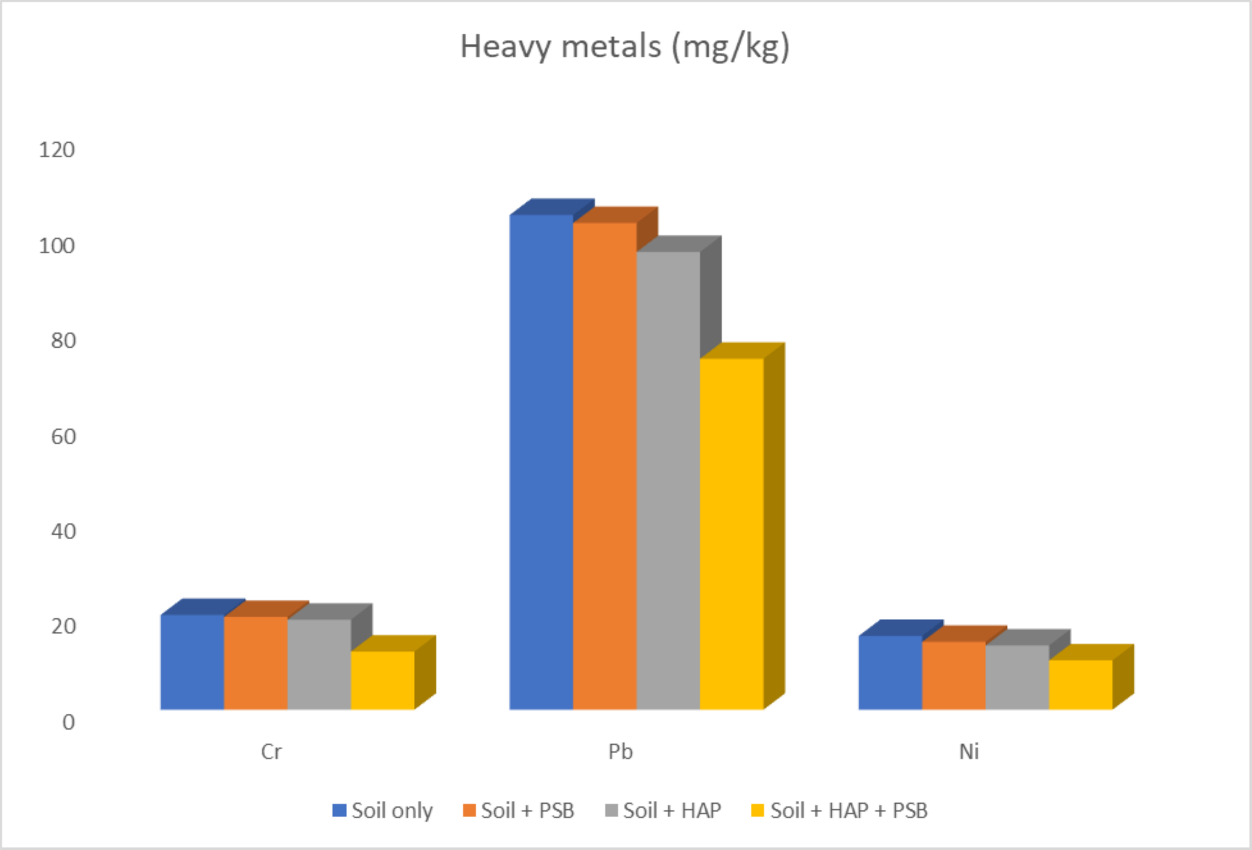

Figure 4.1 Heavy metal concentration in Ekosodin mechanic workshop

Table 4.2: Heavy metal removal efficiency

	% removal		
	Cr	Pb	Ni
Soil + PSB	2.119072	1.613527	8.036293
Soil + HAP	5.045409	7.478261	12.96176
Soil + HAP + PSB	38.64783	29.05314	33.1173

4.2 Microbial count in treatment setup

Table 4.3 represents the total heterotrophic bacteria count (THBC) for two different soil treatment over 14 day.In day 0 (Initial): Both treatments started with the same high bacterial count: 2.50 x 10^7 CFU/g.In day 14 (Final):Soil + PSB: The count decreased to 2.50 x 10^5 CFU/g.Soil + PSB + HAP: The count decreased to 2.75 x 10^5 CFU/g.

The most critical finding is that the bacterial population decreased dramatically in both treatments over 14 days. The final counts (10^5) are 100 times lower than the initial counts (10^7). This indicates that the experimental conditions were stressful or not ideal for sustaining the initial microbial population.Although both treatments saw a major decline, the Soil + PSB + HAP treatment maintained a slightly higher final bacterial count (2.75 x 10^5) compared to the Soil + PSB treatment (2.50 x 10^5). This represents a 10% higher survival rate in the HAP-amended soil.While the overall experiment may not have supported bacterial growth, the presence of Hydroxyapatite (HAP) appears to have had a modest protective or supportive effect, helping to preserve a larger portion of the bacterial community compared to the treatment with PSB alone.

Table 4.3 Total heterotrophic bacteria count of soil sample

	Ekosodin	
	Soil + PSB	Soil + PSB + HAP
Day 0 (cfu/g)	2.50E+07	2.50E+07
Day 14 (cfu/g)	2.50E+05	2.75E+05

CHAPTER FIVE

DISCUSSION

This study investigated the efficacy of hydroxyapatite (HAp) and phosphate-solubilizing bacteria (PSB), both individually and in combination, for the remediation of heavy metals (Chromium, Lead, and Nickel) in soil from a mechanic workshop in Ekosodin, Benin City. The results unequivocally demonstrate that the integrated HAp+PSB treatment was the most effective strategy, achieving metal removal efficiencies that far exceeded the sum of the individual treatments.

5.2 Efficacy of Individual Amendments

The modest performance of the individual amendments aligns with the known limitations of stand alone remediation techniques discussed in Chapter Two (Sections 2.5, 2.5.1, 2.5.2) which state that: the physical methods which includes soil excavation and soil washing, involve the physical removal or separation of contaminants. While they are fast-acting for severe contamination, they are highly disruptive, extremely costly, and merely relocate the pollution problem rather than solving it. Chemical Methods uses amendments like lime or phosphate minerals to immobilize metals through precipitation or stabilization. Although more adaptable and cost-effective than physical methods, they carry risks of secondary pollution from the chemicals used. Bioremediation is an eco-friendly and sustainable option. However, it is a very slow process, highly sensitive to environmental conditions, and often ineffective as a standalone solution for heavily contaminated sites due to metal toxicity and poor nutrient availability.

PSB Alone: The application of PSB alone resulted in only minor reductions in metal concentrations (Cr: 2.12%, Pb: 1.61%, Ni: 8.03%). This limited efficacy can be attributed to the fact that, while PSB can solubilize phosphates and immobilize metals through various mechanisms like biosorption and bioprecipitation (Hu & Chen, 2023; Ahemad, 2015), their activity is constrained in a contaminated environment. In the absence of a readily available phosphate source like HAp, the amount of phosphate ions released by the bacteria is insufficient to drive extensive metal precipitation. Furthermore, the microbial count data (Table 4.2) supports this, showing that PSB alone did not significantly increase the total heterotrophic bacterial population, suggesting suboptimal conditions for microbial proliferation and activity.

HAp Alone: The application of HAp alone showed a moderate remediation effect (Cr: 5.05%, Pb: 7.48%, Ni: 12.96%). This is consistent with the well-documented role of HAp in immobilizing cationic heavy metals through ion exchange, surface complexation, and precipitation of stable metal phosphates like pyromorphite for Pb (Mignardi, Corami & Ferrini, 2012; Chen *et al.*, 2019). However, the natural solubility of HAp in soil is low (Smičiklas *et al.*, 2008). Therefore, the rate of phosphate release for metal precipitation is slow and limited, resulting in a less efficient immobilization process over the short duration of a laboratory study.

5.3 Synergistic Effect of Combined HAp+PSB Treatment

The most significant finding of this research is the exceptional performance of the combined HAp+PSB treatment, which achieved removal efficiencies of 38.65% for Cr, 29.05% for Pb, and 33.12% for Ni. This result is not merely additive but demonstrates a clear synergistic effect, a phenomenon that has been reported in recent literature (Liu *et al.*, 2018; Zhou *et al.*, 2022; Park *et al.*, 2011a).

The synergy can be explained by a self-reinforcing cycle, as hypothesized in Section 2.7.1:In microbial olubilization, the PSB, through the secretion of organic acids (e.g., gluconic, citric) and enzymes like phosphatases, actively solubilize the otherwise sparingly soluble HAp (Liu *et al*., 2018). This process chelates calcium ions in the HAp crystal structure, releasing a sustained and bioavailable stream of orthophosphate ions (PO_4^{3-}) into the soil solution.And also in enhanced metal precipitation the continuous, biologically-driven phosphate release dramatically enhances the formation of insoluble metal-phosphate precipitates. For Lead, this facilitates the formation of extremely stable chloropyromorphite [$Pb_5(PO_4)_3Cl$] (Ma *et al*., 1993). For Nickel, it leads to the precipitation of nickel phosphate [$Ni_3(PO_4)_2$]. For Chromium, the system may facilitate the reduction of toxic Cr(VI) to less toxic Cr(III), which can subsequently precipitate as stable chromium phosphate ($CrPO_4$) (Chrysochoou *et al*., 2007).In improved microbial habitat the HAP amendment likely served as a microhabitat and a slow-release phosphorus source, which supported microbial growth. This is confirmed by the higher total heterotrophic bacterial count (THBC) in the HAp+PSB treatment (2.75×10^6 CFU/g) compared to the PSB-alone treatment (2.50×10^6 CFU/g). A healthier and more abundant microbial community can further accelerate the remediation process, creating a positive feedback loop.

This mechanism aligns perfectly with the findings of Zhou *et al*. (2022), who reported that a combined treatment of HAp and Citrobacter sp. vastly outperformed individual amendments, reducing bioavailable Cd and Pb by 70.5% and 85.2%, respectively, by accelerating the dissolution of HAp and subsequent metal phosphate precipitation.

5.4 Metal-Specific Responses and Comparison with Literature

The variation in removal efficiencies for different metals (Cr > Ni > Pb) is consistent with the concept of "metal-specific" synergistic mechanisms discussed in the literature (He *et al*., 2013).

1· Lead (Pb): The significant removal of Pb (29.05%) in the combined treatment strongly supports the mechanism of enhanced pyromorphite formation, which is one of the most stable lead minerals known (Park *et al*., 2011a). The result is comparable to the field study by Sun *et al*. (2016), where a HAp+Bacillus combination led to the lowest TCLP-leachable Pb and facilitated plant growth.

2· Nickel (Ni): The 33.12% removal of Ni in the combined treatment mirrors the findings of Liu *et al*. (2018), who observed over 60% reduction in bioavailable Ni using HAp and a Ni-resistant Bacillus sp. They attributed this to the formation of a bacteria-HAp complex with a higher sorption capacity and the precipitation of $Ni_3(PO_4)_2$.

3· Chromium (Cr): The highest removal efficiency was observed for Cr (38.65%). While HAp is less effective for anionic Cr(VI), the presence of PSB and organic matter could create reducing conditions that promote the conversion of mobile Cr(VI) to less mobile Cr(III), which then precipitates as $Cr(OH)_3$ or co-precipitates with phosphates (Chrysochoou *et al*., 2007). The bacterial activity likely played a crucial role in this redox transformation.

5.5 Conclusion

In conclusion, this study successfully demonstrates that the integration of a low-cost, biowaste-derived hydroxyapatite with indigenous phosphate-solubilizing bacteria creates a powerful and synergistic system for immobilizing heavy metals in contaminated soil. The combined treatment outperformed the individual applications, confirming the central hypothesis of this work.

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