EFFECTS OF COOKING METHODS IN REDUCING RISK TO ESSENTIAL AND TOXIC METALS EXPOSURE IN RICE (*Oryza Sativa*) – HEALTH RISK ASSESSMENT IN MALAYSIA

BY

GOH NGEE CHUANG

DISSERTATION IS SUBMITTED IN PARTIAL FULFILMENT

OF THE REQUIREMENTS FOR THE

FOR THE DEGREE OF MASTER OF SCIENCE

(HEALTH TOXICOLOGY)

ADVANCED MEDICAL AND DENTAL INSTITUTE
UNIVERSITI SAINS MALAYSIA

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that is not has been previously on concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

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GOH NGEE CHUANG

P-IPM0010/22

Date:......25 July 2023..............

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LIST OF SYMBOLS

>	Greater than
<	Less than
=	Equals to
Σ	Sum of
±	Statistical margin of error

LIST OF ABBREVIATIONS

As	Arsenic
Al	Aluminium
AT	Averaging Time
BW	Body Weight
Cd	Cadmium
C_{metal}	Concentration of metal in rice
Co	Cobalt
Cr	Chromium
CSF	Cancer Slope Factor
Cu	Copper
ED	Exposure Duration
EF	Exposure Frequency
Fe	Iron
HI	Hazard Index
HQ	Hazard Quotient
HCA	Hierarchical Clustering
HRA	Health Risk Assessment
IARC	International Agency for Research on Cancer
ICP-MS/MS	Tandem Inductively Coupled Plasma Mass Spectrometry
IR	Ingestion Rate
LCR	Lifetime Carcinogenic Risk
MOH	Ministry of Health Malaysia
Mg	Magnesium
Mn	Manganese
Ni	Nickel
Pb	Lead
PBA	Washed, parboiled, and absorbed
RfD	Reference Dose
USEPA	United States Environment Protection Agency
USM	Universiti Sains Malaysia
WA	Washed and absorbed
Zn	Zinc

KESAN KAEDAH MEMASAK DALAM MENGURANGKAN PENDEDAHAN KEPADA LOGAM PENTING DAN TOKSIK DALAM BERAS – PENILAIAN RISIKO KESIHATAN DI MALAYSIA

ABSTRAK

Pencemaran logam dalam beras nasi telah didokumentasikan secara berleluasan di seluruh dunia. Malaysia bukan pengecualian, terdapat laporan mengenai pencemaran logam dalam beras nasi dari pelbagai negeri telah muncul baru-baru ini. Banyak kajian telah mengkaji rawatan masakan untuk mengurangkan pencemaran logam dalam beras nasi yang keberkesanannya masih belum dikaji dalam beras Malaysia. Tujuan penyelidikan ini adalah untuk mengkaji keberkesanan rawatan masakan penyerapan dan pra-perebusan sebelum penyerapan masakan (PBA) ke atas sampel-sampel beras yang dibeli dari pasaraya Malaysia. Penentuan logam telah dijalankan melalui spektrometri jisim plasma terkopel berpasangan tandem (ICP-MS/MS) dan penilaian risiko kesihatan (HRA) dijalankan mengikut rangka kerja USEPA untuk penilaian risiko logam. Hasil kajian ini telah menunjukkan bahawa PBA berjaya menghapuskan 77.9% logam toksik (Al, As, Cd, Cr, Ni, dan Pb), sambil mengeluarkan 68.4% logam penting (Co, Cu, Fe, Mg, Mn, Zn). Pentingnya, kaedah ini memastikan bahawa kepekatan As, Cd, dan Pb dalam semua sampel kekal di bawah had yang dibenarkan mengikut Peraturan Makanan Malaysia 1985. Keberkesanan PBA boleh dipertribusikan kepada pembuangan air rebusan berlebihan yang mengandungi logam larut dari beras sebelum beras itu dimasak sepenuhnya. Penilaian risiko kesihatan deterministik (HRA) menunjukkan bahawa PBA secara signifikan mengurangkan Indeks Bahaya (HI) sebanyak 84.5% dan Risiko Karsinogenik Seumur Hidup (LCR) yang berkaitan dengan pendedahan As sebanyak 88.9% berbanding dengan kawalan. PBA berjaya mengurangkan risiko kesihatan bukan karsinogenik yang ditimbulkan oleh Cd, Mn, Ni, dan Zn di bawah had yang boleh diterima (Hazard Quotient (HQ) = 1) untuk semua kumpulan umur, kecuali As dalam kalangan kanak-kanak. Walau bagaimanapun, LCR masih berada di atas had yang dibenarkan (1 x 10-4) untuk semua kumpulan umur. Penilaian risiko HRA berdasarkan kebarangkalian menggunakan simulasi Monte Carlo menunjukkan bahawa majoriti (>95%) kanak-kanak, remaja, dan dewasa akan mengalami risiko kesihatan bukan karsinogenik secara keseluruhan dan karsinogenik dari pendedahan As yang tidak dapat diterima, tidak mengira kaedah masakan yang digunakan. Hasil kajian ini menunjukkan bahawa kaedah PBA berkesan dalam mengurangkan kepekatan logam serta risiko kesihatan yang ditimbulkan oleh logam beracun dalam beras.

EFFECTS OF COOKING METHODS IN REDUCING EXPOSURE TO ESSENTIAL AND TOXIC METALS IN RICE – HEALTH RISK ASSESSMENT IN MALAYSIA

ABSTRACT

The ubiquity of metal contamination in rice grains has been well documented worldwide. Malaysia is no exception, as reports of metal contamination in rice grains collected from various states have surfaced recently. Many studies have investigated cooking treatments to reduce the metal contamination in rice which efficacy were still unexplored on Malaysian sourced rice. The aim of this research was to investigate the effectiveness of cooking treatments, absorption cooking and parboiling before absorption cooking (PBA) on rice samples bought from Malaysian supermarkets. Metal quantification was conducted via tandem inductively coupled plasma mass spectrometry (ICP-MS/MS) and further health risk assessment (HRA) was conducted according to the USEPA framework for metal risk assessment. The findings indicated that PBA successfully eliminated 77.9% of toxic metals (Al, As, Cd, Cr, Ni, and Pb), while removing 68.4% of essential metals (Co, Cu, Fe, Mg, Mn, Zn). Importantly, this method ensured that concentrations of As, Cd, and Pb in all samples remained below the permissible limits according to the Malaysian Food Regulations 1985. The effectiveness of PBA can be attributed to the disposal of boiled excess water which contained dissolved metals from the rice before the rice was fully cooked. Deterministic health risk assessment (HRA) revealed that PBA significantly reduced the Hazard Index (HI) by 84.5% and Lifetime Carcinogenic Risk (LCR) associated with As exposure by 88.9% compared to controls. PBA effectively reduced the non-carcinogenic health risks posed by Cd, Mn, Ni, and Zn below the acceptable limit (Hazard Quotient (HQ) = 1) for all age groups, except As in children. However, LCR remained above the upper permissible limit (1×10^{-4}) for all age groups. Probability-based HRA using Monte Carlo simulations indicated that the majority (>95%) of children, adolescents and adults would experience unacceptable total non-carcinogenic and carcinogenic health risks from As exposure, regardless of the cooking method employed. These results indicate that the PBA method was effective in reducing both concentration of metal as well as health risk posed by toxic metals in rice.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Metals and metalloids are ubiquitous all around us; they are naturally occurring elements found within the Earth's crust and have been extensively used in various industries from chemical manufacturing, agriculture to the mining industry (Zaynab *et al.,* 2022). Its wide use exacerbates the risk of human exposure, which can occur via various routes, including ingestion, inhalation, and dermal route (Zakaria *et al.*, 2021). While some of these metals are considered essential due to the integral roles in biological systems such as iron (Fe) and zinc in trace amounts (Zoroddu *et al.,* 2019), exposure to toxic metals, such as lead (Pb), cadmium (Cd), and arsenic (As), can pose adverse effects to human health (Genchi *et al.,* 2020b; Kumar *et al.,* 2020; Palma-Lara *et al.*, 2020). Anthropogenic contamination of the terrestrial environment, particularly agricultural soil, is a particular concern due to the notable uptake of metals in food crops via root uptake, which can subsequently be translocated to different parts of the plant, including their edible parts (Rai *et al.*, 2019; Zwolak *et al.*, 2019).

Exposure to metals is particularly dangerous for people who consume large amounts of certain foods, making rice a prominent potential source of exposure due to its importance as a staple food, especially in Asia. About 90% of the world's rice is produced in the Asian region, satisfying the caloric needs for approximately 4.4 billion Asians (Bhandari and Mishra, 2018). In Malaysia alone, an estimated 2.5 plates of white rice per day is consumed everyday by the average Malaysian adult (Firdaus *et al.*, 2020). Rice is a crop that is particularly susceptible to contamination with metals in particular cadmium (Cd), arsenic (As), and lead (Pb) (Sharafi *et al.*, 2019b; Zakaria *et al.*, 2021). In Malaysia, recent studies have found rice grains produced in different cultivation areas have exceeded the acceptable health risk limit for both non-carcinogenic and carcinogenic health risks due to metal contamination (Praveena and Omar, 2017; Zulkafflee *et al.*, 2019, 2020, 2022; Sibuar *et al.*, 2022).

The persistence of metal contamination in rice as a global public health issue has prompted further research on the effectiveness of different cooking methods, such as washing, presoaking, absorption cooking, parboiling, and cooking with different volumes of water, on the reduction of metal content in rice (Mwale *et al.*, 2018; Sharafi *et al.*, 2019b; Shariatifar *et al.*, 2020; Menon *et al.*, 2021). These studies have shown that certain cooking methods may reduce metal contamination to a significant rate such as in the study by Menon *et al.* (2021) that demonstrated a removal rate of 73% in inorganic arsenic by parboiling rice before cooking via absorption method. It is important to note that cooking will also inadvertently reduce the concentration of essential micronutrients such as iron (Fe), copper (Cu), cobalt (Co) and zinc (Zn) (Sharafi *et al.*, 2019b; Shariatifar *et al.*, 2020); highlighting the need to identify the appropriate method to reduce toxic metal contamination whilst preserving the nutritional contents in rice.

A health risk assessment can be performed based on the results to allow us to determine whether contamination is detrimental to human health or still within acceptable limits (Praveena and Omar, 2017; Zakaria *et al.*, 2021). In this study, a health risk assessment (HRA) was conducted to evaluate the health risks of consuming metal contaminated rice that are available in Malaysian supermarkets. Two cooking methods, namely absorption cooking and parboiling prior to absorption cooking (PBA), will be evaluated for their effectiveness to reduce toxic metal concentrations to an acceptable level whilst maintaining the concentration of essential metals in rice. The results from the study can be used to identify any rice products that exceeds permissible contaminant limit set by Food Safety and Quality Division, Ministry of Health Malaysia which could warrant further investigations or even the withdrawal of the said product from the market. Effective cooking methods can be disseminated to the public, ensuring their awareness of the appropriate risk-reducing cooking method.

1.2 Problem statement

Metal contamination in rice grains collected in different states of Malaysia have been recently reported (Praveena and Omar, 2017; Zulkafflee *et al.*, 2019, 2020, 2022; Sibuar *et al.*, 2022). These studies have reported that the metal concentration in rice grains has exceeded the acceptable health risk limit for both non-carcinogenic and carcinogenic health risks. Currently, there are a lack of studies in Malaysia that investigated metal contamination in cooked rice which is a more accurate method to determine metal exposure to human population. In addition, the most recent studies were conducted by Praveena and Omar (2017) and Omar *et al.* (2015) which were more than 5 years. While most of the recent local studies focused on rice grains collected from paddy fields (Zulkafflee *et al.*, 2019, 2020, 2022; Sibuar *et al.*, 2022). Many studies have also studied the effectiveness of cooking methods to reduce toxic metals found in rice in other countries such as studies by Mwale *et al.* (2018), Sharafi *et al.* (2019b), Shariatifar *et al.* (2020) and Menon *et al.* (2021). So far, no similar studies on cooking methods have been reported in Malaysia. Therefore, there is a need to investigate if the methods used in the aforementioned papers are as effective as compared to conventional cooking methods on rice available in Malaysia.

1.3 Objectives of the study

The objectives of this study are as follows.

i. To determine the concentration of toxic metals (Al, As, Cd, Cr, Ni, Pb) and essential metals (Cu, Co, Fe, Mg, Mn, Zn) that are found in rice bought at Malaysian supermarkets with the use of tandem inductively coupled plasma mass spectrometry (ICP-MS/MS).

ii. Determine the non-carcinogenic and carcinogenic health risk of consuming rice contaminated with the metals based on the USEPA Health Risk Assessment Framework for Metals.

iii. Determine the best cooking method which can reduce toxic metal concentrations and the levels of health risk associated with toxic metals while maintaining essential metal concentrations.

1.4 Hypotheses

The null hypotheses of this study are as follows.

i. There is not an excessive concentration of metals (As, Cd, Pb, Cr, Ni, Al, Fe, Zn, Cu, Co, Mn, Mg) in rice samples bought from Malaysian supermarkets.

ii. Health risk assessment of the concentration of toxic metals would produce a permissible non-carcinogenic and carcinogenic health risk.

iii. There is no significant difference in concentration of metals between washed rice and cooked rice.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This literature review aims to provide an overview of a few metals of interest and their associated adverse effects or biological importance, known cases of metal contamination of rice, health risk assessments of exposure to metals in rice, as well as the cooking methods that were investigated to reduce metal contamination. This section will summarize the current state of knowledge which would be the foundation for this research.

2.2 Metals and their associated health effects to humans

While some metals are notoriously known for their potential to cause adverse effects to both the environment and animals, some metals play a significant role as trace minerals. It is important to note that even essential elements are only needed in small amounts and when present in excess quantities, could also present adverse health effects.

2.2.1 Toxic metals

Metals are considered dangerous as they tend to accumulate and persist in the environment, animals as well as humans, which would bring about numerous deleterious health effects (Alengebawy *et al.*, 2021). The associated adverse health effects to humans for each toxic metal would be covered in the following subsections.

2.2.1(a) Aluminium

Aluminium (Al) is one of the most abundant elements and can be found naturally in air, water, and soil hence there is potential of exposure to contaminated ambient air and consuming contaminated water or food crops (Affourtit *et al.*, 2020). In addition, its properties make it widely used in many household items such as in cans for food packaging, kitchen utensils, cosmetics, food additives as well as medication such as antacids (Briffa *et al.*, 2020). Signs and symptoms of Al toxicity are kidney and liver dysfunction, colitis, pulmonary fibrosis, osteomalacia, endocrine disruption, and neurological symptoms such as dementia, trembling, and Alzheimer's symptoms (Briffa *et al.*, 2020). The toxicity of Al can be mainly attributed to its tendency to elevate oxidative stress by the generation of free reactive radicals (Igbokwe *et al.*, 2019). Aluminium has an affinity to estrogen receptors and may imitate estrogen receptors hence are termed as metalloestrogen, which have potentially disruptive actions (Igbokwe *et al.*, 2019). The International Agency for Research on Cancer [IARC] (2012b) have deemed Al as Group 1 carcinogen due to sufficient evidence of its carcinogenicity in production settings and its inherent link with urinary bladder and lung cancer.

2.2.1(b) Arsenic

Arsenic (As) is one of the most abundant naturally occurring elements found in the Earth’s crust and exists in various organic and inorganic forms, including various states of oxidation (Palma-Lara *et al.*, 2020). Inorganic As is regarded to be more toxic than the organic counterparts and predominantly exists as either trivalent arsenite (As(III)) or pentavalent arsenate (As(V)) (Ingole *et al.*, 2021). Alengebawy *et al.* (2021) reported that several industries such as smelting and insecticides containing As have contributed to As contamination. Industries, agriculture, mining, and wastewater are also deemed anthropogenic sources of arsenic (Briffa *et al.*, 2020). The primary route of exposure to As is via ingestion whereby it has been reported that roughly 20-300 µg of As is consumed per day via contaminated food or water (IARC, 2012a). One of the most harmful effects of As exposure is cancer. According to the IARC (2012a), As and inorganic As compounds are classified as Group 1 carcinogens, which stipulates that there is sufficient evidence that it will cause cancer upon exposure to humans. As concentrations of >200 µg/L in drinking water have been shown to elevate the risk of developing these cancers (Lamm *et al.*, 2021).

2.2.1(c) Cadmium

Cadmium (Cd) exists primarily in a +2 state in the crust of the Earth and due to its chemical and physical properties, it is widely used in various industries such as phosphate fertilizers and pesticides in the agriculture industry to plastic and nickel-cadmium battery manufacturing (Briffa *et al.*, 2020). The common route of exposure is through inhalation; however, ingestion is also another notable route of exposure particularly the ingestion of Cd contaminated rice leading to significant cadmium poisoning reports in the Japanese population (Ochoa *et al.*, 2020). Non-carcinogenic effects of Cd are nephrotoxicity, infertility as well as alterations in calcium metabolism leading to a condition named as Itai-Itai disease, a prominent issue in Japan characterized by osteoporosis and brittle bones (Briffa *et al.*, 2020). Besides this, Cd compounds are classified as Group 1 carcinogens, as there is sufficient evidence for its carcinogenicity to humans (IARC, 2012a). Ingole *et al.* (2021) reported that Cd may contribute to this effect by stimulating oxidative stress and DNA damage, increased proliferation and inhibition of apoptosis and DNA repair mechanisms.

2.2.1(d) Chromium

Chromium (Cr) exists in 7 different oxidation states from 0 to +6, with the metallic Cr(0), trivalent Cr(III), and hexavalent Cr(VI) states being the most common (DesMarias and Costa, 2019). Cr(III) is found to have more stability in the environment, whereas Cr(VI) mostly derives from its use in various industrial processes such as metal refining, electroplating, and leather tanning (DesMarias and Costa, 2019; Briffa *et al.*, 2020). Currently, more attention is focused on Cr(VI) due to its toxicity and low biological need (DesMarias and Costa, 2019, Pavesi and Moreira, 2020). In the general population, ingestion of contaminated food and water represents the major exposure route whereas inhalation of Cr(VI) dust or particles in air emissions is more commonly implicated in workers or people living in the vicinity of these industries (Pavesi and Moreira, 2020). When consumed, Cr(VI) would cause acute ingestion poisoning with a variety of symptoms including nausea, liver damage, and toxic nephritis whereas when inhaled, it would cause pneumonia, bronchitis, allergy reactions and lung cancer (Briffa *et al.*, 2020).

2.2.1(e) Nickel

Nickel (Ni) naturally exists in a few oxidation states and is most commonly found in its +2 state (Genchi *et al.*, 2020a). Several industries that use nickel are petrochemical, metallurgical, nickel-cadmium batteries manufacturing, and steel fabrication industries (Das *et al.*, 2018). In the general non-smoking population, ingestion of Ni contaminated food and drinking water is the primary route of exposure (IARC, 2012a). Ni is also extremely widespread and is reported to be found in many foodstuffs from nuts, grains, seafood, and soya products (Genchi *et al.*, 2020a). Ingestion of Ni would lead to reproductive toxicity, kidney, and cardiovascular diseases (Genchi *et al.*, 2020a). The IARC has categorized Ni compounds and Ni metals under Group 1 where there is sufficient evidence of carcinogenicity in humans (IARC, 2012a). Ni is known to induce carcinogenesis via inhibition of several biological processes including ROS generation, DNA hypermethylation and mutation as well as inhibition of DNA repair mechanisms (Briffa *et al.*, 2020; Ingole *et al.*, 2021).

2.2.1(f) Lead

Lead (Pb) in the environment can exist in both organic and inorganic forms, which are both inherently toxic; however, the toxicity of organic Pb complexes is significantly more toxic (Kumar *et al.*, 2020). Pb has been used in many industries and can be found in many products and items surrounding us such as inorganic Pb in old paint and organic

Pb in the form of Tetraethyl Pb in leaded gasoline, and pesticides (Briffa *et al.*, 2020; Kumar *et al.*, 2020). Primary exposure to Pb occurs primarily through ingestion of water or foodstuffs contaminated with Pb, such as crops such as vegetables, spices, fruits, and cereal crops that are grown on contaminated soil, while inhalation exposure mainly through occupational settings (Kumar *et al.*, 2020). When absorbed into the body, Pb can cause a myriad of acute symptoms such as hypertension, abdominal pain, miscarriages to more serious long-term disabilities such as peripheral nerve damage, cognitive impairment, development retardation in children including reduced intelligence, attention span and development of anti-social behavior (Briffa *et al.*, 2020). The IARC has categorized inorganic Pb under Group 2A which is probably carcinogenic to humans due to limited evidence in humans while there is sufficient evidence in animals (IARC, 2006). Organic Pb on the contrary is not classifiable as it would be metabolized to ionic Pb in humans and animals which exerts toxicities similar to inorganic Pb (IARC, 2006).

Table 2.1 The various health effects of toxic metals and their anthropogenic source.

Toxic metal	**Anthropogenic Source**	**Non-carcinogenic effects**	**Carcinogenicity**	**References**
Al	Household items e.g., cans for food packaging, kitchen utensils, cosmetics, food additives as well as medication e.g., antacids	liver dysfunction, colitis, pulmonary fibrosis, osteomalacia, endocrine disruption, and neurological symptoms	IARC Group 1 carcinogens: breast, urinary bladder, and lung cancer	(Affourtit *et al.*, 2020; Briffa *et al.*, 2020; IARC, 2012b; Igbokwe *et al.*, 2019; Ingole *et al*,. 2021)
As	Agriculture and mining industries.	Gastro-intestinal tract and lung irritation, skin changes.	IARC Group 1 carcinogens: kidney, liver, and prostate cancer.	(Alengebawy *et al.*, 2021; IARC, 2012a; Igbokwe *et al.*, 2019; Ingole *et al.*, 2021; Lamm *et al.*, 2021; Palma-Lara *et al.*, 2020)
Cd	Agriculture industry, plastic, and nickel-cadmium battery manufacturing.	Nephrotoxicity, infertility, Itai-Itai disease	IARC Group 1 carcinogens: breast, prostate, pancreas, lung, and kidney cancers.	(Briffa *et al.*, 2020; Genchi *et al.*, 2020b; IARC, 2012a; Ingole *et al.*, 2021; Ochoa *et al.*, 2020)

Table 2.1 Continued

Cr	Metallurgical and steel industries, electroplating, leather tanning and dye paints manufacturing.	Nausea. vomiting, fever, liver damage, and toxic nephritis when ingested. When inhaled, it would cause pneumonia, bronchitis, liver and kidney disease, allergy reactions	IARC Group 1 carcinogens: lung and nose cancer.	(Briffa *et al.*, 2020; DesMarias and Costa, 2019; Ingole *et al.*, 2021; Pavesi and Moreira, 2020)
Ni	Petrochemical, metallurgical, ceramics, steel fabrication and rubber industries	Reproductive toxicity, kidney, and cardiovascular diseases	IARC Group 1 carcinogens: lung and nasal cancer.	(Briffa *et al.*, 2020; Das *et al.*, 2018; Genchi *et al.*, 2020a; IARC, 2012a; Ingole *et al.*, 2021)
Pb	Everyday items e.g., paint, leaded gasoline, agrochemicals.	Hypertension, abdominal pain, miscarriages, serious long-term disabilities e.g., peripheral nerve damage, cognitive impairment, development retardation in children including reduced intelligence and attention span	IARC Group 2a carcinogens: limited evidence in humans and sufficient evidence in animals.	(Briffa *et al.*, 2020; IARC, 2006; Kumar *et al.*, 2020)

2.2.2 Essential metals

Essential elements for biological organisms including plants and animals can be grouped into three groups; the major elements (such as carbon, hydrogen, nitrogen, and oxygen), macrominerals (such as Mg, Na and K) and other trace elements (Briffa *et al.*, 2020). Trace elements such as Fe, Zn, Cu, Co, and Mn are required in trace quantities for the proper and health functioning of biological systems where deficiencies of any of these metals are exhibited by numerous disorders and symptoms (Zoroddu *et al.*, 2019). Several biological functions which depend on these elements are the maintenance of skeletal structure, regulation of acid-base equilibrium, constituents of key enzymes, and formation of structural proteins and enzymes such as iron for haemoglobin and selenium for the glutathione peroxidase enzyme (Briffa *et al.*, 2020). Some of the functions of essential metals (Cu, Co, Fe, Mg, Mn, and Zn) are summarized in the following table.

Table 2.2 The biological functions of essential metals, average amount found in an adult male and the dietary recommended intake.

Essential metal	**Biological function**	**Average amount found in a 70 kg man**	**Dietary recommended daily intake for adult**
Cu	Combines with metalloproteins and enzymes which act as a catalyst for various biological activities such as immunity, neural functions, blood health and antioxidant activity (Sousa *et al.*, 2019).	100 mg (Maret, 2016)	1.5–3.0 (Jomova *et al.*, 2022)
Co	It is essential as a component of vitamin B12 (cobalamin) which is integral for several biological function including maintenance of nervous system and erythropoiesis (Zoroddu *et al.*, 2019).	2 mg (Maret, 2016)	Few µg/day (Zoroddu *et al.*, 2019)
Fe	Involved in a numerous metabolic process, which includes oxygen transport, deoxyribonucleic acid (DNA) synthesis as well as being a component of haemoglobin (Sousa *et al.*, 2019)	5 g (Maret, 2016)	15 mg/day (Jomova *et al.*, 2022)
Mg	Important components for over 300 enzymatic reactions including neurotransmission and the synthesis of fatty acids and proteins. Also, a fundamental component for ATP (Zoroddu *et al.*, 2019).	30 g (Zoroddu *et al.*, 2019)	420 mg/day (Jomova *et al.*, 2022)
Mn	Important cofactor in various enzymes involved with metabolism, regulation of cellular energy, reproduction, in the growth of bone and connective tissue. Mn has a protective role in shielding the mitochondria from oxidative stress as it is involved in the synthesis of manganese superoxide dismutase (MnSOD) (Zoroddu *et al.*, 2019).	12–20 mg (Maret, 2016)	1-10 mg/day (Zoroddu *et al.*, 2019)
Zn	Zinc fingers can organize and maintain tertiary structure of proteins, regulates of gene expression by transcription factors via modulation of DNA elements which involves it in multiple intracellular activities including metabolism, maintenance of physiological processes, cell signaling and transduction, as well as cell growth and differentiation (Sousa *et al.*, 2019, Zoroddu *et al.*, 2019).	2 g (Maret, 2016)	15 mg/day (Zoroddu *et al.*, 2019)

2.3 Metal contamination in rice

Rice is a major food and caloric source for more than half of the world's population, and its dependency is even more evident in Asia. About 90 per cent of the world's rice is produced in the Asian region, fulfilling caloric needs for approximately 4.4 billion Asians (Bhandari and Mishra, 2018). In Malaysia alone, an estimated 2.5 plates of white rice per day is consumed everyday by the average Malaysian adult (Firdaus *et al.*, 2020). It is worthwhile to consider the nutritional value of rice which also contains moderate amounts of essential vitamins such as thiamine, riboflavin, vitamin B, as well as the previously mentioned essential metals (Bhandari and Mishra, 2018; Sharafi *et al.*, 2019c).

The rice cultivation methods in Asia and South-East Asia which typically involves flooding paddies in combination with contaminated irrigation water is also noted to be a significant factor in the elevation of toxic metals in rice such in the case of inorganic As (Carey *et al.*, 2019; Biswas *et al.*, 2020). It is also reported that the high volume of rice consumption in Asia has also contributed to high dietary exposure and metal accumulation such as in Japan where its population has the highest Cd in the world (Ochoa *et al.*, 2020). The widespread metal contamination in rice can be seen in the numerous reports published from various countries as seen in the following table.

Table 2.3 Recent studies reporting metal contamination in rice samples and the associated health risks.

Country	**Type of sample**	**Target analytes**	**Metals posing significant non-carcinogenic health risk**	**Metals posing significant carcinogenic health risk**	**Reference**
Malaysia	Cooked rice	Al, As, Cd, Co Cu, Cr, Fe, Pb, Zn.	No significant risk from single metal exposure but significant for total metal exposure.	Significant risk from single As exposure and total exposure.	(Praveena and Omar, 2017)
Thailand	Raw rice (4 different varieties)	As, Cd, Co, Cu, Cr, Mn, Ni, Zn,	Significant single metal exposures for Mn and As for ¾ varieties. Significant total metal exposures for all varieties except for 1 variety.	Significant risk for ¾ varieties from As for all age groups except for consumption of 1 variety in children and adolescents.	(Kukusamude *et al.*, 2021)
India	Raw rice	As, Cd, Fe, Ni, Pb	Significant risk from single metal exposure due to As and Pb and for total metal exposure.	Significant risk due to single Ni, Cd, As exposures and total metal exposures.	(Baruah *et al.*, 2021)
	Raw rice	As, Cd, Ni, Pb + essential metals (Cu, Co, Fe, Mn, Se, Zn)	Significant risk from single metal exposure due to As and Pb, and for total metal exposure.	Significant risk from single Cd, As, and Ni exposures, and total metal exposure.	(Halder *et al.*, 2020)
China	Raw rice	As, Cd, Hg, Pb	Significant risk from single metal exposure due to Cd, and total metal exposure.	Significant risk due to single Pb and Cd exposures, and total metal exposure.	(Lu *et al.*, 2018)
	Raw rice	As, Cd, Cu, Cr, Pb, Zn	Significant risk from single metal exposures due to Cd, As, and Cu, and total metal exposures.	N/A	(Tang *et al.*, 2019)
Peru	Raw rice	As, Cd, Hg, Pb, Sn	No significant risk from single metal exposure but significant risk for total metal exposure (mostly from As and Hg).	Significant risk due to single As and Cd exposures and total exposures.	(Roman-Ochoa *et al.*, 2021)

N/A: Not available.

2.4 Regulatory limit for metals in food and rice

Based on the Food and Agriculture Organization of the United Nations (FAO), Table 2.4 represents the established permissible metal limits that are acceptable in rice which serve as a guideline for national regulatory agencies for their acceptable values to be found in food commodities in their country. In Malaysia, according to the Malaysian Food Regulations 1985 Regulation 38, there is enforcement on the permissible limits of metals that can be found in general foodstuff as stated in Table 2.4. This is similarly seen in Europe and China, whereas the United States of America (USA) under their US Food and Drug Administration (FDA) has not yet established any regulatory limits for metals in finished foodstuffs with the exception of bottled water (Zwolak *et al.*, 2019).

Table 2.4 Permissible metal limits in various food items and their enforcing regulations.

Country/Region/Authority	**Legislation/Regulation no.**	**Food**	**Metal**	**Permissible limits (mg/kg)**	**Reference**
Food and Agriculture Organization of the United Nations (FAO)	CXS 193-1995	Rice	As	0.2	(FAO, 2019)
			Cd	0.4	
			Cr	0.1	
		Cereal grains	Pb	0.2	
Malaysia	Malaysian Food Regulations 1985 Regulation 38	Rice	Cd	0.4	(MOH, 1985)
		Foodstuff	Inorganic As	1	
			Pb	2	
Europe	Regulation EC 1881/2006	Rice	Cd	0.1	(EC, 2006)
		Cereals	Pb	0.2	
China	National Food Safety Standard of Maximum Levels of Contaminants in Foods (GB 2762-2012)	Rice	Cd	0.2	(PRC, 2013)
			Pb	0.2	
			Inorganic As	0.2	
			Total Hg	0.02	
		Hulled grains	As	0.5	
		Grain Products	Cr	1.0	

2.5 Health risk assessments (HRA) of exposure to metals in cooked rice

Under the United States Environmental Protection Agency (USEPA) published Framework for Metal Risk Assessment (USEPA, 2007), health risk assessments (HRA) consist of 4 steps which includes hazard identification, hazard characterization, exposure assessment, and risk characterization and aims to evaluate potential non-carcinogenic and carcinogenic health effects of metal exposure on certain populations after ingestion (USEPA, 2007). The endpoints and formulas used by the USEPA are covered in Chapter 3.

This HRA framework used by the USEPA is deterministic, as it only allows for the determination of risks based on the best approximation of input parameters. Recent development and accessibility to mathematical or stochastic models have further improved this method by addressing the uncertainty, unpredictability and randomness which could occur due to various variabilities from contaminant concentration, individual predisposal, data scarcity, and other parameters differences (Zakaria *et al.,* 2021). An example would be the use of Monte Carlo Simulation (MCS) which have been widely used to integrate the uncertainty factor into both non-carcinogenic and carcinogenic health risks of rice consumption (Li *et al.,* 2018; Shariatifar *et al.,* 2020; Lien *et al.,* 2021; Kukusamude *et al.,* 2021; Ullah *et al.,* 2022).

2.6 Effect of cooking methods in reducing contamination

An important factor that affects metal contamination in rice is the cooking method. Multiple studies have investigated the effectiveness of various cooking methods including pre-soaking rice, absorption cooking method, parboiling cooking method, and rinse cooking in excess water. Some of the results will be summarized in Table 2.5.

Table 2.5 The effectiveness of various cooking methods in reducing metal contamination in rice from different countries.

Country	Cooking methods	Target analytes	Effectiveness of cooking method in reducing metal contamination	Reference
Malaysia	Absorption cooking method	Al, As, Cd, Co Cu, Cr, Fe, Pb, Zn	Non-carcinogenic health risk: insignificant risk from single metal exposure but significant risk for total metal exposure after cooking (HQ < 1, HI > 1). Carcinogenic health risk: significant risk from single As exposure and total exposure after cooking (LCR > 1 x 10^{-4}).	(Praveena and Omar, 2017)
United Kingdom	1. Unwashed and absorbed 2. Washed and absorbed 3. Pre-soaked and absorbed 4. Parboiled and absorbed (PBA)	As	Parboiling and Absorption (PBA) method was most effective in reducing inorganic As concentration by 54% reduction for brown rice & 73% reduction for white rice.	(Menon *et al.*, 2021)
	3 different rice-to-water ratios: 1:3 absorption cooking method 1:6 rinse cooking method 1:10 parboiling cooking method	As + essential metals (Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Ni, Se, Zn)	As decreased the most in parboiling method (44%) followed by rinse method (30%) and absorption method (4.5%). All the essential elements (except Fe, Se, Zn and Cu) experienced a significant loss (>14.5%) using the 1:6 method.	(Mwale *et al.*, 2018)
Iran	1. Raw rice 2. Washing (W) 3. W + soaking (1, 5 and 12 h) 4. W + Kateh cooking method (similar to Praveena and Omar (2017) absorption method) 5. W + rinsed cooking method (similar to parboil method used by Menon *et al.* (2021) except water not preboiled)	As, Cd, Pb	Rinsed method more efficient than Kateh method; rinsed method removed (As=42.3%, Pb=42.9%, Cd =27.6%) compared to (As=26.9%, Pb=26.9%, Cd =20.9%) for Kateh method. Non-carcinogenic health risk: insignificant risk from single metal exposure but significant risk for total metal exposure after cooking (HQ < 1, HI > 1). Carcinogenic health risk: significant risk from As exposure and total exposure (LCR > 1 x 10^{-4}).	(Sharafi *et al.*, 2019c)
Spain	Absorption cooking method	(Al, As, Cd, Pb) + essential elements (Ni, Se, Cr, Cu, Mn, and Zn)	Non-carcinogenic: no significant risk from single metal exposure and total metal exposure (HQ & HI < 1). Carcinogenic: No significant risk after cooking (LCR < 1 x 10^{-4}).	(Aguilera-Velázquez *et al.*, 2023)

CHAPTER 3

METHODOLOGY

3.1 Sample collection

Four types of unpolished (whole grain) rice and six types of polished (white) rice were sampled from supermarkets based on convenience sampling. The random varieties of rice purchased were based on the commonly available varieties as well as preference by Malaysians (Praveena and Omar, 2017). The regional source of rice samples was obtained from the package's label as in the Table 3.1.

Table 3.1 The source, type and category of rice samples used in this study.

Sample no.	**Brand**	**Category of rice**	**Type of Rice**	**Source**	**Weight of sample purchased**
1	A	Polished	Imported calrose rice	Taiwan (TW)	1 kg
2	B	Unpolished	Thai red rice	Thailand (TH)	1 kg
3	C	Unpolished	Multigrain unpolished rice (red, brown, black, purple)	Malaysia (MY)	1 kg
4	D	Polished	Herbal rice	India (IN)	1 kg
5	E	Unpolished	Black glutinous rice	Malaysia (MY)	250 g
6	F	Unpolished	Local brown rice	Malaysia (MY)	5 kg
7	G	Polished	Cambodian hill rice	Cambodia (CD)	5 kg
8	H	Polished	Jasmine fragrant rice	Thailand (TH)	500 g
9	I	Polished	Kashmir basmati rice	Pakistan (PK)	500 g
10	J	Polished	Local white rice	Malaysia (MY)	500 g

3.2 Sample preparation

In this study, the parboiling method used by Menon *et al.* (2021) will be investigated as this method recorded the highest percentage reduction in inorganic As. This cooking method is similar as to the rinse cooking method by Sharafi *et al.* (2019c) which was also effectively used to reduce contamination of Pb and Cd. Another cooking method that will be investigated is the absorption cooking method by Praveena and Omar (2017) and Omar *et al.* (2015) as it is a preferred method for rice cooking in Southeast Asia as excessive water is not required and it is also time and labour effective (Bairagi *et al.,* 2020).

The rice samples were treated according to the 3 following methods as shown in Figure 3.1:

1. Control (Washed): 50 g of rice sample was rinsed 3 times using deionized water. This treatment represented the control group.

2. Washed and Absorbed (WA): 50 g of rice samples were rinsed 3 times using deionized water. The samples were cooked using the absorption method where the rice to water ratio used was 100 mL (1:2 ratio) for white/polished rice and 150 mL (1:3) for unpolished rice (brown, red rice). The samples were cooked in a clean beaker on a hotplate on high power (500°C) then medium power (270°C) once water started to boil. The samples were allowed to cook until no water remained.

3. Washed, Parboiled, and Absorbed (PBA): 50 g of rice samples were rinsed 3 times using deionized water. The rice samples were then parboiled in 200 mL of pre-boiled deionized water in the rice to water ratio of 1:4 for 5 minutes. The excess water was then discarded, and the rice was cooked using the absorption method in fresh deionised water.

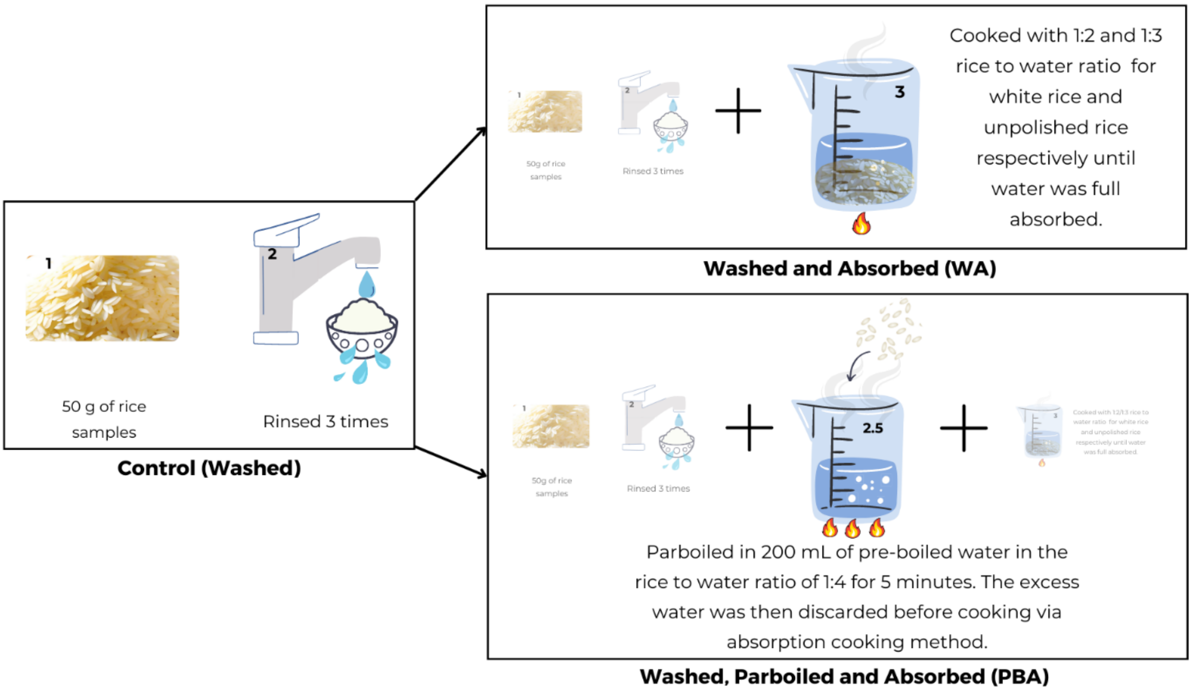

Figure 3.1 The rice treatments used in this study.

After cooking, all beakers containing samples were dried in an oven (65°C) and dried for two days or until a constant dry weight was observed. The dried samples were then grounded manually using a pestle and mortar to avoid any cross contamination. The samples were then allowed to pass a 0.25 mm mesh sieve (No. 60 mesh sieve) and the samples were kept in sealed bags at 4°C until the digestion process took place.

3.3 Acid digestion

Acid digestion of samples were carried out by Milestone Connect ultraWAVE Single reaction chamber microwave digestion using the inbuilt General Method of Environmental Sample. Around 300mg (refer to Table A2 in Appendix A) of homogenized samples were weighed and transferred into a disposable borosilicate glass vessel. 5ml of HNO_3 (68%) is added into the vessel. The digestion system was programmed according to the microwave heating program: step 1 with a temperature of 175°C for 10 minutes at 110 bar pressure; step 2 with a temperature of 175°C for 10 minutes at 110 bar pressure. After complete digestion, the samples were taken out of the vessel and filtered using a 0.22 µm hydrophobic PTFE syringe. The samples were kept in a refrigerator at 4°C before ICP-MS analysis (Praveena and Omar, 2017).

3.4 Tandem inductively coupled plasma mass spectrometry analysis

Before analysis, the samples were diluted 100 times with blank solution (Ultrapure water) before being analyzed in triplicates by tandem inductively coupled plasma mass spectrometry (ICP-MS/MS) (iCAP–TQ, Thermo Fisher Scientific). The (ICP-MS/MS instrument operating conditions are summarized in the Table A3 in Appendix A). To evaluate the accuracy of the method, the external calibration standards (Standard 2A, 10 µg/ml each in 5% HNO3, Inorganic Ventures) were analyzed in the same manner as the samples. Values of limit of detection (LOD) for Mg, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb were found as 0.994, 2.07, 0.0066, 0.0393, 0.0855, 0.0112, 0.0624, 0.117, 4.70, 0.0011, 0.0152, and 0.109 ppb respectively. Values of limit of quantification (LOQ) for Mg, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb were found as 0.208, 0.45, 0.07, 0.011, 0.142, 0.003, 0.016, 0.038, 0.988, 0.009, 0.004 and 0.032 ppb respectively. The correlation coefficients of all the standard calibration curves were in the range of $R^2 > 0.999$.

3.5 Statistical analysis

All statistical analysis including descriptive statistical analysis, normality testing, hypothesis testing, Hierarchical Clustering Analysis (HCA), Principal Component Analysis (PCA) and others were conducted using IBM's SPSS version 27. All hypotheses testing were conducted at 0.05 significance level. HCA and PCA were used to group the metals based on the similarities in chemical properties. HCA was performed with Ward's method for linkage formation and the distances between the clusters were measured using squared Euclidean distances. For PCA, the nonnormally distributed data was first logarithmically transformed into normally distributed data to produce better results (Choi, 2016; Abdul Hamid *et al.*, 2019). The extraction method for PCA was based on eigenvalues more than 1 and Direct Oblimin Method was used to rotate and project the results on the loading plot.

3.6 Health Risk Assessment (HRA)

In this study, the non-carcinogenic health risk for As, Cd, Mn, Ni and Zn, and the carcinogenic health risk for As were evaluated due to the availability of the USEPA's Oral Reference Dose (RfD) and Cancer Slope Factor (CSF) at the time the development of the manuscript. A health risk assessment for non-carcinogenic and carcinogenic health risks for children, adolescents, and adults were determined by using the formulas as stated in the USEPA's Framework for Metal Risk Assessment (USEPA, 2007).

Firstly, the estimated Average Daily Dose (ADD) was calculated using the following formula:

$$ADD = (C_{metal} \times IR \times ED \times EF) / (BW \times AT) \quad \text{Equation (1)}$$

where ADD = Average daily dose (mg/kg-day)

C_{metal} = Average concentration metal in rice (mg/kg)

IR = Ingestion rate (kg/day)

ED = Exposure duration (year)

EF = Exposure frequency (day/year)

BW = Body weight (kg)

AT = Averaging time (ED x 365 days)

The values for each variable can be found in the Table 3.2. Only the mean values without their standard deviations were used for the calculation.

Table 3.2 Input parameters for the estimation of ADD.

Parameters	**Unit**	**Children**	**Adolescents**	**Adults**	**Reference**
C_{metal}	mg/kg	Refer to Table B1 in Appendix B.			Obtained from this study