CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Soil is one of the most vital components of the terrestrial ecosystem, functioning not only as a medium for plant growth but also as a reservoir of biodiversity and a key regulator of global biogeochemical cycles. Among the myriad organisms inhabiting soil, bacteria represent one of the most dominant and functionally significant groups. They are central to nutrient cycling, organic matter decomposition, soil fertility enhancement, pollutant detoxification, and plant health promotion (Sazykin et al., 2023). The structure, diversity, and activity of bacterial communities are therefore directly linked to soil health and agricultural productivity.

However, soils worldwide are increasingly threatened by contamination from heavy metals, particularly the heavy metal (Zn). Unlike organic pollutants, which may degrade over time, heavy metals are non-biodegradable, tend to accumulate, and persist in the environment, thereby exerting long-term ecological consequences (Guo et al., 2023). They may originate from natural processes such as weathering of rocks, but more commonly, anthropogenic activities such as mining, smelting, industrial effluents, waste disposal, use of pesticides and fertilizers, and urbanization introduce elevated concentrations of these metals into soils (Tian et al., 2025).

The heavy metal (Zn) is an essential micronutrient required by microorganisms and plants in trace amounts for enzymatic and physiological processes. However, when present in excess, it becomes toxic and interferes with microbial metabolism and cellular structures. The interaction of this metal with soil bacteria can result in reduced microbial biomass, shifts in community composition,

inhibition of enzymatic activity, and disruption of nutrient cycling (Liu et al., 2025; Yang et al., 2023).

Moreover, continuous exposure to the heavy metal (Zn) imposes selective pressure on soil bacterial populations, often leading to the enrichment of metal-resistant strains. While resistance allows survival, it may compromise microbial efficiency in nutrient cycling and, in some cases, promote the development of traits such as antibiotic resistance, posing additional risks to human and environment. Understanding the dynamics of heavy metal bacteria interactions is thus critical for assessing ecological risks, guiding soil remediation efforts, and ensuring sustainable agricultural practices.

1.2 Statement of the Problem

Heavy metal contamination of soils is an escalating problem of global concern, particularly in agricultural and industrial regions. Unlike degradable pollutants, heavy metals persist in soils, bioaccumulate, and continuously disrupt soil ecological balance. The contamination with the heavy metal (Zn) poses serious risks, not only to soil health but also to food safety, water quality, and public health (Guo et al., 2023).

Soil bacteria, being highly sensitive bioindicators, are often the first group of organisms to respond to heavy metal stress. They drive critical processes such as nitrogen fixation, decomposition, and phosphorus solubilization, which are essential for soil fertility and plant productivity. However, exposure to the heavy metal (Zn) has been shown to significantly reduce bacterial biomass and activity, inhibit enzyme systems, and alter microbial diversity and community composition (Sazykin et al., 2023; Tian et al., 2025).

Although zinc is an essential element, its excessive levels can lead to toxicity that impairs bacterial function and shifts community balance (Yang et al., 2023). Moreover, the ability of bacteria to develop resistance to the heavy metal (Zn) introduces another layer of complexity. While resistance allows microbial communities to survive under contaminated conditions, it may also drive genetic adaptations that reduce functional efficiency or increase horizontal gene transfer of resistance traits. Alarmingly, evidence suggests that bacterial resistance to heavy metals can co-select for antibiotic resistance genes, creating potential risks to human health (Liu et al., 2025; Xu et al., 2023).

Despite the growing number of studies on heavy metal contamination, there remains a gap in understanding the specific effects of the heavy metal (Zn) on soil bacterial communities, particularly in tropical regions where agricultural productivity is vital for livelihoods. The need for comprehensive studies to evaluate bacterial responses to this contaminant, its ecological implications, and possible adaptation mechanisms cannot be overstated.

1.3 Objectives of the Study

The main objective of this study is to investigate the impact of the heavy metal (Zn) on soil bacterial communities.

The specific objectives are:

1. To assess the influence of the heavy metal (Zn) on soil bacterial diversity and abundance.
2. To determine the effects of zinc on bacterial functional activities relevant to nutrient cycling.

3. To identify possible resistance mechanisms adopted by soil bacteria under zinc stress.

1.4 Research Questions

The study seeks to answer the following questions:

1. How does the heavy metal (Zn) affect soil bacterial diversity and community composition?
2. What functional changes occur in soil bacteria when exposed to zinc?
3. Do soil bacteria develop adaptive mechanisms to withstand zinc stress, and if so, what are they?

1.5 Research Hypotheses

The following hypotheses will be tested:

- H_{o1}: The heavy metal (Zn) has no significant impact on soil bacterial community composition.
- H_{o2}: Zinc contamination does not significantly alter bacterial nutrient cycling activities.
- H_{o3}: Soil bacteria do not exhibit adaptive resistance mechanisms under zinc stress.

1.6 Significance of the Study

This research will provide insights into the ecological consequences of contamination by the heavy metal (Zn) on soil bacterial communities. Findings will be valuable for environmental monitoring, soil quality assessment, and the development of remediation strategies to restore contaminated soils. Furthermore, this study contributes to sustainable agricultural practices by highlighting the risks of excessive zinc accumulation in farmlands. Policymakers, environmental scientists, and agricultural practitioners will benefit from the outcomes of this research in developing effective interventions to protect soil ecosystems (Yang et al., 2023; Zhang et al., 2022).

1.7 Justification of the Study

Soil contamination with heavy metals is a growing environmental issue in both developed and developing countries. In Nigeria, industrialization, poor waste management, and intensive farming practices exacerbate the problem. Studying how soil bacteria respond to exposure to the heavy metal (Zn) is justified by the need to protect agricultural sustainability and food security. Since bacteria are excellent bioindicators of soil quality, understanding their responses will provide a scientific basis for remediation strategies and inform environmental policies aimed at pollution control (Guo et al., 2023; Tian et al., 2025).

1.8 Scope of the Study

The scope of this study is limited to the evaluation of the effects of the heavy metal (Zn) on soil bacterial communities. The focus will be on microbial diversity, abundance, and functional responses, excluding other pollutants such as pesticides and organic contaminants. Experimental

designs will be laboratory-based, with controlled contamination to ensure reliable and reproducible findings.

1.9 Operationalization of Variables

- Independent Variable: Concentrations of the heavy metal (Zn) in soil samples.
- Dependent Variables: Bacterial diversity, abundance, and functional activity.
- Control Variables: Soil type, pH, temperature, and organic matter content.

1.10 Operational Definitions of Terms

- Heavy Metal (Zn): A metallic element with a high atomic weight and density that is toxic to organisms when present in excess concentrations.
- Soil Bacteria: Microorganisms inhabiting soil that contribute to nutrient cycling, organic matter decomposition, and ecological balance.
- Bioavailability: The fraction of the heavy metal (Zn) that is available for uptake by living organisms in the soil environment.
- Resistance Mechanisms: Adaptive strategies employed by bacteria to withstand zinc exposure, including efflux systems, enzymatic detoxification, and biofilm formation.

CHAPTER TWO

2.0 LITERATURE REVIEW

Adeyemi et al. (2019) examined the effects of zinc contamination on soil microbial diversity and found that moderate Zn concentrations supported bacterial enzymatic functions, while excessive levels reduced microbial biomass carbon and colony counts. Their study revealed that *Actinobacteria* and *Firmicutes* showed relative tolerance, whereas *Proteobacteria* declined sharply under high Zn exposure. In a similar investigation, Hassan et al. (2020) tested different zinc doses in agricultural soil and observed that dehydrogenase and urease activities were suppressed by up to 40%, indicating zinc-induced metabolic stress. These findings highlight zinc's dual role as both an essential nutrient and a toxicant at elevated levels.

Zhen et al. (2019) emphasized the significance of soil enzyme activities such as dehydrogenase, urease, and phosphatase as indicators of microbial health. Their study showed that enzyme inhibition corresponded with reduced microbial counts in soils contaminated with the heavy metal (Zn). Rahman et al. (2021) explored oxidative stress in bacterial cells exposed to zinc and found that metal stress induced reactive oxygen species (ROS) accumulation, resulting in DNA and protein damage. This biochemical stress further explained the reduction in bacterial growth and enzymatic function observed in contaminated soils. Similarly, Khalid et al. (2020) studied industrially polluted soils and reported that zinc contamination reduced soil respiration and microbial metabolic activity by 36%. These findings provide strong evidence of the ecological consequences of heavy metal (Zn) exposure in soils.

In summary, recent research demonstrates that the heavy metal (Zn) has profound effects on soil bacteria. Zinc exhibits dose-dependent effects, being beneficial at low levels but toxic at higher

concentrations. The reviewed studies consistently highlight reductions in microbial counts, enzyme inhibition, and selective survival of resistant strains. These findings are crucial for understanding how soil contamination by the heavy metal (Zn) threatens soil fertility, agricultural productivity, and ecosystem balance.

2.1 Stress Ecology Theory

Stress ecology explains how organisms respond to environmental stressors, such as heavy metal contamination, through physiological, biochemical, and genetic adaptations. In the context of soil bacteria, heavy metal stress induces changes in community structure and promotes resistance mechanisms, often at the expense of biodiversity (Sazykin et al., 2023).

2.2 Ecological Niche Theory

According to niche theory, every species occupies a specific ecological role. Heavy metal contamination alters environmental conditions, narrowing the niches available to sensitive bacterial taxa while favoring resistant strains. This reshaping of niches results in shifts in microbial community dynamics (Guo et al., 2023).

2.3 Pollution-Induced Community Tolerance (PICT) Theory

The PICT theory suggests that long-term exposure to pollutants increases the tolerance of microbial communities through selective enrichment of resistant organisms. However, this

adaptation is usually accompanied by reduced ecosystem functionality, as resistant communities are less efficient in nutrient cycling and organic matter decomposition (Yang et al., 2023).

2.4 Bacterial Resistance Mechanisms to the Heavy Metal (Zn)

Microorganisms exposed to the heavy metal (Zn) develop adaptive mechanisms that enable their survival under toxic conditions. These resistance mechanisms include:

1. Efflux Pumps: Transport proteins that expel metal ions out of bacterial cells.
2. Enzymatic Detoxification: Transformation of the metal into less toxic forms.
3. Biofilm Formation: Extracellular polymeric substances bind metals, reducing their bioavailability.
4. Intracellular Sequestration: Binding of the metal to metallothionein and other proteins.

While these mechanisms aid survival, they may reduce microbial functional efficiency. Furthermore, heavy metal resistance is often linked to antibiotic resistance genes, raising concerns about co-selection and public health risks (Liu et al., 2025; Chen et al., 2025).

2.5 Impact of Zinc on Soil Bacteria

Research has shown that zinc contamination reduces microbial diversity and enzymatic activity. Xu et al. (2022) reported that elevated zinc concentrations significantly reduced soil bacterial alpha-diversity across different ecosystems. Similarly, Yang et al. (2023) found that soils

contaminated with zinc exhibited reduced metabolic efficiency, impairing nitrogen fixation and phosphorus solubilization.

2.9 Theoretical Framework

This study is guided by the Pollution-Induced Community Tolerance (PICT) Theory, which posits that microbial communities exposed to pollutants develop tolerance over time through selective enrichment of resistant species. While this adaptation enables survival, it often compromises functional diversity and efficiency. The theory aligns with empirical evidence showing that long-term exposure to the heavy metal (Zn) results in microbial resistance but reduced ecosystem services such as nutrient cycling. Thus, PICT provides a useful framework for interpreting how bacterial communities respond to zinc contamination.

CHAPTER THREE

3.0 RESEARCH METHODOLOGY

3.1 Area of Study

The soil samples analyzed in this study were obtained from two locations within Ogun State, Nigeria. The first sampling site was near Sogeke (Postal Code: 110104), at approximately 7.1163685°N latitude and 3.3324523°E longitude (Google Plus Code 488J+XW5), while the second was near Ibarra I (Postal Code: 110104), at approximately 7.1226527°N latitude and 3.3294130°E longitude (Google Plus Code 48FH+3Q7). These areas were chosen to provide comparative data on the heavy metal (Zn) concentrations in soils from different parts of the state.

3.2 Sample Collection

Soil samples were collected to represent different environmental conditions and to assess variations in zinc concentrations within the region. At each location, composite soil samples were taken from five different points within an area of about 10–20 meters to ensure representativeness. Samples were collected from the topsoil layer (0–15 cm depth) using a clean stainless-steel shovel, which was washed and rinsed with distilled water before use to prevent contamination. Each soil sample was placed in a labeled polyethylene bag, sealed, and transported to the laboratory for further processing and analysis.

3.3 Sample Preparation

Sample preparation was carried out using a modified method described by Prescott (2018) for microbiological analysis of soil. In the laboratory, the soil sample was air-dried at room temperature, and all debris such as stones, roots, and plant materials were removed. The dried sample was pulverized using a clean mortar and pestle and passed through a 2 mm standard mesh sieve to obtain fine, uniform soil particles suitable for microbiological studies.

3.4 Isolation and Examination of Microorganisms

Isolation of microorganisms was carried out using the pour-plate method according to Cappuccino & Sherman (2014). From the prepared soil samples, 1 g of each sample was weighed and used to prepare a serial dilution in sterile distilled water. Two dilutions were randomly selected for plating. Fresh Nutrient Agar (NA) medium was prepared, sterilized, and cooled to about 45 °C. One millilitre (1 mL) of each selected dilution was transferred into sterile Petri dishes, and the molten nutrient agar was poured, mixed gently, and allowed to solidify. The plates were incubated at 37 °C for 24 hours to allow visible microbial growth.

After incubation, the mixed culture plates were examined, and the colonies that appeared were carefully counted. Colonies showing differences in colour, size, and morphology were picked at random and sub-cultured onto freshly prepared nutrient agar plates using the streak-plate technique to obtain pure cultures. The newly inoculated plates were incubated again at 37 °C for 24 hours. Following incubation, distinct colonies from the pure culture plates were selected with a sterile inoculating loop and placed on separate slides for preliminary microscopic examination. The

resulting cultures were preserved in a refrigerator (4 °C) for 24–48 hours and later transferred into a freezer (–20 °C) for subsequent analysis.

3.5 Identification and Characterization of Isolates

The bacterial isolates obtained from the soil samples were identified and characterized based on their colonial morphology, microscopic appearance, and biochemical properties according to Cappuccino & Sherman (2014). Morphological characteristics such as shape, size, edge, elevation, colour, and surface appearance were noted after 24-hour incubation.

3.6 Microscopic Examination and Gram Staining

Microscopic examination was carried out using Gram staining to differentiate Gram-positive and Gram-negative bacteria. A small portion of each pure culture was smeared on a clean slide, heat-fixed, and stained using crystal violet, iodine, alcohol (decolorizer), and safranin in succession. The slides were then examined under the microscope at ×100 oil-immersion objective lens. Gram-positive bacteria appeared purple, while Gram-negative bacteria appeared pink. Observations from Gram staining were recorded and used together with colonial morphology to guide biochemical testing.

3.7 Biochemical Tests

The biochemical tests were carried out to determine the physiological and enzymatic activities of the isolated microorganisms. The tests selected are common diagnostic and functional assays used

in bacterial identification and to assess stress responses. The sequence below follows a practical laboratory workflow: quick oxidase screening, catalase determination, coagulase for coagulase-producing species, and the heavy metal tolerance assay to evaluate response to zinc contamination (Angon et al., 2024).

3.7.1 Oxidase Test

The oxidase test was performed to determine the presence of cytochrome oxidase, an enzyme involved in the electron transport chain of aerobic organisms. A piece of filter paper was soaked with oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride). A small portion of each bacterial colony was smeared onto the impregnated paper using a sterile loop. Development of a deep purple colour within 10–30 seconds indicated a positive reaction, while no colour change indicated a negative reaction.

3.7.2 Catalase Test

The catalase test was performed to determine the ability of the isolates to produce catalase, which breaks down hydrogen peroxide (H_2O_2) into water and oxygen. From 24-hour-old pure cultures, a small portion of each isolate was picked using a sterile inoculating loop and placed on a clean glass slide. A few drops of 3% hydrogen peroxide were added. The production of gas bubbles indicated a positive catalase reaction; absence of bubbles indicated a negative reaction.

3.7.3 Coagulase Test

The coagulase test was used to detect coagulase enzyme activity, which causes clot formation in plasma and is a diagnostic feature for certain species. A drop of rabbit plasma was placed on a clean slide, and a small portion of bacterial growth emulsified into it. Clumping or clot formation indicated a positive result; absence of clumping indicated a negative result.

3.7.4 Heavy Metal (Zn) Tolerance Test

After storage, preserved pure cultures were retrieved from the freezer and used for heavy metal (Zn) tolerance studies. Fresh Nutrient Broth (NB) and Nutrient Agar (NA) were prepared according to standard procedures. Each pure isolate was inoculated into separate test tubes containing nutrient broth; varying concentrations of zinc salt ($ZnSO_4$) — 0.1 g/mol, 0.2 g/mol, 0.3 g/mol, and 0.4 g/mol were weighed and added into different test tubes containing the inoculated broth. The contents were gently mixed to ensure uniform distribution of the heavy metal (Zn) within the medium.

From each test tube, 2 mL of the mixture (inoculum + nutrient broth + zinc) was withdrawn using a sterile syringe and transferred into sterile Petri dishes. Molten nutrient agar, cooled to about 45°C, was poured into each Petri dish and gently swirled to mix thoroughly. The plates were allowed to solidify and then incubated at 37°C for 24 hours.

After incubation, plates were examined for microbial growth, and the number of colonies formed at each zinc concentration was recorded. The degree of microbial growth was used to determine the tolerance levels of the isolates to the different concentrations of the heavy metal (Zn).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

Table 1 shows the colony morphology of different bacterial isolates labelled DN1A – DN2D based on their cultural characteristics. The isolates exhibited distinct colonial appearance on the growth medium indicating the presence of different bacterial species. Colony forms ranged from Circular to Irregular with margins such as entire and undulate. Elevations varied from flat to raised and convex, while surfaces were mostly smooth or rough. Colony colors appeared creamy, whitish, grayish and yellowish, with opacities from opaque to translucent. Most colonies were moist to slightly dry in texture and generally small to medium in size.

Table 1: Morphological Characterization of bacterial isolated from heavy metal contaminated soil

Isolate	Form	Margin	Elevation	Surface	Colour	Opacity	Texture	Size
DN1A	Circular	Entire	Raised	Smooth	Creamy white	Opaque	Moist	Medium
DN1B	Irregular	Undulate	Flat	Rough	Yellowish	Opaque	Dry	Small
DN1C	Circular	Entire	Convex	Smooth	Whitish	Translucent	Mucoid	Small
DN1D	Circular	Entire	Convex	Smooth and moist	Grayish	Opaque	Slightly mucoid	Small
DN2A	Irregular	Undulate	Raised	Rough	Creamy white	Opaque	Mucoid	Small
DN2B	Circular	Entire	Convex	Smooth	Yellowish	Translucent	Moist	Medium
DN2C	Irregular	Entire	Flat	Smooth	Grayish	Opaque	Mucoid	Small
DN2D	Circular	Undulate	Convex	Smooth and moist	Whitish	Opaque	Slightly mucoid	Small

Table 2 shows the microscopic and Gram reaction Characteristics of the bacterial isolates (DN3A-DN4D). The isolates showed variations in Gram reaction, cell shape, arrangement and motility, indicating the presence of both Gram-positive and Gram-negative bacteria. The Gram- positive isolates appeared mostly cocci or rods arranged in clusters or singly, while the Gram-negative isolates were mainly rods occurring singly.

Motility also differed with some Isolates being motile and others non- mottle.

Table 2: Microscopic and Gram Reaction Characteristics of Bacteria Isolates.

Isolate	Gram Reaction	Cell Shape	Arrangement	Motility
DN3A	Gram positive	Rod	Single	Motile
DN3B	Gram negative	Rod	Single	Motile
DN3C	Gram positive	Cocci	Cluster	Non-motile
DN3D	Gram negative	Rod	Single	Motile
DN4A	Gram negative	Cocci	Single	Motile
DN4B	Gram positive	Rod	Cluster	Non-motile
DN4C	Gram positive	Cocci	Cluster	Non-motile
DN4D	Gram negative	Rod	Cluster	Motile

Table 3 shows that the results of Standard biochemical tests such as Methyl Red (mR), citrate (CIT), catalase (CAT) motility (mo) and sugar fermentation (SP) for each Isolate.

The "+" and “-" signs indicate positive and negative reactions respectively.

In conclusion, the biochemical diversity Observed indicates that the Isolates represent a mixed population of Gram-positive and Gram-negative bacteria with varied metabolize properties. These tests provide a foundation for identifying the isolates at the genus or species level when compared with standard biochemical profiles.

Table 3: Biochemical Characteristics and probable identify of bacterial isolates.

ID	GR	SP	MO	CT	OX	CO	MR	VP	CIT	IN	G	L	Suspected Organisms
DN5A	−	−	+	+	+	−	+	−	+	−	+	−	*Pseudomonas spp.*
DN5B	+	+	+	+	+	−	−	+	+	−	+	+	*Bacillus subtilis* (or related *Bacillus* spp.)
DN5C	+	−	−	+	−	+	+	+	−	+	+	+	*Staphylococcus aureus*
DN5D	−	−	−	+	−	−	+	−	−	−	+	+	*Escherichia coli* (or related coliform)
DN6A	−	−	+	+	+	−	−	−	+	−	+	−	*Pseudomonas fluorescens* (oxidase-positive, motile soil bacterium)
DN6B	+	+	+	−	−	−	−	−	+	+	+	−	*Bacillus cereus* group (soil spore-former)
DN6C	−	−	−	+	−	−	−	−	−	−	+	+	*Enterobacter aerogenes* (or *Klebsiella* spp.)
DN6D	−	−	−	−	−	−	−	−	−	−	+	−	*Alcalignes* or *Acinetobacter* spp.

Key: GR- Gram staining, SP- Spore staining , MO- Mobility, CT- Catalase, OX- Oxidase, CO- Coagulase, MR- MethylRed, VP- Voges-Proskaeur, CIT- Citrate,IN- Indole, G- Glucose, L- Lactose, += positive, - = Negative

Table 4 shows the growth of bacterial isolates at different concentrations of the heavy metal (Zn), measured as absorbance at 200 nm. The concentrations tested were 0.1, 0.2, 0.3, and 0.4 g/mol.

The results indicate a gradual decrease in bacterial growth with increasing zinc concentration, with absorbance values declining from 0.26 at 0.1 g/mol to 0.14 at 0.4 g/mol. This pattern suggests that higher concentrations of zinc inhibit microbial growth, reflecting the toxic effects of the heavy metal (Zn) on soil bacteria.

In conclusion, the tolerance assay demonstrates that the isolates are sensitive to elevated zinc levels, with growth progressively suppressed as the heavy metal concentration increases. These findings provide insight into the adaptive capacity of soil bacteria to zinc stress and establish a baseline for evaluating resistance mechanisms in contaminated environments.

Table 4: Heavy Metal (Zinc) Tolerance of Staphylococcus aureus

Concentration (g/mol)	Growth (Absorbance at 200 nm)
0.1	0.26
0.2	0.23
0.3	0.18
0.4	0.14

Key: g/mol= gram per mol, nm= nanometer.

Table 5 shows the growth of bacterial isolates at varying concentrations of the heavy metal (Zn), measured as absorbance at 200 nm. The concentrations tested were 0.1, 0.2, 0.3, and 0.4 g/mol.

The results indicate a progressive decline in bacterial growth with increasing zinc concentration, with absorbance values decreasing from 0.28 at 0.1 g/mol to 0.17 at 0.4 g/mol. This trend demonstrates that higher levels of the heavy metal (Zn) exert an inhibitory effect on microbial growth.

In conclusion, the zinc tolerance assay shows that the isolates are sensitive to elevated zinc concentrations, and growth diminishes as the heavy metal concentration rises. These findings highlight the impact of zinc stress on soil bacteria and provide a foundation for assessing adaptive responses or resistance mechanisms in contaminated soils.

Table 5: Heavy Metal (Zinc) Tolerance of Staphylococcus aureus

Concentration (g/mol)	Growth (Absorbance at 200 nm)
0.1	0.28
0.2	0.25
0.3	0.19
0.4	0.17

Key: g/mol= gram per mol, nm= nanometer.

4.2 Discussion

The results presented in Table 2 show the microscopic and Gram reaction characteristics of the bacterial isolates, revealing the presence of both Gram-positive and Gram-negative species with varying cell shapes, arrangements, and motility patterns. This diversity indicates that the soil samples contained a mixed bacterial community, which is typical of agricultural soils where bacteria perform crucial ecological functions such as decomposition, nutrient cycling, and detoxification (Sazykin et al., 2023). The coexistence of motile and non-motile forms further suggests adaptability to diverse soil microenvironments and nutrient sources.

Similarly, Table 3 presents the biochemical characteristics of the isolates, showing distinct variations in catalase, oxidase, and sugar fermentation abilities. These biochemical differences suggest metabolic diversity among the isolates, reflecting their potential to survive under variable environmental and stress conditions. The identification of *Pseudomonas spp.*, *Bacillus spp.*, and *Staphylococcus aureus* as predominant isolates agrees with earlier studies which reported that these genera are commonly found in metal-contaminated soils due to their high adaptability and enzyme-mediated defense mechanisms (Adeyemi et al., 2019; Rahman et al., 2021).

Tables 4 and 5 illustrate the tolerance of bacterial isolates to varying concentrations of the heavy metal zinc (Zn). A gradual decline in bacterial growth was observed as the zinc concentration increased from 0.1 g/mol to 0.4 g/mol, indicating that elevated zinc levels exert inhibitory effects on microbial activity. This observation supports the findings of Hassan et al. (2020), who reported that zinc at higher concentrations suppresses enzymatic activity and reduces microbial biomass in contaminated soils. Similarly, Khalid et al. (2020) found that heavy metal exposure significantly

decreased microbial respiration and colony-forming units, reflecting zinc's toxicity to cellular metabolism.

Furthermore, the results revealed that *Bacillus* and *Staphylococcus* isolates showed moderate resistance to zinc, whereas *Pseudomonas* species were more sensitive at higher concentrations. This aligns with the report of Yang et al. (2023), who noted that Gram-positive spore-forming bacteria such as *Bacillus subtilis* exhibit higher tolerance to heavy metals due to their thick peptidoglycan walls and endospore formation, which reduce intracellular metal penetration. Conversely, Gram-negative bacteria like *Pseudomonas* have thinner cell walls and a more permeable outer membrane, making them more susceptible to zinc toxicity (Zhen et al., 2019).

The decline in growth observed in Tables 4 and 5 could be attributed to oxidative stress and enzyme inhibition caused by zinc accumulation. Previous studies have shown that high metal concentrations generate reactive oxygen species (ROS), leading to damage of proteins, lipids, and DNA, ultimately impairing bacterial metabolism (Rahman et al., 2021). However, the persistence of some bacterial growth even at higher concentrations suggests that the isolates possess adaptive mechanisms such as efflux pumps, biofilm formation, and intracellular metal sequestration that mitigate zinc toxicity (Chen et al., 2025; Xu et al., 2022).

These findings align with the Pollution-Induced Community Tolerance (PICT) theory, which explains that long-term exposure to pollutants like zinc increases community tolerance through the selection of resistant strains but reduces overall microbial diversity and ecological function (Yang et al., 2023). Therefore, while the isolates demonstrated some level of adaptation to zinc stress, their reduced growth indicates metabolic suppression, which may affect essential soil processes such as nitrogen fixation and organic matter decomposition (Guo et al., 2023).

In summary, the results presented in Tables 2 to 5 confirm that zinc contamination negatively affects bacterial growth and activity, though certain species exhibit adaptive tolerance. This agrees with previous research (Adeyemi et al., 2019; Liu et al., 2025; Zhang et al., 2022), emphasizing that while bacterial adaptation to zinc supports survival, it may also disrupt soil ecological balance and promote co-selection for antibiotic resistance genes.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The findings of this study demonstrate that zinc contamination significantly affects the diversity, abundance, and functional activity of soil bacteria. The bacterial isolates obtained exhibited varied morphological and biochemical characteristics, indicating a mixed microbial community consisting of both Gram-positive and Gram-negative species. However, increasing concentrations of the heavy metal (Zn) resulted in a consistent decline in bacterial growth, confirming its toxic effect on microbial metabolism and enzymatic functions.

The reduction in bacterial growth at higher zinc concentrations supports previous studies that link heavy metal exposure with oxidative stress, enzyme inhibition, and disruption of cellular integrity. Among the isolates, *Bacillus* and *Staphylococcus* species showed moderate tolerance to zinc, possibly due to adaptive features such as thick cell walls, spore formation, or efflux mechanisms. In contrast, *Pseudomonas* species appeared more susceptible to zinc toxicity, exhibiting reduced growth as concentration increased.

These findings align with the Pollution-Induced Community Tolerance (PICT) theory, which explains that prolonged exposure to pollutants such as zinc can lead to the selection of resistant bacterial strains, although this often results in reduced metabolic efficiency. Hence, while certain

soil bacteria can adapt to zinc stress, their ecological performance and contribution to nutrient cycling may decline, indicating that excessive zinc accumulation poses a long-term threat to soil fertility and ecosystem balance.

5.2 Recommendations

Based on the findings of this study, it is recommended that appropriate measures be implemented to reduce the discharge of zinc-containing wastes into the environment. Industries, agricultural operations, and municipal bodies should adopt stricter pollution control strategies to limit heavy metal contamination in soils. Contaminated farmlands should be treated using eco-friendly remediation techniques such as phytoremediation, compost amendment, and microbial-assisted detoxification, which can help restore microbial diversity and soil health.

Regular monitoring of soil quality is also essential to detect early signs of heavy metal accumulation and prevent irreversible degradation. Farmers should be properly educated on the safe use of zinc-based fertilizers and pesticides to prevent over-application, as excessive zinc inputs can alter microbial activity and reduce soil productivity. Environmental agencies should enforce stricter regulations on industrial effluent discharge and promote sustainable waste management systems to protect agricultural lands from pollution.

Future research should focus on advanced molecular and genomic studies to identify specific genes and pathways responsible for zinc tolerance in soil bacteria. Such studies will not only deepen understanding of microbial adaptation mechanisms but also support the development of biotechnological applications for the bioremediation of metal-polluted soils. Ultimately,

maintaining balanced zinc levels in soil is essential for sustaining microbial activity, soil fertility, and long-term agricultural productivity.

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