REPUBLIQUE DE COTE D'IVOIRE
Union-Discipline-Travail

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

GRADUATE RESEARCH PROGRAMME: CLIMATE CHANGE AND BIODIVERSITY

Academic year: 2025-2026 **Order number: 3022/2026**

THESIS

Submitted for the Degree of Doctor of Philosophy of

Félix HOUPHOUËT-BOIGNY University, Cocody-Abidjan

Speciality: Climate Change and Biodiversity

Pierre Anthony MENDY

The Potential of Mesophilic Bacteria for Heavy Metal Bioremediation in Agriculture under Climate Change in The Gambia

Public defense on 11th June, 2026

Members of the Jury

Mr. ZIAO Nahosse	Full Professor	UNA	President of the Jury
Mrs. KONE Mawa	Associate Professor	UFHB	Supervisor
Mr. AHMED Ado Baba	Full Professor	UTG	Co-Supervisor
Mr. ADOU Yao Constant Yves	Full Professor	UFHB	Referee
Mr. MEGNASSAN Eugene	Full Professor	UNA	Referee
Mrs. AMBEU N'ta Christelle Mélissa épse LOKO	Associate Professor	UFHB	Examiner

REPUBLIQUE DE COTE D'IVOIRE
Union-Discipline-Travail

Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

ÉCOLE DOCTORALE:
CHANGEMENT CLIMATIQUE ET BIODIVERSITÉ

Année académique: 2025-2026 **Numéro d'ordre: 3022/2026**

THÈSE

Présentée pour l'obtention du grade de Docteur de

L'Université Félix HOUPHOUET-BOIGNY, Cocody-Abidjan

Spécialité: Changement climatique et Biodiversité

Pierre Anthony MENDY

Le Potentiel des Bactéries Mésophiles pour la Biorémédiation des Métaux Lourds en Agriculture dans le contexte de Changement Climatique en Gambie

Soutenance publique le 11 juin 2026

Membre du Jury

Mr. ZIAO Nahosse	Professeur Titulaire	UNA	Président de Jury
Mrs. KONE Mawa	Maître de Conférences	UFHB	Directrice
Mr. AHMED Ado Baba	Professeur Titulaire	UTG	Co-Directeur
Mr. ADOU Yao Constant Yves	Professeur Titulaire	UFHB	Rapporteur
Mr. MEGNASSAN Eugene	Professeur Titulaire	UNA	Rapporteur
Mrs. AMBEU N'ta Christelle Mélissa épse LOKO	Maître de Conférences	UFHB	Examinatrice

Dedication

This thesis is dedicated to my beloved parents (Anthony MENDY & Keita MENDY), all of blessed memory, my beloved wife, Grace Fatou MENDY, and my wonderful children, Deborah Isha Pierre MENDY, Pierre Joshua MENDY, and Grace Susana Pierre MENDY, for their understanding, boundless love, and support that carried me through my studies.

Acknowledgements

All praise and glory are due to Almighty God, the Creator of the heavens and the earth, who has graciously endowed humanity with knowledge and wisdom.

I would like to express my sincere gratitude to the West African Science Service Centre on Climate Change and Adapted Land Use (WASCAL) and the German Federal Ministry of Education and Research (BMBF), Federal Republic of Germany, for their generous financial support throughout this research.

My sincere thanks go to the management and staff of the WASCAL Regional Office in Accra, Ghana, for their continuous administrative and logistical assistance. I am equally grateful to the President of Université Félix Houphouët-Boigny (UFHB), the Dean of the Biosciences Department at UFHB, and the entire management and staff of the University of The Gambia (UTG) for their institutional support during my doctoral program.

I am deeply indebted to my principal supervisor, Associate Professor Mawa KONE, for her unwavering support, patience, motivation, and constructive critiques, which significantly improved the quality of this dissertation. I extend my heartfelt appreciation to my co-supervisor, Professor Ado Baba AHMED, for his invaluable input in shaping the direction of this research and for his meticulous reviews and corrections throughout the process.

It has been an honor to conduct the laboratory component of this work under the guidance of Professor Martin ANTONIO, a distinguished molecular biologist and Lead Principal Investigator at the Medical Research Council Unit, The Gambia, at the London School of Hygiene and Tropical Medicine (MRCG@LSHTM). I am sincerely grateful for his mentorship and generosity. May he be richly blessed. My heartfelt thanks go to the members of the jury for their constructive comments and evaluation of this work.

I would also like to thank Professor Sidat YAFFA, UTG/WASCAL Director, for facilitating my integration into Professor ANTONIO's laboratory team, the Research Molecular Microbiology Group at MRCG@LSHTM. My appreciation further extends to Associate Professor KOUASSI Konan Edouard (WASCAL Director for Climate Change and Biodiversity, Cote d'Ivoire), Professor Sorho FATOGOMA (WASCAL Scientific Coordinator for Climate Change and Biodiversity), and

Dr. Alpha KARGBO, for their guidance and mentorship from the inception to the completion of this thesis.

Special thanks are due to Mr. Dembo KANTEH for his assistance in coordinating my laboratory activities at MRCG@LSHTM. I am especially thankful to my lab colleagues at MRCG@LSHTM, whose collaboration and encouragement were vital to the successful execution of this research: Dr. Isaac Darko Otchere, Mr. Dam KHAN, Mrs. Peggy-Estelle TIENTCHEU, Mr. Shola Able-THOMAS, Mr. Momodou M. DRAMMEH, Ms. Aissatou SECK, Mr. Kouadio N'da Kouame NAZAIRE, and Mr. Nfamara CAMARA. I also want to acknowledge the technical support provided by Mrs. Janet KOUASSI, the secretary at the WASCAL Center in Côte d'Ivoire. Mr. Tahirou TOURE, the logistician at the WASCAL Center in Abidjan, Côte d'Ivoire, for fostering a welcoming and supportive environment.

I am equally appreciative of the camaraderie and emotional support provided by my fellow WASCAL colleagues, particularly Dr. Afua Amponsah AMANKWAH, Dr. Kossi-Messan Jacques AGBOKA, Dr. Blessing Chinomso OKORIE, Dr. Nasradeen Adam Hamed GADALLAH and Dr. François Darius ACCROMBESSI, with whom I shared a valuable social and academic journey in Côte d'Ivoire.

Lastly, I acknowledge the dedicated efforts of my research assistants, Mr. Assan SOWE and Mr. Echene JATTA, for their tireless support during the data collection and experimental phases of this work. I extend my heartfelt gratitude to my family, church members, friends, loved ones, and the kind-hearted people of The Gambia and Côte d'Ivoire for their unwavering support, encouragement, and goodwill throughout this journey. To every one of you, I am deeply grateful. Indeed, the successful completion of a doctoral degree is a collective achievement, for it truly takes a community to nurture a doctoral graduate.

Abstract

Crop and livestock production are critical to food security in The Gambia, yet yields have declined, partly due to the impacts of climate change and environmental pollution. This study investigated the potential of mesophilic bacteria (20-40 °C) for heavy metal bioremediation in agriculture under changing climatic conditions. Given the importance of groundnuts and rice, especially rice, consumed at 117 kg/person/year and cultivated across diverse ecosystems, the study explored farmers' perceptions of climate variability and pollution. A mixed-methods approach was used, with a quantitative sample size of 432 and qualitative data gathered through focus groups and eight key informant interviews across six agricultural regions. Results indicated widespread awareness among farmers of climate change, but limited knowledge of heavy metal pollution and bioremediation. Major constraints to production included poor access to water, credit, quality inputs, and rising salinity. Laboratory experiments isolated *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Bacillus* sp., and *Grimontia hollisae* for heavy metal bioremediation tests. *Bacillus* sp. exhibited the highest bioremediation efficiency, particularly on Cu and Fe media, reaching optical density (OD) values of 1.0 (Cu at 28 and 98 ppm, day 5) and 0.9 (Fe at 50 ppm, day 1) with sustained growth. *P. aeruginosa* showed immediate performance, ranking second in both Cu and Zn bioremediation. It achieved a rapid OD peak of 0.6 on day 1 at 58 ppm and demonstrated substantial growth in Zn media. *P. fluorescens* had the most robust and sustained OD responses across low and high Zn levels, although less than *Bacillus* sp. in Cu and Fe stress media. These performances indicated promise in Zn, Fe, and Cu remediation, thus highlighting their potential application in climate-resilient, pollution-aware farming practices.

Keywords: climate change, pollution, heavy metals, mesophilic bacteria, bioremediation

RESUME

La production végétale et animale est essentielle à la sécurité alimentaire en Gambie, mais les rendements ont diminué, en partie en raison des effets du changement climatique et de la pollution de l'environnement. Cette étude a examiné le potentiel des bactéries mésophiles (20-40 °C) pour la bioremédiation des métaux lourds en agriculture dans un contexte de conditions climatiques changeantes. Étant donné l'importance de l'arachide et du riz, en particulier le riz, consommé à hauteur de 117 kg/personne/an et cultivé dans divers écosystèmes, l'étude a exploré la perception des agriculteurs face à la variabilité climatique et à la pollution. Une approche mixte a été utilisée, combinant un échantillon quantitatif de 432 personnes et des données qualitatives recueillies à travers des discussions de groupes et huit entretiens avec des informateurs clés dans six régions agricoles. Les résultats ont révélé une large connaissance des agriculteurs par rapport au changement climatique, mais une connaissance limitée de la pollution par les métaux lourds et de la bioremédiation. Les principales contraintes à la production comprenaient le manque d'accès à l'eau, au crédit, à des intrants de qualité, ainsi que l'augmentation de la salinité. Des expériences en laboratoire ont permis d'isoler *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Bacillus* sp. et *Grimontia hollisae* pour des tests de bioremédiation des métaux lourds. *Bacillus* sp. a montré la plus grande efficacité de bioremédiation, en particulier dans les milieux contenant du cuivre (Cu) et du fer (Fe), atteignant des valeurs de densité optique (DO) de 1,0 (Cu à 28 et 98 ppm, au jour 5) et de 0,9 (Fe à 50 ppm, au jour 1) avec une croissance soutenue. *P. aeruginosa* a montré une performance rapide, se classant au deuxième rang pour la bioremédiation du Cu et du zinc (Zn). Elle a atteint un pic de DO rapide de 0,6 au jour 1 à 58 ppm et a démontré une croissance substantielle dans les milieux contenant du Zn. *P. fluorescens* a montré les DO les plus robustes et soutenues dans les milieux de faibles et de forts niveaux de Zn, bien qu'inferieures aux DO de *Bacillus* sp. dans le Cu et le Fe. Ces performances indiquent un potentiel prometteur pour la bioremédiation du Zn, du Fe et du Cu, soulignant ainsi leur application possible dans des pratiques agricoles résilientes au climat et conscientes de la pollution.

Mots-clés: changement climatique, pollution, métaux lourds, bactéries mésophiles, bioremédiation.

Table of Contents

List of Figures

List of Tables

List of Appendices

List of Abbreviations

WCR - West Coast Region

NBR - North Bank Region

LRR – Lower River Region

CRR – Central River Region (South & North)

URR – Upper River Region

KP – Knowledge and Perception

ASGM – Artisanal Small-Scale Gold Mining

HMs – Heavy Metals

AAS – Atomic Absorption Spectrophotometry

MTs – Metallothioneins

GSH – Glutathione

PCs – Phytochelatins

PM – Particulate Matter

MF – Microfiltration

UF – Ultrafiltration

NF – Nanofiltration

RO – Reverse Osmosis

FO – Forward Osmosis

ED – Electrodialysis

Na_2EDTA – Disodium Salt Ethylenediaminetetraacetic Acid

MEUP – Micellar-enhanced Ultrafiltration

PEUP – Polymer-enhanced Ultrafiltration

PEI – Polyethyleneimine

PBI – Polybenzimidazole

PVP – Polyvinylpyrrolidone

PES – Polyethersulfone

PACL – Polyaluminium Chloride

SDS – Sodium Dodecyl Sulfate

MIBC – Methyl Isobutyl Carbinol

LIE – Liquid-ion Exchange

MMMs – Mixed Matrix Membranes

IEPs – Isoelectric Points

CFFA – Coal-Fired Fly Ash

LPS – Lipopolysaccharide

MTC – Metal Tolerance Concentration

MIC – Minimum Inhibitory Concentration

OD – Optical Density

AST – Antimicrobial Susceptibility Testing

CLSI – Clinical and Laboratory Standards Institute

EUCAST – European Committee on Antimicrobial Susceptibility Testing

TEM – Transmission Electron Microscopy

EPS – Extracellular Polymeric Substances

GMOs – Genetically Modified Organisms

GEMs – Genetically Engineered Organisms

CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats

UTG – The University of The Gambia

MRCG@LSHTM – Medical Research Council The Gambia at the London School of Hygiene and Tropical Medicine

MoHERST – Ministry of Higher Education, Research, Science and Technology

MoECNR – Ministry of Environment, Climate Change and Natural Resources

NARI – National Agricultural Research Institute

NEA – National Environment Agency

MEAs – Multilateral Environmental Agreements

NEMA – National Environment Management Act

AACCP – Adapting Agriculture to Climate Change Project

GAPs – Good Agricultural Practices

IPPM – Integrated Production and Pest Management

CSA – Climate Smart Agriculture

FFSA – Farmer Field School Approach

SWMU – Soil Water Management Unit

CHAPTER 1

INTRODUCTION

1.1 Background

Heavy metal (HM) pollution is a growing global concern, especially in the context of climate change and agricultural sustainability. Human activities such as mining, industrialization, and the excessive use of agrochemicals (fertilizers, pesticides, fungicides) contribute significantly to the accumulation of toxic metals in soil, water, and air (**Mendy *et al.*, 2021)**. These metals can be absorbed by crops, impairing plant growth and posing serious risks to food safety and human health when consumed. The contamination of agricultural land with heavy metals has therefore raised public health concerns, particularly after several high-profile pollution incidents (**Sandeep *et al.*, 2019**).

Bioremediation, which uses native bacteria to detoxify polluted environments, offers an affordable and eco-conscious solution. Understanding farmers' awareness of HM pollution and exploring microbial potential for remediation is essential for managing agricultural risks in vulnerable regions. In The Gambia, data on HM pollution is limited, though studies have detected trace metal levels in water around Faraba Banta **(Král *et al.*, 2019**) and in connected water bodies near gold mining areas in Senegal's Kedougou region (**Niane *et al.*, 2014**). Moreover, the presence of mineral sands like ilmenite, zircon, and rutile in coastal mining sites in The Gambia, such as Kartong, Sanyang, and Batakonko, suggests additional potential sources of contamination (**Manzini *et al.*, 2024**).

1.2 Problem statement

Heavy metals (HMs) are naturally occurring elements, but their widespread and unregulated use due to anthropogenic activities has resulted in serious environmental contamination. Continuous discharge of HMs into soil and water has posed toxic threats to plants, animals, and humans, with agriculture being one of the most affected sectors (**Martins *et al.*, 2021; Dixit *et al.*, 2015**). The accumulation of heavy metals in soils reduces crop yield and quality, and their absorption by plants allows entry into the food chain, which threatens human health.

Moreover, global climate change, marked by irregular precipitation and reduced freshwater availability, has intensified the impact of heavy metal pollution. Over 70% of freshwater is used in agriculture, mining, and waste disposal, yet climate variability contributes to water scarcity and reduced microbial activity. This worsens land degradation, such as salinization and desertification, and further reduces agricultural productivity and biodiversity (**Arora, 2018**).

Given the limitations of conventional remediation methods, bioremediation using mesophilic bacteria has emerged as a promising alternative. These bacteria can survive in harsh environments, including acidic conditions, and are capable of detoxifying and removing HMs from contaminated environments through processes like bioleaching (**Gu *et al.*, 2018**). However, climate-induced environmental stress and fluctuating metal concentrations may affect their performance, making the search for resilient and efficient bacterial strains crucial for sustainable remediation and agricultural recovery (**Oyewo *et al.*, 2020**; **Xiang *et al.*, 2021**).

1.3 Research Gaps

Despite the increasing global attention on heavy metal (HM) contamination and its consequences for environmental and public health, there is a notable lack of region-specific data and targeted mitigation strategies in The Gambia (**Sandeep *et al.*, 2019**). While trace levels of heavy metals have been detected in surface waters near mining zones such as Faraba Banta and transboundary waters linked to Senegalese gold mining sites, comprehensive assessments of HM contamination in Gambian agricultural soils remain scarce (**Niane *et al.*, 2014**; **Král *et al.*, 2019**). Furthermore, the presence of mineral-rich sands in coastal areas such as Kartong, Sanyang, and Batakonko raises concern about long-term metal leaching into surrounding ecosystems **(Bisht and Martinez-Alier, 2023).**

Importantly, the role of indigenous soil bacteria in mitigating HM toxicity through bioremediation (**Atuchin *et al.*, 2023**) has not been adequately explored within the Gambian context. Likewise, there is limited understanding of local farmers' awareness of HM risks and their practices regarding agrochemical use, which may exacerbate contamination. This creates a critical knowledge gap at the intersection of environmental microbiology, agricultural sustainability, and public health. Addressing this gap is essential to develop locally adapted bioremediation strategies and to inform risk communication and policy decisions in The Gambia.

1.4 Justification

Heavy metals such as mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), zinc (Zn), and copper (Cu) are toxic and persistent contaminants frequently present in soil and aquatic environments, posing significant challenges that can be addressed through bioremediation strategies. Their presence in the environment results from both natural processes, such as weathering of rocks, soil formation, and increasing human activities like industrialization, mining, urbanization, and intensive agriculture (**Yabe *et al.*, 2010**; **Hakeem, 2015**).

Studies in several African countries show that metal concentrations in soil, water, crops, and livestock often exceed international safety limits, indicating a growing environmental and public health concern (**Yabe *et al.*, 2010**). These metals are not only persistent but also non-biodegradable, making their remediation a critical environmental priority.

In Sub-Saharan Africa, research on heavy metal pollution has expanded, supported by advances in analytical techniques for more accurate detection and assessment. However, significant knowledge gaps remain due to the limited availability of comprehensive studies evaluating the extent, distribution, and impacts of heavy metal contamination across the region (**Yabe *et al.*, 2010; Niane *et al.*, 2014**). Mining activities, particularly in Zambia's Copperbelt and West African gold mining regions such as Kedougou (Senegal), have introduced substantial levels of heavy metals into soil and water systems (**Niane *et al.*, 2014**). Inadequate environmental regulation, poor waste disposal, and growing urbanization and agriculture further exacerbate this contamination (**Léopold *et al.*, 2008**).

Moreover, artisanal and small-scale mining (ASGM), textile industries, and tanneries in Africa and other developing regions discharge metal-laden effluents into rivers and farmlands, contaminating the food chain and posing a threat to local communities (**Chabukdhara and Nema, 2012; Alvarez *et al.*, 2017**).

However, climate change introduces new variables that can affect the efficiency of microbial bioremediation. Microbial activity is highly sensitive to environmental factors such as temperature, pH, moisture, and nutrient availability, all of which can be altered by climate change (**Alkorta *et al.*, 2017**). For example, fluctuations in temperature and CO_2 levels can influence the production of key metal-binding peptides like metallothioneins (MTs), glutathione (GSH), and phytochelatins (PCs), thereby impacting metal detoxification pathways (**Niane *et al.*, 2019**).

In agriculture, the problem is more severe due to the extensive use of untreated wastewater and sewage sludge on farmlands, especially in water-scarce regions. These practices have resulted in the accumulation of heavy metals in food crops, compromising food safety and human health. Greenhouse-grown vegetables, for instance, have shown higher metal uptake compared to open-field crops, possibly due to environmental constraints like low light intensity **(Rai *et al.*, 2019**).

Bioremediation, especially using metal-tolerant bacteria, offers an environmentally friendly, cost-effective, and sustainable approach to mitigating heavy metal contamination. However, the rising climate challenges and growing demand for safe agricultural practices, developing effective microbial strategies for metal removal is vital for restoring contaminated soils, protecting food security, and ensuring long-term ecosystem health. In the Gambia River system, **Niane *et al.*** (**2019**) reported mercury-resistant bacteria in sediments impacted by ASGM activities, highlighting the dual issue of contamination and microbial adaptation.

In light of increasing pressures from population growth, land-use alterations, and climate change, there is a pressing need to develop and enhance microbial-based bioremediation approaches for the effective management of heavy metal contamination. Investigating indigenous or mesophilic bacterial species capable of detoxifying heavy metals in sub-Saharan environments could provide sustainable solutions for safeguarding water quality for agricultural, domestic, and ecological use (**Adams *et al.*, 2020; Raimi *et al.*, 2021).**

In summary, this study is justified by the interconnected challenges of climate change, agricultural resilience, and environmental degradation, with bioremediation offering a practical and adaptable strategy for addressing heavy metal pollution in ecologically sensitive areas.

1.6 Research Aim and Objectives

The study aimed to assess farmers' knowledge and perceptions of the effects of climate variability and pollution and their varying adaptation strategies to cope with the menace.

Additionally, it evaluated the potential of mesophilic bacteria for heavy metal bioremediation in agriculture under climate change in The Gambia.

The formulated specific objectives are:

1. Assess farmers' knowledge and perceptions of the effects of climate variability and pollution on crop production and their varying adaptation strategies in The Gambia.
2. Characterize mesophilic bacteria from CRR South of The Gambia.
3. Evaluate the potential of isolated bacterial strains to tolerate and grow under varying heavy metal concentrations using optical density (OD) measurements.

1.7 Research Questions

In the course of this PhD work, it is intended to provide the answers to the following questions:

- What are the perceptions and adaptation strategies of farmers against climate variability and pollution affecting crop production in The Gambia?
- Which mesophilic bacterial species are found in the study area?
- Which mesophilic bacterial species in the study area are good biosorbents for HMs?

1.8 Hypothesis

H_1: Perceptions and adaptation strategies towards HMs pollution on crop production as the results of climate change vary among ethnic and educational backgrounds of individuals in The Gambia.

H_2: There is morphological and genetic variation among the mesophilic bacteria found in the CRR South of The Gambia.

H_3: The extent of mesophilic bacteria species growth in the polluted environment varies among bacteria, with higher bioremediation potential in *Bacillus* species.

1.9 Organization of the Study

This thesis is written in five chapters to ensure a coherent and structured presentation of the research.

Chapter 1: Introduction

The first Chapter is the background of the study, the gaps, and the rationale of the research, the research objectives, the research questions, the hypothesis, and the limitations of the study.

Chapter 2: Literature Review

The second Chapter constitutes a review of related literature on climate change and variabilities, climate change and pollution, climate change and heavy metal pollution, sources of heavy metal pollution in the environment, and effects of heavy metals on microbes, plants, animals, and humans. In this same chapter, methods, bioremediation of heavy metals from contaminated environments, strategies of bioremediation, characteristics, and species of mesophilic bacteria used in the bioremediation of heavy metals were highlighted. The use of the Knowledge and Perception Model to carry out studies on farmers' knowledge and perception of the effects of climate variability and pollution, and their varying adaptation strategies.

Chapter 3: Materials and Methods

The third Chapter explains the research methods used in this study.

Chapter 4: Results and Discussion

The fourth Chapter describes the results and discussions of the research.

Chapter 5: Conclusions, Recommendations, and Future Perspectives

This is the final chapter of the research, which summarizes the main outcomes of the research, addresses the limitations of the study, and offers recommendations and future perspectives.

CHAPTER 2

LITERATURE REVIEW

2.1 Climate Change and Variability

Nowadays, global warming, climate change, and water shortages are having devastating effects throughout all parts of the modern world. Environmental science is now in imminent need of a determined human scientific endeavor (**Raimi *et al.*, 2021**)**.** The harmful and concerning effects of climate change are reflected in the disruption of natural, agricultural, and urban environments, due to higher mean temperatures, longer and more abrupt drought periods, and storms, as well as increased likelihood of heat waves and heavy precipitation scenarios, which can exacerbate heavy metal contamination and highlight the need for effective bioremediation strategies. The injurious effects of these environmental parameters altered by climate change can have an impact on both soil and water microorganisms and, hence, impede their bioremediation performance (**Alkorta *et al.*, 2017**). The potential of soil and water microorganisms in bioremediation is under threat due to alterations in temperature, moisture, wet-drying and freeze-thawing cycles, pH, etc. that may result in modified interactions among microorganisms required for the remediation of environmental contaminants (**Fierer *et al.*, 2003**).

2.2 Climate Change and Pollution

Global warming, climate change, and environmental pollution together create a complex set of stress factors that intensify the challenges in the bioremediation of heavy metals. A combination of several ecological stress factors can potentially affect plants, soil, and microbial populations in a changing climate. Stress factors that can simultaneously affect plants could be grouped as biotic (e.g., viruses, bacteria, insects), abiotic, which is climate-driven (e.g., flooding, drought, heat), abiotic anthropogenic-driven (e.g., pesticides, antibiotics, heavy metals), or biotic/abiotic soil-associated (e.g., nutrient deficiency, salinity, decreased microbial diversity) origin. The earth's temperature is increasing, mainly due to anthropogenic factors (e.g., fossil fuel combustion and greenhouse gas emissions from energy supply, transport, industry, and agriculture), and climate change alters the concentration and distribution of air pollutants and interferes with the seasonal presence of allergenic pollens in the atmosphere by prolonging these periods (**Zandalinas *et al.*, 2021**).

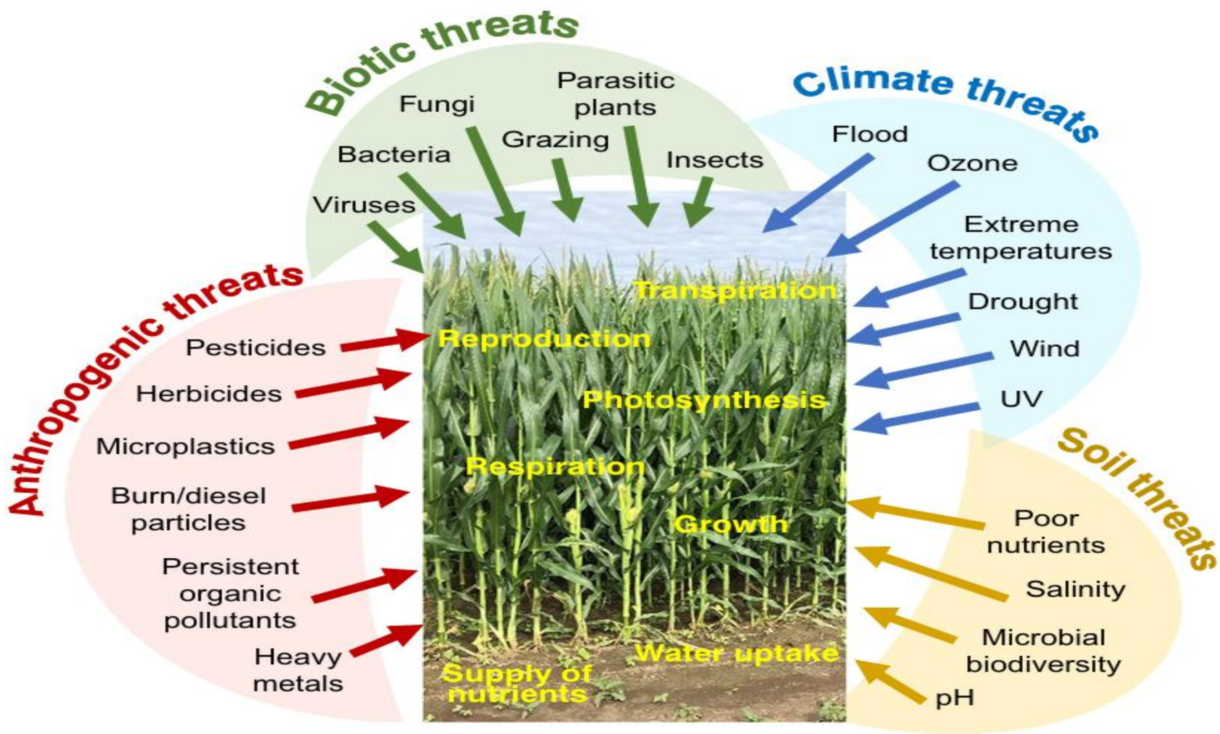


Figure 1: Modified multifactorial stress combination due to climate change and pollution poses potential impacts on plants, soil, and microbial populations (Zandalinas *et al.*, 2021).

2.3 Climate Change and Heavy Metal Pollution

Yearly, almost 100 billion tons of tilling are discharged all around the world. Tailings deposited from mining waste not only destroy the surrounding ecological environment but also pose a threat to water, soil, plants, animals, and human beings by leaching. Several factors are in play, which affect heavy metal release from tailings, such as chemical properties of tailings, temperature, precipitant, microorganisms, and O_2 content. If tailings' leachate is not managed properly, it has the potential to cause considerable environmental degradation as well as water and soil contamination and can have a severe impact on the health of nearby communities, biodiversity loss, and the aquatic ecosystem (**Fu *et al.*, 2023**). Therefore, gaining insight into the mechanisms behind heavy metal leaching from mine tailings is crucial for effective pollution control and proper management of tailings. The release and mobility of heavy metals primarily occur through two processes: the transformation of metal speciation and the production of acid mine drainage. When the sulphide-bearing material is exposed to oxygen and water, acid mine drainage is generated and heavy metals are leached out from the tailings. Although this process occurs naturally, mining and climate change can promote acid mine drainage generation and tailings' leaching, simply by increasing the quantity of sulfide exposed and the reaction rate **(Fu *et al.*, 2023**). There is a broad scientific consensus that ongoing greenhouse gas emissions are steadily warming the planet, and if left unaddressed, this trend could lead to serious environmental consequences, including increased heavy metal contamination, highlighting the urgent need for sustainable solutions such as bioremediation. The scientific community agreed that the Earth is getting warmer daily due to greenhouse gas emissions, and if this trend is not checked, it will result in severe consequences. Industrialization came with many issues that include but are not limited to continuous exposure of the earth's ecosystem to a variety of potentially hazardous elements such as cadmium, copper, nickel, zinc, and lead that are present in large amounts in the soil (**Kim *et al.*, 2015; Oyewo *et al.*, 2020**).

Among the xenobiotic substances associated with climate change and capable of causing environmental degradation are heavy metals. The term "heavy metal" refers to the group of metals and metalloids that have an atomic density of more than 4 g/cm^3, a very high level above that of water (**Priyadarshanee and Das, 2021**). Some of the toxic heavy metals such as lead (Pb), chromium (Cr), zinc (Zn), cadmium (Cd), iron (Fe), copper (Cu), manganese (Mn), and nickel (Ni) are released from various industrial manufacturing or different catalytic processes through their water and solid discharges (**Ananthashankar, 2013).** These recalcitrant metals have the potential to stay longer, contaminate, and degrade the environment because they are non-degradable and continuously accumulate in the food chain, causing severe threats to life (**Tak *et al.*, 2013**). Consumption of crops cultivated in heavily contaminated soils is one of the primary pathways through which humans are exposed to heavy metals. Therefore, soils and sediments can retain and immobilize chemicals until there is a change in the factors that influence their retention in soils and sediments, leading to a change in the bioavailability of the stored chemical and consequently mobilization of the chemical into the environment (**Kaninga *et al.*, 2020; Oyewo *et al.*, 2020**). The effects of climate change on heavy metal contamination have been qualitatively assessed in aquatic organisms. Increased flooding risks associated with climate change may lead to the inundation of contaminated sites, thereby enhancing the likelihood of pollutant remobilization. This can result in the transport of heavy metals through floodwaters and contaminated sediments into freshwater and marine ecosystems. Among the key drivers of heavy metal contamination in the environment is climate change, which influences their mobilization, distribution, and bioavailability. This is because the capacity of soils to absorb and immobilize heavy metals is strongly influenced by certain prevailing properties. These properties include but are not limited to pH, redox potential, salinity, and organic content that directly affect the cation exchange capacity and soil structure (**Wang *et al.*, 2014; Oyewo *et al.*, 2020**). Climate change-induced temperature variations can influence the release of arsenic from flooded contaminated soils, potentially enhancing the mobilization of heavy metals in the environment. Arsenic (As) in soils and sediments is commonly mobilized when anoxic conditions support microbial iron (Fe) and As reduction. Both laboratory and field studies have suggested a decoupling between Fe and As reduction and release, although the links between these processes are still not well understood. In microcosm experiments, the formation of Fe(II) and As(III) in the porewater and the soil solid phase during the flooding of a contaminated floodplain soil at temperatures of 23, 14, and 5 ^oC. At all temperatures, flooding induced the development of anoxic conditions and caused increasing concentrations of dissolved Fe(II) and As(III). Decreasing the temperature from 23 to 14 and 5 ^oC strongly slowed down soil reduction and resulted in a much lower release of dissolved iron (Fe) and arsenic (As). After 33 days at 5 ^oC, the concentrations of dissolved Fe (<0.4 mM) and As (<10 µM) were about 10 times lower than when measured at 23 ^oC. The high-temperature dependence of soil redox dynamics is not surprising, given that the activity of soil microbes normally increases with rising temperature, as many bacteria reach their maximum activity near 25-30 ^oC (**Visser *et al.*, 2012**).

The removal of soil and water containing heavy metals can be highly complicated due to several factors, such as their composition, climate conditions, and the mobility/distribution of species in both solid and liquid phases (**Oyewo *et al.*, 2020**).

2.4 Sources of Heavy Metal Pollution in the Environment

There are different sources through which constantly increasing amounts of heavy metals are annually discharged into the environment, such as improper disposal of industrial solid and liquid waste, mining activities, sewage, and the use of pesticides and fertilizers (**Todorova *et al.*, 2019**). Although several inorganic metals like magnesium (Mg), nickel (Ni), chromium (Cr^{3+}), copper (Cu), calcium (Ca), manganese (Mn), sodium (Na), and zinc (Zn) are vital elements needed in small quantity for metabolic and redox functions, others such as aluminum (Al) lead (Pb), cadmium (Cd), gold (Au), mercury (Hg), and silver (Ag) do not have any biological role and are toxic to living organism (**Igiri *et al.*, 2018**). A significant proportion of these heavy metals enter the environment as a result of airborne emissions and particulate matter produced by transportation, energy generation, metallurgical industries, and construction activities. They also reached into the air in the form of aerosol and were finally deposited in the soil through natural sedimentation and rainfall processes.

Automobile transport has the potential to cause serious heavy metal pollution. Moreover, heavy metals enter the soil through sources such as sewage irrigation, domestic wastewater, industrial effluents, and sludge discharge. Industries release a lot of harmful chemicals, which are directly released into an open area (**Fu and Wang, 2011**). Furthermore, people nowadays use many fertilizers and pesticides for agricultural purposes. Continuous overuse of fertilizers has accelerated soil degradation, creating conditions conducive to heavy metal accumulation and necessitating bioremediation efforts. Heavy metals are the highest reported pollutant in fertilizers. Phosphoric fertilizers generally contain high amounts of toxic heavy metals, whereas nitrogen and potash fertilizers contain considerably lower amounts of heavy metals. Scientists globally have been working tirelessly on the management of environmental pollutants and their control over the past two decades due to their hazardous effects on biodiversity (**Mendy *et al.*, 2021**). Despite notable advancements in human living standards in recent years, the environmental consequences of both anthropogenic and natural activities, particularly the accumulation of heavy metals, must not be overlooked.

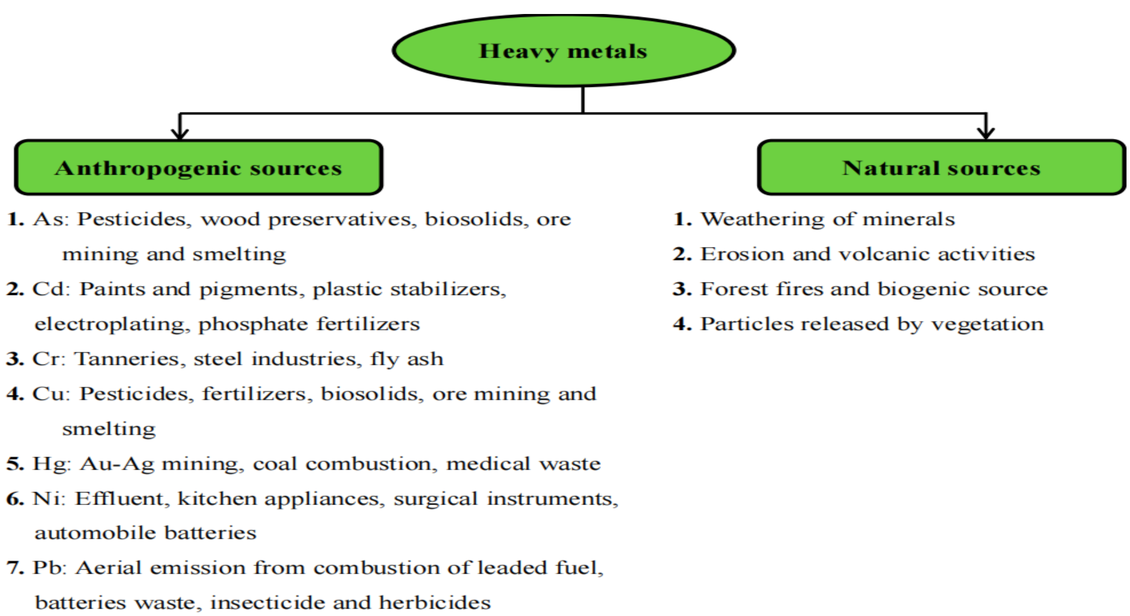


Figure 2: Sources of heavy metal concentration in the environment (Dixit *et al.*, 2015; Abraham *et al.*, 2017).

2.4.1 Natural Sources

Numerous studies have identified various natural sources of heavy metals, which can be released into the environment under specific environmental conditions. Under different and certain environmental conditions, natural emissions of heavy metals occur. Such emissions include volcanic eruptions, sea-salt sprays, forest fires, rock weathering, biogenic sources, and wind-borne soil particles (**Abraham *et al.*, 2017**; **Ermolin *et al.*, 2018**). Natural weathering processes can mobilize heavy metals from their original reservoirs into various environmental compartments. During fire outbreaks, the plant biomass and litter layers are consumed, and the mineral soil is heated up, changing both the physical and biogeochemical properties. These changes are either short-term or long-term, while some may be permanent. The alterations depend mainly on the nature of the forest, the severity and frequency of fire events, and post-fire climatic conditions. Research has it that fire can release and remobilize sequestered metals from plants and soil organic matter through a relatively complex set of processes. Forest fires can significantly change the physical and biogeochemical characteristics of soil, leading to the release, mobilization, and redistribution of heavy metals previously bound to soil organic matter and vegetation into the air, soil, and water systems. This may affect the health of aquatic and terrestrial animals as well as humans (**Abraham *et al.*, 2017**).

Heavy metals occur in various chemical forms such as hydroxides, oxides, sulfides, sulfates, silicates, and organic complexes. Among these, the most prevalent heavy metals include lead (Pb), nickel (Ni), chromium (Cr), cadmium (Cd), arsenic (As), mercury (Hg), zinc (Zn), and copper (Cu). Although the aforementioned heavy metals can be found in trace amounts, they still cause serious health problems to microorganisms, plants, animals, and humans (**Parmar and Thakur, 2013**).

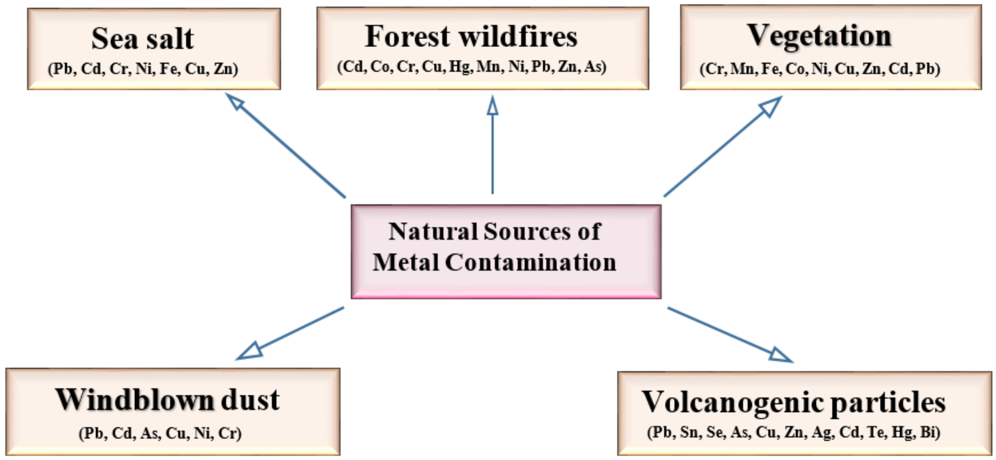


Figure 3: Modified natural sources of metal contamination (Edelstein and Ben-Hur, 2018; Srivastava *et al.*, 2018).

2.4.2 Anthropogenic Sources

Various developmental and agricultural activities, including electroplating, tanning, paper production, mining, smelting, fertilizer use, pesticide application, and electronic manufacturing, have introduced significant amounts of pollutants into the environment, particularly heavy metals and petroleum hydrocarbons. The production of weapons from nuclear power factories and nuclear accidents is capable of releasing enormous amounts of radionuclides into the environment. These recalcitrant pollutants have been reported in many studies to have disrupted the physiological functions of biodiversity in their natural ecosystems (**Amin Al Sulami, 2015; Jacob *et al.*, 2018**). The removal of xenobiotic pollutants, particularly heavy metals, from soil, air, and water in industrial and agricultural zones remains a significant environmental challenge. The main focus on remediation of toxic heavy metals was, but not limited to, the following: mercury, lead, cadmium, and chromium due to their negative impact on the environment. Some of these pollutants have adverse consequences for the ecosystem and human health, including anthropogenic CO_2 and greenhouse gases, which cause acute and chronic malfunctions, such as *itai-itai* disease, renal damage, emphysema, hypertension, and atrophy (**Alkorta *et al.*, 2017; Mendy *et al.*, 2021**).

In Europe, research has shown that there are about 2.5 million regions with potential pollution, out of which 340,000 of these sites are inundated with actual pollution **(Panagos *et al.*, 2013;**

European Environment Agency, 2014). In many of these unhygienic environments, soils are simultaneously polluted with various compounds (heavy metals, mineral oils, aromatic hydrocarbons, etc.), rendering their remediation more difficult (**Panagos *et al.*, 2013; Agnello *et al.*, 2016**) hence posing significant environmental hazards for terrestrial and aquatic ecosystems as they are important sources of pollution which may result in ecotoxicological effects (**Panagos *et al.*, 2013**). With globalization, industries are very active in the generation of effluents, which are mainly disposed of indiscriminately in the environment. Some of the particles in this sewage sludge are heavy metals and, when released to the environment without treatment, can pose devastating threats to living cells, including plants, animals, microbes, and human beings. Currently, bioremediation is one of the most appealing technologies that is employed to detoxify such effluents of heavy metals. Microbes or their enzymes can be used to transform toxic heavy metals into less reactive concentrations to clean up a polluted environment (**Igiri *et al.*, 2018**). Tannery industries use chromium materials, which could be either in chrome liquor or in powder for tanning of leather, and residual chromium, which is eventually discharged in solid or liquid effluents. However, research conducted by **Wionczyk *et al.* (2006**) has shown that only a fraction of the chromium salts used in the process react with the skins; the rest of the salts remain in the tanning exhaust bath and are later moved to a depuration plant, where the chromium salts end up in the sludge. When these effluents are discharged into the environment, untreated would elevate the level of pollution. For example, in India, about 2000-3000 tons of chromium escape into the environment annually from tannery plants, with high levels of chromium concentrations ranging between 2000 and 5000 mg/l in the aqueous effluent as compared to the recommended permissible discharge limits of 2 mg/l (**Altaf *et al.*, 2008**). Ideally, metals can be regarded either as essential or nonessential to a particular organism according to whether or not they exert any beneficial biological function. When biologically active, they can be incorporated into a microbial-cell structure and therein function, for example, as the metallic cofactors of enzymes. However, most metals, even those that are essential, can become toxic at elevated concentrations, posing risks to cells (e.g., inhibition of cell division and enzyme activity, denaturation of proteins, and damage to genetic materials (**Jacob *et al.*, 2018**). The concentration at which these metals become toxic depends on the metal and the physicochemical conditions (such as pH; the oxidation-reduction, or redox, potential [Eh]; the presence of organic matter, other ions, or solid substances with high cation-exchange capacity) that define their chemical speciation as well as on the particular tolerance of the microbes

(**Castro *et al.*, 2019**). Numerous studies have documented the adverse impacts of heavy metals and other toxicants on human health, highlighting the urgency of effective remediation strategies. Once these metals accumulate in the food chain, their ingestion at high levels can trigger harmful health conditions in humans. For instance, consumption of cadmium-contaminated foods has been shown to have caused human diseases, and it has also been evidenced that Pb exposure may result in seizures, mental retardation, and behavioral disorders (**Agwaramgbo *et al.*, 2012**). The threat that heavy metals pose to plants, humans, animal, and microbial activity, especially microbial respiration, is aggravated by their low environmental mobility, even under high precipitation, and their long-term persistence in the environment (**Sobolev and Begonia, 2008**).

In the past, Africa was termed a continent freed from heavy metal pollution (**Biney *et al.*, 1994**). Still, the recent rapid population growth and an insatiable urge for urbanization and industrialization have resulted in an unprecedented expansion without proper planning of cities and indiscriminate disposal of waste (**Yabe *et al.*, 2010**). Therefore, solid waste collection and management have posed a significant threat to biota in many African countries as waste generation surpasses local authorities' capacities for collection and disposal. The United Nations Centre for Human Settlements (UNCHS) opined that only one-third of the solid waste produced in urban areas across Africa is collected, and out of that, only 2% is recovered and recycled (**Yabe *et al.*, 2010**). Although the number of contaminants being released into water bodies is significant, there is an assumption that surface water contamination is insignificant in developing countries of tropical humid regions. There is, therefore, a clear indication that our understanding of freshwater resources is limited, and the need for their exploration and protection is urgent. Thus, the heavy runoff associated with perennial rainfall may worsen the impact of such pollution (**Elzwayie *et al.*, 2017**). Furthermore, it was also observed that the use of leaded gasoline, fugitive dust (**Dimari *et al.*, 2008**), indiscriminate disposal and burning of toxic waste, including nickel/cadmium-based indiscriminate disposal and burning of toxic waste, including nickel/cadmium-based batteries, due to weak pollution legislation, has worsened heavy metal pollution on the continent (**Yabe *et al.*, 2010**).

During the last decades, several studies were conducted in artisanal small-scale gold mining (ASGM) sites (e.g., Amazonia, East Africa, China) and indicated health threats for the indigenous populations who consume Hg-contaminated fish (**Niane *et al.*, 2014**). In sub-Saharan Africa, very little research has been carried out so far to assess Hg-contamination due to ASGM, and little or no data are available for the Kédougou region of Senegal, where ASGM is ongoing. Senegal is one of the countries where the use of Hg for gold (Au) in ASGM amalgamation has been intensified in the last decade. Previously, traditional artisanal gold mining involved mainly mechanical processes (i.e., gravimetric panning without Hg amalgamation). Over 50,000 people are currently engaged in artisanal gold activities in various villages in eastern Senegal (**Niane *et al.*, 2014**). The main activities of ASGM in this region occurred along the shores of the Gambia and Falémé Rivers and in seasonal water ponds located in the interior of the land. The inadequate availability of hydraulic equipment compels people to use water from rivers and wells for routine mining activities. During the process of gold recovery, elemental Hg used for Au amalgamation can be lost directly to the surrounding environment (i.e., soil or river sediment) or released to the atmosphere (i.e., mostly during the burning of amalgams) (**Guedron *et al.*, 2009**). Research has reported that the highest total mercury (THg) concentrations (2 to 6mg kg^{-1}) were recorded in surface sediments collected during the dry season at the Bantako 2 ASGM area along the River Gambia. In the same work, it was revealed that during the seasonal water pond site at Tinkoto, which is a few kilometers away from the riverbank, the samples collected near the surface yielded the highest THg concentration of 9.9 mg kg^{-1}, and slightly lower THg contents in samples from deeper locations. This shows that the depth of water bodies and the seasonality of the environment have a great influence on the availability of metals (**Niane *et al.*, 2014**).

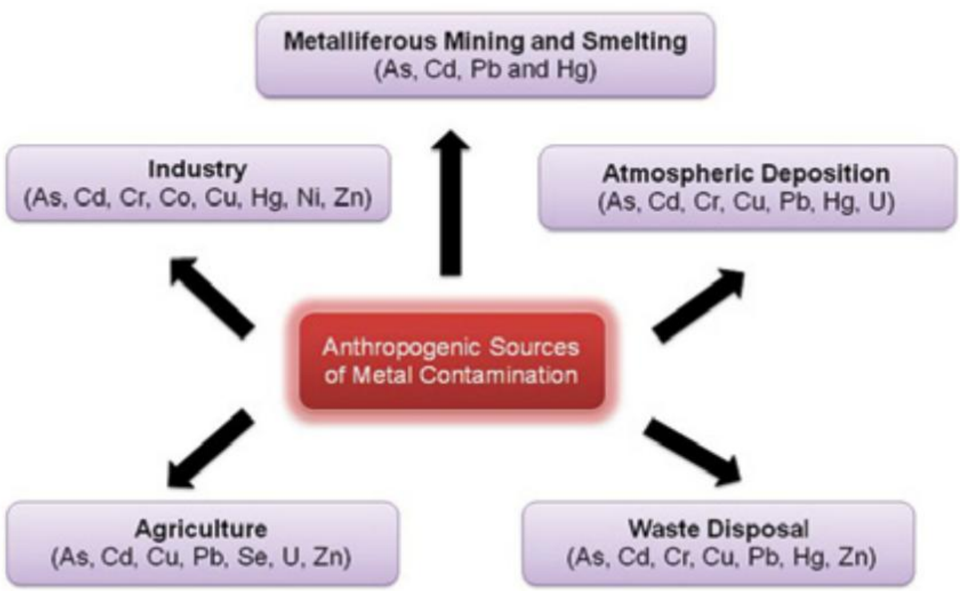


Figure 4: Anthropogenic activities inundate HMs in the environment (Gupta and Joia, 2016).

2.5 Harmful effects of heavy metals on microbes, plants, animals/livestock, and humans

In living organisms, heavy metals contribute to the interference of enzymatic processes and disruption of the function of subcellular structures and can cause damage through free radical processes, as well as via physicochemical properties similar to physiologically active metals. For example, in the cell cytoplasm, metal ions readily bind to functional groups, e.g, –SH, -OH, and –NH, which causes the protein molecule deformation and leads to a complete slowdown of the biological activity of proteins, and consequently apoptosis (**Wróbel *et al*., 2023**).

2.5.1 Impact of Heavy Metals on Microbes

Organisms respond to metal stress by adopting different defense systems, such as exclusion, compartmentalization, formation of complexes, and synthesis of binding proteins, like metallothioneins and phytochelatins (**Yadav *et al.,* 2017**). Mechanisms of metal toxicity are generally considered in terms of metal ion affinity for cellular components and biomolecules, or the stability of metal-biomolecule complexes formed, although the consequences are varied. At high concentrations, metal ions can either completely inhibit the microbial population by inhibiting their various metabolic processes, or organisms can develop resistance or tolerance to the elevated levels of metals (**Yadav *et al.,* 2017**)**.**

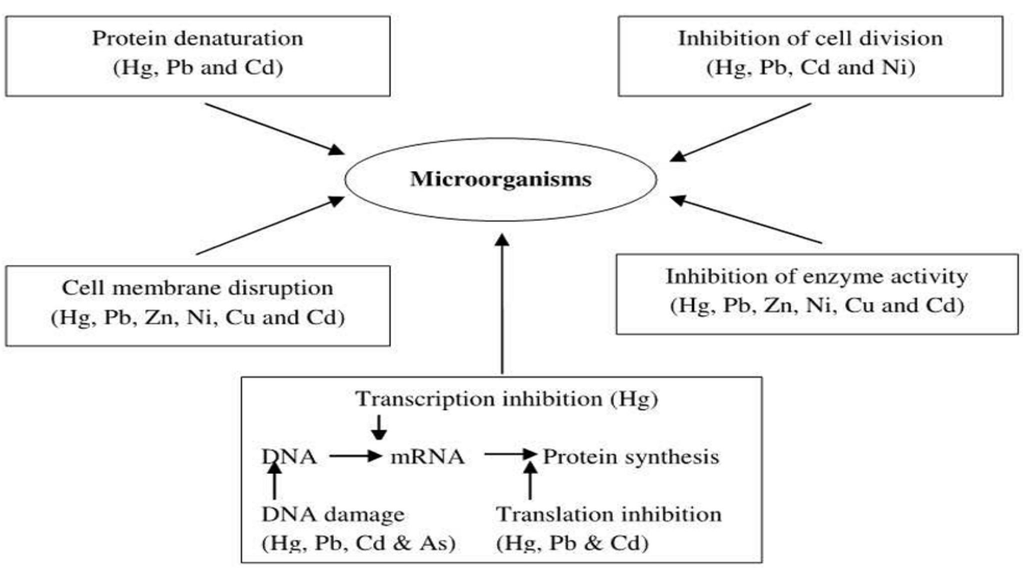


Figure 5: HM toxicity mechanism to microbes (Yadav *et al.,* 2017).

2.5.2 Impact of Heavy Metals on Plants

Heavy metals enter and accumulate in the ecological food chain primarily through absorption by plants, which act as the initial point of contact due to their root systems. These metals are then transferred to higher trophic levels as plants are consumed, highlighting the critical role of plant-soil interactions in the environmental spread of metal pollutants. In aquatic systems, the plant body is exposed to these ions, where heavy metals are absorbed directly into the leaves due to particles deposited on the foliar surfaces (**Asati *et al.*, 2016**).

Plants growing in heavy metal-polluted soils may encounter altered metabolism and physiological and biochemical activities that result in growth reduction, lower biomass production, and metal accumulation, especially when the heavy metals involved do not play any beneficial role in their growth and development (**Edelstein and Ben-Hur, 2018**). Numerous research studies have demonstrated that the accumulation and improper management of heavy metals pose significant toxic threats to plants, animals, and other living organisms once critical thresholds are exceeded. This underscores the urgent need to advance bioremediation research to better understand heavy metal toxicity in biological systems and develop sustainable strategies that protect ecological balance and reduce environmental pollution. There is therefore a need to intensify the research for a better understanding of heavy metal toxicity in plants and other related biological systems to maintain the ecological harmony of our planet. There are two aspects of plant interaction with heavy metals. While heavy metals exert harmful effects on plant growth and development, plants possess inherent resistance mechanisms that enable them to tolerate toxicity and contribute to the detoxification and remediation of heavy metal pollution in the environment. Studies indicate that heavy metal contamination adversely influences both the growth and photosynthetic pigment production of organisms. However, bioremediation using hyperaccumulator plants offers an effective strategy for reducing heavy metal concentrations in polluted soils, as these plants can absorb and accumulate high levels of metals, thereby facilitating environmental cleanup through phytoremediation. The high concentration of heavy metals, which is caused by their accumulation in soil, can be removed by using a hyperaccumulator plant through bioremediation/phytoremediation. Plants employ different mechanisms in the remediation of heavy metal-polluted soils, and phytoextraction is the most common method of phytoremediation used for the treatment of heavy metal-polluted soils, which ensures the complete removal of the pollutant (**Asati *et al.*, 2016**).

2.5.3 Impact of Heavy Metals on Animals/Livestock

Irrespective of the physiological role of heavy metals, when taken in excess beyond the tolerance limit, they are toxic. Animals are exposed to heavy metals via contaminated feed, fodder, forage, and water due to anthropogenic environmental pollution. Soils and water bodies in specific geographic regions are inundated with excess concentrations of heavy metals, which can be a natural source of poisoning in animals. Heavy metals, once introduced into living organisms, tend to accumulate and persist over extended periods, leading to varying degrees of toxicity ranging from acute to chronic effects, which may be clinically apparent or occur without noticeable symptoms depending on the level and duration of exposure. This highlights the critical need for effective bioremediation strategies to reduce environmental contamination and prevent bioaccumulation in ecosystems. Most metals are concentrated in the vital organs such as the liver and kidney, and cause clinical or subclinical toxicities or subtle effects like oxidative stress, immunotoxicity, cardiotoxicity, teratogenicity, enzyme inhibition, reproductive defects, and endocrine disruption (**Gupta *et al.*, 2021**). Acute heavy metal poisoning is associated with typical clinical symptoms, leading to the death of an individual animal or a group of animals with similar necropsy findings. Animals' exposure to chronic toxicities of HMs is characterized by deterioration in general body condition and loss of production. Elevated concentrations of heavy metals in consumable animal products highlight the urgent need for effective bioremediation strategies to mitigate environmental contamination and protect public health. Early diagnosis, specific antidote, and management can effectively reduce the adverse impact of heavy metals on livestock health and production (**Gupta *et al.*, 2021**).

For example, animals that consume high mercury-containing vegetation will be affected and will suffer from alopecia, neuropathy, and visual and gastrointestinal tract disorders (**Gupta *et al.*, 2021**).

2.5.4 Impact of Heavy Metals on Humans

The soil-plant-human food chain is a critical route through which heavy metal contamination in soil can reach humans. This pathway is particularly concerning because the accumulation of toxic metals poses significant health risks to both animals and humans, highlighting the urgent need for effective clean-up strategies to reduce metal transfer and exposure. For example, varying concentrations of heavy metals have been detected in food items such as beverages, juices, wines, and several food and herbal products in both developed and developing countries (**Edelstein and Ben-Hur, 2018**). The highest risk of heavy metals emanates directly from their transport along the food chain and the phenomenon of bioaccumulation: the largest amounts of a substance are delivered to the last link, which is human. Humans exposed to elevated levels of heavy metals may suffer from various diseases, such as cancers, cardiovascular problems, depression, hematological, gastrointestinal, and renal failure, osteoporosis, and tubular and glomerular dysfunction (**Edelstein and Ben-Hur, 2018).** Dietary intake, particularly from plant-based foods, constitutes the primary pathway for human exposure to heavy metals. Therefore, the accumulation of heavy metals in crops for animal feed and direct human consumption should be limited (**Wróbel *et al.*, 2023**).

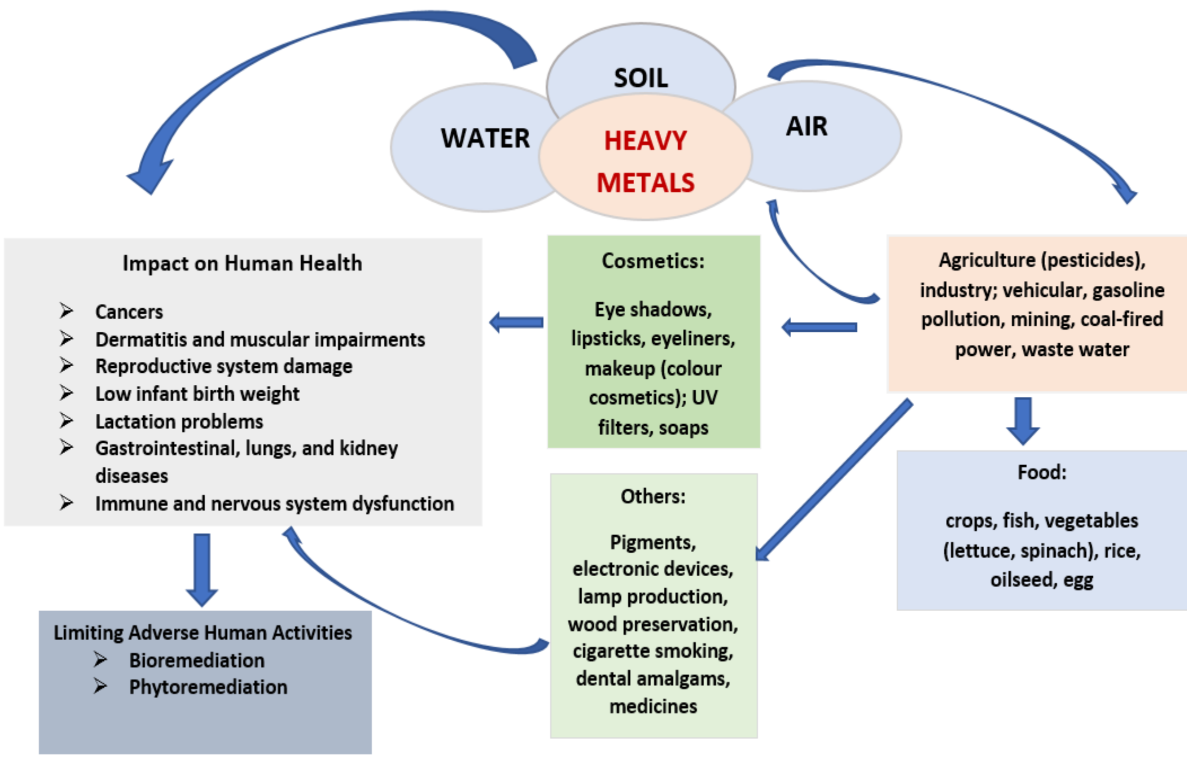


Figure 6: Routes of human exposure to HMs and various health implications (adapted from Anuoluwa *et al.*, 2025).

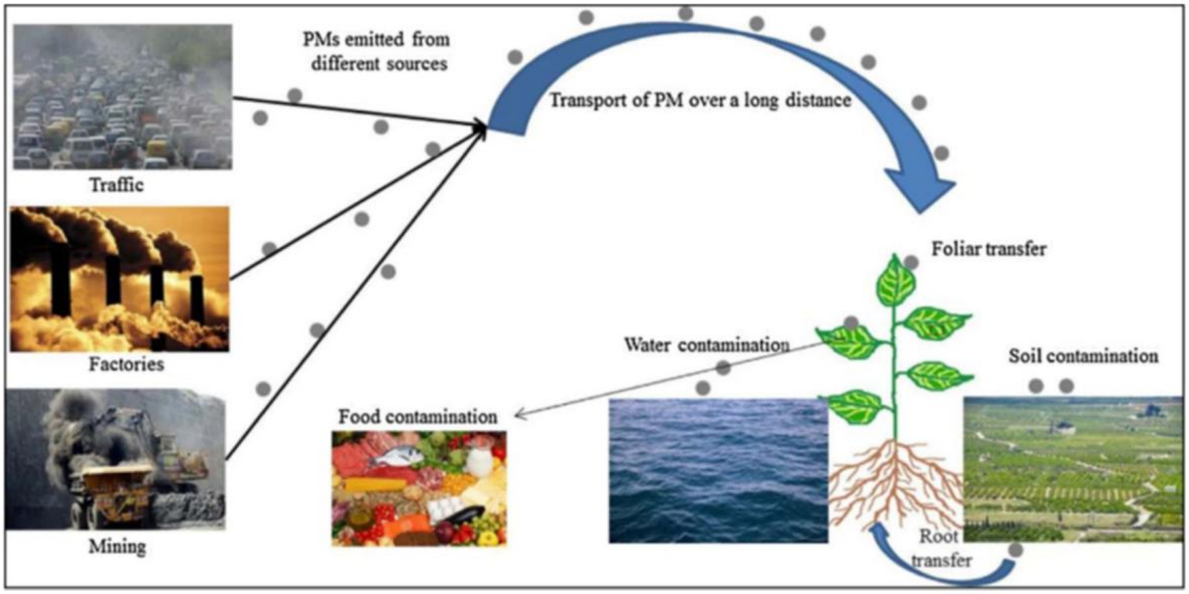


Figure 7: Heavy metal emission into the atmosphere from different industrial activities, transfer over long distances in air, deposition, and absorption by plant foliage, and possible risks of food chain contamination. PM = particulate matter (Edelstein and Ben-Hur, 2018).

2.6 Methods of Heavy Metal Removal from Contaminated Soil and Water

Nowadays, one of the greatest challenges faced by man is environmental pollution by heavy metals. In contrast to organic compounds that undergo natural degradation, heavy metals (HMs) are non-biodegradable and can remain in the environment for extended periods. The remediation of HMs requires strategic interventions to safeguard soil, air, and water quality, as well as the health of humans, animals, and ecosystems. This can be achieved through a range of physical, chemical, and biological treatment approaches. Various traditional techniques used for the remediation of inorganic effluents include chemical precipitation, coagulation, and flocculation. Additionally, methods such as adsorption, ion exchange, solvent extraction, neutralization, membrane filtration, and electrochemical treatment are commonly applied.

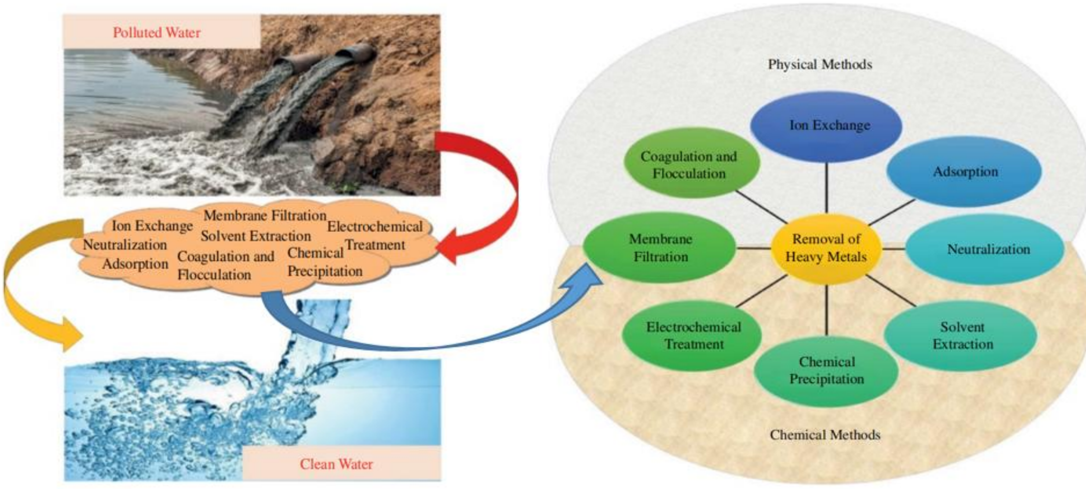


Figure 8: Modified methods used for the removal of heavy metal ions (Singh *et al.*, 2021).

2.6.1 Physical Methods

Physical methods employ the physicochemical properties of the substances for removal. This technology involves adsorption, electrodialysis, membrane filtration, capping, granular activated carbon, photocatalysis, soil washing, etc. (**Jobby *et al.*, 2018**).

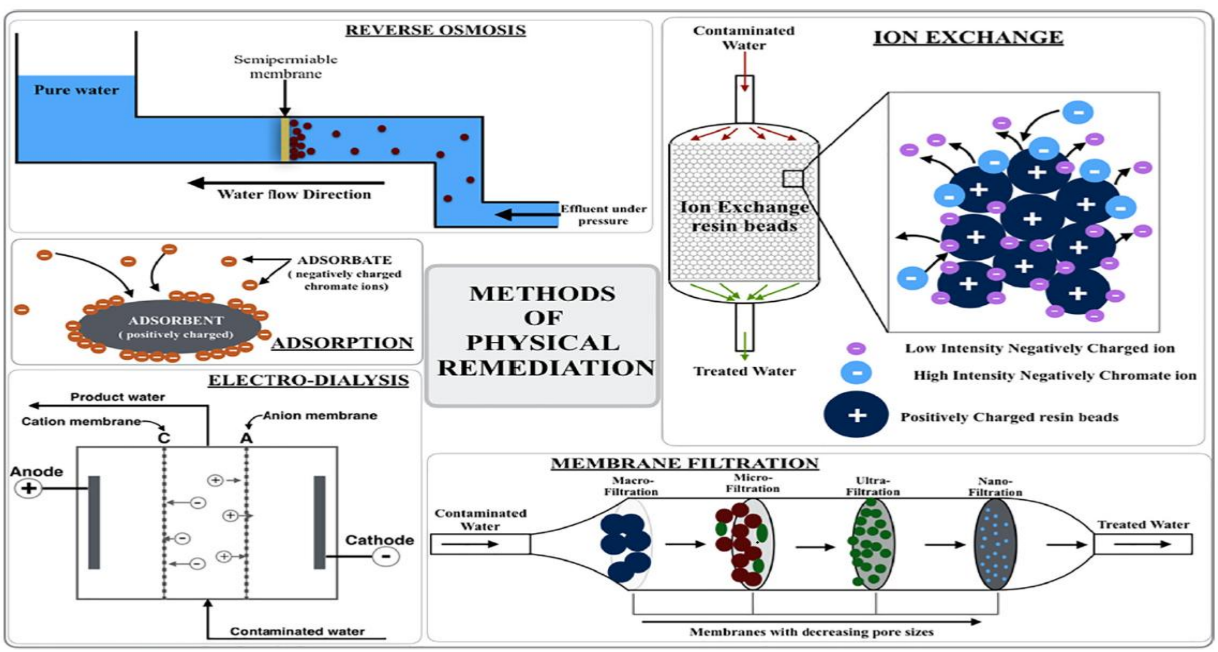


Figure 9: Diagrammatic representation of various methods of physical remediation of HMs (Jobby *et al.*, 2018).

2.6.1.1 Membrane Filtration

In inorganic effluent treatment, various types of porous and thin-film composite membranes are employed. Membrane processes can be driven by various types of forces, including low pressure (as in microfiltration (MF), ultrafiltration (UF), and membrane distillation), high pressure (such as nanofiltration (NF) and reverse osmosis (RO)), and osmotic pressure (found in forward osmosis (FO), electrodialysis (ED), liquid membranes, among others). Key parameters influencing membrane performance include pore size and distribution, hydrophilicity, surface charge, fluid flow dynamics, and the presence of specific functional groups. These factors critically affect the overall membrane operation, influencing both the permeate flux and the removal efficiency of contaminants such as heavy metals. The membrane's effectiveness is primarily evaluated based on its permeability (flux) and selectivity characteristics. Porous membranes such as cellulose acetate, polyamide, and polysulfone are commonly utilized in pressure-driven membrane processes, where feed solutions are forced through the membrane under elevated pressure. Upon passage through the membrane, water is collected at atmospheric pressure. In reverse osmosis (RO) systems, the applied pressure must exceed the osmotic pressure of the feed solution to ensure efficient water permeation.

The membrane filtration technique operates primarily through mechanisms such as size-based exclusion (steric hindrance), electrostatic repulsion due to charge interactions (Donnan exclusion), and adsorption of contaminants onto the membrane surface. Membrane separation has great potential in the treatment of inorganic effluents such as heavy metals, and it can remove organic compounds and suspended solids (**Abdullah *et al.*, 2019**). This technology has attracted great attention and has become a favorable approach to heavy metal remediation due to its cost-effectiveness and high efficiency rate. For the targeted removal of heavy metals, various membrane-based technologies have been engineered to reduce both treatment costs and environmental contamination levels. Metal ions such as Cu^{2+}, Ni^{2+}, Cr(VI), and As(V) have been successfully remediated by reverse osmosis, ultrafiltration, and nanofiltration (NF) (**Singh *et al.*, 2017**).

- **Reverse Osmosis (RO)**: This is a pressure-driven membrane filtration process that uses a semi-permeable membrane to separate water from dissolved contaminants (**Fu and Wang, 2011**). It efficiently eliminates a wide range of dissolved ions and plays a significant role in desalination processes, accounting for over 20% of the global desalination capacity. Additionally, it is extensively applied in wastewater treatment, particularly within the fields of chemical and environmental engineering.

Numerous scientific investigations have confirmed the effectiveness of reverse osmosis (RO) in eliminating heavy metals from wastewater. For instance, **Mohsen-Nia *et al.*** (**2007**) employed disodium ethylenediaminetetraacetic acid (Na_2EDTA) as a chelating agent to improve the removal efficiency of copper and nickel, resulting in metal rejection rates of up to 99.5% through enhanced complexation. Similarly, **Ozaki *et al.*** (**2002**) employed an aromatic polyamide membrane (ES20) and achieved over 95% removal of copper, nickel, and chromium. In another study, **Singh *et al.* (2017**) reported that pre-treatment combined with RO removed 99.2% of nickel and 98.8% of zinc from wastewater.

- **Ultrafiltration (UF) for Heavy Metal Removal**: Ultrafiltration (UF) is a membrane-based separation process utilized for the removal of heavy metals, macromolecules, and suspended solids from wastewater. The technique functions by selectively filtering compounds according to their molecular weight, typically ranging from 1,000 to 100,000 Daltons, through membrane pores sized between 5 and 20 nanometers. This method can attain a removal efficiency of up to 90%.

There are two improved UF techniques for HM removal as shown below (**Huang *et al.*, 2015**):

✓ **Micellar-Enhanced Ultrafiltration (MEUF)**

This method uses surfactants (such as anionic ones) that form micelles in solution. Heavy metal ions are trapped on the outer surface of these micelles due to electrostatic interactions. The micelles, along with the bound metals, are retained by the UF membrane. MEUF is efficient for removing trace contaminants but has higher operational costs.

✓ **Polymer-Enhanced Ultrafiltration (PEUF)**

In PEUF, water-soluble polymers are added to bind with small solutes like heavy metals. These larger polymer-metal complexes are then removed by UF membranes. PEUF allows the removal of low molecular weight metals that are not retained by regular UF. It is energy-efficient and operates under lower pressures compared to reverse osmosis (RO) and nanofiltration (NF) (**Huang & Feng, 2019; Singh *et al.*, 2021**).

Studies have shown that combining UF with chelating agents can significantly improve the removal of metals such as arsenic As(V), Cd(II), Pb(II), Hg(II), Ni(II), and Se(II) from industrial wastewater (**Singh *et al.*, 2021**).

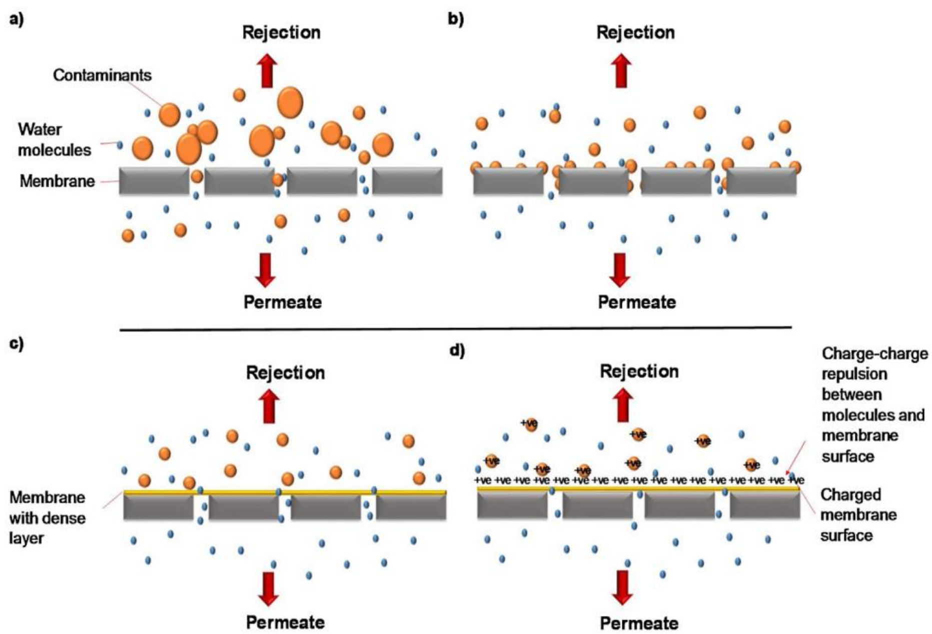


Figure 10: Remediation mechanisms by using a membrane. (a) Size exclusion/steric hindrance mechanism by low pressure; (b) adsorption by a low-pressure membrane; (c) size exclusion/steric hindrance mechanism by TFC membrane, and (d) Donnan exclusion/charge-charge repulsion by TFC membrane (Abdullah *et al.*, 2019).

- **Nanofiltration (NF):** This one is a membrane filtration method that lies between ultrafiltration and reverse osmosis (**Mehdipour *et al.*, 2015**). It is effective in removing heavy metals like nickel, chromium, copper, arsenic, cadmium, and lead (**Fu and Wang, 2011**). Most NF membranes are made from synthetic polymers with charged groups (**Zhu *et al.*, 2014**).

Positively charged nanofiltration membranes, such as polyethylimine (PEI)-crosslinked polyimide, are strong and stable, but they work best for removing multivalent cations. To improve the removal of anions, researchers modified these membranes by adding negatively charged chelating polymers, increasing their performance and achieving over 98% rejection of multivalent anions (**Gao *et al.***, **2014**).

Other membranes made using polybenzimidazole (PBI) and polyethersulfone/polyvinylpyrrolidone (PES/PVP) have also shown high removal rates 95% for cadmium, 98% for dichromate ($Cr_2O_7^{2-}$), and 93% for lead, due to their good chemical resistance and mechanical strength (**Zhu *et al.***, **2014**).

Additionally, embedding ferric oxide nanoparticles into membranes has enhanced lead removal. Polyamide NF membranes have also shown high efficiency (up to 97.5%) for lead removal, especially with increased pressure and feed concentration (**Gholami *et al.*, 2014**).

- **Electrodialysis (ED):** This process employs ion exchange membranes and an applied electric field to selectively separate ions from a solution through a membrane-based mechanism. These membranes are of two types: cation exchange (which allows positive ions to pass) and anion exchange (which allows negative ions to pass). ED is widely used for desalinating brackish and seawater, treating industrial wastewater, recovering valuable materials, and producing salts.

  In bioremediation and heavy metal treatment, ED has shown strong potential. **Nataraj *et al.*** (**2007**) built a pilot ED system that effectively removed hexavalent chromium (Cr^{6+}) to below 0.1 mg/L. Similar findings by **Cifuentes *et al.*** (**2009**) reported efficient separation of copper (Cu) and iron (Fe) during copper electrowinning operations, and **Lambert *et al.*** (**2006**) achieved the separation of trivalent chromium (Cr^{3+}) from sodium using modified membranes. This aligns with the research by **Mohammadi *et al.*** (**2004**), indicating that lead (Pb^{2+}) removal improved with higher voltage and temperature, though performance dropped with faster flow rates. Above 500 mg/L lead, the removal rate no longer depended strongly on concentration.

2.6.1.2 Coagulation and Flocculation

Coagulation-flocculation is a commonly employed physicochemical technique for eliminating heavy metals from aqueous solutions. Coagulation works by neutralizing the charges on colloidal particles using coagulants such as alum, polyaluminium chloride (PACL), magnesium chloride ($MgCl_2$), aluminum hydroxide, and polyethyleneimine (PEI), allowing the particles to destabilize and begin aggregation. Flocculation then promotes the gradual agglomeration of these destabilized particles into larger flocs through gentle mixing and interactions with organic and inorganic polymers.

Biopolymers like chitosan act as eco-friendly coagulant-flocculant agents due to their biodegradability (**Singh *et al.*, 2021**). Once formed, the flocs are separated from the aqueous solutions through processes such as sedimentation, filtration, flotation, or straining. This method is effective for both drinking water purification and industrial wastewater treatment (**Teh and Wu, 2020**).

Studies have shown high metal removal efficiencies—up to 99% lead removal—with optimal pH ranges depending on the coagulant: pH 6.2–7.8 for alum, 8.7–10.9 for $MgCl_2$, and 8.0–9.3 for PACL. Additionally, ferric chloride ($FeCl_3$) has been used effectively to remove metals like copper, zinc, lead, and nickel from synthetic effluents (***Singh et al.*, 2021**).

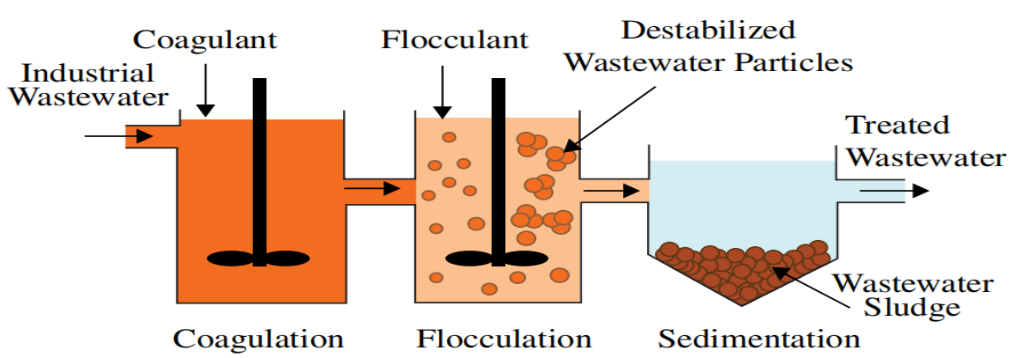


Figure 11: Coagulation and flocculation for effluent treatment (Singh *et al.*, 2021).

2.6.1.3 Floatation

The flotation technique is a widely applied method for the remediation of heavy metals in wastewater due to its high metal selectivity, minimal sludge production, and efficient separation capabilities (**Fu and Wang, 2011**). Cadmium removal was efficiently accomplished by employing sodium dodecyl sulfate (SDS) as a collector, combined with isopropanol or methyl isobutyl carbinol (MIBC) as frothing agents, achieving removal efficiencies of up to 98.8% at a frother concentration of 0.1% v/v. However, **Polat and Erdogan** (**2007**) demonstrated the removal of copper, zinc, and chromium using SDS and hexadecyl trimethyl ammonium bromide (HTAB) as collectors, with ethanol and MIBC as frothers. Their results showed removal rates increasing from 74% to 90% with rising pH.

Ion flotation offers key advantages such as low energy input, fast processing, suitability for trace metal concentrations, compact system design, and cost-effectiveness (**Singh *et al.*, 2017**). **Dorda *et al.*** (**2003**) integrated flotation with membrane filtration for enhanced copper, nickel, and zinc removal, achieving final concentrations below 0.05 mg/L. Zeolite was used as a bonding agent due to its high metal affinity, and HTAB served as a collector to separate the loaded agent via flotation.

Additionally, **Yuan *et al.*** (**2008**) utilized a plant-derived biosurfactant, tea saponin, as a natural collector and frother for the ion flotation of cadmium, lead, and copper. This biosurfactant-based method, which supports biodegradability and specificity, achieved removal efficiencies of 89.95% (Pb), 81.13% (Cu), and 71.17% (Cd) at a collector-to-metal ratio of 3:1 (**Singh *et al.*, 2017**).

2.6.1.4 Ion Exchange

Ion exchange is a cost-effective and efficient technology for removing heavy metals from aqueous solutions (**Fu and Wang, 2011**). It involves the transfer of metal ions from a liquid phase to a solid phase using ion exchange resins, either natural (e.g., zeolites) or synthetic (**Dizge *et al.*, 2009**). The process is reversible and involves the substitution of metal ions in the solution with hydrogen ions or other cations/anions present on the resin surface.

Synthetic organic resins are favored because of their superior efficiency, customizable molecular architecture, and porous three-dimensional polymer framework, which allows for selective ion recognition. These resins, which are insoluble in water, consist of high molecular weight polyelectrolytes capable of exchanging ions based on charge. They are classified as strong or weak acid/base cation or anion exchangers (**Singh *et al.*, 2021**).

Commonly used cationic exchange groups include sulfonic acid ($-SO_3H$) and carboxylic acid ($-COOH$). The ion exchange mechanism follows:

$$nR{-}SO_3H + M^{n+} \rightarrow (R{-}SO_3^-)_nM^{n+} + nH^+$$

$$nR{-}COOH + M^{n+} \rightarrow (R{-}COO^-)_nM^{n+} + nH^+$$

The efficiency of ion exchange is influenced by variables including the solution's pH, temperature, and initial concentration of metal ions, duration of contact, and the ionic charge. Though highly efficient for trace-level metal removal, it is less effective at treating solutions with very high metal concentrations (**Gode and Pehlivan, 2006**).

Industrially, ion exchange processes have been employed for the removal of heavy metals such as cadmium (Cd), chromium (Cr), zinc (Zn), lead (Pb), nickel (Ni), and copper (Cu). Notable resins include Purolite C100, INDION 225H, and AMBERJET 1200 Na, with removal efficiencies reaching up to 99% for specific metals. Despite its high effectiveness, the high cost of resins and process setup limits widespread commercial use (**Singh *et al.*, 2021**).

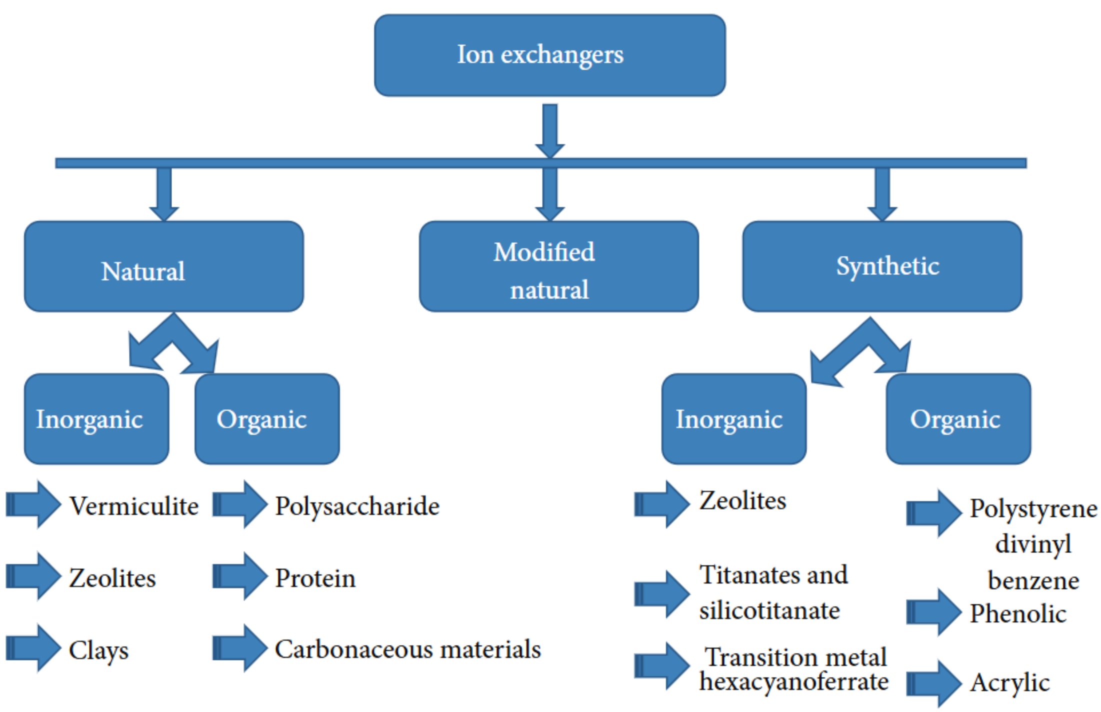


Figure 12: Classification of ion exchangers (Lalmi *et al.*, 2018; Singh *et al.*, 2021).

2.6.1.5 Adsorption

The rising levels of metal ions in industrial effluents have surpassed safe environmental thresholds, highlighting the urgent need for eco-friendly remediation methods. Adsorption, a surface-based process, is widely used to remove metal contaminants from wastewater. This process entails the attachment of contaminants to the external surface of an adsorbent via physical or chemical interactions, including van der Waals forces or hydrogen bonds, without entering the internal pores or structure of the adsorbent.

In composite and mixed matrix membranes (MMMs), adsorptive additives (e.g., –COOH, $–NH_2$, $–SO_3H$ groups) are integrated during fabrication to enhance membrane properties like porosity, hydrophilicity, and structural strength. The presence of these functional groups and porous structures enhances the membrane's ability to adsorb and immobilize metal ions. Although the inclusion of nanomaterials may reduce pore size, metal ions can still permeate due to their small size, indicating that adsorption, not size exclusion, is the primary removal mechanism **(Abdullah *et al.*, 2019**).

According to **Abdullah *et al.*** (**2016**), metal ion adsorption in MMMs occurs in four stages: (1) diffusion into membrane pores, (2) chemisorption on exposed nanoadsorbent surfaces, (3) ion transfer to adsorbent interfaces, and (4) equilibrium. The sorption mechanism is primarily controlled by chemisorption occurring at the external surface sites, with the rate-limiting factor being the transfer of ions to the adsorbent interface.

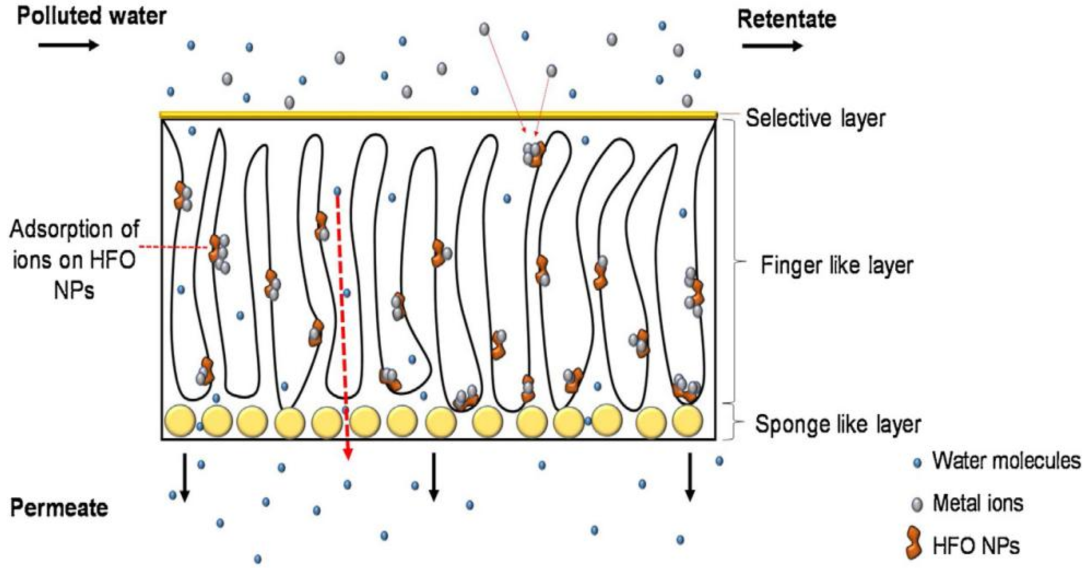


Figure 13: Illustrated mechanism of HMs adsorption on adsorbents in UF MMMs (Abdullah *et al.*, 2019).

Donnan exclusion (charge-charge repulsion)

Dense membranes remove solutes primarily through two mechanisms: sieving (size exclusion) and Donnan exclusion, the latter being a charge-based mechanism. Donnan exclusion is driven by electrostatic forces between charged solutes and the membrane surface, which depend on the membrane's isoelectric point (IEP). At the IEP, the membrane carries no net charge, resulting in solute rejection being determined solely by steric hindrance. Below the IEP, the membrane surface becomes negatively charged and repels anions; above the IEP, it becomes positively charged and repels cations. This surface charge results from the ionization of functional groups on the membrane.

The effectiveness of Donnan exclusion also depends on metal speciation, which varies with pH:

Arsenic (As) exists in multiple forms: AsO_4^{3-}, $HAsO_4^{2-}$, $H_2AsO_4^{-}$, and neutral H_3AsO -, depending on pH. Meanwhile, lead (Pb) exist primarily as Pb^{2+} (pH 2–7), $Pb(OH)^{+}$ (pH 9), $Pb(OH)_2$ (solid, pH 10), and $Pb(OH)^{3-}$ (pH 12). Cadmium (Cd) exists as Cd^{2+} (pH 1–6), $Cd(OH)^{+}$ (pH 8–10), and $Cd(OH)_2$ or $Cd(OH)^{2-}$ at higher pH. Chromium (Cr) is similar to arsenic; its predominant species are anionic compounds such as $HCrO_4^{-}$, CrO_7^{2-} (pH 2–7), and CrO_4^{2-} (pH

7–12). Mercury (Hg) mainly exists in the form of Hg^{2+} (pH 1–2), $HgOH^{+}$ (pH 3), and solid $Hg(OH)_2$ (pH 4–8).

Most commercial polyamide membranes are negatively charged at neutral pH but exhibit amphoteric behavior in water, allowing pH-tuned rejection of specific metal ions via Donnan exclusion (**Abdullah *et al.*, 2019**).

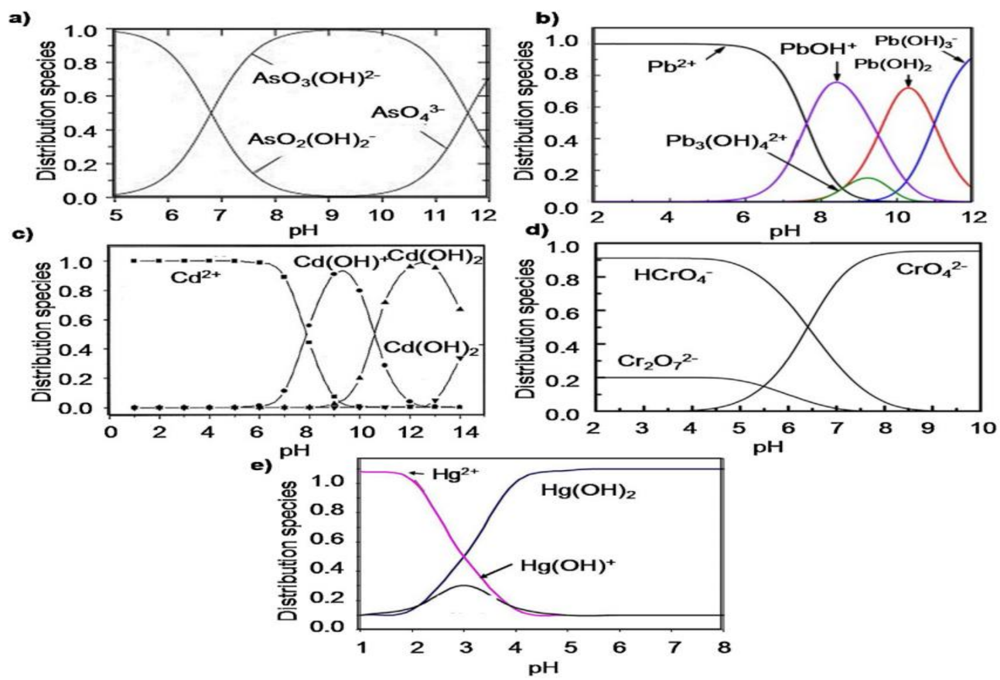


Figure 14: Speciation of metals as a function of pH, (a) arsenic, (b) lead, (c) cadmium, (d) chromium, and (e) mercury (Abdullah *et al.*, 2019).

Adsorption offers several advantages over conventional remediation methods, including reduced sludge production, cost-effectiveness, eco-friendliness, and high efficiency (**Yadav *et al.*, 2019**). It allows for both the regeneration of adsorbents and the recovery of metal ions (**Gunatilake, 2015**). The process relies on solid-liquid equilibrium and mass transfer mechanisms and utilizes a wide range of low-cost adsorbents such as agricultural waste, natural materials, industrial by-products, modified biopolymers, and magnetic materials **(Singh *et al.*, 2021**).

Various adsorbents, including photocatalyst beads, red mud, coal, sludge, nanoparticles, and zeolite, have been successfully used for metal ion removal **(Singh *et al.*, 2021**). Multi-component contamination necessitates the selection of adsorbents with broad removal capacities. Coal-fired fly ash (CFFA) has been effective in removing Cd, Ni, and Cu (**Kumar *et al.* 2019**). Lignocellulosic materials such as sawdust from maple, beech, poplar, and linden trees have shown over 80% removal efficiencies for metals like Cr(VI), Cu, Zn, Cd, Ni, Mn, and Pb (**Božić *et al.* 2009**), attributed to their negatively charged cellulose-based structures.

Advanced materials like cellulose-graft-polyacrylamide/hydroxyapatite hydrogels, disulfide-linked polymers, and amino-functionalized Fe_3O_4/SiO_2 nanoadsorbents have also demonstrated effective removal of metals such as Cu, Pb, Zn, and Cd. Manganese-doped Fe_3O_4 nanoparticles have been used to adsorb Co, Ni, Zn, As, Ag, Cd, Hg, and Tl (**Singh *et al.*, 2021**).

The following materials have been utilized as adsorbents for the removal of heavy metals through adsorption processes.

- **Activated Carbon Adsorbents:** Activated carbon (AC) is extensively utilized for the removal of heavy metals because of its highly porous structure and substantial surface area. Though traditionally derived from high-carbon industrial materials, its high production cost limits widespread use. As a cost-effective alternative, AC can be synthesized from agricultural wastes such as coconut shells, Moso and Ma bamboo, grape residues, olive stones, and lignin **(Singh *et al.*, 2017)**.

  Studies show that AC derived from waste coconut buttons effectively adsorbs Pb(II), Cu(II), and Hg(II), with capacities of 94.35, 82.09, and 75.78 mg/g, respectively **(*Singh et al.*, 2017**). Optimal removal occurred at pH 6.0 for Pb and Cu, and pH 7.0 for Hg, achieving >90% for Pb and >95% for Cu and Hg. This performance surpasses that of red mud, carbon aerogels, and rice husk ash.

  Moso and Ma bamboo-based AC demonstrated excellent remediation efficiency for Pb, Cu, Cr, and Cd, with removal efficiencies reaching 99.9–100%, following the order Pb > Cu > Cr > Cd. Grape waste-derived AC (e.g., stalks, leaves, pomace) achieved capacities of 1.93 mmol/g for Pb and 0.67 mmol/g for Cd (**Sardella *et al.*, 2015**). Similarly, AC from olive stones adsorbs Cu(II) and Cd(II) at 17.667 and 57.098 mg/g, respectively (**Bohli *et al.* 2015**).

Overall, agricultural waste-derived activated carbons present an efficient, low-cost option for heavy metal remediation in wastewater treatment.

- **Carbon Nanotube Adsorbents**: Over the last ten years, carbon nanotubes (CNTs) have garnered considerable attention in the scientific community owing to their remarkable stability, extensive surface area, enhanced adsorption capabilities, and outstanding mechanical and electrical characteristics (**Ihsanullah *et al.*, 2015**). **Sankararamakrishnan *et al.*** (**2014**) fabricated carbon nanotubes using chemical vapor deposition on activated alumina doped with iron and nickel. This process yielded nanofloral cluster structures exhibiting elevated adsorption capacities for chromium and cadmium, measured at 264.5 mg/g and 229.9 mg/g, respectively. Additionally, **Kosa *et al.*** (**2012**) enhanced multi-walled CNTs with 8-hydroxyquinoline, achieving cadmium removal efficiencies exceeding 80%, along with effective removal of copper, lead, and zinc.

- **Agricultural Wastes:** Biosorption is a sustainable and eco-friendly technique for extracting heavy metals from wastewater, employing biological materials as sorbents that contain functional groups with high affinity for binding metal ions. Agricultural wastes, rich in components like cellulose, lignin, hemicellulose, proteins, and lipids, serve as effective biosorbents due to the presence of reactive functional groups (e.g., carboxyl, hydroxyl, amino, sulphydryl) that facilitate metal binding.

  A variety of agricultural by-products such as rice husk, wheat bran, sugarcane bagasse, sawdust, coconut coir, and fruit peels have demonstrated significant metal adsorption potential (**Singh *et al.*, 2021**). For instance, acid-modified oak sawdust and coir pith have achieved over 90% chromium removal. Similarly, sugarcane bagasse has been effectively used in both chemically modified (e.g., with succinic acid) and biologically enhanced forms (e.g., inoculated with Acinetobacter haemolyticus) for Cr(VI) reduction and adsorption, with removal efficiencies exceeding 90% **(Ahmad *et al.*, 2013)**. Eggshells and activated sugarcane bagasse have also shown high chromium removal rates (93% and >87%, respectively), highlighting the versatility and efficiency of biosorbents derived from agricultural residues (**Daraei *et al.*, 2015**).

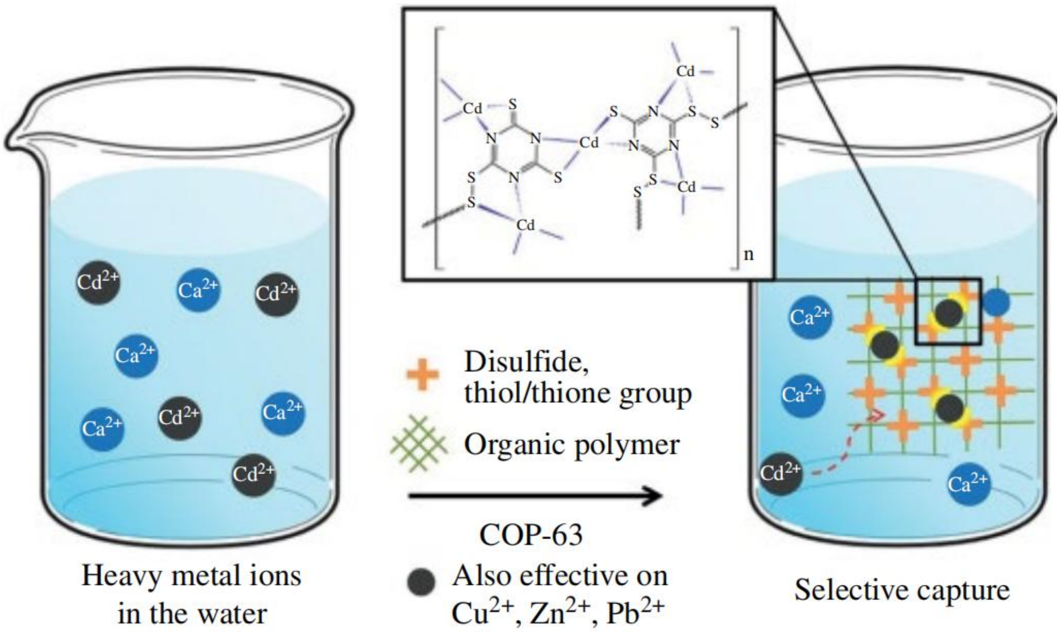


Figure 15: Adsorption method for removal of heavy metals (Singh *et al.*, 2021).

2.6.2 Chemical Methods

Chemical remediation uses sulfur dioxide, sodium metabisulfite, ferrous sulfate, sodium sulfite, barium sulfate, lime, and limestone for reduction and photocatalysis (**Jobby *et al.*, 2018**).

2.6.2.1 Neutralization

Heavy metals in sediments can be removed through precipitation and pH neutralization. Various alkaline agents are commonly employed to elevate the pH of acidic waters and facilitate the precipitation of dissolved metals. These include limestone, lime (CaO), ferrous-based salts, and chemical compounds such as magnesium hydroxide ($Mg(OH)_2$), magnesium carbonate ($MgCO_3$), barium chloride ($BaCl_2$), and calcium chloride ($CaCl_2$). Apatite has demonstrated high efficacy in neutralizing acid mine drainage (AMD), resulting in the near-complete removal of heavy metals. The process generates a phosphorus-enriched precipitate containing trace metals such as Fe, Mn, Cu, and Mg, which holds potential for reuse as an agricultural fertilizer. Apatite itself is recyclable after treatment (**Ghirişan *et al.*, 2007**).

Calcium oxide (CaO) is particularly efficient and cost-effective for neutralizing metal cations by forming hydroxides or complex anions. Its performance is stable across different temperatures. Neutralization begins with lime hydrolysis, which increases pH by releasing hydroxyl ions. This process has demonstrated up to 97.7% removal of lead (Pb) and 98% removal of antimony (Sb) and sulfate (SO_4^{2-}) (**Balladares *et al.*, 2018**).

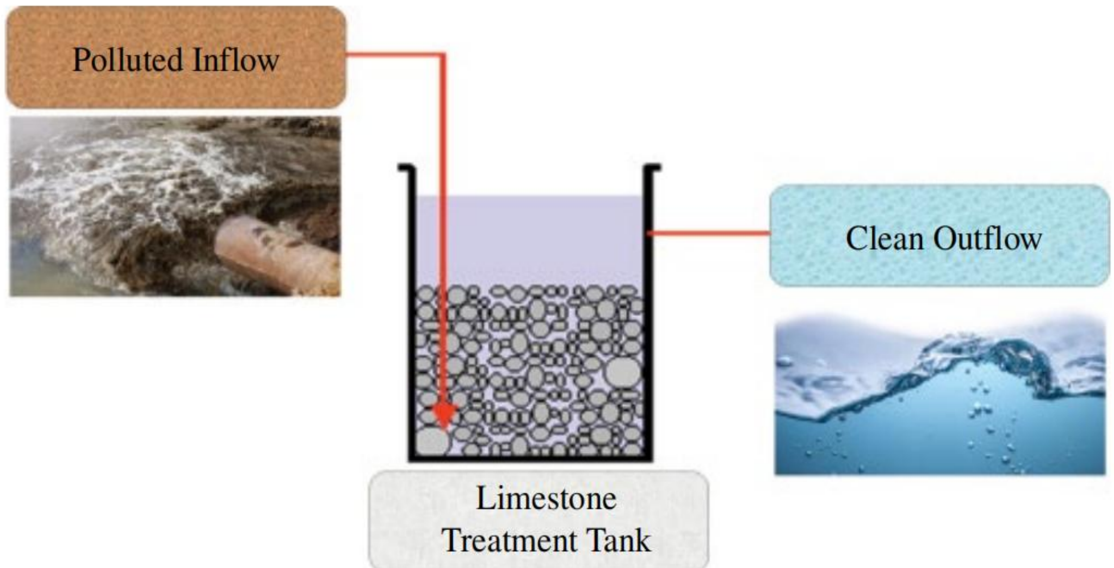


Figure 16: Neutralization method for the removal of pollutants (Singh *et al.*, 2021).

2.6.2.2 Solvent Extraction

Solvent extraction is a well-established technique in hydrometallurgy, widely used for the recovery of metals such as cobalt, nickel, copper, zinc, uranium, molybdenum, vanadium, and others. It has also been applied in nuclear fuel reprocessing, acid purification, and metal separation from complex solutions.

Recent advancements have led to the development of more selective and environmentally friendly extractants **(Wilson *et al.*, 2014**). Organophosphorus compounds, including di-(2-ethylhexyl) phosphoric acid (D2EHPA), are widely utilized as extractants in the separation and recovery of metal ions. D2EHPA has proven effective in recovering zinc and copper from acidic sulfate solutions, nickel from neutral to slightly alkaline sulfate solutions (pH 6–7), and chromium from sulfuric acid solutions **(Singh *et al.*, 2021)**.

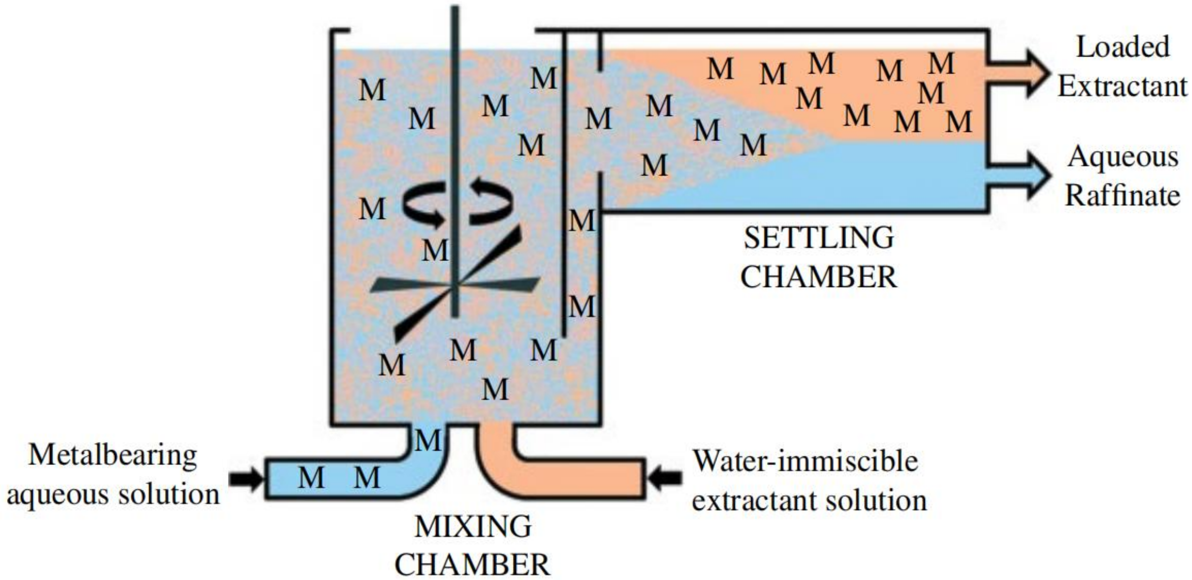


Figure 17: Schematic representation of a mixer-settler for continuous operation of solvent extraction (Wilson *et al.*, 2014).

2.6.2.3 Chemical Precipitation

Chemical precipitation is one of the most widely used and cost-effective methods for removing heavy metals from wastewater. It operates efficiently across various temperatures and is simple to implement. The process involves adjusting the pH to convert dissolved heavy metal ions into less soluble forms, such as sulfides, hydroxides, or carbonates, which can then be removed by physical means like sedimentation, filtration, or flotation.

The effectiveness of removal is influenced by variables such as particle size, density, and surface charge characteristics. Frequently used chemical precipitants include lime, soda ash, caustic soda, sodium bicarbonate, sodium sulfide, and sodium hydrosulfide. These reagents react with metal ions to form insoluble compounds that settle out of the water. After separation, the treated water can be reused or safely discharged **(Fu and Wang, 2011**). Chemical precipitations are categorized as hydroxide and sulfide precipitations.

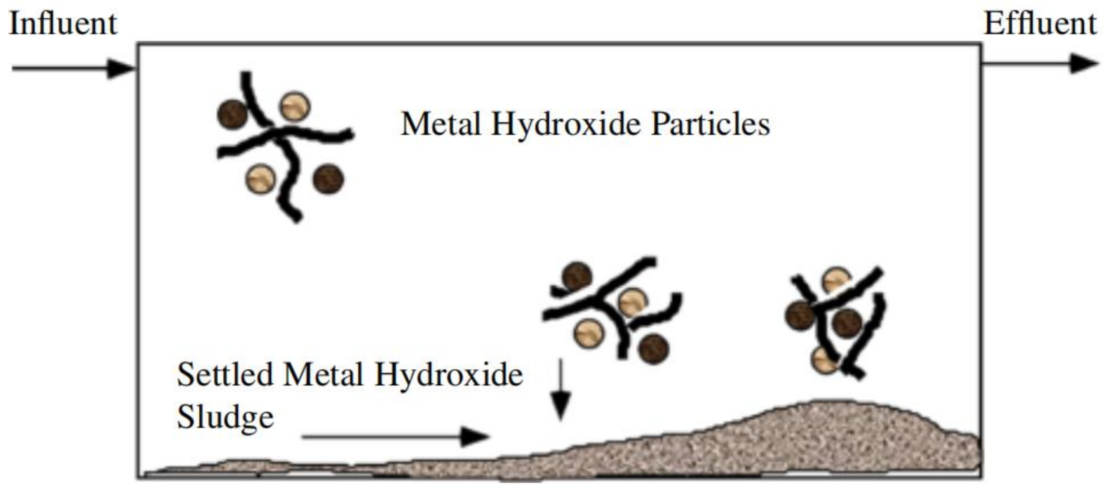


Figure 18: Chemical precipitation for metal ion removal (Chen *et al.*, 2009).

- **Hydroxide Precipitation**: This method removes heavy metals by forming insoluble hydroxides. In a study by **Singh *et al.* (2017)**, calcium hydroxide ($Ca(OH)_2$) and sodium hydroxide (NaOH) were used to precipitate copper (Cu^{2+}) and chromium (Cr^{6+}). Cr^{6+} was first reduced to Cr^{3+} using ferrous sulfate and sulfuric acid, with optimal reduction occurring at pH 2.0-2.3. Maximum Cr^{3+} precipitation occurred at pH 8.7 using $Ca(OH)_2$. The treatment process effectively decreased chromium concentrations from 30 mg/L to 0.01 mg/L and copper from 48.52 mg/L to 0.694 mg/L, corresponding to a removal efficiency ranging between 98.56% and 99.9%.

- **Sulphide Precipitation**: Sulfide precipitation is a method used to remove heavy metals by forming insoluble metal sulfides. This process is effective because it allows for the selective removal of metals, has a fast reaction rate, and produces sludge with good settling and dewatering properties. Unlike hydroxide precipitation, sulfide precipitation works well across a wider pH range, and the resulting metal sulfides can be recovered through smelting (**Azabou *et al.*, 2007**).

  Özverdi and Erdem (2006) studied the use of pyrite and synthetic iron sulfide to remove Cu^{2+}, Cd^{2+}, and Pb^{2+}. They found that at low pH (<3), metal removal occurs through chemical precipitation due to hydrogen sulfide (H_2S) formation:

$$FeS(s) + 2H^+ \rightarrow Fe^{2+} + H_2S\uparrow \quad (1)$$

$$M^{2+} + H_2S \rightarrow MS(s) + 2H^+ \quad (2)$$

At higher pH (3–6), metal ions are also removed through adsorption onto sulfide surfaces.

A newer approach involves sulfate-reducing bacteria (SRB), which convert sulfate to hydrogen sulfide under anaerobic conditions using simple organic compounds:

$$3SO_4^{2-} + 2CH_3CH(OH)COOH \rightarrow 3H_2S + 6HCO_3^- \quad (3)$$

The produced H_2S then reacts with metal ions to form insoluble sulfides as shown above (Eq. 2) **(Fu and Wang, 2011**).

- Additionally, **Matlock *et al.*** (**2001**) further reported that sulfur-containing ligands, including pyridine-thiol derivatives, exhibit high efficacy in the removal of heavy metals such as copper and cadmium by forming thermodynamically stable metal-ligand complexes that precipitate out of solution. Removal efficiencies reached 99.99% for copper at pH 4.5 and 99.88% for cadmium at pH 6.

- **Carbonate Precipitation**: Carbonate and sulfide-based precipitation methods have proven to be efficient alternatives to hydroxide precipitation for the removal of dissolved heavy metals from wastewater. Sodium carbonate (Na_2CO_3), commonly known as soda ash, facilitates the precipitation of metal carbonates at a relatively lower pH (~9). In contrast, sodium sulfide (Na_2S) enables effective sulfide precipitation, allowing for the efficient removal of heavy metals such as zinc, copper, and lead from aqueous solutions **(Chen *et al.*, 2018**). In acid mine drainage treatment, metals are typically removed by neutralization and peroxide-assisted precipitation.

- **Chelating Precipitation:** Excessive use of strong chelating agents like ethylenediaminetetraacetic acid (EDTA) in wastewater poses environmental challenges, as both heavy metals and EDTA must be removed. Conventional methods struggle to remove nickel (Ni^{2+}) from Ni-EDTA complexes. To address this, research employed a sequential treatment process involving the Fenton reaction followed by hydroxide precipitation, which effectively reduced the nickel concentration from 50 mg/L to 3.6 mg/L, corresponding to a removal efficiency of 92.8% **(Singh *et al.*, 2017).**

Later, **Fu *et al.* (2012**) introduced an advanced Fenton-chemical precipitation method using zero-valent iron, which is low-cost, low-toxicity, and easy to handle. This improved process reduced nickel levels to below 1 mg/L, reaching 98.4% removal efficiency. Compared to the earlier method, the advanced Fenton process showed significantly better performance in treating chelated heavy metal wastewater (**Singh *et al.*, 2017**).

2.6.2.4 Electrochemical Treatment

Electrochemical treatment is a proven technique for the efficient removal of heavy metal ions from wastewater. This method involves the application of an electric current to a metal ion-containing solution, where reduction reactions occur at the cathode and oxidation at an inert anode, facilitating the extraction of metal contaminants. This leads to the precipitation of heavy metals as hydroxides, especially in slightly acidic or neutral conditions (**Sahu *et al.*, 2017**). The type of electrode used significantly influences the efficiency of metal removal (**Tran *et al.*, 2017**).

Electrochemical methods produce a compact sludge that is easy to handle. However, the process requires high-energy input, and issues such as electrode corrosion, passivation, and temperature rise can limit its use. Moreover, maintenance costs are high, and the technique is best suited for pollutants that do not form harmful by-products.

Combining electrochemical systems with membrane bioreactors has shown improved removal of heavy metals and phosphorus, better sludge quality, and reduced membrane fouling **(Giwa *et al.*, 2019**). Additionally, integrating electro-adsorption with electrochemistry improves contaminant removal through the action of electric fields and specialized electrodes. Certain metals, such as Cu^{2+}, Zn^{2+}, and Fe^{3+}, act as electroactive catalysts that facilitate the accelerated breakdown of organic contaminants. In situ formation of catalysts like Cu and Cu_2O boosts this effect (**Gao *et al.*, 2015**). Conductive polymers, such as polypyrrole (PPy), have also been used successfully to remove metals like lead, copper, and chromium (**Sun *et al.*, 2020**).

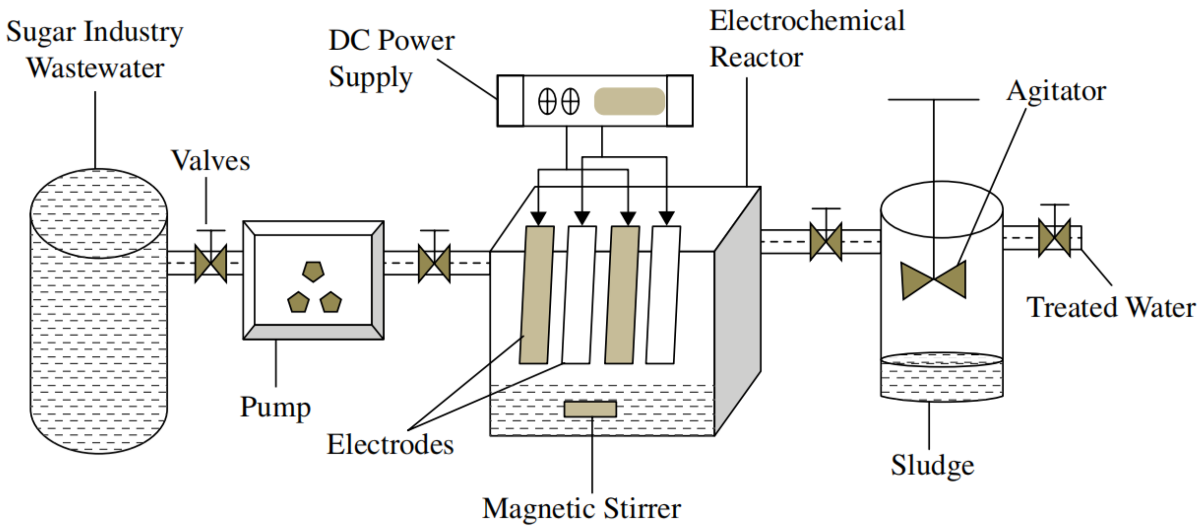


Figure 19: Electrochemical treatment for removal of pollutants (Sahu *et al.*, 2017).

2.7 Bioremediation of Heavy Metals from Contaminated Environment

Scientists explored the use of biomass from agriculture, forestry, biochar, and microbes such as bacteria, fungi, algae, and plants as biosorbents of HMs from contaminated environments **(Jobby *et al.*, 2018)**. Mechanisms employed by such organisms exhibit tolerance or high affinity to the metals in the environment or within laboratory settings, which could establish the basis for using such biota in recovery and bioremediation. Unlike in organic-substance bioremediation, where the microorganisms involved degrade toxicants to simpler and less harmful compounds and eventually mineralize them, in heavy-metal bioremediation, the microbial action is restricted exclusively to mobilization and redox-mediated transformation of the metallic species. Microbial metal mobilization can be used to release (and eventually recover) metals from low-grade mineral ores, solid wastes, ashes, sludge, and even exhausted materials, among others. Mobilization can be affected by different microbial actions that mainly involve metal solubilization through pH or Eh changes and metal complexation. Alternatively, metals can be immobilized to purify polluted water through mechanisms employed by certain microorganisms that are similar to those mentioned above (pH and Eh changes plus complexation) but produce the opposite effect (i.e., immobilization) (**Castro *et al.*, 2019**).

2.7.1 Strategies of Bioremediation

The three main strategies for bioremediation are natural attenuation, biostimulation, and bioaugmentation.

2.7.1.1 Natural Attenuation

'Natural attenuation' refers to the careful monitoring of the natural rate of contaminant breakdown, without any direct intervention (**Leal *et al.,* 2016**). In natural attenuation, contaminants are reduced to less harmful forms, hence the application of a variety of processes such as biodegradation, volatilization, dilution, dispersion, sorption, etc. (**Declercq *et al.,* 2012**). When the technology of such processes is monitored, the strategy is called monitored natural attenuation, which may be used singly or in combination with other technologies.

2.7.1.2 Biostimulation

Biostimulation involves modifying environmental parameters, such as temperature, moisture content, aeration, pH, and redox potential, and supplementing contaminated soils or aquatic systems with nutrients (e.g., nitrogen, phosphorus) and electron acceptors to promote the proliferation of indigenous microbial communities capable of degrading pollutants. This approach enhances the biodegradation process by stimulating native microorganisms already adapted to the contaminated environment, thereby reducing contaminant concentrations effectively. Biostimulation mainly focuses on the addition of nitrogen (N) and phosphorus (P) sources, as the biodegradation of these compounds leads to a quick depletion of the available pools of major inorganic nutrients, such as N and P (**Sarkar *et al.,* 2005**).

2.7.1.3 Bioaugmentation

'Bioaugmentation' refers to the breakdown of xenobiotics either due to the enhanced growth of the indigenous microorganisms and/or due to the addition of adequate microbial consortia to activate their natural potentials and considering the ecological context (**Sprocati *et al.,* 2012**). Genetically modified and microbial inocula, as well as native microorganisms, could be used for the breakdown of pollutants if the appropriate nutritional and environmental conditions for growth have been provided (**Gupta and Joia, 2016**). This bioremediation strategy is considered when native microbial populations are not capable of metabolizing the pollutant or when the amount of degrading microorganisms is not sufficiently large **(Dzionek *et al.*, 2016**). But for bioaugmentation to succeed, inoculated microorganisms must be able to (i) survive and proliferate in the soil or water; (ii) have direct contact with the pollutant, which must be in a bioavailable form, and (iii) express *in situ* their capacity to metabolize the pollutant at a reasonable rate (**Alkorta *et al.*, 2017**).

2.7.2 Bioremediation Mechanisms for Heavy Metals

Many microorganisms can bind hazardous and potentially toxic ions. These microorganisms utilize two primary pathways for the uptake of metal ions. The first pathway, known as biosorption or passive uptake, operates independently of cellular metabolic processes and occurs in both viable and non-viable cells. This mechanism involves the attachment of metal ions to the cell surface and extracellular matrix components. However, the second mode of metal uptake into the cell across the cell membrane is dependent on the cell metabolism and is referred to as intracellular uptake, active or bioaccumulation, and can occur only with living organisms through the transport of contaminants to the cell and the accumulation of metals inside the cell, which participates in the biochemical pathways (**Yadav *et al.*, 2017**). Surface binding of metal ions is very important and usually represents the main source of metal ion binding. The process is enhanced by the abundance of binding sites as the cell membrane is composed of many proteins, lipids, and polysaccharides, which are rich in metal ion binding sites like carboxylates, amines, hydroxyls, phosphates, thiols, as well as others.

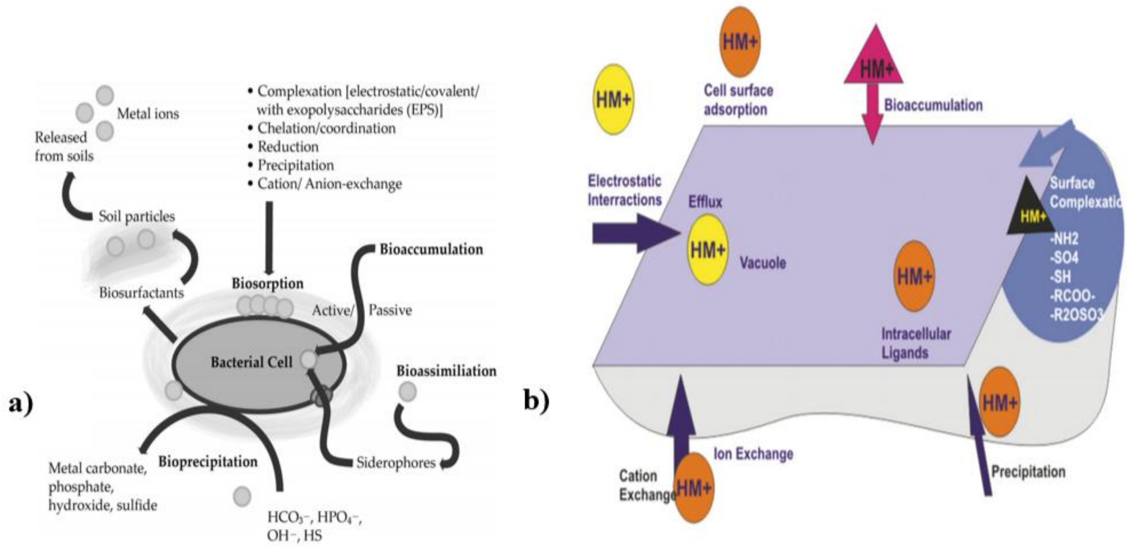


Figure 20: Various types of bacterial interactions with HMs accumulated in soils (Wróbel *et al.*, 2023).

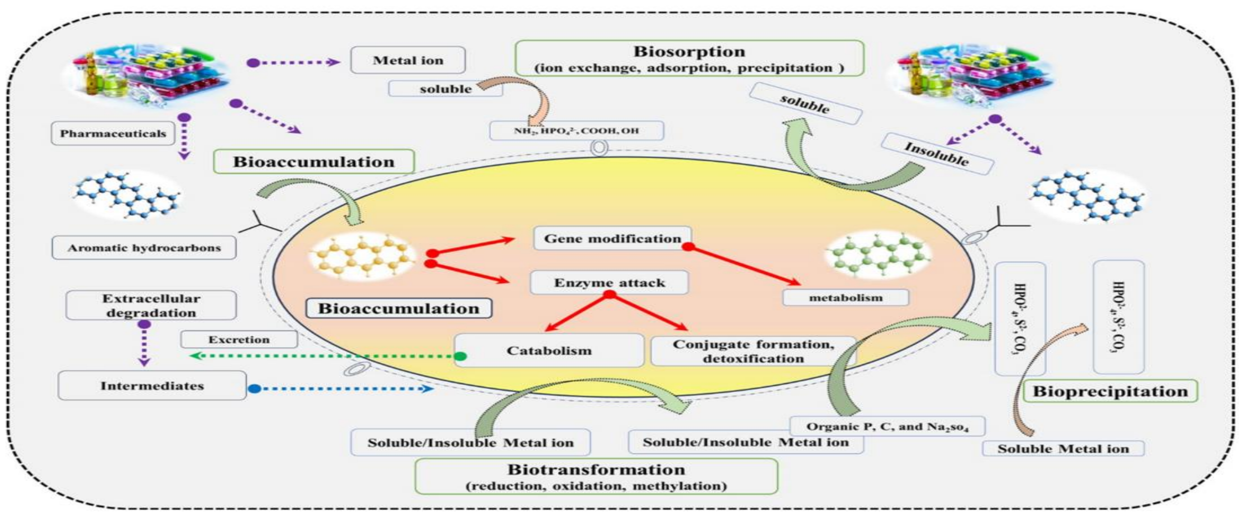


Figure 21: Different approaches adopted by microbes to degrade toxic compounds (Sharma *et al.*, 2024).

2.7.2.1 Biosorption

Biosorption is the technology of ions binding from aqueous solution to non-living biomass. The mechanism depends on the sorption of metal ions from aqueous solution to chemical functional groups on the biomass's cell wall surface (**Dmytryk *et al.*, 2014**). Concisely, it is the adsorption of particles to a biological matrix employing physical interactions (electrostatic forces), complexation, or chelation. At neutral pH, the extracellular surface of microorganisms contains anionic moieties that provide binding sites for cationic HMs (**Diep *et al.*, 2018**). The process can be used either for the removal of contaminants from effluents, or also for the method of production of micronutrient feed additives and fertilizer components. Biosorption is a metabolically passive process and is performed by material of biological origin, not by living biomass (**Castro *et al.*, 2019**).

The intricate architecture of microorganisms indicates multiple pathways through which metals can be assimilated by microbial cells. These diverse and complex biosorption processes are broadly categorized into:

1. Metabolism-dependent mechanisms, and
2. Metabolism-independent mechanisms.

Based on the site of metal removal from the surrounding medium, biosorption can be further classified into:

1. Extracellular accumulation or precipitation,
2. Sorption or precipitation at the cell surface, and
3. Intracellular accumulation.

Metal transport across the cell membrane results in intracellular accumulation, a process that relies on cellular metabolic activity. Consequently, this form of biosorption occurs exclusively in metabolically active (viable) cells. It is often associated with an active defense system of the microorganism, which reacts in the presence of toxic metal (**Ahalya *et al.*, 2003**).

In non-metabolism-dependent biosorption, metal ions are adsorbed through physicochemical interactions with functional groups located on the surface of microbial cells. The microbial cell walls, predominantly consisting of polysaccharides, proteins, and lipids, contain numerous metal-binding sites, including carboxyl, sulfate, phosphate, and amino functional groups. This type of biosorption, i.e., non-metabolism dependent, is relatively rapid and can be reversible **(Ahalya *et al.*, 2003**).

2.7.2.2 Bioaccumulation

Living organisms absorb and retain various kinds of mineral elements from the surrounding environment. Bioaccumulation allows the binding of toxic metals or organic substances inside a cell structure. The biological activity of biomass is crucial in the process of bioaccumulation. Cells absorb metals through metabolic processes, and thus, they have to be alive. During bioaccumulation, the entire cell absorbs ions inside the cells of living organisms through the same routes as nutrients are supplied. In plant nutrition, elevated levels of heavy metals (HMs) in the soil can lead to toxicity, facilitating the uptake of these metal ions through the root system and disrupting essential cellular processes. In phytoremediation technologies, HMs are mobilized in the soil solution and then taken up by plant roots (**Figure 21**) (**Fakhar *et al.*, 2022**).

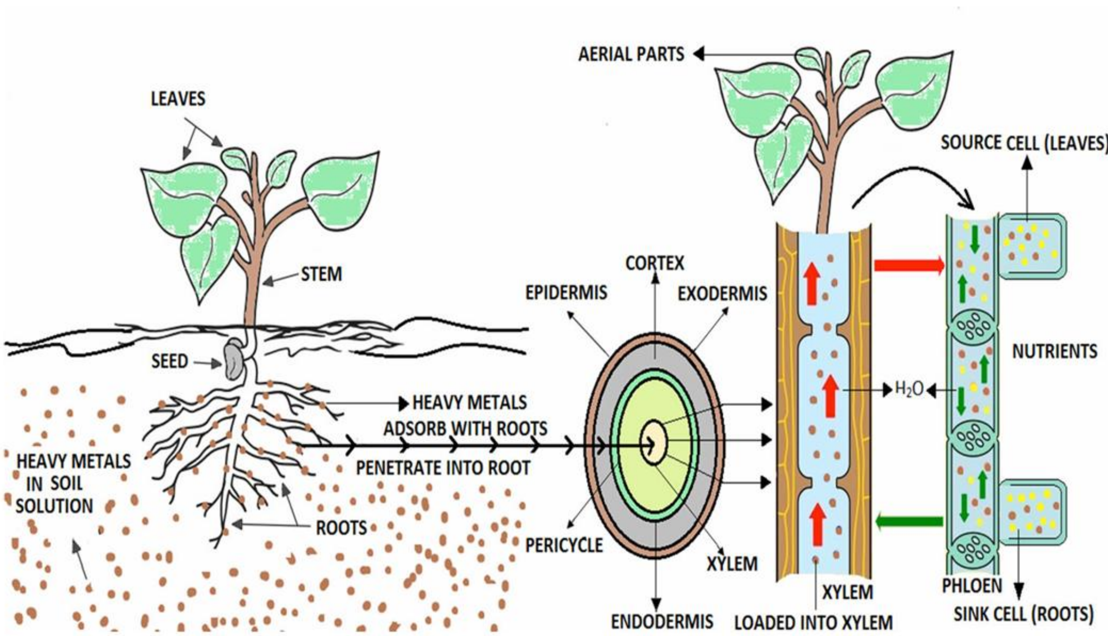


Figure 22: HM uptake and transportation mechanism (Fakhar *et al.*, 2022).

In the initial stage of bioaccumulation, metal ions are passively bound to the cell surface through a process identical to biosorption. The second stage involves the active transport of metals into the cell, which only occurs when cells are metabolically active **(Castro *et al.*, 2019)**. When conditions favor microbial growth during this phase, biomass increases and can bind larger amounts of metal ions compared to biosorption. Although non-essential metals are sometimes taken up by living cells accidentally, their high concentrations, even at low levels, can threaten microbial biomass **(Diep *et al.*, 2018)**. Contaminants are passively bound to extracellular biomass components, similarly to biosorption, then cross the cellular membrane into the intracellular space using energy to assist ion movement. Acidification of the environment, which promotes metal ion precipitation by *Citrobacter* and *Achromobacter denitrificans*, significantly enhances metal accumulation in microbial biomass through the activity of enzymes like phosphatase and processes like denitrification, respectively **(Babák *et al.*, 2012)**. The uptake of contaminants through the lipid bilayer into cells and their subsequent intracellular accumulation are significantly influenced by the physiological and biochemical characteristics of the biomass, interactions between metals and the environment, metal bioavailability and toxicity, as well as factors such as temperature, pH, and metal concentration. Organisms used in bioaccumulation should be chosen from species resistant to high contaminant loads and lacking mechanisms to prevent excessive cellular accumulation **(Giovanella *et al.*, 2020)**.

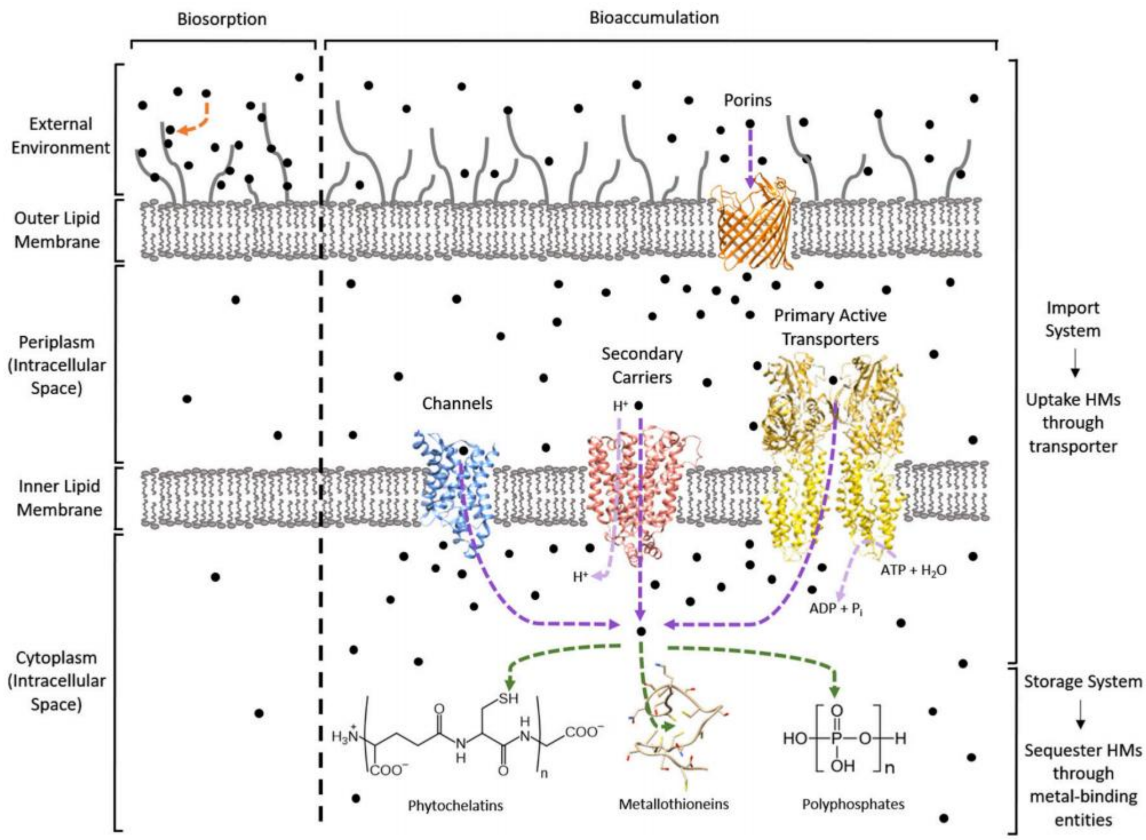


Figure 23: Schematic diagram of both biosorption and bioaccumulation of heavy metals using a gram-negative bacterium (Diep *et al.*, 2018).

2.7.3 Factors Affecting HM Bioremediation

Bioremediation is a multifaceted biological process predominantly mediated by microorganisms. These microbes reduce the bioavailability of heavy metals through mechanisms including the secretion of organic and inorganic acids (e.g., citric and sulfuric acids), redox reactions (reduction and oxidation), and the synthesis of metal-chelating compounds such as cyanide.

Nonetheless, both biotic and abiotic factors significantly impact microbial growth and metabolic functions, thereby limiting their bioremediation efficiency **(Jacob *et al.*, 2018)**. While microorganisms possess innate capabilities to withstand adverse environmental conditions, they face inherent constraints. Consequently, it is critical to identify and understand the factors impeding microbial heavy metal detoxification to devise effective strategies for optimizing bioremediation processes. These influencing factors can be broadly classified into: (i) biotic components and (ii) abiotic parameters, which include physicochemical characteristics and climatic variables. **Figure 23** illustrates the environmental challenges encountered by microbes during bioremediation.

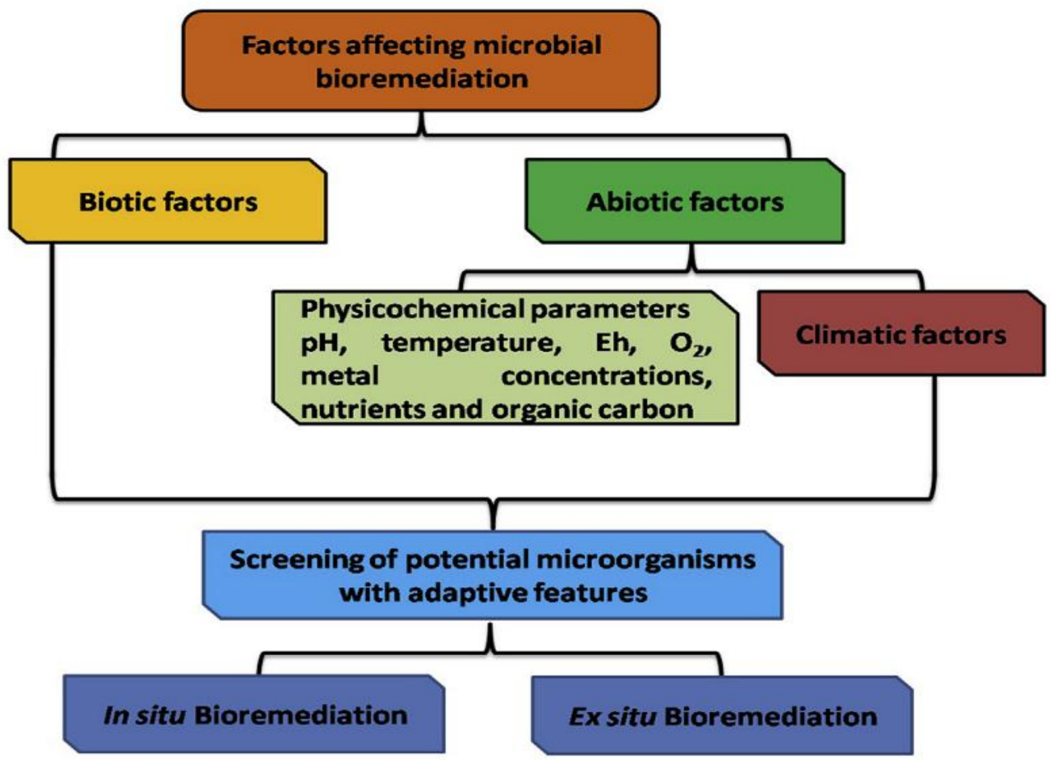


Figure 24: Factors affecting HM bioremediation (Jacob *et al.*, 2018).

2.7.3.1 Biotic Factors

Biotic factors greatly influence the bioremediation efficiency of the microorganisms by changing cell size and composition, extracellular product formation, etc. Research has shown that biomass concentration is one of the most vital biological factors for bioremediation. **Park and Choi (2002)** opined that at low biomass concentrations, the metal recovery efficiency of microbes was higher when the intercellular space was greater. When biomass attained its equilibrium concentrations, HMs in the reduction medium are not only absorbed onto the surface of the biomass but also enter into the intracellular part, facilitated by the concentration gradient of the metal. Nonetheless, the higher concentration of biomass limits the interaction of HMs with the binding sites of the microbial surfaces **(Ismail and Moustafa, 2016).** Electrostatic interactions between cells play vital functions in HM removal by microbes; high biomass induces the shell effect on the outer structure of the microorganisms. Higher biomass concentration is known to cause cell aggregation, which eventually reduces the intercellular space, resulting in decreased metal remediation **(Fadel *et al.*, 2014)**. **Terry and Stone (2002)** inferred that the higher biomass concentration of Scenedesmus abundans reduced the bioremediation of Cu(II) and Cd(II). **Romera *et al.* (2007)** also investigated the removal efficiency of different algal strains for various metal ions and showed that the maximum removal was observed at the lowest biomass concentrations. Similar results have been documented for *Microcystis* **(Pradhan and Rai, 2000)**, *Bacillus subtilis, Pseudomonas aeruginosa***,** and *Saccharomyces cerevisiae* **(Fadel *et al.*, 2014)**.

2.7.3.2 Abiotic Factors

In addition to biotic influences, abiotic factors play a significant role in modulating the efficiency of microbial heavy metal removal. The different abiotic factors in this work are the physicochemical parameters (temperature, pH, metal ion concentration, O_2, redox potential (Eh), nutrients, and organic carbon) and climatic conditions **(Jacob *et al.*, 2018)**.

2.7.3.2.1 Physicochemical Parameters

2.7.3.2.1.1 Effects of Temperature

Temperature plays a critical role in the efficiency of heavy metal (HM) removal within reduction media, primarily by altering the physicochemical properties of the contaminants and secondarily by modulating microbial metabolic processes. As temperature increases toward the microbial optimum, enzymatic activity and overall metabolism are enhanced, facilitating bioremediation. However, temperatures exceeding the optimal range can lead to enzyme denaturation, reduced microbial activity, and potential cell death. Additionally, high temperatures may alter ribosomal structure, thereby impairing protein synthesis. Conversely, suboptimal temperatures can decrease membrane fluidity, hindering the operation of essential transport mechanisms involved in metal uptake and detoxification. These alterations would affect the mobility of substances into the cells, thereby affecting the growth rates **(Jacob *et al.*, 2018)**. Temperature extremes, whether excessively low or high, can lead to microbial mortality and alter gas solubility, thereby affecting microbial activity. Previous studies have shown that optimal heavy metal removal is typically achieved under mesophilic conditions, ranging from 20°C to 40°C. Nonetheless, certain thermophilic microorganisms exhibit peak growth and enhanced metal remediation capabilities at elevated temperatures, specifically within the moderate thermophilic (above 45°C) to hyperthermophilic (up to 80°C) range. In remediation mechanisms using compost heaps or biopiles where the temperature of the soil/sediment may reach 60 ^{o}C or higher during the initial active phase, these thermophiles (*Geobacillus thermantarcticus*, *Anoxybacillus amylolyticus*, etc.) would be the best fit for the process **(Jacob *et al.*, 2018)**.

2.7.3.2.1.2 Effects of pH

pH plays a critical role in the bioremediation of heavy metals (HMs), as it influences microbial growth dynamics, enzymatic activity, metal speciation, and the reactivity of microbial cell surface functional groups. Since heavy metal transformation is predominantly enzyme-mediated, deviations from the optimal pH can alter enzyme ionization states, thereby reducing the bioremediation efficiency of the microorganisms. Several microbial species, including *Acinetobacter junii*, *Escherichia coli*, *Bacillus* spp., *Cellulosimicrobium funkei*, *Micrococcus luteus*, *Lentinula edodes*, *Pannonibacter phragmitetus*, *Pleurotus platypus*, *Pseudochrobactrum saccharolyticum*, *Pseudomonas aeruginosa*, *Trichoderma* spp., and *Virgibacillus* spp., have demonstrated effective reduction of heavy metal bioavailability under neutral pH conditions. The remediation potential and associated enzymatic activities of these microorganisms were notably enhanced when environmental pH was maintained at optimal levels. Alteration in the pH of the reduction medium can significantly reduce the metal bioavailability and solubility **(Jacob *et al.*, 2018)**.

2.7.3.2.1.3 Effects of Metal Ion Concentration

The impact of heavy metals (HMs) on microbial cellular functions is influenced by their concentration, with toxicity intensifying at higher levels. Microorganisms often exhibit enhanced heavy metal remediation efficiency as the initial concentrations of metals increase. While low concentrations of HMs may support certain physicochemical microbial processes, elevated concentrations exert significant inhibitory effects on microbial growth and metabolic activity. Heavy metal bioremediation efficiency is different from one organism to another, and the differences in HM concentrations affect the microbe's ability to removal. For instance, *Micrococcus luteus* demonstrated a high copper removal efficiency of 408 mg/g within 12 hours of exposure, whereas *Desulfovibrio desulfuricans* achieved a copper uptake of 98.2 mg/g following a 168-hour treatment period. Similarly, **Dursun *et al.* (2003)** reported that *A. niger* starin removed 15.6 mg/g of Cu after 1 h incubation. These differences might have been due to the genetic makeup of microorganisms as well as the composition of the reduction medium **(Hobman and Crossman, 2015)**.

2.7.3.2.1.4 Effects of Nutrients, Organic Carbon, and O_2

The bioavailability of essential nutrients is largely influenced by microbial bioremoval activities occurring both in situ and ex-situ. Elements such as carbon, nitrogen, phosphorus, sulfur, calcium, and magnesium are integral to microbial proliferation, serving as substrates for metabolic processes and enzyme synthesis essential for contaminant degradation. Among these, carbon plays a critical role as the foundational element of organic molecules, and its optimal concentration is a key determinant for sustaining microbial growth and metabolic functionality. Nitrogen is required in cellular protein and cell wall component synthesis, while phosphorus is necessary for cell membrane, nucleic acid, and ATP **(Xu *et al.*, 2015)**. A study conducted by **Venil *et al.* (2011)** reported that the optimum concentrations of carbon and nitrogen sources in the growth medium significantly induced the chromium removal efficiency of *Bacillus* sp. REP02. Similarly, **Maheswari and Murugesan, (2009)** also reported the influence of various nutrient sources on arsenic bioremediation by *Aspergillus nidulans*. Oxidation represents a predominant mechanism by which microorganisms facilitate the bioremediation of heavy metals. Oxygen is paramount in bioremediation because it is an energy-yielding process, and it determines the bacterial pattern **(Jacob *et al.*, 2018)**.

2.7.3.3. Climatic Factors

Climate change significantly impacts microbial growth and metabolic activity; however, current evidence does not directly demonstrate a definitive effect of climatic variables on the efficiency of microbial bioremediation processes. However, several studies opined that alterations in the physicochemical nature of the microbial vicinity may change and have the potential to affect the metabolic processes of microorganisms and their bioremediation activity **(Srivastava *et al.*, 2014)**. **Sowerby *et al.* (2005)** indicated that the extracellular enzymatic production from microorganisms is related to microbial activity as well as physicochemical properties of the inhabited soil, which are influenced by climatic conditions. Global climate change is primarily influenced by two key variables: atmospheric carbon dioxide (CO_2) concentration and ambient temperature. Climatic factors, especially CO_2 at higher levels, are associated with the increase in bacterial population and significantly reduced fungal population (**Jacob *et al.*, 2018**). Soil temperature, analogous to atmospheric CO_2 concentration, plays a critical role in regulating microbial proliferation and enzymatic functions. Elevated temperatures coupled with arid conditions have been shown to negatively affect fungal biomass accumulation. Additionally, photosynthetic efficiency constitutes a key factor influencing the effectiveness of bioremediation processes. In a recent study, **Bwapwa *et al.* (2017)** highlighted the prominent role of the photosynthetic efficiency of algae that enhanced the overall heavy metal biosorption. According to **Brake *et al.* (2004),** the optimum removal of heavy metals and contaminants by algae varies with the type of season because of parameters such as light and temperature. Algae exhibit high sensitivity to seasonal environmental factors, particularly variations in temperature and light intensity. Thus, these parameters should be considered when algal bioremediation is involved **(Jacob *et al.*, 2018)**.

2.7.4 Characteristics of Mesophilic Bacteria

Mesophilic bacteria are microorganisms that grow best at moderate temperatures (20-40 °C), a range considered optimal for in situ bioremediation. Studies show that heavy metal removal is often highest under mesophilic conditions due to enhanced microbial enzyme activity.

Examples include *Acinetobacter* sp., *Bacillus cereus*, *Virgibacillus sp.*, *Klebsiella aerogenes*, *Paenibacillus* sp., *Ochrobactrum sp.*, *Rhizobium* sp., etc., all reported to effectively remediate heavy metals at mesophilic temperatures **(Jacob *et al.*, 2018)**.

Mobilization and immobilization of catalyzed microbial actions are key components of environmental biotechnology. Metal ions mobilized from ores, mining wastes, or solid residues can be used to recover metals and/or remediate a polluted environment. Furthermore, immobilization reduces the movement of metals, cleans up effluents and ground and surface water, and can help concentrate and recover metals (**Castro *et al.*, 2019**).

Apart from mesophilic bacteria being cost-effective, simple, and eco-friendly, their ubiquity, small size, energy generation from metals and metalloids, they have the capacity for proliferation under standardized laboratory conditions and the robustness to withstand varying environmental stressors. Furthermore:

1. They can survive in all conditions,
2. They prefer to grow under moderate temperatures of 20-45 ^{0}C,
3. Their optimum growth temperature is 37 ^{0}C,
4. Their microbial metabolism and enzymatic activities are enhanced for bioremediation,
5. They have high metal-resistant genes,
6. Their biomass has a higher metal retention capacity,
7. They have maximum MICs (80-700 mM) (**Yadav *et al.*, 2017; Castro *et al.*, 2019**).

2.7.5 Bacterial Cell Wall Characteristics

The walls of most gram-positive bacteria contain one or more secondary polymers, which are covalently linked to the main wall structural component, peptidoglycan. Peptidoglycan has two components: a polymer of sugars (*N*-acetylglucosamine and *N*-acetylmuramic acid) and crosslinks of short peptides consisting of 4 to 8 residues. These secondary polymers, which are major components of the gram-positive cell wall, include teichoic, teichuronic, and lipoteichoic acids. Peptidoglycan and these cell wall acids are exposed to the external aqueous solution and form the surface of the bacterial cell. The wall of teichoic acids is linked exclusively to the peptidoglycan layer of the cell wall and does not extend to the plasma membrane. In contrast, lipoteichoic acids are anchored to the plasma membrane and extend through the entire peptidoglycan layer. Both types of teichoic acids are composed of glycerol or ribitol polymers connected by negatively charged phosphate groups, which allow them to bind positively charged ions like magnesium and calcium. This binding aids in the physiological functions of stabilizing the cell wall and regulating ion transport **(Yee & Fein, 2001).**

However, gram-negative cell walls lack the thick peptidoglycan layer and have a second membrane (external membrane) within the cellular envelope. The external membrane is a porous structure, rich in protein and lipopolysaccharide (LPS), and is highly permeable. A thin layer of peptidoglycan, as well as various enzymes and structural proteins, is located in the periplasmic space beneath the external membrane. Except in unusual cases, teichoic acid, teichuronic acid, and lipoteichoic acid are not found in gram-negative cell walls **(Yee & Fein, 2001).**

2.8 Species of Mesophilic Bacteria Used in Bioremediation of Heavy Metals

Microorganisms remove metals by the following mechanisms Adsorption, Complexation (microorganisms produce organic acids e.g., citric acid, gluconic acid, formic acid, lactic acid, and malic acid which chelate metal ions), Precipitation (some bacteria produce ammonia, H_2S which precipitates metals as hydroxides or sulfates) and Volatilization (some bacteria cause methylation of Hg^{2+} and converts to dimethyl mercury a volatile compound). Most studies in this discipline involved the isolation of microorganisms, culturing them in various media against xenobiotic substances (such as heavy metals, antibiotics, etc., that can inflict harm on cells) (**Fernandes *et al.*, 2021**), as well as biotechnological and environmental compatibility. Remediation potentials were also investigated in different ecological conditions, which may impede or support microbial activities within a specified concentration range. Based on the bacterial species used in bioremediation, *Aeromonas* spp., *Anthrobacter* spp., *Bacillus* spp., *Escherichia coli*, *Micrococcus* spp., *Aspergillus* spp., and *Pseudomonas* spp. emerge as among the most promising microorganisms in this regard. It is also important to note that the information on the use of these bacteria in aquatic ecosystem pollution and the bioremediation mechanism is very limited (**Yadav *et al.*, 2017; Fakhar *et al.*, 2022**). The stimulatory or inhibitory effects of heavy metals on microorganisms are influenced by the total concentration of metal ions, their chemical speciation, and associated factors such as redox potential. Environmental parameters, including temperature, pH, the presence of low molecular weight organic acids, and humic substances, play critical roles in altering the transformation, mobility, oxidation state, and bioavailability of heavy metals to microbial cells. At acidic pH, the increased formation of free metal ions enhances proton availability, which in turn modifies metal-binding sites. Elevated hydrogen ion concentrations cause the adsorbent surface to become more positively charged, thereby reducing the electrostatic attraction to metal cations and increasing metal toxicity. Temperature significantly affects heavy metal adsorption, as increased temperature promotes faster diffusion of metals through the microbial boundary layer and enhances metal solubility, thereby increasing bioavailability. Nevertheless, microbial metabolic and enzymatic activities rise with temperature only within an optimal range, beyond which bioremediation efficiency declines. The interaction between microbes and heavy metals is also governed by the characteristics of sorption sites, cell wall architecture, and the ionization state of chemical groups present on the microbial cell surface. The efficiency of the degradation process depends on the substrate and several environmental factors (**Igiri *et al.*, 2018**). The injurious effects of these ecological parameters altered by climate change can have an impact on both soil and water microorganisms and, hence, impede their bioremediation performance (**Alkorta *et al.*, 2017**). The potential of soil and water microorganisms in bioremediation is under threat due to alterations in temperature, moisture, wet-drying and freeze-thawing cycles, pH, etc., that may result in modified interactions among microorganisms required for the remediation of environmental contaminants (**Fierer *et al.*, 2003**).

2.8.1 *Aeromonas* Species

Aeromonas belongs to the class Gamma Proteobacteria within the Aeromonadaceae family. It is a Gram-negative, rod-shaped, non-spore-forming, and facultative anaerobe. Aeromonas species are common in soil and aquatic habitats, living on various foods. A few strains have the potential to cause diseases in poikilothermic animals, including mammals, birds, and fish. Human infections by these strains include septicemia, pneumonia, wound infections, urinary tract infections, and gastroenteritis. Furthermore, Aeromonas species have an extraordinarily efficient ability to transform and remove heavy metals and other pollutants from contaminated areas **(Huang *et al.*, 2019).** Research has shown that Aeromonas species can degrade oil hydrocarbons, decolorize triphenylmethane dyes, e.g., malachite green, and remove nitrates from wastewater. A study conducted in the Tanjaro area, southwest of Sulaymaniyah city in the Kurdistan Region of Iraq, showed that *Aeromonas sobria* was able to withstand Zn, Cu, and Ni at concentrations of 5, 6, and 8 mM, respectively, based on maximum tolerance concentration (MTC). Transmission electron microscopy (TEM) revealed that *A. sobria* accumulated both Cu and Ni, whereas biosorption was suggested for the Zn.

Among the chromium-tolerant aeromonads isolated from chromium-contaminated areas with Cr(VI) reduction ability, *Aeromonas dechromatica* and *Aeromonas hydrophila* have been identified (**Huang *et al.*, 2019**).

This is a testament that metal-resistant *A. sobria*, *A. dechromatica*, and *A. hydrophila* could be promising candidates for heavy metal bioremediation in contaminated areas.

2.8.2 *Arthrobacter* Species

Arthrobacter is a group of pleomorphic bacteria, ubiquitous in origin, with effective roles in agriculture, and is a potential candidate for bioremediation. A general classification based on the 16S rRNA sequence phylogeny data by which Arthrobacter genus has been subdivided into many groups such as *Arthrobacter aurescens, Arthrobacter globiformis, Arthrobacter pascens, Arthrobacter oryzae, Arthrobacter humicola, Arthrobacter oxydans, Arthrobacter protophormiae, Arthrobacter sulfureus, Arthrobacter citreus, Arthrobacter agilis, Arthrobacter psychrolactophilus, Arthrobacter pigmenti, Arthrobacter cryoconiti, Arthrobacter ilicis, Arthrobacter cupressi, Arthrobacter gyeryongensis*, etc. **(Hoang *et al.*, 2014).** They are resistant to harsh climates and can utilize xenobiotics and sequester heavy metals, making them novel organisms for the reclamation of polluted land **(Roy & Kumar, 2020).** The metal-bioremediation potential of living or dry cells of *Arthrobacter globiformis* and *Arthrobacter oxidas* was investigated as a function of metal recovery. The linearized absorption isotherms of Cr ions in anion and cation forms for the two kinds of Arthrobacter were obtained by fitting experimental data. The Freundlich parameters evaluated from the isotherms, with the correlation coefficients, were determined. The results showed a significant difference between the binding constants for Cr(VI)-*Arthrobacter oxidas* and Cr(VI)-*Arthrobacter globiformis*. The decrease in bioavailability was determined experimentally for Cr(VI)-*A. globiformis* in comparison to *A. oxidas*. It was established that the carboxyl groups were the main binding sites in the cell walls of gram-positive bacteria. The displacement of protons is attributed to such bond formation and is partly dependent on the extent of protonation, which is determined by the pH value. The results showed that Cr(VI) sorption depended on the species of Arthrobacter bacteria. The difference between Arthrobacter species in metal ion binding may be related to the properties of metal sorbates and the properties of bacteria (functional groups, structure, and surface area), which vary in the species. Functional groups such as amino, carboxylic, sulphydryl, phosphate, and thiol differ in their affinity and specificity for metal binding (**Gelagutashvili, 2013**).

2.8.3 *Bacillus* Species

Bioremediation agents include bacteria of the genus *Bacillus*, among others. They are Gram-positive, spore-forming, rod-shaped, and aerobic or facultative anaerobes. They are mostly found in the soil, but can also be isolated from other sources, such as water, vegetables, food, human, and animal intestines. The uniqueness of *Bacillus* spp. relates to their spore formation in extreme conditions, which enables them to resist high environmental stresses such as high temperature, drought, humidity, and radiation. Their spore-forming ability confers them an advantage over other bacteria and makes them widely used commercially in various fields of industry and agriculture. Among the most common *Bacillus* spp. in terms of having bioremediation potential are *Bacillus subtilis*, *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus carotarum*, *Bacillus lentus*, *and Bacillus licheniformis* (**Pham *et al.*, 2022; Wróbel *et al.*, 2023**). This bacterial genus employed various strategies such as biosorption, extracellular polymeric substance (EPS)-mediated biosorption, bioaccumulation, or bioprecipitation in bioremediation. The strategies enhanced the *Bacillus* spp. strains to reduce the amounts of metals such as lead, cadmium, mercury, chromium, arsenic, or nickel in the environment. Among the mercury-resistant microbes isolated from industrial effluents of tanneries, *Bacillus licheniformis* showed mercury processing ability as it removed 73% of mercury (**Muneer *et al.*, 2013**). Moreover, *Bacillus* species have been shown to enhance phytoremediation processes by promoting plant growth and facilitating the bioaccumulation of heavy metals within the soil matrix. Therefore, *Bacillus* spp. is one of the best sustainable solutions for reducing heavy metals from various environments, especially soil (**Pham *et al.*, 2022; Wróbel *et al.*, 2023**).

2.8.4 *Micrococcus* Species

Micrococcus is a genus of bacteria in the Micrococcaceae family. Micrococci have gram-positive spherical cells ranging from 0.5 to 3 micrometers in diameter and typically appear in tetrads. Research has shown that *Micrococcus lalobius* and *Micrococcus kristinae* isolated from polluted rivers in Basra City, Southern Iraq, exhibited significant resistance to Pb (2200-2600) µg mL^{-1}, Cu (400) µg mL^{-1}, Cd (300-400) µg mL^{-1}, and Ni (200-300) µg mL^{-1}. This indicates that these bacterial strains are candidates for bioremediation of heavy metal ions at different concentrations from aqueous metal solutions (**Anwar *et al.*, 2017**). *Micrococcus luteus* was also tested and proved capable of removing significant amounts of copper and lead; hence, it's a potential candidate in bioremediation technology. In another research, *Micrococcus luteus* DE2008 was used to study the biosorption of Lead and Copper. The results showed that Pb did not affect the biomass of *M. luteus* DEC2008, but unlike Cu, the minimum metal concentration that affects the biomass was 0.1 mM Cu (II). Based on these results, this bacterial strain shows a greater tolerance of Pb than Cu (**Muhammad & Ibrahim, 2018**).

2.8.5 *Pseudomonas* Species

Research has indicated that among the many microbes with tolerance towards toxic levels of HMs that are used to take up metals through the acquisition of specific resistance systems, such as efflux and uptake mechanisms, extracellular precipitation pumps were strains of *Pseudomonas.* While some develop resistance to HMs naturally, others are genetically modified to give high remediation results. *Pseudomonas* is a Gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm. The bacterium thrives in soil, water, and surfaces that are in contact with soil or water. Research has shown that eight copper-resistant *Pseudomonas* strains also demonstrated high levels of zinc, cesium, lead, arsenate, and mercury (Awasthi *et al.*, 2015). Another study also established that *Pseudomonas* species remediated 37% (Pb^{2+}), 32% (Ni^{2+}), 29% (Cu^{2+}), and 32% (Cr^{3+}), and was suggested to be an effective agent in the bioremediation of a polluted environment (**Naz *et al.*, 2016**). It was concluded from the research conducted by **Nagashetti *et al.*** (**2013**) that *Pseudomonas aeruginosa* has great potential to remediate hexavalent chromium, copper, and zinc from contaminated environments. It is, therefore, a good biosorbent for the removal of chromium, copper, and zinc from industrial waste (**Chellaiah, 2018**). A test was conducted to determine the actual interaction of metal ions and biomass accumulation, which showed *Pseudomonas fluorescens* had high-efficiency removal of Cr, whereas *Pseudomonas putida* proved to be effective in removing Cu, Ni, and Cd (**Hussein *et al.*, 2005**). A similar study revealed that *P. aeruginosa* BC15 showed high resistance and the ability of biosorption to Zn and Cd in solid and liquid media, and biosorption with *P. putida* showed 80% of Cd, Cu, Pb, and Zn being effectively removed from aqueous solution (**Edward Raja *et al.*, 2008**).

2.8.6 *Staphylococcus* Species

Staphylococcus species are gram-positive, sphere-shaped (coccal) bacteria that may cause skin, heart valve, and bone infections, and pneumonia, and may be resistant to treatment with some antibiotics. *Staphylococcus* species have demonstrated potential as effective biosorbents for the remediation of heavy metals (HMs) in contaminated soil and water systems. Experimental findings revealed average removal efficiencies of 42% for copper (Cu), 45% for chromium (Cr), and 82.6% for lead (Pb), within an optimal temperature range of 35 °C to 39 °C (**Kumar *et al.*, 2011**). *Staphylococcus aureus* was found to be able to reduce 99% of Cr^{6+} in 24 hrs. at 35 Oc. FTIR results suggested that carboxyl, amino, and phosphate groups of cell walls were involved in complexation with chromium **(Tariq *et al.*, 2019). Ilhan *et al.*** (**2004**) showed in their study that *Staphylococcus saprophyticus* has the potential to remove chromium, lead, and copper ions from industrial sludge (**Kumar *et al.*, 2011**)**.** Another research conducted by **Elizabeth *et al.*** (**2017**) revealed that among the four bacteria tolerant to heavy metals isolated from the Panteka stream, *Staphylococcus epidermidis* was the most effective in removing lead (100%), nickel (100%), cadmium (90.29%, zinc (84.95%), and iron (54.82%). *Staphylococcus lentus* (E3) isolated from soil polluted with industrial effluents at Kasur, Pakistan, exhibited a promising ability for Cr uptake. This bacterial strain enhanced the growth parameters of plants grown under Cr-stressed conditions concerning root, shoot lengths, and wet and dry biomass (**Jamil *et al.*, 2022**).

2.8.7 Genetically Modified Organisms (GMOs)

Advancements in science and technology have significantly contributed to strategies aimed at reducing environmental pollution. One notable development is the application of genetically modified organisms (GMOs), particularly engineered strains of *Escherichia coli*, in bioremediation processes. These modified microbes are tailored to enhance the degradation or removal of specific environmental contaminants that indigenous microbial populations may not effectively process, thereby providing a precise and efficient solution for pollutant remediation. The use of genetically modified *E. coli* in bioremediation has several advantages, such as precision, efficiency, and versatility **(Sharma *et al.*, 2024).**

The genome of microorganisms has been manipulated, keeping specific goals in mind. The term “genetically engineered organisms” (GEMs) refers to microorganisms (bacteria, fungi, and yeast, among others) that have been altered by humans utilizing molecular biology in vitro procedures. Recent research has shown the use of the CRISPR-Cas system with model organisms such as Pseudomonas or *E. coli*. Although *Achromobacter* sp. HZ01 and *Comamonas testosteroni* are non-model organisms; they are potential candidates for bioremediation due to the new technologies such as CRISPR tools and the synthesis of gRNA to express function-specific genes pertinent to degrading pollutants **(Sharma *et al.*, 2024)**.

E. coli is a rod-shaped facultative coliform bacterium in the genus *Escherichia*. As one of the model organisms used in bioremediation, *E. coli* is generally known as the "workhorse" of molecular biology for its fast growth rate in chemically defined media and harbors a genome with features like an organized structure, a remnant of many phages, insertion sequence (IS), and high transport capacity towards the cytoplasm. The incorporation of a gene into bacteria results in the conversion of the bacteria into a distinct strain that possesses the ability to efficiently eliminate hydrocarbon contaminants and other substances from the surrounding environment. The *E. coli* SE5000 strain underwent genetic modification by introducing the nixA gene, which enables the expression of a nickel-transporting system, thereby degrading nickel from an aqueous system. Furthermore, the *E. coli* FACU strain has a significant ability to reduce Cr (IV) to Cr (III), exhibiting great potential as a viable agent in the bioremediation of hazardous chromium species in aerobic environmental conditions (**Sharma *et al.*, 2024**).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

3.1.1 Description of Study Area

The Gambia lies within the tropical sub-humid ecoclimatic zone, receiving annual rainfall between 800 and 1200 mm (**Barrow *et al.*, 2020; Kargbo *et al.*, 2023**). The country experiences two distinct seasons: a rainy season from June to October and a dry season from November to May, during which dry, dusty Harmattan winds from the Sahara prevail (**Kargbo *et al.*, 2021**). During the dry season (November to May), the Intertropical Convergence Zone (ITCZ) shifts southward away from the country because of the seasonal movement of the sun and changes in pressure systems. Consequently, the rain-bearing convergence zone migrates southward, reducing rainfall over the area.

The study area features a heterogeneous landscape comprising coastal wetlands, tidal flats, marshland ecosystems, colluvial slopes, and elevated upland terrains adjacent to the River Gambia. These areas support rice and other crop cultivation, with land management practices (e.g., bunding, ridging, and sluice construction) adapted to soil type, water movement, and tidal influence (**Engel-Di Mauro, 2012**).

This study was conducted in thirteen rural districts across six Agricultural regions of The Gambia: Kombo Central and Foni Kansala (West Coast Region), Jarra West and Kiang East (Lower River Region), Lower Niumi and Jokadu (North Bank Region), Upper Saloum and Niani (Central River Region North), Janjangbureh, Lower Fulladu, and Lower Fulladu West (Central River Region South), and Fulladu East and Wuli West (Upper River Region). The districts were randomly selected to ensure a representative sampling. By randomly selecting districts across all six agricultural regions, the researcher aimed to collect unbiased, representative data from farmers experiencing a variety of climatic conditions, allowing them to understand the general trends in climate change perception and adaptation.

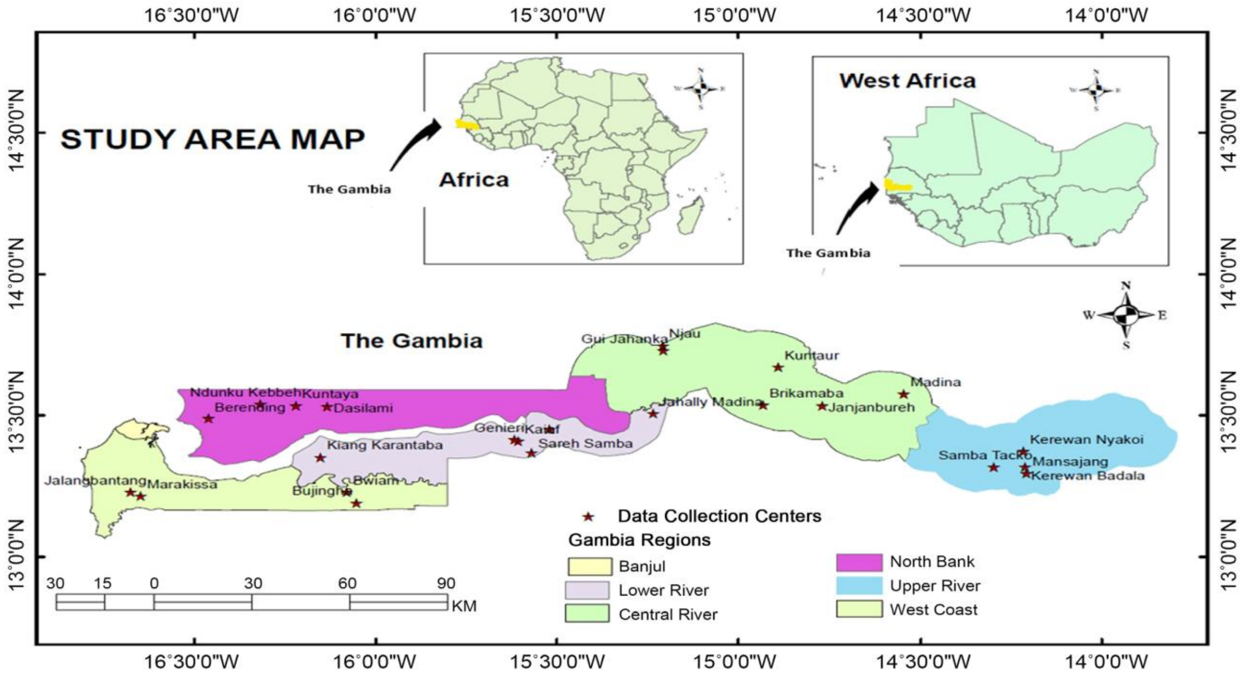


Figure 25: Map of The Gambia showing the study area. The figure was generated and modified using ArcGIS 9.

3.1.2 Study Population

Participants were selected through a randomized sampling method to ensure objectivity and minimize selection bias. Adopting Cochran's sample size formula, where **n** is the sample size, **z** is the standard error associated with the chosen level of confidence (1.96), **p** is the estimated prevalence (0.50), **q** is 1-**p** (0.50), and d is the acceptable error (0.05):

$$\mathbf{n = Z^2 \times p\ (1 - p)/d^2}$$

From the above formula, a sample size of 384 participants was obtained. To facilitate a balanced allocation across the studied sub-regions, the sample size was rounded up by 12.5% to a more logistically convenient and divisible target of 432 participants **(Nanjundeswaraswamy & Divakar, 2021).** This adjustment ensured equal representation across all surveyed groups without compromising statistical precision. As stated earlier on, the survey was conducted across six regions. In five of these regions, two districts were randomly selected from each region. Within each selected district, two farming communities were purposively chosen for participation in the study. This purposive selection was based on the observation that only specific communities were actively engaged in crop production.

However, in the Central River Region South (CRR-S), a different sampling approach was applied. Three districts, such as Janjanbureh, Lower Fulladu, and Lower Fulladu West, were included in the study. From each of these districts, one farming community was selected, but in Lower Fulladu West, two communities were included due to their notably high level of agricultural activity.

Overall, this design ensured representation across six regions while accounting for variations in agricultural engagement at the community level. Data were collected from 228 male and 204 female crop farmers using 432 structured and pre-tested questionnaires administered under the supervision of the PhD candidate, who coordinated the process and trained two research assistants to ensure compliance with the study protocol. The questionnaires were made up of both closed and open-ended questions, which were interpreted orally in the local languages (Mandinka, Fula, and Wollof) to the farmers and later transferred back to the English Language consistently to enhance their applicability in various contexts.

3.1.3 Research Design

A cross-sectional survey was implemented across the six designated Agricultural Regions of The Gambia (**Figure 25**) over twelve months, spanning from March 2022 to March 2023. The investigation relied on primary data sources, which included both quantitative and qualitative methods. Quantitative data were obtained through the administration of structured questionnaires to 432 crop farmers. In contrast, qualitative insights were gathered in 2023 through Focus Group Discussions (FGDs) and Key Informant Interviews (KIIs), utilizing unstructured interview guides tailored to explore deeper perspectives on relevant agricultural issues.

The quantitative and qualitative questionnaires were designed to assess farmers' knowledge and perceptions regarding the impacts of climate variability and environmental pollution on crop production, as well as to explore the diverse adaptation strategies employed across farming communities in The Gambia. The combination of both quantitative and qualitative approaches provides a more holistic understanding of the research question than either approach singly (**Creswell & Angeles, 2006**).

3.2 Data Collection and Analysis in Objective One

3.2.1 Equipment

Questionnaires, a Garmin eTrex 10, a rugged handheld GPS, and a laptop were used during the survey to obtain data, the location of the community being surveyed, and a record of data, respectively.

3.2.2 Ethics Approval and Consent to Participate in the Study

Ethical clearance and research authorization for the initial phase of this study were granted by the Ministry of Higher Education, Research, Science and Technology (MoHERST), The Gambia (Ref: AFG 85/272/02), and the University of The Gambia Research Ethics Committee (UTG REC) (Ref: UTG REC 2023-007). These approvals facilitated the process of securing consent from local community leaders, enabling the field-based survey activities to commence.

Voluntariness: Only crop farmers who voluntarily agreed to participate in the study were interviewed. They were at liberty to withdraw from participation without adverse outcomes.

Beneficence: Crop producers in The Gambia were sensitized on the implications of climate variability and environmental pollution on agricultural productivity, as well as the adaptive measures they employ to mitigate these challenges.

3.2.3 Community Visit

The communities where samples were obtained were visited before the start of the study to obtain consent from their “Alkalos,” or heads of the communities. A checked list of all the communities involved in rice farming in CRR South using the River Gambia as a source of water was obtained, after which three communities were randomly selected for sampling.

3.2.4 Research Assistants

Two research assistants were engaged and trained in the following techniques:

- ✓ How to be able to administer a questionnaire
- ✓ Recording of relevant information in the field

3.2.5 Pre-testing and Validation of Questionnaires

Before the main survey, a pilot test of the data collection tool was conducted. Research Field Assistants were selected from Gambia College, Brikama Campus, to facilitate this pilot phase. These assistants, who are undergraduate students at tertiary institutions, possess proficiency in English and two predominant local languages spoken within the study regions. All assistants received comprehensive training over two days to ensure proper administration of the instrument and to address any arising questions. The reliability of the instrument was evaluated through a pilot survey involving 40 randomly chosen crop farmers from the West Coast and Lower River Regions between October 7 and 10, 2022. Following the pilot, modifications were made by rephrasing certain items and eliminating others to reduce the overall interview duration. Subsequently, the finalized questionnaire was validated at Gambia College, Brikama Campus, on October 28, 2022.

3.2.6 One-to-one interview questionnaires for crop farmers

A cross-sectional investigation was carried out across the six Agricultural Regions of The Gambia between March 2022 and March 2023. The study utilized primary data collected in 2023 via structured quantitative questionnaires. Four hundred and thirty-two (432) crop farmers participated in the survey. The questionnaires focused on assessing the farmers' knowledge and perceptions regarding the impacts of climate variability and environmental pollution on crop productivity, as well as their diverse adaptation strategies employed within The Gambia. The combination of both quantitative and qualitative approaches provides a more holistic understanding of the research question than either approach singly **(Creswell & Angeles, 2006)**. The questionnaire was administered to farmers through oral translation into local indigenous languages, including Mandinka, Fula, and Wolof. The instrument was structured into four primary sections, specifically:

(a) Demography: Comprised of 10 questions for crop farmers

(b) The knowledge probe (KP) assessing the impact of climate variability on crop yield consisted of 22 items, systematically organized into three distinct sections:

- Knowledge of the causes of changes in climate;
- Knowledge of the various climate change parameters;
- Understanding the impacts of climate variability and pollution on environmental systems.

(c) Knowledge Probe on the contamination of farmland:

- Knowledge of soil and water pollution;
- Knowledge and impact of heavy metal pollution.

(d) Factors impeding farmers' responses to climate change in their crop production.

3.2.7 Focus Group Discussions (FGDs)

Six Focus Group Discussions, each comprising eight participants, were conducted. Two districts were selected from each region, and two farming communities in each district were chosen for the interview using semi-structured interview questions (**Agwu *et al.*, 2018**). Two farmers per community participated in the FGDs. These participants were recruited from the one-to-one interview sessions. The age and number of years the farmers were involved in crop production were the main criteria for recruiting them for the discussion (**Agwu *et al.*, 2018; Kargbo *et al.*, 2023**). One FGD was held in each of the six regions using semi-structured interview questions, which lasted for about 20-30 min, depending on the participants' knowledge about the questionnaire. The interview protocol was structured around three key thematic areas: (1) assessing the understanding and perceptions of climate variability within farming communities; (2) investigating the adaptive strategies employed by these communities to mitigate the impacts of climate variability; and (3) examining farmers' awareness and viewpoints regarding environmental pollution and the bioremediation of contaminants in agricultural soils.

3.2.8 Key Informant Interviews (KIIs)

Key Informant Interviews (KIIs) were conducted with representatives from selected government institutions involved in climate change and agriculture. Two representatives were interviewed from each of the following institutions: the School of Agriculture at Gambia College, the School of Agriculture and Environmental Sciences at the University of The Gambia, the National Agricultural Research Institute (NARI), the Extension Service Unit, the Soil and Water Management Unit (SWMU) within the Department of Agriculture, the Ministry of Environment, Climate Change and Natural Resources (MoECCNR), and the National Environment Agency (NEA). Selection of these individuals was conducted by their respective organizations, based on their pertinent roles and specialized expertise.

3.2.9 Statistical Analysis

Raw data were organized using Microsoft Excel, and statistical analyses were performed with Epi Info version 7.2.5.0. Descriptive statistics were employed to characterize the dataset. Relationships between demographic factors, including gender, age, ethnicity, religion, marital status, and educational attainment, and the variables of interest (knowledge and perception) were evaluated using Pearson's chi-square test alongside Binomial Logistic Regression analysis. Statistical significance was determined at a threshold of $p < 0.05$.

3.3 Characterization of mesophilic bacteria from CRR South of The Gambia

3.3.1 Ethical Approval

The University of The Gambia Research Ethics Committee (UTG REC) granted ethical clearance for this research, with the approval documented under reference number UTG REC 2024-075. Before this, the proposal received a favorable opinion from the Scientific Coordinating Committee (SCC) at MRCG@LSHTM. Informed consent was obtained through signed forms from community leaders in the sampled areas.

3.3.2 Sampling Area

The region, CRR South, was purposively selected for sample collection because it is one of the country's rice hubs where rice can be cultivated year-round. Rice farming here does not rely solely on rainfall since it uses water from the river. The sampling process involved creating a checklist of all communities involved in rice production along the riverbank within the study area. Three different communities, such as Janjanbureh (13°32`2.87``N 14°45`58.15``W), Brikama Ba (13°31`60.0``N 14°55`60.0``W), and Kudang (13°40`0``N 15°4`0``W), and one rice field in each were randomly selected for sample collection (see Figure 4). In each of these communities, three plots within the rice fields in CRR South were identified, totaling nine sites or plots where samples were collected in sterile 200 mL plastic bottles. These samples were taken to the laboratory for screening and isolating mesophilic bacteria. At each site or plot, careful precautions were taken during collection to prevent contamination. The water's temperature and pH were recorded, and mobile Google Maps were used to obtain the coordinates of each sampling location within the rice fields.

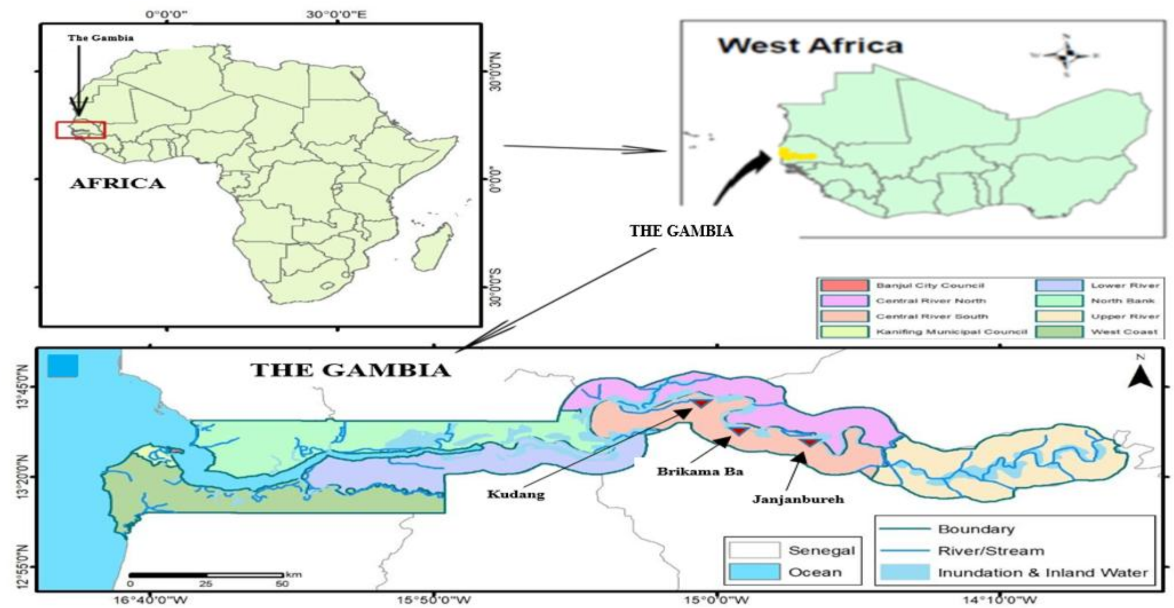


Figure 26: Map of The Gambia showing the sampling sites in CRR-South of The Gambia (generated from microreact.org).

3.3.4 Sampling Methods

Water, sediment/soil solution, and dry soil samples were collected from rice cultivation fields in Janjanbureh (13°32′2.87″N, 14°45′58.15″W), Brikama Ba (13°31′60.0″N, 14°55′60.0″W), and Kudang (13°40′0″N, 15°4′0″W), located along the River Gambia in the Central River Region (CRR) South, by the PhD candidate and two trained research assistants to isolate mesophilic bacteria.

In Janjanbureh, three rice fields, Faraba Lay, Nyantang Lay, and Morroba Lay, were identified, and Faraba Lay was selected randomly. Three sample types were collected from separate plots:

Water (**A1**): 13°31′35.73984″N, 14°46′1.34616″W

Sediment (**A2**): 13°31′43.43844″N, 14°45′58.75488″W

Dry soil (**A3**): 13°31′35.52816″N, 14°45′59.33736″W

In Brikama Ba, samples were collected from the Jahally-Pacharr Rice Fields:

Water (**B1**): 13°33′27.41832″N, 14°56′21.0642″W

Sediment (**B2**): 13°33′25.94268″N, 14°56′51.41652″W

Dry soil (**B3**): 13°33′15.54444″N, 14°55′49.85076″W

In Kudang, the Saikou Farro Rice Field was randomly selected:

Water (**C1**): 13°41′30.76548″N, 15°2′58.53444″W

Sediment (**C2**): 13°41′34.61568″N, 15°2′52.50912″W

Dry soil (**C3**): 13°41′32.92116″N, 15°3′2.60388″W

At each site, environmental parameters including temperature, pH, and salinity were recorded.

3.3.5 Sample Collection and Handling

Water samples were collected in plastic bottles, while sediment samples were collected using sterile McCartney bottles. Dry soil samples were collected using a fork and spatula into measuring cylinders. All containers were washed with 99% absolute ethanol, rinsed with purified water, and air-dried before use. Before collecting samples, the water bottles were rinsed thoroughly three times using purified water. Sediment samples were collected by dipping sterile McCartney bottles into shallow water with sterile forceps, which were disinfected intermittently with 99% ethanol. Dry soil was scooped from the banks of rice fields and transferred into labeled measuring cylinders.

All containers were clearly labeled according to the sample type and location, then transported under sterile conditions to the laboratory for microbial analysis (**Popoola *et al.*, 2024**).

3.3.6 Isolation and Characterization of Mesophilic Bacteria

Samples were initially prepared using 0.9% saline solution and distilled water for colony enumeration via the pour plate method (**Zaky *et al.*, 2011**). Selective media were employed to isolate specific bacterial groups: MacConkey agar for *Pseudomonas* spp., Ryan Aeromonas Isolation Medium for *Aeromonas* spp., and Blood Agar for *Bacillus* spp.

Both water and sediment samples were filtered to remove particulates. Ten milliliters (10 mL) of each sample were stored at room temperature in sterile 50 mL Falcon tubes. The suspensions were homogenized by shaking, and 10 µL aliquots were plated onto sterile nutrient agar under aseptic conditions. Plates were incubated at 37 °C for 24 hours (**Bibi *et al.*, 2020**).

Well-isolated colonies were subcultured for purification. Morphological characterization was carried out based on colony color, elevation, shape, and Gram staining. Colonies displayed various appearances, including greyish, pale, orange-yellow, light yellow, greenish, white, pink, or violet (**Bibi *et al.*, 2020**).

3.3.7 Gram Staining

Requirements

Bacterial isolates, microscopic blank slides, crystal violet, distilled water, cotton wool, Gram's iodine, 70% alcohol, sterile inoculating loop, and a light microscope. Other equipment included a staining rack, a Bunsen burner, and a lighter.

Method

- **Prepare smear**
    - The inoculating was held in a flame until red-hot and then allowed to cool for ~30 sec. Using this sterilized loop, a thin film of the sample was prepared and stained on a glass slide.
    - The smear was air-dried, then heat-fixed by passing the slide over a flame 2 to 3 times.
- **Stain smear**
    - The heat-fixed smear was covered with 1-2 drops of crystal violet solution, and allowed to stand for 30 sec.
    - The stain was washed off gently with flowing water for ~5 sec, and excess water was shaken off.
    - The smear was covered with Gram's iodine solution for 1 min and washed with distilled water as in step 4, and excess water was shaken off.
- **Decolorize and counterstain smear**
    - The slide was decolorized with 70% alcohol by tilting it slightly and slowly dropping the solution above the smear on the slide, and it was allowed to run down across the smear. This was done until the purple color ceased to flow away from the smear.
    - The smear was washed with distilled water as in step 4 for ~5 sec., and excess water was shaken off.
    - The slide was counterstained with 1-2 drops of safranin for 30 sec.
    - The slide was washed with water as in step 4, then shaken off excess water, and it was allowed to air dry while other slides were carefully blotted with bibulous paper.

- **Examine the slide**

  - The bacteria were examined under oil immersion (900× to 1000×) to distinguish between Gram-positive and Gram-negative bacteria (**Okimasi-Henry, 2023**).

3.4 Biochemical Tests

3.4.1 Oxidase Test

The oxidase test is a simple biochemical test to detect the presence of cytochrome c, an enzyme in the bacterial electron transport chain that transfers electrons to oxygen. The oxidase paper was prepared by soaking a filter paper in tetramethyl-p-phenylenediamine dihydrochloride substrate and allowing it to dry overnight. Using a disposable sterile plastic inoculating loop, some colonies of the test organism grown on the nutrient agar for 24 h were collected and smeared on the Whatman filter paper. If the organism is oxidase producing, the phenylenediamine in the reagent will be oxidised to a deep purple colour, while for a negative test, the paper remains colourless within 10-15 seconds (**Okimasi-Henry, 2023**).

3.4.2 Bacterial Identification using Analytical Profile Index (API) 20E Test

In this study, bacterial identification was performed using the API 20E system, a standardized biochemical test panel designed for the rapid identification of non-enteric Gram-negative bacteria. This method was employed as an alternative to conventional biochemical tests to reduce contamination risk, improve processing time, and optimize laboratory workflow. The method is qualitative, relying on positive and negative biochemical reactions **(Artati *et al.*, 2024**).

The API 20E kit consists of 20 miniaturized test chambers containing dehydrated substrates for detecting various enzymatic activities, particularly those related to carbohydrate utilization and amino acid degradation. Before testing, isolates were obtained from water, sediment, and soil samples collected in CRR-South. Cultures were grown on blood agar, MacConkey agar, or *Aeromonas* medium and purified by sub-culturing single colonies onto nutrient and blood agar plates incubated at 37 °C overnight.

Purified isolates were suspended in sterile saline and adjusted to a 0.5 McFarland turbidity standard. The bacterial suspension was then used to inoculate the API 20E strip. Oxidase testing was conducted before strip inoculation, as recommended. After incubation, the results of the 20 biochemical reactions were interpreted to generate a seven-digit numerical profile. This profile was analyzed using the APIweb database to determine the most likely bacterial identity.

Figure 27: The researcher performing Gram staining (A) and API 20E (B) procedures for bacterial identification in the Microbiology Laboratory at MRCG@LSHTM, 2025.

3.4.3 Antimicrobial Resistance Test

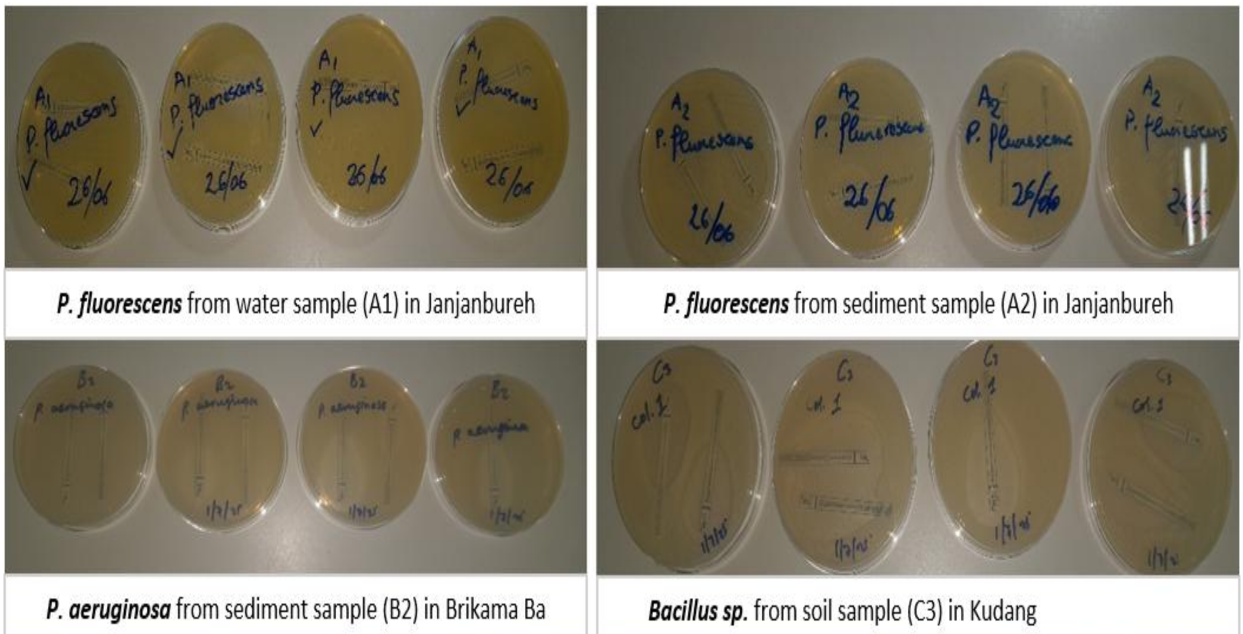
Bacterial infections can be severe and life-threatening. Despite its importance in guiding appropriate treatment, antimicrobial susceptibility testing is not routinely performed in many laboratories. However, increasing global bacterial resistance highlights the need for such testing, as resistance patterns often vary by geographic location **(Infectious and Advanced, 2013)**.

In this study, two strains of *Pseudomonas fluorescens* were tested, one from a water sample (A1) and the other from sediment (A2) in Janjanbureh. Additionally, *Pseudomonas aeruginosa* and *Bacillus* spp., isolated from sediment (B2) and dry soil (C3) samples in Brikama Ba and Kudang, respectively, were included. These isolates were tested for susceptibility to seven antibiotics: meropenem, chloramphenicol, ciprofloxacin, ceftriaxone, tetracycline, gentamicin, and trimethoprim-sulfamethoxazole, using Mueller-Hinton Agar (MHA).

Two other isolates, *Grimontia hollisae* and *Bordetella pertussis*, both from a dry soil sample (A3) in Janjanbureh, were not tested due to the absence of interpretive guidelines or MIC breakpoints in the Clinical and Laboratory Standards Institute (CLSI) documents.

The E-test (Epsilometer test), a quantitative method, was used to determine the Minimum Inhibitory Concentration (MIC) of the antibiotics. Bacterial suspensions were adjusted to match a

0.5 McFarland turbidity standard and uniformly inoculated onto Mueller-Hinton agar (MHA) plates. E-test strips were then carefully placed onto the agar surface to ensure complete adherence, with two strips positioned per plate. The inoculated plates were incubated at 37 °C for 24 hours. Following incubation, the minimum inhibitory concentrations (MICs) were determined by observing the point at which the elliptical zone of inhibition intersected the graduated scale on the E-test strip.



P. fluorescens from water sample (A1) in Janjanbureh

P. fluorescens from sediment sample (A2) in Janjanbureh

P. aeruginosa from sediment sample (B2) in Brikama Ba

Bacillus sp. from soil sample (C3) in Kudang

Figure 28: Antimicrobial Susceptibility Testing for the Isolated Bacterial Species.

3.5 Bioremediation Potential Analysis

3.5.1 Optical Density-Based Assessment of Bacterial Tolerance to Heavy Metals

To evaluate bacterial bioremediation potential, fresh overnight cultures were obtained from Mueller-Hinton Agar (MHA) plates. A 0.5 McFarland standard bacterial suspension was prepared in normal saline. Each test condition consisted of 5 mL of nutrient broth supplemented with varying concentrations of heavy metals in 15 mL Falcon tubes. For zinc and iron, concentrations ranged from 50 to 450 ppm (in 50 ppm intervals), while copper concentrations ranged from 18 to 98 ppm (in 10 ppm intervals).

An aliquot of 200 µL of the bacterial suspension was inoculated into each tube, except for one uninoculated control per setup (empty well). All treatments were performed in duplicate.

Controls included:

- Positive control: nutrient broth + bacteria (no metal)
- Negative control: empty well (blank media)

All cultures were incubated at pH 7.0 and 37 °C for 24 h with shaking to ensure aeration. For optical density (OD) measurements, 200 µL of each treated culture was transferred to a 96-well microplate. OD at 600 nm was measured using a spectrophotometer at day 0 (before incubation) and then every 24 hours for 5 consecutive days.

Bacterial growth in the presence of metals was used to assess tolerance and potential for bioremediation. Isolates with the highest OD values over time were considered the most effective in metal degradation or tolerance **(Abbasi *et al*., 2024**).

Figure 29: The researcher prepared bacterial suspensions in nutrient broth with varying metal concentrations in the Microbiology Laboratory at MRCG@LSHTM, 2025.

3.5.2 Statistical Analysis

Raw data were systematically recorded using Microsoft Excel. Subsequent statistical analyses of bacterial responses to various metal treatments were conducted in RStudio. Visualization and pairwise comparisons of treatment effects were performed utilizing the ggplot2 package. Differences were considered statistically significant at the $p < 0.05$ threshold.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Farmers' knowledge and perceptions of the effects of climate variability and pollution on crop production and their varying adaptation strategies

This objective examined farmers' knowledge and perceptions of climate variability, pollution, and their adaptation strategies in The Gambia. The results show that out of 432 farmers surveyed, 426 (98.61%) were aware of climate change and its effects on crop production, while only 6 (1.39%) were not (**Table 3**). Overall, the findings indicate a very high level of awareness and understanding of climate variability among farmers in the study area. A report from Ethiopia by **Mengistu (2011)** observed that high awareness levels and vulnerability analysis to climate change are closely linked to poverty and the living conditions of farmers, which determine their vulnerability and adaptation to climate change. An increase in the frequency of climate-related risks can lead households to anticipate lower income, potentially pushing them below the poverty threshold in the country. Other researchers have emphasized vulnerability's strong effects on climate change on poor agricultural and rural communities of developing countries because of their low income and deprived adaptive capabilities **(Skoufias *et al.*, 2011)**. In addition, deviations in weather situations are likely to affect the frequency and magnitude of several climatic hazards, such as floods, storms, droughts, etc. **(Khan *et al.*, 2020).**

4.1.1 Social Demographic Characteristics of the Participants

Out of the 432 crop farmers who participated in this study, 228 (52.78%) are males, while 204 (47.22%) are females. About one-quarter of the participants, 92 (21.30%), were between 40 and 49 years old, about half of the respondents, 194 (44.91%), were between 50 and 59 years old, and about a quarter of the respondents, 117 (27.08%), were 60 years and above. The findings showed that the main population engaged in crop farming is the aged population (50 years and above), which accounted for about 72%. This is attributed to the fact that most people believed that farming was meant for the illiterate, the less educated, and the poor. This is in contrast to the study conducted in Uganda by **Ahaibwe *et al.* (2013)**, which revealed that although youth participation in agriculture is declining, the country still has a younger agricultural workforce than The Gambia where farming is largely dominated by an ageing population. The majority of the respondents, 157 (36.34%), are Mandinkas by ethnicity, followed by Fulas, 107 (24.77%), and then Wolof, 75 (17.36%), and others accounted for the rest of the respondents. On marital status, 354 (81.94%) of the respondents are married, while 58 (13.43%) are single, and the rest are either widowed or divorced. Regarding educational status, the highest number, 171 (39.58%) of the respondents were illiterate, followed by those who only attained primary education, 82 (18.98%) (**Table 1**).

Table 1: Demography of Crop Farmers.

Characteristics	**Frequency**	**Percentage**
Gender		
Male	228	52.78
Female	204	47.22
Total	**432**	**100.00**
Age (years		
20-29	10	2.31
30-39	19	4.40
40-49	92	21.30
50-59	194	44.91
60 and above	117	27.08
Total	**432**	**100.00**
Ethnic group		
Mandinka	157	36.34
Fula	107	24.77
Wolof	75	17.36
Others	93	21.53
Total	**432**	**100.00**
Religion		
Islam	403	93.29
Christianity	29	6.71
Total	**432**	**100.00**
Marital status		
Married	354	81.94
Single	58	13.43
Widowed	15	3.47
Divorced	5	1.16
Total	**432**	**100.00**
Highest level of education		
Primary	82	18.98
Secondary	74	17.13
Tertiary/University	32	7.41
Informal/Madarasa	171	39.58
None	73	16.90
Total	**432**	**100.00**

4.1.2 Percentage of Farmers Involved in the Cultivation of Various Crops and Land Tenure Systems Used

Participants in this survey, as shown in **Table 2**, were asked about the main crop they grow, the type of farmland, whether they have changed crops in the past 20 years, and if they have changed crops, what the reason for the change of crops was. The results showed that 138 (31.94%) of the respondents grew rice, 212 (49.07%) grew groundnuts, 21 (4.86%) grew maize, 41 (9.49%) grew millet, 9 (2.08%) grew cassava, and the rest grew one of the following crops; banana, lettuce, okra, and onion as shown in **Table 2** indicating limited crop diversification. This heavy reliance on a few dominant crops can increase vulnerability to pests, diseases, climate change, and market price fluctuations, potentially affecting farmers' incomes and overall agricultural stability. A similar study was conducted in the Central River Region of The Gambia, which indicated that 46.8% of the respondents grew rice, 45.2% grew groundnuts, while 1.4%, 5.3%, and 2.08% grew maize, millet, and cassava, respectively, and the rest grew other crops in the study area **(Badgie, 2018).** About the type of farmland, 6 (1.39%) of the respondents cultivated their crops on state land, 81 (18.75%) cultivated their crops on communal land, 304 (70.37%) cultivated on individual land, and 41 (9.49%) grew on rented land (**Table 2**). Similarly, **Badgie (2018)** revealed that 18.75% of respondents cultivated their crops on communal land, 49.3% on individual land, and 9.49% on rented land.

On the question of change of crops in the last 20 years, 421 (97.45%) of the respondents agreed that they have changed crops in the last 20 years, and 11 (2.55%) said they have not changed crops in the last 20 years. The reasons for the change of crops were as follows: 135 (31.25% of the respondents argued that they changed crops due to short rainfall duration, 43 (9.95%) of them said because of drought, 175 (40.51%) attributed their change of crops to low soil fertility and 79 (18.29%) opined that incidence of pest and disease compelled them to change crops. Although **Seo and Mendelsohn (2008)** in South American farms supported these findings, they pointed out that this may not be the case if the adjustment requires heavy investment. These findings are contrasted by the study conducted by **Crane *et al.* (2011)**, which reported that high climatic variability and unpredictable rain have led not only to changes in crops but also to almost every farmer now practicing mixed agro-pastoralism and may intermittently engage in fishing and hunting when possible. The diversification of crops and other sectors of the economy would minimize the total failure of the enterprises should the farmer encounter extreme climate events.

Table 2: The main crops grown under various land tenure systems.

Characteristics	Frequency	Percentage
Rice	138	31.95
Groundnuts	212	49.07
Maize	21	4.86
Millet	41	9.49
Cassava	9	2.08
Others	11	2.55
Total	**432**	**100.00**
Type of farmland		
State	6	1.39
Communal	81	18.75
Individual	304	70.37
Rented	41	9.49
Total	**432**	**100.00**
Have you changed crops in the past 20 years?		
Yes	421	97.45
No	11	2.55
Total	**432**	**100.00**
If yes, what was the reason for the change of crops?		
Short rainfall duration	130	30.88
Drought	43	10.21
Low soil fertility	171	40.62
Incidence of pests and disease	77	18.29
Total	**421**	**100.00**

4.1.3 Farmers' Perception of the Effects of Climate Variability on Crop Production in The Gambia

The perception of farmers on the effects of climate variability on crop production shows that 426 (98.61%) respondents reported being aware of climate change. Almost all the respondents except for salt intrusion 241 (55.79%) reported that they have observed and experienced a change in temperature 422 (97.69%), rainfall pattern 430 (99.54%), erosion 427 (98.84%), windstorm 426 (98.61%, drought 418 (96.76%) and flood 432 (100.00%) over the past 20 years (**Table 3**). According to meteorological evidence from **Cham *et al.* (2018)**, rainfall has decreased, the period of the rainy season has decreased, minimum temperatures have decreased, maximum temperatures have increased, and the frequency of severe weather events such as drought and dust storms has increased in The Gambia over the past 60 years. This study revealed that crop farmers in The Gambia are very aware of the effects of climate variability on crop production.

Except for salt intrusion, the overwhelming majority of more than 95% of the respondents are aware of climate change and perceive changes in temperature and rainfall patterns, a frequent occurrence in erosion, windstorms, drought, and flood as the main hazards caused by climate variability in the study area. A similar study was conducted in Ebonyi State, Nigeria, which demonstrated that the majority of rice farmers perceived climate change events such as unpredictable rainfall patterns and distribution, prolonged dry season, frequent floods, increased temperature, and severe windstorms, which align with other scientific data, resulting in poor yields and products **(Onyeneke *et al.*, 2021)**. Another similar study in Uganda also revealed that almost 99% of the respondents had perceived a change in the climate in the last decade **(Okonya *et al.*, 2013)**. The farmers' views on the causes of climate variability on crop production in The Gambia in the last 20 years are consistent with the study conducted in South Africa, which shows that the risk of unprecedentedly high January-March (JFM) average temperatures is increasing, posing a growing threat to Agriculture **(Bradshaw *et al.*, 2022)**. The majority (98.61%) of the respondents in The Gambia perceived the effects of climate variability on crop production. This is similar to the study conducted in Pakistan, which reported that 91% of respondents in Peshawar perceived droughts as a major threat caused by weather and climate variability **(Khan *et al.*, 2020)**. During the FGDs and KIIs with stakeholders, it was established that all the informants from the selected communities and institutions, respectively, were fully aware of climate variability and its effects on crop production.

Table 3: Farmers' perception of climate variability in Agriculture in The Gambia in the last 20 years.

Climate variable	Yes	No
Awareness of climate change	426 (98.61%)	6 (1.39%)
Observed changes in temperature pattern	422 (97.69%)	10 (2.31%)
Observed changes in rainfall pattern	430 (99.54%)	2 (0.46%)
Experienced erosion	427 (98.84%)	5 (1.16%)
Experienced windstorm	426 (98.61%)	6 (1.39%)
Experienced salt intrusion on arable land	241 (55.79%)	191 (44.21%)
Experienced drought in this region	418 (96.76%)	14 (3.24%)
Experienced flood	432 (100.00%)	0 (0.00%)

Under the causes of climate variability, 71 (16.67%), 76 (17.59%), and 76 (17.59%) of respondents perceived climate change, temperature change, and change in rainfall to natural causes, whereas 100 (23.47%), 105 (24.31%), and 101 (23.38%) opined that it is due to human-induced activities. In contrast, the other respondents 245 (57.51%), 244 (56.48%), and 251 (58.10%) argued that both natural and human-induced activities are the causes of change in climate, change in temperature and change in rainfall respectively while 10 (2.34%), 7 (1.62%) and 4 (0.93%) said that they do not know the cause of climate change, change in temperature and change in rainfall respectively (**Table 4**).

Table 4: Perceived causes of climate variability on crop production in The Gambia.

Climate variable	**Natural**	**Human-induced**	**Natural & Human-induced**	**I don't know**
Change in temperature	76 (17.59%)	105 (24.31%)	244 (56.48%)	7 (1.62%)
Change in rainfall	76 (17.59%)	101 (23.38%)	251 (58.10%)	4 (0.93%)

The findings, as shown in **Table 4**, revealed that more than 50% of the farmers perceived the causes of climate variability, such as temperature and rainfall, to be the interplay of both natural and human-induced activities. A similar study was conducted in Nigeria, which opined that changes in climate and environmental conditions occur as a result of both natural and human factors **(Nwankwoala, 2015)**. This agrees with the study conducted by **Hartter *et al.* (2018)** in eastern Oregon (USA), where most of the respondents did not accept human causation as the only agent of climate change. However, a large majority (85%-86 %) of them agree that the climate is changing, either due to natural or human-induced causes **(Hartter *et al.*, 2018)**. In contrast to the study conducted by **Perkins-Kirkpatrick *et al.* (2022),** which posits that extreme events that influence climate change are perceived as anthropogenic activities.

The perception of crop farmers towards the effects of elevated temperature on crop production shows that 400 respondents (92.59%) reported a decrease in crop production, 20 (4.63%) stated an increase, 8 (1.85%) believed that the elevated temperature has no effect, and 4 (0.93%) were unsure of any effects (**Figure 30**). This aligns with the research conducted in Slovakia by **Varga (2021)**, which argued that the impacts of climate change would be felt in agriculture, leading to reduced production in warmer areas due to temperature stress, risk of erosion as a result of more extreme weather conditions, the occurrence of new pests, etc.

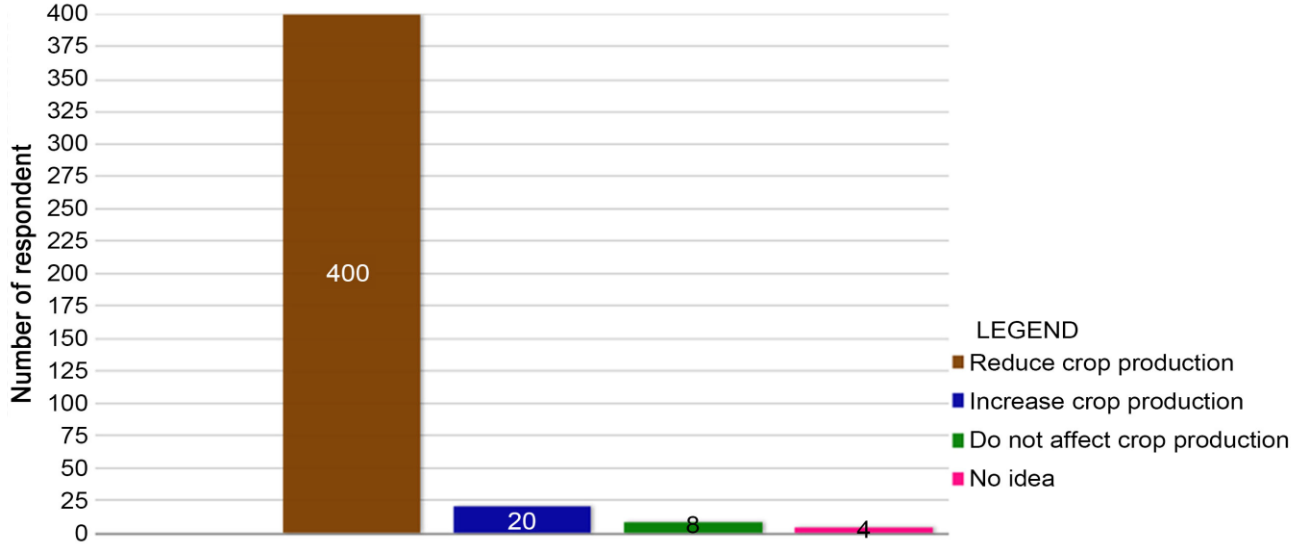


Figure 30: Effects of elevated temperature on crop production.

The crop farmers were asked about the effects of changes in rainfall patterns on crop growth, development, and production. The results show that 403 (93.29%) reported that they observed reduced crop growth, development, and production, 22 (5.09%) stated that there was an increase in crop growth, development, and production, and 3 (0.69%) said that it did not affect crop growth, development, and production. In comparison, 4 (0.93%) reported that they had no idea about the resultant effects of changes in rainfall pattern on crop growth, development, and production (**Figure 31**).

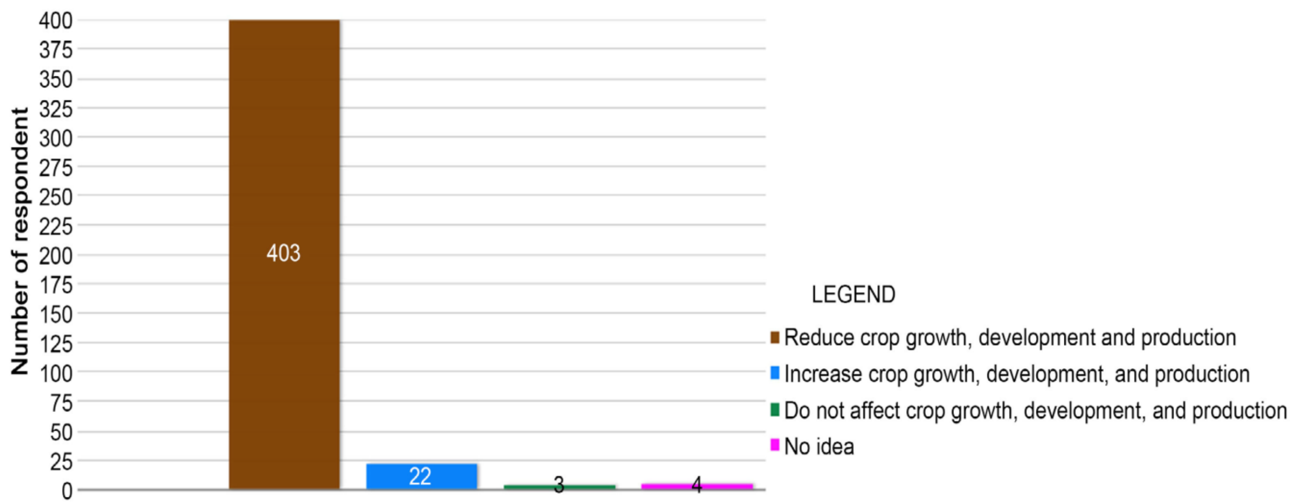


Figure 31: Effects of changes in rainfall pattern on crop growth, development, and production.

Figure 32 shows the various effects of climate variability and pollution on the environment such as high temperatures 198 (45.83%), less or erratic rainfall 48 (11.11%), floods increase on farms 28 (6.48%), late onset of rains 34 (7.87%), less crop yield 79 (18.29%), increased winds before rains 17 (3.94%) and land degradation 28 (6.48%). A similar study in Nigeria pointed out that the majority (more than 70%) of farmers perceived an increase in temperature and a decrease in precipitation patterns **(Sofoluwe *et al.*, 2011)**.

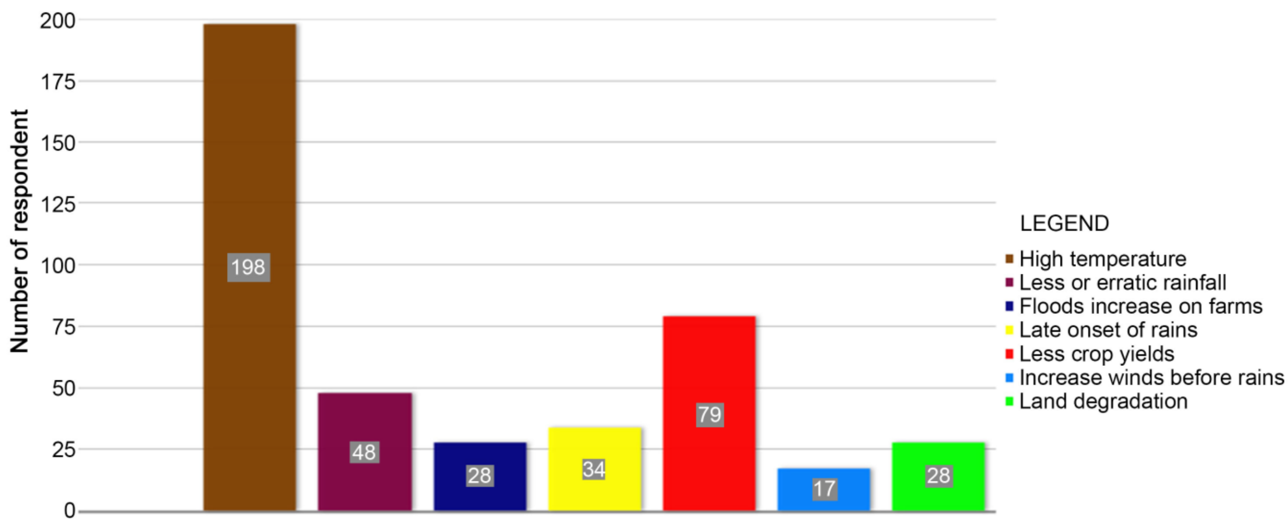


Figure 32: Observed changes in climate variability and pollution in the environment.

A study conducted by **Agwu *et al.* (2018)** showed that farmers in Nigeria are well aware of climate change and its impact on crop productivity, stressing that the variability of the Harmattan season influences the fruit production of the *Garcinia kola*. **Mertz *et al.* (2009)**, who analyzed the perceptions of climate change and the strategies for coping and adaptation in the savannah zone of central Senegal, agreed that households are aware of climate variability and identified wind and occasional excess rainfall as the most destructive climate events. The findings of **Odewumi *et al.* (2013)** in Ibadan, South-western Nigeria, on farmers' perception of the effect of climate change and variations on urban agriculture indicated that 89.6% of the respondents agreed that the climate is changing. Similarly, research has revealed that about 89% of the farmers perceived a significant increase in temperature, 72% perceived high evapotranspiration rates, 68% showed that there had been violent rain and hailstorms, and 65% experienced delayed rainfall and early cessation **(Chalchisa & Sani, 2016)**.

4.1.4 Factors Associated with Farmers' Perception of the Effects of Climate Variability on Crop Production in The Gambia Using Pearson Chi-Square Test

The results of the Pearson chi-square test (**Table 5**) indicate significant associations between the demographic profiles of farmers and their perceptions regarding the effects of climate variability on agricultural productivity in The Gambia. Notably, marital status emerged as the only demographic variable significantly associated with all assessed dimensions of climate variability, suggesting that household structure and family responsibilities may influence awareness and concern about agricultural risks. This may reflect the role of married individuals in ensuring household food security and long-term livelihood planning.

In addition, education level was significantly associated with farmers' perception of specific climate-related challenges, including erosion, windstorms, and salt intrusion. This finding supports the notion that educational attainment enhances cognitive ability and access to climate information, enabling better recognition of environmental threats and adaptation needs.

The study further shows that age and ethnic background were significantly linked to perception of salt intrusion, indicating that both life experience and sociocultural identity shape how farmers understand and interpret environmental changes. This finding aligns with the work of **Ishaya and Abaje (2008),** indicating that older farmers rely on accumulated knowledge, while ethnic affiliations may influence access to land, local knowledge systems, or traditional adaptation practices.

In contrast, **Deressa *et al.* (2011)** reported in the Nile Basin of Ethiopia that only age was a significant predictor of farmers' perception of climate change, highlighting regional variations in the role of sociodemographic factors. This variation highlights the critical need for context-specific evaluations to accurately capture community-level perceptions and adaptive responses to climate fluctuations.

The results indicate that while Gambian crop producers demonstrate a general understanding of the adverse effects of climate variability on agricultural productivity, this level of awareness is significantly influenced by socio-demographic variables, particularly age, educational attainment, marital status, and ethnic background. Such insights are crucial for designing targeted extension programs and climate adaptation policies.

Table 5: Pearson chi-square results on farmers' perception of the effects of climate variability on crop production in The Gambia.

Items	Factors	X^2 values	P-value
Have you ever experienced erosion in the last 20 years in The Gambia?	Age	3.1008	0.541^{ns}
	Ethnic group	0.6677	0.716^{ns}
	Marital status	15.7696	0.001*
	Highest level of education	13.1948	0.010*
Have you experienced windstorms in the past 20 years?	Age	3.2733	0.513^{ns}
	Ethnic group	0.6955	0.706^{ns}
	Marital status	9.2015	0.027*
	Highest level of education	9.7389	0.044*
Have you experienced salt intrusion for the past 20 years on your arable land?	Age	9.8123	0.044*
	Ethnic group	26.7692	<0.001*
	Marital status	9.9964	0.019*
	Highest level of education	17.6778	0.001*
Have you ever experienced drought in the last 20 years in this region?	Age	2.4970	0.645^{ns}
	Ethnic group	5.1251	0.077^{ns}
	Marital status	8.5958	0.035*
	Highest level of education	8.3664	0.079^{ns}

*****= significant difference at $p < 0.05$; $^{\mathbf{ns}}$ = not significant.

4.1.5 Farmers' Perceptions of the Contamination of Agricultural Land

Table 6 presents the results of a binomial logistic regression analysis conducted to evaluate the key socio-demographic factors influencing crop farmers' perception of heavy metal (HM) contamination of agricultural land in The Gambia. The findings reveal that farmers' age and ethnic group were statistically significant predictors of both their general perception of land contamination and their awareness of the specific threats posed by HM pollutants.

Notably, age was negatively associated with perception and awareness. For instance, the confidence limits (CL: −0.135 to −0.020; P = 0.008) indicate that older farmers were significantly less likely to perceive contamination as a problem. Similarly, the stronger association observed for age and awareness of HM threats (CL: −0.167 to −0.054; P ≤ 0.001) reinforces that younger farmers tend to be more environmentally conscious or informed regarding HM risks to soil and water.

In contrast, the ethnic group variable showed a positive association, with significant confidence limits (e.g., CL: 0.068 to 0.188 and CL: 0.053 to 0.171; both P ≤ 0.001), suggesting that certain ethnic groups were more likely to recognize HM contamination and its potential impacts. These results may reflect underlying differences in cultural practices, education levels, access to extension services, or historical land-use patterns among ethnic groups.

Taken together, these results highlight that awareness and perception of environmental contamination are not uniformly distributed among farmers and are influenced by both age and ethnic background. This implies a need for targeted awareness campaigns and educational interventions, particularly aimed at older farmers and specific ethnic communities, to improve understanding of HM pollution and promote safer agricultural practices.

Table 6: Binomial logistic regression of Farmers' perceptions on the contamination of agricultural land.

Variables		**95% C**	**Limits**	**P-Value**
Outcome	**Independence**			
Have you experienced soil and water pollution from farming?	Gender	-0.037	0.099	0.375
	Age	-0.021	0.061	0.344
	Ethnic group	-0.003	0.083	0.070
	Religion	-0.044	0.845	0.077
	Marital status	-0.051	0.068	0.772
	Highest level of education	-0.011	0.039	0.283
Are you aware of heavy metal (HM) pollution?	Gender	-0.149	0.039	0.251
	Age	-0.135	-0.020	0.008*
	Ethnic group	0.068	0.188	<0.001*
	Religion	-0.516	0.715	0.750
	Marital status	-0.045	0.120	0.368
	Highest level of education	-0.018	0.051	0.348
Are you aware of threats posed by HM contamination in soil and water?	Gender	-0.152	0.034	0.213
	Age	-0.167	-0.054	<0.001*
	Ethnic group	0.053	0.171	<0.001*
	Religion	-0.549	0.666	0.849
	Marital status	-0.049	0.113	0.437
	Highest level of education	-0.013	0.055	0.231

The age of the respondents represents experience in farming, and research has shown that experienced farmers are more likely to perceive climate change (**Deressa *et al.*, 2011)**.

Table 7 shows the responses of crop farmers about their perceptions of the contamination of agricultural land. The majority of 358 (82.87%) of the informants had experienced soil and water pollution on their farms. These findings revealed that although the farmers in The Gambia had never experienced HM pollution on their farms, 109 (25.23%) of them are aware of the threats posed by these recalcitrant substances to the environment (**Table 7**). This may be because there is little or no analysis of the content of heavy metals in the fertilizers, chemicals, and pesticides used on the arable soil. With this little knowledge of HM contamination in The Gambia, bioremediation technology, which is not only meant for HM removal but also those contaminants of organic substance contaminants, is an exotic phenomenon. In contrast with the study conducted in Lankao County, China, by **Ren *et al.* (2018)**, which showed that the content of heavy metals in various arable soils is closely related to the input of fertilizer, pesticide, farmyard manure, etc. Similarly, the study conducted by **Lu (2019)** opined that farmers in China believe that their cultivated land is moderately polluted by heavy metals, and they have a strong awareness of HM pollution in locally cultivated land. Research conducted by **Ren *et al.* (2018)** revealed that remediation of HM contamination from arable soil indicates that the adoption of appropriate treatment measures can reduce the HM content of arable soil to some extent, such as the adjustment of planting patterns, deep plowing for soil amelioration, formula fertilization, and the adoption of phytoremediation (an aspect of bioremediation), etc.

Table 7: Farmers' perceptions of the contamination of agricultural land.

Variables	Yes	No
Have you experienced soil and water pollution on your farm?	358 (82.87%)	74 (17.13%)
Are you aware of heavy metal (HM) pollution?	109 (25.23%)	323 (74.77%)
If yes, have you experienced HM pollution on your farmland in the last 20 years?	…………….	109 (100.00%)
Are you aware of HM contamination of soil and water?	109 (25.23%)	323 (74.77%)

Figure 33 shows the main soil and water pollution in The Gambia. The findings revealed that the main soil and water pollution emanates from both organic and inorganic contaminants: 163 (45.53%) attributed the pollution to chemical fertilizers, 39 (10.89%) said that pesticides cause the pollution, 81 (22.63%) argued that industrial and agricultural wastes cause the pollution, 74 (20.67%) of them opined that their farmlands are polluted by sewage and 1 (0.28%) of the farmers said that other agents cause the pollution (**Figure 33**). Similarly, the research of **Srivastav (2020)** showed that several researchers have reported the risks to environmental systems due to overuse and prolonged application of chemical fertilizers and pesticides, which would invariably promote soil health deterioration along with environmental pollution.

It was also revealed in the FGD sessions that most of the upstream farmers were less affected by pollution than those farmers in the coastal and riverine agricultural areas. Contrastingly, the research conducted in the United States by **Chakraborti (2021)** concluded that point sources have a significant negative impact on ambient water quality than the non-point sources of contamination at upstream locations. However, during the FGD sessions, the coastal and riverine farmers complained of pollution emanating from the indiscriminate disposal of plastic bags, agrochemicals, pesticides, salt intrusion, oil spillage, and runoff from dump sites, to name a few. HM pollution was not mentioned, which could be attributed to the fact that there is little or no evidence of any study on HM pollution in The Gambia.

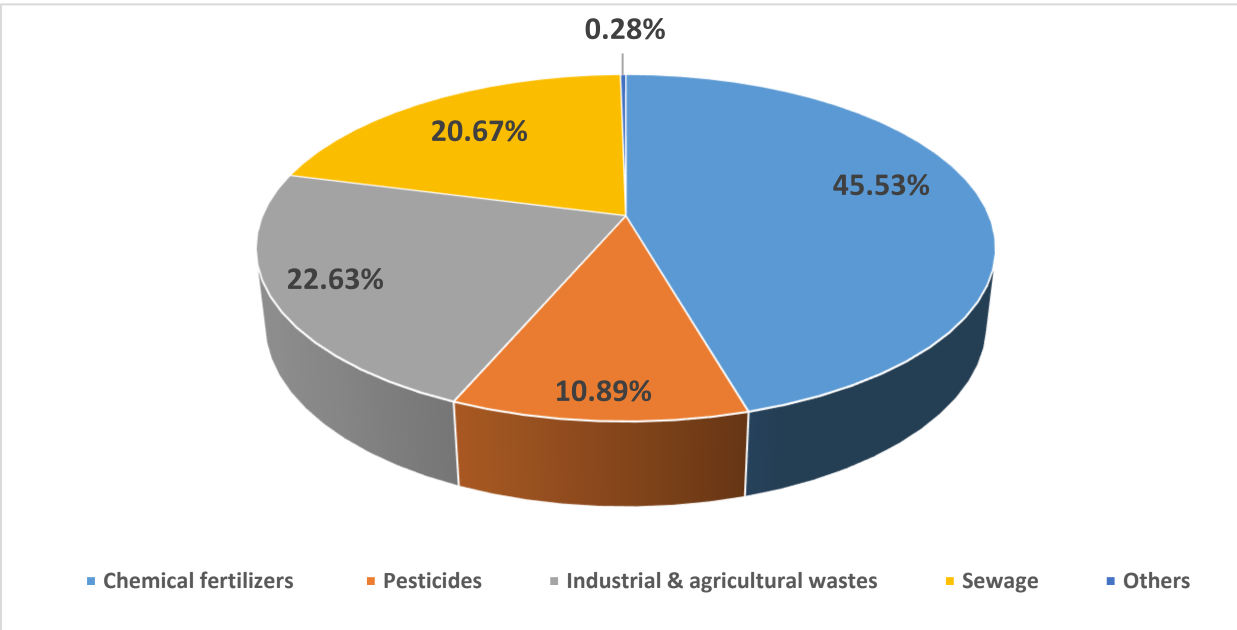


Figure 33: The main soil and water pollutants on farmland in The Gambia.

4.1.6 Constraints Militating against Farmers' Response in The Gambia in Their Crop Production toward Climate Change

The field surveys showed (**Figure 34**) the challenges faced in responding to climate variability in the study area. Inadequate access to credit facilities and efficient inputs was ranked first, with a high number of respondents, 288 (66.67%), each, militating against farmers in their response to climate change. This was followed by labor constraints, inadequate access to the market, inadequate access to water and irrigation facilities, inadequate access to information, use of poor skills, salt intrusion, and inadequate access to ideal land as they ranked third 240 (55.56%), fourth 239 (55.32%), fifth 222 (51.39%), sixth 165 (38.19%), seventh 134 (31.02%) and eighth 101 (23.38%) respectively. These findings align with those of **Idrisa (2012)**, who reported that inadequate financial resources (credit), inadequate access to extension services, poor access to technologies required for adaptation, and inadequate weather information were the challenges confronting farmers' adaptation to climate change in Borno State, Nigeria. However, research conducted in India by **Satishkumar *et al.* (2013)** had a divergent perspective, which revealed that personal constraints like the small size and fragmented landholdings, low literacy level, and inadequate knowledge of how to cope or build resilience are the main hurdles confronting the agrarian industry. The study further identified that farmers encountered several institutional and technological barriers, including inadequate extension services related to climate risk management, limited availability of drought-resistant crop varieties, restricted access to meteorological forecasting tools, low confidence in forecast accuracy, and a heavy reliance on unpredictable monsoon patterns.

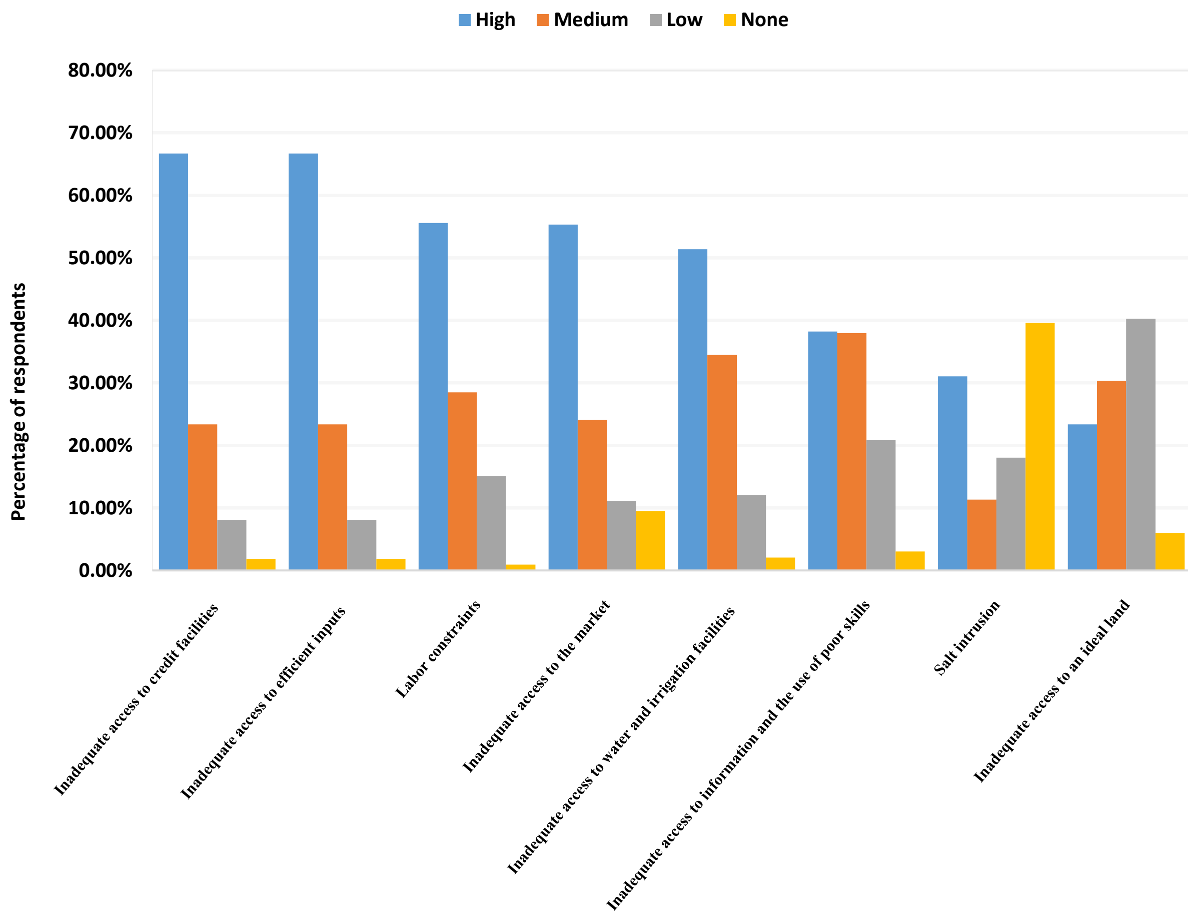


Figure 34: Constraints militating against farmers' response in The Gambia in their crop production toward climate change.

4.1.7 Adaptation Strategies Employed by Farmers in The Gambia toward Climate Change

Over time, the agricultural sector has demonstrated its capacity to adapt to climate variability through the implementation of context-specific strategies and technological innovations. Findings from the survey reveal the predominant adaptive measures employed by farmers in response to evolving climatic conditions (**Figure 35**).

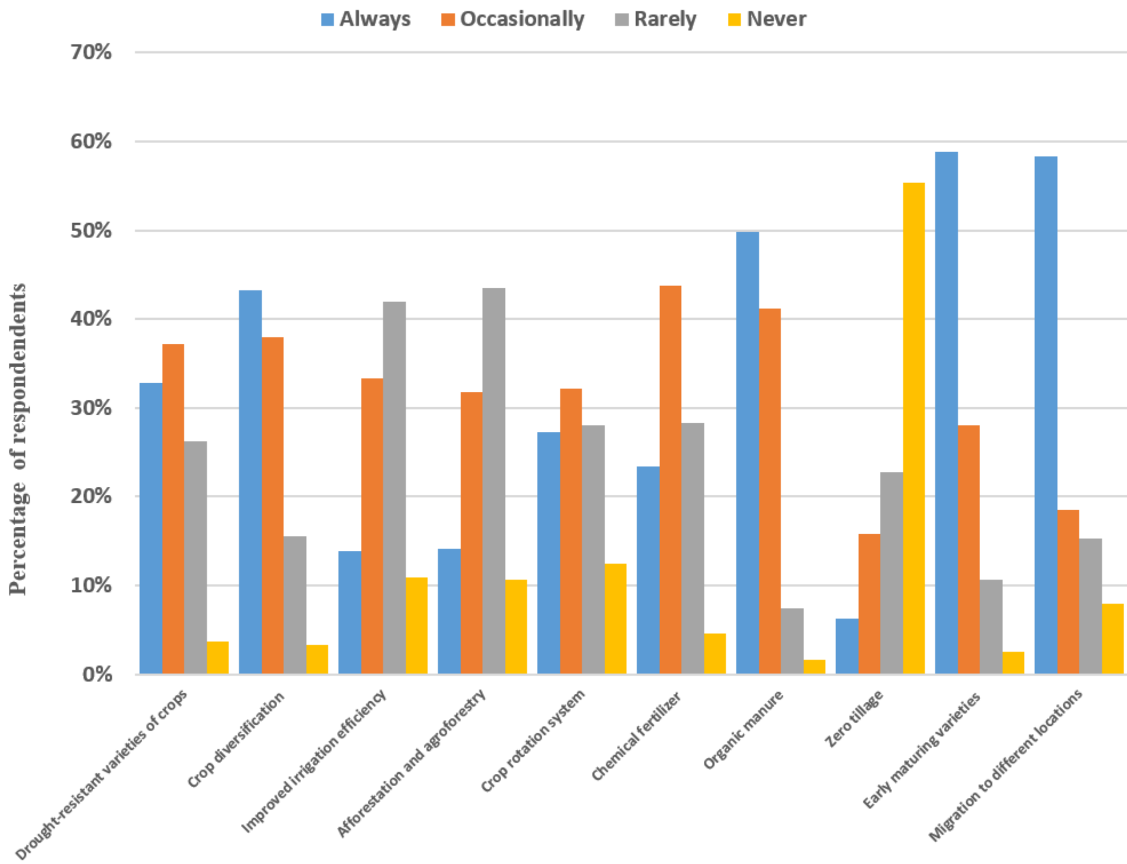


Figure 35: Agricultural crop production adaptation strategies employed by farmers in The Gambia toward climate change.

The possible adaptation strategies employed by farmers in The Gambia towards climate change indicated that about 141 (32.79%) of the farmers specified that planting drought-resistant crop varieties would help solve challenges and increase their crop yields. This is in agreement with research conducted in Nigeria, Senegal, Burkina Faso, and Ghana, which pointed out that smallholder farmers have used drought-resistant crop varieties as an adaptation method to climate change (Akinnagbe & Irohibe, 2015). About 187 respondents, representing 43.29% of the participants, reported that the implementation of crop diversification strategies significantly mitigated the recurrent yield losses previously experienced on their farms. They emphasized that governmental support in the form of access to diverse crop varieties would enhance resilience to adverse climatic conditions, as the failure of some crops could be offset by the successful growth of others. Similar research conducted in Tanzania revealed that farmers diversified crop varieties to spread risks on the farm **(Orindi & Eriksen, 2005)**. Crop diversification can serve as insurance against rainfall variability **(Akinnagbe & Irohibe, 2015).**

A subset of participants (n = 60; 13.89%) reported that enhancing irrigation efficiency would facilitate year-round cultivation of crops. This continuous agricultural production was perceived as a key strategy for ensuring consistent food availability and mitigating seasonal food insecurity. A study conducted by **Akinnagbe and Irohibe (2015)** emphasized that the impacts of climate change have led to increased crop water demand due to high evapotranspiration **(FAO, 2006)**. Additionally, 61 (14.12%) of the respondents argued that afforestation and agroforestry would help prevent erosion and maintain soil fertility, ultimately boosting crop yields. Research has revealed that a practice similar to this has been described in the southwestern part of Nigeria to raise shade-tolerant crops, such as *Dioscorea* spp. and cocoyam, in essentially permanent forest settings. In the drier parts of the Sahel, African baobab (*Adansonia digitata*) and acacia (*Acacia* spp.) trees are usually planted by local farmers since they are also economically valuable trees, especially during the hot and dry parts of the year **(Akinnagbe & Irohibe, 2015)**. Another 118 (27.31%) of the respondents opined that the practice of a crop rotation system would help replenish the depleted soil nutrients, thereby improving crop yield. Further, 101 (23.38%) of the respondents pointed out that the use of chemical fertilizers could also help enhance their adaptation to climate change and reduce the losses incurred on farms. They cried out that the price of fertilizers set by the government was too high for average farmers; hence, most of them could not afford them. Almost half, 215 (49.77%) of the respondents, settled for the use of organic manure as a means of adaptation strategy to mitigate the effects of climate change. Although zero tillage is not a familiar method used by farmers in The Gambia to reduce the adverse effects of climate change on their crop production, only 27 (6.25%) of them embraced the strategy to improve crop yields. The majority, 254 (58.80%) and 252 (58.33%) of the respondents employed early maturing varieties and migration to different locations, respectively, as crop adaptation strategies towards climate change. These findings are in line with a survey conducted in the Zou Department of South Benin, which revealed that farmers adopted many strategies such as mulching, organic fertilizer, the use of improved varieties, chemical fertilizers, and pesticides in response to climate change: agroforestry and perennial plantation diversification of income-generating activities (Fadina & Barjolle, 2018). Although the findings of this research show some similarities with research conducted by **Akinnagbe and Irohibe (2015)**, the common agricultural adaptation strategies adopted by farmers in Nigeria were the use of drought-resistant varieties of crops, crop diversification, changes in cropping patterns and calendar of planting, conserving soil moisture through appropriate tillage methods, improving irrigation efficiency, and afforestation and agro-forestry. In the West African Sahel, recent studies have shown that the region has suffered a prolonged drought for much of the past three decades, and one way that farmers have adapted is by sending young men and women in search of wage labor after each harvest. However, how far they travel depends, in part, on the success of the harvest (Akinnagbe & Irohibe, 2015). More generally, environmental migrants are, most of the time, people affected by long-term and permanent climate variations in temperature and precipitation; hence, they consider the migration process as a long-run adaptation strategy **(Falco *et al.*, 2018)**. This study showed that adaptation strategies such as improved irrigation efficiency, afforestation and agroforestry, and zero tillage were not always used (**Figure 35**).

4.2 Local Knowledge of Climate Change and Variability from Farmers' Perspective in The Gambia

In an attempt to address the challenges caused by the effects of climate change, farmers in the FGDs were asked to itemize and briefly explain some local indicators or indigenous knowledge they employed to predict weather conditions and climate variability in The Gambia (**Table 8**). The results obtained from the FGDs showed that 38 (79.17%) of the farmers used the life behavior of insects to predict the climate and the weather conditions of the approaching season. The red velvet mites served as local indicators for climate variability in most communities consulted during the study. About 18 (37.50%) of the farmers in the communities argued that the emergence of these insects at the beginning of the rainy season indicates that there would be enough rain and a bumper harvest in that particular season. They stressed that these kinds of insects are rarely seen nowadays, indicating that the rains have been declining over the past years. The locusts (red and brown) and armyworms registered 8 (16.67%) and 12 (25%), respectively. Opined that whenever these insects appear at the beginning of a stormy season, there shall be little rain and scarcity of food. This serves as a warning and encourages the farmers to prepare for any eventuality to cope with the impacts of droughts and food scarcity. These findings align with the study conducted by **Ahmed *et al.* (2016)**, which discovered that the indigenous people of Tharparkar district in Sindh province in Pakistan use insects as a main source to foretell the nature of the rainfall. They argued that whenever some insects appear and move to the upper areas of land and plants, there would be a greater probability of rainfall. These predictions are very important for the people because they depend on field cultivation, which is only possible with rainfall. Another local indicator used by the people in the communities to determine and predict the nature of the weather conditions and the yield of the crops is the local trees/shrubs.

Table 8: Indicators used by local communities to predict the climate variability in The Gambia.

Perceive causes		No.FGDs (N=48)	Percentage (%)
Insects	Red velvet	18	37.50
	Red & Brown Locusts	8	16.67
	Armyworms	12	25
Trees/Shrubs	*Acacia nigrescens*	20	41.67
	Parkia biglobosa	7	14.58
	Adansonia digitata	15	31.25
Abiotic factors	Wind (strong, cold, or dusty)	8	16.67
	Unusual flooding	3	6.25
	Rainbow	12	25
Amphibians	Toad/Frogs	29	60.42
Birds	Red bishop	27	56.25
	Senegal Coucal bird	11	22.92
Vegetables/herbs	*Rumex acetosa*, i.e., sorrel	13	27.08
No idea	-	3	6.25

During the field survey, 87.5% (n = 42) of participating farmers in the focus group discussions reported utilizing specific characteristics of indigenous trees and shrubs as traditional indicators of local weather patterns. Notably, *Acacia nigrescens* was mentioned by 41.67% (n = 20) of respondents, who observed that leaf shedding from this species typically signals the impending onset of the rainy season. Conversely, the emergence of new foliage was associated with the commencement of the rainy season, reflecting improved moisture availability and favorable growing conditions. Such ethnobotanical knowledge serves as an important local forecasting tool, allowing farmers to align their agricultural activities with anticipated climatic changes and optimize planting schedules accordingly. The finding revealed that 7 (14.58%) used *Parkia* *biglobosa* to predict weather and climatic conditions by observing the fruiting nature of the tree. Whenever the tree produces a large quantity of fruit before the onset of the rainy season, the rainy season is predicted to have a good prospect. On the other hand, if fruit production were low, the indications are that the subsequent rainy season would experience inadequate rains and poor crop performance and yields. These observations and predictions enable the local farmers to diversify their crop production so that, whatever happens, they will not operate in total failure. The research showed that 15 (31.25%) of the farmers stressed that the features (sprouting of the leaves, the pattern of flowering and fruiting) of *Adansonia digitata* (common name: baobab tree) are used by the communities as indicators to predict weather and plan before the onset of the rainy season. They argued that when the sprouting leaves on the canopy of the baobab tree cover the vulture perched on the branches, it is an indication that the rains will commence within a month. The respondents also pointed out that when the pattern of flowering and fruiting starts from the branches at the base of the tree upwards, it indicates that the rainy season will be short in that particular year. When these signs are seen, farmers automatically plant early-maturing varieties to minimize loss and achieve appreciable yields. On the other hand, if the flowering and fruiting start from the top branches, coming down to the lower branches at the base of the tree, it is an indication that the rainy season will last longer. Therefore, late varieties, such as sorghum, late millet, etc., can be grown in that particular season. The study also revealed that 29 (60.42%) of the farmers used amphibians, such as toads/frogs, to determine and predict the weather and climatic conditions. Community members observed that a high abundance of toads and frogs at the onset of the rainy season is traditionally perceived as a biological indicator of anticipated heavy rainfall and favorable agricultural yields during that period. They were quick to note that the high number of these amphibians is declining compared to the previous decades, which indicates that there is a decline in rainfall nowadays. This enables the farmers to grow more drought-tolerant crops. About 38 (79.17%) of the farmers said that birds served as local climate variability indicators for the onset of rains and the success of that particular year. Farmers who said that birds such as red bishops (*Euplectes orix*) and Senegal Coucal (*Centropus senegalensis*) registered 27 (56.25%) and 11 (22.92%), respectively. According to them, the appearance of the red bishops and Senegal Coucal birds serves as an indication of the approaching start of the rainy season. Their appearance in large numbers depicts that the rainy season is about to commence and has the prospect of a bumper harvest. This aligns with the research conducted by **Ahmed *et al.* (2016)**, which showed that various birds in the community were used to predict the weather.

For example, the aged persons of Tharparkar and other countries of Asia, such as India, and parts of Africa, such as Zimbabwe and Tanzania, use the marbled teal bird for the prediction of the monsoon season. The Menghwar community relies heavily on the behavior and movement of these birds for the prediction of the weather because agriculture is one of their main sources of survival. According to them, whenever these birds sing during the monsoon season, the people of Thar would understand that the rain has come. Another important local climate variability indicator to predict the situation of the incoming farming season for crop production is *Rumex acetosa* (sorrel). The communities, according to the findings, showed that 13 (27.08%) of the farmers observed that whenever there is plenty of sorrels on the farm and in the wild in a particular season, it shows that there will be a high yield of groundnuts at the end of the farming season. They also pointed out that the amount of sorrel in the wild is declining considerably compared to the previous decades because the rains have also decreased. This has necessitated the abandonment of groundnut cultivation by many farmers in The Gambia for other crops. This situation creates a scarcity of groundnuts in the country, which alternately results in price hikes in the market. Farmers who opined that abiotic factors such as wind, floods, and rainbows were also employed by the farming communities to predict the prospect and nature of weather and climatic conditions were 23 (47.92%). The results indicated that 8 (16.67%) of crop farmers in the study area opined that whenever the rainy season begins with the blowing of strong or cold and dusty wind, it is an indication of short and inadequate rains in that particular year. The Focus Group Discussions revealed that 3 participants (6.25%) reported that atypical early-season flooding of farmlands, particularly rice fields in riverine zones, is interpreted as a traditional signal of a potentially favorable agricultural season. These communities have established baseline flood thresholds, and when exceeded before the rainy season, they anticipate sufficient and prolonged rainfall conducive to crop cultivation. Such local meteorological knowledge is well understood by residents cultivating rice in areas subject to strong tidal influence. This observation aligns with the findings of **Slegers (2008)**, who reported that early-season flooding is used as a predictor of favorable rainfall conditions in both the Kondoa District (Tanzania) and Asfachew (Ethiopia). Additionally, 12 farmers (25%), predominantly rice producers, reported using the appearance of a rainbow at the onset of rains as a traditional weather forecasting tool. They collectively affirmed that the presence of a rainbow serves as an indicator for estimating the likely duration and productivity of the growing season. According to the FGD sessions, they could tell if the rainbow comes from the south towards the north, the incoming season is predicted to be of adequate rains and good crop performance. On the other hand, if the rainbow emerges from the north towards the south, the season is predicted not to be good. In a similar research conducted in Ghana by **Gyampoh and Asante (2011)**, it has been revealed that whenever a rainbow is seen formed in the east in the early morning shows that there will be less rainfall for the season. The same study opined that when the rainbow is formed in the west in the early morning, the season would experience more rainfall.

Participants in both the Focus Group Discussions (FGDs) and Key Informant Interviews (KIIs) emphasized that implementing strategies such as adopting clean energy sources (e.g., solar, wind turbines, hydrogen-fueled vehicles), promoting afforestation, practicing selective resource exploitation, minimizing excessive fertilizer use, enforcing anti-littering policies, employing crop rotation and bush fallowing, and integrating climate-smart agricultural techniques (including minimum tillage, improved crop varieties, use of farmyard manure, water conservation technologies, and biopesticides) can foster positive environmental attitudes. Additionally, raising awareness through targeted campaigns and effective formulation and enforcement of environmental policies were identified as crucial measures to mitigate climate change impacts. Participants in the Focus Group Discussions (FGDs) highlighted that commonly practiced agricultural adaptation methods include Integrated Production and Pest Management (IPPM), Climate Smart Agriculture (CSA), the Farmer Field School Approach (FFSA), afforestation, agroforestry integration, cultivation of early maturing and disease-resistant crop varieties, as well as the construction of dikes and irrigation infrastructure. These strategies are widely recognized and employed by most farmers to mitigate the impacts of climate variability. Conversely, nearly all respondents from both FGDs and key informant interviews (KIIs) reported limited or no awareness of bioremediation techniques, such as the use of algae, fungi, bacteria, and plants, for environmental pollution remediation. Only a minority indicated having encountered information about bioremediation through literature.

4.2 Bacterial Identification

4.2.1 Isolation and Characterization of Mesophilic Bacteria

Screening and isolation of mesophilic bacteria were done targeting *Aeromonas* spp., *Bacillus* spp., and *Pseudomonas* spp. Using *Aeromonas* isolation medium base, blood agar, and

MacConkey media, respectively. Bacterial species were observed growing in all the plates with different media, except in some plates where *Aeromonas* species were expected to grow, as shown in **Table 10**. Even though bacteria were growing in Aeromonas isolation medium, further analyses established that they were not *Aeromonas* species.

4.2.2 Gram Staining

Following the successful isolation and purification of bacterial colonies, Gram staining was employed as a preliminary step for bacterial classification. Microscopic observation of the stained isolates revealed that 14 out of the initial 15 isolates examined were Gram-positive, with only one showing a Gram-negative reaction. The Gram-positive isolates predominantly grew on blood agar (BA), exhibiting diverse colony morphologies, including large or tiny, flat, opaque, grey, irregular, dry, and shiny appearances. In contrast, isolates that grew on MacConkey agar (MAC) and *Aeromonas* Isolation Medium (AIM) presented a wider range of colony traits, pale, flat, greenish, or whitish, and some appeared tiny, suggestive of Gram-negative characteristics.

Overall, 22 isolates were confirmed as Gram-positive, with their colony morphology and cultural characteristics closely resembling members of the genus *Bacillus*. Seven isolates were Gram-negative, displaying phenotypic features commonly associated with *Aeromonas* and *Pseudomonas* species (**Table 9**). These findings emphasize the importance of Gram staining in differentiating bacterial groups at an early stage of identification.

The present study differs from earlier findings by **Popoola and Ogwerel (2024)**, where sample collection was limited to a single environmental type (e.g., only water) across various locations. In contrast, the current work employed a broader environmental sampling strategy collecting from water, sediment, and soil across three distinct communities, which may explain the observed diversity in Gram reaction and bacterial composition.

These results reinforce the role of Gram staining as a fundamental step in bacterial taxonomy. It provides essential differentiation between Gram-positive and Gram-negative groups and guides the selection of subsequent biochemical or molecular identification procedures. Its utility lies not only in differentiating cell wall types but also in supporting the interpretation of environmental influences on microbial diversity and distribution.

Table 9: Isolation and Characterization of Mesophilic Bacteria.

Sample	Media	Colony Morphology	Gram staining		Oxidase
			Gram +ve	Gram -ve	
A1	BA	Flat grey colonies	Gram +ve filaments		
	MAC	NLF-pale colour		Gram –ve rods	+ve
	RYAN	Greenish brown	Gram +ve rods	Gram –ve rods	+ve
A2	BA	Colony 1- flat Colony 2- tiny white	Gram +ve rods Gram +ve rods		
	MAC	Flat colony	Gram +ve rods		
	RYAN	Green colony (1 CFU)		Gram –ve rods	+ve
A3	BA	Colony 2- irregular borders Colony 3- slightly flat Colony 4- moucoid	 Gram +ve rods Gram +ve rods	Gram –ve rods	+ve
	MAC	Grey colony? Bacillus	Gram +ve rods		
	RYAN	whitish		Gram –ve rods	+ve
B1	BA	Colony 1- whitish Colony 2- yel	Gram +ve rods Gram +ve rods		
	MAC	NLF - pale colour & raise	Gram +ve rods		-ve
	Ryan	No growth			
B2	BA	Colony 2- slightly convex	Gram +ve rods		
	MAC	Colony 2- tiny grey Colony 3- pale	Gram +ve rods Gram +ve rods		
	Ryan	Green colony (1 CFU)		Gram –ve rods	+ve
B3	BA	Colony 2- comma-shaped & irregular borders	Gram +ve rods		
	MAC	NLF - pale colour & round		Gram –ve rods	+ve
	Ryan	No growth			
C1	BA	Colony 2- flat grey colonies	Gram +ve rods		
	MAC	NLF - pale colour	Gram +ve rods		-ve
	Ryan	No growth			
C2	BA	Colony 1- flat Colony 2- tiny white	Gram +ve rods Gram +ve rods		
	MAC	NLF- pale colour	Gram +ve rods		-ve
	Ryan	No growth			
C3	BA	Colony 1- flat, dry, and shiny Colony 2- greyish	Gram +ve rods Gram +ve rods		
	MAC	Colony 1- tiny pale	Gram +ve rods		
	Ryan	No growth			

4.3 Biochemical Test

4.3.1 Oxidase Test

Ten (10) of the bacterial isolates were tested for oxidase reagents. All those bacterial isolates that were Gram-negative and three that were Gram-positive were involved in the oxidase test, and the results indicated that the Gram-negative and Gram-positive isolates were oxidase positive and negative, respectively.

4.3.2 API 20E Test

Bacteria from CRR South were analysed for bioremediation potential using various media. Ryan Medium revealed a high occurrence of *Pseudomonas fluorescens* in water (A1) and sediment (A2) samples, respectively, while *Bordetella pertussis* (43.1%) and *Pseudomonas aeruginosa* (67.2%) dominated soil and sediment samples from sites A3 and B2, respectively. Blood agar supported the growth of *Bacillus* spp. in all samples and *Grimontia hollisae* from the soil sample in site A3. On MacConkey agar, most isolates showed Gram-positive reactions, except A1 (*P. fluorescens*, 94%) and B3, which is pending API confirmation.

Overall, API and Gram staining identified 22 *Bacillus* spp., 3 *P. fluorescens*, 1 each of *P. aeruginosa*, *G. hollisae*, and *B. pertussis*. These findings confirm the effectiveness of API 20E for rapid bacterial identification **(Artati *et al.*, 2024),** supporting its relevance in selecting bacteria for zinc bioremediation.

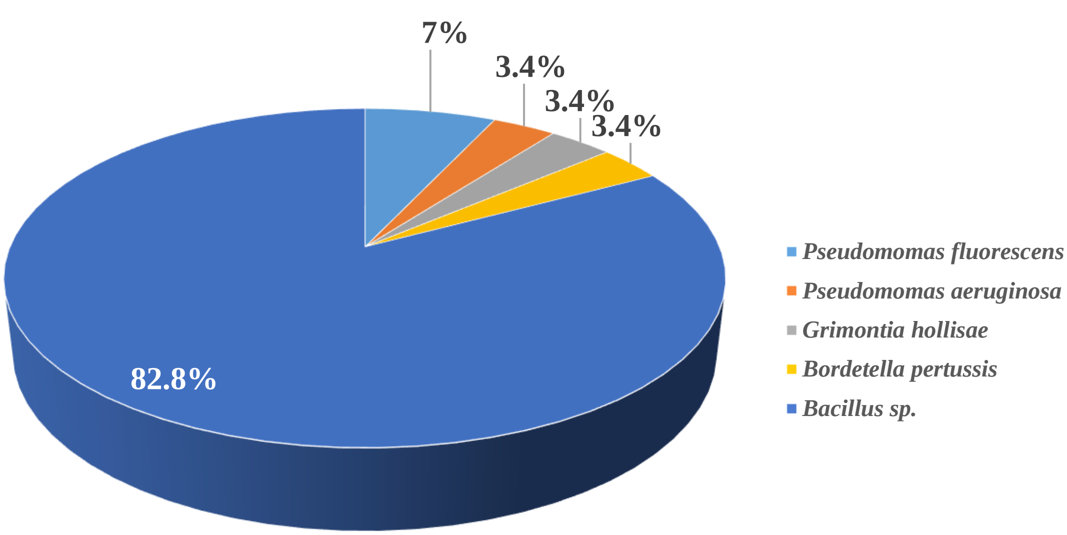


Figure 36: Percentage Occurrences of the identified Bacterial Isolates.

4.3.3 Antimicrobial Susceptibility Testing

Antibiotic susceptibility of four bacterial isolates was evaluated using the Epsilometr test (E-test) against seven different antibiotics. Isolates were first subcultured on Mueller-Hinton agar (MHA) to assess the susceptibility profiles of presumptive bacterial species. Single colonies from overnight cultures were selected and suspended in normal saline to achieve a turbidity equivalent to the 0.5 McFarland standard. A 100-µL aliquot of each bacterial suspension was evenly spread onto MHA plates to produce a uniform lawn. Two E-test strips were applied per plate, except for the last plate, which received a single strip, all using sterile forceps. The inoculated plates were incubated at 37 ^{o}C for 24 h, after which the minimum inhibitory concentrations (MICs) were recorded.

Interpretation of MIC values followed Clinical and Laboratory Standards Institute (CLSI) guidelines. The two strains of *Pseudomonas fluorescens* were interpreted using breakpoints for Other Non-Enterobacterales (CLSI M100, 32nd edition, 2022), while *Pseudomonas aeruginosa* was assessed using both zone diameter and MIC breakpoints from the same document. For *Bacillus* sp., interpretation followed the CLSI guidelines for fastidious or infrequently isolated bacteria (CLSI M45, 3rd edition, 2015). The results of antimicrobial susceptibility testing (AST) are presented in **Table 10.**

Table 10: Antimicrobial Susceptibility Testing for the Selected Bacterial Isolates.

Antibiotics	*Pseudomonas Fluorescen s A2*	*Pseudomonas fluorescens A1*	*Pseudomonas aeruginosa*	***Bacillus sp.*** C3
Meropenem	Suceptible	Suceptible	Suceptible	Suceptible
Chloramphenicol	Suceptible	Resistant	Not applicable	Suceptible
Ciprofloxaxin	Suceptible	Suceptible	Suceptible	Intermediate
Ceftriaxone	Resistant	Intermediate	Not applicable	Not applicable
Tetracycline	Resistant	Suceptible	Not applicable	Suceptible
Gentamicine	Resistant	Suceptible	Suceptible	Suceptible
Trimethoprim-Sulfamethoxazole SXT25	Suceptible	Resistant	Not applicable	Suceptible

Legend
- Suceptible
- Resistant
- Intermediate
- Not applicable

Antibiotic susceptibility results in **Table 10** show that the two *Pseudomonas fluorescens* strains (A2 and A1) have different resistance profiles. Strain A2 was resistant to ceftriaxone, tetracycline, and gentamicin, while strain A1 was resistant to chloramphenicol and trimethoprim-sulfamethoxazole. These differences, despite both strains coming from the same location, may be caused by environmental pressures in different microhabitats, genetic differences (such as resistance genes or mutations), biofilm formation, phenotypic adaptations, or past exposure to antimicrobials in the environment.

Pseudomonas aeruginosa and *Bacillus* sp. were generally sensitive to the tested antibiotics, except *Bacillus sp*., which showed intermediate susceptibility to ciprofloxacin. According to CLSI and EUCAST guidelines, certain antibiotics, namely chloramphenicol, ceftriaxone, tetracycline, and trimethoprim-sulfamethoxazole, are intrinsically ineffective against *P. aeruginosa* and are therefore not recommended for susceptibility testing using the E-test. Intrinsic resistance to ceftriaxone was also observed in *Bacillus* sp., suggesting its limited utility against both organisms.

These findings align with **Milligan *et al.* (2023),** who reported that *Pseudomonas* species, commonly found in wastewater and aquatic environments, often harbor resistance genes and act as opportunistic pathogens.

Due to limited CLSI and EUCAST guidelines, antimicrobial susceptibility (AST) for *Grimontia hollisae* and *Bordetella pertussis* was not performed, as standardized MIC breakpoints are unavailable for these rarely encountered, fastidious organisms.

Finally, *Grimontia* spp. and *Bordetella* spp. are known pathogens associated with aquatic environments and the human respiratory tract, respectively. Their detection at the study sites highlights potential public health risks **(Mahmoud *et al.*, 2021)**.

4.4 Bioremediation Experiments

4.4.1 Optical Density-Based Assessment of Bacterial Tolerance to Heavy Metals

4.4.1.1 Empty Well Control/Air Blank (OD)

Figure 37 shows empty wells (without media, metal, or bacteria) used as a negative control. When measured with a spectrophotometer, the optical density (OD) was close to zero. This is expected, as OD reflects the amount of light absorbed or scattered by the contents. Since the wells were empty, there was nothing to absorb or scatter light, resulting in minimal absorbance.

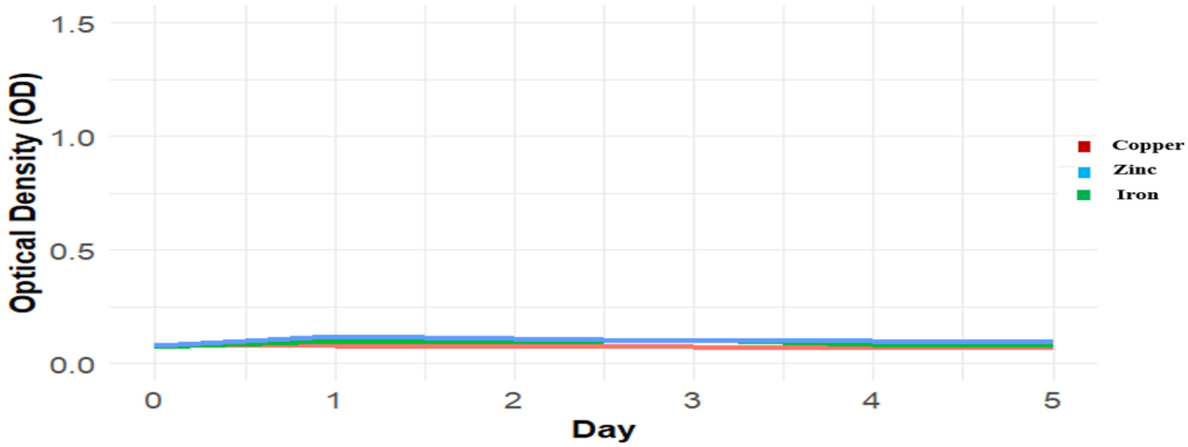


Figure 37: Growth in Empty Wells.

4.4.1.2 Control Group: Bacterial Growth in Broth without Heavy Metals

Figure 38 illustrates the optical density (OD) changes of four bacterial strains: *Bacillus* sp., *Grimontia hollisae*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens* over five days in nutrient broth without metal stress. *Bacillus* sp. and *G. hollisae* showed similar growth patterns, with steady increases until day 4, followed by a stationary phase likely due to nutrient and other factors such as oxygen or ion requirements. *G. hollisae*, a marine bacterium, may have grown better with added NaCl, as it requires Na^+ ions for optimal growth. Without metal contaminants, all strains utilized the nutrients freely. *P. aeruginosa* exhibited rapid growth, peaking at OD 0.5 by day 1, and then declined, likely due to production of biofilms or cell lysis from nutrient exhaustion. In contrast, *P. fluorescens* showed slow but continuous growth, reaching an OD of 1.4 by day 5. These differences highlight that even closely related bacteria may have distinct nutrient needs and stress responses. Similar findings by **Nie *et al.* (2013)** emphasized that microbial growth depends on sample source, genetic traits, and environmental conditions.

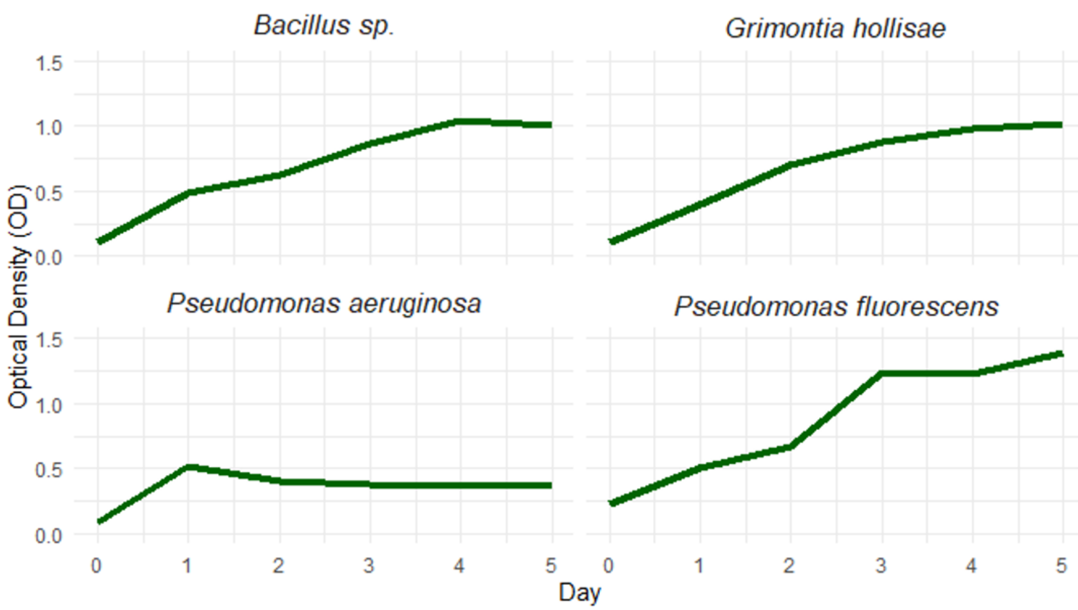


Figure 38: Control Group: Bacterial Growth in Broth without Heavy Metals.

4.4.1.3 Bacterial Growth in Copper over Time

Figure 39 illustrates the growth patterns of the four bacterial species in treated media containing copper. The results indicated that *Bacillus* sp. reached its highest growth rate on day 5 with an OD of 1 at a metal concentration of 28 ppm. Although increasing the concentration to 98 ppm resulted in the same peak growth rate, this was only achieved on day 5. *Grimontia hollisae* achieved its highest growth at 98 ppm, with an OD of 0.76 on day 3, after which it declined. *Pseudomonas aeruginosa* grew rapidly at 58 ppm and reached its peak within a short period (day 1) with an OD of 0.6, then declined to 0.48 by day 3, before starting to grow again, reaching an OD of 0.58 by day 5. *Pseudomonas fluorescens* showed its best growth rate at 98 ppm, with an OD of 0.9 on day 3. Although a notable growth rate (OD = 1.1) was also observed at 38 ppm, this was achieved on day 5.

Among the four bacterial species tested under copper stress, *Bacillus* sp. showed the highest growth rate, indicating the greatest potential for bioremediation, followed by *Pseudomonas aeruginosa*. This finding supports previous research by **Abdullahi *et al.* (2020)** showing that *Bacillus* and *Pseudomonas* species exhibited the strongest plant growth-promoting (PGP) traits under heavy metal stress among bacterial isolates from ex-mining soil. These strains were also identified as key contributors to the survival of *Mimosa pudica* in abandoned mining sites, indicating their potential use as native bacteria for bioremediation of heavy metal-contaminated soils. Similar findings by **Andreazza *et al*. (2010)**, showed that *Bacillus* and *Pseudomonas* species are among the most copper-resistant bacteria in Cu-contaminated soils. These results suggest their strong potential as biosorbents for copper removal from soil and water.

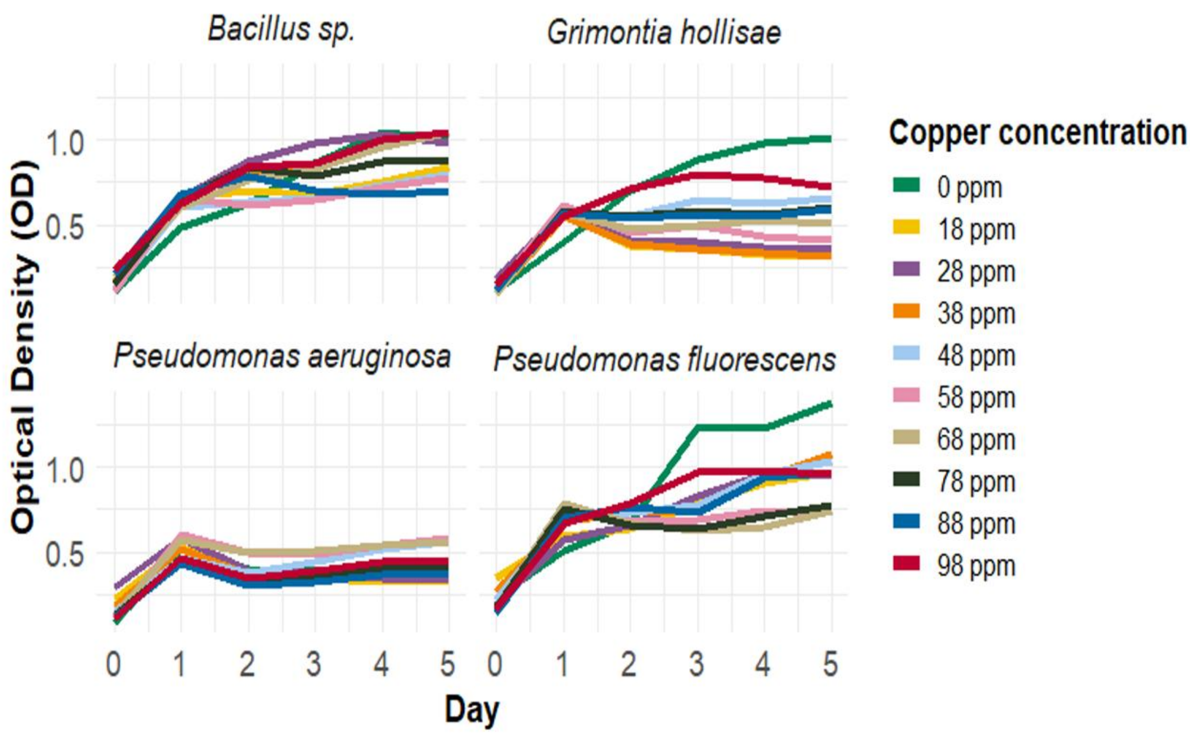


Figure 39: Bacterial Growth in Copper.

4.4.1.4 Bacterial Growth in Iron over Time

In **Figure 40**, bacteria isolates growing in media supplemented with iron concentrations above 50 ppm exhibited atypical growth patterns characterized by an initially high optical density (OD), followed by a decline and subsequent stabilization. This phenomenon is likely due to physicochemical interactions between iron salts and the media components, rather than true bacterial growth.

Upon addition of iron salt ($FeCl_2$), ferrous ions (Fe^{2+}) rapidly oxidize to ferric forms (e.g., $Fe(OH)_3$), leading to precipitation and increased turbidity independent of bacterial presence. Furthermore, iron hydrolysis (e.g., Fe^{3+}) can lower the pH of the medium as shown below:

- $Fe^{3+} + 3H_2O \rightarrow Fe(OH)_{3\downarrow} + 3H^+$

A lower pH may stress or inhibit bacterial growth, depending on the organism.

The observed OD pattern can be interpreted as follows:

- **Initial High OD**: This is attributed to the chemical precipitation of iron compounds and the formation of colloidal particles. Iron may also complex with organic molecules such as phosphates, amino acids, peptides, or vitamins in the nutrient broth, contributing to turbidity.

- **Declining OD**: As precipitates aggregate or settle, turbidity decreases. Bacteria may also interact with these particles, either by adsorption or by altering iron solubility through redox activity.

- **Stationary OD**: A stable turbidity phase follows, reflecting equilibrium in precipitation. Bacterial cells, if viable, may remain metabolically active but exhibit limited growth due to iron toxicity or nutrient depletion.

The results of this study demonstrated that low concentrations of iron (50 ppm) support bacterial growth, which is critical for metabolic activity and bioremediation potential. At this concentration, all four bacterial isolates entered the exponential growth phase. *Bacillus* sp. exhibited the most rapid growth, reaching an OD of 0.9 by day 1, after which growth stabilized. *Grimontia hollisae* reached a peak OD of 1.20 on day 2, followed by a decline. *Pseudomonas aeruginosa* showed a moderate increase, reaching an OD of 0.4 by day 1, declining by day 2, and then stabilizing. *Pseudomonas fluorescens* displayed a gradual increase to OD 0.5 on day 1, followed by a slight decline to OD 0.4 on day 2, and then entered a second exponential phase, reaching OD 0.8 by day 5. These findings suggest that all isolates have potential for bioremediation of iron-contaminated environments, with *Bacillus sp*. showing the most robust growth at low iron levels, followed by *Grimontia hollisae*. This finding supports the work of **Fakhar *et al.* (2022**) and **Lyng *et al.* (2024)**, who showed that *Bacillus* species can tolerate heavy metals like Fe, Cr, and Zn through plasmid-encoded resistance genes. While they chelate iron to support growth, their bioremediation efficiency is optimal only at low iron concentrations due to potential toxicity at higher levels.

High concentrations, on the other hand, may become toxic, especially due to reactive oxygen species (ROS) generation through Fenton reactions:

- $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + .OH + OH^-$ (hydroxyl radicals)

Some bacteria may respond by producing siderophores to scavenge iron or by reducing Fe^{3+} back to Fe^{2+}.

This study explored the potential of *Grimontia hollisae* in bioremediation and found promising results. However, no published evidence currently supports its effectiveness as a heavy metal biosorbent. As a marine bacterium, it may tolerate saline and contaminated environments, and its pathogenicity to humans remains a concern.

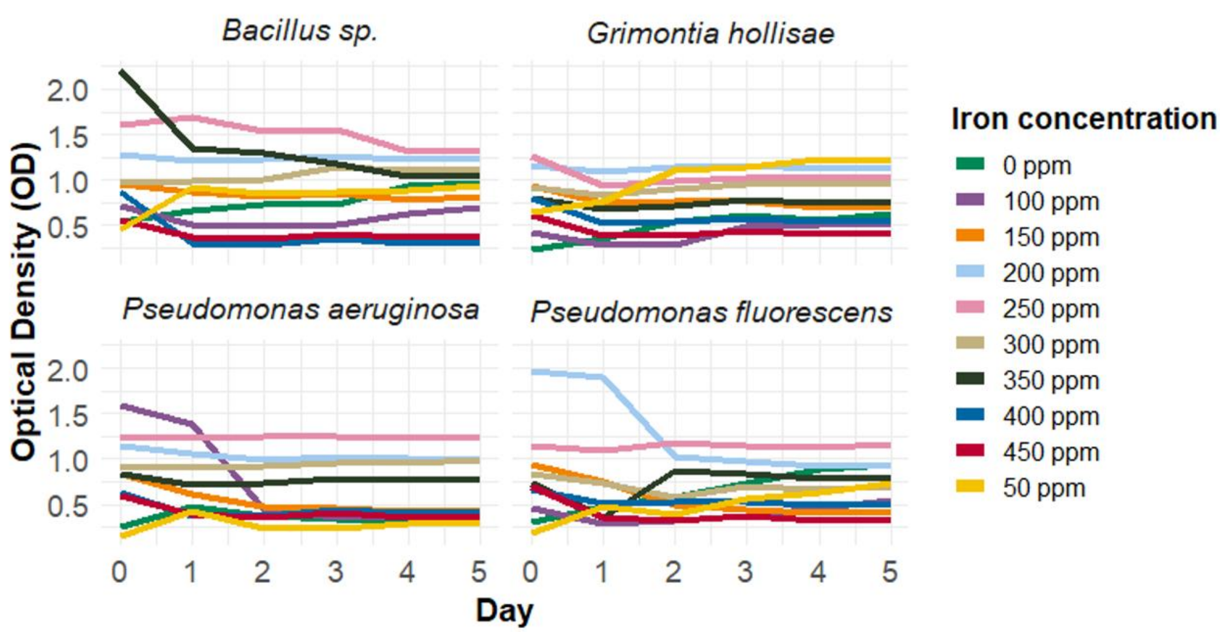


Figure 40: Bacterial Growth in Iron.

4.4.1.5 Bacterial Growth in Zinc Over Time

Figure 41 illustrates the growth response of isolated bacterial species exposed to varying zinc ion concentrations:

- *Bacillus* sp. showed rapid growth (log phase) from day 0 to day 1 at high zinc levels (400 & 450 ppm), reaching an OD of 0.25. Growth declined by day 3 (OD 0.1) and then stabilized, indicating moderate zinc tolerance.

- *Grimontia hollisae* exhibited optimal growth at low zinc concentrations (150 ppm), achieving an OD of 0.28 by day 1. Its growth decreased to an OD of 0.15 by day 3 and plateaued, suggesting sensitivity to higher zinc levels.

- *Pseudomonas aeruginosa* grew well at higher concentrations (400 & 450 ppm), with a peak OD of 0.24 on day 1, followed by a decline to OD 0.15 by day 3 and subsequent stabilization, indicating good zinc tolerance.

- *Pseudomonas fluorescens* demonstrated strong growth at both low (50 ppm) and high (400 & 450 ppm) zinc concentrations, reaching OD values of 0.25 on day 2 and day 1, respectively. In both cases, OD declined to **~0.**18 by day 3 and then stabilized, reflecting a broad adaptability across zinc levels.

Kumar *et al.* (2010) found that among four microbial strains, such as *Pseudomonas* sp., *Staphylococcus* sp., *Bacillus* sp., and *Aspergillus niger*, tested for bioremediation of metal ions, *Pseudomonas* sp., isolated from soil, showed the highest capacity for heavy metal (including Zn) biosorption. This highlights the ability of *Pseudomonas* species to tolerate and detoxify heavy metals through various resistance mechanisms and metabolic pathways, making them valuable agents for bioremediation and environmental cleanup. **Joo *et al.* (2010)** compared the zinc biosorption capacities of *Pseudomonas aeruginosa* ASU 6a and *Bacillus cereus* AMUC B52, reporting maximum uptake values of 83.3 mg/g and 66.6 mg/g, respectively. The results indicated that *P. aeruginosa* ASU 6a was more efficient in zinc ion removal. Despite the influence of factors such as initial metal concentration, pH, and contact time, both strains proved to be effective biosorbents for Zn(II) from contaminated soil and water.

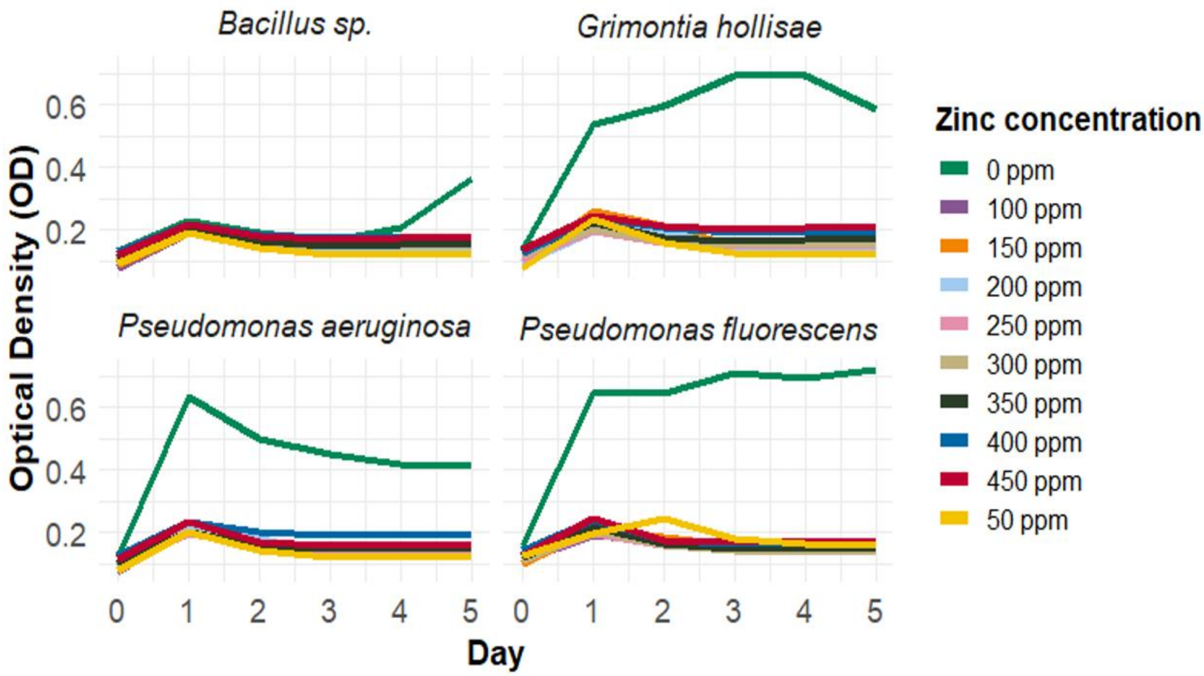


Figure 41: Bacterial Growth in Zinc.

4.4.1.6 Comparative Biosorption Efficiency of Bacterial Isolates for Copper, Iron, and Zinc

The optical density (OD) trends over five days under metal stress conditions revealed distinct biosorptive efficiencies among the tested bacterial strains. *Bacillus* sp. consistently showed the highest tolerance and biosorption potential in copper and iron-treated media, reaching peak OD values of 1.0 (Cu at 28 and 98 ppm, day 5) and 0.9 (Fe at 50 ppm, day 1), with sustained growth afterward. This stability across varying concentrations highlights its strong adaptability and metabolic activity, making *Bacillus* sp. the most efficient biosorbent for the three metals.

Pseudomonas fluorescens emerged as the most effective zinc biosorbent, showing robust and sustained OD responses across low and high Zn levels, suggesting superior zinc detoxification ability. However, it was less competitive under copper and iron stress, where *Bacillus* sp. outperformed all others.

Pseudomonas aeruginosa showed intermediate performance, ranking second in both copper and zinc bioremediation. It achieved a rapid OD peak of 0.6 on day 1 at 58 ppm Cu, indicating a swift metabolic response to copper stress, and also demonstrated substantial growth in zinc media, albeit slightly lower than *P. fluorescens*.

Grimontia hollisae, while initially performing well under iron exposure with a peak OD of 1.2 on day 2, exhibited a sharp decline thereafter, indicating limited long-term biosorptive efficiency and lower tolerance compared to the other strains.

4.4.1.7 Pairwise Comparative Analysis of Bacterial Response to Heavy Metals Over Time

4.4.1.7.1 Initial Impact (Day 0): Strong Influence of Iron vs. Other Metals

At day 0, all four bacterial species, *Pseudomonas fluorescens*, *P. aeruginosa*, *Grimontia hollisae*, and *Bacillus* species, demonstrated a highly significant difference ($p < 0.001$) in growth response when exposed to Fe and Cu, as well as Zn and Fe. These differences indicate a strong inhibitory or stimulatory contrast between Fe and the other metals at the onset of exposure. Notably, Zn vs. Cu comparisons showed no significant difference (ns) across all species on Day 0, suggesting that the immediate impact of Zn and Cu on bacterial growth was relatively similar.

4.4.1.7.2 Progressive Response Shift (Day 1): Emergence of Zn Influence

By Day 1, Fe vs. Cu differences became statistically non-significant, especially for *P. fluorescens*, *G. hollisae*, and *Bacillus* sp., implying acclimatization or reduced differential stress from Fe and Cu. However, Zn vs. Fe continued to show significant differences ($p < 0.001$) in all species, highlighting the persistent and distinct impact of Zn compared to Fe. Interestingly, Zn vs. Cu became significant for *P. fluorescens*, *G. hollisae*, and *Bacillus* sp., suggesting that zinc began to exert a more pronounced selective pressure compared to copper from Day 1 onward.

4.4.1.7.3 Zn-Focused Divergence (Days 2-5): Sustained Discriminatory Effect of Zinc

From Day 2 to Day 5, a clear trend emerged where Zn vs. Fe and Zn vs. Cu consistently showed significant differences ($p < 0.05$) across all bacteria. This highlights zinc's unique and sustained impact on bacterial growth dynamics. On the contrary, Fe vs. Cu remained non-significant in all species throughout this period, reinforcing the earlier trend that Fe and Cu exerted a relatively similar or balanced effect on growth after initial exposure. The distinction between Zn and the other metals became even more prominent, particularly for *P. fluorescens*, *Bacillus* sp., and *G. hollisae*, suggesting an increased sensitivity or adaptation mechanism specific to zinc exposure.

4.4.1.7.4 Species-Specific Sensitivity Patterns

Species-wise, *P. aeruginosa* consistently showed no significant difference in Fe vs. Cu and Zn vs. Cu until Day 4, where Zn vs. Cu finally became significant ($p = 0.033$). This delay indicates a slower response or tolerance development compared to the other species. *Bacillus* sp., however, demonstrated the most consistent and early significant responses to Zn exposure, possibly indicating a higher susceptibility or stronger regulatory adaptation mechanism toward zinc. Meanwhile, *G. hollisae* exhibited a progressive increase in sensitivity to Zn vs. Cu from Day 1 onward, which may reflect a time-dependent gene expression or metal sequestration response.

4.4.1.7.5 General Interpretation and Implications

Overall, the OD-based pairwise comparisons highlight zinc as the most distinct and influential metal in terms of bacterial growth inhibition or modulation, particularly when compared to iron and copper. The initial strong effect of iron becomes less distinctive over time, while zinc's influence becomes increasingly significant. These findings suggest that for bioremediation or microbial tolerance studies, zinc stress response mechanisms should be prioritized, especially when dealing with multimetal-contaminated environments. The differential time-dependent responses among species also emphasize the need to tailor microbial selection based on specific metal exposure profiles and time-course behavior.

Table 11: Statistical Comparison of Metal Pair Effects (Fe-Cu, Zn-Cu, and Zn-Fe) on Four Bacterial Isolates over 5 days.

Day	Bacteria	Comparison	P-Value
0	*Pseudomonas fluorescens*	Fe-Cu	<0.001*
0	*Pseudomonas fluorescens*	Zn-Cu	0.864^{ns}
0	*Pseudomonas fluorescens*	Zn-Fe	<0.001*
0	*Pseudomonas aeruginosa*	Fe-Cu	<0.001*
0	*Pseudomonas aeruginosa*	Zn-Cu	0.928^{ns}
0	*Pseudomonas aeruginosa*	Zn-Fe	<0.001*
0	*Grimontia hollisae*	Fe-Cu	<0.001*
0	*Grimontia hollisae*	Zn-Cu	0.994^{ns}
0	*Grimontia hollisae*	Zn-Fe	<0.001*
0	*Bacillus* sp.	Fe-Cu	<0.001*
0	*Bacillus* sp.	Zn-Cu	0.961^{ns}
0	*Bacillus* sp.	Zn-Fe	<0.001*
1	*Pseudomonas fluorescens*	Fe-Cu	0.931^{ns}
1	*Pseudomonas fluorescens*	Zn-Cu	0.034*
1	*Pseudomonas fluorescens*	Zn-Fe	0.014*
1	*Pseudomonas aeruginosa*	Fe-Cu	0.086^{ns}
1	*Pseudomonas aeruginosa*	Zn-Cu	0.117^{ns}
1	*Pseudomonas aeruginosa*	Zn-Fe	<0.001*

1	*Grimontia hollisae*	Fe-Cu	0.428^{ns}
1	*Grimontia hollisae*	Zn-Cu	0.015*
1	*Grimontia hollisae*	Zn-Fe	<0.001*
1	*Bacillus* sp.	Fe-Cu	0.160^{ns}
1	*Bacillus* sp.	Zn-Cu	0.017*
1	*Bacillus* sp.	Zn-Fe	<0.001*
2	*Pseudomonas fluorescens*	Fe-Cu	0.765^{ns}
2	*Pseudomonas fluorescens*	Zn-Cu	<0.001*
2	*Pseudomonas fluorescens*	Zn-Fe	<0.001*
2	*Pseudomonas aeruginosa*	Fe-Cu	0.093^{ns}
2	*Pseudomonas aeruginosa*	Zn-Cu	0.105^{ns}
2	*Pseudomonas aeruginosa*	Zn-Fe	<0.001*
2	*Grimontia hollisae*	Fe-Cu	0.158^{ns}
2	*Grimontia hollisae*	Zn-Cu	0.015*
2	*Grimontia hollisae*	Zn-Fe	<0.001*
2	*Bacillus* sp.	Fe-Cu	0.820^{ns}
2	*Bacillus* sp.	Zn-Cu	<0.001*
2	*Bacillus* sp.	Zn-Fe	<0.001*
3	*Pseudomonas fluorescens*	Fe-Cu	0.434^{ns}
3	*Pseudomonas fluorescens*	Zn-Cu	<0.001*
3	*Pseudomonas fluorescens*	Zn-Fe	<0.001*
3	*Pseudomonas aeruginosa*	Fe-Cu	0.065^{ns}
3	*Pseudomonas aeruginosa*	Zn-Cu	0.062^{ns}
3	*Pseudomonas aeruginosa*	Zn-Fe	<0.001*
3	*Grimontia hollisae*	Fe-Cu	0.167^{ns}
3	*Grimontia hollisae*	Zn-Cu	0.004*
3	*Grimontia hollisae*	Zn-Fe	<0.001*
3	Bacillus sp.	Fe-Cu	0.862^{ns}
3	Bacillus sp.	Zn-Cu	<0.001*
3	Bacillus sp.	Zn-Fe	<0.001*
4	*Pseudomonas fluorescens*	Fe-Cu	0.125^{ns}
4	*Pseudomonas fluorescens*	Zn-Cu	<0.001*
4	*Pseudomonas fluorescens*	Zn-Fe	<0.001*
4	*Pseudomonas aeruginosa*	Fe-Cu	0.150^{ns}
4	*Pseudomonas aeruginosa*	Zn-Cu	0.033*
4	*Pseudomonas aeruginosa*	Zn-Fe	<0.001*
4	*Grimontia hollisae*	Fe-Cu	0.236^{ns}
4	*Grimontia hollisae*	Zn-Cu	0.008*
4	*Grimontia hollisae*	Zn-Fe	<0.001*
4	*Bacillus* sp.	Fe-Cu	0.947^{ns}
4	*Bacillus* sp.	Zn-Cu	<0.001*
4	*Bacillus* sp.	Zn-Fe	<0.001*
5	*Pseudomonas fluorescens*	Fe-Cu	0.106^{ns}
5	*Pseudomonas fluorescens*	Zn-Cu	<0.001*
5	*Pseudomonas fluorescens*	Zn-Fe	<0.001*
5	*Pseudomonas aeruginosa*	Fe-Cu	0.191^{ns}
5	*Pseudomonas aeruginosa*	Zn-Cu	0.025*
5	*Pseudomonas aeruginosa*	Zn-Fe	<0.001*
5	*Grimontia hollisae*	Fe-Cu	0.163^{ns}
5	*Grimontia hollisae*	Zn-Cu	0.005*
5	*Grimontia hollisae*	Zn-Fe	<0.001*
5	*Bacillus* sp.	Fe-Cu	0.940^{ns}
5	*Bacillus* sp.	Zn-Cu	<0.001*
5	*Bacillus* sp.	Zn-Fe	<0.001*

* = significant difference at $p < 0.05$; ^{ns} = not significant.

CHAPTER 5

CONCLUSIONS, RECOMMENDATIONS, AND PERSPECTIVES

5.1 Conclusions

This research provided an in-depth evaluation of farmers' understanding and perspectives regarding the impacts of climate variability and environmental pollution on agricultural productivity. It also documented the range of adaptation measures implemented within farming communities in The Gambia. Simultaneously, mesophilic bacteria were isolated and examined to explore their potential for heavy metal bioremediation under changing climate conditions. The integration of socio-environmental perspectives with microbiological investigations offered a holistic framework for identifying key challenges and informing potential strategies to enhance sustainable agricultural practices in the region. The results emphasize the urgent need for climate-resilient farming techniques and underscore the promising role of native bacterial strains in reducing soil and water pollution. These findings contribute valuable information for developing environmentally sustainable, microbiologically based agricultural strategies amid climate change. The study underscores sustainable agricultural solutions and climate-smart policy development by integrating local knowledge, native bioremediation, and interdisciplinary approaches, as reflected in the following findings:

- The declining involvement of the youth in agricultural practices underscores a growing dependency on food imports to sustain the increasing population. This trend poses a critical challenge to national strategies aimed at achieving food self-sufficiency and threatens the long-term sustainability of domestic food systems.

- The study reveals that structural and institutional barriers, particularly limited access to credit and quality inputs, significantly hinder farmers' adaptive capacity to climate variability in the study area. These findings underscore the urgency for targeted, context-specific policy responses that address both systemic constraints and localized challenges such as land fragmentation, low literacy, and inadequate extension services in The Gambia.

- This study highlights that Gambian farmers, particularly in coastal and riverine areas, attribute heavy metal (HM) contamination of farmlands to chemical fertilizers, pesticides, effluents, and poor waste disposal practices. Perceptions of HM risks varied by age and ethnicity, with older farmers generally less aware and certain ethnic groups more informed, while gender, religion, marital status, and education showed no influence. These findings emphasize the need for age and culturally responsive awareness strategies to support informed decision making and sustainable farming practices.

- The observed differences in antibiotic resistance between *Pseudomonas fluorescens* strains, despite originating from the same site, underscore the influence of microhabitat variability, genetic factors, and prior antimicrobial exposure on resistance profiles. The intrinsic resistance patterns of *Pseudomonas aeruginosa* and *Bacillus* sp., particularly against agents such as ceftriaxone and chloramphenicol, reflect established limitations in the use of certain antibiotics per CLSI and EUCAST recommendations. The environmental presence of *Grimontia* and *Bordetella* species with known pathogenic potential, along with the lack of standardized AST protocols, raises important concerns for public health monitoring and highlights gaps in current diagnostic frameworks.

- The optical density trends over five days revealed that *Bacillus* sp. was the most efficient biosorbent across copper, iron, and zinc, followed by *Pseudomonas fluorescens* and then *P. aeruginosa*. These findings highlight the superior metal tolerance and biosorption performance of *Bacillus* sp., emphasizing its potential as the leading candidate for native bioremediation in heavy metal-contaminated environments.

5.2 Recommendations

5.2.1 Recommendations for Government and Policymakers

1. Implement climate-focused education and incentive-based agricultural training to engage youth, enhance food security, and reduce reliance on imports.

2. Improve farmer resilience through expanded access to agricultural credit, quality inputs, secure land tenure, and climate-adaptive extension services.

3. Promote the adoption of climate-resilient seeds, irrigation systems, and early warning mechanisms to support adaptation to climate variability.

4. Support rural development by investing in farmer literacy, agricultural education, and strengthened extension networks to enhance awareness of the impact of climate variability on agriculture.

5. Integrate local farmer knowledge into climate-smart and pollution-sensitive policy design to ensure culturally and environmentally appropriate interventions.

5.2.2 Recommendations for Tertiary Institutions and Non-Governmental Organisations

1. Develop and implement climate-responsive agricultural curricula to train farmers in resilient farming practices and seed improvement.

2. Support research and application of mesophilic bacteria such as *Bacillus* and *Pseudomonas* species for heavy metal remediation in sustainable agriculture.

3. Foster inclusive partnerships to address educational disparities and expand localized agricultural extension services, especially in marginalized areas.

5.2.3 Recommendations for Farmers and Local Communities

1. Engage in ongoing climate education initiatives to build awareness of climate variability and its impact on agriculture.

2. Adopt adaptive farming practices such as drought-tolerant crops, soil conservation methods, and water-efficient systems to enhance resilience at the community level.

3. Encourage the use of native, metal-tolerant microbes like *Bacillus* and *Pseudomonas* species in bioremediation strategies to address heavy metal contamination and promote sustainable agrochemical practices.

5.3 Perspectives

To build on the findings of this study, future research should focus on:

1. Genetic characterization of promising mesophilic bacterial strains involved in heavy metal bioremediation.
2. Assessment of heavy metal contamination levels in agricultural systems across The Gambia.
3. Investigation of the molecular mechanisms responsible for the high biosorption capacity of *Bacillus* spp. and *Pseudomonas* spp.
4. Expansion of regional collaborations to promote bioremediation technologies in West Africa.
5. Integration of artificial intelligence and capacity building to support large-scale monitoring of heavy metal pollution across Africa.

REFERENCES

Abbasi, M., Khan, I., Rehman, A., Hayat, A., Ur Rehman, M., Shah, T. A., Khan, A. A., Ul Haq, T., Aziz, T., Alharbi, M., Alasmari, A. F., & Albekairi, T. H. (2024). Bioremediation of Heavy Metals Contaminated Soil By Using Indigenous Metallotolerant Bacterial Isolates. *Applied Ecology and Environmental Research*, *22*(2), 1623–1648. https://doi.org/10.15666/aeer/2202_16231648

Abdullah, N., Yusof, N., Lau, W. J., Jaafar, J., & Ismail, A. F. (2019). Recent trends of heavy metal removal from water/wastewater by membrane technologies. *Journal of Industrial and Engineering Chemistry*, *76*, 17–38. https://doi.org/10.1016/j.jiec.2019.03.029

Abdullahi, S., Haris, H., Zarkasi, K. Z., & Amir, H. G. (2020). Beneficial bacteria associated with Mimosa pudica and the potential to sustain plant growth-promoting traits under heavy metal metal stress. *Bioremediation Journal, 25(1), 1-21. https://doi.org/10.1080/10889868.2020.1837724*

Abraham, J., Dowling, K., & Florentine, S. (2017). Risk of post-fire metal mobilization into surface water resources: A review. *Science of the Total Environment*, *599–600*, 1740–1755. https://doi.org/10.1016/j.scitotenv.2017.05.096

Agnello, A. C., Bagard, M., van Hullebusch, E. D., Esposito, G., & Huguenot, D. (2016). Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation. *Science of the Total Environment*, *563–564*, 693–703. https://doi.org/10.1016/j.scitotenv.2015.10.061

Agwaramgbo, L., Thomas, C., GrayS, C., Small, J., & Young, T. (2012). An Evaluation of Edible Plant Extracts for the Phytoremediation of Lead Contaminated Water. *Journal of Environmental Protection*, *03*(08), 722–730. https://doi.org/10.4236/jep.2012.38086

Agwu, O. P., Bakayoko, A., Jimoh, S. O., & Stefan, P. (2018). Farmers' perceptions on cultivation and the impacts of climate change on goods and services provided by Garcinia kola in Nigeria. *Ecological Processes*, *7*(1). https://doi.org/10.1186/s13717-018-0147-3

Ahaibwe, G., Mbowa, S., & Lwanga, M. M. (2013). Youth engagement in agriculture in Uganda: Challenges and prospects.

Ahmad, W. A., Wan Ahmad, W. H., Karim, N. A., Santhana Raj, A. S., & Zakaria, Z. A. (2013). Cr(VI) reduction in naturally rich growth medium and sugarcane bagasse by Acinetobacter haemolyticus. *International Biodeterioration and Biodegradation*, *85*, 571–576. https://doi.org/10.1016/j.ibiod.2013.01.008

Ahmed, C. I., Hu, W. B., & Kumar, S. (2016). Indigenous knowledge about prediction in climate change. *International Journal of Humanities and Social Science,* 5(1), 45-62.

Akinnagbe, O., & Irohibe, I. (2015). Agricultural adaptation strategies to climate change impacts in Africa: a review. *Bangladesh Journal of Agricultural Research*, *39*(3), 407–418. https://doi.org/10.3329/bjar.v39i3.21984

Alkorta, I., Epelde, L., & Garbisu, C. (2017). Environmental parameters altered by climate change affect the activity of soil microorganisms involved in bioremediation. *FEMS Microbiology Letters*, *364*(19), 1–9. https://doi.org/10.1093/femsle/fnx200

Altaf, M. M., Masood, F., & Malik, A. (2008). Impact of long-term application of treated tannery effluents on the emergence of resistance traits in Rhizobium sp. isolated from Trifolium alexandrinum. *Turkish Journal of Biology*, *32*(1), 1–8.

Alvarez, A., Saez, J. M., Davila Costa, J. S., Colin, V. L., Fuentes, M. S., Cuozzo, S. A., Benimeli, C. S., Polti, M. A., & Amoroso, M. J. (2017). Actinobacteria: Current research and perspectives for bioremediation of pesticides and heavy metals. *Chemosphere*, *166*, 41–62. https://doi.org/10.1016/j.chemosphere.2016.09.070

Amin Al Sulami, R. J. (2015). Biosorption and Bioaccumulation of Some Heavy Metals by

Deinococcus Radiodurans Isolated from Soil in Basra Governorate- Iraq. *Journal of Biotechnology & Biomaterials*, *05*(02). https://doi.org/10.4172/2155-952x.1000190

Andreazza, R., Pieniz, S., Wolf, L., Lee, M., Camargo, F. A. O., & Okeke, B. C. (2010). Science of the Total Environment Characterization of copper bioreduction and biosorption by a highly copper resistant bacterium isolated from copper-contaminated vineyard soil. *Science of the Total Environment, The*, *408*(7), 1501–1507. https://doi.org/10.1016/j.scitotenv.2009.12.017

Anuoluwa, I. A., & Anuoluwa, B. S. (2025). *Bioremediation of Heavy Metal Contaminated Soils in Sub-Saharan Africa : Implications for Food Safety and Public Health Nutrition*. *16*(1), 49–76.

Anwar, M., Ali, S., & Asaad, A. T. (2017). Evaluation of Heavy Metals Resistant Micrococcus sp. Isolated from Rivers in Basra, Iraq. *Journal of Bioremediation & Biodegradation*, *08*(01), 8–11. https://doi.org/10.4172/2155-6199.1000383

Arora, N. K. (2018). Bioremediation: a green approach for restoration of polluted ecosystems. *Environmental Sustainability*, *1*(4), 305–307. https://doi.org/10.1007/s42398-018-00036-y

Artati, D., Oman, M., Sukarta, D., & Robisalmi, A. (2024). Validation of Analytical Method for Aeromonas hydrophila Identification using Analytical Profile Index (API) 20E KIT Method. *Jurnal Medik Veteriner*, *7*(1), 88–104. https://doi.org/10.20473/jmv.vol7.iss1.2024.88-104

Asati, A., Pichhode, M., & Nikhil, K. (2016). Effect of heavy metals on plants: An overview. *International Journal of Application or Innovation in Engineering & Management*, *5*(3), 56–66. https://doi.org/10.13140/RG.2.2.27583.87204

Atuchin, V. V., Asyakina, L. K., Serazetdinova, Y. R., Frolova, A. S., Velichkovich, N. S., & Prosekov, A. Y. (2023). Microorganisms for Bioremediation of Soils Contaminated with Heavy Metals. *Microorganisms*, *11*(4), 1–19. https://doi.org/10.3390/microorganisms11040864

Awasthi, G., Chester, A., Chaturvedi, R., & Prakash, J. (2015). Study on role of Pseudomonas aeruginosa on heavy metal bioremediation. *International Journal of Pure and Applied Bioscience*, *3*(4), 92–100. http://www.ijpab.com/form/2015 Volume 3, issue 4/IJPAB-2015-3-4-92-100.pdf

Azabou, S., Mechichi, T., & Sayadi, S. (2007). Zinc precipitation by heavy-metal tolerant sulfate-reducing bacteria enriched on phosphogypsum as a sulfate source. *Minerals Engineering*, *20*(2), 173–178. https://doi.org/10.1016/j.mineng.2006.08.008

Babák, L., Šupinová, P., Zichová, M., Burdychová, R., & Vítová, E. (2012). Biosorption of Cu, Zn and Pb by thermophilic bacteria - Effect of biomass concentration on biosorption capacity. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *60*(5), 9–18. https://doi.org/10.11118/actaun201260050009

Badgie, D. (2018). *Implications of farmers' perception, local knowledge and climate change on rice production in the Central River Region, The Gambia*. http://ir.ucc.edu.gh/jspui/handle/123456789/3408

Balladares, E., Jerez, O., Parada, F., Baltierra, L., Hernández, C., Araneda, E., & Parra, V. (2018). Neutralization and co-precipitation of heavy metals by lime addition to effluent from acid plant in a copper smelter. *Minerals Engineering*, *122*(March), 122–129. https://doi.org/10.1016/j.mineng.2018.03.028

Barrow, A., Badjie, M., Touray, J., Kinteh, B., Nget, M., Touray, E., Kinteh, S. L. S., Sillah, S. O., Darboe, L., Jallow, Y., Badjan, M., Gaye, M., & Jatta, S. P. S. (2020). Knowledge, Attitude, and Practice of Provincial Dwellers on Prevention and Control of Schistosomiasis: Evidence from a Community-Based Cross-Sectional Study in the Gambia. *Journal of*

Tropical Medicine, *2020*. https://doi.org/10.1155/2020/2653096
Bibi, A., Ahmed, A., Batool, K., & A-Rahman, J. (2020). Isolation and Characterization of Mesophilic Bacteria from Rhizosphere of Plant Rice (Oryza sativa) from Lodhran, Pakistan. *Biosciences, Biotechnology Research Asia*, *17*(1), 73–78. https://doi.org/10.13005/bbra/2811
Biney, C., Amuzu, A. T., Calamari, D., Kaba, N., Mbome, I. L., Naeve, H., Ochumba, P. B. O., Osibanjo, O., Radegonde, V., & Saad, M. A. H. (1994). Review of Heavy Metals in the African Aquatic Environment. In *Ecotoxicology and Environmental Safety* (Vol. 28, Issue 2, pp. 134–159). https://doi.org/10.1006/eesa.1994.1041
Bohli, T., Ouederni, A., Fiol, N., & Villaescusa, I. (2015). Evaluation of an activated carbon from olive stones used as an adsorbent for heavy metal removal from aqueous phases. *Comptes Rendus Chimie*, *18*(1), 88–99. https://doi.org/10.1016/j.crci.2014.05.009
Božić, D., Stanković, V., Gorgievski, M., Bogdanović, G., & Kovačević, R. (2009). Adsorption of heavy metal ions by sawdust of deciduous trees. *Journal of Hazardous Materials*, *171*(1–3), 684–692. https://doi.org/10.1016/j.jhazmat.2009.06.055
Bradshaw, C. D., Pope, E., Kay, G., Davie, J. C. S., Cottrell, A., Bacon, J., Cosse, A., Dunstone, N., Jennings, S., Challinor, A., Chapman, S., Birch, C., Sallu, S. M., King, R., & Macdiarmid, J. (2022). Unprecedented climate extremes in South Africa and implications for maize production. *Environmental Research Letters, 17*(8). https://doi.org/10.1088/1748-9326/ac816d
Brake, S. S., Hasiotis, S. T., & Dannelly, H. K. (2004). Diatoms in acid mine drainage and their role in the formation of iron-rich stromatolites. *Geomicrobiology Journal*, *21*(5), 331–340. https://doi.org/10.1080/01490450490454074
Burger, J., & Gochfeld, M. (2006). Mercury in fish available in supermarkets in Illinois: Are there regional differences. *Science of the Total Environment*, *367*(2–3), 1010–1016. https://doi.org/10.1016/j.scitotenv.2006.04.018
Bwapwa, J. K., Jaiyeola, A. T., & Chetty, R. (2017). Bioremediation of acid mine drainage using algae strains: A review. *South African Journal of Chemical Engineering*, *24*(July 2016), 62–70. https://doi.org/10.1016/j.sajce.2017.06.005
Castro, C., Urbieta, M. S., Cazón, J. P., & Donati, E. R. (2019). Bioresource Technology Metal biorecovery and bioremediation : Whether or not thermophilic are better than mesophilic microorganisms. *Bioresource Technology*, *279*(November 2018), 317–326. https://doi.org/10.1016/j.biortech.2019.02.028
Chabukdhara, M., & Nema, A. K. (2012). Assessment of heavy metal contamination in Hindon River sediments: A chemometric and geochemical approach. *Chemosphere*, *87*(8), 945–953. https://doi.org/10.1016/j.chemosphere.2012.01.055
Chakraborti, L. (2021). Impact of upstream plant level pollution on downstream water quality: evidence from the clean water act. *Journal of Environmental Planning and Management*, *64*(3), 517–535. https://doi.org/10.1080/09640568.2020.1776227
Chalchisa, T., & Sani, S. (2016). Farmers' perception, impact and adaptation strategies to climateChalchisa, T., & Sani, S. (2016). Farmers' perception, impact and adaptation strategies to climate change among smallholder farmers in Sub-Saharan Africa: A systematic review. Journal of Reso. *Journal of Resources Development and Management*, *26*(January 2016), 1–8. https://www.academia.edu/download/87020672/234696369.pdf
Cham, F. O., Secka, A., Yaffa, S., & Sawanneh, M. (2018). Climate varaibility, perception and adaptation: differences between male and female cattle owners. *International Journal of Agriculture and Environmental Research,* 4(1).
Chellaiah, E. R. (2018). Cadmium (heavy metals) bioremediation by Pseudomonas aeruginosa:

a minireview. *Applied Water Science*, *8*(6), 1–10. https://doi.org/10.1007/s13201-018-0796-5

Chen, Q., Yao, Y., Li, X., Lu, J., Zhou, J., & Huang, Z. (2018). Comparison of heavy metal removals from aqueous solutions by chemical precipitation and characteristics of precipitates. *Journal of Water Process Engineering*, *26*(May), 289–300. https://doi.org/10.1016/j.jwpe.2018.11.003

Cifuentes, L., García, I., Arriagada, P., & Casas, J. M. (2009). The use of electrodialysis for metal separation and water recovery from CuSO4-H2SO4-Fe solutions. *Separation and Purification Technology*, *68*(1), 105–108. https://doi.org/10.1016/j.seppur.2009.04.017

Crane, T. A., Roncoli, C., & Hoogenboom, G. (2011). Adaptation to climate change and climate variability: The importance of understanding agriculture as performance. *NJAS - Wageningen Journal of Life Sciences*, *57*(3–4), 179–185. https://doi.org/10.1016/j.njas.2010.11.002

Creswell, J. W., & Angeles, L. (2006). *2Nd Edition Designing and Conducting Mixed Methods Research*.

Daraei, H., Mittal, A., Noorisepehr, M., & Mittal, J. (2015). Separation of chromium from water samples using eggshell powder as a low-cost sorbent: kinetic and thermodynamic studies. *Desalination and Water Treatment*, *53*(1), 214–220. https://doi.org/10.1080/19443994.2013.837011

Declercq, I., Cappuyns, V., & Duclos, Y. (2012). Monitored natural attenuation (MNA) of contaminated soils: State of the art in Europe-A critical evaluation. *Science of the Total Environment*, *426*, 393–405. https://doi.org/10.1016/j.scitotenv.2012.03.040

Deressa, T. T., Hassan, R. M., & Ringler, C. (2011). Perception of and adaptation to climate change by farmers in the Nile basin of Ethiopia. *Journal of Agricultural Science*, *149*(1), 23–31. https://doi.org/10.1017/S0021859610000687

Diep, P., Mahadevan, R., & Yakunin, A. F. (2018). Heavy metal removal by bioaccumulation using genetically engineered microorganisms. *Frontiers in Bioengineering and Biotechnology*, *6*(OCT). https://doi.org/10.3389/fbioe.2018.00157

Dimari, G. A., Hati, S. S., Waziri, M., & Maitera, O. N. (2008). Pollution synergy from particulate matter Sources: The Harmattan, fugitive dust and combustion emissions in Maiduguri metropolis, Nigeria. *European Journal of Scientific Research*, *23*(3), 465–473.

Dixit, R., Wasiullah, Malaviya, D., Pandiyan, K., Singh, U. B., Sahu, A., Shukla, R., Singh, B. P., Rai, J. P., Sharma, P. K., Lade, H., & Paul, D. (2015). Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. *Sustainability (Switzerland)*, *7*(2), 2189–2212. https://doi.org/10.3390/su7022189

Dizge, N., Keskinler, B., & Barlas, H. (2009). Sorption of Ni(II) ions from aqueous solution by Lewatit cation-exchange resin. *Journal of Hazardous Materials*, *167*(1–3), 915–926. https://doi.org/10.1016/j.jhazmat.2009.01.073

Dmytryk, A., Saeid, A., & Chojnacka, K. (2014). Biosorption of microelements by spirulina: Towards technology of mineral feed supplements. *Scientific World Journal*, *2014*. https://doi.org/10.1155/2014/356328

Dorda, J., Mavrov, V., Chmiel, H., Lazaridis, N. K., & Matis, K. A. (2003). *Hybrid flotation — membrane filtration process for the removal of heavy metal ions from wastewater*. *37*, 4018–4026. https://doi.org/10.1016/S0043-1354(03)00314-2

Dursun, A. Y., Uslu, G., Cuci, Y., & Aksu, Z. (2003). Bioaccumulation of copper(II), lead(II) and chromium(VI) by growing Aspergillus niger. *Process Biochemistry*, *38*(12), 1647–1651. https://doi.org/10.1016/S0032-9592(02)00075-4

Dzionek, A., Wojcieszyńska, D., & Guzik, U. (2016). Natural carriers in bioremediation: A review. *Electronic Journal of Biotechnology*, *23*, 28–36. https://doi.org/10.1016/j.ejbt.2016.07.003

Edelstein, M., & Ben-Hur, M. (2018). Heavy metals and metalloids: Sources, risks and strategies to reduce their accumulation in horticultural crops. *Scientia Horticulturae*, *234*(December), 431–444. https://doi.org/10.1016/j.scienta.2017.12.039

Edward Raja, C., Sasikumar, S., & Selvam, G. S. (2008). Adaptive and cross resistance to cadmium (II) and zinc (II) by Pseudomonas aeruginosa BC15. *Biologia*, *63*(4), 461–465. https://doi.org/10.2478/s11756-008-0095-y

Elizabeth, C. N., Victoria, M. Y., Nkechi, E. E., & Godwin, B. O. (2017). Isolation and characterization of heavy metal tolerant bacteria from Panteka stream, Kaduna, Nigeria and their potential for bioremediation. *African Journal of Biotechnology*, *16*(1), 32–40. https://doi.org/10.5897/ajb2016.15676

Elzwayie, A., Afan, H. A., Allawi, M. F., & El-Shafie, A. (2017). Heavy metal monitoring, analysis and prediction in lakes and rivers: state of the art. *Environmental Science and Pollution Research*, *24*(13), 12104–12117. https://doi.org/10.1007/s11356-017-8715-0

Engel-Di Mauro, S. (2012). Minding history and world-scale dynamics in hazards research: The making of hazardous soils in the Gambia and Hungary. *Journal of Risk Research*, *15*(10), 1319–1333. https://doi.org/10.1080/13669877.2011.591500

Ermolin, M. S., Fedotov, P. S., Malik, N. A., & Karandashev, V. K. (2018). Nanoparticles of volcanic ash as a carrier for toxic elements on the global scale. *Chemosphere*, *200*, 16–22. https://doi.org/10.1016/j.chemosphere.2018.02.089

European Environment Agency. (2014). *Progress in management of contaminated sites* (Issue Lsi 003). https://doi.org/10.2788/4658

Fadel, M., Hassanein, N. M., Elshafei, M. M., Mostafa, A. H., Ahmed, M. A., & Khater, H. M. (2017). Biosorption of manganese from groundwater by biomass of Saccharomyces cerevisiae. *HBRC Journal*, *13*(1), 106–113. https://doi.org/10.1016/j.hbrcj.2014.12.006

Fadina, A. M. R., & Barjolle, D. (2018). Farmers' adaptation strategies to climate change and their implications in the Zou department of South Benin. *Environments - MDPI*, *5*(1), 1–17. https://doi.org/10.3390/environments5010015

Fakhar, A., Gul, B., Gurmani, A. R., Khan, S. M., Ali, S., Sultan, T., Chaudhary, H. J., Rafique, M., & Rizwan, M. (2022). Heavy metal remediation and resistance mechanism of Aeromonas, Bacillus, and Pseudomonas: A review. *Critical Reviews in Environmental Science and Technology*, *52*(11), 1868–1914. https://doi.org/10.1080/10643389.2020.1863112

Falco, C., Donzelli, F., & Olper, A. (2018). Climate change, agriculture and migration: A survey. *Sustainability (Switzerland)*, *10*(5), 1–21. https://doi.org/10.3390/su10051405

FAO. (2006). Livelihood adaptation to climate variability and change in drought-prone areas of Bangladesh: developing institutions and options. In *Case Study - Institutions for Rural Development, FAO* (Issue No.5). cabi:20083073694

Fernandes, J. P., Almeida, C. M. R., Salgado, M. A., Carvalho, M. F., & Mucha, A. P. (2021). Pharmaceutical compounds in aquatic environments— occurrence, fate and bioremediation prospective. *Toxics*, *9*(10), 1–26. https://doi.org/10.3390/toxics9100257

Fierer, N., Allen, A. S., Schimel, J. P., & Holden, P. A. (2003). Controls on microbial CO2 production: A comparison of surface and subsurface soil horizons. *Global Change Biology*, *9*(9), 1322–1332. https://doi.org/10.1046/j.1365-2486.2003.00663.x

Fu, S., Lu, J., Walder, I., & Wu, D. (2023). Effect of temperature on the leaching of heavy metals from nickel mine tailings in the arctic area, Norway. *International Journal of Agricultural* *and Biological Engineering*, *16*(2), 152–158. https://doi.org/10.25165/j.ijabe.20231602.7216

Gao, H., Zhang, J., Wang, R., & Wang, M. (2015). Highly efficient hydrogen production and formaldehyde degradation by Cu2O microcrystals. *Applied Catalysis B: Environmental*, *172–173*, 1–6. https://doi.org/10.1016/j.apcatb.2015.02.004

Ghirişan, A. L., Drăgan, S., Pop, A., Simihăian, M., & Miclăuş, V. (2007). Heavy metal removal and neutralization of acid mine waste water - Kinetic study. *Canadian Journal of Chemical Engineering*, *85*(6), 900–905. https://doi.org/10.1002/cjce.5450850611

Gholami, A., Moghadassi, A. R., Hosseini, S. M., Shabani, S., & Gholami, F. (2014). Preparation and characterization of polyvinyl chloride based nanocomposite nanofiltration-membrane modified by iron oxide nanoparticles for lead removal from water. *Journal of Industrial and Engineering Chemistry*, *20*(4), 1517–1522. https://doi.org/10.1016/j.jiec.2013.07.041

Giovanella, P., Vieira, G. A. L., Ramos Otero, I. V., Pais Pellizzer, E., de Jesus Fontes, B., & Sette, L. D. (2020). Metal and organic pollutants bioremediation by extremophile microorganisms. *Journal of Hazardous Materials*, *382*(January 2019), 121024. https://doi.org/10.1016/j.jhazmat.2019.121024

Giwa, A., Dindi, A., & Kujawa, J. (2019). Membrane bioreactors and electrochemical processes for treatment of wastewaters containing heavy metal ions, organics, micropollutants and dyes: Recent developments. *Journal of Hazardous Materials*, 172–195. https://doi.org/10.1016/j.jhazmat.2018.06.025

Gode, F., & Pehlivan, E. (2006). Removal of chromium(III) from aqueous solutions using Lewatit S 100: The effect of pH, time, metal concentration and temperature. *Journal of Hazardous Materials*, *136*(2), 330–337. https://doi.org/10.1016/j.jhazmat.2005.12.021

Gu, T., Rastegar, S. O., Mousavi, S. M., Li, M., & Zhou, M. (2018). Advances in bioleaching for recovery of metals and bioremediation of fuel ash and sewage sludge. *Bioresource Technology*, *261*(February), 428–440. https://doi.org/10.1016/j.biortech.2018.04.033

Guedron, S., Grangeon, S., Lanson, B., & Grimaldi, M. (2009). Mercury speciation in a tropical soil association; Consequence of gold mining on Hg distribution in French Guiana. *Geoderma*, *153*(3–4), 331–346. https://doi.org/10.1016/j.geoderma.2009.08.017

Gunatilake, S. K. (2015). *Methods of Removing Heavy Metals from Industrial Wastewater*. *1*(1), 12–18.

Gupta, A. & Joia, J. (2016). Microbes as Potential Tool for Remediation of Heavy Metals: A Review. *Journal of Microbial & Biochemical Technology, 8(4), 364-372. https:doi.org/10.4172/1948-5948.10000310*

Gyampoh, B. A., & Asante, W. A. (2011). *Mapping and Documenting Indigenous Knowledge in Climate Change*. *January 2011*, 139. https://doi.org/10.13140/RG.2.1.4818.6640

Hakeem, K. R. (2015). Crop production and global environmental issues. *Crop Production and Global Environmental Issues*, 1–598. https://doi.org/10.1007/978-3-319-23162-4

Hartter, J., Hamilton, L. C., Boag, A. E., Stevens, F. R., Ducey, M. J., Christoffersen, N. D., Oester, P. T., & Palace, M. W. (2018). Does it matter if people think climate change is human caused? *Climate Services*, *10*(June), 53–62. https://doi.org/10.1016/j.cliser.2017.06.014

Hoang, V. A., Kim, Y. J., Nguyen, N. L., & Yang, D. C. (2014). Arthrobacter gyeryongensis sp. nov., isolated from soil of a Gynostemma pentaphyllum field. *International Journal of Systematic and Evolutionary Microbiology*, *64*(PART 2), 420–425. https://doi.org/10.1099/ijs.0.053967-0

Hobman, J. L., & Crossman, L. C. (2015). Bacterial antimicrobial metal ion resistance. *Journal of Medical Microbiology*, *64*, 471–497. https://doi.org/10.1099/jmm.0.023036-0

Huang, Y., & Feng, X. (2019). Polymer-enhanced ultrafiltration: Fundamentals, applications and recent developments. *Journal of Membrane Science*, *586*(May), 53–83. https://doi.org/10.1016/j.memsci.2019.05.037

Hussein, H., Farag, S., Kandil, K., & Moawad, H. (2005). Tolerance and uptake of heavy metals by Pseudomonads. *Process Biochemistry*, *40*(2), 955–961. https://doi.org/10.1016/j.procbio.2004.04.001

Idrisa, Y. (2012). Analysis of Awareness and Adaptation to Climate Change among Farmers in the Sahel Savannah Agro-ecological Zone of Borno State, Nigeria. *British Journal of Environment and Climate Change*, *2*(2), 216–226. https://doi.org/10.9734/bjecc/2012/1475

Iftikhar Ahmed, C., Bang, W. H., & Kumar, S. (2016). Indigenous Knowledge About Prediction in Climate Change. *International Journal of Humanities and Social Sciences (IJHSS) ISSN(P): 2319-393X; ISSN(E): 2319-3948 Vol. 5, Issue 1, Dec – Jan 2016, 45-62 © IASET*, *December*. www.iaset.us

Igiri, B. E., Okoduwa, S. I. R., Idoko, G. O., Akabuogu, E. P., Adeyi, A. O., & Ejiogu, I. K. (2018). Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater: A Review. *Journal of Toxicology*, *2018*. https://doi.org/10.1155/2018/2568038

Ihsanullah, Al-Khaldi, F. A., Abusharkh, B., Khaled, M., Atieh, M. A., Nasser, M. S., Laoui, T., Saleh, T. A., Agarwal, S., Tyagi, I., & Gupta, V. K. (2015). Adsorptive removal of cadmium(II) ions from liquid phase using acid modified carbon-based adsorbents. *Journal of Molecular Liquids*, *204*, 255–263. https://doi.org/10.1016/j.molliq.2015.01.033

Ilhan, S., Nourbaksh, M., Kilicarslan, S., & Ozdag, H. (2004). Removal of chromium, lead and copper ions from industrial waste waters by. *Journal, Turkish Electronic Vol, Biotechnology*, *2*(January 2004), 50–57.

Infectious, C., & Advance, D. (2013). Cr Ipt Ce Pt Cr Ipt. *Clinical Infectious Diseases*, *419*, 1–31.

Ishaya, S., & Abaje, I. . (2008). Indigenous people's perception on climate change and adaptation strategies in Jema'a local government area of Kaduna State, Nigeria. *Journal of Geography and Regional Planning*, *1*(8), 138–143.

Ismail, I., & Moustafa, T. (2016). Biosorption of heavy metals. *Heavy Metals: Sources, Toxicity and Remediation Techniques*, *October*, 131–174.

Jacob, J. M., Karthik, C., Saratale, R. G., Kumar, S. S., Prabakar, D., Kadirvelu, K., & Pugazhendhi, A. (2018). Biological approaches to tackle heavy metal pollution: A survey of literature. *Journal of Environmental Management*, *217*, 56–70. https://doi.org/10.1016/j.jenvman.2018.03.077

Jamil, N., Hyder, S., Valipour, M., Yasir, M., Iqbal, R., Roy, R., Zafar, M. U., & Ahmed, A. (2022). Evaluation of the Bioremediation Potential of Staphlococcus lentus Inoculations of Plants as a Promising Strategy Used to Attenuate Chromium Toxicity. *Sustainability (Switzerland)*, *14*(20), 1–12. https://doi.org/10.3390/su142013056

Jobby, R., Jha, P., Yadav, A. K., & Desai, N. (2018). Biosorption and biotransformation of hexavalent chromium [Cr(VI)]: A comprehensive review. *Chemosphere*, *207*, 255–266. https://doi.org/10.1016/j.chemosphere.2018.05.050

Joo, J., Hassan, S. H. A., & Oh, S. (2010). International Biodeterioration & Biodegradation Comparative study of biosorption of Zn 2 þ by Pseudomonas aeruginosa and Bacillus cereus. *International Biodeterioration & Biodegradation*, *64*(8), 734–741. https://doi.org/10.1016/j.ibiod.2010.08.007

Kaninga, B. K., Chishala, B. H., Maseka, K. K., Sakala, G. M., Lark, M. R., Tye, A., & Watts, M. J. (2020). Review: mine tailings in an African tropical environment—mechanisms for the bioavailability of heavy metals in soils. *Environmental Geochemistry and Health*, *42*(4), 1069–1094. https://doi.org/10.1007/s10653-019-00326-2

Kargbo, A., Jawo, E., Amoutchi, A. I., Ndow, M., Bojang, A., Zainabou, D., Jessica A, K. F., Koua, H. K., & Kuye, R. (2023). Perceptions and impacts of climate variability on livestock farming in The Gambia. *Journal of Applied Animal Research*, *51*(1), 366–374. https://doi.org/10.1080/09712119.2023.2203765

Khan, I., Lei, H., Shah, I. A., Ali, I., Khan, I., Muhammad, I., Huo, X., & Javed, T. (2020). Farm households' risk perception, attitude and adaptation strategies in dealing with climate change: Promise and perils from rural Pakistan. *Land Use Policy*, *91*(October 2019), 104395. https://doi.org/10.1016/j.landusepol.2019.104395

Kim, R. Y., Yoon, J. K., Kim, T. S., Yang, J. E., Owens, G., & Kim, K. R. (2015). Bioavailability of heavy metals in soils: definitions and practical implementation—a critical review. *Environmental Geochemistry and Health*, *37*(6), 1041–1061. https://doi.org/10.1007/s10653-015-9695-y

Kosa, S. A., Al-Zhrani, G., & Abdel Salam, M. (2012). Removal of heavy metals from aqueous solutions by multi-walled carbon nanotubes modified with 8-hydroxyquinoline. *Chemical Engineering Journal*, *181–182*, 159–168. https://doi.org/10.1016/j.cej.2011.11.044

Král, A., Cham, B., Cham, B., & Oyelakin, O. (2019). Assessment of water quality in the university of the Gambia environs for environmental and human health. *Malaysian Journal of Chemistry*, *21*(1), 47–53.

Kumar, A., Tehri, C. B., & Garhwal, K. (2011). *Bioremediation potential of three acclimated bacteria with reference to*. *2*(2), 896–908.

Lalmi, A., Bouhidel, K. E., Sahraoui, B., & Anfif, C. el H. (2018). Removal of lead from polluted waters using ion exchange resin with Ca(NO3)2 for elution. *Hydrometallurgy*, *178*(2017), 287–293. https://doi.org/10.1016/j.hydromet.2018.05.009

Lambert, J., Avila-Rodriguez, M., Durand, G., & Rakib, M. (2006). Separation of sodium ions from trivalent chromium by electrodialysis using monovalent cation selective membranes. *Journal of Membrane Science*, *280*(1–2), 219–225. https://doi.org/10.1016/j.memsci.2006.01.021

Leal, A. J., Rodrigues, E. M., Leal, P. L., Júlio, A. D. L., Fernandes, R. de C. R., Borges, A. C., & Tótola, M. R. (2017). Changes in the microbial community during bioremediation of gasoline-contaminated soil. *Brazilian Journal of Microbiology*, *48*(2), 342–351. https://doi.org/10.1016/j.bjm.2016.10.018

Léopold, E. N., Jung, M. C., Auguste, O., Ngatcha, N., Georges, E., & Lape, M. (2008). Metals pollution in freshly deposited sediments from river Mingoa, main tributary to the Municipal lake of Yaounde, Cameroon. *Geosciences Journal*, *12*(4), 337–347. https://doi.org/10.1007/s12303-008-0034-5

Lu, H. (2019). Impact of non-agricultural employment and environmental awareness on farmers' willingness to govern the heavy metal pollution of Farmland: A case study of China. *Sustainability (Switzerland)*, *11*(7). https://doi.org/10.3390/su11072068

Lyng, M., Jørgensen, J. P. B., Schostag, M. D., Jarmusch, S. A., Aguilar, D. K. C., Lozano-Andrade, C. N., & Kovács, Á. T. (2024). Competition for iron shapes metabolic antagonism between Bacillus subtilis and Pseudomonas marginalis. *ISME Journal*, *18*(1), 1–13. https://doi.org/10.1093/ismejo/wrad001

Maheswari, S., & Murugesan, A. G. (2009). Remediation of arsenic in soil by Aspergillus nidulans isolated from an arsenic-contaminated site. *Environmental Technology*, *30*(9), 921–926. https://doi.org/10.1080/09593330902971279

Mahmoud, H., Jose, L., & Eapen, S. (2021). Grimontia sedimenti sp. Nov., isolated from benthic sediments near coral reefs south of Kuwait. *International Journal of Systematic and Evolutionary Microbiology*, *71*(3), 1–8. https://doi.org/10.1099/ijsem.0.004720

Manzini, A., Sadio, L., & Descroix, L. (2024). *Peasant resistances value for global sustainability : three failed coastal grabs of metal mining projects in West Africa*. *March*.

Matlock, M. M., Howerton, B. S., Henke, K. R., & Atwood, D. A. (2001). A pyridine-thiol ligand with multiple bonding sites for heavy metal precipitation. *Journal of Hazardous Materials*, *82*(1), 55–63. https://doi.org/10.1016/S0304-3894(00)00353-8

Mehdipour, S., Vatanpour, V., & Kariminia, H. R. (2015). Influence of ion interaction on lead removal by a polyamide nanofiltration membrane. *Desalination*, *362*, 84–92. https://doi.org/10.1016/j.desal.2015.01.030

Mendy, P. A., Kargbo, A., & Entonu, M. E. (2021). Bioremediation of heavy metal ions from contaminated soil and water by microbes: A review. *African Journal of Biological Sciences*, *3*(2), 1. https://doi.org/10.33472/afjbs.3.2.2021.1-8

Mengistu, D. K. (2011). Farmers' perception and knowledge on climate change and their coping strategies to the related hazards: case study from Adiha, central Tigray, Ethiopia. *Agricultural Sciences*, *02*(02), 138–145. https://doi.org/10.4236/as.2011.22020

Mertz, O., Mbow, C., Reenberg, A., & Diouf, A. (2009). Farmers' perceptions of climate change and agricultural adaptation strategies in rural sahel. *Environmental Management*, *43*(5), 804–816. https://doi.org/10.1007/s00267-008-9197-0

Milligan, E. G., Calarco, J., Davis, B. C., Keenum, I. M., Liguori, K., Pruden, A., & Harwood, V. J. (2023). A Systematic Review of Culture-Based Methods for Monitoring Antibiotic-Resistant Acinetobacter, Aeromonas, and Pseudomonas as Environmentally Relevant Pathogens in Wastewater and Surface Water. *Current Environmental Health Reports*, *10*(2), 154–171. https://doi.org/10.1007/s40572-023-00393-9

Mohammadi, T., Razmi, A., & Sadrzadeh, M. (2004). Effect of operating parameters on Pb2+ separation from wastewater using electrodialysis. *Desalination*, *167*(1–3), 379–385. https://doi.org/10.1016/j.desal.2004.06.150

Mohsen-Nia, M., Montazeri, P., & Modarress, H. (2007). Removal of Cu2+ and Ni2+ from wastewater with a chelating agent and reverse osmosis processes. *Desalination*, *217*(1–3), 276–281. https://doi.org/10.1016/j.desal.2006.01.043

Muhammad, L. H., & Ibrahim, M. B. (2018). Biosorption of iron by heavy-metal tolerant Micrococcus sp. *Bayero Journal of Pure and Applied Sciences*, *11*(1), 102–109. https://doi.org/10.4314/bajopas.v11i1.19

Muneer, B., Iqbal, M. J., Shakoori, F. R., & Shakoori, A. R. (2013). Tolerance and biosorption of mercury by microbial consortia: Potential use in bioremediation of wastewater. *Pakistan Journal of Zoology*, *45*(1), 247–254.

Nagashetti, V., Mahadevaraju, G. ., Muralidhar, T. ., Aamir, J., Dwijendra, T., & Bhusal, K. P. (2013). Biosorption of Heavy Metals from Soil by Pseudomonas Aeruginosa. *International Journal of Innovative Technology and Exploring Engineering*, *2*(6), 22–24. https://www.ijitee.org/portfolio-item/f0758052613/#:~:text=Abstract%3A Pseudomonas aeruginosa is known to accumulate metal,metals from the soil to reduce its toxicity.

Nanjundeswaraswamy, T. S., & Divakar, S. (2021). Determination of sample size and sampling methods in applied research. Proceedings on engineering sciences, 3(1), 25-32. doi:10.24874/PES03.01.003

Naz, T., Khan, M. D., Ahmed, I., Rehman, S. U., Rha, E. S., Malook, I., & Jamil, M. (2016). Biosorption of heavy metals by Pseudomonas species isolated from sugar industry. *Toxicology and Industrial Health*, *32*(9), 1619–1627. https://doi.org/10.1177/0748233715569900

Niane, B., Moritz, R., Guédron, S., Malick, P., Rudolf, H., Mall, I., & Poté, J. (2014). Effect of recent artisanal small-scale gold mining on the contamination of surface river sediment : Case of Gambia River , Kedougou region , southeastern Senegal. *Journal of Geochemical Exploration*, *144*, 517–527. https://doi.org/10.1016/j.gexplo.2014.03.028

Nie, M., Pendall, E., Bell, C., Gasch, C. K., Raut, S., Tamang, S., & Wallenstein, M. D. (2013). Positive climate feedbacks of soil microbial communities in a semi-arid grassland. *Ecology Letters*, 16(2), 234-241. https://doi.org/10.1111/ele.12034

Nwankwoala, H. N. L. (2015). Causes of Climate and Environmental Changes: The Need for Environmental-Friendly Education Policy in Nigeria. *Journal of Education and Practice*, *6*(30), 224–234. http://ezproxy.wheaton.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=eric&AN=EJ1081366&site=ehost-live

Odewumi, O. (2013). Farmers perception on the effect of climate change and variation on urban agriculture in Ibadan Metropolis, South-western Nigeria. *Journal of Geography and Regional Planning*, *6*(6), 209–217. https://doi.org/10.5897/jgrp2013.0370

Okimasi-henry, O. V. (2023). *Effect Of Refrigeration on The Microbial Quality of Vegetable Salads*.

Okonya, J. S., Syndikus, K., & Kroschel, J. (2013). Farmers' Perception of and Coping Strategies to Climate Change: Evidence From Six Agro-Ecological Zones of Uganda. *Journal of Agricultural Science*, *5*(8). https://doi.org/10.5539/jas.v5n8p252

Omokhagbor Adams, G., Tawari Fufeyin, P., Eruke Okoro, S., & Ehinomen, I. (2020). Bioremediation, Biostimulation and Bioaugmention: A Review. *International Journal of Environmental Bioremediation & Biodegradation*, *3*(1), 28–39. https://doi.org/10.12691/ijebb-3-1-5

Onyeneke, R. U., Amadi, M. U., Njoku, C. L., & Osuji, E. E. (2021). Climate change perception and uptake of climate-smart agriculture in rice production in ebonyi state, nigeria. *Atmosphere*, *12*(11), 1–21. https://doi.org/10.3390/atmos12111503

Orindi, V. A., & Eriksen, S. H. (2005). Mainstreaming adaptation to climate change in the development process in Uganda. In African Centre for Technology Studies (ACTS), Ecopolicy Series no. 15. https://www.africaportal.org/publications/mainstreaming-adaptation-to-climate-change-in-the-development-process-in-uganda/

Oyewo, O. A., Adeniyi, A., Bopape, M. F., & Onyango, M. S. (2020). Heavy metal mobility in surface water and soil, climate change, and soil interactions. In *Climate Change and Soil Interactions*. LTD. https://doi.org/10.1016/b978-0-12-818032-7.00004-7

Ozaki, H., Sharma, K., & Saktaywin, W. (2002). Performance of an ultra-low-pressure reverse osmosis membrane (ULPROM) for separating heavy metal: Effects of interference parameters. *Desalination*, *144*(1–3), 287–294. https://doi.org/10.1016/S0011-9164(02)00329-6

Panagos, P., Hiederer, R., Van Liedekerke, M., & Bampa, F. (2013). Estimating soil organic carbon in Europe based on data collected through an European network. *Ecological Indicators*, *24*, 439–450. https://doi.org/10.1016/j.ecolind.2012.07.020

Pardeep Singh, by, Singh, R., Kumar Singh, V., Bhadouria, R., Yadav, M., Singh, G., & Jadeja, R. (2021). Pollutants and Water Management: Resources, Strategies and Scarcity, First Edition. Edited 15 15.1 Introduction Physical and Chemical Methods for Heavy Metal Removal. *Pollutants and Water Management: Resources, Strategies and Scarcity*, *1*(1), 377–397.

Park, J. K., & Choi, S. B. (2002). Metal Recovery Using Immobilized Cell Suspension from a Brewery. *Korean Journal of Chemical Engineering*, *19*(1), 68–74.

https://doi.org/10.1007/BF02706876

Parmar M., T. L. S. (2013). Received : 15 th June-2013 Accepted : 22 nd June-2013 Review article HEAVY METAL CU , NI AND ZN : TOXICITY , HEALTH HAZARDS AND THEIR REMOVAL TECHNIQUES BY LOW COST ADSORBENTS : A SHORT OVERVIEW Revised : 20 th June-2013 1 M . Tech Research Scholar , Depa. *International Journal of Plant, Animal and Environmental Sciences*, *3*(3), 143–157.

Perkins-Kirkpatrick, S. E., Stone, D. A., Mitchell, D. M., Rosier, S., King, A. D., Lo, Y. T. E., Pastor-Paz, J., Frame, D., & Wehner, M. (2022). On the attribution of the impacts of extreme weather events to anthropogenic climate change. *Environmental Research Letters*, *17*(2). https://doi.org/10.1088/1748-9326/ac44c8

Pham, V. H. T., Kim, J., Chang, S., & Chung, W. (2022). Bacterial Biosorbents, an Efficient Heavy Metals Green Clean-Up Strategy: Prospects, Challenges, and Opportunities. *Microorganisms*, *10*(3), 1–16. https://doi.org/10.3390/microorganisms10030610

Polat, H., & Erdogan, D. (2007). *Heavy metal removal from waste waters by ion flotation*. *148*, 267–273. https://doi.org/10.1016/j.jhazmat.2007.02.013

Popoola, B. M., & Ogwerel, J. P. (2024). Bacterial isolates from drinking water river sources exhibit multi - drug resistant trait. *Environmental Monitoring and Assessment*. https://doi.org/10.1007/s10661-024-13117-9

Pradhan, S., & Rai, L. C. (2000). Optimization of flow rate, initial metal ion concentration and biomass density for maximum removal of Cu2+ by immobilized Microcystis. *World Journal of Microbiology and Biotechnology*, *16*(6), 579–584. https://doi.org/10.1023/A:1008987908001

Priyadarshanee, M., & Das, S. (2021). Biosorption and removal of toxic heavy metals by metal tolerating bacteria for bioremediation of metal contamination: A comprehensive review. *Journal of Environmental Chemical Engineering*, *9*(1), 104686. https://doi.org/10.1016/j.jece.2020.104686

R Ananthashankar, A. G. (2013). Production, Characterization and Treatment of Textile Effluents: A Critical Review. *Journal of Chemical Engineering & Process Technology*, *05*(01), 1–18. https://doi.org/10.4172/2157-7048.1000182

Rai, P. K., Lee, S. S., Zhang, M., Tsang, Y. F., & Kim, K. H. (2019). Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environment International*, *125*(November 2018), 365–385. https://doi.org/10.1016/j.envint.2019.01.067

Raimi, M. O., Odubo, T. V., & Omidiji, A. O. (2021). Creating the Healthiest Nation: Climate Change and Environmental Health Impacts in Nigeria: A Narrative Review. *Sustainability in Environment*, *6*(1), p61. https://doi.org/10.22158/se.v6n1p61

Ren, S., Li, E., Deng, Q., He, H., & Li, S. (2018). Analysis of the impact of rural households' behaviors on heavy metal pollution of arable soil: Taking Lankao County as an example. *Sustainability (Switzerland)*, *10*(12). https://doi.org/10.3390/su10124368

Romera, E., González, F., Ballester, A., Blázquez, M. L., & Muñoz, J. A. (2007). Comparative study of biosorption of heavy metals using different types of algae. *Bioresource Technology*, *98*(17), 3344–3353. https://doi.org/10.1016/j.biortech.2006.09.026

Roy, P., & Kumar, A. (2020). *Pratiti Roy, Ashok Kumar*. 3–11. https://doi.org/10.1016/B978-0-12-823414-3.00001-0

Sahu, O., Rao, D. G., Gopal, R., Tiwari, A., & Pal, D. (2017). Treatment of wastewater from sugarcane process industry by electrochemical and chemical process: Aluminum (metal and salt). *Journal of Water Process Engineering*, *17*, 50–62. https://doi.org/10.1016/j.jwpe.2017.03.005

Sandeep, G., Vijayalatha, K. R., & Anitha, T. (2019). Heavy metals and its impact in vegetable

crops. ~ *1612 ~ International Journal of Chemical Studies*, *7*(1), 1612–1621.
Sankararamakrishnan, N., Jaiswal, M., & Verma, N. (2014). Composite nanofloral clusters of carbon nanotubes and activated alumina: An efficient sorbent for heavy metal removal. *Chemical Engineering Journal*, *235*, 1–9. https://doi.org/10.1016/j.cej.2013.08.070
Sardella, F., Gimenez, M., Navas, C., Morandi, C., Deiana, C., & Sapag, K. (2015). Conversion of viticultural industry wastes into activated carbons for removal of lead and cadmium. *Journal of Environmental Chemical Engineering*, *3*(1), 253–260. https://doi.org/10.1016/j.jece.2014.06.026
Sarkar, D., Ferguson, M., Datta, R., & Birnbaum, S. (2005). Bioremediation of petroleum hydrocarbons in contaminated soils: Comparison of biosolids addition, carbon supplementation, and monitored natural attenuation. *Environmental Pollution*, *136*(1), 187–195. https://doi.org/10.1016/j.envpol.2004.09.025
Satishkumar, N., Tevari, P., & Singh, A. (2013). A Study on Constraints Faced by Farmers in Adapting to Climate Change in Rainfed Agriculture. *Journal of Human Ecology*, *44*(1), 23–28. https://doi.org/10.1080/09709274.2013.11906639
Seo, S. N., & Mendelsohn, R. (2008). An analysis of crop choice: Adapting to climate change in South American farms. *Ecological Economics*, *67*(1), 109–116. https://doi.org/10.1016/j.ecolecon.2007.12.007
Sharma, S., Pathania, S., Bhagta, S., Kaushal, N., Bhardwaj, S., Bhatia, R. K., & Walia, A. (2024). Microbial remediation of polluted environment by using recombinant E . coli : a review. *Biotechnology for the Environment*, 1–21. https://doi.org/10.1186/s44314-024-00008-z
Singh, K., Renu, N. A., & Agarwal, M. (2017). Methodologies for removal of heavy metal ions from wastewater: an overview. *Interdisciplinary Environmental Review*, *18*(2), 124. https://doi.org/10.1504/ier.2017.10008828
Skoufias, E., Rabassa, M., & Olivieri, S. (2011). *The Poverty Impacts of Climate Change A Review of the Evidence The World Bank Poverty Reduction and Economic Management Network Poverty Reduction and Equity Unit*. *April*.
Slegers, M. F. W. (2008). Exploring farmers' perceptions of drought in Tanzania and Ethiopia. In *Doctoral Thesis*.
Sobolev, D., & Begonia, M. F. T. (2008). Effects of heavy metal contamination upon soil microbes: Lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. *International Journal of Environmental Research and Public Health*, *5*(5), 450–456. https://doi.org/10.3390/ijerph5050450
Sofoluwe, N. A., Tijani, A. A., & Baruwa, O. I. (2011). Farmers' perception and adaptation to climate change in Osun State, Nigeria. *African Journal of Agricultural Research*, *6*(20), 4789–4794.
Sowerby, A., Emmett, B., Beier, C., Tietema, A., Peñuelas, J., Estiarte, M., Van Meeteren, M. J. M., Hughes, S., & Freeman, C. (2005). Microbial community changes in heathland soil communities along a geographical gradient: Interaction with climate change manipulations. *Soil Biology and Biochemistry*, *37*(10), 1805–1813. https://doi.org/10.1016/j.soilbio.2005.02.023
Sprocati, A. R., Alisi, C., Tasso, F., Marconi, P., Sciullo, A., Pinto, V., Chiavarini, S., Ubaldi, C., & Cremisini, C. (2012). Effectiveness of a microbial formula, as a bioaugmentation agent, tailored for bioremediation of diesel oil and heavy metal co-contaminated soil. *Process Biochemistry*, *47*(11), 1649–1655. https://doi.org/10.1016/j.procbio.2011.10.001
Srivastav, A. L. (2020). Chemical fertilizers and pesticides: role in groundwater contamination. In *Agrochemicals Detection, Treatment and Remediation: Pesticides and Chemical*

Fertilizers. LTD. https://doi.org/10.1016/B978-0-08-103017-2.00006-4

Sun, J., Liu, L., & Yang, F. (2020). A WO3/PPy/ACF modified electrode in electrochemical system for simultaneous removal of heavy metal ion Cu2+ and organic acid. *Journal of Hazardous Materials*, *394*(November 2019), 122534. https://doi.org/10.1016/j.jhazmat.2020.122534

Tak, H. I., Ahmad, F., & Babalola, O. O. (2013). Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. *Reviews of Environmental Contamination and Toxicology*, *223*, 33–52. https://doi.org/10.1007/978-1-4614-5577-6_2

Tariq, M., Waseem, M., Rasool, M. H., Zahoor, M. A., & Hussain, I. (2019). Isolation and molecular characterization of the indigenous Staphylococcus aureus strain K1 with the ability to reduce hexavalent chromium for its application in bioremediation of metal-contaminated sites. *PeerJ*, *2019*(10), 1–20. https://doi.org/10.7717/peerj.7726

Teh, C. Y., & Wu, T. Y. (2020). *The Potential Use of Natural Coagulants and Flocculants in the Treatment of Urban Waters*. *39*(2014), 1603–1608. https://doi.org/10.3303/CET1439268

Terry, P. A., & Stone, W. (2002). Biosorption of cadmium and copper contaminated water by Scenedesmus abundans. *Chemosphere*, *47*(3), 249–255. https://doi.org/10.1016/S0045-6535(01)00303-4

Todorova, K., Velkova, Z., Stoytcheva, M., Kirova, G., Kostadinova, S., & Gochev, V. (2019). Novel composite biosorbent from Bacillus cereus for heavy metals removal from aqueous solutions. *Biotechnology and Biotechnological Equipment*, *33*(1), 730–738. https://doi.org/10.1080/13102818.2019.1610066

Tran, T. K., Chiu, K. F., Lin, C. Y., & Leu, H. J. (2017). Electrochemical treatment of wastewater: Selectivity of the heavy metals removal process. *International Journal of Hydrogen Energy*, *42*(45), 27741–27748. https://doi.org/10.1016/j.ijhydene.2017.05.156

Varga, A. (2021). Climate Change and Its Impact on Agriculture. *Acta Horticulturae et Regiotecturae*, *24*(s1), 50–57. https://doi.org/10.2478/ahr-2021-0010

Venil, C. K., Mohan, V., Lakshmanaperumalsamy, P., & Yerima, M. B. (2011). Optimization of Chromium Removal by the Indigenous Bacterium Bacillus spp. REP02 Using the Response Surface Methodology . *ISRN Microbiology*, *2011*, 1–9. https://doi.org/10.5402/2011/951694

Visser, A., Kroes, J., Van Vliet, M. T. H., Blenkinsop, S., Fowler, H. J., & Broers, H. P. (2012). Climate change impacts on the leaching of a heavy metal contamination in a small lowland catchment. *Journal of Contaminant Hydrology*, *127*(1–4), 47–64. https://doi.org/10.1016/j.jconhyd.2011.04.007

Wang, L., Sun, X., Li, S., Zhang, T., Zhang, W., & Zhai, P. (2014). Application of organic amendments to a coastal saline soil in north China: Effects on soil physical and chemical properties and tree growth. *PLoS ONE*, *9*(2), 1–9. https://doi.org/10.1371/journal.pone.0089185

Warner, C. L., Chouyyok, W., Mackie, K. E., Neiner, D., Saraf, L. V., Droubay, T. C., Warner, M. G., & Addleman, R. S. (2012). Manganese doping of magnetic iron oxide nanoparticles: Tailoring surface reactivity for a regenerable heavy metal sorbent. *Langmuir*, *28*(8), 3931–3937. https://doi.org/10.1021/la2042235

Wilson, A. M., Bailey, P. J., Tasker, P. A., Turkington, J. R., Grant, R. A., & Love, J. B. (2014). Solvent extraction: The coordination chemistry behind extractive metallurgy. *Chemical Society Reviews*, *43*(1), 123–134. https://doi.org/10.1039/c3cs60275c

Wionczyk, B., Apostoluk, W., & Charewicz, W. A. (2006). Solvent extraction of chromium (III) from spent tanning liquors with Aliquat 336. *Hydrometallurgy*, *82*(1–2), 83–92.

https://doi.org/10.1016/j.hydromet.2006.03.055
Wróbel, M., Śliwakowski, W., Kowalczyk, P., Kramkowski, K., & Dobrzyński, J. (2023). Bioremediation of Heavy Metals by the Genus Bacillus. *International Journal of Environmental Research and Public Health*, *20*(6). https://doi.org/10.3390/ijerph20064964
Xiang, M., Li, Y., Yang, J., Lei, K., Li, Y., Li, F., Zheng, D., Fang, X., & Cao, Y. (2021). Heavy metal contamination risk assessment and correlation analysis of heavy metal contents in soil and crops. *Environmental Pollution*, *278*. https://doi.org/10.1016/j.envpol.2021.116911
Xu, X., Hui, D., King, A. W., Song, X., Thornton, P. E., & Zhang, L. (2015). Convergence of microbial assimilations of soil carbon, nitrogen, phosphorus, and sulfur in terrestrial ecosystems. *Scientific Reports*, *5*(October), 1–8. https://doi.org/10.1038/srep17445
Yabe, J., Ishizuka, M., & Umemura, T. (2010). Current levels of heavy metal pollution in Africa. *Journal of Veterinary Medical Science*, *72*(10), 1257–1263. https://doi.org/10.1292/jvms.10-0058
Yadav, K. K., Gupta, N., Kumar, V., & Singh, J. K. (2017). Bioremediation of heavy metals from contaminated sites using potential species: A review. *Indian Journal of Environmental Protection*, *37*(1), 65–84.
Yee, N., & Fein, J. (2001). Cd adsorption onto bacterial surfaces: A universal adsorption edge? *Geochimica et Cosmochimica Acta*, *65*(13), 2037–2042. https://doi.org/10.1016/S0016-7037(01)00587-7
Zaky, M. M. M., Salem, M. A. M., Persson, K. M. M., & Eslamian, S. (2011). Incidence of Aeromonas species isolated from water and fish sources of Lake Manzala in Egypt. *International Journal of Hydrology Science and Technology*, *1*(1–2), 47–62. https://doi.org/10.1504/IJHST.2011.040740
Zandalinas, S. I., Fritschi, F. B., & Mittler, R. (2021). Global Warming, Climate Change, and Environmental Pollution: Recipe for a Multifactorial Stress Combination Disaster. *Trends in Plant Science*, *26*(6), 588–599. https://doi.org/10.1016/j.tplants.2021.02.011
Zhu, W. P., Sun, S. P., Gao, J., Fu, F. J., & Chung, T. S. (2014). Dual-layer polybenzimidazole/polyethersulfone (PBI/PES) nanofiltration (NF) hollow fiber membranes for heavy metals removal from wastewater. *Journal of Membrane Science*, *456*, 117–127. https://doi.org/10.1016/j.memsci.2014.01.001

APPENDICES

Appendix 1: List of publication

4. Mendy, P. A., Kone, M., Ahmed, A. B., Yaffa, S., Kargbo, A., Amankwah, A. A., Agboka, K.-M. J., Okorie, B. C., Sowe, A., & Jatta, E. (2024). Farmers' Knowledge and Perceptions of the Effects of Climate Variability and Pollution on Crop Production and Their Varying Adaptation Strategies in The Gambia. *American Journal of Climate Change*, *13*, 543-566. https://doi.org/10.4236/ajcc.2024.133025

Appendix 2: The Map of the Sampling Sites

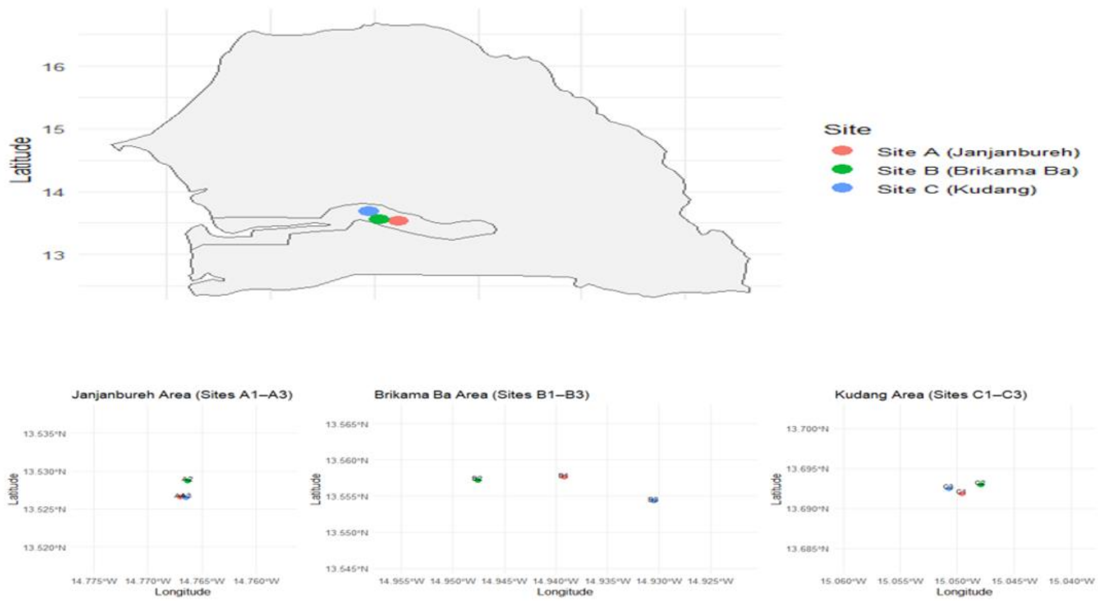


Figure 1: The map of the sampling sites in the Central River Region South (Generated by Dam Khan, 2025)

Appendix 3: Pictures of Sampling Sites

Figure 2: Field Assistants at Kudang, Brikama Ba, and Janjanbureh (*Photo by Pierre Anthony Mendy*, 2025)

Appendix 4: Pictures of Microbiology Laboratory at Medical Research Council, The Gambia, at the London School of Hygiene and Tropical Medicine

Figure 3: Research Team at Medical Research Council, The Gambia at London School of Hygiene and Tropical Medicine (*Photo by Pierre Anthony Mendy*, 2025).

Appendix 5: Bacterial Isolation and Microscopy Characterization

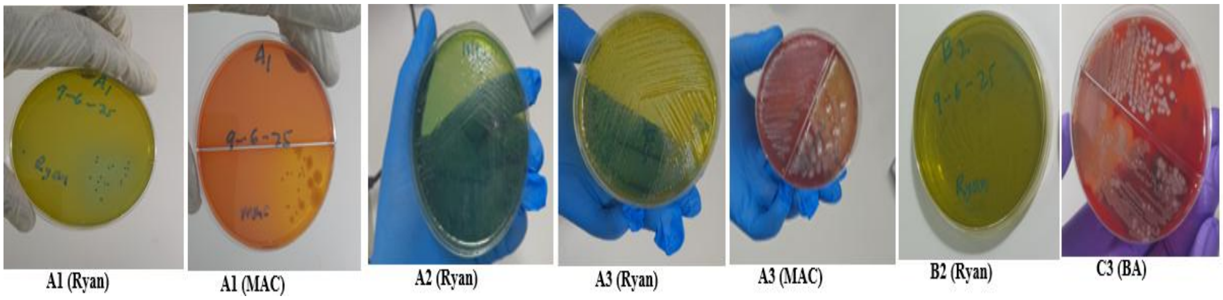


Figure 4: Bacterial colonies on Nutrient Agar after 24h incubation.

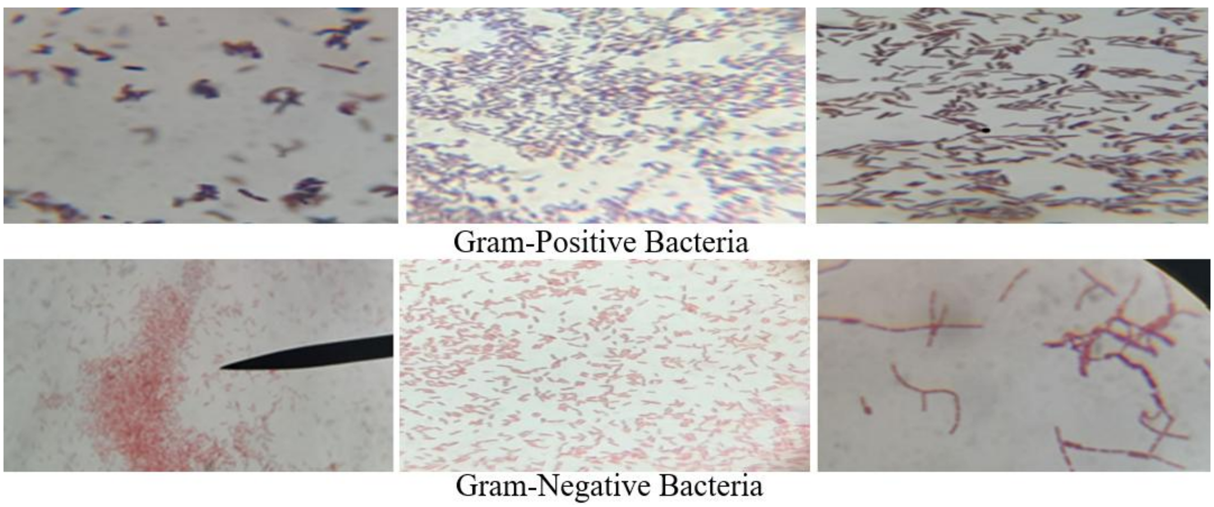


Figure 5: Gram Stain Microscopy of Isolated Bacteria.

PUBLICATION