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**Bioaccessibility of Some Heavy Metals and Total  
Phosphorus in Cooked and Uncooked Rice of Al-  
Diwaniyh Governorate**

Thesis  
Submitted to the Council of the College of Science, University of  
Al-Qadisiyah, Partial Fulfillment of the Requirements for  
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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((فَلْيَنْظُرِ الْإِنْسَانُ إِلَى طَعَامِهِ))

صدق الله العلي العظيم

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# **Dedication**

## **To:**

✚ With profound gratitude to God Almighty, whose guidance and mercy made this work possible.

✚ To my beloved parents, the true role models of my life, whose unwavering support and encouragement have been the foundation of my journey.

✚ To my esteemed supervisor and honorable professors, whose wisdom and generosity in sharing knowledge illuminated my path.

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## Summary

This study examined the concentrations, bioaccessibility, cooking reduction, and potential health effects of certain heavy metals in rice grown in Al-Diwanyah Governorate, Iraq. Rice samples were obtained from four principal agricultural regions: Mahanawiyah, Salahiyah, Shamiyah, and Ghamas. The contents of cadmium (Cd), lead (Pb), zinc (Zn), and chromium (Cr) were quantified via flame Atomic Absorption Spectrophotometry (FAAS) following acid digestion. The bioaccessibility of heavy metals was examined using the Simple Bioaccessibility Extraction Test (SBET), which replicates gastrointestinal circumstances, while health hazards were evaluated by Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI).

Spatial heterogeneity in heavy metal concentration was observed across multiple site locations. The maximum concentration of Cd was seen in Shamiyah (0.30 mg/kg), but the greatest concentrations of Pb and Zn were noted in Mahanawiyah (6.47 and 9.01 mg/kg, respectively). All locations had lead levels exceeding the FAO/WHO permitted limits, but cadmium levels above the acceptable threshold were recorded alone in Shamiyah. The concentration of metals in the cooked samples was markedly diminished, in the following order: Cd (96.02%), Pb (93.91%), Zn (84.60%), and Cr (84.22%). The relative bioaccessibility results indicated that the gastrointestinal availability of the element was greatest for Pb (45%) and least for Cd (25%). The health risk assessment indicated that youngsters faced a greater risk than adults due to their increased exposure relative to body weight. The HI values of raw rice samples across all locations above the permitted threshold ( $HI > 1$ ), while cooked rice often mitigated the danger.

Nonetheless, several locations exhibited the presence of Pb in bioaccessible fractions, posing a potential non-carcinogenic concern, particularly for young infants. Multivariate statistical analysis indicated that agricultural techniques, irrigation water quality, and environmental pollution were the principal factors affecting heavy metal deposition in rice grains. A correlation was identified between Cd and P ( $r = 0.98$ ). Additionally, a high positive correlation was noted between Zn and Pb ( $r = 0.79$ ). Conversely, a very strong negative correlation was detected between the concentrations of P and Cd, with correlation coefficients of  $-0.78$  and  $-0.66$ . The dendrogram and heatmap exhibited distinct clustering patterns, indicating variations and resemblances in the behavior of the elements within the studied samples. The dendrogram categorized the analyzed variables into two primary groups.

The variables with the greatest similarity and the shortest linkage distance were Cd and P, which were grouped together in the first cluster. The significant proximity is associated with the correlation analysis ( $r = 0.98$ ) and PCA results, suggesting that Cd and P originate from the same source. Multivariate statistical analysis indicated that agricultural techniques, irrigation water quality, and environmental pollution were the principal factors affecting heavy metal deposition in rice grains.

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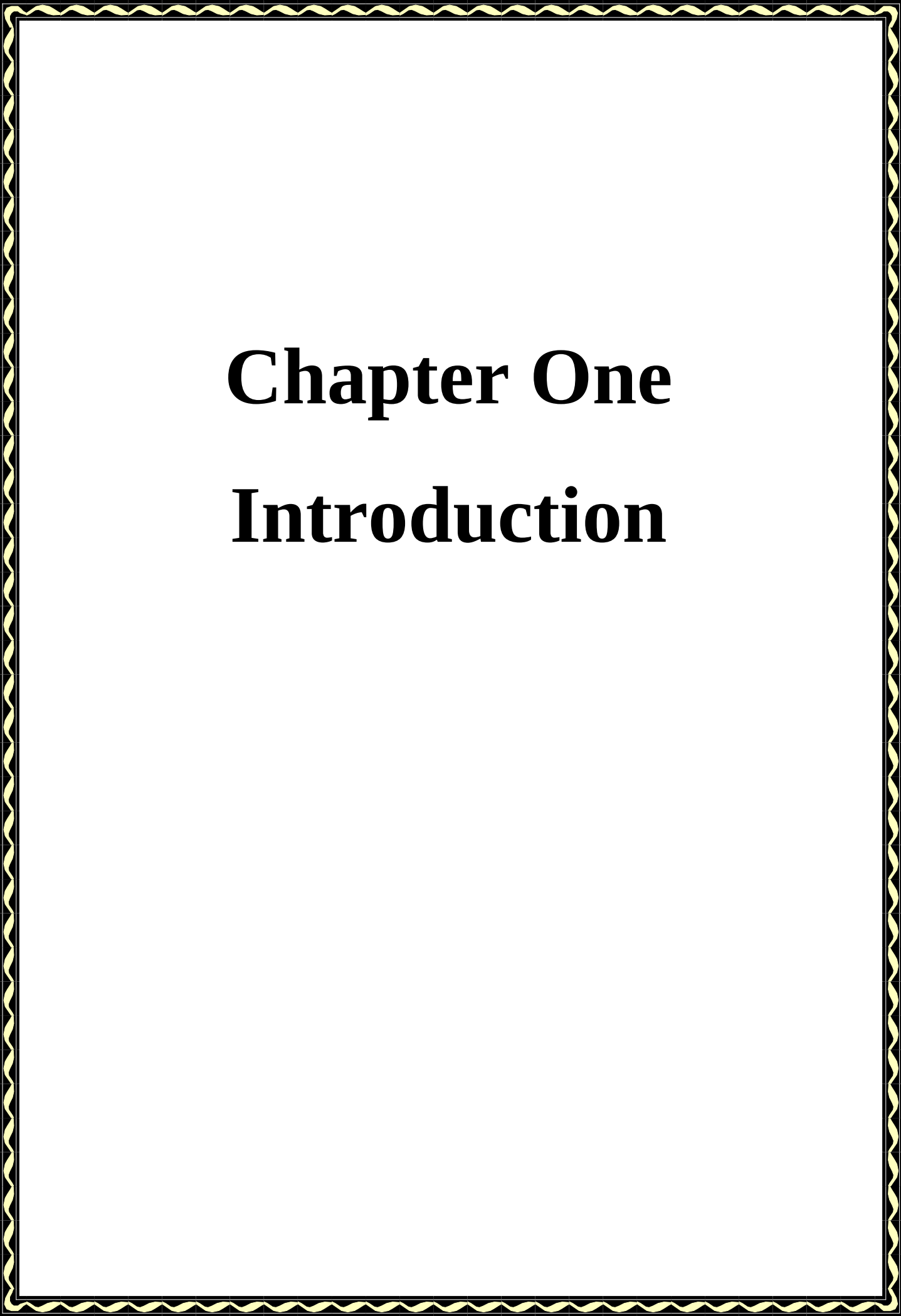
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### List of Abbreviations

| Abbreviation | Full Term                                 |
|--------------|-------------------------------------------|
| <b>FAAS</b>  | Flame Atomic Absorption Spectrophotometer |
| <b>Cd</b>    | Cadmium                                   |
| <b>Pb</b>    | Lead                                      |
| <b>Zn</b>    | Zinc                                      |
| <b>Cr</b>    | Chromium                                  |
| <b>Ni</b>    | Nickel                                    |
| <b>SBET</b>  | Simple Bioaccessibility Extraction Test   |
| <b>EDI</b>   | Estimated Daily Intake                    |
| <b>THQ</b>   | Target Hazard Quotient                    |
| <b>HI</b>    | Hazard Index                              |
| <b>CR</b>    | Cancer Risk                               |
| <b>TCR</b>   | Total Cancer Risk                         |
| <b>PCA</b>   | Principal Component Analysis              |
| <b>HCA</b>   | Hierarchical Cluster Analysis             |
| <b>QA/QC</b> | Quality Assurance / Quality Control       |
| <b>FP</b>    | Flame Photometer                          |



# **Chapter One**

## **Introduction**

## 1.1. Introduction

Environmental pollution remains one of the most critical global challenges. It is estimated that nine million people died due to environmental pollution in 2015, a figure surpassing the combined fatalities from HIV/AIDS, malaria, and tuberculosis (Niohuru,2023). Furthermore, reducing or avoiding exposure to chemical pollutants could prevent approximately 42% of strokes, which represent the second leading cause of death and disability worldwide (Landrigan *et al.*, 2018; WHO, 2016). Among various food crops, rice is a cornerstone of global food security, supplying more than half of the world's population with their primary daily caloric intake and nutritional source (Rezvi *et al.*,2023). However, rice possesses a high capacity to accumulate large amounts of toxic heavy metals and metalloids in its grains, including cadmium (Cd), lead (Pb), zinc (Zn), nickel (Ni), and chromium (Cr). (Zakaria *et al.*,2021). These contaminants typically originate from industrial discharges, untreated wastewater irrigation, and the intensive application of chemical fertilizers and pesticides, raising serious concerns about food safety and public health (Mao *et al.*, 2022; Khalid *et al.*,2018; Kesari *et al.*,2021).

Studies conducted in Iraq and neighboring regions confirm that Pb and Cd concentrations in rice frequently exceed the permissible limits established by the FAO/WHO, posing severe risks of neurological, renal, and carcinogenic outcomes (Dehghani and Mosaferi, 2016; Kumar *et al.*,2020; Zafarzadeh and Shahryari, 2025). For instance, a recent survey in Erbil markets reported median levels of Pb (0.495 mg/kg) and Cd (0.143 mg/kg), with cumulative Hazard Index (HI) values surpassing safe thresholds, particularly for younger age groups (Hassan *et al.*,2023). In Iraq, these environmental drivers are multifaceted: untreated wastewater discharge into the Tigris and Euphrates rivers, fertilizer-driven Cd inputs, and industrial expansion have collectively elevated heavy metal burdens in

paddy soils (Jarallah and Husseini, 2021). This underscores the urgency of evaluating rice contamination in Iraq's major producing areas, such as the Al-Qadisiyah Governorate, where rice is a dietary staple consumed daily (Qasim,2024).

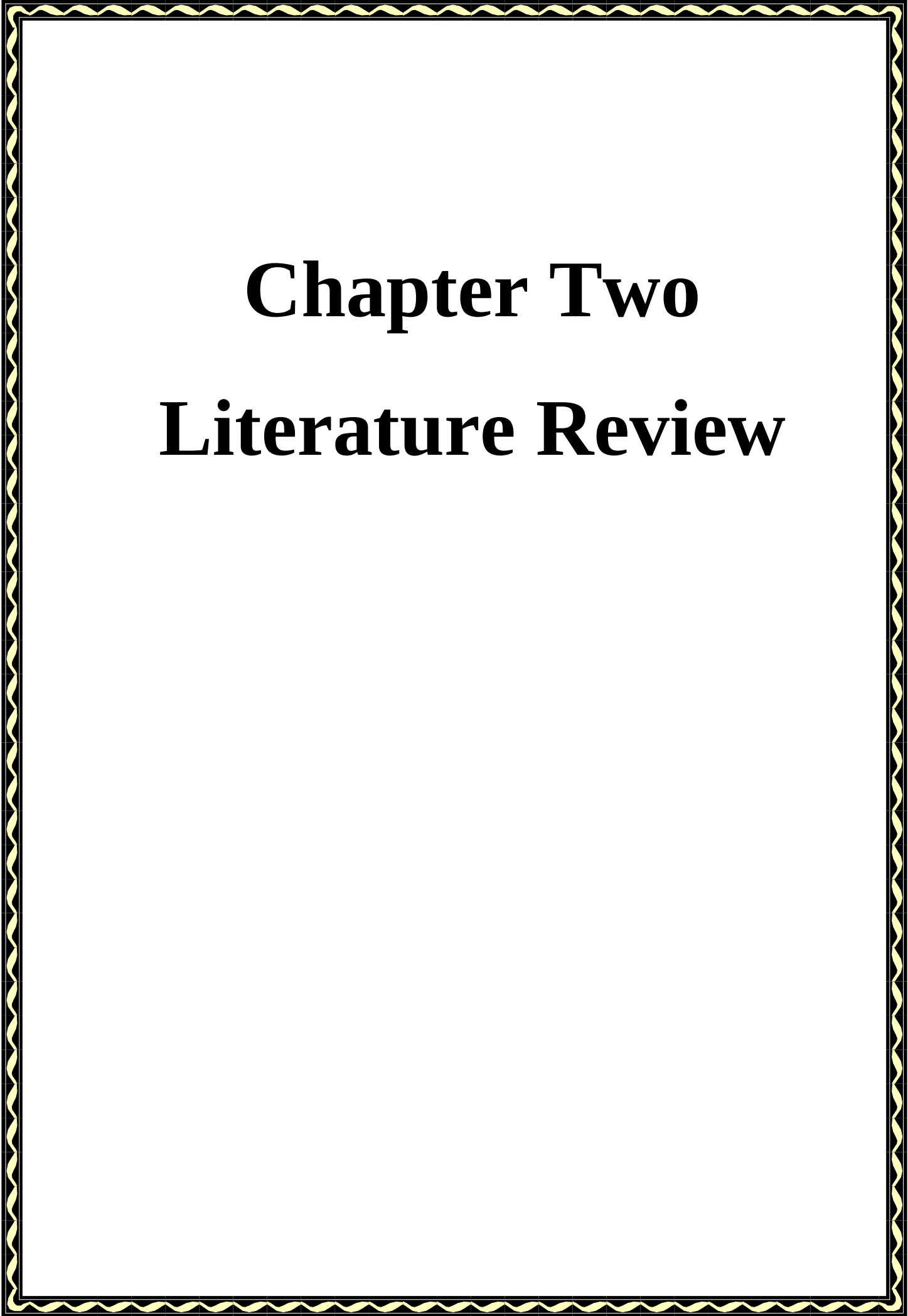
To establish a robust health risk assessment, contemporary scientific paradigms emphasize two complementary approaches (Brady *et al.*,2020). On one hand, evaluating risks based on total heavy metal concentrations provides a highly conservative and protective safety framework (Chen *et al.*,2021). Ensuring that potential hazards are not underestimated (Iwasaki and Yang,2020). On the other hand, focusing on bioaccessibility—defined as the percentage of a substance released from the food matrix into the gastrointestinal tract—provides a more realistic estimation of the actual toxicological burden available for physiological absorption (Zheng *et al.*, 2020). This distinction is critical because the total concentration of metals does not inherently correspond to the fraction that enters systemic circulation and causes biological harm ( Jadaa and Mohammed, 2023).

Furthermore, household processing practices play a vital role in modulating both the final concentration and the bioaccessibility of these metals (Rousseau *et al.*,2020). Standard cooking approaches, including washing, soaking, and boiling, can either reduce or redistribute heavy metals depending on water quality, temperature, and heat exposure time (Li *et al.*, 2023; Powers *et al.*, 2023). While washing and boiling may leach a portion of the total metal content into the discarded cooking water, the bioaccessible fraction might not decrease proportionally; in some contexts, it may even increase due to chemical and structural transformations that render the metals more soluble during digestion (Sharafi *et al.*,2019; Liu *et al.*,2020). Despite the critical importance of these shifting dynamics, there remains a profound scarcity of comprehensive studies in Iraq that simultaneously investigate total concentrations, cooking effects, and the

subsequent bioaccessibility of heavy metals in locally cultivated rice (Rasouli *et al.*,2022).

## **1.2. Objectives of the Study**

1. Determine the concentrations of selected heavy metals (Cd, Pb, Zn, Cr, Hg, and P) in cooked and uncooked rice samples collected from Al-Qadisiyah Governorate using appropriate analytical methods.
2. Evaluate the bioaccessibility of selected heavy metals and phosphorus P using the standardized (SBET) method that simulate human digestive conditions.
3. Compare the bioaccessible fractions of selected heavy metals and phosphorus P in cooked and uncooked rice samples
4. Assess the health risks of total and bioaccessible heavy metals in rice consumption..



# **Chapter Two**

## **Literature Review**

## 2. Literature Review

### 2.1. Heavy Metals

Heavy metals, the name has so many definitions based on various parameters. Based on density the metals which are having a density values greater than 5 g/cm<sup>3</sup> are considered as heavy metals (Tadele, 2022). Some of the heavy metals are having so much of biological importance in trace amounts (Ali *et al.*, 2019). particularly the elements that are present in the 4th period in the modern periodic table (Rayner-Canham, 2020). The biological importance of these metals is enzyme functioning (vanadium and manganese), hormone functioning, production (selenium), cellular growth (nickel), and metabolic growth (arsenic) (Teschke, 2022). But these metals are required for the human in trace amounts only if their amount in the body increases they cause adverse effects on human health (Mehri, 2020). Overall the heavy metal should be considered as having high density and also biological importance in trace amounts(Koptsik and Koptsik, 2022).

#### 2.1.1. Types of Heavy Metals

Based on the survey of literature the metals that are considered as heavy metals are chromium, lead, cadmium, iron, arsenic, cobalt, mercury, copper, and zinc ( Bilal-Mood *et al.*, 2021).

Heavy metals have been classified in to two types as essential and non- essential( Bibi *et al.*,2023 ). Essential Heavy metals are less toxic at low concentrations and they act as coenzyme in biological process (Bharti and Sharma,2022). For example Hemoglobin and Myoglobin consist of Iron, Vitamin B12 consist of cobalt (Kostova,2023). Non-essential heavy metals are highly toxic even at very low concentrations, they are non -biodegradable and cause severe toxic effects to living organisms(Cuschieri *et al.*,2023).

Humans are exposed to trace element and heavy metal through the daily ingestion of food and beverages( Mehri,2020). Trace element and

heavy metal ingestion through rice is a crucial to be investigated as rice has been dominating staple food for more than two billion people especially from Asia (Zakaria *et al.*,2021). World Health Organization (2003) has indicated that average daily intake of rice per person range from 9g in west countries and 600g in Asia which clearly showed that rice contribution to toxic elements exposure in human is crucial to be investigated(Laurent and Jolliet,2012). International Agency for research on Cancer (2012) has classified arsenic (As), cadmium (Cd) and lead (Pb) are both carcinogenic and non-carcinogenic to humans while chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), aluminium (Al) and zinc (Zn) are non carcinogenic to humans(Marufi *et al.*, 2024).

Toxic elements in rice studies have attracted a great concern worldwide involving paddy field and marketed rice samples( Sharma and Nagpal,2021). Rice has ability to absorb toxic element from soil and water in paddy field more effectively than other crops( Sharma *et al.*,2021). This is due to paddy plants is grown in water flooded conditions which permit toxic elements to be taken up by its root and accumulated in rice (Uddin *et al.*, 2021). Anaerobic condition in paddy field soil has resulted in higher mobilization rates and increase bioaccessible form of toxic elements in rice (Duan *et al.*, 2022). Since toxic elements have the ability to accumulate and nonbiodegradable, the presence of toxic element concentrations in marketed rice is largely unavoidable (Sharma *et al.*, 2021).

In Malaysia, most of the rice studies were conducted involving field rice samples related to pesticides, insecticide, fertilizers and organic contamination (Mohd Nizam *et al.*,2023). Although study done by Salim, Elias and Wood (2010) has focused on multiple toxic elements in rice varieties yet limited information was available on human health in the context of population and consumers exposure risks (Idris, N. S. U, 2018).

Heavy metals, particularly Cd and As, represent significant environmental and public health concerns owing to their potent toxicity (Mitra *et al.*,2022). persistent nature, and capacity for bioaccumulation and biomagnification within ecological systems, ultimately leading to severe health risks for humans through the food chain ( Edo *et al.*,2024).

### **2.1.2. Heavy Metal Toxicity**

Some heavy metals are essential to the human biological process, but depending upon their dosage intake leads some unexpected hazardous effects on health and the physiological system (Ohiagu *et al.*, 2022). According to studies shows that despite of its beneficiary health effects, heavy metals are acting as carcinogenic agents (Mitra *et al.*, 2022). Dissolved forms of these metals through different forms as soil pollutants, water pollutant and air pollutants entering into food chain and finally ending in humans (Angon *et al.*, 2024). These are leading to severe damage to the cellular system and leading to expose towards cancer (Zhao *et al.*, 2023). According to the reports of the International agency for research on cancer non-essential heavy metals (As, Cd, Cr) are major cancer- causing agents (Bibi *et al.*, 2023).

## **2.2. Mechanism of Heavy Metals Uptake in Plants**

Many researchers have investigated contaminant uptake and its mechanism by plants( Zeb *et al.*,2022). It might be useful to regulate the contributing factors to enhance the mechanism of plant Uptake ( Ullah *et al.*,2023). The plants normally play its role both as “accumulators” and “excluders”. Accumulators sustain even with concentrating contaminants in their aerial parts (Siromlya and Zagurskaya,2022). The plants biotransform or biodegrade the contaminants into inactive forms in their tissues (Erguc *et al.*,2021).

The excluders, in principle, limit the uptake of contaminant into their biomass (Guérin *et al.*,2020). Plants have developed very specific and highly efficient mechanisms to attain crucial micronutrients from the environment,

albeit when existing at low ppm and ppb levels (Rahman *et al.*,2024).The plant roots, which are assisted by plant-producing chelating agents and plant-inducing pH changes and redox reactions, can dissolve and absorb micronutrients from down levels in the soil, even from partly not soluble sediments (Zakaria *et al.*,2021).

Plants have also changed very explicit mechanisms to translocate and store micronutrients in their parts (Alaoui *et al.*,2022). The same mechanisms are also embraced in the uptake, translocation, and storage of heavy metals, where the nature of the chemical property's mimics those of essential elements (Hussain *et al.*,2024). Therefore, mechanisms of micronutrient uptake are of great attention to phytoremediation (Fan *et al.*,2021).

The array of known transport mechanisms set in the plant cell plasma membrane included in ion uptake and translocation involve (i) co- and anti-transporters (proteins that utilize the electrochemical gradients that are produced by ATPases to initiate the active uptake of ions), proton pumps (ATPases that occupy energy and produce electrochemical gradients), and channels (proteins that ease the transport of ions into the cell) (Zakaria *et al.*,2021).

A range of ions is likely will be taken up by each transport mechanism. The ionic species interaction during several heavy metal contaminants uptake is the primary problem in the mechanism of uptake (Kaur *et al.*,2021). Following roots uptake, the translocation of contaminants into shoots is required, since the harvest of biomass from root is generally not achievable (Rahman *et al.*,2024). There were lack of information concerning which forms of metal ions are translocated from the roots to the shoots (Tang *et al.*,2023).

Heavy metals uptake mechanisms by plants are expected to be strictly controlled. (Kosakivska *et al.*,2021). Plants, in general, do not accumulate trace elements over their metabolic needs (Mehri,2020). Such settings are

trivial in the range of 10 to 15ppm of most trace elements suitable for most needs (Zakaria *et al.*,2021). The exemptions are plants that are classified as “hyperaccumulator”, which can absorb toxic metal ions at a concentration in the thousands of ppm. In addition, other matter is the form of which toxic metal ions are accumulated in the plants, specifically in hyperaccumulator, and how these plants are resistant to metal toxicity (Memon *et al.*,2022).

In principle, manifold mechanisms are involved; including the storage in the plant’s vacuole seems to be a major one (Cui *et al.*,2020). Evapotranspiration is a term used to explain the process of water evaporating from plant leaves into plant roots (Ghiat *et al.*,2021). The water serves as a pump to disseminate nutrients absorbed to the plant roots and shoots (Arsova *et al.*,2020). The original soil was leaved undisturbed in order to remove contamination from roots to shoots (Romdhane *et al.*,2021). The plants that execute a shoot-to-a metalconcentration ratio that is greater than one are called “hyperaccumulators”, and these plants are normally used in phytoextraction strategies (Zakaria *et al.*,2021). Otherwise, the plants with shootto-root ratio less than one are categorized as non-accumulating plants (Kumar *et al.*,2020).

Theoretically, toxic environment should be flourished with hyperaccumulators, where it requires less maintenance and generates high biomass (Cao *et al.*,2025). Heavy metals, like Cd, Zn, Co, Mn, Ni, and Pb, can be accumulated by metal accumulating plant species concentrated up to 100 or 1000 times higher than the excluder (non-accumulator) plants (Memon *et al.*,2022).

Typically, microorganisms living in the rhizosphere, like bacteria and fungi, may promote mobilizing metal ions and increase the bioavailable fraction (Barra Caracciolo and Terenzi,2021). The proposedmechanismofAs uptake in paddy plants by (Ai *et al.*,2022).The As speciation in rice grain is dominated by dimethylarsinic acid (DMAV) and inorganic As (iAs), which

are majorly composed of arsenite, As (III) (Mahida *et al.*,2024). The total iAs is generally reported by the sum of two species through the conversion between As (III) and arsenate, As (V), which may occur during some extraction procedures (dos Santos Costa and Coelho,2021). In the biotransformation process, these iAs are converted to methylated arsenicals by enzymes, which are the biomarker of chronic arsenic exposure and the end metabolites (Hirano,2020).

Reported that the phosphate transporters in As translocation manage the influx of arsenate, whereas the arsenite is taken up via aquaporin nodulin 26-like intrinsic proteins (Tang and Zhao,2021). In the xylem sap of plants, inorganic As (V) and As (III) are the major arsenicals found (Rosa *et al.*,2022). A very small proportion of As is translocated to shoot tissue (leaf), where similar reduction and sequestration mechanisms exist and, via the phloem, some of the total As content ceases in the vacuoles and other parts of edible tissue, such as rice grain (Rahman *et al.*,2024).

Different rice varieties and rice genotypes have shown significant differences in accumulating As at its higher concentration (Dwiningsih and Alkahtani,2022). This might be partly due to the surface characteristics of rhizosphere, which also play an important role in iron plaque formation of reddish-brown coating on the root surface (Zandi *et al.*,2023).

Besides As, the irrigation water in paddy field often also causes loads of Cd in soil (Mou *et al.*,2020).It accumulates in grains following four major transport processes, namely (1) the uptake of Cd by root, (2) root-to-shoot translocation by xylem flow, (3) redirection of nodes, and (4) remobilization from leaves (Zheng *et al.*,2023). When the element Cd binds to cysteine-rich protein,such as metallothionein, their concentrations are increased to 3,000-fold (Permana and Akbarsyah,2021).

Phytochelatin (PCS) has been classified as class III metallothioneins and Cd is known as the strongest inducer for their biosynthesis (Seregin and

Kozhevnikova,2023). The Cd element is able to form complexes with PCS and is transported to vacuoles (Thévenod *et al.*,2019). PCS-metal complexes possibly form a more complex aggregation in vacuole by isolating toxic metals from various metal sensitive enzymes in plant cell cytoplasm (Seregin and Kozhevnikova,2023).

### **2.3. Human Health Associated with Heavy Metal Contamination in Rice**

Because there is an increasing trend of population growth and demand of rice consumption, the use of an escalating number of pesticides by producers, in order to protect their crop, may influence the level of heavy metals in soils (Alengebawy *et al.*,2021).Heavy metal pollution on a paddy field has recently been of great concern as an environmental pollutant due to of its bioaccumulation in the environment and non-biodegradable properties (Bhoi *et al.*,2025). Heavy metals have been commonly discussed as a potential pollutant in rice and it is classified as one of the major important toxic substances, due to its high potential risk to the ecosystem and human health (Zakaria *et al.*,2021).

The International Agency for Research on Cancer (IARC) (2012) (Wild *et al.*,2020). has classified arsenic (As), cadmium (Cd), chromium (Cr), and nickel (Ni) as group 1 carcinogen, since long-term exposure leads to increased risk of various type of cancer, including disruptions in tumor suppressor gene expression, damage repair processes, and enzymatic activities that are concerned in metabolism through oxidative damage (Bánfalvi, 2011). By nature, heavy metals can be divided into two forms, which are organic and inorganic (Borah *et al.*, 2020). For example, inorganic As is more harmful than organic form due to the pentavalent inorganic compound of As that solubilizes in water to the weak acid form and produce arsenate (Genchi *et al.*,2022). that affects people by inducing ground water contamination (Hilili *et al.*,2021).

Heavy metals in the form of organic pollutant may gradually degrade into less harmful components through chemical or biological processes (Zhang *et al.*,2023). Environmental contamination of the biosphere with heavy metals have caused intensive agricultural and other anthropogenic activities and posed critical problems for safe use of agricultural land (Alengebawy *et al.*,2021). Agricultural soils are potentially contaminated with essential and nonessential heavy metals through existing agriculture practice with arbitrarily use of agrochemicals, such as pesticide and fertilizers, along with mechanical cultivation that change the speciation and the mobility of heavy metals (Selvam *et al.*, 2024).

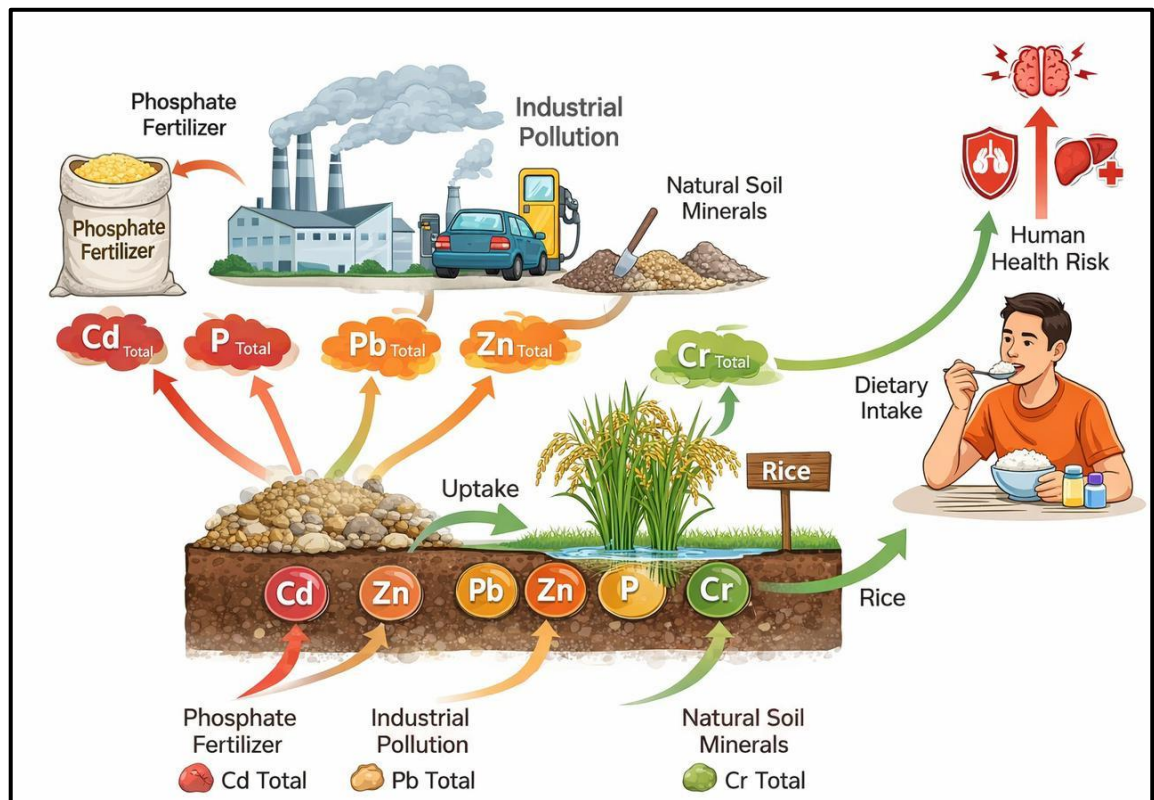
In subsistence farms of Asia, there is a clear verification of the association between Cd poisoning and human renal dysfunction (Awuchi *et al.*,2022). and, certainly, rice has been recognized as one of the prime sources of Cd and Pb to human intake especially in Japan (Sharma *et al.*,2021). Plant organs have different abilities to adsorb, translocate, and accumulate heavy metals, and the speed and magnitude of these processes vary between plant species and cultivars (Rahman *et al.*,2024). Other than that, the adsorption and accumulation of heavy metals in soil is driven by soil properties, such as pH, and the concentration of organic matter (Yu *et al.*,2023).

The direct transfer of heavy metals to human body from plant parts of interest is rice grain, which is an edible part for consumption that may cause a threat to human health (Uddin *et al.*,2021). Because heavy metals are proclaimed to amass in living organisms, especially toxic metals, such as As, Cd, and Pb, health risk assessments are conducted on the basis of heavy metal concentration found in paddy plants and soils to evaluate the potential health risk (Zulkafflee *et al.*,2020).

The heavy metal taken up from plants may transfer into the food chain and adversely affect human health, depending on their carcinogenicity (Angon *et al.*,2024). People are potentially exposed to toxic metals and

metalloids from rice, especially those who take it as staple food for daily energy requirement (Sharma *et al.*,2021). The tolerable daily intake (TDI) for Cd, Hg, and As, which are highly toxic to human exposure, are recommended to be 1.0 µg/kg body weight (bw)/day, 0.57 µg/kg (bw)/day, and 2 to 7 µg/kg (bw)/day, respectively (Demir and Ağaoğlu,2023). Understanding the soil-metals uptake mechanisms in food crop is essential for devising the effective remediation process (Wang *et al.*,2021).

In order to achieve this goal, this review initially describes the mechanism of heavy metal uptake in paddy plants and soils and their contributing factors with respect to bioaccumulation (Jayakumar *et al.*,2021). Impressively, this review tries to provide an overview on the human risk assessment in relation to non-carcinogenic and carcinogenic risk (Marufi *et al.*,2024). In this regard, it also covers information regarding *in vitro* digestion models that can be conducted to determine the bioaccessible form of heavy metals (Dima *et al.*,2020).As shown in the figure.(2.1).



**Figure(2.1): Heavy Metal Bioaccessibility from Soil to the Rice and Human Effect**

## 2.4. Sources of Heavy Metal Contamination in Rice

Heavy metal contamination in rice mainly originates from both natural and anthropogenic sources (Zakaria *et al.*,2021). The most common toxic metals found in paddy fields include cadmium (Cd), arsenic (As), and lead (Pb) (Mlangeni *et al.*,2023).Major contamination pathways involve mining activities, industrial effluents, wastewater irrigation, and the excessive use of chemical fertilizers and pesticides (Padhiary and Kumar,2024).

Soil characteristics such as pH and organic matter content further influence metal mobility and plant uptake, allowing significant amounts of Cd, As, and Pb to accumulate in rice grains (Siddique *et al.*,2022). Since rice is a major dietary staple in many regions, including Al-Qadisiya Province, contamination in local soils or irrigation water directly impacts human exposure to these metals.

Heavy metals have been categorised as one of the major chemical hazards due to the potential risk they pose to the environment and to human health (Mitra *et al.*,2022). Modern agricultural practices such as non-selective application of agrochemicals such as pesticides and fertilisers in addition to mechanical cultivation may possibly contaminate the agricultural soils with essential and nonessential heavy metals (Nunes da Silva *et al.*,2022).

The adsorption and accumulation of heavy metals in the layer of soil are probably due to relatively high organic matter, which has a direct effect on the cation exchange capacity and buffer capacity, as well as the retention of heavy metals (Aljumaily and Al-Hamandi, 2022). Moreover, different plants, as well as different parts of the plants, have different capacities in the absorption and accumulation of heavy metals including the metal uptake and translocation between plant species and even between cultivars of the same plant species (Rahman *et al.*,2024). As an important environmental compartment, soil acts as a sink for heavy metals, ultimately resulting in heavy metal accumulation in soils (Jayakumar *et al.*, 2021).

Previous investigations have found that the sources of heavy metals in soils include commercial fertilizers, irrigation water, animal manure, sewage sludge, and atmospheric deposition (Salem *et al.*, 2020). Thus, the diversity of HMs sources presents challenges in accurately identifying and tracking the sources of HM contamination in agricultural soil (Angon *et al.*, 2024).

Recently, stable metal isotope technology, positive matrix factorization (PMF) models, absolute principal component scores-multiple linear regression model (APCS-MLR) and geographic information system (GIS) mapping have gained attention for their effectiveness in identifying and tracing sources of HM pollution in the environment (Zhang *et al.*, 2020).

#### **2.4.1. Agricultural Sources (Fertilizers, Pesticides, Irrigation Water)**

Inorganic and organic fertilizers are crop food considered as indispensable sources of nutrients to the agriculture ecosystem (Rashmi *et al.*, 2020). These crop food, besides supplying essential nutrients and acting as soil conditioners, pose potential pollution risk in agriculture and might contain significant amount of contaminants (Rashid *et al.*, 2023).

Indiscriminate use of fertilizer and manures, improper handling and storage facilities, etc (Shukla *et al.*, 2022). often result in degradation of natural resources, releasing contaminants into soil, air, and water which directly impact human health (Akhtar *et al.*, 2021).

Inorganic fertilizers are subjected to easy breakdown in soil compared to organic manures and, therefore, easily contaminate soil, water, and air (Pahalvi *et al.*, 2021). Major source of contamination is from nitrogenous and phosphatic fertilizers, which affect soil properties (Tyagi *et al.*, 2022). Runoff cause water contamination, or sometime escape to atmosphere affecting air quality thereby enhanced contribution to greenhouse gases contributing to climate change (Singh and Yadav, 2021).

In addition, contaminants from both inorganic and organic fertilizers rich in nutrient lost via runoff and soil erosion result in eutrophication of aquatic bodies( Tiwari and Pal,2022). High build up of heavy metals like Cr, Cd, Ni, Hg in soil are often associated with excess application rate of manures and fertilizers in agriculture (Wei *et al.*,2020).

Scientists have defined pesticides as synthesized chemical compounds used in many areas, including in the agricultural sector, to control pests (Khan *et al.*,2023). Therefore, pesticides are considered as efficient, economical, and effective weapons in integrated pest management systems (IPMs) (Deguine *et al.*,2021). The uncontrolled use of pesticides causes their bioaccumulation in food chains, which leads to high risk to mammals and other non-target organisms (Akhter *et al.*,2024).

In addition, the direct or indirect effect of pesticides on non-target organisms leads to an imbalance of the surrounding ecosystem (Serrão *et al.*,2022). Moreover, the pesticide residues remain in the plant parts, soil, air, and even penetrate into water (Ali *et al.*,2020). Such residues are considered as one of the most destructive threats the ecosystem faces; these can exist in the environment for a long time, with carcinogenic effects (Suvorov and Stancu,2021).

On the other hand, pesticides are an essential component of rice cultivation that are used to enhance yields and manage pests and diseases (Fahad *et al.*,2021). Commonly used pesticides include organophosphorus compounds, pyrethroids, and herbicides (Ahamad *et al.*,2023). However, the improper or excessive use of these chemicals can result in elevated pesticide residue levels in rice (Yura *et al.*,2021). These residues contaminate soil, air, and water and may be absorbed by rice (Goswami *et al.*,2020). Through its root system, leading to cumulative exposure and increased health risks over time (Smith *et al.*,2022).

In addition to pesticide residues, heavy metals such as Cd, arsenic (As), Pb, chromium (Cr), and mercury (Hg) also accumulate in rice due to industrial emissions, the use of heavy-metal-containing fertilizers, and the natural composition of soil (Kelle *et al.*,2021). Pesticide residues and heavy metal contaminants in rice not only degrade its quality and taste but also pose serious health risks (Huda *et al.*,2024). Recent studies have shown that the prolonged consumption of contaminated rice significantly increases the risk of diseases such as kidney disease, neurological disorders, and cancers (Zakaria *et al.*,2021).

Rice paddies are regularly sprayed with pesticides to limit herbicidal, fungicidal, and insecticidal activity (Calha *et al.*,2022). Pesticides are heavily used in the Indian rice industry, which comes second for pest demand after the cotton industry (Nayak and Solanki, 2021). There are over 230 registered pesticides by the Indian Agricultural Authority, many of which are prohibited in the European Union (EU) and the United States (US) (Khandelwal *et al.*,2022).

Pesticides are so overused in India that the Integrated Pest Management had concerns about the hazards pesticides may have on the environment (Rajmohan *et al.*,2020). In fact, acephate, carbendazim, thiamethoxam, triazophos, and tricyclazole have all been documented to exceed EU maximum residue limits (MRLs) in Indian rice (EUEuropean Commission,, EUEuropean Commission,)( Khandelwal *et al.*,2022). Oral exposure to these pesticides can cause gene dysfunction, endocrine disorders, and cancer (Salazar-Flores *et al.*,2022).

Long-term irrigation water creates heavy metal deposition in soils (Suska-Malawska *et al.*,2022). The main problem of crop irrigation with wastewater is heavy metals in wastewater, which might deposit in the soil and eventually end up in cultivated plants (TABATABAEI *et al.*,2020).

Prolonged usage of wastewater effluents for irrigation often increases soil heavy metal content (Soleimani *et al.*,2023).

Toxic metal buildup decreases soil fertility and crop quality while interfering with the soil's ecological function and effects on other ecosystem elements( Rashid *et al.*,2023). Furthermore, HMs can be released as solutions, which the rhizosphere of the plants can then absorb when the soil's capacity to contain heavy metals declines due to a rise in HMs at the soil level (Jayakumar *et al.*,2021).

On the other hand, using treated wastewater instead of groundwater for irrigation purposes in arid and semi-arid regions is crucial since it boosts treated wastewater's reusability and decreases the need for agrochemicals (Khan *et al.*,2022).Treated wastewater is more cost-effective than groundwater and chemical fertilizers, following all associated standards. Moreover, controlling and monitoring contaminants added to the soil, including heavy metals, is necessary through irrigation with these alternative sources (Hashem and Qi,2021).

#### **2.4.2. Environmental Pollution Sources (Industrial, Mining, Sewage)**

Discharges of industrial wastewater into the sewer network from industrial organizations require an official permit (Buras and Solano Donado, (2021). These official permits include the maximum levels for a limited number of certain substances, restrictions on the level of pH and temperature, as well as the maximum allowed volume of oil and grease, for example (Dias *et al.*,2022). Radioactive material and persistent chemicals, among others, are explicitly mentioned and they must not exceed concentrations that are typical for domestic wastewater (Koul *et al.*,2022).

A study was conducted of industrial pollution in lowland rice areas in the district of Rancaekek, West Java (Rachman *et al.*,2022). These areas are being polluted by heavy metals from sewage sludge produced by the textile

industry (Li *et al.*,2021). This waste is disposed of directly into three rivers, all of which are used to irrigate lowland rice (Elbasiouny and Elbehiry,2020). About 720 ha of lowland rice fields were polluted in this way (Chen *et al.*,2022). Soil surveys by Kurnia (1999) revealed that there were very high concentrations of boron, cadmium and lead in three villages in the Rancaekek district (Susanto *et al.*,2021). Falling soil productivity in these areas caused a reduction in rice yields and farmers' incomes (Dhanda *et al.*,2022).

After 20 years of contamination, the average rice yield had decreased by about 80%. (Ren *et al.*,2020).The initial rice yield of about 4-6 mt/ha had become 1 mt/ha (Mgendi *et al.*,2022). However, the heavy metal content in the soil had increased by about 18% - 98%, compared to unpolluted soil (Zhao *et al.*,2022). A greenhouse study using polluted soil from this area showed that high concentrations of lead, cadmium, copper, chromium and boron were found in the plant tissue, roots and grain of rice (Sharma *et al.*,2021). Most of the pollutants had accumulated in the root system Previous mining activities in No (Smorkalov and Vorobeichik,2022).

Uranium mining area resulted in release of radioactive waste into the adjoining environment leading to distribution of contaminants into different ecological compartments such as surface water and paddy soil (Adeola *et al.*,2023). Sewage refers to wastewater and excrement conveyed in sewers (Ghangrekar,2022). These include waste matter such as water or human urine or solid waste, faeces or dirty water from homes which flows away through sewers (Alda-Vidal *et al.*,2020).

Sewage also includes faecal sludge which refers specifically to a mixture of human excreta, water and solid wastes such as toilet paper or other anal cleansing materials conveyed in sewers (Jothinathan and Singh,2023). Inside the onsite septic tank, sewage is separated into three components, liquids which are the effluents, solids or sludge that settles to the bottom of the tank, and grease and other scum that floats to the top (Rai, 2024).

Domestic sewage containing heavy metals from body lotions, skin cream, and poisonous substances discharged in the septic tanks can be passed into the environment during overflow of the facility (Oduah and Ogunye,2023).

## 2.5. Impact of Cooking on Heavy Metals

Very limited studies are available in the literature on the fate of toxic heavy metals during food cooking operations (Perello *et al.*,2021). Studied the effect of food processing unit operations on concentrations of arsenic, mercury, cadmium and lead in food products (Annar,2022). They found that cooking operations exerted a limited impact on the removal of heavy metals from the foods under investigation and concluded that the hypothetical reduction in heavy metals depends upon the cooking conditions (time, temperature and medium of cooking) (Karimi and Goli,2021).

Some studies on the impact of rice cooking conditions on arsenic levels have been carried out by a few researchers (Atiaga *et al.*,2020). Food is generally subjected to cooking prior to consumption in order to increase the palatability of the product (Lester *et al.*,2022). In comparison with raw rice, cooked rice is the best to be used as sample in HRA studies as the sample must be on the basis product it was ingested by customers so that risk evaluation will reflect real situation of human exposure( Sabbagh, 2023).Nevertheless, cooking was found to be a minor exposure route for heavy metal in rice (Bielecka *et al.*,2020).

Numerous studies have been performed to examine the effects of common food processing procedures on the levels of heavy metals in food (Nkwunonwo *et al.*,2020). It is known that the cooking process may, under certain condition, alter the concentration or speciation of contaminants in food matrices (Guo *et al.*,2024). Little is known about the effects of rice cooking on the bioaccessibility of Cd and its human health risk due to Cd exposure from rice intake (Mohajer *et al.*,2024).

Heavy metals do not evaporate or disintegrate into harmless substances but rather when detached during cooking and processing will only migrate into the processing medium and retained in the food (Lucky *et al.*,2023). Hence there is no established consensus on how different cooking processes contribute to the variations in the heavy metal contents of the final products (Inobeme *et al.*,2020). It is against this backdrop that this review focuses on some of the studies carried out and their findings with regards to the effect of cooking methods on heavy metal contents (Mohajer *et al.*,2024).

Attempts was also made to highlight some of the different factors they adduced to be responsible for the reported findings ( Pasko *et al.*, 2021). In their study reported that method of processing plays a significant role in affecting the content of heavy metals in food (Scutarașu and Trincă,2023).

The contents of the potentially toxic metals investigated were observed to follow the trend: Freeze dry > sundry > oven dry (Smeds *et al.*,2022). They attributed this observation to the higher water and other volatile materials present in the sample studied ( Zhang *et al.*,2022).

These groups of metals are of concern in that unlike organic contaminants they cannot be degraded by microorganisms their transformation only occurs in terms of changes in oxidation number ( Mbachu *et al.*,2020). Although these metals are ubiquitous hence found in all aspects of the environment, under such conditions they do not constitute harms since they are less bio-available (Raklami *et al.*,2022).

Food substances such as meat and fish are known to have remarkable nutritional values due to their high proteins contents notwithstanding, their complex nature makes it possible for the bioaccumulation of toxic heavy metals (Acharya, 2024). Reduction of heavy metals contents in food could occur during processing (Adjei-Mensah *et al.*,2021).

Some food substance could also absorb some metals when the medium, water and oil are contaminated with metals( Munir *et al.*,2021). The metallic material used for grilling may also be a potential source for this rise (Ajayi and Abegunde,2022). Contacts of food with surfaces already contaminated by these metals also occur ( DeFlorio *et al.*,2021).

Metal contamination has also been pointed to the inappropriate storage system, such as putting acidic food materials in metallic materials rather than plastic, which gradually results to rise in metal contents(Lebelo *et al.*,2021). Most people come in contact with heavy metals through contaminated food (ABDULLAHI *et al.*,2021). The concentrations of these heavy metals can be reduced through precooking processes, such as washing and soaking, prior to consumption (El-Sharnouby and Asiri,2025). Important factors influencing rice pretreatment and cooking, which can help mitigate rice contaminants, include washing, soaking in water, and the specific cooking method utilized( Shahriar *et al.*,2022). There have been some studies on efficiently reducing heavy metals by washing, soaking, and cooking processes (Inobeme *et al.*,2020).

Studies have indicated that precooking processes prior to cooking, or cooking with an increased volume of water, may aid in the removal of heavy metals from food( Li *et al.*,2023). The removal efficiency of arsenic from rice through the washing process is reported to be 10–20% (Collivignarelli *et al.*,2022). have reported 15, 7%, and 28% of arsenic removal using the Kateh cooking method (Menon *et al.*,2021). In another study, it was found that combined with rinse washing and soaking in salt reduced cadmium by 96.4, nickel by 67.9%, and lead by 90.11% from raw rice (Ziarati *et al.*,2022).

## 2.6. Heavy Metal Bioaccessibility

Bioaccessibility refers to simulation of the gastrointestinal to determine the fraction of ingested compound that is potentially available for absorption (Dima *et al.*, 2020). bioaccessibility of trace metals is typically evaluated using in vivo studies i.e. animal investigations, which are expensive time-consuming, and harmful to the animals (Zhuang *et al.*, 2020). Therefore, in vitro testing may be preferable to in vivo tests for determining the bioaccessibility of toxic metals due to their lower cost and ethical implications(Akimzhanova and Guney,2022).

Understanding bioavailability of heavy metals in rice is helpful for estimating the amount of heavy metals which can be absorbed by the human body (Uddin *et al.*,2021). Thus, there is a need to determine the oral bioaccessibility of heavy metals in rice (Zakaria *et al.*, 2021). Oral bioaccessibility is defined as the fraction of contaminant that is released from the food matrix into the digestive juice chyme and becomes available for absorption, i.e., enters the blood stream (Dima *et al.*,2020). Therefore it is used as an indicator of maximal oral bioavailability of the contaminant in food (Tian *et al.*,2020).

Bioavailability refers to the rate and extent of an administered contaminant that becomes available for utilization and distribution to a living biota (Spacie *et al.*,2020). Several animal models (mice, swine, and rabbits, etc.) have been used for the assessment of in vivo bioavailability of inorganic or organic pollutants in contaminated soils (Wijayawardena *et al.*,2023). To date, there is a few researches on measuring Cd relative bioavailability (RBA) in food matrices, especially contaminated rice (Xiao *et al.*,2024).

Other researchers have focused on the factors influencing the Hg bioaccessibility in fish; e.g., the cooking mode used can decrease the Hg bioaccessibility, because the heat applied denatures the protein structures (Costa *et al.*,2022). Yet, concerning rice, the factors impacting THg and

MeHg bioaccessibility are seldom reported (Wu *et al.*,2022). To fill this knowledge gap, in this study we collected 34 rice samples to investigate their THg and MeHg bioaccessibility, and different sample groups were set to explore the affection of Hg bioaccessibility (Langasco, 2021). Three factors of interest were tested: rice cultivar, planting location, and cooking, with appropriate statistical analyses implemented to investigate their effects (Kumar *et al.*,2023). Both are dependent on digestion and release from the food matrix, but bioavailability is also dependent on absorption by intestinal cells and transport to body cells (Dima *et al.*,2020).

In this review, bioavailability will be used to explain both terms despite the difference in the meaning as recognized by most of the studies referenced in here (Stielow *et al.*,2023). Moreover, in order for a nutrient to be bioavailable it has to be bio-accessible( Rodríguez *et al.*,2020).

### **2.6.1. In-vitro Methods of Investigating Bioavailability**

#### **2.6.1.1. Advantages of In-vitro Methods**

As mentioned above, *in-vitro* methods are considered as an appealing alternative to human and animal *in-vivo* studies (Huang *et al.*,2021). They are used as a tool to measure the iron bioavailability in various samples because of the simple, low cost and rapid technique that can be replicated inter-and intra-laboratory (Serhan, 2020).

Moreover, human studies present higher variability due to potentially large variability between subjects' iron status (Stoffel *et al.*,2020). *In-vitro* methods are also useful to assess the effects of processing, bio-fortification, and enhancers on iron bioavailability (Mattar *et al.*, 2022).

#### **2.6.1.2. Application of In-vitro Methods in Food Studies**

Previous studies have used various types of *in-vitro* methods for cereals, legumes and fruits and vegetables (Ceccanti *et al.*, 2022). Each method uses different constituents of simulated gastro-intestinal juice and extraction time (Mihaylova *et al.*, 2021).

### 2.6.2. In-vitro Assessment of Heavy Metals in Rice

The bioaccessibility of cadmium (Cd) and arsenic (As) in rice was reported in this study and the *in vitro* method was adopted from (Zhuang *et al.*, 2020). The U.S. Food and Drug Administration (USFDA)- (Mukherjee, 2023).

### 2.6.3. In-vitro Methods in Health Risk Assessment

Evaluated the health risk of As in rice based on bioaccessibility and bioavailability data derived from *in vitro* and *in vivo* studies (Lin *et al.*, 2023). In this study, four static methods, namely In Vitro Gastrointestinal (IVG), Solubility Bioavailability Research Consortium (SBRC), Physiologically Based Extraction Test (PBET), and Deutsches Institut für Normung (DIN) were applied for an accurate Hg health risk assessment since these methods have not been applied for Hg in rice (Wu *et al.*, 2022).

### 2.6.4. Research Gap in Cd Bioaccessibility Studies

For Cd bioaccessibility in food matrices, little research has focused on vegetables and seafood products (Marmelo *et al.*, 2020). To date, relatively few studies exist about the Cd bioaccessibility or bioavailability in rice, with only a few data based on *in vitro* digestion (Lv *et al.*, 2019). However, limited research has examined the *in-vitro* bioaccessibility of heavy metals in rice from Iraq, particularly in Al-Qadisiyah.

## 2.7. Amber Rice (*Oryza sativa* L)

Rice (*Oryza sativa* L.) is amongst the most important cultivated food widely consumed worldwide, particularly in Asia, as it is an important source of energy obtained by the individual (Mohapatra and Sahu, 2021).

Rice occupies a special place in the daily meals of the Iraqi people; it is an indispensable dish in any home (Canard, 2022). Rice is widely cultivated in Iraq, especially in the center and south of the country, Amber the variety is known is one of the most important cultivars (Badro *et al.*, 2019). Amber rice

is a traditional aromatic rice cultivar widely cultivated in central and southern Iraq, particularly in regions such as Najaf and Al-Diwaniyah (Al-Tameemi,2019).

It is characterized by its distinctive aroma, long grains, and high consumer preference among Iraqi populations (Muhialdin *et al.*,2022). And because of its unique taste and aroma, it is distinguished by that makes it desirable for both Iraqis and neighboring countries (Savvaidis *et al.*,2022). Amber rice represents a traditional, aromatic local cultivar that has been cultivated across Iraq for centuries (Spengler,2020).

### 2.7.1. Classification of Rice Cultivars

Rice seeds' length and width are the two principal quantitative milling and classification traits, and rice cultivars are classified as long, medium, or short grain (Li *et al.*,2022 ; Kiratiratanapruk *et al.*,2020).The size and shape of seeds are stable varietal properties that can be used to identify rice cultivars (Rezk *et al.*,2025). Agronomic and morphological characterization are fundamental in providing information about Iraqi aromatic rice cultivars (Islam *et al.*,2018).

Amber rice cultivated in Iraq can be generally classified as a medium-grain aromatic rice type (Von Bargaen,2025). It belongs to the aromatic rice group characterized by the presence of fragrance compounds, particularly 2-acetyl-1-pyrroline (2AP), which is responsible for its distinctive aroma (Okpala *et al.*,2019). Morphologically, it exhibits relatively long and slender grains compared with non-aromatic local rice cultivars, and it is distinguished by its soft texture after cooking and strong consumer preference in Iraq (Verma and Srivastav,2022).

Accordingly, Amber rice is considered a distinct local landrace within the aromatic rice group cultivated mainly in central and southern Iraq (Badro *et al.*,2019). Reflecting both its genetic uniqueness and long-term adaptation to local environmental conditions (de Lafontaine *et al.*,2018).

### 2.7.2. Environmental and Water Scarcity Challenges

The agricultural sector in Iraq suffers from drought, lack of water resources and water consumption, which directly affected the production of agricultural crops (Salman *et al.*, 2021). As the yield per unit area decreased, and it led to the abolition of the cultivation of some economically important crops such as rice and other crops (Bray, 2023).

Traditional cultivars like Amber rice are characterized by their exceptionally high water demand (Villalobos-López *et al.*, 2022). Consequently, modern research emphasizes optimizing water consumption and evaluating the ecological sustainability of this staple cultivar (Lakhia *et al.*, 2024).

#### 2.7.2.1. Sources of Contamination and Bioaccumulation

The presence of heavy metals is attributed to environmental and technical factors; it mainly depends on soil pollution with these minerals depending on its geographical location, in addition to other important factors such as the rice variety and agricultural techniques that include the use of pesticides (Wan *et al.*, 2024; Rashid *et al.*, 2023; Zhang and Wang, 2020).

Fertilizers, water source watering, industrial emissions and pollution that may occur during harvesting, storage, transportation and crop sales also contribute to this contamination (Zahoor and Mushtaq, 2023).

HMs cannot be degraded after entering the soil and polluting nearby soil and rivers through surface runoff and groundwater (Sahu *et al.*, 2023). This results in an elevation of HM content within the vicinity of the mining site, particularly in the soil, leading to environmental pollution (Wei *et al.*, 2018).

Meanwhile, it has been demonstrated that rice exhibits a greater capacity for bioaccumulation and translocation of HMs compared to other grain crops, primarily due to its botanical characteristics and planting environment (Zakaria *et al.*, 2021). Therefore, rice is considered one of the

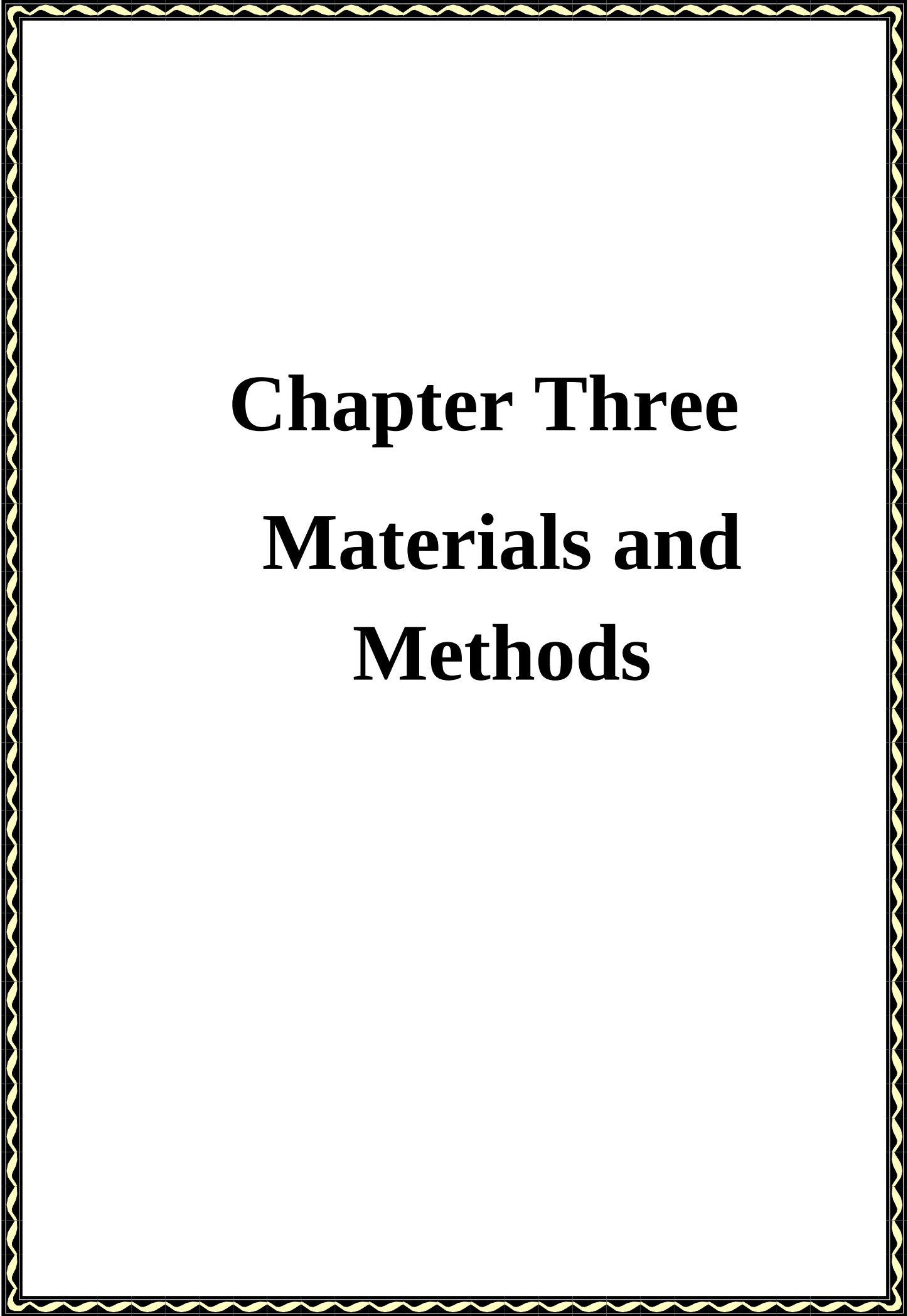
most significant model plants for assessing the ecological health risks posed by HMs due to its widespread cultivation and high sensitivity to contaminants (Uddin *et al.*, 2021).

After uptake by rice roots, HMs are translocated and accumulated in the rice grains (Cao *et al.*, 2018). For example, Cd is absorbed by rice roots, transported upward through the xylem, redistributed between stems and leaves, and transferred to the grains via the phloem (Zhang *et al.*, 2022). It has been recently calculated that Cd absorption from rice ingestion accounted for 50% and 60% in Indonesia and Japan, respectively ( Zakaria *et al.*, 2021).

#### **2.7.2.2. Human Exposure and Cooking Effects**

In the core of Asia's largest karst region, this increases the likelihood of human exposure to HMs and poses a potential risk to both ecosystem and human health (Mallongi *et al.*, 2022). More importantly, HM pollution may endanger human health through the soil–crop–food pathway (Angon *et al.*, 2024).

Therefore, studying the levels of heavy metals in Amber rice and evaluating the effect of washing, soaking and cooking processes on their concentration and bioaccessibility (Abu-Almaaly, 2020). The fraction of a substance that is released from food and available for absorption in the human gastrointestinal tract is of particular importance for assessing potential health risks to Iraqi consumers (Hawrami *et al.*, 2020).



# **Chapter Three**

## **Materials and Methods**

### 3. Materials and Methods

#### 3.1. Equipment and Instruments

**Table (3.1) Equipment of Present Study**

| NO | Device name                                | Supplying company | Origin  |
|----|--------------------------------------------|-------------------|---------|
| 1. | Hot plate                                  | Gallen Kamp       | Korea   |
| 2. | Sensitive Balance                          | Sartorius         | Germany |
| 3. | Water Bath                                 | Guohua            | Germany |
| 4. | Ph meter                                   | CRISON-Dasic20    | Germany |
| 5. | Flame Atomic Absorption Spectrophotometer  | AA7000 Shimadzu   | Japan   |
| 6. | Sieves                                     | EUROLAB           | Germany |
| 7. | UV-Visible Spectrophotometer (UVmini-1240) | Shimadzu          | Japan   |
| 8. | Flame photometer                           | Genway            | Uk      |

**Table (3.2) Materials of Present Study**

| NO | Chemical Materials                             |
|----|------------------------------------------------|
| 1. | Nitric acid (HNO <sub>3</sub> )                |
| 2. | Hydrochloric acid (Hcl)                        |
| 3. | Perchloric acid(HClO <sub>4</sub> )            |
| 4. | Glycine (NH <sub>2</sub> CH <sub>2</sub> COOH) |
| 5. | Distill Water                                  |
| 6. | Filter paper 0.45µm                            |

### 3.2. Study Area

Al-Diwaniyah Governorate is among the most significant cities in Iraq where rice is cultivated since it has the right land to grow rice and it has plenty of water that promotes the process of germination. It is the most important exporter of rice to Iraq (Mohamed& Salman,2023). It has also firms that produce rubber and textiles, and with the construction of many oil stations in Iraq, it is becoming a major petroleum storage city (Kuruüzüm,2022).

The population was first estimated to be one million in the census of 2014, and the capital of the population is Diwaniyah. It is a governorate in the Euphrates region of Iraq, where a tributary of the Euphrates River, the Shatt al-Hillah, flows, entering Diwaniyah at the Shatt al-Diwaniyah. It is surrounded by Babylon Governorate to the north, Najaf Governorate to the east, Wasit and Dhi Qar Governorates to the southeast and Muthanna Governorate to the south. The samples were collected in the following location in Diwaniyah Governorate: Mahanawiyah (L1) (32°02'47.9"N 44°33'59.5"E) , Salahiyah (L2) (31.972823 N, 44.590764E), Shamiyah (L3) (31.930747, 44.603368) , and Ghamas (L4) (31.725186, 44.596523) Figure (3.1).

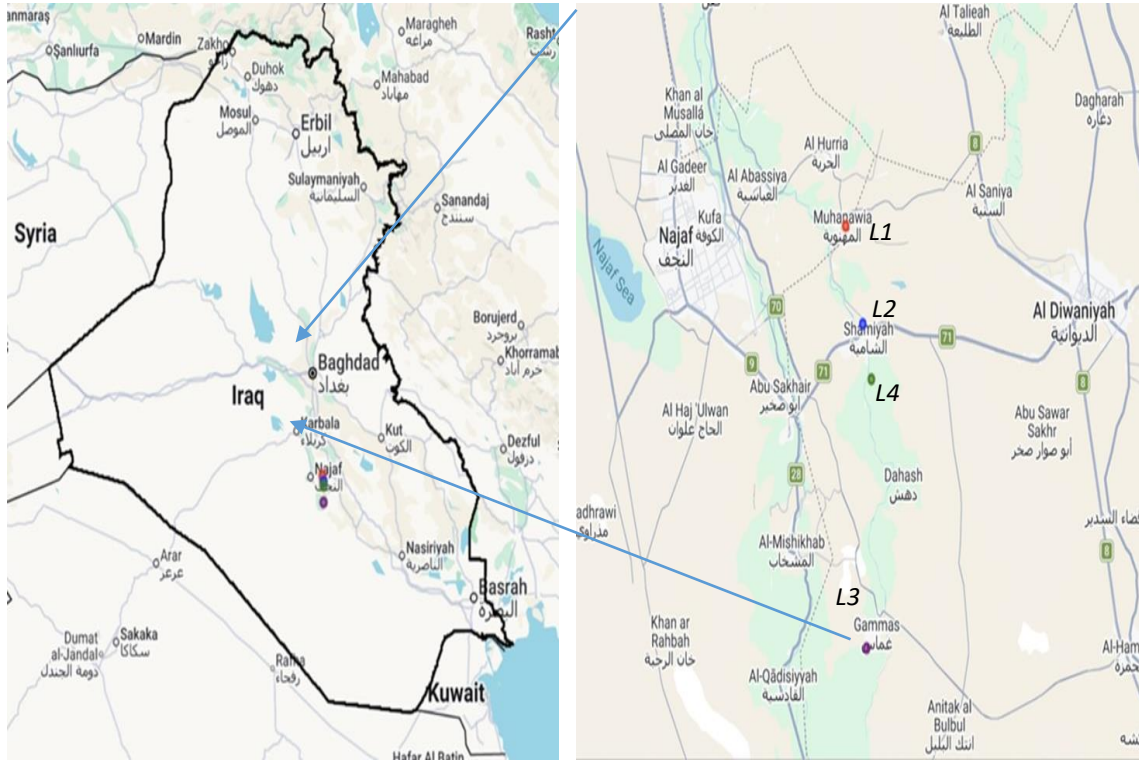


**Figure (3.1) Iraq map and samples locations in Al-Qadisiyah Governorate (Google Earth Engine)**

### 3.3. Rice Samples Collection

A total of forty rice samples ( $n = 40$ ) were systematically collected from four agricultural regions within Al-Diwaniyah Governorate, Iraq, during the harvest season of October and November 2025. From each site, representative grains were obtained to ensure coverage of spatial variability in cultivation practices and environmental conditions. The collected samples were immediately placed in pre-cleaned polyethylene bags, carefully labeled to indicate site and date of collection, and stored in refrigerated containers to preserve their integrity during transport to the laboratory.

Upon arrival, the rice grains were processed to remove husks using a specialized de-husking machine, ensuring uniform preparation across all samples. The de-husked rice was then subjected to standardized laboratory protocols for drying, milling, and homogenization, thereby minimizing variability and ensuring reproducibility of subsequent analytical procedures.

This rigorous sampling and preparation strategy was designed to provide reliable data on both total concentrations and bioaccessible fractions of heavy metals in rice cultivated across the study area.

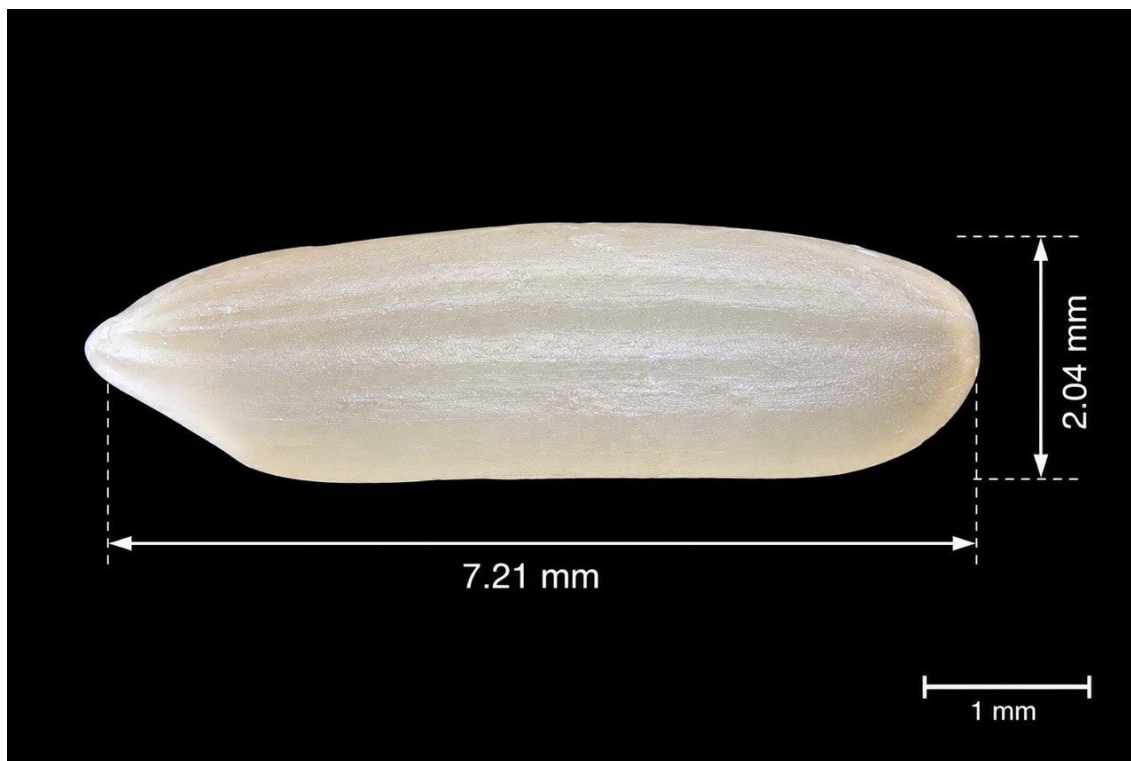


Figure (3.2) Raw Amber Rice Grains Used in the Study

### 3.4. Digestion and Analysis

#### 3.4.1. Rice Cooking Methods

The rice samples were washed with distilled water three times, and then boiled in 400 mL of distilled water. Then, when the process of cooking rice was finished, the rice was washed with a lot of water to remove the excess (Sharaf et al., 2019). To analyze the hazardous metals, 2 g of the cooked rice samples above were cooked using the ASTM method (ASTM, 1999).

#### 3.4.2. Sample Digestion and Preparation for Analysis

Sample preparation is a significant step in spectroscopic analysis to achieve reliable and accurate results (Pawliszyn, 2012). Wet digestion methods are basically used for the dissolution of rice samples prior to

elemental analysis (Pohl et al.,2024). Acid digestion has a substantial effect on the recovery of various analyte contents in highly complex matrices (Williams et al.,2023). For this purpose, concentrated nitric acid ( $\text{HNO}_3$ ) and perchloric acid ( $\text{HClO}_4$ ) were utilized as oxidant reagents to ensure complete matrix destruction and minimize interference (Haghaninia et al.,2025).

The samples were dried under room temperature; the rice of each region was milled individually. The samples had been dried at room temperature; the rice of each region had been dried separately. Then 2g milled rice was digested in 10ml of an acid mixture ( $\text{HNO}_3\text{:HClO}_4$ ) in 4:1 proportion. The mixture was then subjected to a heating device at a low temperature of 40 C and then 3 hours at a low temperature of 140 C (Shi et al.,2020). The digested samples were allowed to cool and after that diluted to 40 ml with double-distilled water (DDW) and filtered using Whatman No. 1 filter paper in pre-cleaned volumetric flasks. Trace metal content of the materials was determined using Atomic Absorption Spectrophotometer (SHIMADZU AA-7000).The systematic steps for the acid digestion process of rice samples are illustrated in Figure(3.3). (Lawi et al.,2023).

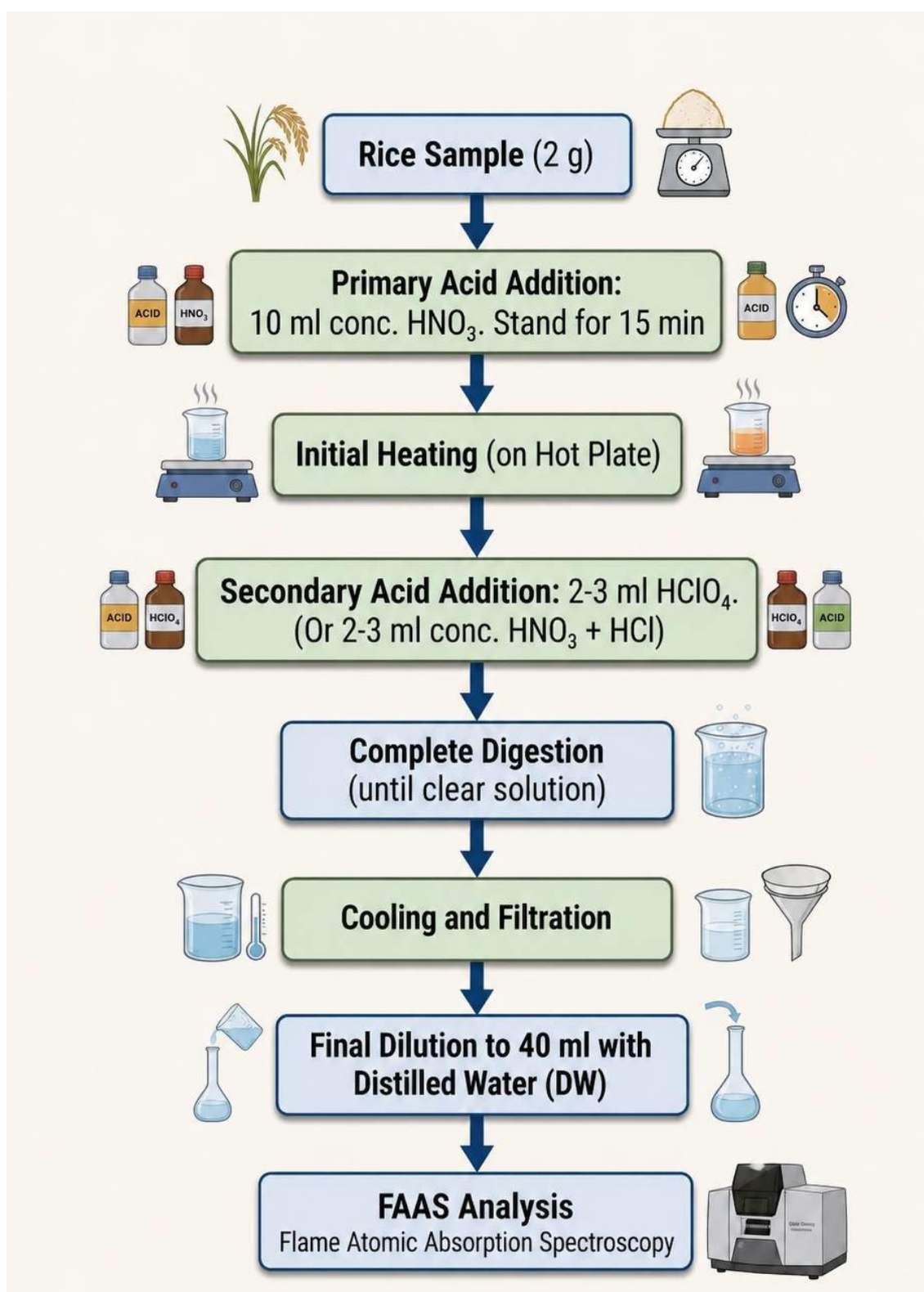


Figure (3.3) Flow Chart of Heavy Metal Acid Digestion of Rice Samples

### 3.4.3. Determination of Heavy Metals Using AAS

The determination of heavy metals (Zn, Pb, Cr, Cd, and Hg) was performed using Atomic Absorption Spectroscopy (Shimadzu AA-7000) (Figure 3.4) (Hossain et al., 2022). The working principle of the device is based on measuring the absorption of optical radiation by free atoms in the gaseous state at a wavelength specific to each element (Cowan, 2023). Light emitted from a Hollow Cathode Lamp (HCL) passes through the atomized sample, where the ground-state atoms absorb energy proportional to their concentration, following the Beer-Lambert law:

$$A = \log \left( \frac{I_0}{I} \right) = \varepsilon \cdot b \cdot c$$

where:

A is the absorbance

$I_0$  is the incident light intensity

I is the transmitted light intensity

$\varepsilon$  is the molar absorptivity

b is the path length

c is the analyte concentration

The analytical procedure involved operating and stabilizing the system, installing the Hollow Cathode Lamp, and setting the specific wavelength for each element (Schmidt et al., 2022). Standard solutions (0.5, 1, 2, and 5 ppm) were prepared and measured to establish a linear calibration curve by

plotting absorbance (A) versus concentration (c). The concentration of heavy metals in the digested sample solutions was determined by interpolating their measured absorbance values into the calibration equation ( $A = m \cdot c + b$ ) obtained from the calibration curve (Xiao et al., 2020). Blank samples and standard reference solutions were analyzed periodically to ensure the accuracy and precision of the analytical procedure (Ng et al., 2020). The detection limits of the instrument were considered according to the manufacturer's specifications (Saadati et al., 2013).



**Figure (3.4) Flame Atomic Absorption Spectrophotometer (Shimadzu AA-7000) used in this Study**

#### **3.4.4. Quality Assurance and Quality Control (QA/QC)**

Strict QA/QC measures were implemented throughout the study to ensure the reliability and accuracy of the analytical results (Kirwan et al.,2022). All glassware and equipment were cleaned with 2% HNO<sub>3</sub> and rinsed with deionized water prior to use to avoid contamination (Brown et al.,2019).

Procedural blanks were prepared and analyzed alongside the samples, and the same procedure without samples was used as a control (Munno et al.,2023).Three replications were conducted for each sample (Foroud et al.,2000).The instrument was calibrated daily, and the calibration curves for the analyzed metals showed correlation coefficients ( $r^2$ ) greater than 0.99, indicating good linearity and reliability of the analytical measurements (Krzebietke et al.,2023).

### **3.5. Heavy Metals Bioaccessibility**

#### **3.5.1. In Vitro Gastrointestinal Digestion for Bioaccessibility Assessment**

Bioaccessibility is used to describe the proportion of the ingested contaminants in food that are released from the matrix into digestive juice in the gastrointestinal tract (Shahidi & Pan,2022 ). With in vitro digestion models, the digestion process in the human gastrointestinal tract is simulated in a simplified manner by applying physiologically based conditions (Dixit et al.,2024).

In this study, an in vitro model simulating human digestion was carried out according to the procedure described by Hur et al.,2011). The bioaccessibility of heavy metals in rice was determined using the SBET which is a one step in vitro digestion assay, which does not involve pepsin (Aluko,2012).

### 3.5.2. SBET (Simple Bioaccessibility Extraction Test)

The systematic procedure for the SBET technique is illustrated in the flow chart below Fig.3. The procedure of this technique was to weigh 2 g of dry rice and put it in a centrifuge tube and mix it with 50 ml of gastric solution (0.4 M glycine; pH 1.5 adjusted with concentrated HCl). The tube was moved end over end at 30 rpm during 1 hour at 37 °C, centrifuged at 3500 g/15 minutes and the suspension so obtained filtered through the 0.45 µm cellulose acetate disk filter. The material was then subjected to pH test followed by the determination of the material by atomic absorption spectrophotometer. (SHIMADZU AA-7000).

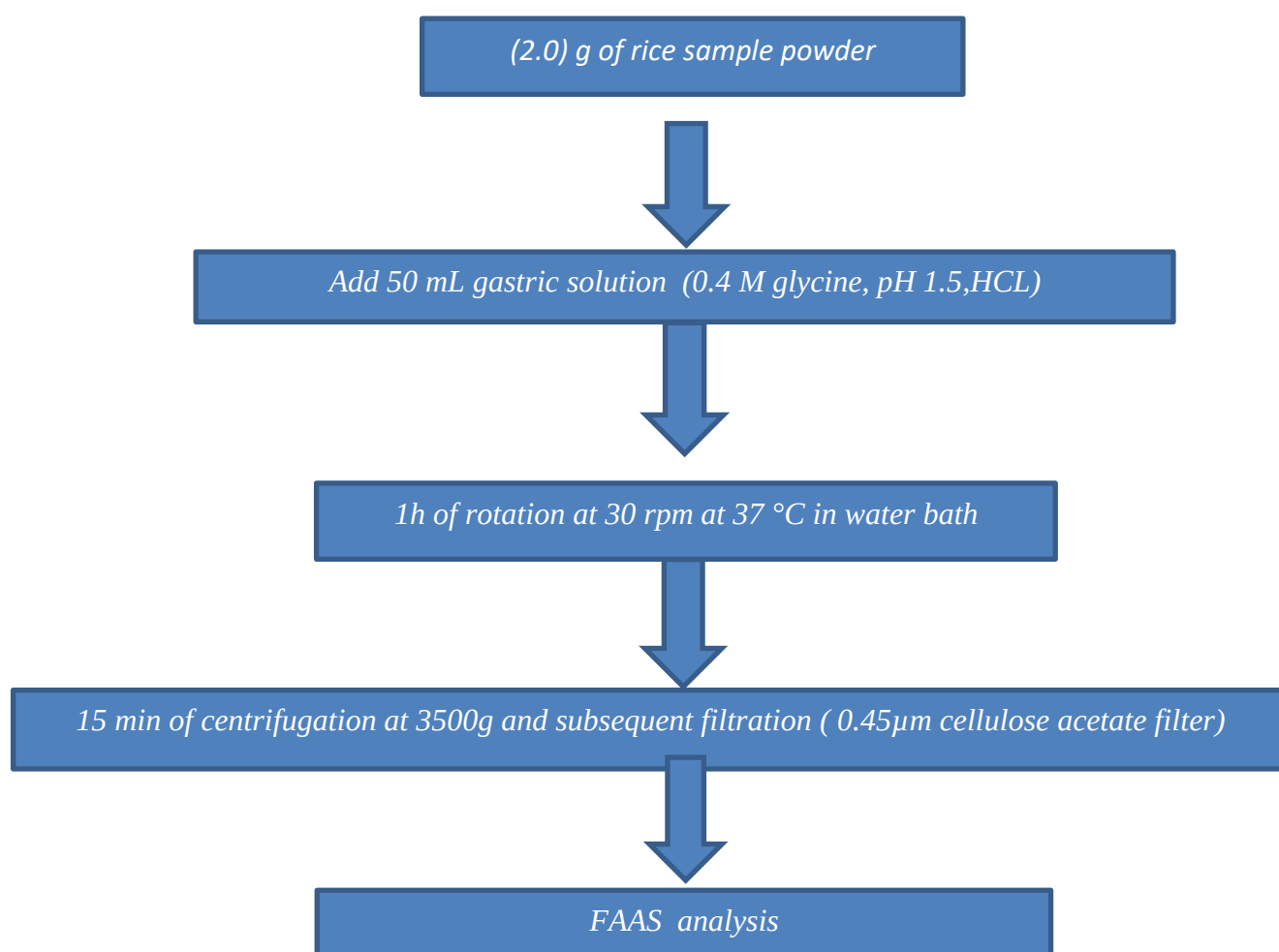


**Figure (3.5) Flow chart of Simple Bioaccessibility Extraction Test (SBET) for rice samples.**

The relative bioaccessibility of heavy metals in rice was determined with the help of the following equation:

$$\text{Metals bioaccessibility of rice (\%)} = \frac{SBET (rice)}{Total\ metals (rice)} \times 100$$

(Alpofead et al., 2023).

### 3.6. Health Risk Assessment

Risk assessment is defined as the methods of evaluating the probability of occurrence of harmful health impacts over a determined time period (Rausand, 2013). Human activities have progressively increased in recent years, leading to environmental deterioration and the accumulation of toxic elements in agricultural systems (Alengebawy et al., 2021).

Recently, concern has been raised about possible contamination of rice by heavy metals due to their persistence and toxicity (Zakaria et al., 2021). Therefore, evaluating dietary intake is considered a critical step in determining the exposure level to toxic elements through food consumption (Sanaei et al., 2021).

#### 3.6.1. Estimated Daily Intake (EDI)

The Estimated Daily Intake (EDI) of the heavy metals that were consumed by rice was determined based on the rules outlined by the USEPA (1989) by using the equation below:

$$EDI = \frac{C \times IR \times EF \times ED}{BW \times AT} \quad (1)$$

Where C is the concentration of metal in rice (mg/kg), IR is ingestion rate, EF is the exposure frequency, ED is the exposure duration, BW is the body weight and AT is the averaging time.

Average body weight (BW)=68.4 kg for adults and 15 kg for children, Ingestion rate of rice (IR)=0.110 kg/day for adults and 0.033 kg/day for children, Exposure frequency (EF)= 350 day/year for adults and children, Exposure duration (ED)= 30 years for adults and 6 years for

children (Amarloei et al., 2025), Average time (AT) in day for non-carcinogens:  $ED \times 365$  (USEPA, 1989).

### 3.6.2. Target Hazard Quotient (THQ)

The risk of THQ is not carcinogenic and the EDI/oral reference dose (RfD) of a certain metal (Kormoker et al., 2021):

$$THQ = \frac{EDI}{RfD} \quad (2)$$

The RfD values used were 0.001, 0.3, 0.0035, and 1.5 mg/kg/day for Cd, Zn, Pb, and Cr, respectively (US EPA, 2023; Shaheen et al., 2024). A THQ greater than 1 means that there is a possibility of negative health outcomes.

### 3.6.3. Hazard Index (HI)

The cumulative non-carcinogenic risk of consumption of rice, which contains several metals, was calculated as the Hazard Index (HI), as the sum of the separate THQs (Zakir et al., 2021):

$$HI = \sum_{i=1}^n THQ_i \quad (3)$$

$HI > 1$  is a serious cumulative health hazard to the exposed populace.

### 3.6.4. Cancer Risk (CR)

Potential cancer risks associated with exposure to heavy metals through rice consumption were estimated using the Incremental Lifetime Cancer Risk (ILCR) (Eslamizad & Alehashem, 2025). ILCR represents the probability of an individual developing cancer over a lifetime due to continuous exposure to carcinogenic contaminants (Cani et al., 2023). According to the United States Environmental Protection Agency (US EPA), the acceptable cancer risk range is between  $1 \times 10^{-6}$  and  $1 \times 10^{-4}$

$$CR = CDI \times CSF \quad (4)$$

where CR is the Cancer Risk, and CSF is the Cancer Slope Factor. In this study, the CDI ( $\text{mg/kg/day}$ ) was assumed to be equal to the Estimated Daily Intake (EDI). The CSF values used for the health risk

calculations were 0.38, 0.50, and 0.0085 mg/kg/day for Cd, Cr, Pb ,Zn and Hg respectively (Aendo et al., 2022).

### 3.6.5. Total Cancer Risk(TCR)

Total Cancer Risk (TCR) was calculated as the sum of the individual cancer risks of carcinogenic heavy metals to estimate the overall lifetime carcinogenic risk associated with rice consumption (Rezaei et al.,2021).

$$TCR = \sum CR_i \quad (5)$$

## 3.7. Statistical analysis

Three multivariate statistical methods were used to assess the relationships, sources, and patterns of distribution of heavy metals (Zn, Pb, Cr, Cd, and P) in rice with the help of R Statistical Software. Statistical significance was considered at  $p < 0.05$ .

### 3.7.1. Correlation Analysis

Pearson Correlation Coefficient ( $r$ ) was used to establish inter-metal relationships. This analysis determines the strength and direction of the linear relationship between variables indicating whether the metals have a common origin or geochemical behavior or not. The corrplot package was used to visualize the correlation matrix (Liao & Wang,2023). The intensity of colors and the size of the circle reflect how much the correlation is strong and blue color signifies a positive correlation whereas red color signifies the negative correlation.

### 3.7.2. Principal Component Analysis (PCA)

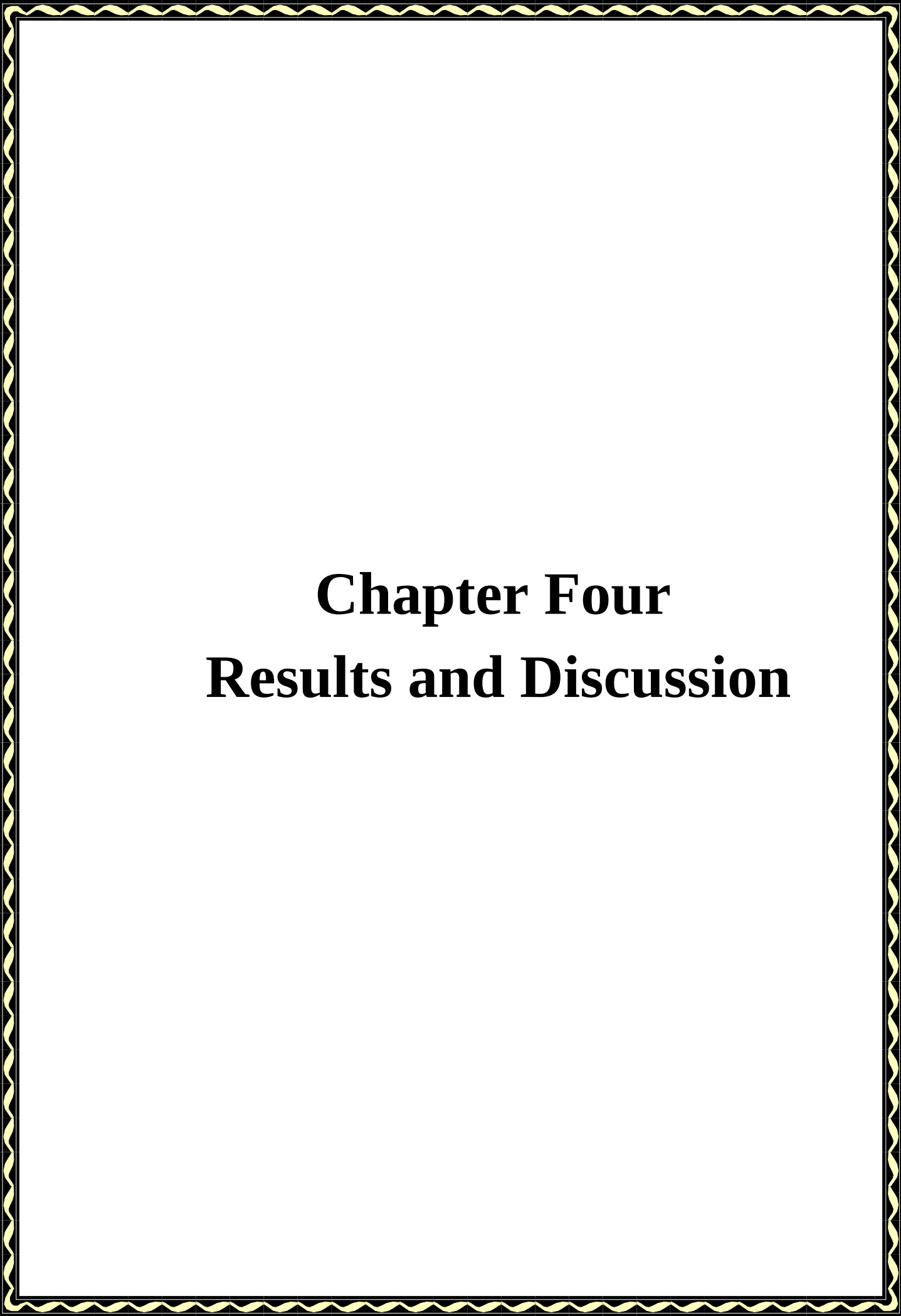
Principal Component Analysis (PCA) was conducted to reduce the data dimensionality and determine the latent variables that control the distribution of the metals. The data were brought to unit variance to make sure that all the metals had equal contributions to the model. PCA was performed with the FactoMineR package (Lê et al., 2008) and the findings were plotted as a biplot with factoextra (Kassambara and Mundt, 2020).

### 3.7.3. Cluster Analysis (HCA)

The Hierarchical Cluster Analysis (HCA) was performed to cluster metals and samples according to their similarity. Agglomerative method was employed where Euclidean distance was taken as the measure of similarity and the minimum variance technique of Ward was employed to reduce the similarity within a cluster. They are shown in the form of a dual-dendrogram heatmap, created with the pheatmap (Kolde, 2019) or gplots package, which illustrates the clustering of variables (top) and samples (left) and a color-coded scale of standardized concentrations (z-scores).



**Figure (3.6) Laboratory Work and Experimental Setup during Sample Analysis**



# **Chapter Four**

## **Results and Discussion**

## 4. Results and Discussion

### 4.1. Heavy Metals in Rice

The concentrations of the investigated elements varied considerably among the four agricultural regions of Al-Qadisiyah Governorate. This spatial variation may be associated with differences in soil properties, irrigation-water quality, fertilizer application, and other anthropogenic activities. To provide a clearer interpretation of the findings, the concentrations of each element are discussed separately, considering the total concentration in rice, the highest and lowest recorded concentrations, possible sources of variation, comparison with previous studies, and changes following cooking and gastrointestinal digestion.

Cadmium (Cd) showed considerable spatial variation among the investigated locations. The highest total Cd concentration was recorded in Shamiyah (0.30 mg/kg), whereas the lowest concentration was observed in Salahiyah (0.05 mg/kg). The relatively high Cd concentration in Shamiyah may be associated with human activities and differences in the physical and chemical properties of the agricultural soil. In particular, agricultural practices and fertilizer application may contribute to Cd accumulation in agricultural soils and subsequently increase its uptake by rice plants.

The presence of Cd in agricultural soils is of particular concern because Cd contamination can adversely affect both crop productivity and food quality. Cadmium stress can alter panicle and spikelet fertility, consequently reducing rice yield (Kanu et al., 2017). In addition, heavy-metal contamination may affect the quality and palatability of rice grains, which can negatively influence the economic value of agricultural crops (Xia et al., 2024).

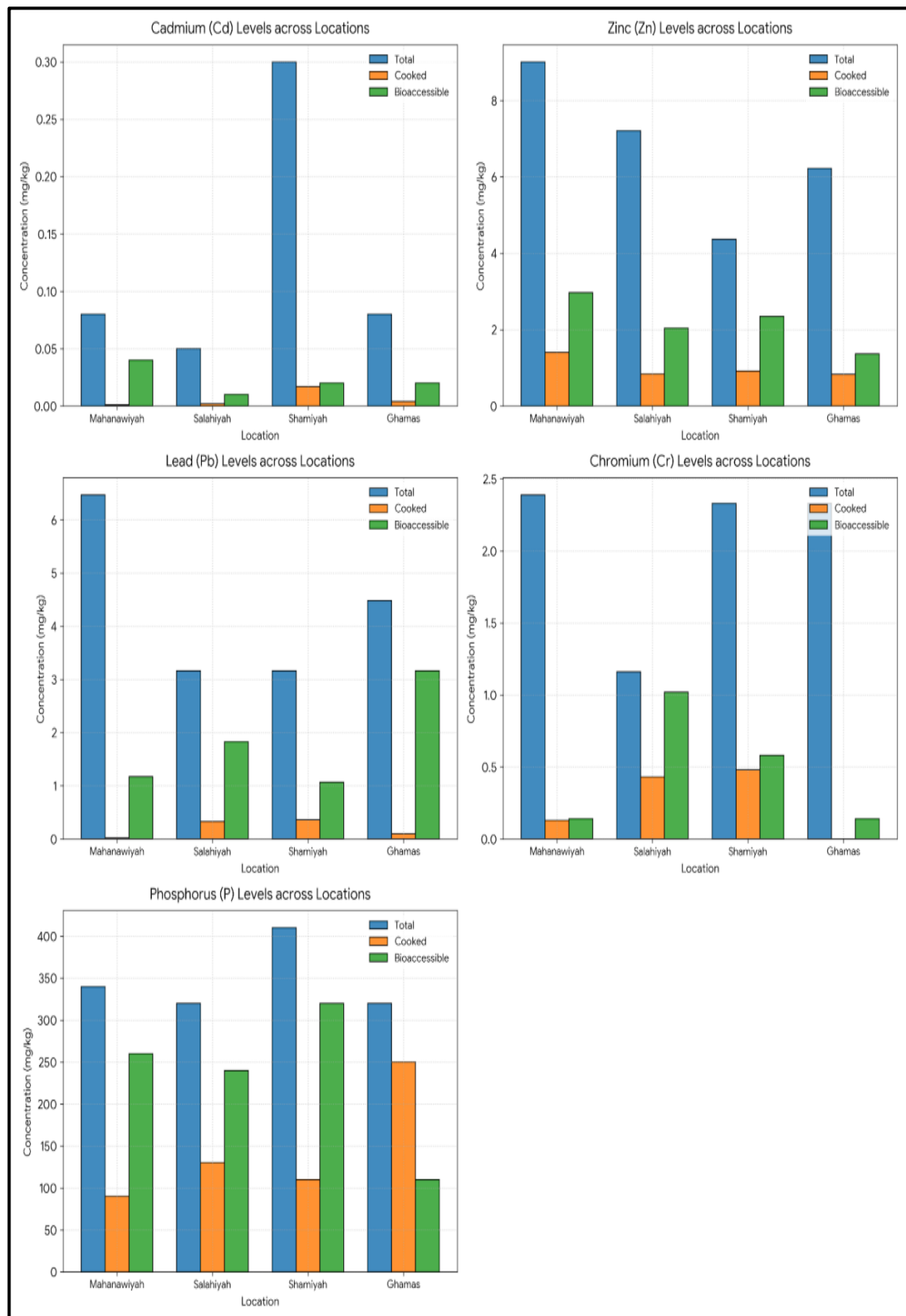


**Figure (4.1) Total, Bioaccessibility and Cook of Heavy Metals and Permissible Limits of Trace Metals in Rice of Al-Qadisiyah Governorate FAO/WHO standard (Cd=0.2; Pb=0.2; Zn=20) (Wasim et al., 2019; Zeeshan et al., 2023)**

The Cd concentration detected in Shamiyah was compared with the permissible value used in this study, as presented in Figure (4.1). The comparison indicated that Cd represented an important contaminant requiring consideration in the investigated rice samples. Similar observations have been reported in previous studies. Dehghani and Mosaferi (2016) reported high concentrations of Cd and Pb in imported Pakistani rice grains available in Iranian markets. Similarly, Tariq et al. (2013) reported the presence of Cd, Cr, Ni, Fe, and Pb in rice-growing areas of Shakargarh Tehsil, District Sialkot, Pakistan. Elevated concentrations of heavy metals in rice have also been reported in Sari, northern Iran (Zafarzadeh and Shahryari, 2025).

Cooking substantially reduced Cd concentrations in the rice samples. The concentration of Cd in cooked rice ranged from 0.001 to 0.017 mg/kg, with the highest residual concentration recorded in Shamiyah and the lowest in Salahiyah. The marked decrease following cooking may be related to the dissolution and leaching of Cd into the cooking water. Nevertheless, the detection of residual Cd after cooking indicates that some Cd remained associated with the rice matrix, suggesting that contamination from agricultural soil or irrigation water may contribute to its presence.

The bioaccessible Cd concentration was relatively low compared with the other investigated elements. The highest bioaccessible Cd concentration was recorded in Mahanawiyah (0.04 mg/kg). The relatively low bioaccessibility may be associated with the binding of Cd to components of the rice matrix, which can limit its release during gastrointestinal digestion. Therefore, although cooking considerably reduced Cd concentrations, the remaining bioaccessible fraction should still be considered when evaluating dietary exposure.

Lead (Pb) showed substantial variation among the investigated regions. The highest total Pb concentration was recorded in Mahanawiyah

(6.47 mg/kg), whereas the lowest concentrations were observed in Salahiyah and Shamiyah at approximately 3.15 mg/kg. The relatively high Pb concentration in Mahanawiyah may reflect differences in environmental contamination and agricultural conditions, particularly the quality of irrigation water, soil contamination, and surrounding anthropogenic activities.

Lead contamination in rice-growing areas is of particular concern because irrigation water can represent an important pathway for the transfer of Pb into agricultural soils and subsequently into rice plants. In Iraq, the Tigris and Euphrates rivers represent major sources of irrigation water, and the release of untreated wastewater into these river systems may contribute to heavy-metal contamination. In addition, oil refineries, chemical plants, and tanneries may discharge heavy metals into surrounding water systems. Pb accumulation in paddy soils has also been identified as an important consequence of irrigation-water contamination in studies conducted along the Al-Diwaniyah River (Jarallah and Husseini, 2021).

The Pb concentrations detected in all investigated locations exceeded the permissible value of 0.2 mg/kg used in this study, indicating a potential food-safety concern and the need for routine monitoring. Although the Pb concentration detected in the present rice samples was lower than the  $8.03 \pm 1.07$  mg/kg reported by Payus and Talip (2014) in paddy soil in Malaysia, the concentrations observed in the present study remain considerably higher than the permissible level used for comparison.

Cooking substantially decreased Pb concentrations. The concentration of Pb in cooked rice ranged from 0.02 to 0.36 mg/kg, with relatively high residual concentrations observed in Ghamas and Mahanawiyah. The reduction following cooking indicates that a considerable proportion of Pb can be removed during boiling. However, some Pb remained detectable after cooking, which may be explained by the

strong binding of Pb to organic components and structural constituents of rice grains, making complete removal during household processing difficult. This residual Pb is important because Pb is a highly toxic, non-essential metal that can adversely affect the nervous system and other organs.

The highest bioaccessible Pb concentration was observed in Ghamas (3.16 mg/kg). This finding is particularly important because the bioaccessible fraction represents the proportion of the element that can potentially be released during gastrointestinal digestion. Thus, despite the considerable reduction achieved by cooking, the relatively high bioaccessible Pb concentration in Ghamas indicates that dietary exposure may remain a concern.

Zinc (Zn) concentrations also varied among the investigated locations. The highest total Zn concentration was recorded in Mahanawiyah (9.01 mg/kg), while the lowest concentration was observed in Shamiyah (approximately 4.4 mg/kg). The relatively high concentration in Mahanawiyah may be associated with differences in soil characteristics, irrigation-water quality, fertilizer application, and other environmental inputs.

Unlike Cd and Pb, Zn is an essential trace element required for normal physiological functions. However, excessive Zn exposure may have adverse effects; therefore, its concentration in food should also be monitored. The Zn concentrations observed in the present study were lower than those reported by Sarkar et al. (2023), who recorded concentrations of 12 mg/kg in Narsingdi and 10 mg/kg in Jhenaidah. This indicates that the Zn concentrations in the investigated rice samples were comparatively lower than those reported in these previously studied regions.

According to the permissible value presented in Figure (4.1), the highest Zn concentration observed in the present study remained below the

20 mg/kg reference value used for comparison. Therefore, although Zn was detected in all investigated rice samples, its total concentration did not indicate an exceedance of the specified reference level.

Cooking reduced the Zn concentration substantially. The concentration of Zn in cooked rice ranged from approximately 0.83 to 1.40 mg/kg. This reduction may be attributed to the dissolution of minerals into the cooking water and their diffusion from the rice surface during boiling (Herath et al., 2020). Because Zn is an essential nutrient, its reduction during cooking may also represent a loss of nutritionally beneficial minerals. Nevertheless, detectable Zn remained in cooked rice after processing.

The highest bioaccessible Zn concentration was recorded in Mahanawiyah (2.97 mg/kg). The relatively high bioaccessible concentration indicates that a considerable proportion of the remaining Zn could potentially be released during gastrointestinal digestion. Because Zn is an essential micronutrient, this finding should be interpreted in relation to both its nutritional importance and the potential adverse effects of excessive exposure.

Chromium (Cr) concentrations were relatively consistent among several of the investigated regions. The highest total Cr concentrations were recorded in Mahanawiyah and Shamiyah, at approximately 2.4 and 2.3 mg/kg, respectively, while the lowest concentration was observed in Salahiyah (approximately 1.2 mg/kg). Ghamas also showed a relatively high total Cr concentration of approximately 2.3 mg/kg.

The spatial distribution of Cr may be influenced by differences in soil characteristics and environmental conditions. Factors such as soil pH, organic matter content, redox conditions, and mineral composition can influence the mobility, chemical behavior, and uptake of Cr by rice plants. Therefore, the relatively similar Cr concentrations observed in

Mahanawiyah, Shamiyah, and Ghamas may reflect comparable environmental conditions affecting Cr availability.

Cooking considerably reduced Cr concentrations in the rice samples. The concentration of Cr in cooked rice ranged from non-detectable to approximately 0.41 mg/kg, with the highest residual concentration observed in Salahiyah. The reduction in Cr following cooking may be associated with leaching into the cooking water during boiling. Razzak et al. (2023) similarly reported that cooking can reduce Cr concentrations in rice. In addition, changes in the chemical associations of Cr during cooking may contribute to its reduction (Liu et al., 2025). However, the presence of residual Cr after cooking indicates that some Cr fractions may remain relatively strongly associated with the rice matrix (Xia et al., 2022).

The highest bioaccessible Cr concentration was observed in Salahiyah (1.02 mg/kg). This finding demonstrates that total concentration and bioaccessibility do not necessarily show the same spatial pattern. The gastrointestinal release of Cr may depend on its chemical form and its interactions with components of the rice matrix.

Mercury (Hg) was not detected in rice samples from any of the investigated locations. The absence of detectable Hg suggests that Hg contamination was relatively low in the investigated agricultural regions and that rice from these areas may represent a relatively low contribution to dietary Hg exposure. However, continued monitoring is important because Hg contamination can vary according to environmental conditions and contamination sources.

The non-detection of Hg also contrasts with the measurable concentrations of Cd, Pb, Zn, and Cr observed in the investigated rice samples, suggesting that the sources and environmental behavior of Hg may differ from those of the other elements.

Phosphorus (P) showed the highest total concentration in Shamiyah (410 mg/kg), whereas the lowest concentrations were approximately 320 mg/kg in Salahiyah and Ghamas. Unlike Cd, Pb, Zn, Cr, and Hg, phosphorus is an essential macronutrient naturally present in rice and should therefore be interpreted primarily as a nutritional element rather than solely as a contaminant.

The relatively high P concentration observed in Shamiyah may be associated with agricultural fertilizer application and differences in soil nutrient status. This interpretation is supported by the strong positive relationship between Cd and P observed in the present study, which may indicate a common agricultural source. Phosphate fertilizers may contribute to both P enrichment and Cd accumulation because phosphate fertilizers can contain Cd impurities.

Cooking reduced P concentrations, although the reduction was lower than that observed for most of the investigated heavy metals. The cooked P concentrations ranged from approximately 90 to 250 mg/kg, with the highest cooked concentration observed in Ghamas. The relatively greater retention of P after cooking may be related to its association with the rice matrix. Because phosphorus is an essential nutrient, the retention of a proportion of P following cooking may have nutritional importance.

The highest bioaccessible P concentration was observed in Shamiyah (320 mg/kg). The relatively high bioaccessibility of P indicates that a substantial proportion remained available following gastrointestinal digestion. This finding is nutritionally relevant because phosphorus is an essential mineral involved in several physiological functions.

Overall, the results demonstrated clear spatial variation in the concentrations of the investigated elements across the four agricultural regions of Al-Qadisiyah Governorate. Shamiyah recorded the highest total concentrations of Cd (0.30 mg/kg) and P (410 mg/kg), whereas

Mahanawiyah recorded the highest concentrations of Zn (9.01 mg/kg) and Pb (6.47 mg/kg). Chromium showed relatively similar concentrations among Mahanawiyah, Shamiyah, and Ghamas, while mercury was not detected in any of the investigated samples.

The differences among locations may be related to variation in soil properties, irrigation-water quality, fertilizer application, and surrounding anthropogenic activities. The findings are particularly important for Pb, because its concentrations exceeded the permissible value used in this study in all investigated locations. In contrast, the Zn concentrations remained below the reference value presented in Figure (4.1).

Cooking substantially reduced the concentrations of the investigated elements, although the extent of reduction differed according to the chemical properties of each element and its interaction with the rice matrix. The persistence of residual Pb and Cr after cooking indicates that cooking cannot completely eliminate heavy-metal exposure. Furthermore, the bioaccessible results demonstrate that the potential dietary exposure cannot be assessed solely from total concentrations or cooking-related reductions. In particular, the relatively high bioaccessible Pb concentration observed in Ghamas highlights the importance of considering gastrointestinal bioaccessibility when evaluating the potential health implications of heavy-metal contamination in rice.

## **4.2. Reduction after Cooking and RB of Metals**

The effect of cooking on the concentrations of the investigated elements is presented in Figure (4.2) and summarized in Table (4.1). The percentage reduction varied among the analyzed elements, indicating that the effectiveness of cooking in reducing elemental concentrations depends on their chemical properties, solubility, mobility, and interactions with the rice matrix (Shahriar et al., 2022; Aguilera-Velazquez et al., 2023). Several studies have emphasized the importance of rice preparation before

consumption, including washing, soaking, and cooking, as practical approaches for reducing contaminants and improving food safety (Al-Saleh et al., 2017; AlMulla et al., 2022; El-Sharnouby and Asiri, 2024).

**Table (4.1). Average reduction in element concentrations after cooking**

| Element | Average reduction (%) |
|---------|-----------------------|
| Cd      | 96.02                 |
| Pb      | 93.91                 |
| Zn      | 84.60                 |
| Cr      | 84.22                 |
| P       | 56.99                 |

Cadmium showed the highest average reduction after cooking, reaching 96.02%. This marked reduction suggests that Cd has a relatively high potential for leaching into the cooking water because of its mobility and solubility. Therefore, household cooking may substantially reduce dietary exposure to Cd. The reduction observed in the present study was considerably higher than that reported by Zhuang et al. (2016), who documented a 10% reduction in Cd when rice was cooked using a rice-to-water ratio of 1:2. Similarly, Shahriar et al. (2022) reported a 35% reduction in Cd when rice was washed five times and subsequently cooked using a rice-to-water ratio of 1:6. In contrast, Khan et al. (2010) reported no decrease in Cd concentration after cooking with an excessive amount of water. These differences suggest that the effectiveness of cooking in reducing Cd may depend on the cooking conditions, particularly the rice-to-water ratio and washing procedure, in addition to the chemical properties of Cd.

Lead showed the second-highest average reduction after cooking, at 93.91%. The substantial decrease indicates that boiling removed a considerable proportion of Pb from the rice grains. However, the presence of residual Pb after cooking suggests that some Pb may remain strongly associated with the rice matrix. Zakaria et al. (2021) reported that Pb can

interact strongly with organic components of the rice grain, which may contribute to its persistence following household processing. Therefore, although cooking substantially reduces Pb concentrations, it may not completely eliminate Pb from cooked rice.

The average reduction in Zn concentration following cooking was 84.60%. Although Zn is an essential trace element required for normal physiological functions, excessive exposure may be undesirable. Therefore, the reduction observed after cooking may decrease potential exposure to excessive Zn but may also result in the loss of a nutritionally important mineral.

The reduction in Zn may be attributed to the dissolution of minerals into the cooking water and their diffusion from the rice surface during cooking (Herath et al., 2020). The substantial reduction observed in the present study indicates that cooking can considerably alter the mineral composition of rice. However, because Zn is nutritionally important, excessive removal may also reduce its contribution to dietary mineral intake.

Chromium showed an average reduction of 84.22% following cooking. This finding indicates that cooking was also effective in reducing Cr concentrations in rice. Similar observations have been reported by Razzak et al. (2023), who found that cooking could reduce Cr levels in rice.

The reduction in Cr may be associated with partial leaching into the cooking water and changes in the chemical associations of Cr during cooking. Liu et al. (2025) suggested that thermal changes may contribute to the destabilization of chromium compounds associated with rice grains. However, the presence of residual Cr after cooking indicates that some Cr fractions may remain relatively strongly bound within the rice matrix (Xia et al., 2022).

Among all the investigated elements, phosphorus showed the lowest average reduction after cooking, at 56.99% (Huang et al., 2024). The relatively lower reduction compared with the heavy metals may reflect differences in the chemical form and binding of phosphorus within the rice matrix. Xu et al. (2023) suggested that the relatively strong association of phosphorus with components of the rice grain may contribute to its greater retention during cooking.

Unlike the toxic heavy metals, phosphorus is an essential macronutrient naturally present in rice. Therefore, the retention of a considerable proportion of phosphorus after cooking may have nutritional importance. Vici et al. (2021) emphasized the nutritional role of phosphorus, indicating that its retention during cooking may be beneficial from a nutritional perspective.

The average reduction percentages followed the order Cd (96.02%) > Pb (93.91%) > Zn (84.60%) > Cr (84.22%) > P (56.99%). The differences in reduction efficiency reflect the physicochemical properties of the elements and their interactions with rice tissues (Farooq and Yu, 2025). The particularly high reductions observed for Cd and Pb indicate that cooking can be an effective household practice for reducing exposure to potentially toxic metals. However, the persistence of residual concentrations after cooking demonstrates that cooking does not completely eliminate elemental contamination.

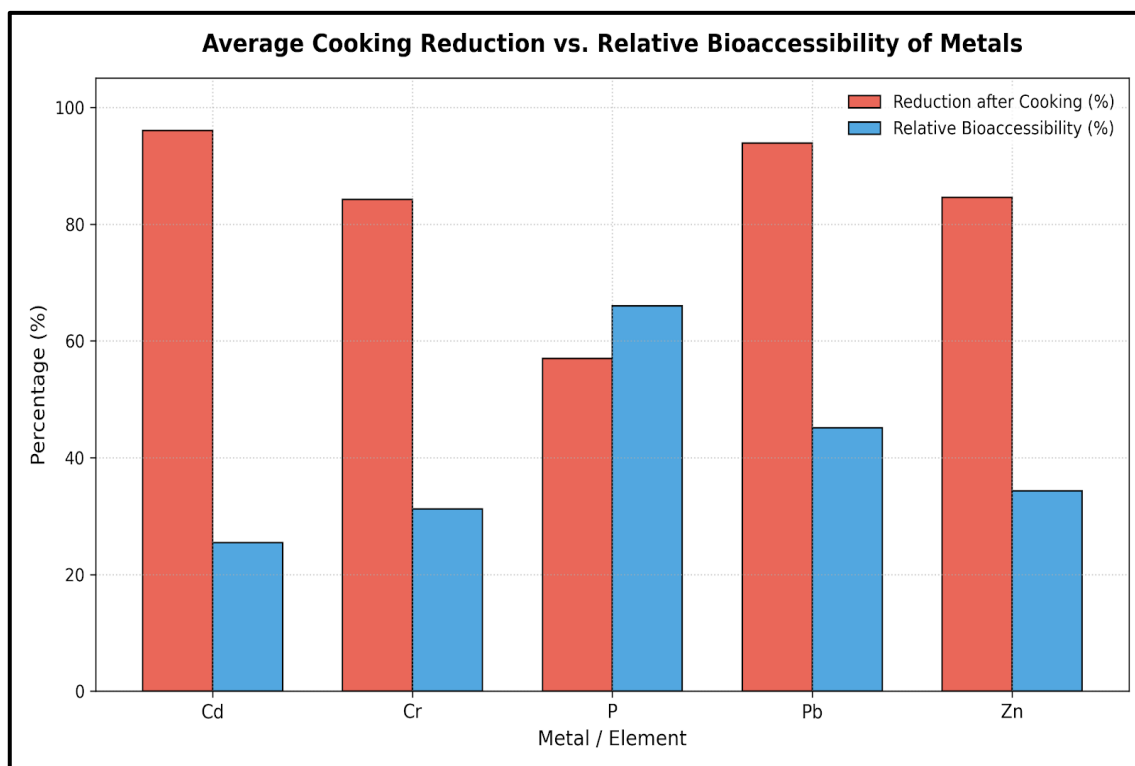


**Figure (4.2).** Average reduction after cooking and relative bioaccessibility of Cd, Cr, P, Pb, and Zn in rice samples from Al-Qadisiyah Governorate.

### 4.3. Relative Bioaccessibility (RB)

The relative bioaccessibility (RB) of the investigated elements varied considerably, indicating differences in their release and potential availability during gastrointestinal digestion. The average RB values are presented in Table (4.2).

**Table (4.2).** Average relative bioaccessibility of the investigated elements

| Element | Average relative bioaccessibility (%) |
|---------|---------------------------------------|
| Pb      | 45                                    |
| Zn      | 34                                    |
| Cr      | 31                                    |
| Cd      | 25                                    |
| P       | 65                                    |

Lead showed the highest relative bioaccessibility among the investigated heavy metals, with an average value of 45%. This finding is particularly important from a food-safety perspective because approximately 45% of the Pb remaining after cooking may potentially be released during gastrointestinal digestion. The relatively high RB of Pb indicates that the reduction achieved during cooking should not be considered equivalent to complete elimination of potential dietary exposure. Therefore, both the cooking reduction and the bioaccessible fraction should be considered when evaluating the potential health risks associated with Pb-contaminated rice.

The average relative bioaccessibility of Zn was 34%, indicating moderate gastrointestinal availability. This value was higher than that observed for Cd and Cr but lower than that observed for Pb. Because Zn is an essential trace element, its bioaccessibility may contribute to its nutritional availability following consumption. Nevertheless, the observed value also reflects the influence of cooking and gastrointestinal digestion on the release of Zn from the rice matrix.

The average relative bioaccessibility of Cr was 31%, indicating moderate gastrointestinal release. This finding suggests that a proportion of the residual Cr remaining after cooking could potentially be released during gastrointestinal digestion. The observed RB may be influenced by the chemical characteristics of Cr and its interactions with components of the rice matrix.

Cadmium showed the lowest relative bioaccessibility among the investigated heavy metals, at 25%. Although cooking resulted in a substantial reduction in Cd concentration, approximately one-quarter of the remaining Cd was potentially released during gastrointestinal digestion. The relatively low RB may be associated with stronger interactions between Cd and components of the rice matrix, which may restrict its

release during digestion. Nevertheless, the presence of a bioaccessible Cd fraction indicates that cooking does not completely eliminate the potential for dietary exposure.

Phosphorus showed the highest overall relative bioaccessibility, with an average value of 65%. This relatively high bioaccessibility is consistent with the physiological importance of phosphorus as an essential nutrient. Therefore, unlike the toxic heavy metals, the relatively high RB of phosphorus may be considered nutritionally favorable because it indicates that a considerable proportion of phosphorus remains potentially available following gastrointestinal digestion.

The relative bioaccessibility values followed the order P (65%) > Pb (45%) > Zn (34%) > Cr (31%) > Cd (25%). These differences demonstrate that total concentration alone may not adequately represent potential dietary exposure. Gastrointestinal digestion can modify the fraction of an element that becomes potentially available for absorption, depending on its chemical properties and interactions with the food matrix. Omar et al. (2013) emphasized the importance of considering digestion-related changes in metal availability to avoid overestimating potential human exposure.

Dietary components may also influence the bioaccessibility of metals. Dietary fiber, for example, may act as a chelating agent and reduce the bioavailability of metallic elements through inhibition of their release and intestinal reabsorption (Borowska et al., 2020; Kachenpukdee and Oonsivil, 2019; Aguilera-Velazquez et al., 2022). Therefore, the differences in RB observed among Cd, Pb, Zn, and Cr may reflect differences in their chemical behavior and interactions with the rice matrix during gastrointestinal digestion.

Overall, the findings demonstrate that cooking substantially reduced the concentrations of all investigated elements, with the greatest reduction observed for Cd (96.02%) and Pb (93.91%), whereas phosphorus showed

the lowest reduction (56.99%). However, the subsequent bioaccessibility assessment demonstrated that a proportion of the residual elements remained potentially available during gastrointestinal digestion, particularly Pb. Therefore, evaluation of dietary exposure to heavy metals in rice should consider both the reduction achieved through cooking and the relative bioaccessibility of the residual contaminants, rather than relying solely on concentrations measured in raw rice.

#### **4.4. Health Risk Assessment**

The Health Hazard Index (HI) values associated with rice consumption in the four investigated agricultural regions are presented in Figure (4.3). Three exposure scenarios were considered: (1) total metal concentrations in raw rice, (2) metal concentrations in cooked rice, and (3) the bioaccessible fraction following gastrointestinal digestion. The dashed horizontal line in Figure (4.3) represents the acceptable risk threshold of  $HI = 1$ , above which a potential non-carcinogenic health risk may be indicated.

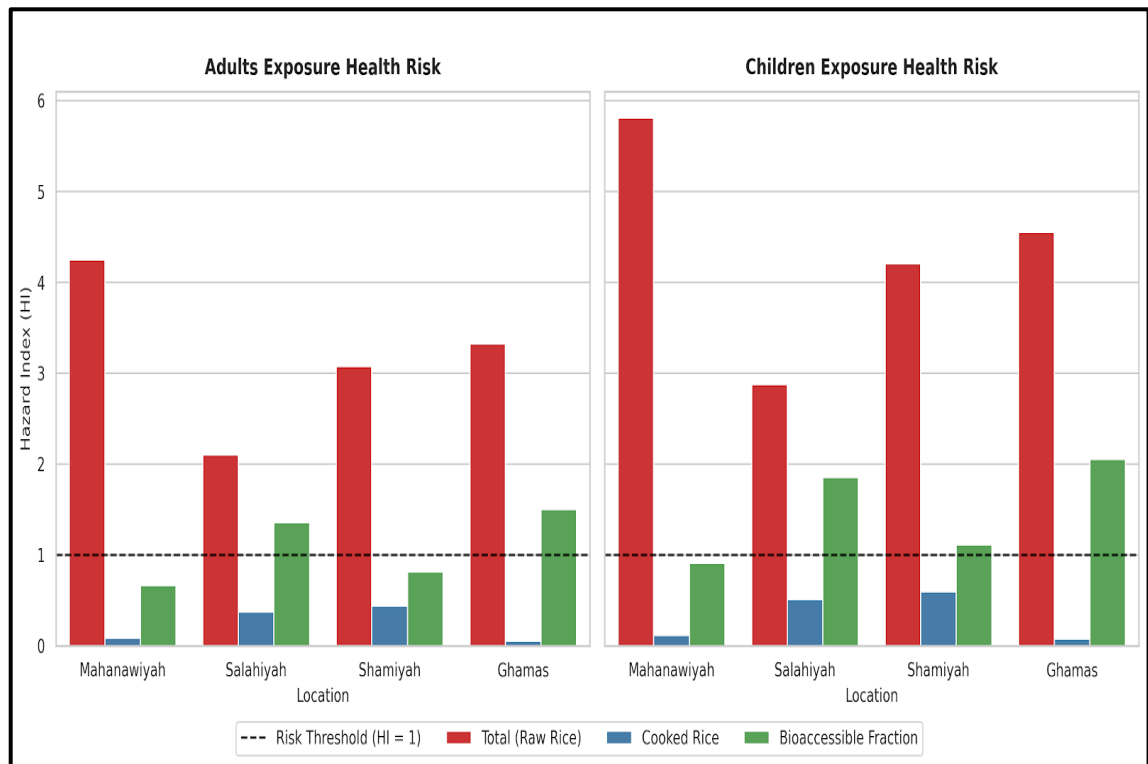


**Figure (4.3). Hazard Index (HI) of heavy metals in rice in Al-Qadisiyah Governorate under raw, cooked, and bioaccessible exposure scenarios for adults and children.**

#### 4.4.1. Health Risk Associated with Raw Rice Consumption

The HI values calculated for raw rice were consistently higher in children than in adults across all investigated locations. This difference indicates a greater potential exposure among children, which may be attributed to their lower body weight and higher food intake relative to body weight. Consequently, the same concentration of heavy metals may result in a greater exposure dose per unit body weight in children than in adults (Hauptman and Woolf, 2017).

For adults, the HI values associated with raw rice ranged from 2.1 in Salahiyah to 4.2 in Mahanawiyah, and therefore exceeded the critical value of 1 at all four locations. The highest adult HI was observed in Mahanawiyah (4.2), followed by Ghamas, Shamiyah, and Salahiyah. This indicates a potential non-carcinogenic health risk associated with long-term

consumption of the investigated raw rice under the exposure assumptions used in this study.

The potential risk was even higher among children. The HI values for raw rice reached approximately 5.8 in Mahanawiyah, while values of approximately 2.8 or higher were observed in the other investigated regions. The consistently higher HI values in children emphasize their greater vulnerability to dietary exposure to heavy metals.

The elevated HI values observed for raw rice may be related to the relatively high concentrations of heavy metals detected in the rice samples. Soil contamination may contribute to the accumulation of metals in rice; however, soil metal concentration is not necessarily the only factor controlling metal accumulation in rice grains. Liu et al. (2016) demonstrated that other environmental factors may also influence the uptake and accumulation of heavy metals in rice plants. In addition, the extensive use of fertilizers and pesticides in agricultural fields may contribute to the accumulation of metals in agricultural soils. Plants can also acquire heavy metals from atmospheric sources in addition to soil-derived sources (Bhoi et al., 2025). These factors may contribute to the elevated metal concentrations observed in rice cultivated in the agricultural regions of Al-Diwaniyah Governorate.

#### **4.4.2. Health Risk after Cooking**

A marked reduction in HI was observed following cooking treatment. The HI values for adults decreased to 0.05–0.44, while the corresponding values for children ranged from 0.10 to 0.58. Importantly, all HI values calculated for cooked rice were below the critical value of 1 for both adults and children.

The reduction in HI following cooking is consistent with the substantial decreases in metal concentrations observed after cooking. Washing and boiling can facilitate the removal of water-soluble fractions of

metals through dissolution and leaching into the cooking water (Sharafi et al., 2018). Therefore, the results indicate that the cooking procedure used in this study can substantially reduce the potential non-carcinogenic risk associated with consumption of the investigated rice.

The reduction was particularly important because the HI values calculated for raw rice exceeded 1 at all investigated locations, whereas all values for cooked rice fell below this threshold. This difference demonstrates the important influence of food preparation on estimated dietary exposure to heavy metals.

#### **4.4.3. Health Risk Based on Bioaccessible Fractions**

The bioaccessible fraction provides an additional perspective on potential exposure because it represents the fraction of metals released during gastrointestinal digestion and potentially available for absorption. The bioaccessible HI values showed a different pattern from those observed for cooked rice. For adults, bioaccessible HI values ranged from approximately 0.65 in Mahanawiyah to 1.50 in Ghamas. Thus, although cooking reduced the estimated risk substantially, the bioaccessible fraction remained above the critical value of 1 in some locations. Among children, the bioaccessible HI values were higher, ranging from approximately 0.90 to 2.05.

The highest bioaccessible HI was observed in Ghamas, reaching approximately 1.50 in adults and 2.05 in children. In contrast, Mahanawiyah showed the highest HI for raw rice but a lower bioaccessible HI in comparison with Ghamas. This difference indicates that total metal concentration alone does not necessarily determine the magnitude of the estimated bioaccessible risk. The amount of metal released during gastrointestinal digestion can vary according to its chemical characteristics and interactions with the rice matrix. For adults, the bioaccessible HI exceeded the threshold of 1 in Ghamas and Salahiyah, whereas

Mahanawiyah remained below the threshold. For children, values above the threshold were observed in Ghamas, Salahiyah, and Shamiyah, while Mahanawiyah remained below the critical value. These findings indicate that, despite the substantial reduction achieved through cooking, the bioaccessible fraction may still contribute to potential non-carcinogenic exposure in certain locations, particularly among children.

#### **4.4.4. Comparison between Adults and Children**

The consistently higher HI values observed in children demonstrate the importance of considering age-related differences in health-risk assessment. Children may be more susceptible to the toxic effects of heavy metals because of their immature organs, developing physiological systems, and differences in metabolism and exposure relative to body weight (Bair, 2022). Consequently, an exposure level associated with a relatively lower risk in adults may represent a greater potential risk in children.

In the present study, this age-related difference was evident across the raw, cooked, and bioaccessible exposure scenarios. The greater HI values observed among children were particularly pronounced for raw rice and the bioaccessible fraction, emphasizing the importance of considering children as a potentially vulnerable population when evaluating dietary exposure to contaminated rice.

#### **4.4.5. Spatial variation in health risk**

The health-risk assessment also demonstrated considerable variation among the investigated agricultural regions. Mahanawiyah had the highest raw-rice HI for both adults and children, with values of approximately 4.2 and 5.8, respectively. This finding is consistent with the relatively high concentrations of Pb and Zn observed in rice from this location and suggests a greater potential exposure when raw rice concentrations are used for risk estimation.

In contrast, Ghamas showed the highest bioaccessible HI values, reaching approximately 1.50 in adults and 2.05 in children. This difference between the raw-rice and bioaccessible assessments indicates that the potential risk is influenced not only by the total concentration of metals but also by their release and availability during gastrointestinal digestion.

The spatial differences in HI may be related to variation in soil properties, irrigation-water contamination, fertilizer application, and surrounding anthropogenic activities (Vici et al., 2021). Such environmental differences can affect both the concentration of metals accumulated by rice plants and their subsequent behavior during cooking and digestion.

Overall, the health-risk assessment demonstrated that consumption of raw rice resulted in HI values above the critical threshold of 1 at all investigated locations for both adults and children, indicating potential non-carcinogenic health concerns under the exposure conditions assumed in this study. The risk was consistently higher in children than in adults.

Cooking substantially reduced the estimated HI values, with all cooked-rice HI values falling below 1 for both population groups. This finding supports the effectiveness of cooking as a practical measure for reducing dietary exposure to heavy metals in rice.

However, the bioaccessible assessment provided a more conservative indication of potential exposure in some locations. Bioaccessible HI values exceeded 1 in Ghamas and Salahiyah for adults and children, and in Shamiyah for children, while Ghamas showed the highest bioaccessible risk. Therefore, although cooking substantially reduces heavy-metal exposure, the potential risk cannot be evaluated solely on the basis of cooked-rice concentrations. Incorporating the bioaccessible fraction provides additional information about the proportion of metals that may become available during gastrointestinal digestion.

Taken together, these findings highlight the importance of considering raw concentrations, cooking-related reductions, and gastrointestinal bioaccessibility when assessing the potential health risks associated with heavy-metal exposure through rice consumption. The consistently higher HI values observed in children further emphasize the need to consider vulnerable population groups in food-safety assessments.

## **4.5. Multivariate Analysis**

### **4.5.1. Correlation Analysis**

Pearson correlation analysis was performed to evaluate the relationships among the total concentrations of Zn, Pb, Cr, Cd, and P in rice samples collected from the four agricultural regions of Al-Qadisiyah Province. As shown in Figure (4.4), both positive and negative correlations were observed among the investigated elements, with correlation coefficients ranging from  $-0.78$  to  $0.98$ . The observed relationships provide useful information about the possible common sources, accumulation pathways, and interactions among the elements in the rice-growing environment.

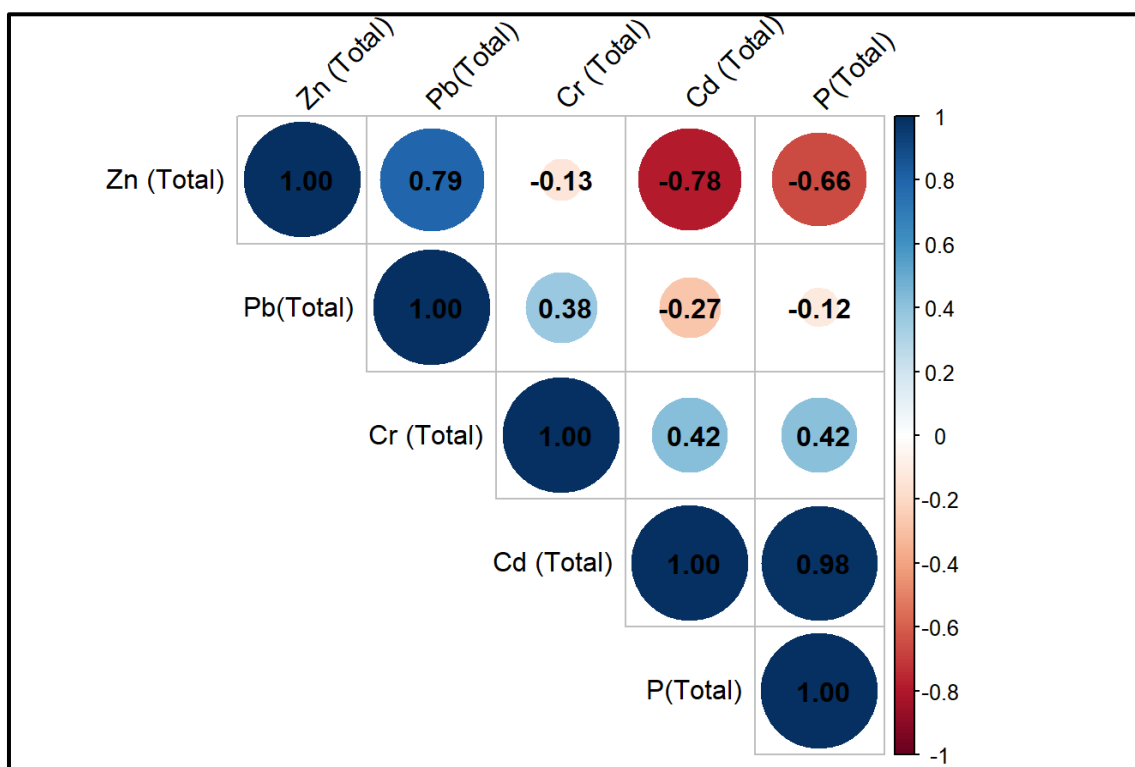


**Figure (4.4). Pearson correlation analysis among the total concentrations of Zn, Pb, Cr, Cd, and P in rice samples collected from Al-Qadisiyah Province, Iraq. Positive correlations are represented by blue circles and negative correlations by red circles, with circle size indicating the strength of the correlation.**

The strongest positive correlation was observed between Cd and P ( $r = 0.98$ ). This very strong association suggests that Cd and P may have common sources or may undergo similar accumulation processes in rice grains. One possible explanation is the application of phosphorus-containing fertilizers, because phosphate fertilizers may contain Cd impurities. Therefore, the strong Cd–P relationship may reflect the contribution of agricultural inputs to the accumulation of both elements in the investigated agricultural areas (Lee et al., 2025). This interpretation is further supported by the PCA results, in which Cd and P were positioned closely together.

A strong positive correlation was also observed between Zn and Pb ( $r = 0.79$ ). This relationship may indicate a common geogenic or anthropogenic source or similar environmental behavior. Both elements may enter agricultural soils through fertilizer application, irrigation water,

soil parent material, or atmospheric deposition. The simultaneous introduction of these elements from such sources could contribute to their accumulation in rice plants (Tepanosyan et al., 2022).

In contrast, strong negative correlations were observed between Zn and Cd ( $r = -0.78$ ) and between Zn and P ( $r = -0.66$ ). These inverse relationships may reflect differences in the uptake and translocation of these elements by rice plants. In particular, Zn is an essential micronutrient and may interact with Cd at soil adsorption sites and during root uptake and translocation. Consequently, variation in Zn availability may influence Cd accumulation in rice plants. The negative relationship observed between Zn and Cd is consistent with the possibility of competitive interactions between these elements and has also been reported in rice production systems (Zhang et al., 2026).

The relationships between Pb and the other elements were generally weak to moderate. Pb showed a positive correlation with Cr ( $r = 0.38$ ), whereas its correlations with Cd and P were weakly negative ( $r = -0.27$  and  $-0.12$ , respectively). These relatively weak relationships suggest that Pb may have accumulation pathways that differ from those of Cd and P. Similarly, the correlation between Zn and Cr was very weak and negative ( $r = -0.13$ ), suggesting limited overlap between their accumulation mechanisms.

Moderate positive relationships were also observed among several of the remaining elements. The correlations between Cr and Cd and between Cr and P were both approximately  $r = 0.42$ , while the relationships involving P and other elements ranged from moderate to strong depending on the element. These associations may indicate partially shared environmental pathways, although other factors such as soil pH, organic matter content, and redox conditions may influence the behavior and availability of Cr in agricultural soils.

Overall, the correlation analysis identified two major positively associated elemental groups: Cd–P and Zn–Pb, while Cr showed weaker and more independent relationships with several of the other elements. The strong Cd–P correlation may indicate a common agricultural source, particularly fertilizer application, whereas the Zn–Pb association may reflect common geogenic or anthropogenic inputs. These findings provide an initial indication of the possible sources and environmental pathways of heavy metals and phosphorus in rice cultivated in Al-Qadisiyah Province.

#### 4.5.2. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was used to further investigate the relationships among Zn, Pb, Cr, Cd, and P and to identify patterns that may provide information about their possible sources in rice samples collected from the four study areas. The first two principal components explained 91.0% of the total variance, with PC1 accounting for 59.1% and PC2 accounting for 31.9%, respectively. Therefore, the PCA provided a useful summary of the major patterns in the elemental dataset.

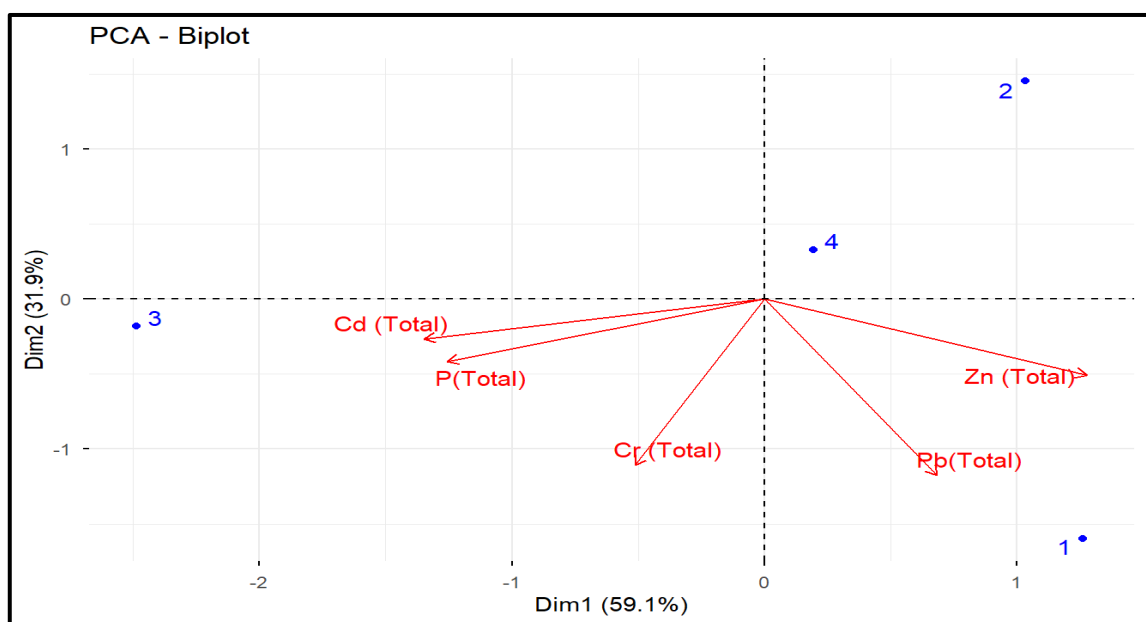


**Figure (4.5).** Principal Component Analysis (PCA) biplot showing the distribution of total Zn, Pb, Cr, Cd, and P concentrations and sampling sites in rice samples collected from Al-Qadisiyah Province, Iraq. PC1 and PC2 explained 59.1% and 31.9% of the total variance, respectively.

The PCA biplot showed two major groups of elements. Zn and Pb were positioned in a similar direction and were strongly associated with the positive region of PC1. This pattern agrees with the strong positive correlation between Zn and Pb ( $r = 0.79$ ) observed in the Pearson correlation analysis. Their close association may indicate a common source or similar accumulation behavior. Possible sources include agricultural inputs, irrigation water, atmospheric deposition, and geogenic contributions from soil parent materials.

In contrast, Cd and P were positioned close to each other on the negative side of PC1, with their vectors showing a similar direction. This pattern is consistent with the very strong positive correlation observed between Cd and P ( $r = 0.98$ ). The close association between these two elements suggests that they may share a common source or environmental pathway. In particular, phosphate fertilizer may represent an important source of Cd introduction into agricultural soils because Cd can occur as an impurity in phosphate rock used in fertilizer production (Mar and Okazaki, 2012).

Chromium showed a comparatively different pattern in the PCA biplot. The Cr vector was separated from the main Zn–Pb and Cd–P groups and was associated more strongly with the negative direction of PC2. This relative separation suggests that Cr may have a different source or may be controlled by different environmental processes. The mobility and uptake of Cr can be influenced by soil redox conditions, organic matter content, and mineral composition.

The distribution of the sampling sites further supported the elemental patterns observed in the PCA. Site 1 was located in the positive region of PC1 and the negative region of PC2, close to the Zn and Pb vectors, suggesting a stronger association with these elements. Site 2 was located in the positive region of both PC1 and PC2 and was separated from

the other sampling sites, indicating differences in its elemental composition and/or environmental conditions. Site 3 was positioned on the negative side of PC1 and close to the Cd and P vectors, indicating a stronger association with these two elements. Site 4 was positioned relatively close to the origin, indicating an intermediate elemental profile compared with the other sites. These spatial patterns are consistent with the interpretation of different environmental influences among the study areas (Rehman et al., 2026).

The angles between the elemental vectors provide additional information about their relationships. The small angle between Cd and P indicates a strong positive association, consistent with their correlation coefficient of  $r = 0.98$ . In contrast, the opposing directions of Zn relative to Cd and P are consistent with the negative correlations observed between these elements. The relatively greater separation of Cr from the other elements indicates weaker associations and potentially different environmental controls.

Overall, the PCA identified two principal elemental groupings, Zn–Pb and Cd–P, while Cr appeared comparatively independent. The Cd–P grouping may reflect the influence of agricultural fertilizer inputs, particularly phosphate fertilizers, whereas the Zn–Pb grouping may be associated with agricultural, irrigation-related, atmospheric, or geogenic sources. The relatively independent behavior of Cr suggests that additional environmental factors, including soil properties and geochemical conditions, may influence its accumulation.

The PCA findings are consistent with the Pearson correlation analysis and provide additional evidence regarding the potential sources and environmental behavior of the investigated elements. Together, the correlation and PCA results indicate that heavy-metal accumulation in rice

from Al-Qadisiyah Province is likely influenced by multiple environmental and agricultural pathways rather than a single contamination source.

#### 4.5.3. Heat Map and Hierarchical Cluster Analysis (HCA)

Hierarchical Cluster Analysis (HCA) was performed to investigate the relationships among the total concentrations of Zn, Pb, Cr, Cd, and P in rice samples and to identify similarities in their distribution and potential common contamination sources. The dendrogram and heatmap presented in Figure (4.6) revealed distinct clustering patterns among the investigated elements, indicating differences and similarities in their environmental behavior and accumulation in rice.

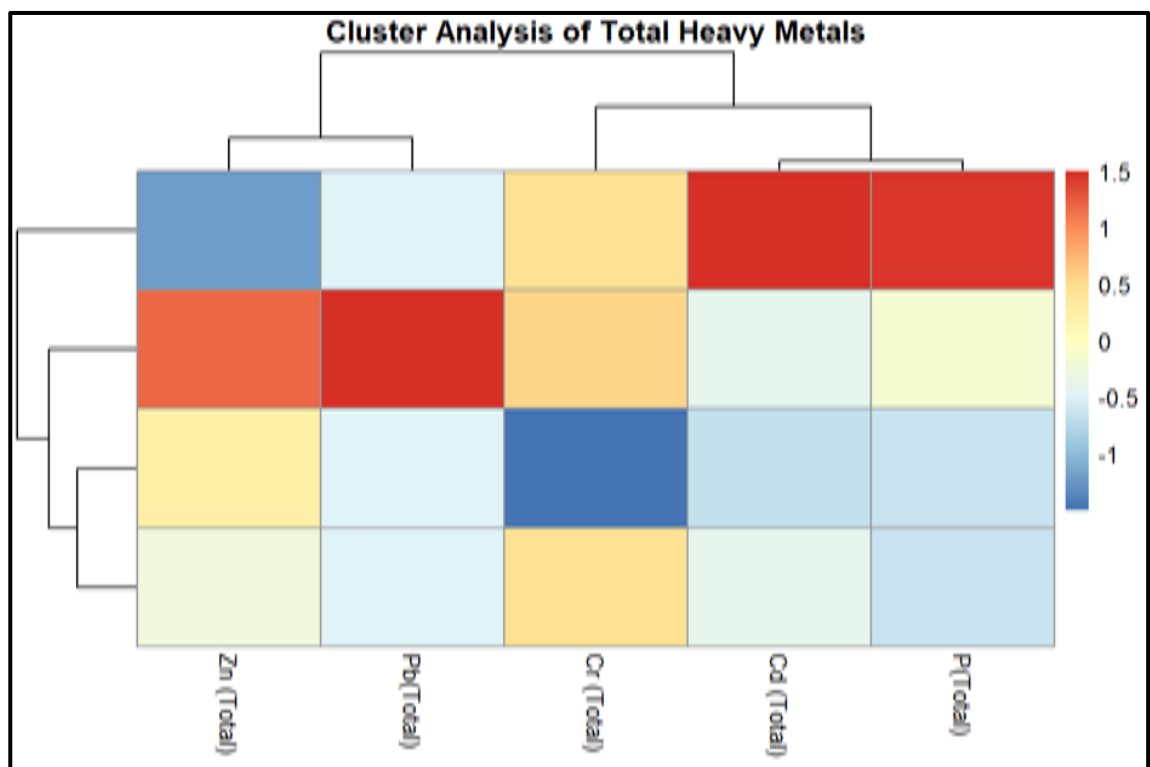


**Figure (4.6).** Hierarchical Cluster Analysis (HCA) heatmap and dendrogram of total Zn, Pb, Cr, Cd, and P concentrations in rice samples from Al-Qadisiyah Province, Iraq. The heatmap represents relative standardized concentrations, while the dendrogram illustrates the similarity among the investigated elements.

The HCA showed that Cd and P formed the closest cluster, as indicated by their shortest linkage distance in the dendrogram. This close association is consistent with the strong positive correlation observed

between Cd and P ( $r = 0.98$ ) in the Pearson correlation analysis and their close positioning in the PCA biplot. The consistent association among the three statistical approaches suggests that Cd and P may share a common source or environmental pathway in the investigated rice-growing areas. One possible explanation is the use of phosphate fertilizers, because phosphate fertilizers may contain Cd impurities. These impurities can accumulate in agricultural soils and subsequently be taken up by rice plants, contributing to the simultaneous occurrence of Cd and P in rice grains (Lee et al., 2026).

The second major cluster consisted of Zn, Pb, and Cr, although the relationships among these elements were not equally strong. Within this cluster, Zn and Pb showed the closest association, which is consistent with their strong positive correlation ( $r = 0.79$ ) observed in the Pearson correlation analysis. Their close clustering may indicate a common source or similar environmental behavior. Possible sources include soil parent material, irrigation water, agrochemical inputs, and atmospheric deposition associated with nearby anthropogenic activities. These potential sources may introduce Zn and Pb simultaneously into agricultural soils and contribute to their accumulation in rice grains.

In contrast, Cr was positioned at a greater linkage distance from Zn and Pb, indicating a weaker relationship with these elements. This observation is consistent with the PCA results, in which Cr appeared relatively independent from the Zn–Pb and Cd–P groups. The relatively separate behavior of Cr may reflect differences in its geochemical characteristics and environmental controls. Factors such as soil mineralogy, pH, redox conditions, and organic matter may influence the mobility, availability, and uptake of Cr by rice plants.

The heatmap further demonstrated variation in the relative concentrations of the investigated elements across the rice samples. The

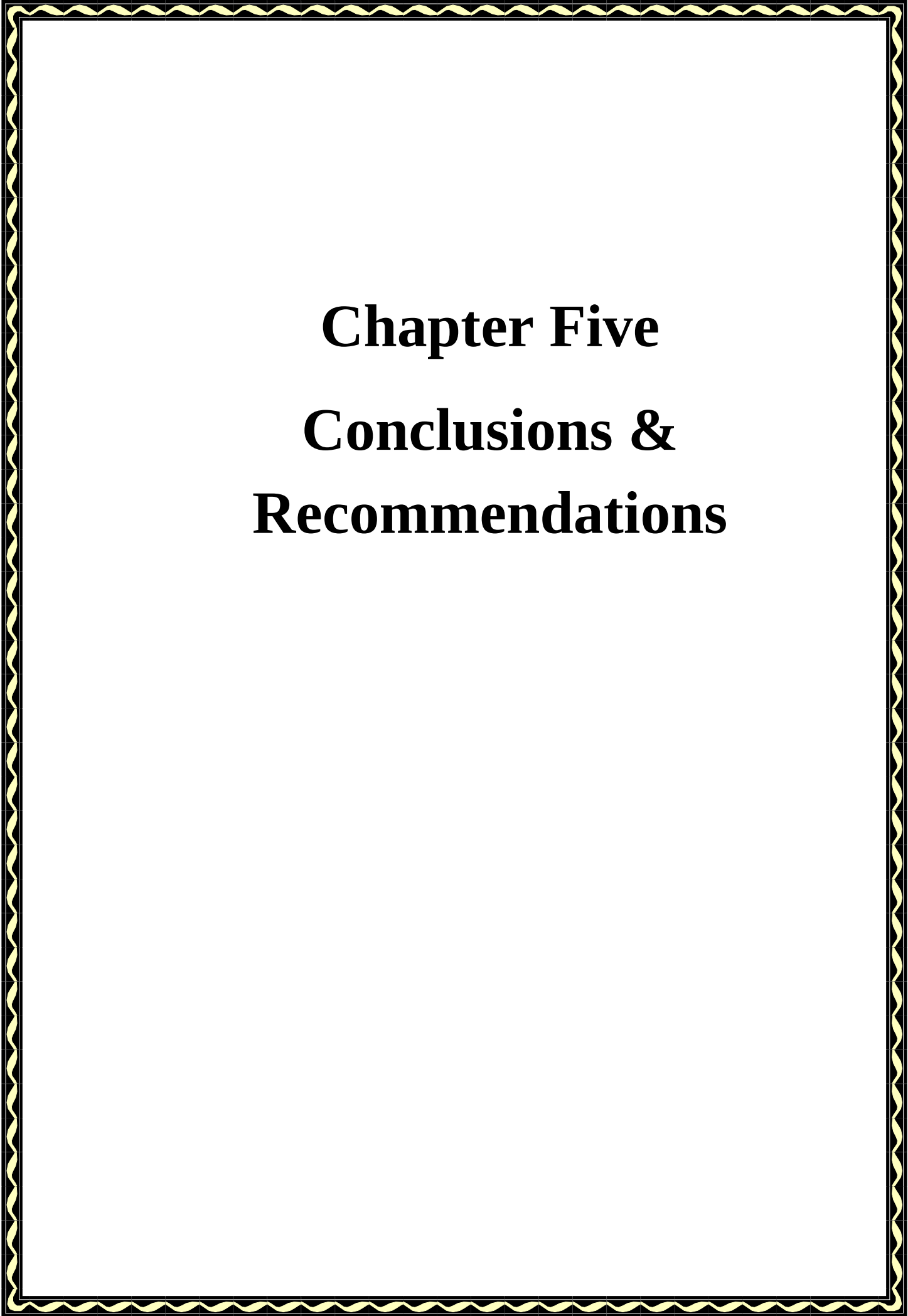
standardized concentration pattern showed areas of relatively higher and lower elemental concentrations, reflecting differences in the accumulation of the investigated elements among the samples. The pattern was particularly consistent with the separation of Cd and P from the Zn–Pb group, while Cr showed a comparatively distinct distribution. These differences may reflect variation in natural and anthropogenic sources affecting the agricultural environment.

The clustering pattern also provides further support for the potential role of agricultural activities in the accumulation of Cd and P. The close association between these elements suggests that fertilizer application, particularly phosphate fertilizers, may contribute to Cd loading in agricultural soils and its subsequent transfer to rice grains. In contrast, the Zn–Pb association may reflect another contamination pathway related to soil characteristics, irrigation, agricultural inputs, or other environmental sources. The relatively separate position of Cr suggests that its accumulation may be influenced by different environmental processes.

Importantly, the HCA results were generally consistent with the findings obtained from both Pearson correlation analysis and PCA. The three multivariate approaches consistently identified Cd–P as a strongly associated group and Zn–Pb as another closely related group, whereas Cr showed a comparatively independent behavior. The agreement among these analyses strengthens the interpretation that the investigated elements may originate from different sources or may be controlled by different environmental processes.

Overall, the HCA provides additional evidence for the presence of distinct elemental associations in rice cultivated in Al-Qadisiyah Province. The Cd–P cluster may be associated mainly with agricultural fertilizer inputs, particularly phosphate fertilizers, whereas the Zn–Pb cluster may reflect shared agricultural, irrigation-related, geogenic, or anthropogenic

sources. The relatively independent behavior of Cr suggests that its accumulation may be controlled by additional geochemical and soil-related factors. These findings contribute to a better understanding of the behavior and potential sources of heavy metals in rice-growing environments and may assist in developing appropriate strategies for contamination control and food-safety management.



# **Chapter Five**

## **Conclusions & Recommendations**

## 5. Conclusions and Recommendation

### 5.1. Conclusions

1. The results of this study showed that there are different concentrations of heavy metals in rice crops grown in Al-Qadisiyah Governorate, and the highest concern was found as lead (Pb). All the explored areas showed high concentration of Pb which was above the FAO/WHO recommended limits and the same applied to cadmium (Cd) in Shamiyah. Results also indicated that bioaccessible metal concentrations were generally below total concentrations, highlighting the need to consider bioaccessibility in human health risk assessments to prevent overestimation of exposure risks.
2. Concentration of heavy metals in rice was significantly reduced upon cooking, especially Cd and Pb, suggesting that the ability to reduce dietary exposure to toxic elements through common household cooking methods. However, some metals (particularly lead, Pb) were still measurable post cooking and had relatively high bioaccessibility, implying that chronic rice consumption may still be a non-carcinogenic health risk, with children identified as being at higher risk than adults on Hazard Index (HI) value.
3. Environmental pollution, irrigation water quality and agricultural practices are highlighted as the factors influencing the accumulation of heavy metals in rice.
4. Heatmap and Hierarchical Cluster Analysis (HCA) revealed strong associations between Cd and P, and between Zn and Pb, while Cr showed a more independent behavior. These findings suggest different potential sources of heavy metal accumulation in rice and were consistent with the PCA results.

5. Principal Component Analysis (PCA) explained 91.0% of the total variance and identified two main groups, Zn–Pb and Cd–P, while Cr showed a more independent behavior, suggesting different potential sources and environmental controls.

## 5.2. Recommendations

1. Environmental monitoring: Establish continuous monitoring programs for soil, irrigation water, and rice crops in Al-Qadisiyah Governorate to detect heavy metal contamination early and prevent accumulation in the food chain.
2. Agricultural practices: Promote environmentally friendly agricultural practices, including minimizing the use of chemical fertilizers and pesticides that contribute to cadmium and lead contamination, and encouraging organic alternatives.
3. Wastewater management: Implement stricter regulations on wastewater discharge into rivers and irrigation systems to reduce industrial and municipal pollution sources that elevate heavy metal levels in paddy soils.
4. Cooking and dietary education: Raise public awareness about the role of household cooking methods (washing, soaking, boiling) in reducing heavy metal concentrations, while emphasizing that bioaccessibility may remain high, especially for lead.
5. Health risk assessment: Conduct regular health risk assessments for both children and adults, focusing on Hazard Index (HI) and Carcinogenic Risk (CR) values, to guide dietary recommendations and public health interventions.
6. Future research: Encourage further longitudinal and multi-site studies with larger sample sizes to validate findings, explore additional metals,

and refine bioaccessibility methodologies for more accurate exposure estimates.

7. Mercury (Hg) may be excluded from routine analysis, as most Iraqi environments are generally free from mercury contamination and its major sources.

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# Indexes

| Location        | Scenario          | Meta<br>l | Concentratio<br>n (mg/kg) | EDI<br>(mg/kg/day) | THQ                  | HI                   |
|-----------------|-------------------|-----------|---------------------------|--------------------|----------------------|----------------------|
| Mahanawiya<br>h | Raw               | Cd        | 0.0800                    | 0.000169           | 0.1688               | 2.27×10 <sup>6</sup> |
|                 |                   | Zn        | 9.0100                    | 0.019007           | 0.0634               |                      |
|                 |                   | Pb        | 1.02×10 <sup>6</sup>      | 0.013649           | 2.59×10 <sup>6</sup> |                      |
|                 |                   | Cr        | 7.31×10 <sup>5</sup>      | 0.005042           | 1.79×10 <sup>6</sup> |                      |
|                 | Cooked            | Cd        | 0.0010                    | 0.000002           | 0.0021               | 0.1154               |
|                 |                   | Zn        | 7.67×10 <sup>5</sup>      | 0.002953           | 0.0098               |                      |
|                 |                   | Pb        | 0.0200                    | 0.000042           | 0.0121               |                      |
|                 |                   | Cr        | 0.1300                    | 0.000274           | 0.0914               |                      |
|                 | Bioaccessib<br>le | Cd        | 0.0400                    | 0.000084           | 0.0844               | 0.9089               |
|                 |                   | Zn        | 2.85×10 <sup>6</sup>      | 0.006265           | 0.0209               |                      |
|                 |                   | Pb        | 1.1700                    | 0.002468           | 0.7052               |                      |
|                 |                   | Cr        | 0.1400                    | 0.000295           | 0.0984               |                      |
| Salahiya        | Raw               | Cd        | 0.0500                    | 0.000105           | 0.1055               | 2.51×10 <sup>6</sup> |
|                 |                   | Zn        | 7.32×10 <sup>4</sup>      | 0.015210           | 0.0507               |                      |
|                 |                   | Pb        | 3.1600                    | 0.006666           | 2.61×10 <sup>6</sup> |                      |
|                 |                   | Cr        | 1.1600                    | 0.002447           | 0.8164               |                      |
|                 | Cooked            | Cd        | 0.0020                    | 0.000004           | 0.0042               | 0.5114               |
|                 |                   | Zn        | 0.8400                    | 0.001772           | 0.0059               |                      |
|                 |                   | Pb        | 0.3300                    | 0.000696           | 0.1989               |                      |
|                 |                   | Cr        | 0.4300                    | 0.000907           | 0.3024               |                      |
|                 | Bioaccessib<br>le | Cd        | 0.0100                    | 0.000021           | 0.0211               | 2.43×10 <sup>6</sup> |
|                 |                   | Zn        | 2.0400                    | 0.004304           | 0.0143               |                      |
|                 |                   | Pb        | 2.34×10 <sup>6</sup>      | 0.003861           | 1.1030               |                      |
|                 |                   | Cr        | 1.0200                    | 0.002152           | 0.7173               |                      |
| Shamiyah        | Raw               | Cd        | 0.3000                    | 0.000633           | 0.6329               | 6.11×10 <sup>4</sup> |
|                 |                   | Zn        | 6.58×10 <sup>5</sup>      | 0.009219           | 0.0308               |                      |
|                 |                   | Pb        | 3.1600                    | 0.006666           | 2.61×10 <sup>6</sup> |                      |
|                 |                   | Cr        | 5.11×10 <sup>5</sup>      | 0.004915           | 1.64×10 <sup>6</sup> |                      |
|                 | Cooked            | Cd        | 0.0170                    | 0.000036           | 0.0359               | 0.5969               |
|                 |                   | Zn        | 0.9200                    | 0.001941           | 0.0065               |                      |
|                 |                   | Pb        | 0.3600                    | 0.000760           | 0.2170               |                      |
|                 |                   | Cr        | 0.4800                    | 0.001013           | 0.3375               |                      |
|                 | Bioaccessib<br>le | Cd        | 0.0200                    | 0.000042           | 0.0422               | 1.1115               |
|                 |                   |           |                           |                    |                      |                      |
|                 |                   |           |                           |                    |                      |                      |
|                 |                   |           |                           |                    |                      |                      |

**Indxe (A): Heavy Metal Exposure in Children Consuming Rice  
from Al-Qadisiyah Governorate**

**129**

|        |               | Zn | $5.84 \times 10^5$ | 0.004958 | 0.0165             | a                  |
|--------|---------------|----|--------------------|----------|--------------------|--------------------|
| Ghamas | Raw           | Pb | 1.0700             | 0.002257 | 0.6449             |                    |
|        |               | Cr | 0.5800             | 0.001224 | 0.4079             |                    |
|        |               | Cd | 0.0800             | 0.000169 | 0.1688             | $1.32 \times 10^6$ |
|        |               | Zn | $1.10 \times 10^5$ | 0.013122 | 0.0438             |                    |
|        |               | Pb | $1.06 \times 10^6$ | 0.009451 | $1.87 \times 10^6$ |                    |
|        |               | Cr | $5.11 \times 10^5$ | 0.004915 | $1.64 \times 10^6$ |                    |
|        | Cooked        | Cd | 0.0040             | 0.000008 | 0.0084             | 0.0745             |
|        |               | Zn | 0.8300             | 0.001751 | 0.0058             |                    |
|        |               | Pb | 0.1000             | 0.000211 | 0.0603             |                    |
|        | Bioaccessible | Cd | 0.0200             | 0.000042 | 0.0422             | 2.0549             |
|        |               | Zn | $6.57 \times 10^5$ | 0.002890 | 0.0096             |                    |
|        |               | Pb | 3.1600             | 0.006666 | $2.61 \times 10^6$ |                    |
|        |               | Cr | 0.1400             | 0.000295 | 0.0984             |                    |

**Indxe (B): Heavy Metal Exposure in Adults Consuming Rice from Al-Qadisiyah Governorate**

**130**

| Location        | Scenario          | Meta<br>l | Concentratio<br>n (mg/kg) | EDI<br>(mg/kg/day) | THQ                  | HI                   |
|-----------------|-------------------|-----------|---------------------------|--------------------|----------------------|----------------------|
| Mahanawiya<br>h | Raw               | Cd        | 0.0800                    | 0.000123           | 0.1234               | 2.15×10 <sup>5</sup> |
|                 |                   | Zn        | 9.0100                    | 0.013894           | 0.0463               |                      |
|                 |                   | Pb        | 1.02×10 <sup>6</sup>      | 0.009977           | 2.41×10 <sup>6</sup> |                      |
|                 |                   | Cr        | 7.31×10 <sup>5</sup>      | 0.003686           | 1.41×10 <sup>5</sup> |                      |
|                 | Cooked            | Cd        | 0.0010                    | 0.000002           | 0.0015               | 0.0844               |
|                 |                   | Zn        | 7.67×10 <sup>5</sup>      | 0.002159           | 0.0072               |                      |
|                 |                   | Pb        | 0.0200                    | 0.000031           | 0.0088               |                      |
|                 |                   | Cr        | 0.1300                    | 0.000200           | 0.0668               |                      |
|                 | Bioaccessib<br>le | Cd        | 0.0400                    | 0.000062           | 0.0617               | 0.6644               |
|                 |                   | Zn        | 2.85×10 <sup>6</sup>      | 0.004580           | 0.0153               |                      |
|                 |                   | Pb        | 1.1700                    | 0.001804           | 0.5155               |                      |
|                 |                   | Cr        | 0.1400                    | 0.000216           | 0.0720               |                      |
| Salahiyah       | Raw               | Cd        | 0.0500                    | 0.000077           | 0.0771               | 2.10×10 <sup>6</sup> |
|                 |                   | Zn        | 7.32×10 <sup>4</sup>      | 0.011119           | 0.0371               |                      |
|                 |                   | Pb        | 3.1600                    | 0.004873           | 7.39×10 <sup>5</sup> |                      |
|                 |                   | Cr        | 1.1600                    | 0.001789           | 0.5963               |                      |
|                 | Cooked            | Cd        | 0.0020                    | 0.000003           | 0.0031               | 0.3738               |
|                 |                   | Zn        | 0.8400                    | 0.001295           | 0.0043               |                      |
|                 |                   | Pb        | 0.3300                    | 0.000509           | 0.1454               |                      |
|                 |                   | Cr        | 0.4300                    | 0.000663           | 0.2210               |                      |
|                 | Bioaccessib<br>le | Cd        | 0.0100                    | 0.000015           | 0.0154               | 6.08×10 <sup>5</sup> |
|                 |                   | Zn        | 2.0400                    | 0.003146           | 0.0105               |                      |
|                 |                   | Pb        | 2.34×10 <sup>6</sup>      | 0.002822           | 0.8063               |                      |
|                 |                   | Cr        | 1.0200                    | 0.001573           | 0.5243               |                      |
| Shamiyah        | Raw               | Cd        | 0.3000                    | 0.000463           | 0.4626               | 3.08×10 <sup>6</sup> |
|                 |                   | Zn        | 6.58×10 <sup>5</sup>      | 0.006739           | 0.0225               |                      |
|                 |                   | Pb        | 3.1600                    | 0.004873           | 7.39×10 <sup>5</sup> |                      |
|                 |                   | Cr        | 5.11×10 <sup>5</sup>      | 0.003593           | 2.81×10 <sup>4</sup> |                      |
|                 | Cooked            | Cd        | 0.0170                    | 0.000026           | 0.0262               | 0.4363               |
|                 |                   | Zn        | 0.9200                    | 0.001419           | 0.0047               |                      |
|                 |                   | Pb        | 0.3600                    | 0.000555           | 0.1586               |                      |
|                 |                   | Cr        | 0.4800                    | 0.000740           | 0.2467               |                      |
|                 | Bioaccessib<br>le | Cd        | 0.0200                    | 0.000031           | 0.0308               | 0.8125               |
|                 |                   |           |                           |                    |                      |                      |
|                 |                   |           |                           |                    |                      |                      |
|                 |                   |           |                           |                    |                      |                      |

|        |               | Zn | $5.84 \times 10^5$ | 0.003624 | 0.0121             |
|--------|---------------|----|--------------------|----------|--------------------|
| Ghamas | Raw           | Pb | 1.0700             | 0.001650 | 0.4714             |
|        |               | Cr | 0.5800             | 0.000894 | 0.2981             |
|        |               | Cd | 0.0800             | 0.000123 | 0.1234             |
|        |               |    |                    |          | $5.00 \times 10^5$ |
|        |               | Zn | $1.10 \times 10^5$ | 0.009592 | 0.0320             |
|        |               | Pb | $1.06 \times 10^6$ | 0.006909 | $2.86 \times 10^6$ |
|        | Cooked        | Cr | $5.11 \times 10^5$ | 0.003593 | $2.81 \times 10^4$ |
|        |               | Cd | 0.0040             | 0.000006 | 0.0062             |
|        |               |    |                    |          | 0.0545             |
|        |               | Zn | 0.8300             | 0.001280 | 0.0043             |
|        | Bioaccessible | Pb | 0.1000             | 0.000154 | 0.0441             |
|        |               | Cd | 0.0200             | 0.000031 | 0.0308             |
|        |               |    |                    |          | $1.14 \times 10^6$ |
|        |               | Zn | $6.57 \times 10^5$ | 0.002113 | 0.0070             |
|        |               | Pb | 3.1600             | 0.004873 | $7.39 \times 10^5$ |
|        |               | Cr | 0.1400             | 0.000216 | 0.0720             |

## الخلاصة

هدفت هذه الدراسة إلى تحديد تراكيز بعض المعادن الثقيلة، وقابليتها الحيوية للإتاحة، ومقدار انخفاضها نتيجة الطهي، وتأثيراتها الصحية المحتملة في الأرز المزروع في محافظة القادسية، العراق. جُمعت عينات الأرز من أربع مناطق زراعية رئيسية، هي: المهناوية، والصلاحية، والشامية، وغماس. تم تقدير تراكيز الكاديوم (Cd)، والرصاص (Pb)، والزنك (Zn)، والكروم (Cr) باستخدام مطيافية الامتصاص الذري (AAS) بعد إجراء الهضم الحمضي للعينات. كما تم تقييم القابلية الحيوية للإتاحة للمعادن الثقيلة باستخدام اختبار الاستخلاص البسيط للقابلية الحيوية للإتاحة (SBET)، الذي يحاكي الظروف المعدية المعوية، في حين تم تقييم المخاطر الصحية باستخدام المدخول اليومي المقدّر (EDI)، ونسبة الخطر المستهدف (THQ)، ومؤشر الخطر (HI).

أظهرت النتائج وجود تباين مكاني في تراكيز المعادن الثقيلة بين مواقع الدراسة المختلفة. سُجل أعلى تركيز للكاديوم في منطقة الشامية (0.30 ملغم/كغم)، في حين سُجلت أعلى تراكيز للرصاص والزنك في منطقة المهناوية، إذ بلغت 6.47 و 9.01 ملغم/كغم، على التوالي. تجاوزت تراكيز الرصاص في جميع المواقع الحدود المسموح بها وفق معايير منظمة الأغذية والزراعة/منظمة الصحة العالمية (FAO/WHO)، في حين سُجلت تراكيز الكاديوم التي تجاوزت الحد المقبول في منطقة الشامية فقط. انخفضت تراكيز المعادن في عينات الأرز المطبوخ بشكل ملحوظ، وبالنسبة الآتية: الكاديوم (96.02%)، والرصاص (93.91%)، والزنك (84.60%)، والكروم (84.22%). وأظهرت نتائج القابلية الحيوية النسبية للإتاحة أن أعلى نسبة إتاحة معدنية معوية كانت للرصاص (45%)، في حين كانت أقل نسبة للكاديوم (25%). وأشار تقييم المخاطر الصحية إلى أن الأطفال كانوا أكثر عرضة للمخاطر مقارنةً بالبالغين نتيجة لارتفاع مستوى التعرض بالنسبة إلى وزن الجسم. تجاوزت قيم مؤشر الخطر (HI) في عينات الأرز الخام في جميع المواقع الحد المسموح به ( $HI > 1$ )، في حين أسهم طهي الأرز في الحد من المخاطر في معظم الحالات. ومع ذلك، أظهرت عدة مواقع وجود الرصاص ضمن الكسور القابلة للإتاحة الحيوية، مما يشكل مصدر قلق محتمل من ناحية المخاطر غير السرطانية، ولا سيما لدى الأطفال الصغار.

أشارت التحليلات الإحصائية متعددة المتغيرات إلى أن الممارسات الزراعية، ونوعية مياه الري، والتلوث البيئي كانت من العوامل الرئيسية المؤثرة في ترسب المعادن الثقيلة في حبوب الأرز. وُجد ارتباط بين الكاديوم (Cd) والفوسفور (P) ( $r = 0.98$ ) كما لوحظ ارتباط موجب

مرتفع بين الزنك (Zn) والرصاص (Pb) ( $r = 0.79$ ) وعلى العكس، لوحظ ارتباط سالب قوي جدًا بين تراكيز الفوسفور (P) والكاديوم (Cd) ، بمعاملات ارتباط بلغت -0.78 و-0.66. أظهر مخطط الشجرة العنقودية (Dendrogram) والخريطة الحرارية (Heatmap) أنماطًا واضحة من التجمع، مما يشير إلى وجود اختلافات وتشابهات في سلوك العناصر ضمن العينات المدروسة. وقسم مخطط الشجرة العنقودية المتغيرات التي تم تحليلها إلى مجموعتين رئيسيتين. وكانت المتغيرات الأكثر تشابهًا والأقصر مسافة ارتباطية هي الكاديوم (Cd) والفوسفور (P) ، إذ تجمعت معًا ضمن العنقود الأول. ويرتبط هذا التقارب الكبير بنتائج تحليل الارتباط ( $r = 0.98$ ) ونتائج تحليل المكونات الرئيسية (PCA) ، مما يشير إلى أن الكاديوم والفوسفور قد يكونان ناتجين من مصدر مشترك. كما أشار التحليل الإحصائي متعدد المتغيرات إلى أن الممارسات الزراعية، ونوعية مياه الري، والتلوث البيئي كانت من العوامل الرئيسية المؤثرة في ترسب المعادن الثقيلة في حبوب الأرز.



جمهورية العراق  
وزارة التعليم العالي والبحث العلمي  
جامعة القادسية  
كلية العلوم

التوافر الحيوي لبعض المعادن الثقيلة والفسفور الكلي في الرز  
المطبوخ وغير المطبوخ في محافظة الديوانية

رسالة

مقدمة الى مجلس كلية العلوم – جامعة القادسية  
كجزء من متطلبات نيل شهادة الماجستير في علوم البيئة

تقدمت بها

نوره محمد ضياء نور

بكلوريوس علوم البيئة – جامعة القادسية (2017)

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