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Specialty: Microbial Biotechnology and health

The meta-omic approach in the bioremediation of heavy metals

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Dedication

To my dear parents, for your unconditional love, endless support, and unwavering belief in me. Your sacrifices and guidance have been the foundation of my journey.

To my sister and brother, for always being by my side, encouraging and inspiring me every step of the way.

To my husband, for your patience, encouragement, and unwavering support throughout this journey.

To my precious daughter, you are my greatest joy and motivation.

May this work serve as a testament to the importance of perseverance and dedication.

With all my love and gratitude.

Abstract

Heavy metal contamination poses a significant threat to ecosystems. This study initially explored the toxicological effects of heavy metals (Cr, Ni, and Al) on rats. 35 male Wistar rats were treated with two doses of LD_{50}: 1/100 and 1/50 of $K_2Cr_2O_7$, $NiCl_2$, and $AlCl_3$. During the 3-month experiment, heavy metal exposure reduced weight gain in all treated groups compared to the control group. Weight gains in the Cr_{100}, Ni_{100}, and Al_{100} groups were 0.78g, 0.89g, and 0.9g, respectively, while in the Cr_{50}, Ni_{50}, and Al_{50} groups, they were 0.61g, 0.7g, and 0.7g, respectively. The Cr_{50} group exhibited anaerobic bacterial levels of 5.39 log CFU/g, while the Ni_{100} and Ni_{50} groups showed aerobic/anaerobic bacterial levels of 5.14/6 and 5.36/5.36 log CFU/g, respectively. In the Al_{50} group, *Lactobacillus* spp levels were 2.27 log CFU/g.

In the second part, seven bacterial strains were isolated from agricultural soils and tested for their resistance and bioremediation capacity of heavy metals. Based on morphological, cultural, biochemical, and molecular characterization, the isolates were identified as follows: strain S1B10 as *Pseudomonas aeruginosa*, S1B26 as *Pseudomonas fluorescens*, S5B16 as a *Bacillus* sp, S2B1 and S6B3 as *Bacillus cereus*, strain S4B31 as *Rhodopseudomonas palustris*, and strain S5B23 as a *Planomicrobium* sp. The results revealed the MICs of the three heavy metals studied, ranging from 900 to 1600 µg/mL. AAS analysis showed that *Bacillus* sp. was the most efficient at removing Cr and Al, with bioaccumulation rates of 42.57% and 59.50%, respectively. *Pseudomonas fluorescens* exhibited the highest bioaccumulation rate for Ni, at 62.37%. When comparing the two consortia, bioremediation of Ni in soil was more efficient in C1, with a rate of 38.02%, while C2 demonstrated a higher bioremediation rate for Al, at 36.42%.

Keywords:

Heavy metals, toxicity, bacteria, bioremediation, soil.

Résumé

La contamination par les métaux lourds représente une menace importante pour les écosystèmes. Cette étude a d'abord exploré les effets toxicologiques des métaux lourds (Cr, Ni et Al) sur des rats. 35 rats mâles Wistar ont été traités avec deux doses de LD_{50} : 1/100 et 1/50 de $K_2Cr_2O_7$, $NiCl_2$ et $AlCl_3$. Au cours de l'expérience de 3 mois, l'exposition aux métaux lourds a réduit le gain de poids dans tous les groupes traités par rapport au groupe témoin. Les gains de poids dans les groupes Cr_{100}, Ni_{100} et Al_{100} étaient respectivement de 0,78 g, 0,89 g et 0,9 g, tandis que dans les groupes Cr_{50}, Ni_{50} et Al_{50}, ils étaient respectivement de 0,61 g, 0,7 g et 0,7 g. Le groupe Cr_{50} a montré des niveaux de bactéries anaérobies de 5,39 log UFC/g, tandis que les groupes Ni_{100} et Ni_{50} ont montré des niveaux de bactéries aérobies/anaérobies de 5,14/6 et 5,36/5,36 log UFC/g, respectivement. Dans le groupe Al_{50}, les niveaux de *Lactobacillus* spp étaient de 2,27 log UFC/g.

Dans la deuxième partie, sept souches bactériennes ont été isolées de sols agricoles et testées pour leur résistance et leur capacité de bioremédiation des métaux lourds. Sur la base de la caractérisation morphologique, culturelle, biochimique et moléculaire, les isolats ont été identifiés comme suit : la souche S1B10 comme *Pseudomonas aeruginosa*, S1B26 comme *Pseudomonas fluorescens*, S5B16 comme *Bacillus* sp, S2B1 et S6B3 comme *Bacillus cereus*, la souche S4B31 comme *Rhodopseudomonas palustris* et la souche S5B23 comme une espèce *Planomicrobium* sp. Les résultats ont révélé les CMI des trois métaux lourds étudiés, allant de 900 à 1600 µg/mL. L'analyse SAA a montré que *Bacillus* sp. était le plus efficace pour éliminer le Cr et l'Al, avec des taux de bioaccumulation de 42,57% et 59,50%, respectivement. *Pseudomonas fluorescens* a montré le taux de bioaccumulation le plus élevé pour le Ni, à 62,37%. En comparant les deux consortiums, la bioremédiation du Ni dans le sol était plus efficace dans le C1, avec un taux de 38,02%, tandis que le C2 a montré un taux de bioremédiation plus élevé pour l'Al, à 36,42%.

Mots clés :

Métaux lourds, toxicité, bactérie, bioremédiation, sol.

الملخص

يُشكل تلوث المعادن الثقيلة تهديدًا كبيرًا للأنظمة البيئية. استكشفت هذه الدراسة في البداية الآثار السمية للمعادن الثقيلة (الكروم، النيكل، والألمنيوم) على الجرذان. عولج 35 جرذًا ذكرًا من جرذان ويستار بجرعتين من الجرعة المميتة DL_{50}: 1/50, 1/100 من $K_2Cr_2O_7$, $NiCl_2$, $AlCl_3$. خلال التجربة التي استمرت ثلاثة أشهر أدى التعرض للمعادن الثقيلة إلى انخفاض في زيادة الوزن في جميع المجموعات المعالجة مقارنةً بالمجموعة الضابطة. بلغت زيادة الوزن في مجموعات Cr_{100} , Ni_{100} , Al_{100} الى 0.78، 0.89، 0.9 غرام على التوالي، بينما بلغت في مجموعات Ni_{50}, Cr_{50} Al_{50} الى 0.61، 0.7، 0.7 غرام على التوالي. أظهرت مجموعة Cr_{50} مستويات بكتيرية لاهوائية بلغت 5.39 logCFU/g، بينما أظهرت مجموعتا Ni_{100} و Ni_{50} مستويات بكتيرية هوائية/لاهوائية بلغت 6/5.14 و5.36/5.36 log CFU/g، على التوالي. في مجموعة Al_{50} بلغت مستويات بكتيريا *Lactobacillus* spp 2.72 logCFU/g .

في الجزء الثاني، عُزلت سبع سلالات بكتيرية من الترب الزراعية واختُبرت مقاومتها وقدرتها على المعالجة الحيوية للمعادن الثقيلة. بناءً على التوصيف المورفولوجي ، الكيميائي الحيوي والجزيئي، تم تحديد العزلات على النحو التالي: السلالة S1B10 كـ *Pseudomonas aeruginosa* ، السلالة S1B26 كـ *Pseudomonas fluorescens* ، السلالة S5B16 كـ *Bacillus* sp ، السلالة S2B1 و S6B3 كـ *Bacillus cereus* ، السلالة S4B31 كـ *Rhodopseudomonas palustris* و السلالة S5B23 كـ *Planomicrobium* sp.

كشفت النتائج عن التركيزات المثبطة الدنيا MICs للمعادن الثقيلة الثلاثة المدروسة، تراوحت بين 900 و1600 ميكروغرام/مل. أظهر تحليل AAS ان *Bacillus* sp كانت الأكثر كفاءة في إزالة الكروم والألمنيوم، بمعدلات تراكم حيوي بلغت 42.57% و59.50% على التوالي . أظهرت *Pseudomonas fluorescens* أعلى معدل تراكم حيوي للنيكل بنسبة 62.37%.

عند مقارنة consortia، كان العلاج البيولوجي للنيكل في التربة أكثر كفاءة في C1 بنسبة 38.02% ، في حين أظهر C2 معدل علاج بيولوجي أعلى للألمنيوم، بنسبة 36.42%.

الكلمات المفتاحية

المعادن الثقيلة، السمية، البكتيريا، المعالجة البيولوجية، التربة.

List of abbreviations

- **µl :** Microliter.
- **µm :** Micrometer.
- **AAS :** Atomic Absorption Spectrometry.
- **Al :** Aluminum.
- **API :** Analytical Profile Index.
- **CFU :** Colony Forming Unit.
- **Cr :** Chromium.
- **DNA :** Deoxyribonucleic Acid.
- **EC :** Electrical Conductivity.
- **H_2S :** Hydrogen Sulfide.
- **kg :** Kilogram.
- **LD :** Lethal Dose.
- **mg :**.Miligram.
- **MH :** Mueller–Hinton.
- **MIC :** Minimum Inhibitory Concentration.
- **ml :** Milliliter.
- **NaCl :** Sodium Chloride.
- **Ni :** Nickel.
- **OD :** Optical Density.
- **P :** Available Phosphorus.
- **pH :** Hydrogen Potential.
- **RNA :** Ribonucleic Acid.
- **TN :** Total Nitrogen.

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Part 1. Study of Heavy Metal Toxicity

Part 2. Study of Heavy Metal Bioremediation

Introduction

Introduction

Massive industrialization and technological advancements in the previous century have significantly burdened the environment by releasing large quantities of hazardous waste, heavy metals, metalloids, and organic contaminants, causing substantial damage to ecosystems **(Xu *et al*., 2024; Ayangbenro & Babalola, 2017)**. Heavy metals are naturally occurring inorganic chemical hazards **(Tonelli & Tonelli, 2020; Mendy *et al*., 2021)** that are typically toxic even at low concentrations. Metals such as nickel (Ni), chromium (Cr), copper (Cu), lead (Pb), zinc (Zn), mercury (Hg), cadmium (Cd), and arsenic (As) are included in the WHO list of chemicals of public concern **(Manzoor *et al*., 2020)**.

Metals occur naturally at various levels in the earth's crust **(Fan *et al*., 2017)** and can enter living organisms through natural processes such as the weathering of parent materials, volcanic eruptions, and forest fires **(Masindi *et al*., 2021)**. However, in terms of pollution, the most significant sources are anthropogenic activities such as mining, tanneries, and agriculture (e.g., fertilizers and pesticides). Additionally, coal mills, coal power plants, casting factories, and metallurgical processes alter the geochemical cycle of the atmosphere and disrupt biochemical equilibrium. Human intervention can lead to the accumulation of these elements in proportions considered dangerous, depending on the properties of the metals and climatic conditions **(Moghadas *et al*., 2022; Tonelli & Tonelli, 2020)**.

Heavy metal pollution is considered as the most severe environmental issue since these pollutants are persistent in nature and capable of penetrating deep into the bed of groundwater sources and surface water, causing public health problems **(Oladimeji *et al*., 2024)**.

These heavy metals enter the food chain and bioaccumulate, transferring from one food chain to the next. Since heavy metals are non-biodegradable and have a long residence time in the environment, they can accumulate in living organisms and cause toxicity, which can persist

for a long time, leading to serious health problems for many forms of life **(Arora *et al*., 2025; Verma, 2020)**. Heavy metals are predicted to induce several health issues in humans, including cancer, cardiovascular illness, mental disorders, chronic weariness, renal and neurological damage, as well as complications affecting the skin and bones **(Rachmawati *et al*., 2025)**.

Soil contamination by heavy metals is one of the most important apprehensions throughout the industrialized world. Factors include soil characteristics, precipitation patterns, groundwater movement, plant cover, and human activity mostly affect heavy metal contamination loads **(Zhang *et al*., 2025)**. Heavy metals pollution not only results in adverse effects on various parameters relating to plant quality and yield but also causes changes in the size, composition, diversity and activity of the microbial community **(Abiodun *et al*., 2023; Chen *et al*., 2018)**.

To protect human and environmental health, it is necessary to minimize the risks of contamination and exposure by degrading pollutants into less toxic or even harmless products **(Manzoor *et al*., 2020)**. To date, a wide variety of physical-chemical and biological treatments are available to remove heavy metals from the environment **(Bodor *et al*., 2020)**. However, the majority of physical and chemical procedures (electrokinetics, solidification, vapour extraction, soil flushing, and stabilization) rendered the soil unfit for plant development. On the other hand, the biological approach, known as “bioremediation,” has an advantage over chemical methods as it relies on natural processes and promotes the use of remediated soil for plant development. It is also economical and energy-efficient **(Senthil Rathi *et al*., 2024)**. The bioremediation process relies on the action of either fungal, algal, bacterial, yeast or plant (phytoremediation) species, to neutralize pollutants and, thus, can be performed either ex situ or in situ **(Medfu Tarekegn *et al*., 2020)**. Bioremediation techniques now appear as real alternatives to conventional techniques that can be very invasive and expensive.

As a result, the primary objective of this research is to evaluate the toxicological impact of heavy metal exposure in rats and to explore environmentally friendly bioremediation strategies using isolated bacterial strains.

The first phase involves assessing the effects of heavy metal exposure on male Wistar rats by monitoring changes in body weight, organ weights, and gut microbiota composition, providing insight into systemic and microbiological responses to metal toxicity. In the second phase, the focus shifts to isolating, identifying, and characterizing bacterial strains with the potential to tolerate and remove heavy metals from agricultural soil, as well as evaluating their remediation potential as a solution for restoring heavy metal-contaminated environments.

This integrative approach seeks to establish a link between heavy metal toxicity and microbiological bioremediation solutions, with the broader goal of contributing to sustainable and effective methods for mitigating environmental pollution.

Literature review

Part 1. Heavy Metals Pollution

1. Heavy metals

Heavy metals are defined as metals and metalloids that have relatively high atomic weights with densities above >5g cm^{-3} **(Shadman *et al*., 2019)**. These metals and metalloids are divided into two categories: essential and non-essential heavy metals. Essential elements, also known as micronutrients, are frequently required by organisms in trace amounts of 10-15ppm to carry out fundamental processes such as growth, metabolism, and organ development. Others are non-essential heavy metals that have no biological role and can harm living organisms even at low concentrations **(Raychaudhuri *et al*., 2021; Bothe, 2011)**.

2. Sources of heavy metals contaminants in Soils

2.1. Nature sources

Heavy metals can be found naturally at various levels in the earth's crust since the Earth's formation. They are distributed in the environment through various natural processes such as volcanic emissions, erosion, the transport of continental dusts, spring waters ,decomposition, and seismic activities **(Briffa *et al*., 2020; DalCorso *et al*., 2019; Godwill *et al*., 2015)**.

2.2. Anthropogenic sources

Heavy metal pollution has emerged as result of anthropogenic activity, which is the primary cause of pollution, such as industrial waste, fossil fuel combustion, mining and smelting, and fertilizer and pesticide application (Table 1). The use of fertilizers, insecticides, pesticides, and other inputs has been identified as a lesser cause of HM pollution, which is exacerbated by the use of heavy metals in agriculture **(Dagdag *et al*., 2023; J.-J. Kim *et al*., 2019)** (Figure 1).

Table 1. Anthropogenic sources and uses of heavy metals. Adapted from **(Bradl, 2005)**.

Metal	Sources
Arsenic (As)	Additive in animal feed, wood preservative, ceramics, pesticides, electronic components, metallurgy, textiles, and pigments.
Cadmium (Cd)	Ni/Cd batteries, pigments, anti-corrosive metal coatings, plastic stabilizers, alloys, and coal combustion
Cobalt (Co)	Metallurgy, ceramics, glass, and paints.
Chromium (Cr)	Manufacturing of ferro-alloys, plating, pigments, textiles, passivation of corrosion in cooling circuits, and wood treatment.
Copper (Cu)	Good conductor of heat and electricity; used in water pipes, roofing, kitchenware, chemicals and pharmaceutical equipment and pigments.
Iron (Fe)	Cast iron, wrought iron, steel, alloys, construction, transportation, and machine manufacturing.
Mercury (Hg)	Extraction of metals by amalgamation, electrical and measuring apparatus, fungicides, pharmaceuticals, and scientific instruments.
Nickel (Ni)	As an alloy in the steel industry, arc-welding rods, pigments for paints and ceramics, surgical and dental prostheses and computer components.
Lead (Pb)	Antiknock agents, lead-acid batteries, pigments, glassware, ceramics, plastics, alloys, sheets, cable sheathings and solder.
Zinc (Zn)	Zinc alloys (bronze, brass), anti-corrosion coatings, batteries, cans, medicines and chemicals, the rubber industry and paints

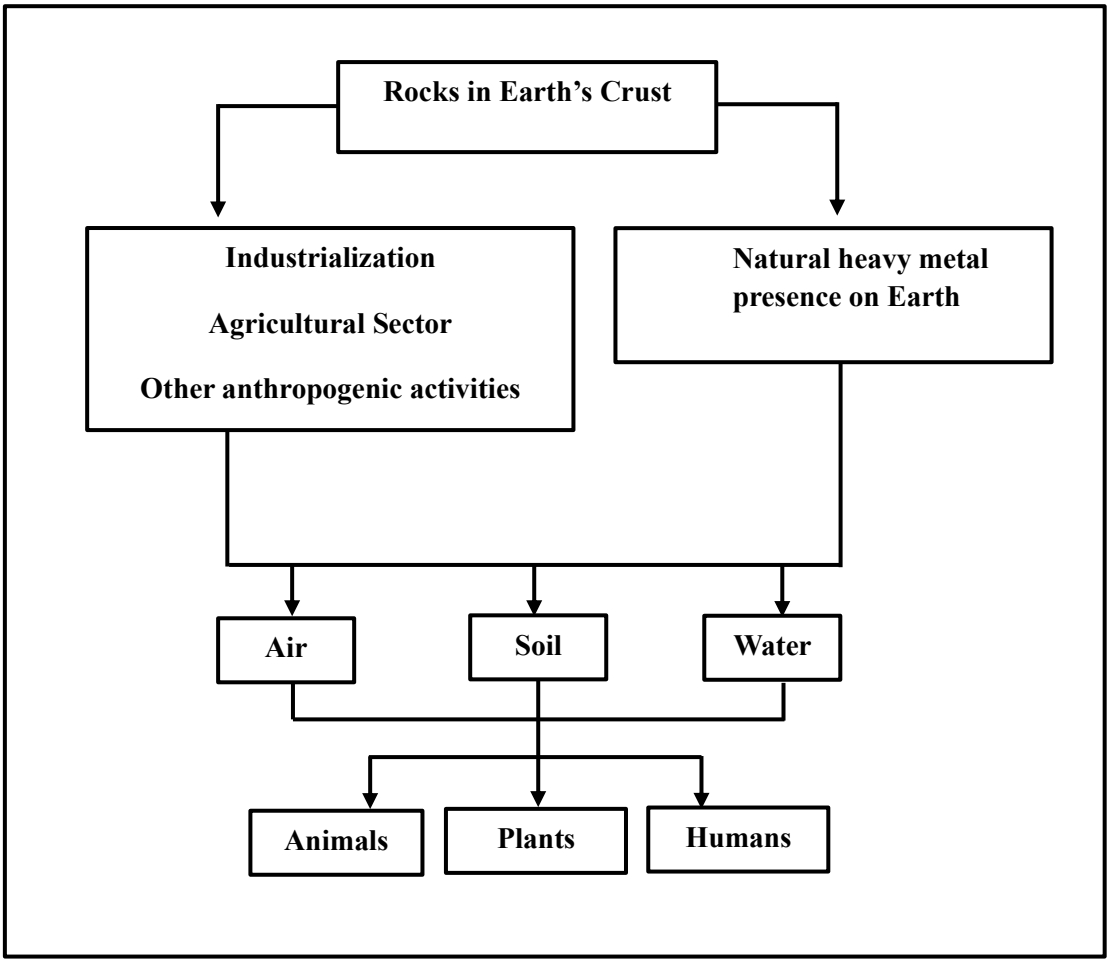

Figure 1. Sources of Heavy Metals and Their Pathways into the Environment and Human Exposure. Adapted from **(Zaimee *et al*., 2021)**.

3. Heavy metal speciation, mobility, and bioavailability in soil

The speciation, mobility, and bioavailability of heavy metals in soil are key parts of understanding their behavior and potential environmental impact.

Speciation refers to the chemical forms or species of heavy metals found in soil. Heavy metal speciation can occur in a variety of chemical forms in soil, including free ions, gaseous phases, complexes with organic matter, adsorbed soil particles, and precipitated minerals **(Roberts *et al*., 2005)**.

Mobility refers to heavy metals' ability to move within the soil matrix or migrate from one area to another. Heavy metals immobilization and mobility in soil environments are significantly affected by their interactions with solid soil components, particularly minerals, organic matter, and microorganisms, which are the principal constituents of soil aggregates **(J. Li *et al.*, 2020)**. Different factors that control the speciation and mobility of heavy metals include soil texture, pH, organic matter content, and physicochemical interactions (redox chemistry, complexation, and sorption) with various solid phase compartments (carbonates, mineral lattices, oxides, and organics) **(Aguirre Gómez & Eugenia Gutiérrez Ruiz, 2023)**.

Bioavailability is a complex, dynamic process that relates to the amount of heavy metals in soil that can be absorbed by plants, microbes, and other living beings (Figure 2) **(Kim *et al.*, 2015; Mirecki *et al.*, 2015)**. Heavy metal bioavailability is influenced by metal speciation, mobility, soil characteristics, and biological processes **(A. Li *et al.*, 2021; Ashraf *et al.*, 2012)**.

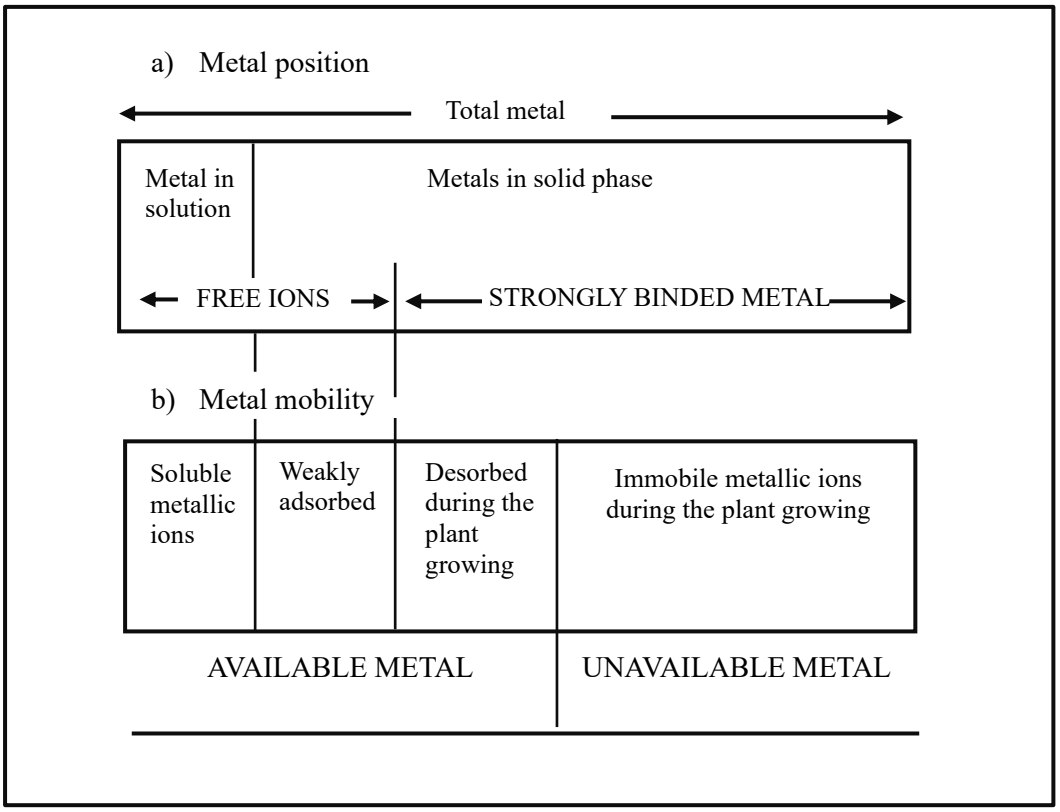

Figure 2. Demonstrating model, the availability of heavy metals in soil. Adapted from **(Smical *et al.*, 2008)**.

4. Microbial resistance towards heavy metals

Certain heavy metals are essential micronutrients. However, higher concentrations of these metals often are cytotoxic. Therefore, some microorganisms inhabiting metal-polluted environments have developed adaptive mechanisms to these contaminants that allow for efficient detoxification and transformation of toxic forms into non-toxic forms **(Srivastava *et al.*, 2014; Giovanella *et al.*, 2017)**. Heavy metal resistance in bacteria is characterized by five mechanisms:

- Metal expulsion through a permeability barrier

Metal ions may be prevented from entering the cell by the capsule, cell wall, or plasma membrane. Metal ions can be absorbed by bacteria via ionazable groups in the cell wall or capsule (carboxyl, amino, phosphate, and hydroxyl groups) **(Ianieva, 2009)**.

- Extracellular sequestration

The accumulation of metal ions by various biological structures such as, siderophores, bio-surfactants, glutathione, and extracellular polymeric substances is known as extracellular sequestration **(Leong & Chang, 2020)**.

- Intracellular sequestration

Intracellular physical sequestration of metal by binding to protein or other ligands to avoid damage to metal-sensitive cellular targets **(Prabhakaran *et al.*, 2016)**.

- Active metal expulsion from a cell (efflux)

Active transport, or efflux, is the most common type of bacterial heavy metal resistance system. These systems are used by bacteria to export metal ions from cells. This reduces the accumulation and concentration of a specific heavy metal in a bacterial cell **(Nanda *et al.*, 2019)**.

- Transformation and detoxification

Biotransformation, enzymatic reduction, or chemical modification of heavy metal ions from a highly toxic form to a less toxic form by enzyme contributes significantly to microorganism resistance to heavy metal ions **(Nanda *et al*., 2019; Leong & Chang, 2020)**.

Part 2. Environmental and Health Impacts of Heavy Metal Pollution

1. Heavy Metals' Effects on Soil and Microbial Dynamics

Heavy metals are regarded as components of the soil; however, when highly concentrated, they cause severe damage to the soil and plants. As a result, they are assumed to be toxicants **(Alengebawy *et al*., 2021)**. Heavy metal pollution is the most serious problem in soil due to their irreversibility, long residual period, small transfer amount, severe toxicity, concealment, complex chemical properties, and ecological response **(Zhang & Wang, 2020)**.

Heavy metal pollution in soil is multifaceted. Heavy metals primarily affect biological characteristics by changing microorganism total content, species diversity, and the intensity of basic microbiological processes and soil enzyme activity. Furthermore, heavy metals reduce the specific adsorption of other cations by increasing saturation or supersaturation of cation exchange sites with heavy metal cations, which displaces protons in the soil solution and leads to a reduced pH **(Nyiramigisha *et al*., 2021; Chibuike & Obiora, 2014)**.

These processes eventually result in a loss of soil quality and fertility, which can be partial or complete in some cases. Any increase in contamination emissions may have a negative impact on crop productivity **(Mohammad Ali *et al*., 2021)**.

2. Heavy Metals' Effects on Plants

Although some heavy metals are required for normal plant growth and metabolism, excessive amounts can be toxic Heavy metals have a negative impact on a number of physiological and biochemical processes in plants, including photosynthesis, mitosis, and water absorption and balance **(Qin *et al*., 2021; Ghori *et al*., 2019)** which causes weak plant growth and yield depression and may even be accompanied by reduced nutrient uptake, plant metabolism disorders, and a reduced ability to fix molecular nitrogen in leguminous plants **(Alengebawy *et al*., 2021; Anas *et al*., 2020; Emamverdian *et al*., 2015)**.

3. Heavy Metals' Effects on Human health

Heavy metals can affect human health in various ways, such as through dermal contact, inhalation of soil dust particulates, and direct ingestion of contaminated drinking water and food. Persistent heavy metals exposure can lead to an imbalance in the body and are used as substitutes for essential elements, such as zinc replaced by cadmium, calcium by lead, and most trace elements by aluminum **(Fu & Xi, 2020)**.

Heavy metals have a variety of acute and chronic toxic effects on various body organs. Heavy metal toxicity can cause gastrointestinal and kidney dysfunction, vascular damage, skin lesions, immune system dysfunction, nervous system disorders, and cancer **(Chai *et al.*, 2021; Balali-Mood *et al.*, 2021)** (Figure 3).

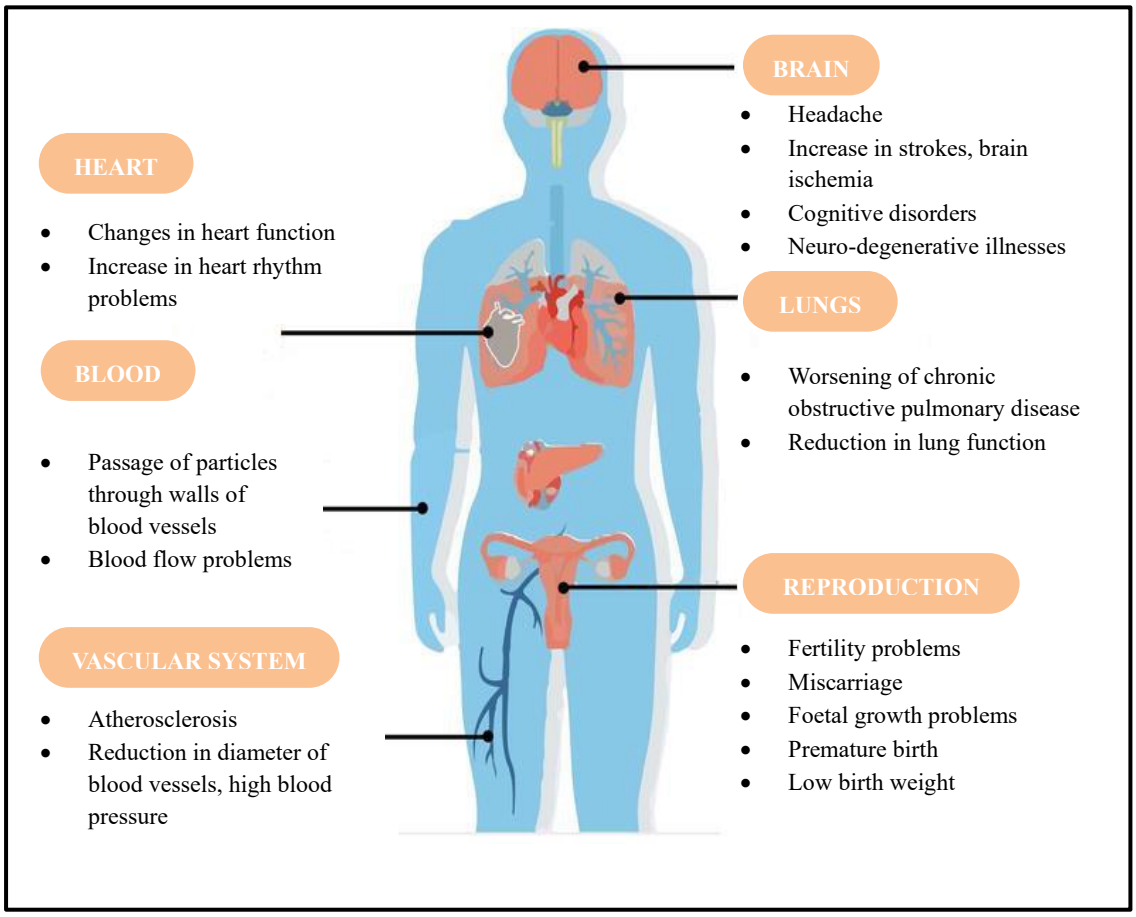

Figure 3. The impact of heavy metals on various vital organs of human health. Adapted from **(Mohammad Ali *et al.*, 2021)**.

3.1. Chromium

Chromium is a chemical element with the symbol Cr and atomic number 24. It belongs to Group 6 of the periodic table and is classified as a transition metal. Chromium is one of the most abundant elements in the environment and industrial settings, with a density of 7.15 g/cm³. This transition metal exhibits seven oxidation states, ranging from Cr(0) to Cr(VI) (hexavalent chromium). Cr(III) is an essential trace element involved in glucose and lipid metabolism, whereas Cr(VI) is highly toxic and recognized as a human carcinogen **(DesMarias & Costa, 2019)**. Chromium predominantly exists in two oxidation states: trivalent chromium (Cr^{3+}) and hexavalent chromium (Cr^{6+}), both of which exhibit toxicity to animals, humans, and plants **(Mohanty & Kumar Patra, 2013)** (Figure 4).

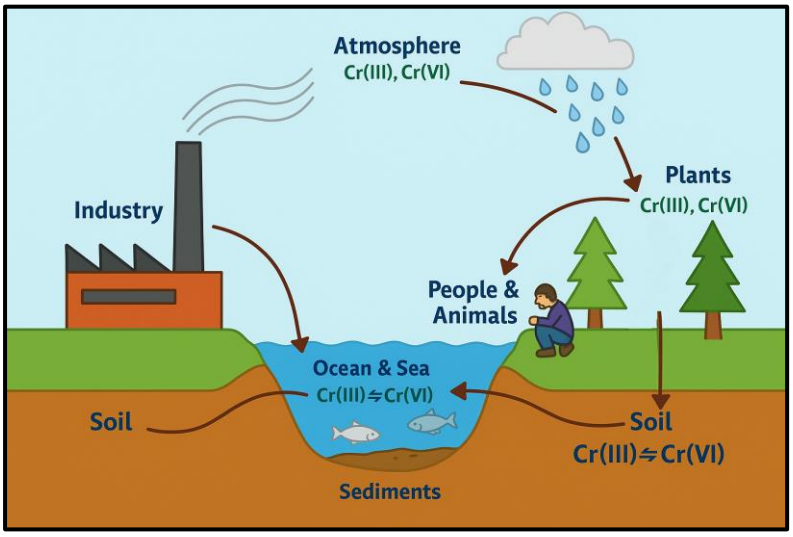

Figure 4. Circulation of chromium in contaminated environments. Adapted from **(Bielicka *et al.*, 2005)**.

Naturally, chromium is emitted through the combustion of coal and oil, as well as from petroleum refining, ferrochromate refractories, pigments, oxidizing agents, catalysts, chromium-based steel, fertilizers, oil drilling operations, and metal plating in tanneries. From anthropogenic sources, chromium enters the environment primarily via sewage discharge and

fertilizer application. In its reduced trivalent form, Cr(III) is relatively immobile and poorly soluble in water, whereas the oxidized hexavalent form, Cr(VI), is highly water-soluble and thus more mobile **(Jaishankar *et al*., 2014)**.

- **Chromium Toxicity: Mechanisms and Health Impacts**

Chromium exists primarily in two biologically relevant forms: trivalent chromium Cr(III) and hexavalent chromium Cr(VI). While Cr(III) is an essential trace element involved in glucose metabolism, Cr(VI) is highly toxic and recognized for its carcinogenic potential **(Costa & Klein, 2006)**. The absorption of Cr(VI) occurs efficiently through the gastrointestinal tract, respiratory system, and skin, facilitated by non-specific anion transporters, whereas Cr(III) is poorly absorbed due to its low solubility and limited membrane permeability **(Zhitkovich, 2011)**. The health effects of chromium in humans depend on several factors, including the dose, route, and duration of exposure. Chromium may exert its effects locally at the site of contact or be distributed to other tissues within the body **(Wilbur *et al*., 2012)**.

Once inside the cell, Cr(VI) undergoes intracellular reduction to Cr(III) via intermediates such as Cr(V) and Cr(IV), generating reactive oxygen species (ROS) in the process. This redox cycling induces oxidative stress, DNA strand breaks, protein crosslinking, and lipid peroxidation, ultimately leading to apoptosis or necrosis **(Sedman *et al*., 2006)**. Through bioaccumulation in the human body, Cr can cause toxicity and a variety of pathophysiological defects such as allergic contact dermatitis and eczema, irritation of mucous membranes, liver and kidney disease, gastrointestinal ulceration, pneumonia, and lung cancer Chronic exposure, particularly via inhalation, has been strongly associated with lung cancer, and Cr(VI) is classified as a Group 1 carcinogen by the International Agency for Research on Cancer **(Balali-Mood *et al*., 2021; Hossini *et al*., 2022)**.

Chromium (VI) exposure has been linked to heart damage, as demonstrated in animal studies showing oxidative stress and inflammation in cardiac tissue, and in exposed workers who exhibited altered myocardial function, particularly those with respiratory issues **(Rager *et al*., 2019; Li *et al*., 2019)**. Chromium toxicity also affects the liver by increasing oxidative stress and inducing cell damage, and severely impacts kidney function by damaging renal tubular cells, potentially leading to dialysis. Additionally, systemic effects like coagulopathy and hemolysis may further worsen renal injury **(Wu *et al*., 2019; Banerjee *et al*., 2017)**.

Chronic or high exposure to hexavalent chromium is associated with an increased risk of cancer in both animals and humans. Long-term exposure can lead to cancers of the stomach, lungs, bladder, pancreas, and other organs. Epidemiological studies show that workers exposed to Cr(VI) have about a 7% higher risk of developing cancer, particularly respiratory, oral, throat, prostate, and gastric cancers, compared to unexposed individuals of similar age and sex **(G. Yan *et al*., 2023)**.

3.2. Nickel

Nickel (Ni) is the 28th element in the periodic table, with a density of 8.9 g/cm³. It is a ductile, hard, silvery-white transition metal that exists in several oxidation states (ranging from -1 to +4); however, the +2 oxidation state (Ni^{2+}) is the most prevalent in the environment and biological systems. Nickel is known for its hardness, ductility, and ferromagnetic properties at room temperature. It exhibits excellent resistance to corrosion and oxidation, making it valuable in various industrial applications. Its face-centered cubic crystal structure contributes to its high ductility and toughness. Additionally, nickel is notable for its ability to form alloys with many metals, enhancing their strength and corrosion resistance **(Wang *et al*., 2020; Duda-Chodak &Aszczyk, 2008)**.

Nickel (Ni) naturally occurs in the Earth's crust, primarily in the form of compounds with sulfur and oxygen—namely sulfides and oxides. It is also found in association with other elements in soil, meteorites, and volcanic emissions, and is present in significant amounts in seawater. Human activities contribute notably to environmental nickel levels through the combustion of diesel, fuel oil, coal, and the incineration of waste and sewage. Additional sources include tobacco smoke, stainless steel cookware and utensils, jewelry manufacturing, and certain food items such as vegetables, chocolate, cocoa, and nuts, which may contain appreciable levels of nickel **(Genchi *et al*., 2020; Cempel & Nikel, 2006)**.

- **Nickel Toxicity: Mechanisms and Health Impacts**

Exposure to environments highly contaminated with nickel (Ni) can result in a range of pathological conditions in humans. Elevated levels of Ni and its compounds in the body are associated with several health issues, including pulmonary fibrosis, renal and cardiovascular disorders, and cancerous developments in the respiratory system **(Duda-Chodak & Aszczyk, 2008)**.

Nickel toxicity can arise through several exposure routes, including parenteral administration, ingestion, inhalation, and dermal absorption. Among these, nickel carbonyl represents the most hazardous form, primarily encountered in occupational settings, and is known to induce respiratory tract irritation along with a range of nonspecific systemic symptoms. Chronic exposure to nickel is associated with a spectrum of adverse health outcomes, such as chronic sinusitis, occupational asthma, and allergic contact dermatitis. Furthermore, prolonged inhalation of nickel compounds has been implicated in the development of respiratory tract malignancies, particularly lung and nasal cancers **(Gates *et al*., 2023)**.

Nickel (Ni) toxicity occurs through several mechanisms, primarily by inducing oxidative stress that damages cellular components and disrupts mitochondrial function. It interferes with enzymes and proteins by binding to them or displacing essential metals like zinc and iron, impairing DNA repair and other key processes. Nickel also triggers inflammation and alters gene expression through epigenetic changes, promoting carcinogenesis **(L. Zhao *et al*., 2022; Guo *et al*., 2019)**. Furthermore, nickel's ability to cross cell membranes via divalent metal transporters facilitates its accumulation in various tissues, particularly in the lungs and kidneys, leading to long-term toxic and immunological effects. These complex interactions highlight nickel's potential to contribute to chronic health conditions such as respiratory tract cancers, cardiovascular diseases, and renal dysfunction **(Menon *et al*., 2016)**.

In addition to its systemic toxicity, one of the most frequently observed outcomes of nickel exposure is allergic contact dermatitis, especially among sensitive individuals. Research has demonstrated that nickel not only acts as a common allergen but also possesses immunomodulatory and immunotoxic properties. Based on extensive human and animal studies, the International Agency for Research on Cancer (IARC) and the U.S. Department of Health and Human Services have classified nickel compounds as carcinogenic to humans **(Das *et al*., 2019; Kumar & Trivedi, 2016)**.

Nickel compounds have been shown to exhibit strong teratogenic effects in experimental studies. Research by Leonard et al. indicated that prenatal exposure to nickel can increase prenatal and neonatal mortality and cause various embryonic malformations, potentially due to disruptions in mitosis leading to cell death **(Saini *et al*., 2013)**.

Systemic absorption of nickel can affect the renal and hepatic systems. Studies have indicated that nickel exposure may lead to kidney dysfunction and liver toxicity, although the exact mechanisms remain under investigation **(Haidar *et al*., 2023)**. Emerging research

suggests a link between nickel exposure and cardiovascular issues. Nickel-induced oxidative stress and inflammation may contribute to endothelial dysfunction, a precursor to various cardiovascular diseases **(Alissa & Ferns, 2011)**. Additionally, nickel has been shown to significantly impact the nervous system by causing cognitive and behavioral impairments, disrupting presynaptic neurotransmission, and affecting various brain regions through distinct mechanisms **(Anyachor *et al*., 2022)**.

3.3. Aluminum

Aluminum (Al), with an atomic number of 13 and a density of 2.7 g/cm³, is the most abundant chemical element in the Earth's crust, accounting for approximately 7% of its composition. It is a lightweight, silvery-white metal known for its versatility and widespread industrial applications **(L. Yan *et al*., 2019; X. Zhao *et al*., 2017)**. Aluminum naturally exists in its trivalent form (Al^{3+}), commonly found as silicates, oxides, and hydroxides, but it can also bind with elements like chlorine, sulfur, and fluorine, and form complexes with organic substances **(Igbokwe *et al*., 2019)**.

- **Aluminum Toxicity: Mechanisms and Health Impacts**

Aluminum (Al) enters the environment through both natural weathering of rocks and anthropogenic activities, though weathering contributes more significantly. Occupational exposure occurs in industries like mining, metal processing, recycling, and manufacturing where aluminum is handled or processed. Populations near industrial waste sites may also face elevated exposure. Numerous aluminum compounds such as aluminum chloride, hydroxide, sulfate, and silicate are widely used across many industries. These uses include petroleum refining, cookware manufacturing, water treatment, pharmaceuticals, cosmetics, and food processing, among others, highlighting aluminum’s extensive industrial and commercial applications **(Mudge *et al*., 2011; Exley, 2003)**.

Al enters the body primarily through inhalation and ingestion. Once inhaled, aluminum compounds often in the form of poorly soluble particles like aluminum silicates; accumulate in the lungs. This accumulation increases with age and can lead to respiratory complications. The extent of tissue buildup and potential toxicity depends on the balance between aluminum intake, absorption, and elimination **(Taiwo, 2014)**. The gastrointestinal tract, particularly the duodenum, is the primary route for systemic aluminum (Al) accumulation after ingestion, although absorption is generally low. Factors influencing Al absorption include age, individual variation, pH, stomach contents, and the type of Al compound **(Zhou *et al*., 2008; Steinhausen *et al*., 2004)** (Figure 5).

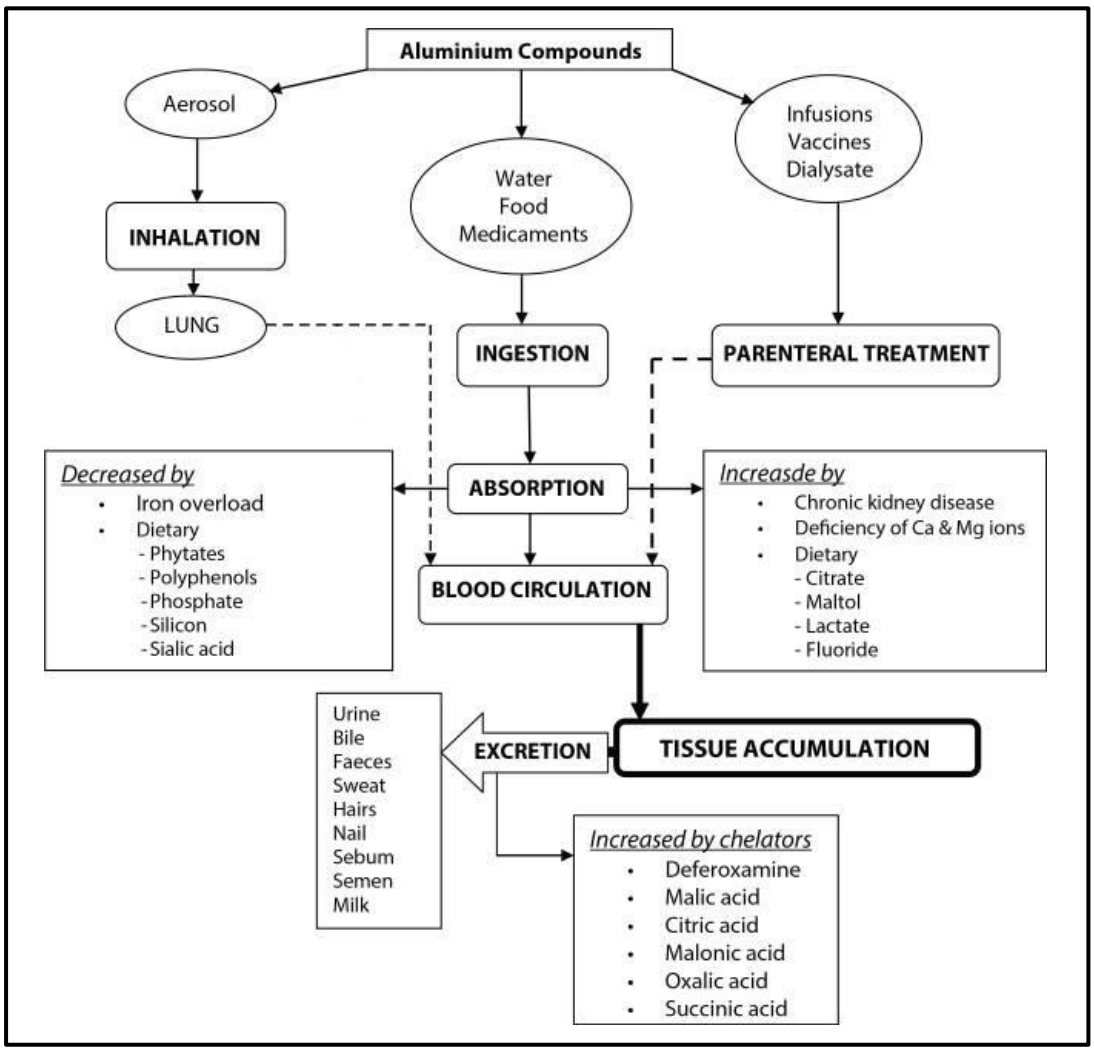

Figure 5. The Effects of Aluminum on the Human Body and the Development of Toxicosis. Adapted from **(Igbokwe *et al*., 2019)**.

Aluminum toxicity involves increased inflammation and oxidative stress, resulting in the production of reactive oxygen species and impairment of antioxidant enzymes. It also disrupts enzyme functions, alters protein synthesis, and interferes with nucleic acid activity **(Rahimzadeh *et al*., 2022)**. The central nervous system is the primary site affected by aluminum toxicity. Elevated levels of aluminum oxide are associated with a higher incidence of headaches, vertigo, emotional instability, difficulty concentrating, insomnia, mood swings, anxiety, and fear. Many studies have shown that these neurological symptoms are closely linked to aluminum oxide exposure. Additionally, aluminum toxicosis has been reported to contribute to the development of conditions such as Alzheimer's disease, autism, osteoporosis, diabetes mellitus, and inflammatory bowel disease. Other observed symptoms include disorientation, altered mental status, anxiety, and acute hypoxic encephalopathy **(Kondaiah *et al*., 2024; Exley, 2016)**.

Aluminum negatively affects multiple organ systems, including the lungs, cardiovascular, and urogenital systems **(Aghashahi *et al*., 2020)**. As a result, it can lead to dementia, lethargy, kidney and liver dysfunction, leukocytosis, colitis, lung damage and pulmonary fibrosis, as well as osteomalacia **(Briffa *et al*., 2020; Exley & House, 2011)**.

Aluminum toxicosis has been associated with various cardiovascular effects, including toxic myocarditis, myocardial dysfunction, and thrombosis, particularly in cases of aluminum phosphide poisoning. Some studies have also reported congenital heart defects, such as ventricular malformations and septal anomalies, especially following prenatal aluminum exposure **(El Hangouche *et al*., 2017; N. Wang *et al*., 2012)**. Additionally, aluminum exposure is linked to muscle and bone disorders. A notable aluminum-induced muscle condition is macrophagic myofasciitis, commonly associated with chronic fatigue and muscle pain **(Gherardi *et al*., 2016)**.

Furthermore, aluminum affects bone health by increasing the risk of conditions such as osteoporosis, osteomalacia, rickets, and osteodystrophy. These adverse effects are primarily due to aluminum's ability to disrupt bone formation by inhibiting osteoblast proliferation, differentiation, and mineralization, ultimately reducing bone density and structural integrity **(Klein, 2019)**.

Part 3. Bioremediation of Heavy Metals: A Natural Path to Pollution Control

1. Remediation techniques for heavy metal-polluted soil environments

In recent years, heavy metal pollution has garnered increasing attention from scientists. Substantial progress has been made in research on heavy metals in soil, including advancements in remediation technology innovation and optimization, as well as studies on the forms and migration of heavy metals within soil **(Zheng *et al*., 2024; Rongxin *et al*., 2021)**.

Various methods have been developed for the remediation of heavy metal-polluted soil, including widely used physical, chemical, and biological approaches. These techniques aim to either completely eliminate contaminants or convert them into less harmful forms. Each method operates through different mechanisms to remove or degrade pollutants from the soil **(H. Kim *et al*., 2022; Raffa *et al*., 2021)**. The selection of a remediation method depends on factors such as the type and severity of contamination, as well as cost and accessibility. A thorough assessment of the situation and consultation with experts are crucial in determining the most effective approach **(Priya *et al.*, 2023)**.

Various physical approaches have been used to remove heavy metals based on their physicochemical properties. These methods include adsorption, membrane filtration, electrokinetic treatment, photocatalysis, granular activated carbon, and soil washing. The chemical process involves methods such as chemical precipitation, ion exchange, flotation, flocculation, and coagulation. While these techniques are effective in removing heavy metals, they are costly, can negatively impact the soil's natural bio-physicochemical properties, and excessive chemical use may lead to challenges in sludge disposal and the risk of secondary pollution **(Akhtar *et al*., 2020; Liu *et al*., 2018)**. Therefore, bioremediation is regarded as the most effective approach for restoring heavy metal-polluted soils due to its ecofriendly, low-cost, and high public acceptance **(Zheng *et al*., 2024)**.

2. Bioremediation

The term bioremediation is derived from two words: bio which means life, indicating that we are talking about live organisms, and "to remediate," which means to solve a problem **(P. Kumar *et al*., 2019)**. Bioremediation is therefore a technique that uses natural biological activity in the environment to remove or render certain contaminants harmless. The method is based on the capacity of microorganisms, or their metabolisms, to decrease (convert, mineralize, degrade, and detoxify) pollution concentrations and restore the environment to its original state **(Raja Sathendra et al., 2018; Azubuike et al., 2016)**.

The bioremediation process is a natural alternative to methods such as incineration, catalytic destruction, the use of absorbents and physical removal and destruction of contaminants; because the procedure can be more efficient at low metal concentrations and the cost of transporting and incinerating the contaminants is at least ten times higher than in situ biological treatment **(Kulshreshtha *et al*., 2014; De *et al*., 2008)**. The volume of effluent generated by bioremediation is much smaller, which reduces the problem of sludge disposal. In addition, because this technology is based on natural processes, it is considered as the most acceptable and greener than other technologies by the public **(Yadav *et al*., 2017)**.

Bioremediation technologies can be broadly classified as ex situ or in situ. Ex situ technologies are methods of removing pollutants at a separate treatment facility. In situ bioremediation methods treat pollutants in their natural environment **(Iwamoto & Nasu, 2001)**.

3. Principle of bioremediation

The main principle of this technique is the use of indigenous or non-indigenous microbial population to remove pollutants from the environment and/or convert pollutants to a less harmful product **(I. Sharma, 2021)**. The microorganisms act against the contaminants only

when they have access to a variety of materials, which they use as organic compounds to help them generate energy and nutrients to build more cells. In certain situations, the natural conditions at the polluted site offer all of the necessary elements in sufficient quantities for bioremediation to occur without the need for human intervention, a process known as intrinsic bioremediation **(Bamforth & Singleton, 2005)**.

4. Factors affecting bioremediation

Several variables influence the bioremediation process, including biotic factors (the activities of aerobic or anaerobic heterotrophic microorganisms) and abiotic factors (physicochemical, environmental parameters and climatic conditions) highlights the environmental challenges faced by microorganisms during bioremediation process **(Jacob *et al*., 2018; Srivastava *et al*., 2014)**.

4.1. Biotic factors: Organism related factors

Organism related factors include population density, composition, inter and intraspecific interactions **(Tekere, 2019)**. The biomass concentration is an essential biological component in microbial bioremediation. Heavy metals in the reduction medium are not only adsorbed to the biomass surface but also penetrate the intracellular section, which is promoted by the metal's concentration gradient, when biomass concentration is at its equilibrium level **(Jacob *et al*., 2018)**.

There are various inherent microbial characteristics that influence the degradation of the substrate; e.g. plasmid-encoded genes provide specificity for substrates and encode the specific enzymes (proteins), mutation, horizontal gene transfer, and interaction (competition, succession, and predation) **(Abatenh *et al*., 2017; Srivastava *et al*., 2014)**.

4.2. Abiotic factors

Soil Structure

The soil structure ranges from low clay or silt content, which is effective delivery of air, water, and nutrients to the microorganisms in situ bioremediation. Furthermore, soil type is an important influence in metal bioavailability in soil. Metal ion availability is intimately connected to the texture of soil particles. Fine-textured clay soils have the lowest availability, followed by clay loam, while loam and sand have the highest availability **(Zhang *et al*., 2020; Sivakumar *et al*., 2014)**.

- ***pH***

pH of the soil is essential for the survival of most microbial species and are limited to a certain level. Their growth and development are restricted to a particular pH range and it is one of the main factors influencing metal adsorption **(Dwivedi, 2012)**. The majority of bioremediation operations are carried out in pH range of 5.5 to 8. The majority of microorganisms, particularly heterotrophic bacteria, are utilised in many bioremediation processes within this optimal pH range. There is a possibility of pH change during pollutant bioremediation, so the regular monitoring is required. The acidic or basic substances are added to adjust the pH in the desired range **(Senthil Kumar & Gunasundari, 2018)**.

- ***Temperature***

Temperature is an important factor that can influence degradation rates by regulating enzymatic processes inside microorganisms. There is always a temperature optimum at which biochemical activities occur in order for each microbe to accomplish the required bio treatment. Temperature extremes (too low or too high) have an impact on both microbial growth and enzyme-catalysed microbial processes. The majority of microorganisms grow successfully at temperatures ranging from 10 to 38 °C. Temperature management of in situ processes is

extremely difficult, whereas temperature of ex situ processes can be slightly affected **(Senthil Kumar & Gunasundari, 2018; Tekere, 2019)**.

- ***Moisture***

Moisture impacts soil permeability, the characteristics and amount of soluble elements, the pH of the soil solution, and the hydraulic conductivity of unsaturated soils, all of which affect the bioremediation of contaminated soils **(Zhou & Hua, 2004)**. Low soil moisture inhibits microbial development and metabolism, whereas high amounts reduce soil aeration. Metal uptake is often more visible at greater soil moisture levels **(Zhang *et al*., 2020)**.

- ***Nutrients***

Nutrient availability is critical for microbial development. Nutrients are insufficient in polluted environments for cellular metabolism and microbiological growth. Because organic carbons are abundant in polluted areas with high rate of depletion during microbial metabolism, introducing nutrients to the contaminated area, such as nitrogen, phosphate, and potassium, may stimulate cellular metabolism and microbial growth, hence increasing bioremediation. For bioremediation, the carbon-to-nitrogen ratio (C:N) must be 10:1, and the carbon-to-phosphorous ratio must be 30:1 **(Mani & Kumar, 2014)**.

- ***O_2 and CO_2***

A sufficient amount of oxygen is essential for leaching bacteria to grow and function properly. Oxygen can be supplied using aerators and pipes. Mechanical agitation is another useful approach for providing a consistent air supply and mixing the contents. CO_2 is the only carbon source required; however, it is not necessary to add CO_2 **(Mahajan *et al.*, 2017)**.

5. In situ and ex situ bioremediation

Bioremediation technologies can be broadly classified as ex situ or in situ. Ex situ technologies are methods of removing pollutants at a separate treatment facility. In situ bioremediation methods treat pollutants in their natural environment **(Iwamoto & Nasu, 2001)**.

The use of biological treatment to clean up hazardous substances present in the subsurface under natural circumstances to either carbon dioxide and water or an attenuated transformation product is known as in situ bioremediation. It is involving low cost, low maintenance and environment friendly **(Megharaj *et al*., 2011)**.

One of the primary constraints of this technique is the depth of soil that oxygen may reach, which is normally confined to the superficial layer (average value of 30 cm from the surface) **(Tomei & Daugulis, 2013)**. In addition, in situ bioremediation is a time-consuming process with seasonal variations in microbial activity that is uncontrolled and minimum manageable **(Senthil Kumar & Gunasundari, 2018)**.

Ex situ bioremediation approaches, involve excavating soil from a polluted site, transporting the contaminated material to an off-site treatment facility, and disposing of the treated soil at permitted places **(Liu *et al*., 2018)**. The main disadvantages of ex situ bioremediation are the high costs compared to in situ treatments **(Megharaj *et al*., 2011)** and the risk of contaminated dispersion during excavation and transport **(Tomei & Daugulis, 2013)**.

However, ex situ technologies on the other hand, have various benefits that make them competitive. It is a simple and effective treatment for a wide range of pollutants, and the most significant advantage is the ability to better control the remediation process since the enclosed

reaction environment is more controlled and the treatment process is more predictable than in situ treatment **(Senthil Kumar & Gunasundari, 2018; Tomei & Daugulis, 2013)**.

6. Microbial Remediation of Heavy Metal-Contaminated Soil

Microbial remediation is a sort of remediation technology that uses soil microorganisms to render pollutants harmless **(Ye *et al*., 2017)** (Table 2). Bioremediation can be successful at a particular site through the designer microbe method and an understanding of the mechanism controlling the growth and activity of microorganisms at polluted areas, their metabolic capacities and their reaction to environmental changes **(Dixit *et al*., 2015)**. Bioremediation processes include biosorption, bioaccumulation, biomineralization, biotransformation, and bioleaching **(Choudhary *et al*., 2017)** (Figure 6).

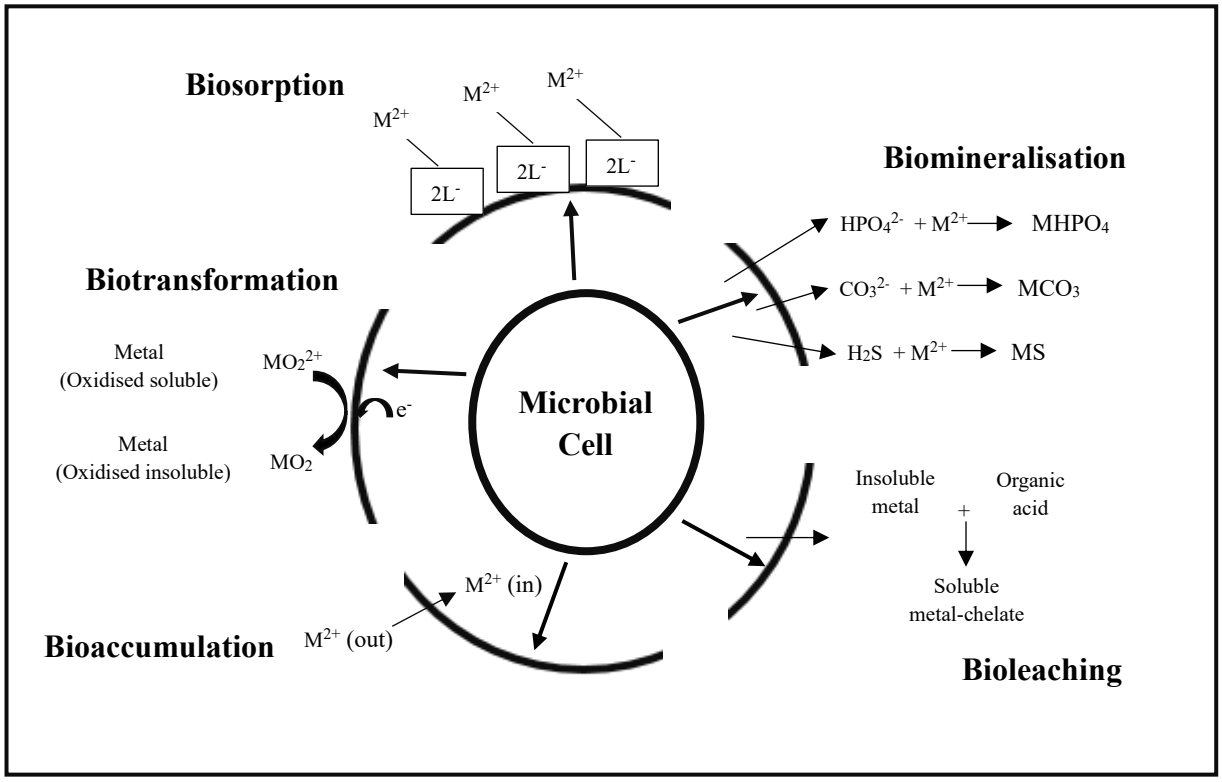

Figure 6. Bioremediation processes in a microbial cell. Adapted from **(Tabak *et al*., 2005)**.

Table 2. Microbial species used in heavy metal bioremediation.

Microbial Group	Species	Target metals	References
Bacteria	*Bacillus sp*	Cr (VI)	**(Kanmani *et al*., 2012)**
	Bacillus sp	Pb (II)	**(Ren *et al*., 2015)**
	Pseudomonas aeruginosa	Pb	**(Ahmady-Asbchin *et al*., 2015)**
	Pseudomonas aeruginosa	Hg (II)	**Yin *et al*., 2016)**
	Enterobacter cloacae	Pb, Cd, Ni	**(Banerjee *et al*., 2015)**
	Kocuria rhizophila	Cd, Cr	**(Haq *et al*., 2016)**
	Sporosarcina ginsengisoli	As (III)	**(Achal *et al*., 2012)**
	Deinococcus radiodurans	Co	**(Gogada *et al*., 2015)**
	Lactobacillus sp	Cu	**(Schut *et al*., 2011)**
	Ochrobactrum intermedium	Cu (II), Cr (VI)	**(Fan *et al*., 2014)**
	Cupriavidus metallidurans	Cu (II), Cr (VI)	**(Fan *et al*., 2014)**
	Cellulosimicrobium sp	Cr (VI)	**(Bharagava & Mishra, 2018)**
	Vibrio fluvialis	Hg	**(Saranya *et al*., 2017)**
	Sphaerotilus natans	Cd, Fe, Pb	**(Ashokkumar *et al*., 2017)**
	Rhodobacter capsulatus	Zn (II)	**(Magnin *et al*., 2014)**
Fungi	*Aspergillus versicolor*	Ni, Cu	**(Taştan *et al*., 2010)**
	Aspergillus lentulus	Cu (II), Pb (II), Cr (III), Ni (II)	**(A. Mishra & Malik, 2012)**

6.1. Biosorption

Biosorption is the capacity of dead or inactive biological materials or living organisms to acquire heavy metals or metalloid species, both soluble and insoluble, via metabolically mediated or physicochemical uptake pathways such as adsorption **(Mustapha & Halimoon, 2015; Gadd, 2004)** Physical adsorption, ion exchange, complexation, chelation, precipitation, and entrapment in inner space are all examples of metal biosorption interactions **(Abbas *et al*., 2014)** (Figure 7).

Biosorption is a complicated process that is influenced by several factors such as organism type, cell physiology, microorganism cell wall composition, and physicochemical factors such as pH, temperature, contact time, ionic strength, metal concentration, and metal ion chemistry **(Castro *et al*., 2019)**.

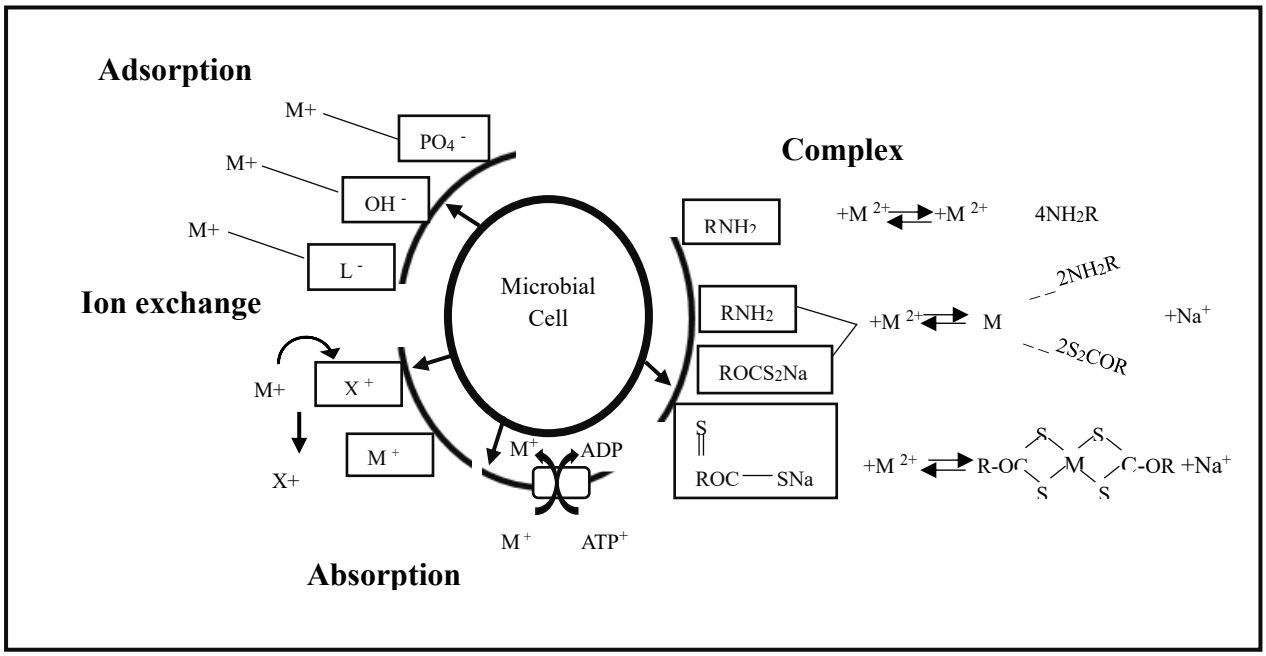

Figure 7. Biosorption mechanisms of microorganisms. Adapted from **(Jin *et al*., 2018)**.

6.2. Bioaccumulation

The term 'bioaccumulation' refers to the coexistence of adsorptive and metabolism-dependent processes by actively developing cells, as opposed to 'biosorption,' which does not require metabolic contribution and can be achieved by non-viable biomass **(Juwarkar & Yadav, 2010; Aksu, 2003)**. Bioaccumulation is a toxicokinetic mechanism that influences the chemical sensitivity of living organisms **(Medfu Tarekegn *et al*., 2020)**.

Heavy metal bioaccumulation is a metabolically active process in which solutes are transferred from the microbial cells outside into the cytoplasm, where the metal is sequestered. This process involves metal binding to intracellular molecules, intracellular precipitation, metal binding proteins, methylation, and other activities **(Diep *et al*., 2018; Tabak *et al*., 2005)**.

6.3. Biotransformation

Biotransformation, also inaccurately referred to as "xenobiotic metabolism," is responsible for minor structural modifications in exogenous substances through the use of biological catalysts such as microbial cells or enzymes isolated from microorganisms, resulting in the formation of molecules with relatively greater polarity **(Hegazy *et al*., 2015; Bianchini *et al*., 2015)**. Biotransformation is the most important method for removing heavy metals from soil, water and sediment **(Chaturvedi *et al*., 2015)**.

Microbial transformation is regarded as an enzymatic reaction that utilizes microorganisms' metabolic activity **(Cano-Flores *et al*., 2020)**. This transformation can be congregated under the categories: oxidation, reduction, hydrolysis, methylation/demethylation, isomerisation, condensation, formation of new carbon bons, and introduction of functional groups **(Smitha *et al*., 2017; M. A. Rahman & Hassler, 2014; Bolan *et al*., 2013)**.

6.4. Bioleaching

Bioleaching is defined as the dissolving of metals from their mineral source that happens in nature whenever favourable circumstances for the development of microorganisms are available **(V. Kumar *et al*., 2019)**. It is an innovative, ecologically friendly, simple, economical and effective method **(Li *et al*., 2020)**. Since 1980, bioleaching has been used efficiently on an industrial scale for mining in several areas due to a greater knowledge of the microbes involved **(P. Kumar *et al*., 2019)**.

6.5. Biomineralization

Biomineralization is a naturally and widely known process in which living organisms (mainly microbes) drive mineral production, and the mineral phase is immobilized by coordination with microbial cells and/or bioprecipitation. This approach has been successfully used in heavy metal bioremediation under the impact of redox reactions, metabolic activities (via the production of inorganic (i.e., CO2, Fe(II), and sulfide) and organic metabolites), and enzymes (oxalic acid, urease, and phosphatase) by various microorganisms **(Z. Rahman & Singh, 2020; Dhami *et al*., 2018; Verma & Sharma, 2017)**.

7. OMICS in bioremediation

Bioremediation strategies by microorganisms have a high potential for effective restoration of contaminated environments. However, the extent of contamination management is determined by a number of factors, including microbial composition, the nature or extent of pollutants, and the surrounding environmental circumstances **(M. Mishra *et al*., 2021; Rodríguez *et al*., 2020)**. Therefore, Omics studies are essential to generating relevant information about the mechanisms involved in contamination management and developing solutions to manage these contaminants in an environmentally benign manner **(P. Sharma *et al*., 2022)**. Omics technology is a molecular biological technique that allows for the

simultaneous analysis of biomolecules such as DNA, RNA, proteins, and metabolites from individual organisms and the entire community **(Chandran *et al*., 2020)**. These technologies include genomics, proteomics, transcriptomics, and metabolomics.

- **Genomics:** Genomics is the study of an organism's entire genetic component. It makes use of recombinant DNA technologies, molecular biology, and bioinformatics **(Rawat & Rangarajan, 2019)**. Metagenomics technology is helpful in understanding activities, interactions, cooperation, and growth in a variety of contexts by researching uncultured organisms involved in bioremediation **(P. Sharma *et al*., 2022)**.

- **Transcriptomic:** A transcriptomic approach examines genome-wide transcriptional activity, discovers regulons and stimulations, delineates operon structures, identifies DNA-binding sites, and conducts comparative genotyping on a diverse range of microbiological species **(Hasin *et al*., 2017)**.

- **Proteomic:** A proteome is an organism's whole set of protein content. The term "proteomics" refers to an Omics technology that studies the proteome expressed in a given biological sample under specific conditions **(Rodríguez *et al*., 2020; Rawat & Rangarajan, 2019)**. Metaproteomics, also known as community proteomics, is the study of the entire protein composition of microbial communities living in a specific habitat. Metaproteomics aids in comprehending the physiological reactions of microbes and the investigation of variations in protein abundance during the bioremediation process, as all the proteins present inside a cell can be analyzed **(Chandran *et al*., 2020)**.

- **Metabolomic:** Certain metabolites are released in the proximal environment by microorganisms throughout their physiological and metabolic functions. Metabolomics refers to the simultaneous quantification of multiple small molecule types, such as amino acids,

carbohydrates, or other products of these metabolic functions **(Hasin *et al*., 2017; Fukushima *et al*., 2009)**.

Materials and Methods

Part 1. Study of Heavy Metal Toxicity

1. Heavy metals applied

This toxicological study focuses on Chromium (Cr), Nickel (Ni), and Aluminum (Al), chosen for their widespread use in industry, potential environmental impact, and known health effects.

Table 3. Lethal dose (LD_{50}) of heavy metals

Heavy metal	LD_{50}	$LD_{50/100}$	$LD_{50/50}$	Reference
Potassium dichromate $K_2Cr_2O_7$	26 mg/kg	0.26mg/kg	0.52mg/kg	**(CE, 2001)**.
Nickel chloride $NiCl_2$	175mg/kg	1.75 mg/kg	3.5 mg/kg	**(Henderson *et al.*, 2012)**.
Aluminium chloride $AlCl_3$	370 mg/kg	3.7mg/kg	7.4mg/kg	**(Llobet *et al.*, 1987)**.

2. Biological material

The biological material used in this study consisted of male Wistar strain laboratory rats, aged between 8 and 12 weeks and weighing between 110 and 180g. The rats were randomly divided into groups and housed in controlled temperature and lighting conditions (22°C, with a 12-hour light/dark cycle), and they had ad libitum access to food and water.

3. Animal Exposure Procedures and Experimental Setup

For the experiment, 35 male rats were randomly selected and divided into seven groups of five rats each: one control group and six experimental groups. The experimental groups were given two doses of LD_{50}: 1/100 and 1/50 of an aqueous solution of $AlCl_3$, $K_2Cr_2O_7$, and $NiCl_2$ for 3 months. The control group was treated with water only.

All treatments were administered by oral gavage at a volume of 1 ml per day each morning during the experimental period. Each week, new solutions were prepared, taking into account the weight gained by each animal in the group.

- **G1:** Control group where the animals received distilled water by gavage (1 ml/day).
- **G2:** Group where the animals received distilled water enriched with potassium dichromate ($K_2Cr_2O_7$) by gavage (1 ml/day), or 1/100th of the LD_{50}.
- **G3:** Group where the animals received distilled water enriched with nickel chloride ($NiCl_2$) by gavage (1 ml/day), or 1/100th of the LD_{50}.
- **G4:** Group where the animals received distilled water enriched with aluminum chloride ($AlCl_3$) by gavage (1 ml/day), or 1/100th of the LD_{50}.
- **G5:** Group where the animals received distilled water enriched with potassium dichromate ($K_2Cr_2O_7$) by gavage (1 ml/day), or 1/50th of the LD_{50}.
- **G6:** Group where the animals received distilled water enriched with nickel chloride ($NiCl_2$) by gavage (1 ml/day), or 1/50th of the LD_{50}.
- **G7:** Group where the animals received distilled water enriched with aluminum chloride ($AlCl_3$) by gavage (1 ml/day), or 1/50th of the LD_{50}.

After 3 months, the rats were sacrificed, and the liver and kidneys were immediately removed, washed with 0.9% NaCl physiological saline, and weighed.

4. Sacrifices and Sample Collection

After 3 months of experimentation, the rats were sacrificed in the morning following a 12-hour fast, using anesthesia induced by a piece of cotton soaked in chloroform. The organs (liver and kidneys) were immediately removed, washed with 0.9% NaCl physiological saline,

and weighed. The dissection was performed as aseptically as possible in a sterile field under a Bunsen burner, using sterile dissection equipment.

5. Body Weight and Relative Organ Weight

The body weight of the rats was measured weekly during the experiment, as well as on the day of sacrifice. The weight obtained allows us to evaluate the weight gain of the rats compared to the first day, according to the following formula **(Sana *et al*., 2020)**:

$$\textbf{Weight gain} = \frac{\textbf{Wf} - \textbf{W0}}{\textbf{Total days}}$$

The organ weights of the different animal groups were recorded. Using these values, the relative index of each organ was determined using the following formula **(Silué *et al*., 2024)**:

$$\textbf{Relative organ weight} = \frac{\textbf{Organ weight}}{\textbf{Body weight}} \textbf{ x 100}$$

6. Enumeration of Microbial Profiling

Samples of small intestine contents were collected and placed in sterile tubes. The gut samples were re-suspended (1:10 vol/vol) in a saline solution (0.9% NaCl) and diluted serially with diluent in several 10-fold steps.

Dilution samples were homogenized and plated on MRS agar for *Lactobacillus* spp. and on NA agar for anaerobic and aerobic bacteria. The plates were then incubated at 37 °C for 48–72 hours: under aerobic conditions for aerobic bacteria, and under strictly anaerobic conditions using anaerobic jars for anaerobic bacteria and *Lactobacillus* spp. The resulting colonies were counted and expressed as CFU/mL of intestinal samples.

7. Statistical Analysis

All experimental results are expressed as arithmetic means obtained from at least three replicates. Statistical analyses were performed using XLSTAT software.

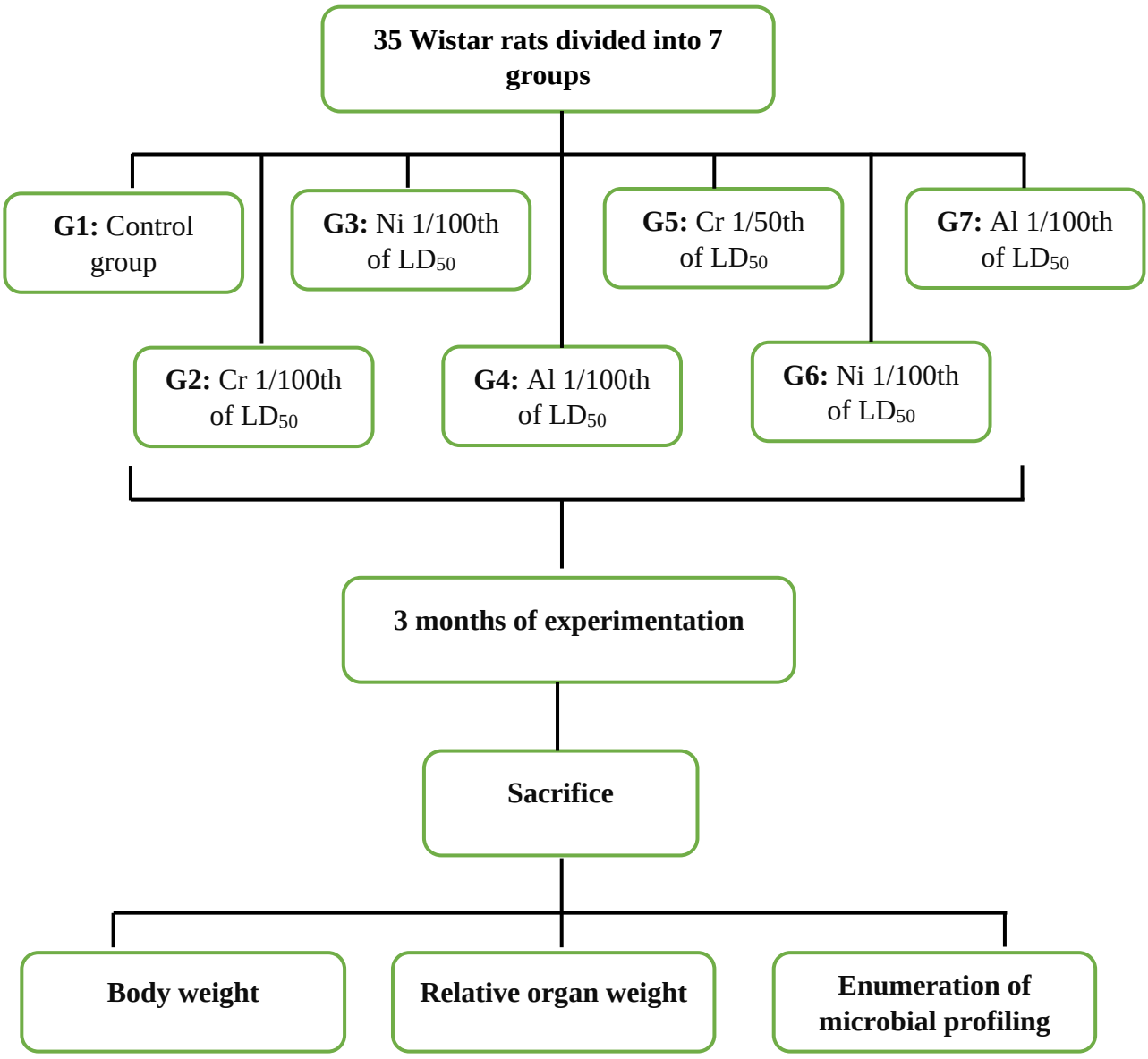

Figure 8. Summary Diagram of the Experimental Protocol.

Part 2. Study of Heavy Metal Bioremediation

1. Study area and sampling

The Daïra of Ghriss, located in Mascara Province, Algeria, includes the town of Ghriss and nearby localities such as Maoussa and Makdha. Ghriss is approximately 18 km from Mascara city and is situated at a moderate altitude, with an elevation of about 500 meters, within the plains of Ghriss (Figure 9).

Soil samples were collected from the rhizosphere of wheat and barley plants in agricultural fields (designated as Soils 1 to 6) located between the coordinates 35°14′–35°16′ N and 0°10′–0°09′ E in Ghriss (Figure 9). The root system and bulk soil were removed to a depth of about 20 cm, and rhizospheric soil was carefully extracted. The collected soil samples were placed in sterilized polyethylene bags and transported to the laboratory, where they were stored at 4 °C until further analysis.

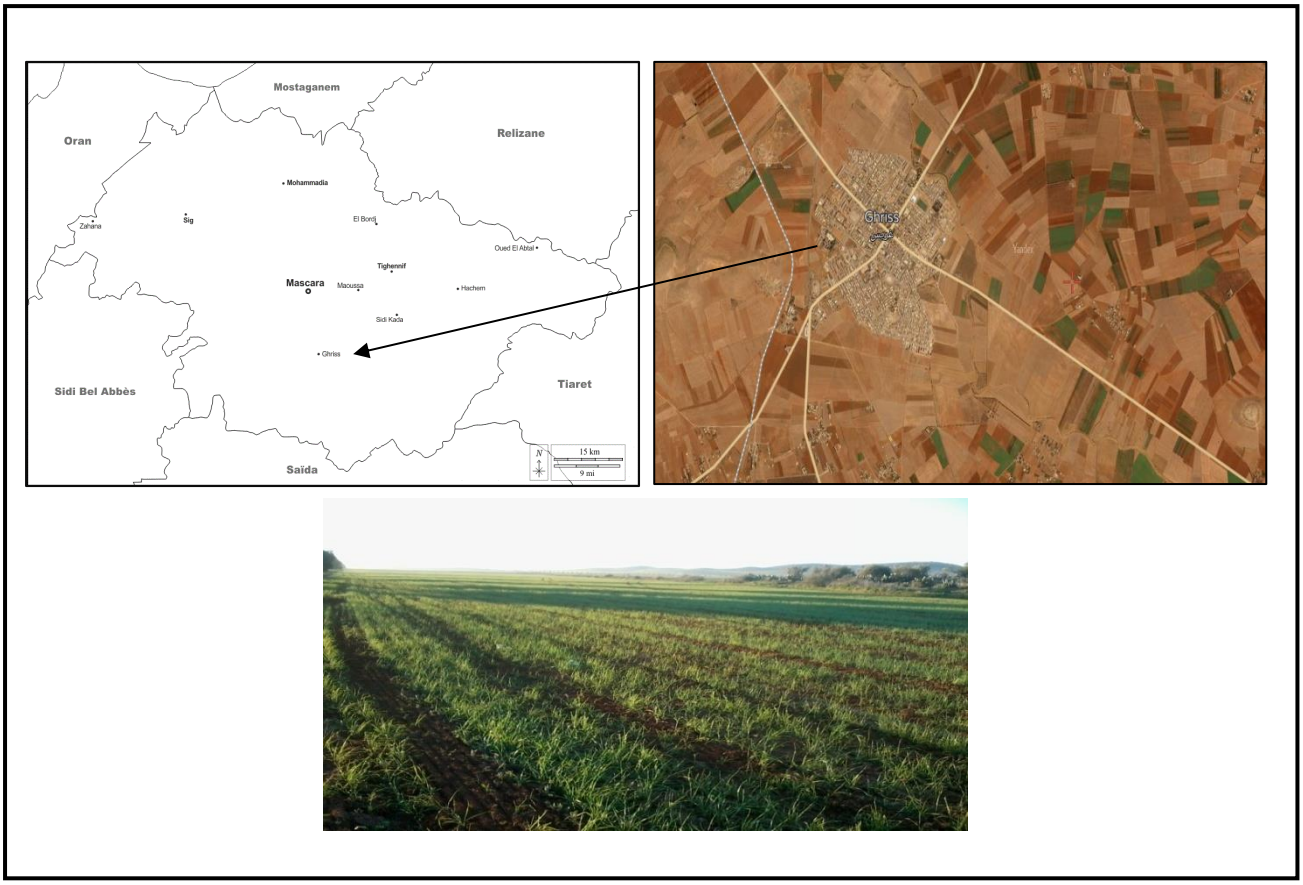

Figure 9. Geographic location of the study area.

2. Physicochemical characteristics of soils

The preparation of soil samples for physicochemical analyses was conducted following their collection. The soil samples were air-dried, then crushed and sieved to 2 mm **(Baize, 2018)**.

The analysis of the pedological parameters of the soil samples was performed at the Regional Laboratory for Soil Analysis and Irrigation Water INSID (Matmar-Relizane), El Feth Quality Analysis Laboratory (Oran) and the Laboratory of Biochemistry and Biotechnology (LR01ES05) at the University of Tunis El Manar, Tunisia.

To characterize the samples, standardized protocols established by AFNOR and additional methods from the literature were applied.

2.1. Granulometric analysis

The granulometric analysis determines the percentage of the soil's constituent materials: clay, silt, and sand. The soil texture was identified using the international Robinson pipette method. After removing organic matter and treating the fine soil with a dispersing agent to break up aggregates, the fractionation of clays and silts was conducted using the Robinson pipette, following a sedimentation period that varies with temperature **(NF P 94-057- AFNOR, 1992)**.

2.2. pH

A 10 g soil sample is suspended in 25 mL of distilled water. The suspension is stirred with a magnetic bar for 60 minutes at at approximately 20 °C, and the pH is measured using a pH meter once stabilization is achieved **(X 31-103- AFNOR, 1988)**.

2.3. Electrical conductivity (EC)

The electrical conductivity of the soil sample was measured using a digital conductivity meter. A 10 g sample of soil was mixed with 50 mL of distilled water to prepare a 1:5 (w/v) slurry. The mixture was thoroughly shaken to ensure complete dissolution of soluble salts. After the soil settled, the conductivity cell was inserted to record the readings. The results were expressed in μS/cm **(Zaiad, 2010)**.

2.4. Total organic carbon (TOC)

Organic carbon is determined using the **Walkley & Black method (1934)**. This method employs wet oxidation of organic matter without external heating, utilizing a mixture of potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid (H_2SO_4). Any unreacted potassium dichromate is subsequently titrated with a ferrous sulfate solution (Mohr's salt). The amount of Mohr's salt required for titration is used to calculate the organic carbon content present in the soil.

2.5. Total nitrogen (TN)

The total nitrogen content in the soil was determined using the **Dumas method (1831)** in a fully automated system. This technique involves the complete combustion of crushed soil under an oxygen-rich environment at high temperatures. During the process, nitrogen is quantitatively converted into N_2 through oxidation and reduction tubes. Other volatile combustion products are either trapped or separated. A thermal conductivity detector is then used to measure the gaseous nitrogen. The results are expressed either as a percentage or in milligrams of nitrogen**.**

2.6. Available phosphorus (P)

Phosphorus is extracted using the **Olsen *et al*. (1954)** method, which employs a 0.5 N sodium bicarbonate solution at a pH of 8.5 as the extracting agent.

2.7. Determination of Total Trace Metal Contents

The mineralization of trace metal elements was performed under heat (on a hot plate for three hours) using a mixture of hydrochloric acid, nitric acid, and hydrofluoric acid. The analysis was conducted using Atomic Absorption Spectrometry (AAS). The results are expressed in mg/kg of dry weight of the soil **(Ye *et al*., 2021)**.

3. Isolation Strategy

Composite soil samples (10 g each) were introduced into 90 mL of sterile saline solution (0.9% NaCl) in 250-mL conical flasks. The mixtures were agitated with a magnetic stirrer at 150 rpm for 5 minutes to ensure effective disintegration of the particles. Standard serial dilutions ranging from 10^{-1} to 10^{-6} were prepared by transferring 1 mL of the suspension into test tubes containing 9 mL of sterile saline solution. Subsequently, 100 µL of each dilution was spread onto nutrient agar (NA) plates and incubated at 37 °C for 48 hours. Bacterial colonies displaying different shapes and colors were selected and purified using the streaking technique on nutrient agar (NA) medium.

4. Screening of heavy metal resistant bacteria

A ‘screening’ was conducted to identify microorganisms capable of growing on media containing heavy metals. The obtained isolates were tested for their resistance to chromium, nickel, and aluminum using the agar dilution method.

The isolated bacterial strains were screened for resistance using the agar diffusion method **(Nokman *et al.*, 2019)**. A 100 µL of the final culture was inoculated onto LB medium separately supplemented with 100 mg/L of heavy metals ($K_2Cr_2O_7$, $NiCl_2$, $AlCl_3$). Inoculation of the LB agar plates was performed using bacterial suspensions (inocula) adjusted to 10^6 cellules/mL. The cultures were incubated for 48 h at 37 °C. Control plates were also prepared using LB medium without the addition of heavy metals for comparison.

The resistance of bacterial strains to heavy metals was determined by their growth on the culture medium.

5. The purification and preservation of isolated strains

After preliminary screening, morphologically distinct colonies were isolated and purified using the streaking method. A Gram stain was performed after each purification. The colonies were preserved at 4 °C on slanted GN medium for short-term storage, while long-term preservation was done in glycerol (30%) at -50 °C.

6. Determination of minimum inhibitory concentration (MIC).

The Minimum Inhibitory Concentration (MIC) is defined as the concentration at which no visible growth occurs on the corresponding agar plates after 48 hours of incubation **(Nokman *et al.*, 2019; Marzan *et al.*, 2017)**. The MIC of heavy metals ($K_2Cr_2O_7$, $NiCl_2$, $AlCl_3$).) was determined by exposing each isolate to varying concentrations (100 µg/mL to 1800 µg/mL). 100 µL of bacterial suspension adjusted to 10^6 cells/mL was spot-inoculated onto LB agar plates supplemented with the respective heavy metal salts. The readings were recorded 48 hours after incubation at 37 °C.

7. Phenotypic and biochemical characterization of heavy metal resistant bacteria

Heavy metal-resistant bacteria were identified based on cultural and morphological characteristics, including colony color, shape, Gram staining, and motility tests. The biochemical characteristics were determined using classical tests according to *Bergey's Manual of Determinative Bacteriology* **(Holt, 1994)** and the API 2NE biochemical gallery. These tests were performed according to the manufacturer's instructions (Biomerieux, France), and the results were interpreted using the Bacterial Identification Program software **(Bryant, 2004)**.

8. Molecular identification of selected heavy metal resistant bacteria

The molecular analysis of heavy metal-resistant bacteria was performed at the Laboratory of Biochemistry and Biotechnology (LR01ES05) at the University of Tunis El Manar (Tunisia) and the Laboratory of Industrial Biotechnology and Systems Biology (IBSB) at Marmara University (Turkey).

The genomic DNA was extracted from the bacterial isolates using the NucleoSpin Soil Kit (Macherey-Nagel, USA) following the manufacturer's instructions. The quantity and purity of the DNA extracts were checked by using Nanodrop spectrophotometer (Thermo ScientificTM, USA). The amplification of 1500 bp fragment of 16S rRNA gene was assessed by PCR using the universal bacterial primers for 16S rRNA 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Janssen, 2006).

The conditions for thermal cycling were as follows: initial denaturation at 96 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 1 min 30 s, and a final extension at 72 °C for 5 min. The PCR products were run in the gel electrophoresis using 1 % agarose gel 1xTE buffer for 45 min at 100 V. The 16S rRNA PCR products were purified

using the Wizard SV Gel and PCR Clean-up System (Promega, New England) and sequenced by an ABI-PRISM 3700 DNA automated sequencer (Applied Biosystems).

To determine the identity of the sequences, they were initially edited with the 4peaks V1.8, and later submitted to BLASTN (National Center for Biotechnology Information; http://blast. ncbi.nlm.nih.gov/Blast.cgi), comparing them with sequences published on GenBank, according to identity ranking (> 97 %) and E-values (0.0). In addition, Maximum likelihood clustering analysis was developed, using as ingroup several 16S rDNA sequences of nominal species matching with our isolates within a percent sequence similarity threshold of 97 % (Nguyen *et al*., 2016). The nucleotide sequences generated during this study were deposited in the GenBank database.

9. Determination of antibiotic resistance

Antibiotic resistance of bacterial isolates was determined using the disk diffusion method **(Lennette *et al*., 1985)**. Bacterial strains were grown overnight in LB liquid medium and plated on Mueller-Hinton agar using sterile swabs. The turbidity of the medium was adjusted to match the 0.5 McFarland standard. Antibiotic disks were placed on the agar plates, which were then incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured after 24 hours. The bacteria were classified as resistant (R), intermediate (I), or susceptible (S) according to the standard antibiotic disk chart. The following antibiotic discs were used: Aztreonam (ATM) 30 µg, Bacitracin (BA) 0,05 UI, Fosfomycin (FC) 200 µg, Cefepime (FEP) 30 µg, Ampicillin (AMP) 10 g, Streptomycin (S) 10 g, Tetracycline (TE) 30 g, and Tobramycin (TOB) 10 g **(Benmalek & Fardeau, 2016)**.

10. Optimization of Physicochemical Parameters for Bacterial Growth

Cultures of the bacterial strain, obtained after incubation for 18–24 hours at 37 °C in LB broth, were centrifuged. The pellets were washed with distilled water and resuspended in

100 mL of sterile distilled water. The bacterial suspension was adjusted to match the 0.5 McFarland standard. Sterile 96-well microplates were used for these experiments.

- **pH**

The pH of LB liquid media was adjusted to 4.0, 5.5, 7.2, and 9.0 using 2 mol/L HCl and 2 mol/L NaOH solutions. A total of 200 µL of the adjusted media was carefully dispensed into each well of the microplate. Subsequently, 10 µL of bacterial suspension from each isolate was inoculated into the wells and incubated at 30 °C for 24 hours.

- **Temperature**

A total of 200 µL of sterile LB liquid media was dispensed into each well of the microplate and inoculated with 10 µL of bacterial suspension from each isolate. Temperature optimization was conducted by incubating the cultures at various temperatures **(**8, 22, 37, 40 et 55 °C).

- **Salinity (NaCl)**

The same volume of bacterial suspension from each isolate was inoculated into 200 µL of the adjusted media with varying NaCl concentrations (5, 10, 20, 40) and cultured at 30 °C for 24 hours.

The OD values of bacterial suspensions under different parameters were measured using a ELISA Microplate Reader at 600 nm (OD600) **(Jiang *et al.*, 2017)**.

11. Assessing the Effects of Heavy Metals on Microbial Growth

10 µL of each bacterial strain culture, obtained after incubation for 18–24 hours at 37 °C in LB broth and adjusted to 0.5 McFarland, were inoculated into 200 µL of sterile LB liquid

media dispensed into each well of the microplate**.** The media were supplemented with 300 µg/mL of heavy metals separately.

Optical density (OD) was measured at 600 nm using an ELISA microplate reader after 2, 8, 24, 48, and 72 hours of incubation. The effect of heavy metal concentration on bacterial growth was evaluated **(Afzal *et al*., 2017)**.

12. Heavy metal accumulation assay

Bacterial isolates were cultivated in LB broth medium (pH 7) in shake flasks placed on a rotary shaker at 150 rpm and 37 °C. Once the cultures reached an optical density of 0.6 at 600 nm, 100 µg/mL of sterilized heavy metals (Cr, Al, or Ni) were added individually to each flask. The cultures were incubated for 7 days under the same conditions. To determine the residual concentration of each metal, 10 mL of each bacterial culture was centrifuged at 6000 rpm for 10 minutes, and the supernatant was analyzed using Atomic Absorption Spectrometry **(Benmalek and Fardeau, 2016)**. The percentage of metal removal capacity (%R) was calculated by comparing the results with a control using the formula:

$$\%R = \frac{(Co - Cf)}{Co} * 100$$

Where %R represents the percentage of heavy metal removed, C_0 is the initial concentration of metal added to the LB broth (µg/mL), and C_f is the final metal concentration remaining in the LB broth (µg/mL) **(Vélez *et al*., 2021; Yan *et al*., 2021)**.

13. Elimination of Heavy Metals in Soil Microcosm

Following the experiments in liquid LB medium, the seven bacterial isolates were selected for a study on heavy metal degradation in contaminated soil samples. These

experiments were conducted in the laboratory using controlled soil microcosms, where the isolates were applied to the contaminated soils as a consortium.

- **Soil preparation**

The soil sample used for the experiment was taken from the Spa Granu-Ouest Froha Crushing Station in Mascara (35°25′N, 0°12′E). The samples were analyzed for the possible amounts of Cr(VI), Ni(II), and Al(III).

The soil sample was initially air-dried for several days at ambient temperature, then ground and sieved through a 2 mm sieve to remove particles larger than this size. To eliminate the indigenous microbial population that could interfere with the growth and activity of the isolates during the incubation period, the soil was sterilized by autoclaving for 1 hour at 120 °C, repeated three times at 24-hour intervals **(Tenover, 2009)**.

- **Selection and preparation of the consortium**

To select the isolates for the consortium, an antagonism test was conducted between the seven bacterial isolates that had been previously chosen with Disk Diffusion Method. In this test, a sterile paper disc was soaked with the bacterial culture and placed on an MH agar plate inoculated with a different bacterial strain. The plate was then incubated, and the growth or inhibition of the bacteria around the disc was observed **(Tenover, 2009)**.

- **Microcosm device**

The microcosm was was created following the methods described by **Lafuente *et al.* (1996)** and **Tirry *et al.* (2018)** with some modifications. Soil microcosms were prepared in 8 cm Petri plates using sterilized soil. 15 of dried and sieved soil were weighed, placed in the Petri dishes, and spread into a uniform layer. Sterile distilled water was added to the soil to achieve a final moisture content of 20% (vol/wt). 3 ml of the bacterial consortium, pre-cultured

in LB medium, were used as the inoculum and thoroughly mixed into the soil using a sterile spatula. Control microcosms were prepared using the same method but with 3 mL of sterile LB medium instead of the bacterial consortium. The percentage of removal for the three metals (Cr(VI), Ni(II), and Al(III)) from the soil was measured using AAS after 7 days of incubation at 37°C under aseptic conditions.

- **Seedling Germination Bioassay Test**

A seedling germination bioassay was conducted to assess the impact of heavy metal removal by the bacterial consortium in soil. The effect of treated and untreated soil on lentil seed germination was evaluated. Lentil seeds were surface-sterilized with alcohol for 2 minutes and then rinsed thoroughly three times with sterilized distilled water. Twenty seeds were placed in each Petri plate, and germination was carried out in the dark at 30°C. Results of the seed germination experiments were recorded after 2 days of sowing. A parallel control experiment was conducted under the same conditions.

14. Statistical Analysis

All experimental results are expressed as arithmetic means obtained from at least three replicates. Statistical analyses were performed using XLSTAT software.

Results and Discussion

Part 1. Study of Heavy Metal Toxicity

1. Results

1.1. Variations in the Body Weight and Relative Organ Weight of Rats

The effect of three heavy metals on the body weight of different groups of rats is illustrated in Table 4.

During the 3-month experiment, monitoring the evolution of the rats' body weight revealed a significant difference between the experimental groups. A decrease in weight gain was observed in all heavy metal-treated groups compared to the control group. This decrease was particularly noticeable in the groups exposed to a dose of 1/50 of the LD_{50}, especially in the Cr_{50} group, which had a weight gain of only 0.61%. In comparison to the T Control group, the weight gain in the Cr_{100}, Ni_{100}, and Al_{100} groups was 0.78, 0.89, and 0.9g, respectively. In the Cr_{50}, Ni_{50}, and Al_{50} groups, the weight gains were 0.61 0.7, and 0.7g, respectively.

Regarding the effect of metals on the relative organs weight, it was observed that nickel, at a dose of Ni_{50}, caused a significant decrease in liver and kidney weight compared to the control group.

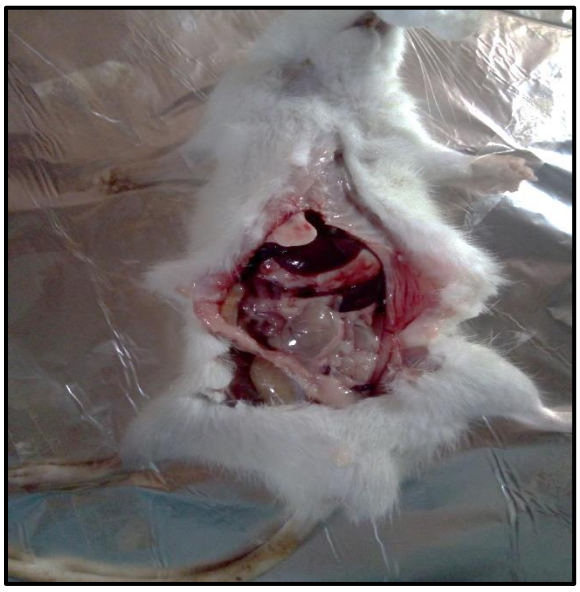

Figure 10. Model of a rat dissection

Table 4. Heavy Metals' Effects on Body Weight and Relative Organ Weight

Parameters	Control	Cr_{100}	Ni_{100}	Al_{100}	Cr_{50}	Ni_{50}	Al_{50}
Initial body weight (g)	139,5	133	145,2	133,7	143	160,2	145,2
Final body weight (g)	255,2	203,6	225,5	217,3	198,3	230,2	211,2
Weight gain (g)	1,28	0,78	0,89	0,92	0,61	0,777	0,733
Relative liver weight (%)	4,33	3,681	3,716	4,22	3,8	3,58	4,3
Relative kidney weight (%)	1,2	1,016	1,028	1,179	1,014	0,98	1,15

1.2. Microbiological approach

The effect of different heavy metals on the microbiological profile in the small intestine of different groups of rats is illustrated in Figure 12.

Exposure to Cr increased the proliferation of aerobic bacteria compared to the control group (T). This increase was more pronounced in the treated groups given a dose of 1/50 DL_{50}. However, the same treatment reduced the growth of anaerobic bacteria, with levels reaching 5.39 log CFU/g in the small intestinal content.

The proliferation of aerobic and anaerobic bacteria in the small intestinal samples of the Ni100 group was 5.14 and 6 log CFU/g, respectively, while in the Ni_{50} group, it was 5.36 and 5.36 log CFU/g, respectively. Our results showed that exposure to $AlCl_3$ significantly reduced anaerobic bacterial growth compared to controls (T) at both concentrations. All intoxicated groups, regardless of dose, exhibited significantly lower levels of *Lactobacillus* spp. compared to controls, with the lowest levels observed in the Al_{50} group.

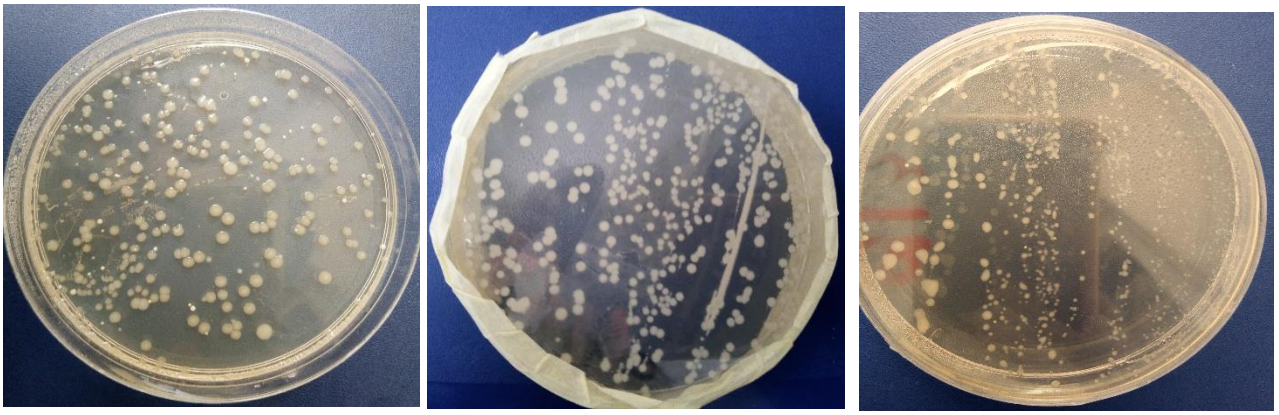

Figure 11. Macroscopic Aspects of Isolated Intestinal Flora.

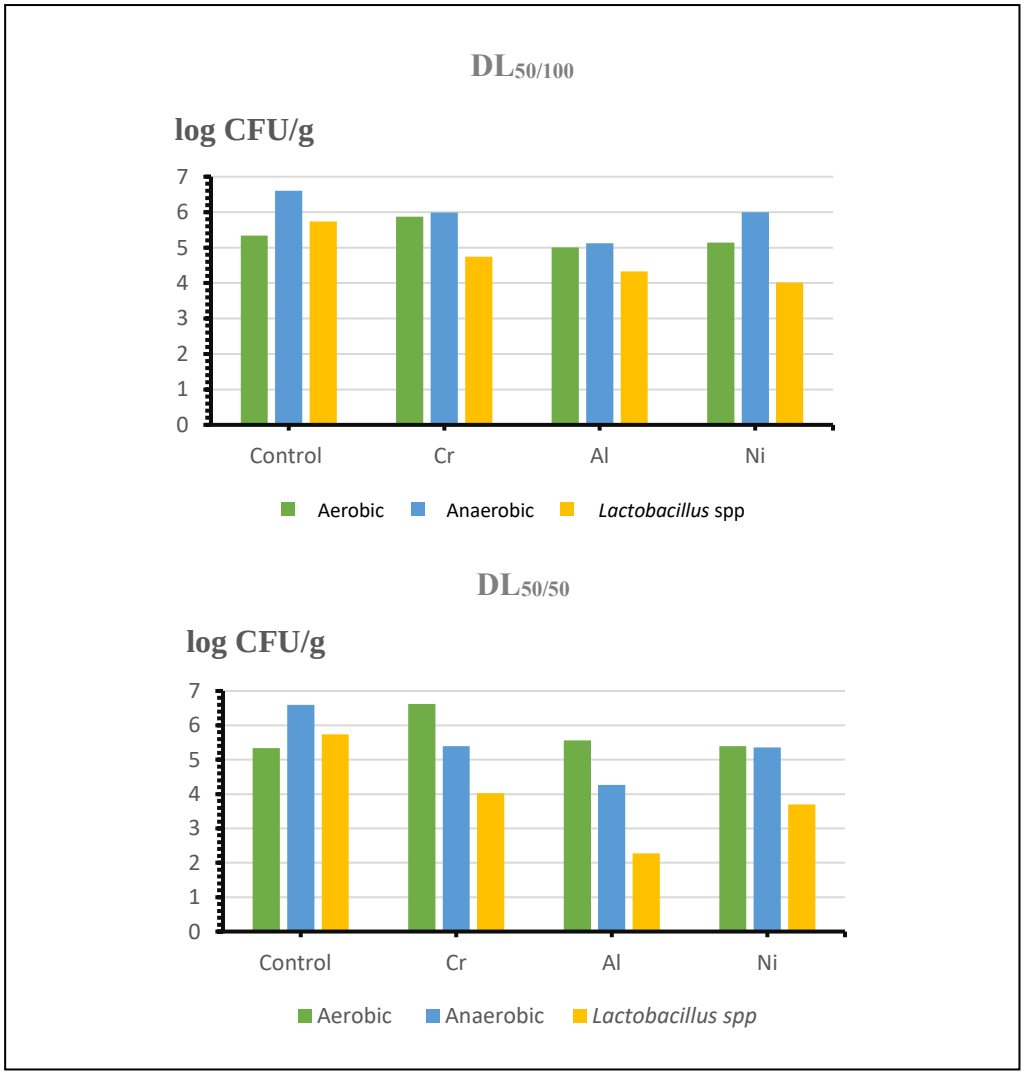

Figure 12. Heavy metals' effects on the microbiological profile of the small intestine in different groups of rats.

2. Discussion

Exposure to heavy metals has emerged as a global public health issue, as certain metals can act as systemic toxicants even at low concentrations within the human body **(Porru *et al.*, 2024)**.

In the present study, groups of adult rats were exposed to heavy metals such as chromium, nickel, and aluminum for up to 3 months. During the 13 weeks of the experiment, evaluations of the rats' body weight revealed significant differences among the experimental groups, with a noticeable decrease in the intoxicated group. This weight loss is attributed to the anorexigenic effects induced by these heavy metals.

These results align with previous studies that reported a decrease in food intake among rats exposed to heavy metals. For instance, **Balgoon, (2019)** observed a notable weight gain decrease of approximately 3.51% in rats treated with $AlCl_3$, which was linked to the metal's impact on intestinal absorption. Aluminum affects the pathways for synthesizing serotonin and dopamine, two neurotransmitters crucial for regulating digestion, eating habits, and feelings of fullness **(Belmokhtar *et al.*, 2020)**.

A substantial reduction in body weight was also noted in the group exposed to nickel, consistent with numerous earlier studies that reported weight loss in rats subjected to nickel exposure **(Dahdouh *et al.*, 2013; Djemli *et al.*, 2012)** . The groups exposed to chromium also experienced weight loss, particularly those receiving $DL_{50/50}$, aligning with the findings of **Stout *et al.* (2009)**. Research indicates that chromium treatment disrupts biochemical parameters affecting glucose, insulin, and lipid metabolism **(Saidi *et al.*, 2020)**.

Long-term exposure to heavy metals can negatively impact organ structure and function **(Vielee *et al.*, 2024; Yang *et al.*, 2022; Farag & El-Shetry, 2020)**. Our findings revealed that

chromium significantly reduced the relative weights of the liver and kidneys, consistent with the study by **Karaulov *et al*. (2019)**. Other research has indicated that the liver is the primary organ affected by Cr(VI) toxicity, leading to liver damage **(N. Li *et al*., 2024)**.

Additionally, research conducted by **Houamria *et al*. (2019)** found that nickel accumulation in the liver and kidneys causes tissue structural damage, resulting in stunted growth and impaired organ function. Our investigation showed that the effect of aluminum on the relative weights of the liver and kidneys increased with higher exposure levels, consistent with **Mokrane's *et al*., (2020)** study.

The gut microbiota plays a crucial role in maintaining intestinal balance. Research has shown that exposure to environmental pollutants, like heavy metals, can contribute to the development of various diseases, which may disrupt gut health and lead to dysbiosis in gut microbiota **(Bist & Choudhary, 2022; Wang *et al*., 2022)**.

In this study, we noted a clear decrease in anaerobic bacteria and *Lactobacillus* spp. in the intoxicated group compared to the control group, especially in the Al group, and this decrease increases with rising concentrations of the metal. However, the same treatment led to an increase in aerobic bacteria. Previous research has shown that prolonged exposure to Cr (VI) can lead to considerable alterations in the composition of gut microbiota **(Zhang *et al*., 2020)**.

According to **Li *et al*. (2021)**, long-term exposure to Cr (VI) induces a decrease in certain anaerobic bacteria in chickens, such as *Butyricimonas*, *Blautia*, *Oscillospira*, *Lachnospiraceae*, *Ruminococcus*, and *Ruminiclostridium*. Research has shown that *Proteobacteria*, particularly those in the *Enterobacteriaceae* family, significantly increase following exposure to nickel in rats **(Richardson *et al*., 2018)**. Other studies have reported that oral exposure to aluminum affects the structure of the gut microbial community, with *Lactobacillus* levels decreasing as the dosage increases **(Feng *et al*., 2024; Shang *et al*., 2023)**.

The toxic effect of heavy metals on intestinal microflora extends beyond the cause of dysbiotic diseases; it can also worsen intestinal infections, affect metabolic processes, and influence antibiotic resistance development **(Zhu *et al*., 2024; Delyukina *et al*., 2023)**.

In summary, heavy metal exposure poses a serious risk to body weight regulation, organ health, and gut microbiome integrity, highlighting the need for continued research and public health measures to mitigate these effects.

Part 2. Study of Heavy Metal Bioremediation

1. Results

1.1. Physicochemical characteristics of the soil

After sieving to 2 mm, the agricultural soil samples collected from the Ghriss site were analyzed for pH, electrical conductivity (EC), organic carbon, total nitrogen, phosphorus, and trace metal elements. The soil texture was determined through granulometric analysis. The results are presented in Table 5.

Table 5. Physicochemical Analysis Results of the Studied Soils

Parameters	Sol 1	Sol 2	Sol 3	Sol 4	Sol 5	Sol 6
Clay (%)	35.34	17.48	13.94	30.10	39.45	19.42
Silt (%)	52.25	27.54	44.69	54.12	47.79	32.49
Sand (%)	13.40	54.98	41.37	15.78	12.76	48.09
pH	7.68 ± 0.05	7.45 ± 0.09	7.14 ± 0.06	7.28 ± 0.06	7.60 ± 0.07	7.56 ± 0.05
EC (µs/cm)	0.16	0.17	0.11	0.08	0.19	0.17
TOC (%)	0.77 ± 0.16	0.82 ± 0.11	0.51 ± 0.21	0.59 ± 0.09	0.81 ± 0.08	1.01 ±0.12
TN (%)	0.35 ± 0.01	0.18 ± 0.00	0.12 ± 0.00	0.16 ± 0.02	0.15 ±0.03	0.24 ±0.1
P (mg/kg)	10.50 ± 0.09	9.01 ± 0.05	10.04 ± 0.07	6.99 ± 0.1	10.01 ± 0.8	9.89 ± 0.01
Cr (VI) (mg/kg)	4.46	27.38	17.53	3.43	7.98	11.87
Al (III) (mg/kg)	27.4	49.87	31.45	25.5	30.43	32.85
Ni (II) (mg/kg)	1.02	0.53	2.01	1.09	0.76	1.07

1.2. Isolation and Screening of heavy metal resistant bacteria

From the soil samples, a total of 222 single bacterial colonies with various visible characteristics and colony morphologies were isolated. The distribution of the isolated bacteria based on their Gram classification is shown in the figure 7. The isolated bacteria exhibited a wide variety of species, with a higher abundance of Gram-positive rods (53%) and Gram-negative rods (21%). After the initial screening of bacterial colonies on LB medium containing

heavy metals at a concentration of 100 µg/mL, 185 bacterial strains were found to be resistant to the tested heavy metals (Cr, Ni, and Al).

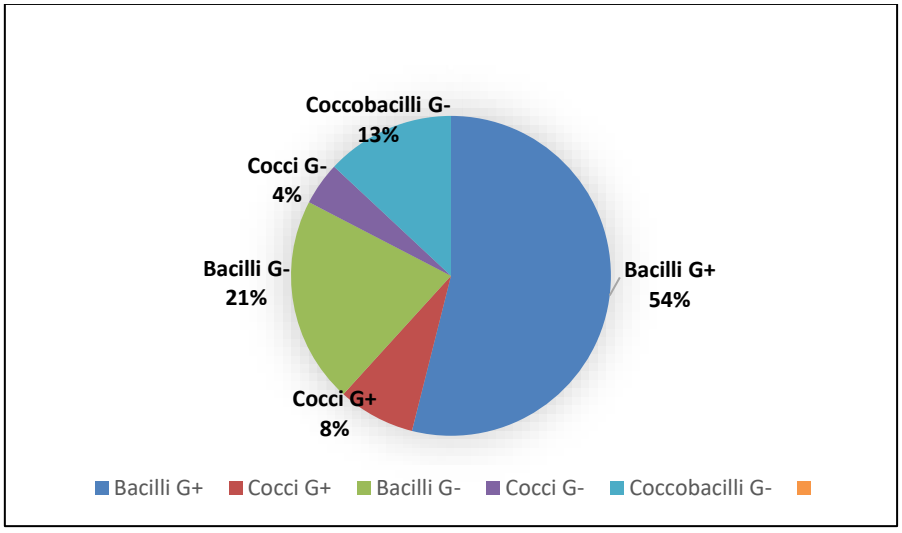

Figure 13. Percentage of each type of bacteria isolated.

1.3. Determination of minimum inhibitory concentration (MIC)

As shown in Figure 14, bacterial resistance to heavy metals decreases as the concentration of metals in the medium increases. A comparison of the three metals shows that bacterial resistance to aluminum was higher than to nickel and chromium, respectively, with no resistance observed above 1400 µg/mL for chromium and 1600 µg/mL for nickel and aluminum.

Seven isolates showed high resistance to heavy metals, with the S4B31 isolate having the highest resistance to Cr (1300 µg/mL). At a Ni concentration of 1600 µg/mL, isolat S2B1 and S5B16 had the highest resistance.

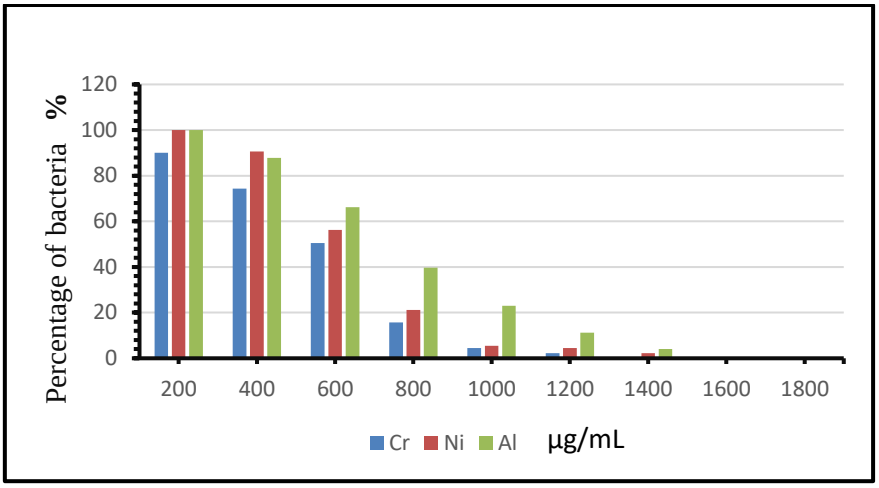

Figure 14. Percentage of bacterial resistance to chromium, nickel, and aluminum.

Table 6. Minimum inhibitory concentration (MIC) of the most effective bacteria.

	S1B10	S1B26	S2B1	S4B31	S5B16	S5B23	S6B3
Cr (V) µg/mL	1200	1000	1200	1300	1100	900	1100
Ni (II) µg/mL	1400	1000	1600	1000	1600	1300	1500
Al (III) µg/mL	1400	1200	1500	1300	1500	1400	1500

1.4. Phenotypic and biochemical characterization of heavy metal resistant bacteria

The phenotypic and biochemical characterization of selected heavy metal-resistant bacteria is presented in Table 7. The morphological identification of the isolated bacteria was confirmed through molecular identification using PCR-amplified ribosomal DNA ITS sequences. Based on the sequencing of 16S rDNA ITS fragments, the isolates were identified by comparing them to the closest species in the GenBank database.

Strain S1B10 exhibited 98% sequence similarity to *Pseudomonas aeruginosa*, whereas strain S1B26 displayed 97% similarity to *Pseudomonas fluorescens*. Isolate S5B16 was identified as a *Bacillus* species with 98% similarity. Similarly, isolates S2B1 and S6B3 showed 98% sequence similarity to *Bacillus cereus*. Strain S4B31 was found to have 98% sequence

similarity to *Rhodopseudomonas palustris*, while strain S5B23 was identified as a *Planomicrobium* species with 98% similarity.

Table 7. Identification of selected heavy metal-resistant bacteria

	Isolat						
	S1B10	**S1B26**	**S2B1**	**S4B31**	**S5B16**	**S5B23**	**S6B3**
Morphological characteristics							
Colony color	Green	Transparent cream	White	Orange	Cream white	Yellow	White
Cell shape	Rod	Rod	Rod	Rod	Rod	Short rod	Rod
Gram nature	-	-	+	-	+	+	+
Motility	+	+	+	-	+	+	+
Biochemical characteristics							
Oxidase	+	+	-	+/-	-	-	-
Catalase	+	+	+	+	+	-	+
Nitrate	+	+	+	+	+	-	+
Citrate	+	+	+	+	+		+
Glucose	+	+	+	-	+	-	+
Lactose	-	-	-	-	-	+	-
Gaz	-	-	+	-	+	-	+
H2S	-	-	-	-	+	-	-

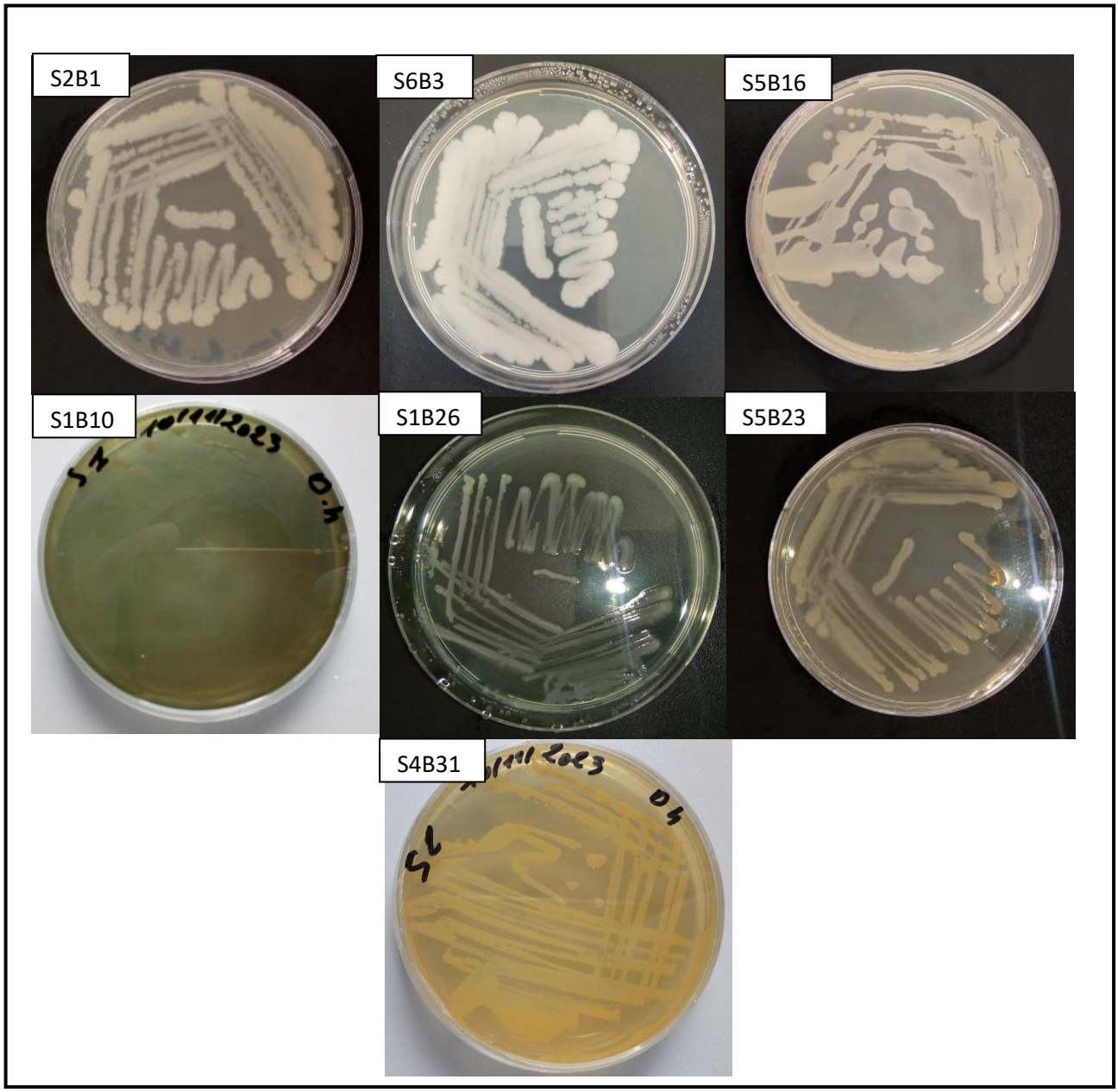

Figure 15. Morphological properties of isolated strains

1.5. Antibiotic resistance

The inhibition zone measurements presented in Figure 16 were used to evaluate the antibiotic sensitivity of the seven isolates. As shown in Table 8, *Pseudomonas* sp. was resistant to three antibiotics: bacitracin, fosfomycin, and tetracycline. Regarding *Bacillus* sp., it showed resistance to aztreonam, bacitracin, and fosfomycin. The isolate *Planomicrobium* sp.

demonstrated resistance to aztreonam and fosfomycin. In contrast, *Rhodopseudomonas palustris* was the most sensitive strain, with no resistance to the antibiotics tested.

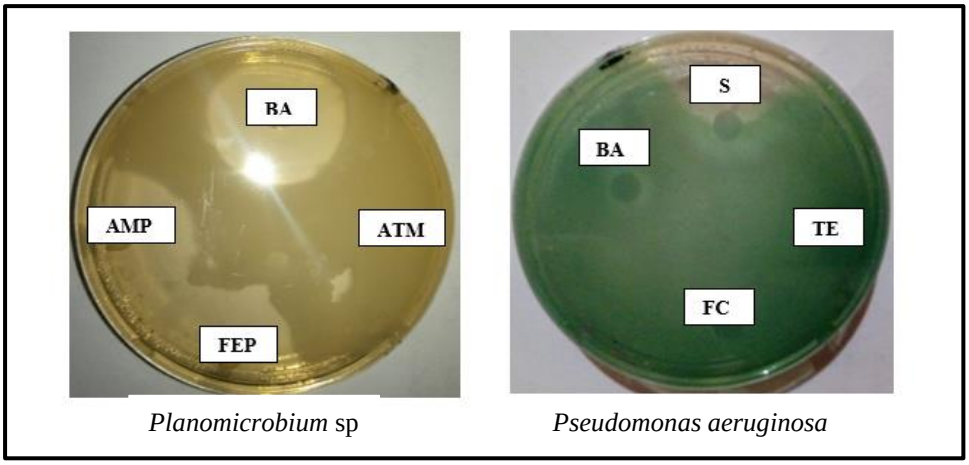

Figure 16. Results of the Antibiotic Susceptibility Test.

Table 8. Antibiotic susceptibility and resistance of the isolated strains.

	ATM	BA	FC	FEP	AMP	S	TE	TOB
Pseudomonas aeruginosa	S	R	R	S	S	S	R	S
Pseudomonas fluorescens	S	R	R	I	S	S	R	S
Bacillus cereus	R	R	R	I	I	I	S	I
Rhodopseudomonas palustris	S	S	S	I	I	S	S	S
***Bacillus* sp**	R	R	I	I	S	I	S	I
***Planomicrobium* sp**	R	S	R	S	S	I	I	S
Bacillus cereus	R	R	I	I	S	I	S	I

S= sensitive , I= intermediare , R= resistant

1.6. Optimization of Physicochemical Parameters for Bacterial Growth

- **pH**

It is evident from Figure 17 that pH significantly influenced the growth of the strains. As the pH gradually increased, the optical densities of the bacteria initially increased, then declined, reaching a maximum value at pH 7.2 for isolate *Bacillus* sp, with an optical density

of 1.276. The strain most resistant to pH 4 was *Rhodopseudomonas palustris*, with an optical density of 0.282, followed by strains *Bacillus* sp and *Planomicrobium* sp, which exhibited optical densities of 0.205 and 0.2, respectively. However, under highly alkaline conditions (pH 9), strain *Bacillus cereus* exhibited the highest resistance, with an optical density of 1.006, followed by *Bacillus* sp, with an optical density of 0.998. The remaining strains (*Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Bacillus cereus*, *Rhodopseudomonas palustris*, and *Planomicrobium* sp) demonstrated moderate resistance, with optical densities ranging between 0.78 and 0.943.

- **Temperature**

As shown in Figure 18, the optical density of the isolated strain increased and then decreased with the gradual rise in temperature. The highest growth was observed at 37 °C, with strain *Planomicrobium* sp exhibiting an optical density of 1.265. Strain *Pseudomonas fluorescens* demonstrated significant resistance to low temperatures, with an optical density of 0.881. However, it was the most sensitive to higher temperatures. At 55 °C, the most resistant bacteria were strains *Bacillus* sp and *Bacillus cereus*, with optical densities of 0.723 and 0.679, respectively.

- **Salinity**

Estimation of bacterial strain tolerance to salinity stress (ranging from 5 to 40 g/L) revealed variable growth intensities, reflecting differing levels of resistance (Figure 19). A decrease in growth, measured in terms of optical density, was observed as the NaCl concentration increased. At a concentration of 5 g/L, all strains exhibited maximal growth. Strain *Pseudomonas fluorescens* recorded the highest growth values at 5 g/L and 20 g/L, with

optical densities of 1.27 and 0.8706, respectively. The strain most resistant to higher NaCl concentrations was *Rhodopseudomonas palustris*, with an optical density of 0.495.

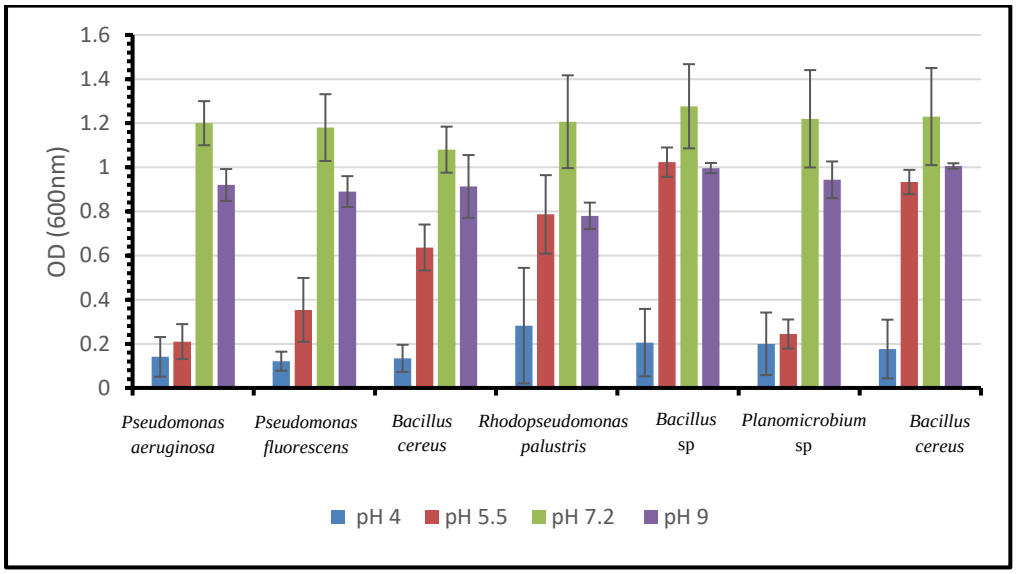

Figure 17. Effect of pH on bacterial growth.

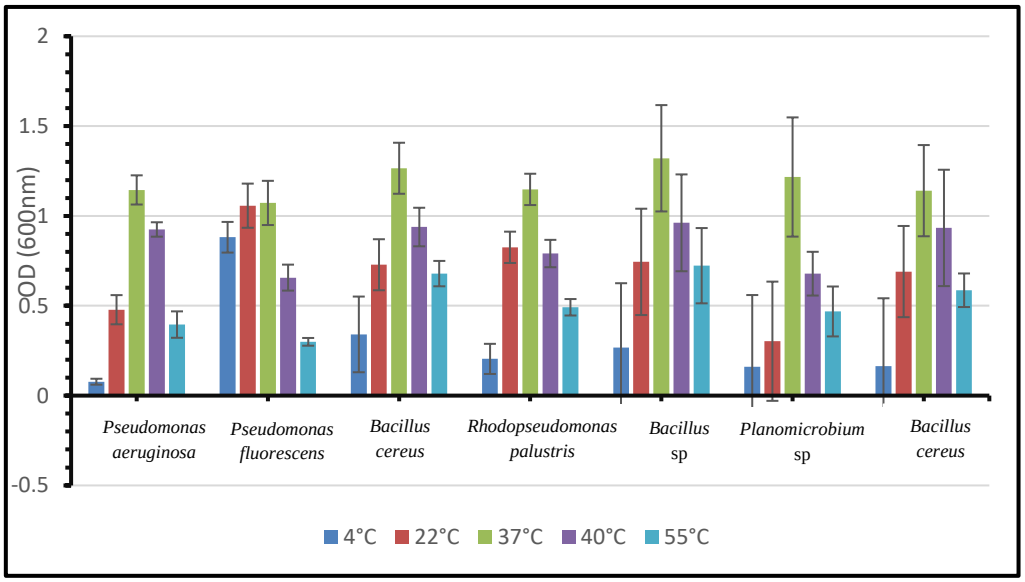

Figure 18. Effect of temperature on bacterial growth

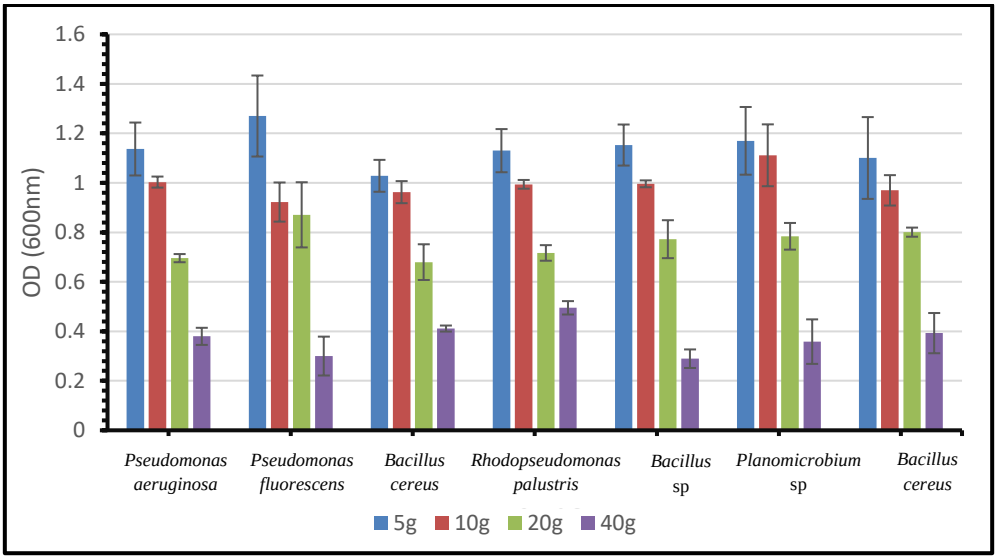

Figure 19. Effect of salinity (NaCl) on bacterial growth.

1.7. Assessing the Effects of Heavy Metals on Microbial Growth

The impact of the three heavy metals on the growth of isolated strains was assessed by comparing their growth profiles to the control. As illustrated in Figure 21, 22, and 23, the isolated strains displayed varying growth patterns when exposed to 100 µg/mL of different heavy metals. A reduction in growth, measured by optical density, was observed compared to the control group without heavy metals. After 72 hours of incubation in LB broth supplemented with 300 µg/mL of Cr, the isolated strain S1B10 exhibited the highest growth rate, with an optical density (OD) of 0.99. Similarly, strain S2B1 showed the highest growth on Ni with an OD of 1.00, while strain S6B3 demonstrated the highest growth on aluminum, reaching an OD of 1.8.

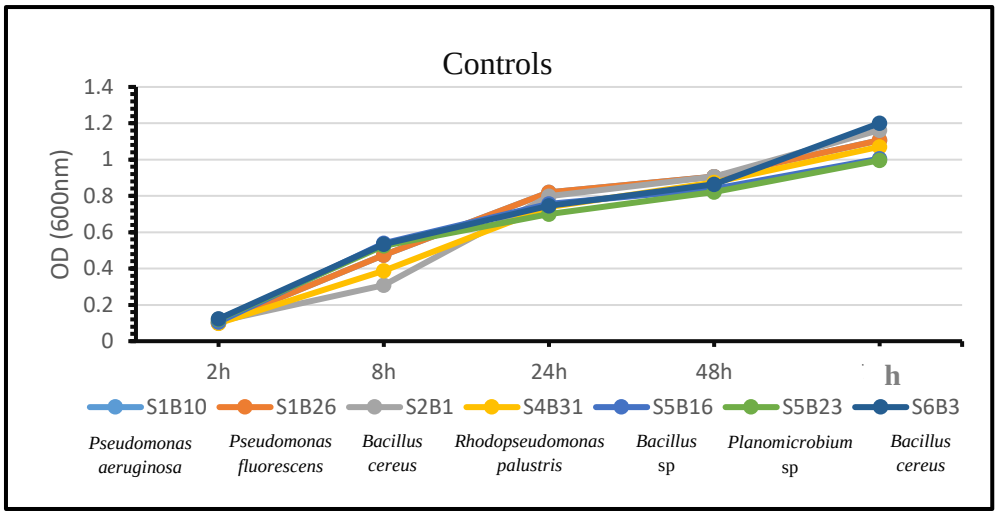

Figure 20. Growth kinetics of isolates.

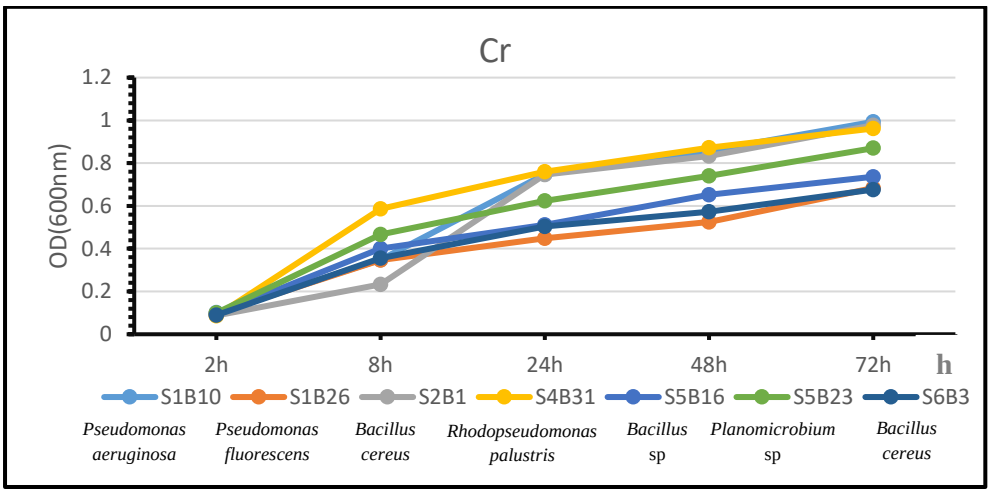

Figure 21. Effect of chromium on the growth of isolates.

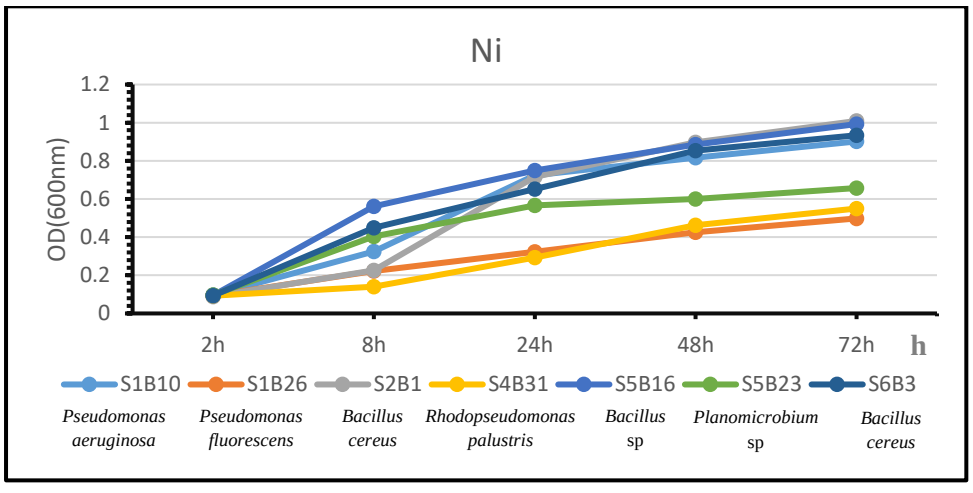

Figure 22. Effect of nickel on the growth of isolates.

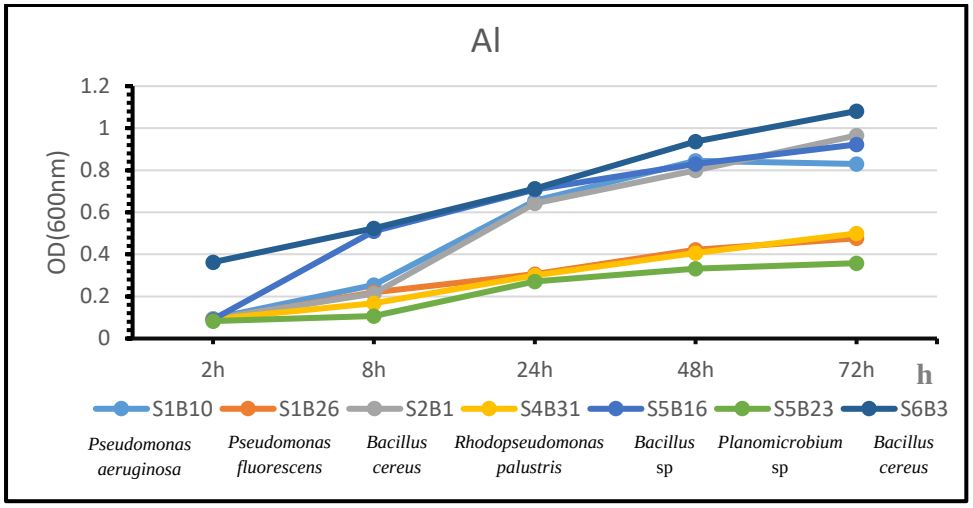

Figure 23. Effect of aluminum on the growth of isolates.

1.8. Heavy metal accumulation assay

Figure 24 illustrates the ability of microorganisms to remove heavy metals, tested using AAS. The results showed that isolate S5B16 was the most efficient at removing Cr and Al, with bioaccumulation rates of 42.57% and 59.50%, respectively, while isolate S1B26 exhibited the highest bioaccumulation rate for Ni, estimated at 62.37%. In contrast, isolate S1B10 demonstrated the lowest removal rate for Cr at 32.99%, and isolate S4B31 had the lowest rates for Ni and Al, at 41.15% and 42.44%, respectively.

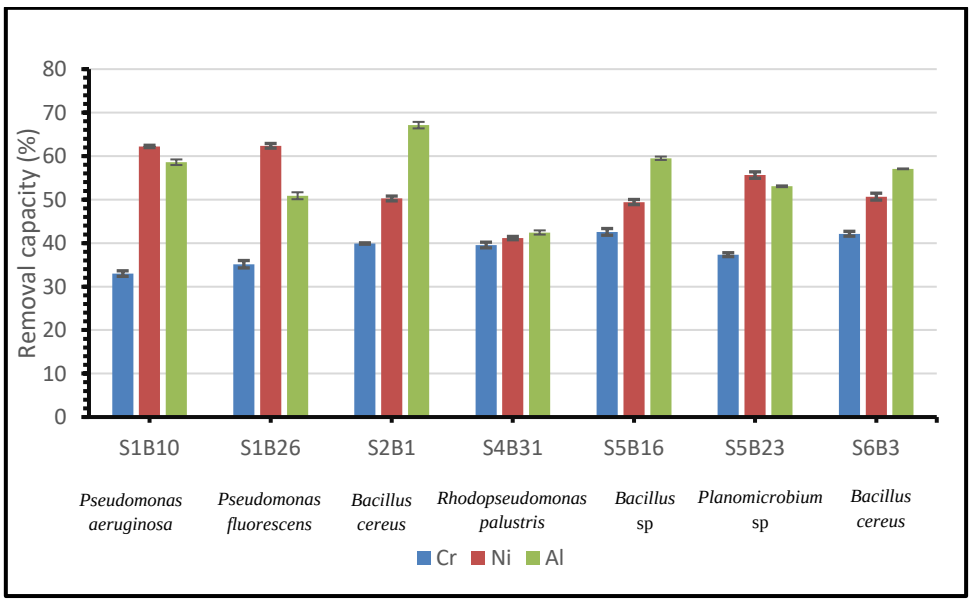

Figure 24. Heavy metals removal capacity of isolated bacteria.

1.9. Elimination of Heavy Metals in Soil Microcosm

From the results of the biocompatibility study of the microorganisms, we can conclude that there are two types of consortia. The first consortium is composed of *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Bacillus cereus* (S2B1), *Rhodopseudomonas palustris*, and *Bacillus cereus* (S6B3), while the second consortium consists of *Bacillus cereus* (S2B1), *Bacillus* sp, *Planomicrobium* sp, and *Bacillus cereus* (S6B3). In the comparison of the two consortia, bioremoval of Ni was more efficient in C1, with a rate of 38.02%, while C2 demonstrated a higher bioremoval rate for Al, estimated at 36.42%. However, C1 and C2 exhibited nearly equal bioaccumulation rates for Cr, at 34.94% and 35.1%, respectively. Figure 24 shows that seed germination in sterilized soil without bacterial culture (control) was approximately 25%, while the germination rate increased to 85% with bacterial consortium 1 (C1) and to 80% with bacterial consortium 2 (C2).

Table 9. Biocompatibility of the studied microorganisms.

	Pseudomonas aeruginosa	***Pseudomonas fluorescens***	***Bacillus cereus (S2B1)***	***Rhodopseudomonas palustris***	***Bacillus sp***	***Planomicrobium sp***	***Bacillus cereus***
Pseudomonas aeruginosa		+	+	+	+	-	+
Pseudomonas fluorescens	+		+	+	+	-	+
Bacillus cereus (S2B1)	+	+		+	+	+	+
Rhodopseudomonas palustris	+	+	+		+	-	+
Bacillus sp	-	-	+	+		+	+
Planomicrobium sp	-	-	+	-	+		+
Bacillus cereus	+	+	+	+	+	+	
Consortium 1 (C1)	***Pseudomonas aeruginosa/ Pseudomonas fluorescens/ Bacillus cereus (S2B1)/ Rhodopseudomonas palustris / Bacillus cereus (S6B3)***						
Consortium 2 (C2)	***Bacillus cereus (S2B1)/ Bacillus sp / Planomicrobium sp/ Bacillus cereus (S6B3)***						

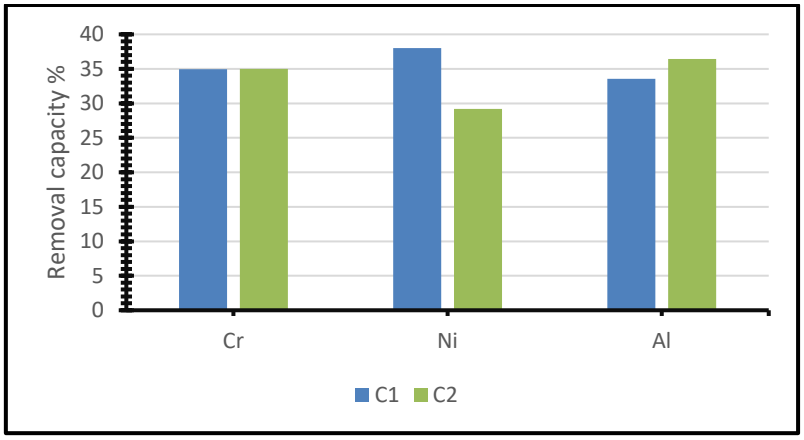

Figure 25. Elimination of heavy metals in soil microcosm.

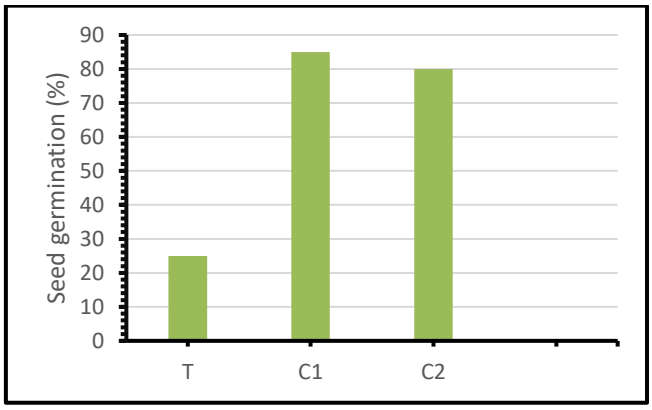

Figure 26. Percentage of seed germination in soil microcosm.

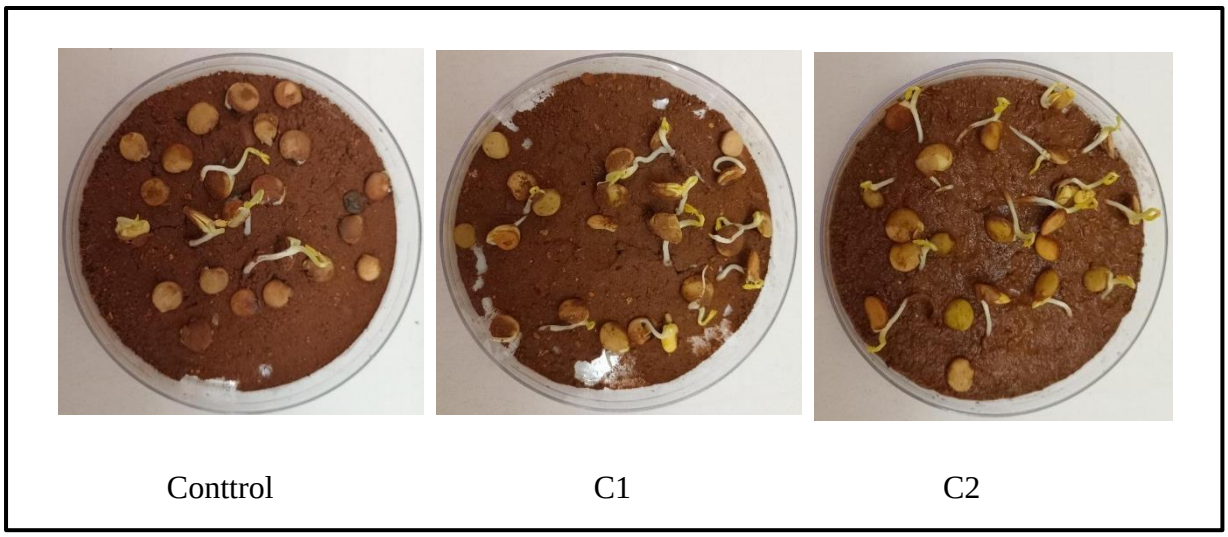

Figure 27. Seedling Germination in soil microcosm.

2. Discussion

With the rapid advancement of technology, both ecosystems and humans have recently been exposed to various chemical toxicants. Heavy metals, in particular, contribute to the contamination of agricultural soils and crops, resulting in severe environmental issues due to their toxicity and persistence in the ecosystem **(Angon *et al*., 2024; Rashid *et al*., 2023; Zaakour *et al*., 2022)**. Numerous studies have demonstrated the efficiency of native microbial strains in soil at reducing the concentration and toxicity of various heavy metals, enabling them to persist under heavy metal stress conditions and contribute to the removal of metals from the environment **(Firincă *et al*., 2024; Atuchin *et al*., 2023; Mohan *et al*., 2022)**.

In this study, we identified and characterized heavy metal-resistant bacteria isolated from agricultural soil. Initial screening of the bacterial colonies revealed that 185 strains successfully grew on LB medium supplemented with 100 µg/mL of heavy metals. However, our results indicated that bacterial resistance to heavy metals decreases as the concentration of metals increases. A comparison of the three metals revealed that bacterial resistance to aluminum was higher than to nickel and chromium, respectively.

Based on morphological, biochemical, and molecular identification, seven isolate strains were identified as *Pseudomonas aeruginosa* (S1B10), *Pseudomonas fluorescens* (S1B26), *Bacillus* sp. (S5B16), *Bacillus cereus* (S2B1 and S6B3), *Rhodopseudomonas palustris* (S4B31), and *Planomicrobium* sp. (S5B23). These strains were selected for further study.

The results revealed that *Pseudomonas sp.* exhibited MICs ranging from 1000 to 1200 µg/mL against Cr, 1000 to 1400 µg/mL against Ni, and 1200 to 1400 µg/mL against Al. Meanwhile, *Bacillus cereus* and *Bacillus* sp. were found to have MICs of 1100 to 1200 µg/mL, 1500 to 1600 µg/mL, and 1500 µg/mL against Cr, Ni, and Al, respectively. Concerning

Rhodopseudomonas palustris, it was characterized by MICs of 1300 µg/mL against Cr and Al, and 1000 µg/mL against Ni. The MICs of *Planomicrobium* sp. were found to be 900 µg/mL, 1300 µg/mL, and 1400 µg/mL against Cr, Ni, and Al, respectively. These findings align with previous studies. For instance, **Nayak *et al*. (2018)** reported that *Bacillus* sp. exhibited tolerance to 1500 mg/L of Cr, while **Hussain & Al-Saadi, (2021)** confirmed the ability of *Pseudomonas* sp. and *Bacillus* sp. strains to tolerate Cr at the same concentration.

In terms of Al, *Pseudomonas* sp. and *Bacillus* sp. isolates remained active at concentrations exceeding 1000 µg/mL. Similarly, **Purwanti *et al*. (2019)** demonstrated that *Pseudomonas* spp. could tolerate Al concentrations up to 500 µg/mL, while **Dhanarani *et al*. 2016** reported *Bacillus* spp. tolerance at 100 mg/L of Al. Additionally, *Pseudomonas aeruginosa* BC15 exhibited resistance to 700 mg/L of Ni **(Raja *et al*., 2006a)**. Furthermore, **Nguyen *et al*. 2016** determined that the MIC of *Rhodopseudomonas palustris* for aluminum under aerobic conditions was 850 µg/mL.

The ability of bacteria to tolerate various heavy metals has been attributed to multiple mechanisms, including membrane protein pumps encoded by either genomic DNA or bacterial plasmids. These pumps regulate the transport of metals across the cell membrane through active or passive mechanisms. This process involves resistance-nodulation-cell division family transporters and exopolysaccharides, as observed in many gram-negative bacteria **(Kang & Gross, 2005)**.

The behavior of the isolated strains against the studied antibiotics varies from one strain to another, indicating differences in bacterial antibiotic resistance. Resistance to antibiotics and tolerance to heavy metals in the environment represent an escalating global public health concern **(Edet *et al*., 2023)**. Many studies have highlighted a link between metal resistance and antibiotic resistance. Microorganisms that are resistant to antibiotics and tolerant to heavy

metals often emerge due to exposure to metal-contaminated environments. This exposure facilitates the coincidental selection of resistance factors for both heavy metals and antibiotics **(Benmalek *et al*., 2012)**. The overlapping presence of antibiotics and heavy metals, along with similarities in their resistance mechanisms, suggests a shared evolutionary history. Genes responsible for metal resistance are often genetically associated with antibiotic resistance genes. These resistance elements are assembled and horizontally transferred through plasmids, transposons, and integrons **(Gillieatt & Coleman, 2024; Perelomov *et al*., 2023; Fawwaz Alfarras *et al*., 2022)**.

The physiological analysis of the isolated strains conducted in this study confirmed that environmental factors, such as pH, temperature, and osmolarity, have a direct impact on their growth capacity. Bacterial growth rates are influenced by environmental conditions through specific response mechanisms. A two-component system, activated by signals such as pH, and temperature, regulates the production of secondary metabolites **(Jiménez-Delgadillo *et al*., 2018)**. pH, temperature, and salinity are key factors influencing growth during heavy metal remediation. Proper optimization of these parameters can enhance bioremediation effectiveness and reduce industrial production costs **(G. Fan *et al*., 2024; Atuchin *et al*., 2023)**.

The effect of heavy metals on the growth of the isolated strains observed in this study showed a decrease in optical density for all strains compared to the control. Similar findings have been reported in previous studies **(Megharaj *et al*., 2003; Srinath *et al*., 2002)**. Heavy metals cause environmental modifications that create unfavorable growth conditions, disrupting cellular physiology and consequently being perceived as stress **(Dressaire, 2009)**. Their impact on the growth of microorganisms may be attributed to detrimental effects on cell division, inhibition of protein synthesis, and significant morphological abnormalities. While metals can function as either micronutrients or toxicants, their availability for uptake by bacterial cells is

crucial. The specific metal species affects solubility, bioavailability, and membrane transport, as well as plays a role in processes such as adsorption, oxidation/reduction, and exposure duration **(Benmalek & Fardeau, 2016)**.

Our results revealed that *Bacillus sp.* was the most effective at removing Cr (42.57%) and Al (59.50%), while isolate S1B26 showed the highest bioaccumulation rate for Ni (62.37%). In contrast, *Pseudomonas aeruginosa* had the lowest removal rate for Cr (32.99%), and *Rhodopseudomonas palustris* had the lowest rates for Ni (41.15%) and Al (42.44%)

Purwanti et al. (2019) demonstrated that *Pseudomonas spp.* could remove up to 45.04% of Al from an initial concentration of 100 mg/L. Similarly, **Dhanarani *et al*. (2016)** reported that *Bacillus* spp. achieved a maximum Al biosorption of 79 mg/L at optimal temperature. **Rajkumar *et al*. (2005)** found that *Pseudomonas* spp. was capable of removing over 87% of Cr(VI) at an initial concentration of 200 mg/L. Additionally, studies have shown that *Bacillus* sp. reduced Cr(VI) by 80% at 40 µg/mL **Elangovan *et al*. (2006)** and by 93% at a starting concentration of 64 mg/L **(Wróbel *et al*., 2023)**. High concentrations of Cr(VI) negatively impact microbial growth by causing oxidative stress and damaging DNA and proteins in bacterial cells **(Nayak *et al*., 2018b)**. Studies have shown that the two main mechanisms for Cr(VI) removal are extracellular reduction (75% removal rate) and cell wall adsorption (24% removal rate) **(Pang *et al*., 2022)**. For Ni, **Naskar *et al*. (2020)** found that *Bacillus cereus* M16 could absorb up to 80% of Ni(II) in aqueous solution, while **Raja *et al*. (2006)** reported that *Pseudomonas aeruginosa* achieved a biosorption capacity of 93% for Ni at an initial concentration of 100 mg/L. The efficiency of bioremediation is highly dependent on the cell population and their resistance mechanisms, which enable metal absorption, transport, and efflux **(Guo *et al*., 2010)**.

Neumerous studies have doccumented the ability *Rhodopeudomonas palustris* to tolerate, assimilate and detoxify heavy metals from envirement. The heavy metal bioremoval profile in soil microcosms with bacterial consortia shows differences between the two consortia **(Li *et al.*, 2022)**. Bacterial survival and stability are enhanced when they exist as a mixed culture, and this depends on the species involved. Additionally, consortia cultures are more efficient in terms of metabolic activity and metal removal capability. Furthermore, consortia are more suitable for field applications, considering factors such as competition and survival **(Tahri Joutey *et al.*, 2015)**.

Conclusion and Perspectives

Conclusion and Perspectives

Environmental contamination by heavy metals poses a serious threat to human and ecological health. The remediation of environmental media using biological methods is a rapidly developing research field. Potential metal-resistant microbes can be utilized to remove metal pollutants from various contaminated areas due to their diverse metal–microbe interactions.

The first part of the study focused on evaluating the toxicological impacts of heavy metals (chromium, nickel, and aluminum) on weight regulation, organ health, and gut microbial balance.

The study demonstrates that heavy metal exposure (Al, Ni, Cr) leads to significant weight loss in rats by disrupting metabolism and intestinal absorption while causing gut microbiota dysbiosis, including a decrease in beneficial bacteria like *Lactobacillus* spp. It also damages the liver and kidneys, impairing their function. These effects contribute to disease development, metabolic disruption, and antibiotic resistance.

The second part of the study investigated heavy metal-resistant bacteria isolated from agricultural soils, focusing on their potential for bioremediation of chromium, nickel, and aluminum. Seven strains were identified, showing varying resistance and removal capacities. The study also highlighted the connection between heavy metal and antibiotic resistance, the influence of environmental factors on bacterial growth, and the enhanced efficiency of mixed bacterial consortia for practical field applications.

Looking ahead, integrating meta-omique approaches into bioremediation strategies could significantly improve our understanding of microbial communities involved in heavy metal detoxification. By leveraging high-throughput sequencing and functional genomics,

researchers can gain deeper insights into the genetic mechanisms that allow these bacteria to tolerate and remove metals, paving the way for the optimization of bioremediation processes. This approach could also facilitate the monitoring of microbial community dynamics, improving the efficiency and sustainability of metal removal in agricultural soils and other contaminated environments.

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Annexes

ANNEXES

Nutrient Agar

- Peptone ……..…… 5 g
- Meat Extract……..…….. 1 g
- Yeast Extract……..…….. 2 g
- Sodium Chloride…….. 5 g
- Agar……. 15 g
- pH = 7,5 7,5

LB (Luria Bartani)

- Dextrose anhydrate ……..…… 10 g
- Peptone ……..…….. 10 g
- yeast extract ……..…….. 5 g
- Sodium Chloride ….…… 5 g
- Agar ……..……..…… 15 g
- pH= 7.00

MRS

- Peptone ……...……...…… 10 g
- Sodium acetate ……...……...…….. 5 g
- Meat extract ……...……...…….. 10 g
- Magnesium sulfate …….. 0,10 g
- Yeast extract …… 5 g
- Manganese sulfate …… 0,05 g
- Glucose ……. 20 g
- Disodium phosphate …… 2 g
- Polysorbate 80…….. 1 g
- Ammonium citrate…… 2 g
- Agar…….. 15 g
- pH = 6,5

Procedure for using the API 20 NE gallery

1. Principle

The API 20 NE strip consists of 20 microtubes containing dehydrated substrates.

The conventional tests are inoculated with a saline bacterial suspension which reconstitutes the media. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents.

The assimilation tests are inoculated with a minimal medium and the bacteria grow if they are capable of utilizing the corresponding substrate.

The reactions are read according to the Reading Table and the identification is obtained by referring to the Analytical Profile Index

2. Technique

2.1. Preparation of the Gallery

Combine the base and lid of an incubation box and distribute water into the wells to create a humid atmosphere. Sterilely place the gallery into the incubation box.

2.2. Preparation of the Inoculum

Prepare a bacterial suspension in a 0.85% NaCl medium ampoule or in a tube of sterile distilled water, with turbidity equal to the 0.5 McFarland standard.

2.3.Inoculation of the Gallery

Fill the tubes (not the wells) of the NO3 to PNPG tests with the prepared suspension, avoiding the formation of air bubbles. Transfer 200 µl (4 to 8 drops) of the same suspension into an AUX Medium ampoule and mix thoroughly. Fill both the tubes and wells of the GLU to PAC tests. Cover the wells of the GLU, ADH, and URE tests with paraffin oil.

Incubate for 24 hours at 30°C.

3. Reading

After incubation, the reading of the gallery should be done by referring to the Reading Table (Tab. 1). Perform the tests that require the addition of reagents: see the results table.

4. Identification

Identification is done using APIWEB (Biomerieux).

Table 1. Reading Table for the Miniaturized API 20 NE Gallery

Tests	Active ingredients	Reaction/Enzymes	Results	
			Negative	**Positive**
NO3	Potassium nirate	reduction of nitrates to nitrites	NIT 1 + NIT 2 / 5 min	
			colorless	pink-red
TRP	L-tryptophane	indole production (TRyptoPhane)	JAMES / immediate	
			colorless pale green / yellow	pink
GLU	D-glucose	fermentation (GLUcose)	blue to green	yellow
ADH	L-arginine	Arginine DiHydrolase	yellow	orange / pink / red
URE	urea	UREase	yellow	orange / pink / red
ESC	esculin ferric citrate	hydrolysis (-glucosidase) (ESCulin)	yellow	grey / brown / black
GEL	gelatin (bovine origin)	hydrolysis (protease) (GELatin)	no pigment diffusion	diffusion of black pigment
PNPG	4-nitrophenyl-Dgalactopyranoside	-galactosidase (Para-NitroPhenyl-ßDGalactopyranosidase)	colorless	yellow
GLU	D-glucose	Assimilation (GLUcose)	transparent	opaque
ARA	L-arabinose	Assimilation (ARAbinose)	transparent	opaque
MNE	D-mannose	Assimilation (ManNosE	transparent	opaque
MAN	D-mannito	Assimilation (MANose)	transparent	opaque
NAG	N-acetyl-glucosamine	Assimilation (N-Acetyl-Glucosamine)	transparent	opaque
MAL	D-maltose	Assimilation (MALtose)	transparent	opaque
GNT	potassium gluconate	Assimilation (potassium GlucoNaTe)	transparent	opaque
CAP	capric acid	Assimilation (CAPric acid	transparent	opaque

ADI	adipic acid	Assimilation (ADIpic acid)	transparent	opaque
MLT	malic acid	Assimilation (MaLaTe)	transparent	opaque
CIT	trisodium citrate	Assimilation (trisodium CITrate	transparent	opaque
PAC	phenylacetic acid	Assimilation (Phenylacetic acid)	transparent	opaque
OX	tetraméthyl-phenylène diamine	cytochrome oxidase	colorless	violet

Table 2. Identification Results of Isolates S1B10 and S1B26 Using the API 20 NE

	NO3	TRP	GLU	ADH	URE	ESC	GEL	PNPG	GLU	ARA	MNE	MAN	NAG	MAL	GNT	CAP	ADI	MLT	CIT	PAC	OX
S1B10	+	-	-	+	-	-	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+
S1B26	+	-	-	+	-	-	+	-	+	+	+	+	+	-	+	+	-	+	+	-	+

S1B10: *Pseudomonas aeruginosa*

S1B26: *Pseudomonas fluorescens*

Table 3. Physico-chemical analysis of polluted soil

Paramaters	**Cr (mg/kg)**	**Ni (mg/kg)**	**Al (mg/kg)**	**pH**	**EC(µs/cm)**
Sol 7	71,02	30,15	129,47	6,9	2,32

Table 4. Critical values of inhibition zone diameters (EUCAST, 2024).

Antibiotique	Zone of inhibition (mm)	
	S	**R**
Aztreonam (ATM) 30 µg	≥ 23	< 17
Bacitracin (BA) 30 µg	≥ 15	<14
Fosfomycin (FC) 200 µg	≥14	<14
Cefepime (FEP) 30 µg	≥ 21	<15
Ampicillin (AMP) 10 µg	≥ 19	< 14
Streptomycin (S) 10 µg	≥ 17	<14
Tetracycline (TE) 30 µg	≥ 22	<18
Tobramycin (TOB) 10 µg	≥ 23	<20

Table 5. The nutritional composition of the rats' feed

Ingredients	
Luzerne Corn, Wheat bran, Soybean meal, Soybean oil, Calcium, Monocalcium phosphate, Salt.	
Additives	
Vitamins	
Vitamin A	1000 IU/kg
Vitamin D3	120.0 IU/kg
Trace Element	
Copper	
Analytical concentration	
Crude Protein	16%
Crude Fat	2.6%
Crude Ash	10%
Crude Cellulose	12%