

**INVESTIGATION AND ISOLATION OF
CADMIUM, FERUM, AND MANGANESE
RESISTANT MICROBES FROM TASIK
HARAPAN, UNIVERSITY SAINS MALAYSIA**

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UNIVERSITI SAINS MALAYSIA

2025

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CADMIUM, FERUM, AND MANGANESE
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HARAPAN, UNIVERSITY SAINS MALAYSIA**

by

AKTER RIMI

**Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science**

August 2025

ACKNOWLEDGEMENT

First and foremost, I extend my deepest gratitude to the Almighty for granting me the strength, wisdom, and perseverance to complete this research. I am eternally grateful to Dr. Husnul Azan Tajaruddin, my supervisor, for his invaluable guidance, unwavering support, and insightful feedback throughout my research journey. His expertise and encouragement have been instrumental in the completion of this thesis. I am also sincerely grateful to his team, “Ifa, Sofia, Hani, Ramisah, and Dr. Azura,” for their constructive suggestions and assistance. This thesis would not have been possible without the unwavering support of my family. My late father remains my constant inspiration. My mother's love and encouragement provided me with the strength to overcome challenges. To my only brother, Mina Asaduzzaman, I extend my heartfelt appreciation for his generous financial support, which made this journey possible. To my dear friends, Kavita and Sanjida, thank you for always being there, offering support and encouragement when I needed it the most. A special mention to Arifa for her generous help in the writing process of my thesis. I would like to express my sincere appreciation to every department, faculty member, staff member, and administration of Universiti Sains Malaysia (USM) for their academic resources, infrastructure, especially the lab, and kind cooperation throughout my academic journey. Their contributions have played a crucial role in making this thesis a reality. Lastly, I acknowledge all individuals, directly or indirectly involved, who contributed to the completion of this research.

Thank you all for being a part of this journey.

Akter Rimi

(March 2022)

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LIST OF SYMBOLS

°C	Degree Celsius
%	Percentage
±	Plus, minus
-	Minus
+	Plus
x	Multiply
°K	Degree Kelvin
μ	Specific growth rate
μ _{max}	Maximum growth rate

LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
SEM	Scanning Electron Microscopy
EDX	Energy-Dispersive X-Ray Spectroscopy
XRD	X-ray diffraction
Cd	Cadmium
Mn	Manganese
Fe	Ferum
h ⁻¹	Per hour
CEMACS	Centre for Marine and Coastal Studies
H ₂ SO ₄	Sulphuric acid
CDW	Cell dry weight
ROS	Reactive oxygen species
HMs	Heavy Metals
HM	Heavy Metal
GR	Growth Rate
μ/h ⁻¹	Growth per hour
mg/L ⁻¹	Milligrams per liter
g/cm ³	Gram per cubic centimetre
mg/kg	Milligram per kilogram
g/L	Gram per litre
Fe ₂ O ₃	Fe oxide
H ₂ O ₂	Hydrogen peroxide
FAO	Food and Agriculture Organization
PPAG	Centre for Global Archaeological Research
NCBI	National Center for Biotechnology Information
BLAST	Basic Local Alignment Search Tool
t _d	Doubling time
USM	Universiti Sains Malaysia
OD	Optical density
hr	Hour

ppm	Parts per million
NaCl	Sodium chloride
g	Gram
mL	Millilitre
CAC	Calcium aluminate cement
EDTA	Ethylenediaminetetraacetic acid
DMT1	Divalent metal transporter 1
O ₂	Oxygen
OEC	Oxygen-evolving complex
ATP	Adenosine triphosphate
SND	Short-cut nitrification and denitrification
EU	European Union

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**PENYIASATAN DAN PENGASINGAN MIKROB TAHAN KADMIUM,
FERUM, DAN MANGAN DARI TASIK HARAPAN, UNIVERSITI SAINS
MALAYSIA**

ABSTRAK

Pencemaran alam sekitar akibat logam berat merupakan salah satu masalah yang paling serius dan berterusan di seluruh dunia. Pelbagai teknologi telah digunakan untuk mengurangkan pencemaran ini, namun bioremediasi telah muncul sebagai salah satu kaedah yang berkesan dan mesra alam. Kajian terdahulu telah menumpukan perhatian kepada bakteria dalam merawat dan menstabilkan pencemaran logam berat. Dalam kajian ini, Tasik Harapan, USM telah dipilih sebagai tapak kajian kerana tahap pencemarannya yang tinggi. Analisis awal mengesahkan bahawa tasik ini mengalami pencemaran logam berat, oleh itu, sampel tanah diambil dari tiga lokasi berbeza di sekitar tasik. Sampel tanah ini kemudian dianalisis untuk parameter utama seperti pH, karbon, nitrogen, dan fosforus menggunakan pelbagai kaedah saintifik. Selepas rawatan awal, populasi bakteria diisolasi menggunakan medium yang mengandungi logam berat (0.01 ppm Cd, Fe & Mn). Sebanyak 14 strain bakteria berjaya dikultur dan dikaji berdasarkan ciri morfologi dan pewarnaan Gram. Strain bakteria ini diuji ketahanannya terhadap logam berat pada pelbagai kepekatan (0, 0.3, 0.6, 0.9, dan 1.2 ppm) bagi besi (Fe), kadmium (Cd), dan mangan (Mn). Daripada 14 strain yang diuji, 6 strain mampu bertahan pada 1.2 ppm Fe. Kadar pertumbuhan (μ) strain ini menunjukkan variasi: strain 2 (0.07 h^{-1}), strain 5 (0.08 h^{-1}), strain 8 (0.18 h^{-1}), strain 9 (0.07 h^{-1}), strain 10 (0.12 h^{-1}), dan strain 12 (0.11 h^{-1}). Ketahanan terhadap Cd diuji, dan hanya 2 strain menunjukkan pertumbuhan pada 1.2 ppm Cd. Strain 8 mencatat kadar pertumbuhan 0.11 h^{-1} , manakala strain 12 menunjukkan 0.60 h^{-1} . Hanya satu

strain (strain 8) mampu bertahan dalam medium yang mengandung Mn pada 1.2 ppm, dengan kadar pertumbuhan 0.10 h^{-1} . Seterusnya, strain yang menunjukkan ketahanan tertinggi dipilih untuk pengenalpastian molekul melalui penjujukan gen 16S rRNA menggunakan perisian MEGA 7, yang mengenal pasti bakteria tersebut sebagai daripada genus *Enterobacter*. Kajian pengoptimuman pertumbuhan *Enterobacter* spp. menunjukkan bahawa syarat optimum adalah pH 7 (0.11 h^{-1}), suhu 35°C (0.11 h^{-1}), dan pengudaraan 150 rpm (0.10 h^{-1}). Semua eksperimen dijalankan dalam triplikat, dan analisis statistik SPSS digunakan untuk menentukan kepentingan hasil kajian. Selain itu, ujian SEM (Scanning Electron Microscopy), EDX (Energy Dispersive X-ray Spectroscopy), dan XRD (X-ray Diffraction) dilakukan untuk mengesahkan kehadiran logam berat dalam sampel bakteria. Kesimpulannya, kajian ini menunjukkan bahawa strain bakteria yang diisolasi dari Tasik Harapan, USM mempunyai potensi besar sebagai agen bioremediasi. Keupayaan mereka untuk bertahan dalam persekitaran tercemar logam berat menjadikan mereka calon yang berpotensi tinggi dalam usaha mengawal pencemaran logam berat dalam ekosistem yang terdegradasi.

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ABSTRACT

Environmental pollution caused by Heavy Metals (HMs) has become one of the most serious and persistent problems worldwide. Various technological interventions have been utilized to remove pollution; however, bioremediation is one of them. Several studies were done on bacteria for the remediation and immobilization of contaminants. Tasik Harapan, USM, has been chosen for the present study. Initial studies confirmed the polluted status of the lake; hence, soil samples were analyzed from three different sites around the lake. The soil samples were first analyzed for key parameters such as pH, carbohydrate, nitrogen, and phosphorus content using different methods. After the preliminary treatments, bacterial populations were isolated using HM-containing media (0.01 ppm of Cd, Fe & Mn), where 14 strains were successfully cultivated based on their morphological characteristics and Gram-staining results. The isolates underwent HM tolerance tests with different concentrations (0, 0.3, 0.6, 0.9, and 1.2 ppm) of pure ionic solutions of Fe, Cd, and Mn. Out of 14 strains, 6 isolates could grow at a concentration of 1.2 ppm of Fe. The growth rates (μ) of these isolates showed variation: isolate 2 was 0.07 h^{-1} , isolate 5 was 0.08 h^{-1} , isolate 8 was 0.18 h^{-1} , isolate 9 was 0.07 h^{-1} , isolate 10 was 0.12 h^{-1} , and isolate 12 was 0.11 h^{-1} . These 6 isolates were further screened for Cd tolerance, and only 2 strains showed growth at 1.2 ppm. Among all the isolates, strain 8 recorded a specific growth rate of 0.11 h^{-1} , while isolate 12 showed 0.60 h^{-1} . Only one isolate (strain 8) grew in the presence of Mn at 1.2 ppm, with a growth rate of 0.10 h^{-1} . Thereafter, that isolate was selected for

molecular identification by 16S rRNA genes, and the phylogenetic tree was made by using MEGA 7 software, where the *Enterobacter* genus was identified. Studies on the optimization of *Enterobacter* spp. growth identified optimum conditions as pH 7 (specific growth rate of 0.11 h^{-1}), temperature at 35°C (0.11 h^{-1}), aeration at 150 rpm (0.10 h^{-1}), and the biomass of bacteria. All the experiments were done in triplicate, and SPSS analyses were done to determine the significance of the results. Then, SEM, EDX, and XRD were done to confirm that traces of metals were found in the bacterial samples. The study reveals that microbial strains isolated from Tasik Harapan, USM, can become excellent agents for bioremediation, with substantial implications in controlling HM pollution in degraded ecosystems.

CHAPTER 1

INTRODUCTION

1.1 Research Background

Tasik Harapan is an important ecological and recreational feature of Universiti Sains Malaysia, which is located on Penang Island, Malaysia. The lake is situated in a lush, tropical setting, surrounded by dense greenery and rolling hills characteristic of Penang's landscape. Having an approximate surface area of 6,070 square meters, 1.5 acres, and with varying depths between 1.0 to 1.5 meters, Tasik Harapan has become a focal point for environmental studies, biodiversity preservation, and campus beautification. Nestled between Desasiswa Harapan and Pusat Islam USM, the lake covers an area of about 3,156 square meters. It is a flood retention pond constructed originally to address the flash flood problems in the campus area. It acts as a recreational outlet for students and staff (Basharudin, 2021). Generally, most water bodies in urban areas face serious environmental challenges, including HM contamination. Wastewater from different schools, offices, restaurants, and hostels could be the major source of HMs in the lake. Some HMs, such as Cd, Mn, and Fe, present in sediment and water, have posed considerable ecological and health risks in the lake (Loh, 2019).

It falls into a tropical rainforest climatic region, a characteristic of which is very high humidity and, for most times of the year, more or less regular temperatures. Its vegetation mainly involves a composition of native flora species together with man-made plants within the premises to make this man-made lake representative of the ecosystem, as it supports small-scale species life. Situated inside the campus, it is highly accessible for study trips, research programs, and casual purposes (G. Li et al., 2024). The main inflow into Tasik Harapan is stormwater runoff through the Sungai Gambir, a small stream linked to the lake. However, due to the limited flushing of the water in

the lake, there has been an accumulation of nutrients such as nitrogen (N) and phosphorus (P), leading to eutrophication. This condition has been exacerbated by urban development and anthropogenic activities in the area, which introduce pollutants and sediments into the lake. These factors combined to make the lake a perfect site for the study of nutrient loading and eutrophication effects within freshwater ecosystems (Rossi et al., 2025).

Geologically, this lake is underlain by alluvial soil, which is typically found in the general lowland areas of Penang. These sediments, comprising silt, clay, and organic matter, favor the growth of aquatic plants but also undergo specific processes such as sedimentation and nutrient cycling. Tasik Harapan is proximal to most of the facilities at USM; therefore, this lake has become an important USM natural resource and research site that allows continuous monitoring and management accordingly (Fitri, 2012). Tasik Harapan has experienced challenges with HM pollution. HMs, such as Cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu), often originate from nearby industrial activities, urban runoff, and wastewater discharge, which can accumulate in sediments and water systems. These pollutants can harm aquatic ecosystems and pose risks to human health through bioaccumulation (Md Salleh, 2020).

A study on related water bodies in Penang, including areas near USM, revealed significant levels of HMs in sediments, with some exceeding permissible limits during certain seasons. These contaminants were linked to both point sources, like industrial discharges, and non-point sources, such as agricultural runoff and urban development (Loheswar, 2025). Moreover, incidents like fish deaths in nearby Teluk Bahang have highlighted broader eutrophication issues and HM contamination in Penang waters. Researchers from USM's Centre for Marine and Coastal Studies (CEMACS) identified high levels of nickel, lead, and Cadmium in surrounding waters, contributing to

environmental stress and oxygen depletion, which can have cascading effects on aquatic life (Sekaran, 2025). The presence of these HMs poses significant risks to aquatic life and can disrupt the lake's ecosystem. For instance, high concentrations of nickel and lead can be toxic to fish and other aquatic organisms, leading to reduced biodiversity and potential bioaccumulation in the food chain (Loh, 2019).

Ferum (Fe) is an essential micronutrient for numerous physiological processes in plants, animals, and humans. However, excessive Fe concentrations can lead to toxicity, adversely affecting biological systems. In contrast, Fe is crucial for chlorophyll synthesis and various enzymatic functions, and its excess can be detrimental (Deka et al., 2024). High Fe levels can cause oxidative stress by generating reactive oxygen species (ROS), leading to cellular damage. Symptoms of Fe toxicity in plants include impaired leaf and root growth, bronzing of leaves, and reduced crop yields. This condition is particularly prevalent in flooded soils, such as those used for rice cultivation, where low oxygen levels increase Fe solubility, exacerbating plant uptake (Harish et al., 2023). Animals exposed to excessive Fe may experience a range of health issues. Acute Fe toxicity can result in gastrointestinal distress, including anorexia and diarrhea, as well as systemic effects like hypothermia, metabolic acidosis, and shock. Chronic exposure may lead to organ damage due to Fe deposition in the liver, heart, and other vital organs (M. Hou et al., 2024). In humans, Fe homeostasis is tightly regulated; however, excessive intake or genetic disorders can disrupt this balance. Acute Fe poisoning, often resulting from accidental ingestion of Fe supplements, can cause symptoms such as nausea, vomiting, abdominal pain, and, in severe cases, organ failure. Chronic Fe overload, as seen in conditions like hemochromatosis, leads to Fe accumulation in organs, increasing the risk of liver diseases, heart complications,

diabetes, and certain cancers. Moreover, excessive Fe can catalyze the formation of ROS, contributing to oxidative stress and cellular damage (Isidori et al., 2018).

Cadmium (Cd) is a non-essential, highly toxic HM that poses significant risks to flora, fauna, and humans, even at very low concentrations. In plant systems, Cd exposure adversely affects shoot and root development, disturbs the physiological balance, obstructs nutrient uptake, and accumulates in major agricultural species, leading to stunted growth and reduced productivity (Biswal et al., 2025). Cd penetrates the body mainly through the food chain in both animals and humans and may cause a number of serious health problems. It is an embryotoxic, carcinogenic, mutagenic, and teratogenic metal. Exposure to Cd has been related in various studies to anemia, hyperglycemia, osteomalacia, renal dysfunction, lung cancer, DNA damage, vertebral osteoporosis and fractures, peripheral arterial disease, neurotoxicity, liver, cardiovascular system, and reproductive function damage (H. Zhang & Reynolds, 2019). Moreover, Cd exposure can also suppress immune function by interfering with Fe metabolism and affecting apoptosis, differentiation, and proliferation, while increasing the chances of oncogene activation. Cd has a very long biological half-life; thus, it is one of the most persistent environmental pollutants (Okerefor et al., 2020). The International Agency for Research on Cancer (IARC) and the Maximale Arbeitsplatz-Konzentration (MAK) Commission have classified Cd as a human carcinogen. Contemporary research has strong evidence associating Cd exposure with lung tumor development, while some new studies are showing possible links with prostate and kidney cancers as well as females' endometrial and breast cancers (Genchi et al., 2020). It has been known from experimental studies in animals that Cd could induce neoplasms in the testis and prostate glands and lung carcinoma following either inhalation or injection. Given the high health risks associated with Cd exposure,

environmental pollution caused by this metal has drawn significant attention from environmentalists and public health officials worldwide. Reducing Cd pollution is critical to protecting ecosystems and human health (Haider et al., 2021).

Manganese (Mn) is an essential trace element for many biological activities involving activating enzymes and participating in photosynthesis and metabolism. Its excessive occurrence, be it by environmental pollution or by occupational environments in humans, animals, and plants, manifests serious toxicological results (P. Chen et al., 2018). Normally, the condition usually reveals itself in plants when growing in soils with acidic characteristics, where high solubility determines elevated uptake by such plants. This interferes with physiological processes such as chlorophyll synthesis, enzyme activities, and homeostasis, resulting in stunted growth, brown leaf spots, and reduced yields in crops (J. Li et al., 2019). This neurodegenerative effect associated with exposure to high levels of Mn feed or water contaminants also causes disorders in the reproduction and metabolic system of animals (Foster et al., 2015). Prolonged exposure to Mn may lead to a neurodegenerative disorder in humans called Mn, which is similar to Parkinson's disease and characterized by motor dysfunction, tremors, and intellectual impairments (Garrett & Lawrence, 2023). High levels of Mn also cause disturbances in Fe metabolism, leading to anemia and oxidative stress. Mn is among the prominent environmental pollutants due to its emissions from mining, industrial effluents, and agricultural runoff. Its tendency to deposit in aquatic and terrestrial environments poses a great threat to ecosystems (Charan et al., 2025). In humans, exposure to Mn usually occurs through the food chain. Long-term Mn exposure has been associated with the impairment of the central nervous system. At the same time, evidence has pointed out that it can take part in neuroinflammation, apoptosis, and oxidative stress-induced damage (Yoo et al., 2025). More recent studies have found

potential links between overexposure to Mn and reproductive toxicity and child development disorders. Despite being a fundamental element, the narrow spectrum between deficiency and toxicity emphasizes the need to regulate Mn concentrations in biological systems and the environment to avoid adverse effects (Tuschl et al., 2025).

The increasing awareness of the adverse effects of HMs on ecological systems, coupled with the increased deposition of these metals, has given impetus to serious research concerning their removal, recovery, and handling practices (Abo-Alkasem et al., 2023). HMs such as Cd, Mn, and Fe are particularly hazardous to soil, water, and biota because of their persistence, potential for bioaccumulation, and toxicological effects. Various conventional methods have been used to remove HMs from industrial effluents, such as electrochemical techniques, chemical precipitation, ion exchange, reverse osmosis, membrane filtration, phytoremediation, and reduction and oxidation methods (Pande et al., 2022). However, these processes have often been associated with several limitations, such as high expenses, generation of toxic sludge, low removal efficiency, and inherent technical problems, which make them less practicable and environmentally friendly for large-scale applications. For example, chemical precipitation is a common practice; however, it usually fails to meet the requirements of regulatory agencies with regard to the removal of HMs and also produces secondary waste that requires further treatment (Syarifuddin et al., 2025). Similarly, ion exchange is highly effective in the removal of some HMs but relies on costly adsorbent materials, therefore economically infeasible for large-scale application. These limitations bring into focus the dire need for environmentally friendly, economically feasible, and sustainable alternatives for the removal of HMs, such as Cd, from industrial effluents and contaminated environments (Kuppan et al., 2024).

Microorganisms offer a promising approach for HM remediation that is eco-sustainable and economically viable. Bacteria, algae, fungi, molds, protozoa, and yeast are just a few microorganisms that have already shown potential for HM from polluted wastewater and contaminated soil environments (Thai et al., 2023). This comes in handy due to the characteristic chemical structure in their cell walls, which are rich in functional groups like carboxyl, carbonyl, hydroxyl, and sulfhydryl, offering them a high affinity to form bonds with metal ions (Alabssawy & Hashem, 2024). Besides, microorganisms use several other mechanisms to remove HMs from their environment. Firstly, complexation by exopolysaccharides, where microbial exopolysaccharides complex bind with metal ions, reduces their bioavailability. Secondly is adsorption to cell surfaces; metal ions bind to microbial cell walls through physical and chemical interactions (Yu et al., 2021). Thirdly, binding with bacterial cell envelopes, microbial cell walls bind HM, thus sequestering them from the environment. Fourth is intracellular accumulation, which occurs when some bacteria accumulate HMs within the cell for detoxification or storage (Uqab et al., 2022). Then, in the biosynthesis of metallothioneins or certain proteins synthesized by microbes, they bind and detoxify HMs. Next is precipitation and transformation; microbes precipitate or convert metals to less toxic or volatile forms. Ultimately, the formation of protective proteins, where particular proteins sequester HMs, renders them less toxic (Pande et al., 2022).

The ability to tolerate and sequester HM has been studied in detail in only a few bacterial types, including *Enterobacter* spp., *Pseudomonas putida*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. Among these, the bacteria *Ralstonia metallidurans*, *Alcaligenes eutrophus*, and *Serratia liquefaciens* were found to be highly effective at HM removal due to their potent resistance mechanisms and high metal-binding efficiency (Tang et al., 2024). Investigations have

shown that bioremediation of HMs is effective and sustainable, as it reduces environmental impact and presents a cheap alternative to conventional methods. The continuous isolation and characterization of bacterial strains showing Cd resistance are an active area of research with great potential to change the face of bioremediation and provide practical solutions for managing HM pollution (Kuppan et al., 2024).

The present study presents a new approach to dealing with HM pollution by isolating, identifying, and characterizing microorganisms showing tolerance and potential growth in Fe, Mn, and Cd derived from Tasik Harapan soil. Unlike the traditional research mainly focusing on HM accumulation or remediation, the current study focused on growth kinetics and environmental adaptability of tolerant microorganisms in the context of these particular metals. This investigation into the ability of these microorganisms to survive and multiply at various concentrations of Fe, Mn, and Cd opens up their potential use in bioremediation and management practices related to the environment. Furthermore, it also discusses the optimization of growth parameters, such as pH, temperature, aeration, and duration of exposure for these microorganisms, resulting in important knowledge that increases their applicability in practical scenarios. A proper understanding of the mechanisms through which microbes tolerate different HMs under controlled conditions is a tremendous advance, allowing new approaches to treating HM pollution and promoting sustainable environmental practices.

1.2 Problem statement

At present, HM contamination in the environment, particularly in soil, is a growing ecological concern due to its profound negative impacts on soil health, agricultural productivity, and overall ecosystem stability. HMs, such as Fe, Mn, and Cd, are toxic at elevated concentrations and can persist in the environment, leading to bioaccumulation and biomagnification across the food chain. These contaminants not only degrade soil quality but also inhibit plant growth, disrupt microbial diversity, and pose significant risks to human health through water and food contamination.

In recent years, bioremediation using HM-resistant bacteria has been a potential, eco-friendly method of decreasing HM contamination. Metal-tolerant microorganisms with biotransformation ability can successfully remove or detoxify HMs in contaminated water by biosorption, bioaccumulation, and enzymatic detoxification. The efficiency of the bacteria is influenced by environmental conditions, species-specific tolerance, and metal removal mechanisms. The native microbial community of Tasik Harapan is not yet fully discovered, and its bioremediation potential against HMs is not well understood.

To begin with, Tasik Harapan, a lake subjected to significant anthropogenic impact, suffers from contamination and eutrophication. This condition is exacerbated by drainage discharge from Universiti Sains Malaysia (USM) into the lake, increasing the likelihood of HM pollution. Tasik Harapan, a region potentially exposed to HM pollution, exemplifies the challenges of HM accumulation. Over time, the build-up of these metals in the soil can adversely affect microbial ecosystems and hinder soil fertility, posing challenges to sustainable land use and environmental conservation.

Microorganisms capable of tolerating and thriving in HM-contaminated soils are critical for mitigating these effects. These microbes can utilize various mechanisms such as bioaccumulation, biosorption, and enzymatic transformation to reduce metal toxicity and restore ecological balance. However, despite their potential, there is a lack of comprehensive research on isolating and characterizing HM-tolerant microbes in Tasik Harapan. Additionally, understanding the growth kinetics and optimizing conditions for these microbes to function effectively in contaminated environments remains unexplored.

This knowledge gap highlights the need for in-depth research to isolate and identify HM-tolerant microorganisms from Tasik Harapan soil, evaluate their growth kinetics under controlled conditions, and determine optimal environmental factors for their activity. Such efforts are vital for developing efficient bioremediation strategies to address HM contamination, enhance soil health, and promote ecological sustainability.

1.3 Objectives-

The objectives of this research are-

- 1.3.1 To isolate, identify, and characterize potential tolerant microbes with Fe, Mn, and Cd from the soil at Tasik Harapan.
- 1.3.2 To investigate Heavy Metal-tolerant (Fe, Mn, and Cd) bacteria that can be able to utilize or grow in different concentrations of that type of Heavy Metal.
- 1.3.3 To identify the kinetic growth of selected tolerant microbe with Fe, Mn, and Cd in a controlled environment.
- 1.3.4 To investigate optimum conditions such as pH, temperature, aeration, concentration (Fe, Mn, and Cd), and time of growth for the tolerant microbe.

1.4 Scope of the study

This study is designed to investigate the potential of HM-tolerant microorganisms isolated from the soil at Tasik Harapan. The focus will be on understanding their ability to thrive in environments contaminated with Fe, Mn, and Cd, assessing their suitability for bioremediation applications. The specific areas of focus include:

1.4.1 Isolation and Characterization of HM-Tolerant Microbes (Locally isolated)

The study will systematically collect soil samples from Tasik Harapan and subsequently isolate microbial strains capable of tolerating HMs. Isolated microbes will be subjected to a detailed characterization process, including morphological, biochemical, and molecular analyses. These steps aim to identify and classify potential microbial candidates demonstrating resilience to Fe, Mn, and Cd stress.

1.4.2 Assessment of HM Tolerance

The study will evaluate the ability of the isolated microbes to tolerate different concentrations of Fe, Mn, and Cd. This involves testing the growth and survival of the microbes in environments with varying levels of these metals to determine their tolerance thresholds. The findings will provide insights into the mechanisms employed by microbes to cope with metal-induced stress.

1.4.3 Kinetic Growth Analysis of Selected Microbes

Once potential microbial candidates are identified, their growth kinetics will be studied in a controlled laboratory environment. This includes analyzing parameters such as lag phase, exponential growth rate, and stationary phase under HM stress. Understanding the growth dynamics will help in predicting their behaviour in real-world conditions and assessing their efficiency in HM remediation.

1.4.4 Optimization of Growth Conditions

To enhance the effectiveness of the microbes, the study will optimize critical environmental factors such as pH, temperature, aeration, metal concentrations, and incubation time. By determining the optimum conditions for microbial growth and metal tolerance, the study aims to maximize their bioremediation potential. This step is crucial for designing strategies to apply these microbes in contaminated environments effectively.

By addressing these areas, the research is expected to contribute significantly to understanding HM-tolerant microbes and their applications. The outcomes of this study will provide a foundation for developing innovative strategies to mitigate the environmental impacts of HM contamination, enhance soil quality, and promote ecological restoration.

1.5 Thesis outline

This thesis is organized into five comprehensive chapters, each addressing specific aspects of the research in detail. Chapter one, “Introduction,” introduces the research by outlining the background and context of the study. It highlights the environmental issues caused by HM contamination, particularly in soil, and discusses its detrimental effects on ecosystem health, agricultural productivity, and microbial diversity. The problem statement, research objectives, and significance of the study are clearly defined.

Then, the literature review provides an in-depth exploration of the research topic, synthesizing existing knowledge and identifying gaps in the field. It includes discussions on HM contamination and its environmental impacts, with a specific focus

on metals such as Cd, Fe, and Mn. The chapter also examines microbial tolerance mechanisms to HMs, highlighting the role of microorganisms in bioremediation.

The Third chapter is “Methodology.” This chapter provides a detailed description of the experimental design and methodology employed in the study. It outlines the process of soil sampling at Tasik Harapan and the isolation of HM-tolerant microbes. The chapter documents the materials used and the laboratory techniques applied, including microbial culturing, morphological characterization, molecular identification, and growth kinetics studies. Experimental protocols for evaluating HM tolerance and optimizing environmental factors, such as pH, temperature, and metal concentration, are also elaborated. This chapter ensures the reproducibility of the study by presenting all methodologies systematically and comprehensively.

The fourth chapter, “Results and Discussion,” presents the findings of the study, organized into clear sections for analysis and interpretation. It begins with the isolation and identification of HM-tolerant microbes from the soil at Tasik Harapan. The tolerance levels of microbes to Cd, Fe, and Mn are detailed, along with their growth patterns under different metal concentrations. Kinetic growth analysis of selected microbes in a controlled environment is discussed, highlighting the influence of various environmental parameters. The findings are compared with existing studies to assess their significance and implications for bioremediation. This chapter integrates data visualization, such as graphs and tables, to illustrate results effectively.

The final chapter summarizes the key findings of the research and their relevance to addressing HM contamination in soils. It discusses the potential applications of the identified microbes in bioremediation and their contribution to sustainable environmental management. Limitations of the study are acknowledged, and recommendations for future research are proposed. This chapter concludes by

emphasising the broader significance of the study for ecological restoration and environmental sustainability. This thesis structure provides a clear and logical progression of ideas, ensuring that each chapter builds on the previous one to provide a cohesive understanding of the research objectives and findings.

LITERATURE REVIEW

2.1 Introduction

Tasik Harapan, also known as Harapan Lake, is located on Penang's Universiti Sains Malaysia (USM) main campus, which was described in Figure 2.1. It was constructed as a flood retention pond to help mitigate flash flood problems in the area. The lake is connected to the Gambir River through an inlet and outlet channel, which helps manage the water flow and reduce flood levels (Munirah et al., 2021).

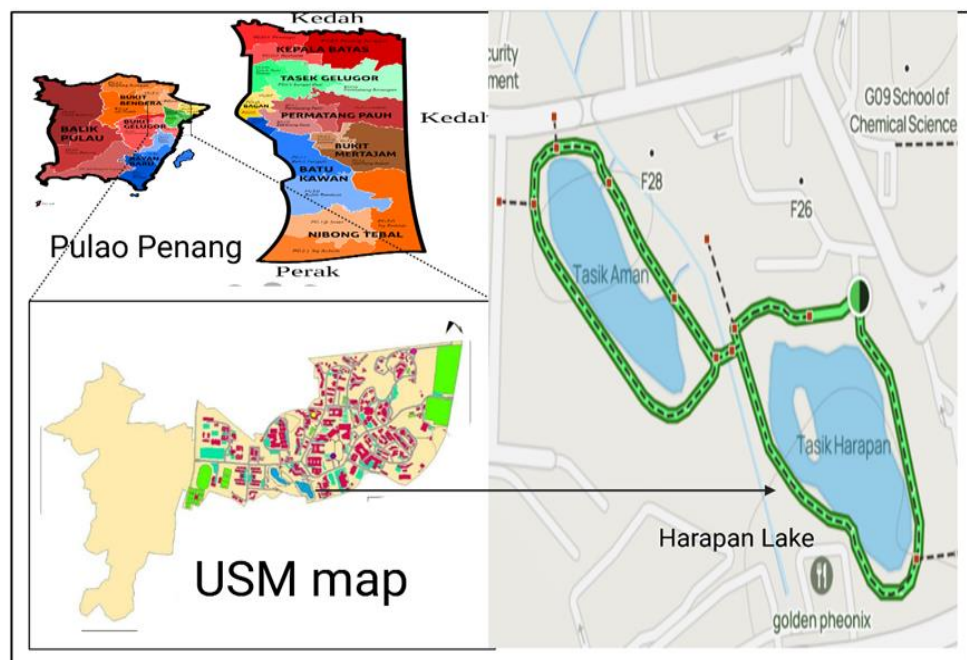


Figure 2.1 Location of Tasik Harapan

Geologically, this lake is underlain by alluvial soil found in the general lowland areas of Penang. These sediments, comprised of silt, clay, and organic matter, favour the growth of aquatic plants but also undergo certain processes such as sedimentation and nutrient cycling. Tasik Harapan is proximal to most of the facilities at USM; therefore, this lake has become an important USM natural resource and research site that allows continuous monitoring and management accordingly (Basharudin, 2017).

Tasik Harapan has experienced challenges with HM pollution. HMs, such as Cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu), often originate from nearby industrial activities, urban runoff, and wastewater discharge, which can accumulate in sediments and water systems. These pollutants can harm aquatic ecosystems and pose risks to human health through bioaccumulation (Hama Aziz et al., 2023).

A study on related water bodies in Penang, including areas near USM, revealed significant levels of HMs in sediments, with some exceeding permissible limits during certain seasons. These contaminants were linked to both point sources, like industrial discharges, and non-point sources, such as agricultural runoff and urban development (Ruzi et al., 2023).

Moreover, incidents like fish deaths in nearby Teluk Bahang have highlighted broader eutrophication issues and HM contamination in Penang waters. Researchers from USM's Centre for Marine and Coastal Studies (CEMACS) identified high levels of nickel, lead, and Cd in surrounding waters, contributing to environmental stress and oxygen depletion, which can have cascading effects on aquatic life (Sekaran, 2025).

The presence of these HMs poses significant risks to aquatic life and can disrupt the lake's ecosystem. For instance, high concentrations of nickel and lead can be toxic to fish and other aquatic organisms, leading to reduced biodiversity and potential bioaccumulation in the food chain (Gristwood, 2019).

2.2 Heavy Metal-induced soil contamination

Soil is a complex combination of elements, including minerals (such as clay) and organic compounds (such as humic substances), as well as aqueous and gaseous components. HMs are a group of naturally occurring elements that exhibit metallic properties such as high density, malleability, and conductivity, including metals and

metalloids with densities exceeding 5 g/cm³. Although some are essential micronutrients for plants and animals in trace amounts (e.g., zinc, copper, Mn, and Fe), their elevated concentrations in soil can lead to significant environmental and ecological harm. These metals typically exist at trace levels (<1000 mg/kg) and are rarely toxic under natural conditions. HM soil pollution has become a pressing global concern due to its persistence, toxicity, and potential for bioaccumulation and biomagnification (Rizvi et al., 2020).

The persistence of HMs in soils is particularly concerning due to their non-biodegradable nature, leading to accumulation over time. HM contamination in soils arises from natural processes and human activities shown in Fig 2.2, leading to significant environmental and health challenges. Geological phenomena such as volcanic eruptions, weathering of metal-rich rocks, and forest fires contribute to the natural deposition of HMs like lead, arsenic, and mercury into the soil. For instance, the weathering of rocks releases metals into the environment over time, gradually increasing soil metal concentrations (Priya et al., 2023).

Human activities, shown in Fig. 2.2, have markedly intensified HM pollution in soils. Industrial operations, including mining, smelting, and manufacturing, discharge substantial amounts of metals into the environment through waste emissions and effluents. Agricultural practices further exacerbate this issue; the application of phosphate fertilisers and pesticides introduces metals such as Cadmium, lead, and arsenic into the soil. Additionally, the use of sewage sludge as fertiliser can contribute to soil contamination (Xu et al., 2024).

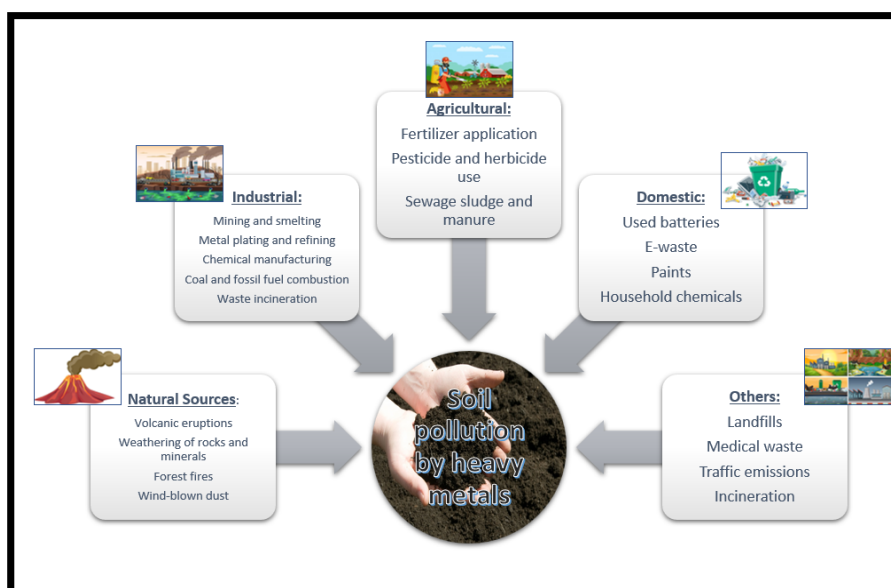


Figure 2.2 Causes of soil pollution by HMs

Urbanisation and the expansion of transportation networks also play significant roles, as exhibited in Fig. 2.2. Historically, the use of leaded gasoline resulted in the deposition of lead along roadways, contaminating adjacent soils. Although the use of leaded gasoline has been phased out in many countries, residual contamination persists. Moreover, industrial emissions and improper waste disposal in urban areas contribute to elevated levels of HMs in soils (Wan et al., 2024).

2.3 Heavy Metal

Naturally occurring elements with high atomic weight and density above 5 g/cm³ are HMs. Widely scattered throughout the crust of the Earth, they have great importance in both industrial and biological spheres. Although some HMs such as ferum (Fe), zinc (Zn), copper (Cu), and manganese (Mn) are vital micronutrients for living things, others include lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are quite poisonous even at modest levels (Rajasekar et al., 2025).

2.3.1 Ferum (Fe)

One of the most abundant and extensively used elements on Earth is ferum (Fe), which has an atomic number of 26, as shown in Figure 2.3. It is classified as a transition metal and is distinguished by its metallic lustre and silvery-grey appearance. Fe is relatively substantial due to its high density of 7.874 g/cm³. Its stability under extreme temperatures is facilitated by its melting point of 1538°C and boiling point of 2862°C (Mishima et al., 2025).

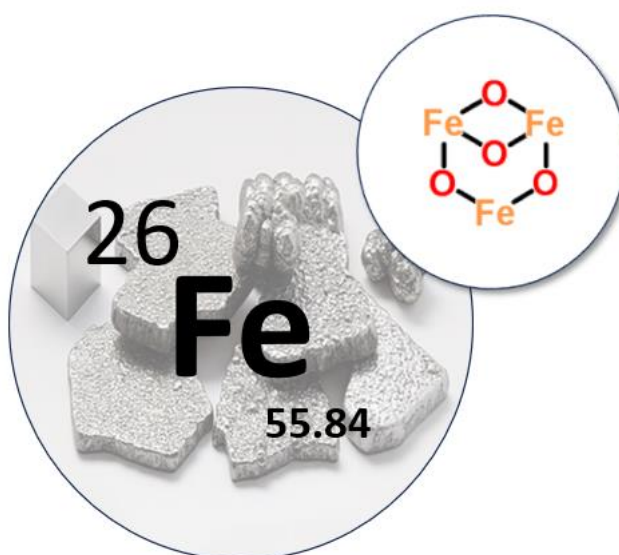


Figure 2.3 Physical and chemical properties of Fe.

Fe is a critical component of data storage devices, electrical transformers, and magnets due to its ferromagnetic properties, which enable it to be magnetised. Fe is capable of forming a variety of compounds due to its ability to exhibit multiple oxidation states, predominantly +2 (ferrous) and +3 (ferric). Rust, which is commonly referred to as Fe-oxide (Fe₂O₃), is readily formed when it reacts with oxygen and moisture in the air. Fe structures are weakened by this corrosion process as time

progresses. Fe is also highly reactive with acids, where it can form Fe salts and complex coordination compounds that are essential in industrial applications such as steel production, catalysis, and pigments (C. Li et al., 2016).

Standards for the concentration of Fe in soil have been developed by a number of organisations in order to guarantee the safety of the environment, the fertility of the soil, and the health of humans. Although Fe is naturally plentiful in soil, too high amounts resulting from industrial contamination may endanger water quality, plant development, and soil condition. Although national regulations vary, Fe concentrations above 50,000 mg/kg are usually regarded as high and may call for environmental evaluation. In accordance with environmental considerations and the kind of land use (agricultural, industrial, or residential), these regulations are subject to variation (Sofu et al., 2015). In Table 2.1, listed below are the Fe standards put forward by a variety of organisations:

Table 2.1 Standards of Fe in soil from several organisations

Organisation	Fe standards	Reference
United States Environmental Protection Agency (US EPA)	Residential soil: 55,000 mg/kg (ppm). Industrial Soil: 230,000 mg/kg (ppm).	(Strezov & Chaudhary, 2017)
World Health Organisation (WHO)	The range is from 31.55 to 187.47 mg/kg.	(S. Akhtar et al., 2022)
Indian Standards (Bureau of Indian Standards - BIS)	The range is from 10,000 to 50,000 mg/kg.	(J.P. et al., 2024)
China Soil Environmental Quality Standards	Class I (Natural soil quality): 20,000–60,000 mg/kg. Class II (Agricultural land): Up to 50,000 mg/kg Class III (Industrial land): Over 100,000 mg/kg	(CHEN et al., 2018; Delang, 2018)
Food and Agriculture Organisation (FAO) of the United Nations	The range is between 2% and 5% (20,000 to 50,000 mg/kg) for optimal plant growth.	(Dagne & Endale, 2019)
Canadian Environmental Quality Guidelines (CEQG) - Canadian Council of Ministers of the Environment (CCME)	Agricultural soil: 35,000 mg/kg. Residential/parkland soil: 50,000 mg/kg. Industrial soil: 100,000 mg/kg	(Sulfonate, 2021)

Recent studies have emphasised the harmful impact of these contaminants, revealing their presence across various land uses, including agricultural, urban, and industrial areas. Additionally, they have pinpointed the environmental factors that facilitate the accumulation of metals in soil. Fe may naturally occur in various soil types, resulting from the materials, including parent rock, volcanic ash, organic matter from decaying plants and animals, and sediments transported by wind or water that contributed to soil formation thousands of years ago (Durairaj & Dharmackan, 2025). In the majority of the reviewed articles shown in Table 2.2, the HM content in soil was attributed to various anthropogenic activities.

Table 2.2 Fe content variations in diverse soils from earlier investigations

Location	Sampling site	Fe Concentration	Reference
Poland	Agricultural site	1747-2037 mg/kg	(Łukowski, 2025)
Saudi Arabia	Farm	11,480 mg/kg	(El-Sorogy et al., 2025)
Iran	Non-paddy soil	4.60-21.43 mg/kg	(Azadi et al., 2021)
	Paddy soil	18.36-37.63 mg/kg	
China	Paddy soil	11.98-43.08 mg/kg	(B. Xu et al., 2021)
Southern Indiana	Agricultural soil	0.92- 18.60 mg/kg	(Jiang et al., 2021)

2.3.1(a) Effects of Fe toxicity on the plant

Fe is an essential ingredient for plant growth and development, significantly contributing to several physiological activities, including photosynthesis, respiration, and nitrogen metabolism. Plants require Fe concentrations ranging from 10^{-4} to 10^{-9} M for optimal growth (Ghouri et al., 2024). Excessive exposure to Fe may culminate in Fe toxicity, damaging the plant's cell membranes, diminishing growth and yield, and adversely impacting the plant's general health. Fe toxicity could happen in several soils, particularly those characterised by high pH, substantial organic matter content, or

increased availability of Fe (L. Xu et al., 2025). These conditions result in an elevated concentration of free Fe ions, which can attach to cellular components, impairing cellular function and inducing plant stress. Elevated Fe concentrations in the soil may result in the creation of harmful compounds, such as hydroxyl radicals, which have the potential to induce oxidative damage in plant cells. This process is referred to as the Fenton reaction, which entails the interaction of Fe, hydrogen peroxide (H₂O₂), and various reactive oxygen species (ROS) where the entire mechanism is shown in Figure 2.4 (Pérez-Hernández et al., 2025).

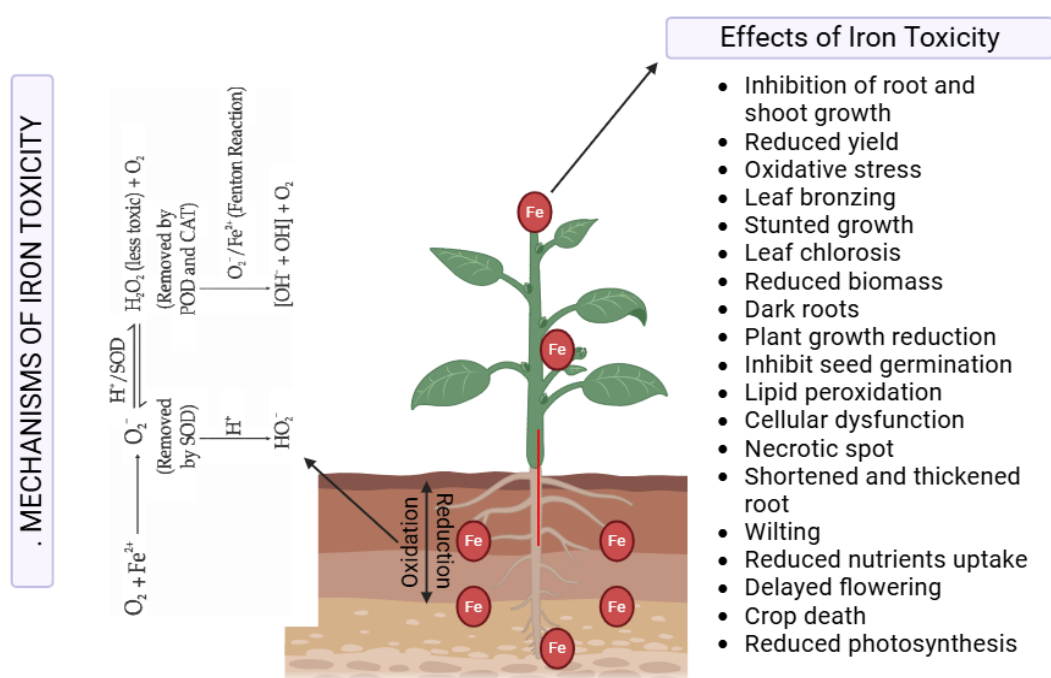


Figure 2.4 Mechanism and toxicity of Fe in plants (modified from an internet picture).

Moreover, an excess of Fe can disrupt the balance of nutrients in plants, therefore producing shortages of other essential minerals, such as manganese, copper, and zinc. A deficit of nutrients can aggravate Fe poisoning consequences by reducing plant development, chlorosis, and necrosis (Zheng et al., 2025). There can be a rather significant influence on crop quality and output. Apart from impeding plant development, it could also cause lower yield and quality of the produced crops.

Furthermore, negatively impacting soil microbial populations is the excess Fe present in the ground, which affects plant development and output (Rout & Sahoo, 2015).

2.3.1(b) Effects of Fe Toxicity on the human body

The regulation of Fe metabolism in the human body is a crucial process that underpins numerous physiological functions, such as oxygen transport, DNA synthesis, and energy production. Since the body doesn't have a direct way to get rid of Fe, it mostly controls its levels by recycling it, storing it, and excreting it in a controlled way. The duodenum and proximal jejunum are where Fe is absorbed. Enterocytes control uptake based on the body's needs. Non-heme Fe found in plant-based foods is absorbed in its ferric (Fe^{3+}) form. It is then changed to ferrous (Fe^{2+}) form by duodenal cytochrome b (Dcytb) before being taken into enterocytes by divalent metal transporter 1 (DMT1) (Eid et al., 2017).

When Fe enters an enterocyte, it can either be stored as ferritin or sent out into the bloodstream by ferroportin, which is the only known Fe exporter. Hephaestin or ceruloplasmin helps turn Fe into Fe^{2+} so it can bind to transferrin, which is the main protein that moves Fe around the body. Transferrin helps Fe get to cells through transferrin receptors. Fe is mostly used for making haemoglobin by erythroid precursor cells in the bone marrow (Mejia & Erdman, 2025).

The liver is essential for managing Fe levels through the production of hepcidin, a peptide hormone that restricts ferroportin and thus regulates the release of Fe into the bloodstream. Macrophages play a role in maintaining Fe balance by recycling Fe from aging red blood cells via phagocytosis and thus making it available for reuse. Disruption in Fe metabolism may result in conditions such as Fe deficiency anaemia or Fe overload disorders, including hemochromatosis (Tan et al., 2025).

The toxicity of Fe in humans arises from an accumulation of too much Fe in the body, negatively impacting various organs and metabolic processes (Sane et al., 2018). This could lead to either acute or chronic toxicity, based on the amount and period of exposure to Fe. Acute Fe poisoning is common, this can lead to acute gastrointestinal symptoms such as nausea, vomiting, diarrhoea, and abdominal pain. Excessive Fe overload can lead to systemic involvement such as metabolic acidosis, liver damage, shock, and multi-organ failure, which can prove to be lethal if not treated (Deumić et al., 2025).

Chronic Fe intoxication is shown in Figure 2.5. On the other hand, it builds up over time through prolonged exposure to Fe ingestion, often compounded by genetic hemochromatosis, as one of several disorders of inherited defects in Fe handling. The disease follows due to a breakdown in body regulation to curb Fe intake from dietary sources, resulting in a constant buildup of Fe within tissue and organ structures (Yang et al., 2025).

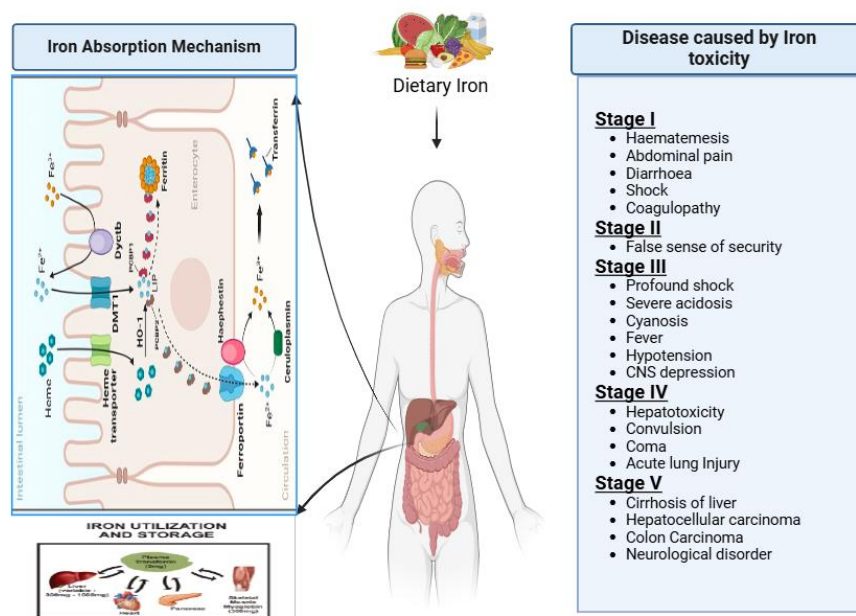


Figure 2.5 Mechanism of Fe absorption and diseases caused by the toxicity of Fe (modified from an internet picture).