HUMAN HEALTH RISK ASSESSMENT OF ARSENIC, CADMIUM, AND ZINC EXPOSURE THROUGH RICE CONSUMPTION IN BANGKOK

Miss Supanad Hensawang

จุฬาลงกรณ์มหาวิทยาลัย
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การประเมินความเสี่ยงทางสุขภาพจากการรับสัมผัสสารหนู แคดเมียม และสังกะสี
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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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By	Miss Supanad Hensawang
Field of Study	Hazardous Substance and Environmental Management
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ศุภนาถ เห็นสว่าง : การประเมินความเสี่ยงทางสุขภาพจากการรับสัมผัสสารหนู แคดเมียม และสังกะสีจากการบริโภคข้าวในกรุงเทพมหานคร (HUMAN HEALTH RISK ASSESSMENT OF ARSENIC, CADMIUM, AND ZINC EXPOSURE THROUGH RICE CONSUMPTION IN BANGKOK) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ดร. เพ็ญรดี จันทร์ภิวัฒน์, 83 หน้า.

วัตถุประสงค์ของการศึกษานี้ประกอบด้วย 1) วิเคราะห์ปริมาณของสารหนู, แคดเมียม และ สังกะสีในข้าวสารที่จำหน่ายในตลาดกรุงเทพมหานคร โดยใช้เครื่องมือ inductively coupled plasma – mass spectrometry (ICP-MS) และ inductively coupled plasma – optical emission spectrometry (ICP-OES) ในการตรวจวิเคราะห์ 2) ประเมินการได้รับสารหนู แคดเมียมและสังกะสีผ่านการบริโภคข้าวในชีวิตประจำวัน และการประเมินความเสี่ยงต่อการเกิดโรคมะเร็งและโรคอื่นๆที่ไม่ใช่มะเร็งที่มีสาเหตุมาจากการได้รับสัมผัสโลหะหนัก และ 3) วิเคราะห์ปริมาณโลหะในรูปแบบที่สิ่งมีชีวิตสามารถดูดซึมได้ในข้าว ผลการศึกษาพบปริมาณของโลหะที่ตรวจพบในตัวอย่างข้าวจำนวน 97 ตัวอย่าง (ค่าเฉลี่ย ± ค่าคลาดเคลื่อนมาตรฐาน) ดังนี้ 0.2049 ± 0.0081 มิลลิกรัมต่อกิโลกรัม สำหรับ สารหนู, 0.0189 ± 0.0012 มิลลิกรัมต่อกิโลกรัม สำหรับแคดเมียม และ 19.79 ± 0.80 มิลลิกรัมต่อกิโลกรัม สำหรับสังกะสี ผลการศึกษาแสดงให้เห็นว่าประเภทของข้าวมีผลต่อปริมาณความเข้มข้นของสารหนูในเมล็ดข้าว ทั้งนี้พบว่าข้าวกล้องหอมมะลิมีระดับความเข้มข้นของสารหนูสูงที่สุด ผลการประเมินความเสี่ยงต่อสุขภาพจากการรับสัมผัสโลหะหนักทั้ง 3 ชนิดผ่านการบริโภคข้าว พบว่า สารหนูเป็นโลหะเพียงชนิดเดียวที่มีค่าระดับความเสี่ยงเกินระดับเกณฑ์ที่ยอมรับได้ (HQ > 1) โดยค่าความเสี่ยงต่อสุขภาพจากสารหนูในผู้ใหญ่ที่บริโภคข้าวประเภทต่าง ๆ พบว่าอยู่ระหว่าง 1.17 ถึง 2.61 และค่าความเสี่ยงต่อสุขภาพจากแคดเมียมและสังกะสีในผู้ใหญ่มีค่าอยู่ระหว่าง 0.04 ถึง 0.09 และ 0.11 ถึง 0.28 ตามลำดับ นอกจากนั้นแล้ว ผลการศึกษาแสดงให้เห็นว่าค่าความเสี่ยงต่อสุขภาพจากการบริโภคข้าวจะมีค่าสูงที่สุดในประชากรวัยเด็กจึงสามารถสรุปได้ว่าเด็กมีความเสี่ยงที่จะได้รับผลกระทบจากการบริโภคข้าวมากที่สุด และยังพบอีกว่าค่าความเสี่ยงต่อสุขภาพในผู้ชายมักจะมีค่ามากกว่าผู้หญิง ในส่วนความเสี่ยงต่อสุขภาพที่จะได้รับผลกระทบจากมะเร็ง (AELCR) พบว่ามีเพียงความเสี่ยงจากการรับสัมผัสสารหนูเท่านั้น โดยมีค่าความเสี่ยงเท่ากับ 3×10^{-8} (ช่วงค่าที่ยอมรับได้คือ 10^{-6} -10^{-4}) ดังนั้นความเสี่ยงในการเกิดโรคมะเร็งจากการรับสัมผัสสารหนูผ่านการบริโภคข้าวในกรุงเทพมหานครจึงอยู่ในระดับต่ำ และท้ายที่สุดพบว่า ร้อยละ15.7 และ 33.3 ของสารหนูในข้าวขาวหอมมะลิและข้าวกล้องหอมมะลินั้นเป็นสารหนูที่อยู่ในรูปแบบที่สิ่งมีชีวิตสามารถดูดซึมได้

สาขาวิชา การจัดการสารอันตรายและสิ่งแวดล้อม ลายมือชื่อนิสิต ....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

5787544520 : MAJOR HAZARDOUS SUBSTANCE AND ENVIRONMENTAL MANAGEMENT
KEYWORDS: RICE / ARSENIC / CADMIUM / ZINC / HEALTH RISK ASSESSMENT / METAL BIOAVAILABILITY

SUPANAD HENSAWANG: HUMAN HEALTH RISK ASSESSMENT OF ARSENIC, CADMIUM, AND ZINC EXPOSURE THROUGH RICE CONSUMPTION IN BANGKOK. ADVISOR: PENRADEE CHANPIWAT, Ph.D., 83 pp.

The objectives of this study were to 1) determine the levels of arsenic (As), cadmium (Cd), and zinc (Zn) in rice sold in local markets of Bangkok using an inductively coupled plasma – mass spectrometry (ICP-MS) and an inductively coupled plasma – optical emission spectrometry (ICP-OES) 2) assess both non-carcinogenic and carcinogenic effects as a result of rice consumption on a daily basis, and 3) determine the bioavailability of metals in rice. The average total metal concentrations (average ± SE) in rice (n=97) were 0.2049 ± 0.0081 mg kg^{-1} for As, 0.0189 ± 0.0012 mg kg^{-1} for Cd, and 19.79 ± 0.80 mg kg^{-1} for Zn. Concentrations of As in rice grain were influenced by type of rice in which brown jasmine rice was found with the highest As contents. In addition, As was the only substance showing the hazard quotient (HQ) value above the threshold level (HQ > 1). HQ values of As in adults ranging from 1.17 to 2.61 for different types of rice consumption. The average HQ values of Cd and Zn exposure in adults ranged from 0.04 to 0.09 and 0.11 to 0.28, respectively. Comparing to the other age groups, the highest HQ values for each rice type consumption were found in children. Moreover, HQ values of male were usually in the higher level than female. The annual excess lifetime cancer risk (AELCR) caused by As exposure with the value of 3×10^{-8} (acceptable range 10^{-6} -10^{-4}) indicated a low possibility of cancer development. Finally, the bioavailable As concentrations were found to be 15.7% in white jasmine and 33.3% in brown jasmine rice.

Field of Study:	Hazardous Substance and Environmental Management	Student's Signature	....
		Advisor's Signature	....
Academic Year:	2015		

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Finally, I must express my very profound gratitude to my family for providing me with unfailing support and continuous encouragement throughout my years of study.

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CHAPTER I

INTRODUCTION

1.1 Background of the study

As a source of carbohydrate, essential nutrients, vitamin, and amino acid, rice is a staple food of many countries around the globe (Bouman et al., 2007). Consumption of rice and rice products estimated by Wailes and Chavez (2015) ranges from 6.3 kg to 244.3 kg per capita per year. This wide variation in rice consumption was mainly due to food culture and cultivation of each country. In case of Thailand, in particular, the consumption rate of rice for adult is about 161.3 kg per capita per year. Due to its high consumption rate and food safety issues, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme (JFST) has recommended maximum levels of several contaminants such as pesticides, mycotoxin and some heavy metals contained in rice (CODEX, 2014;Ahmed et al., 2015). Recently, arsenic (As) and cadmium (Cd) in rice with levels exceeding the Codex guideline of 0.2 mg kg^{-1} and 0.4 mg kg^{-1}, respectively, were reported in several countries such as Bangladesh (Ahmed et al., 2015), Iran (Naseri et al., 2015), Japan (Tsukahara et al., 2003) and China (Qian et al., 2010). Moreover, few researchers found that type of rice can also affect the concentration of heavy metals in rice grain (Meharg et al., 2008). Therefore, heavy metals especially As, Cd, and Zinc (Zn) contained in the main type of rice consumed in Bangkok were taking into account in this recent research.

Once consumed, these metals can be accumulated and cause negative health impacts. For examples, As could cause melanosis, hyperkeratosis, restrictive lung, and peripheral vascular diseases (Das et al., 2004). Chronic Cd exposure may lead to renal dysfunction and itai-itai disease (Nordberg, 2004) while, Zn can cause genetic activity dysfunction (ATSDR, 2005b). Therefore, human health risk of rice consumption should be assessed using the exposure rate of metals to the oral reference dose (RfD) suggested by the Integrated Risk Information System (IRIS) of US EPA. In general, adverse health impacts are expected when the hazard quotient (HQ) is higher than 1 (IPCS, 2004). Though, risk assessment is basically based on total concentrations of contaminants of health concerned, for this case, As, Cd, and Zn, these exposures and risk assessment can only provide a gross idea of health impacts to individuals who generally consume rice on a daily basis. Hence, analysis and assessment of bioavailable concentrations of metals were introduced for the more accurate concentrations of metals exposed by any individual via rice consumption (Omar et al., 2013). This present study was conducted to assess the human health risk of As, Cd, and Zn exposure through rice consumption. Hypotheses and objectives of this study are presented as follow.

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1.2 Objectives

- To determine total As, Cd, and Zn concentrations in rice sold in Bangkok.
- To assess both non-carcinogenic and carcinogenic human health risks of As, Cd, and Zn exposure through rice consumption on a daily basis.
- To determine the bioavailability of As in rice.

1.3 Hypotheses

- Ranges of As, Cd, and Zn contained in different types of rice will be different.
- The highest concentrations of all metals of interests will be detected in brown (non-polished) rice.
- Bioavailable concentrations will be accounted for more than 50% of their total concentrations.
- Hazard index (HI) higher than 1 with the negative health effects of As, Cd, and Zn exposure through rice consumption are expected to be determined in population who consume rice on a daily basis.

1.4 Scope of Study

This research can be divided into three parts (Figure 1) as following.

First, the determination of total concentrations of As, Cd, and Zn in raw rice was firstly conducted. Rice samples were randomly collected from 8 representative local markets in Bangkok. Types of rice samples collected were selected based on the amount of consumption. The total number of samples collected was calculated following the sample size estimation recommended by Israel (2013). Once collected, samples were ground and acid digested for the analyses of total concentrations.

After that, human health risk assessment was conducted using total concentrations obtained from the first step of the study to indicate the non-carcinogenic and carcinogenic risks developed from rice consumption.

Finally, representative samples were selected and analyzed for bioavailable concentrations using *in-vitro* digestion method recommended by Versantvoort et al. (2004)

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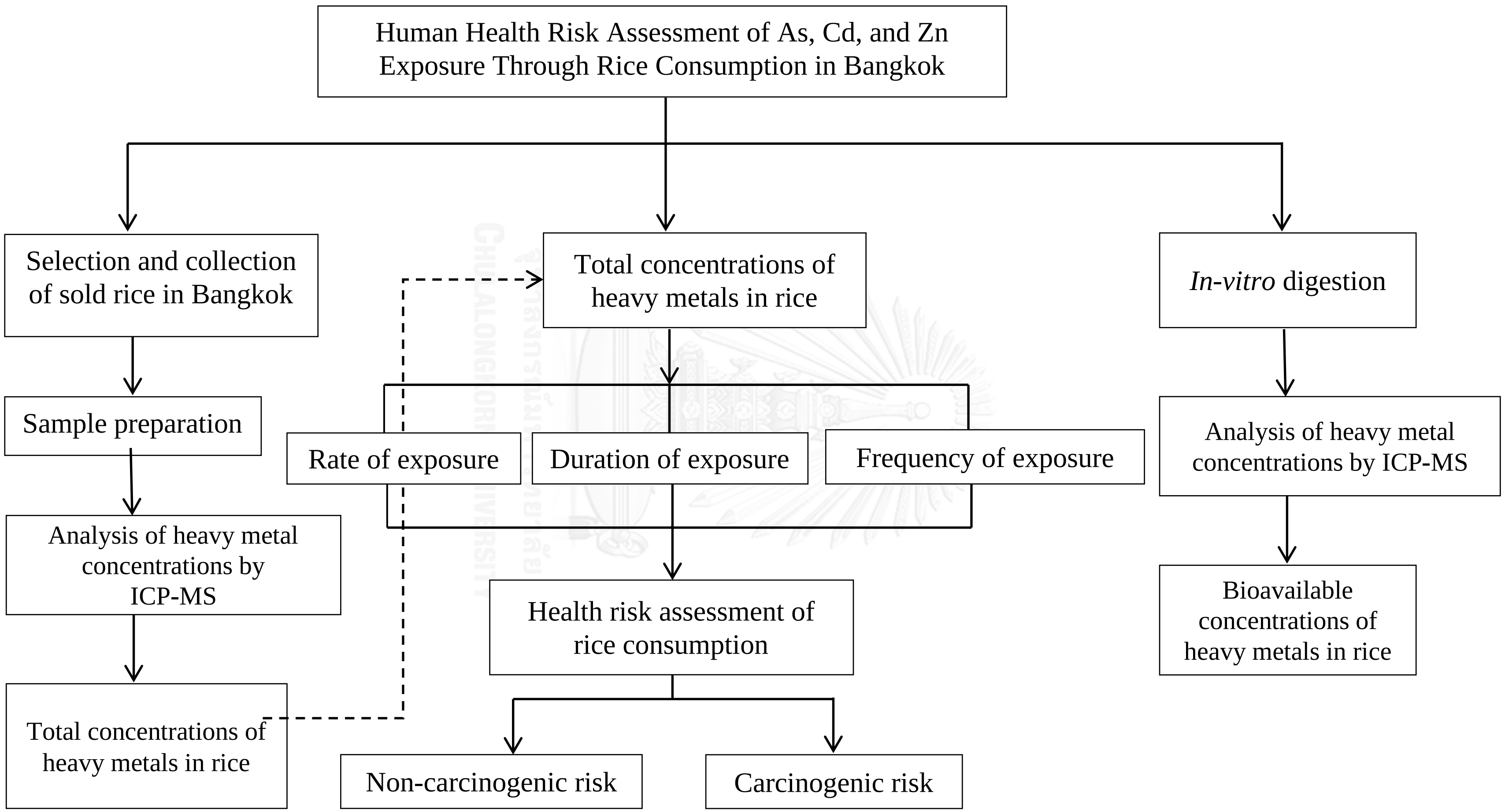

Figure 1 Schematic diagram of a research framework

CHAPTER II

LITERATURE REVIEW

There are five main parts of the following topics in the literature review: i) heavy metal contamination in the environment, ii) heavy metal exposure, iii) heavy metal contamination in rice, iv) health risk assessment of rice consumption, and v) bioavailability of heavy metal in rice.

2.1 Heavy metal contamination in the environment

Even though metals can occur naturally as mixtures in various environmental media (U.S. EPA, 2007), they are mainly released into the environment by human activities such as mining, industrial, and transportation (Bhat and Gómez-López, 2014). Due to their toxicity and abundance, EPA has listed following elements as the metals of concern; aluminum (Al), antimony (Sb), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), thallium (Tl), vanadium (Va), and zinc (Zn) (Langmuir et al., 2004). Six of these elements were described in this study because of their poisonous toxicity. In addition, there have been epidemiological reports on adverse health impacts from their contamination around the globe.

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2.1.1 Arsenic (As)

As can be found in the environment. It is released to the environment through either natural or human activities. It is usually found as combined substances in both inorganic and organic form (ATSDR, 2007). An urgent issue about As contamination in drinking water in Bangladesh reported by the World Health Organization (WHO) estimated that millions of population have died from cancer developed in skin, lung, liver, and bladder from chronic As exposure (Smith et al., 2000). A darkening of the skin or melanogenesis on the palms and soles is the well-known effects of long-term low level exposure. Not only chronic effects of As exposure, but low level of As exposure also cause acute health effects such as nausea, vomiting, diarrhea, decreasing production of red and white blood cells, and a sensation of pins and needles in hand and feet (ATSDR, 2007). To the worst case, very high level of As exposure can possibly result in death (Martin and Griswold, 2009). Therefore, this element is known as one of the highest toxic elements.

Not only groundwater contamination, but As can also contaminate the air, soil, and food and consequently cause human health impacts. It has been reported that the toxicity of As is depending on its forms and oxidation states. In general, the toxicity of As decreases following these order; As (III) > MMAO (III) > DMA (III) GS > DMA (V) > MMA (V) > As (V) (Mandal and Suzuki, 2002).

2.1.2 Cadmium (Cd)

Though, it can be found in the earth's crust, it is easily released to the environment from the industrial processes, mining, fossil fuel combustion, and waste disposal. When Cd is emitted, it can be accumulated in plant and aquatic organisms and affected to human health (ATSDR, 2012a). Recently, there are several reports about Cd contamination in edible plants such as, rice (Fang et al., 2014), soybean (Sriprachote et al., 2012) and some tubers, legumes, and cereals (Akinyele and Shokunbi, 2015). Because of its long half-life, Cd is able to remain in human body more than decades (Yuan et al., 2014). Its chronic effect is related to renal dysfunction and bone fragility. Itai-itai disease is the well-known health impact of chronic Cd exposure. Moreover, this element is likely to increase blood pressure in animals (Satarug et al., 2006).

2.1.3 Chromium (Cr)

Cr can also be found naturally in the environment. It can also be released from the industrial processes, for example metal processing, tannery facilities, chromate production, stainless steel welding, and chrome pigment production (KotasÂ and Stasicka, 2000). Normally, Cr presents in the environment in the form of Cr (III) and Cr (VI). The toxicity of Cr (VI) is higher than Cr (III) due to the uptake ability of human body (ATSDR, 2012b). Moreover, solubility, mobility and bioavailability are the main factors that cause Cr (VI) to be easily distributed and transported in the environment when compare to Cr (III) (KotasÂ and Stasicka, 2000). In generally, Cr causes acute effects to respiratory system including shortness of breath, coughing, and wheezing. Chronic effects of Cr are perforations and ulcerations of the septum, bronchitis, decreased pulmonary function, pneumonia, and other respiratory effects (U.S. EPA, 1998)

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2.1.4 Nickel (Ni)

Ni contamination in the environment can be caused by both natural sources (such as volcano explosion) and anthropogenic sources. Ni is released to the atmosphere through mining and industrial processes. Ni is frequently used in the production of battery, stainless steel, coin, and jewelry. Furthermore, a minute amount of Ni is essential for the production red blood cells (ATSDR, 2005a). However, Ni has been reported, in some cases, to damage DNA through reactive oxygen species (Bhat and Gómez-López, 2014). In human who are sensitive to Ni, the most common harmful acute health effect is an allergic skin reaction. For chronic effect, Ni can cause many impacts to body functions including systemic, immunologic, neurologic, reproductive, developmental, or carcinogenic effect (Das et al., 2008).

2.1.5 Selenium (Se)

Se is not often found in its elemental form, but it can be found in complex form with the other metals, for example, Se in rocks is combined with sulfide minerals or with Ag, Cu, Pb, and Ni minerals. Therefore, Se is usually a by-product of copper refinery. This metal is generally used to produce some photographic devices, plastics, paints, anti-dandruff shampoo, vitamin and mineral supplements, fungicides, and certain types of glass (ATSDR, 2003). In addition, Se is an essential trace element for human. However, if human exposes to high concentrations of Se, damage to the nervous system, fatigue, and irritability can be developed (Bhat and Gómez-López, 2014).

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2.1.6 Zinc (Zn)

Zn is one of the most common elements in the earth's crust and it is also an essential element to human body. It can be found in soil, air, water, and food. This metal compound is mainly used in pharmaceutical and personal care industry (e.g. ingredient of supplements, deodorant, sun block and antidandruff shampoo) (ATSDR, 2005b). Even, Zn is needed for human health, high dose of its might potentially induce dysfunction of genetic activity as an acute effect (Bhat and Gómez-López, 2014). While a decrease in erythrocyte Cu - Zn superoxide dismutase in adult male and female is reported to be the chronic effect of Zn exposure (U.S. EPA, 2005).

Once those heavy metals are released and circulated in the environment, they can consequently be accumulated in soil, water, and air. Due to their long half-life and persistent characteristic, heavy metals can be contaminated to various types of food such as crops, insects, or seafood. Thus, these heavy metals can, then, be transferred and accumulated in humans, as a top consumer, as well. Owing to contaminated heavy metals in several environmental media, routes of exposure are also the important factor affecting to the toxic effects of heavy metals on human health.

2.2 Heavy metal exposure

The definition of exposure explained by U.S. EPA (1992) is a chemical condition contact with a human's outer boundary. As heavy metals, presently, are contaminated to air, water, soil, and other environmental media, human may expose to heavy metals when comes into contact with these environments (U.S. EPA, 1992).

Exposure routes in which heavy metals can enter human body are including (UNL, 2003);

2.2.1 Inhalation

The main exposure route of vapors, gases, mists, and particulates is an inhalation. Normally, the inhaled chemical is usually excreted from human body through exhalation except for small particles. The particles with the size of less than or equal to ten micrometers in diameter (PM_{10}) can be transferred into the respiratory tract and affect human health (U.S. EPA, 2007). Some metals, in particular, with longer half-life cannot be excreted or removed from the respiratory tract and finally cause serious health impacts especially to the sensitive organs.

For example, smoking is the major source of human exposure of Cd via air. A review reported that an approximately 50% of inhaled Cd containing cigarette smoke is absorbed in the lung (Järup and Åkesson, 2009).

Another example of heavy metal exposure via inhalation is a chronic occupational exposure of Cr (VI) which may cause lung cancer in workers. In order to limit adverse effects, the Occupational Safety & Health Administration (OSHA) has set up the permissible Cr (VI) exposure limit (PEL) of 5 µg m^{3-} (OSHA, 2009).

2.2.2 Dermal absorption

Heavy metals can also cause adverse health effects to human health by crossing the skin barrier and being absorbed into the blood system (UNL, 2003). Though, heavy metals are rarely absorbed through the skin, there were some researchers reported that metals may induce toxic and sensitization effects on the skin.

For example, Cr (VI) compound can cause skin burning due to their corrosive characteristic (U.S. EPA, 2007).

2.2.3 Ingestion

Any substance which is swallowed or eaten can get into a human gastrointestinal tract. Generally, the substance, except a corrosive and irritating chemical, does not directly affect the tract. The ingested substance is absorbed through the gastrointestinal tract, transported by the blood system, and finally causes impacts to human health (UNL, 2003). U.S. EPA reported that ingestion pathway is the main route of metal exposure especially from the ingestion of contaminated drinking water and food (U.S. EPA, 2007).

For example, it was reported that consumption of As contaminated groundwater with concentration more than 50 μg L^{-1} has caused As poisoning effects in millions of population. In addition, another source of As exposure was consumption of contaminated food.

Food is a major exposure route of heavy metal (Choudhury et al., 2001). Once uptake, metals can be accumulated and magnified in both animals and plants (Shahbaz et al., 2013). Humans as the top consumer of food chain are, therefore, at risk of higher dose of metals accumulation. Recently, there are many studies reported about heavy metals contamination in various types of foods. For example, total As, toxic (As (III) and As (V)), and non-toxic (MA, DMA, AB, and AC) As species have been found in different raw seafood samples including white fish, cold water fish, and mollusks. The range of total As in seafood was 0.37 mg kg^{-1} – 34.9 mg kg^{-1} (dry weight). The major group of As species in seafood is organic ones. The bioavailable As (III) and As (V) ranged between 87% – 106% and 90% – 113%, respectively

(Moreda–Piñeiro et al., 2012). In addition, fish was also found to be contaminated with heavy metals. Ten fish species of Bangshi River in Bangladesh was contaminated with eight heavy metals including Pb, Cd, Ni, Cr, Cu, Zn, Mn, and As. The study reported that Zn was the most accumulated metal in fish muscles. However, the concentrations of other seven metals (except Pb) did not exceed the safe limits proposed by various agencies. (e.g. Australian National Health and Medical Research Council, Australian and New Zealand Food Standards, Western Australian Food and Drug Regulations etc.) (Rahman et al., 2012). Not only, heavy metal is accumulated in animals, but it can also be concentrated in plant and fungi. Even concentrations of most metals were below the threshold levels of the food quality standard, high consumption rate of heavy metals contaminated food might cause adverse effects to human health (Fang et al., 2014;Akinyele and Shokunbi, 2015;Ye et al., 2015). Rice, one of edible plant species, is a staple food of the world. Due to the high consumption rate, human may intake higher concentrations of the heavy metals via its ingestion.

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2.3 Heavy metal contamination in rice

The consumption of a contaminated crop is the major route in which human can be exposed to metals because crop is a staple food of the world. Particularly, rice (*Oryza sativa L.*), a crop which is cultivated in many areas of the world, is a main energy source of human body function. However, rice, sometimes, can accumulate heavy metal as a result of irrigation of contaminated water and cultivation on contaminated land (Meharg and Rahman, 2003). A heavy metal of most concerned which is contaminated in rice is As. There are many scientific reports about As contaminated rice in various regions. For instance, As contamination in rice was the major route of metal exposure in West Bengal of India. The concentration of As in raw and cooked rice were 0.13 mg kg^{-1} and 0.17 mg kg^{-1} respectively. Risks of cancer developed from rice consumption in that area were found to be higher than the threshold levels recommended by U.S. EPA (Mondal and Polya, 2008).

After the discovery As contamination in rice, concerns on the other heavy metals contamination in rice grain have been raised. For the past ten years, a number of countries have investigated the contamination of several heavy metals in rice. As shown in Table 1, heavy metal concentrations in rice studied in seven countries were reported. As clearly shown in Table 1, it can be concluded that concentrations of heavy metals in rice are site specific depending on country of origin. Wide ranges of metal concentrations were found. In addition, different levels of concentrations can be observed in rice collected from different countries. For instance, Australian rice was found with the lowest concentration of Cd. Rice in Bangladesh was reported with highest concentration of As, while, rice of China and Spain were found with the same magnitude of As. In the case of Zn concentrations, Australian rice contains higher Zn level than Bangladesh rice.

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Table 1 Concentrations of heavy metals in raw rice

Country	Elements	Average concentration (mg kg^{-1})	Range (mg kg^{-1})	References
Australia	Cd	0.008	<0.005 – 0.017	Rahman et al. (2014)
	Cr	0.144	0.015 – 0.465	
	Pb	0.375	0.016 – 1.248	
	Ni	0.166	0.061 – 0.356	
	Zn	17.1	10.9 – 24.5	
Bangladesh	As	0.321	–	Ahmed et al. (2015)
	Cd	0.088	–	
	Cr	0.183	–	
	Zn	13.2	–	
	Se	0.026	–	
China	As	0.119	<0.008 – 0.490	Qian et al. (2010)
	Cd	0.050	<0.001 - 0.740	
	Pb	0.062	<0.005 - 0.40	
	Hg	0.006	<0.00002 - 0.031	

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Table 1 Concentrations of heavy metals in raw rice *(Cont.)*

Country	Elements	Average concentration (mg kg^{-1})	Range (mg kg^{-1})	References
Kuwait	As	–	0.053 – 0.380	Jallad (2015)
	Cd	–	0.005 – 0.170	
	Pb	–	< 0.010	
	Hg	–	< 0.010	
Qatar	As	0.096	0.010 – 0.258	Rowell et al. (2014)
	Se	0.103	<0.006 – 0.422	
	Zn	0.013	0.003 – 0.030	
Spain	As	0.188	0.058 – 0.406	Torres-Escribano et al. (2008)
United State	As	0.280	0.162 – 0.710	Zavala and Duxbury (2008)

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Moreover, the localization of heavy metals in rice grain was reported. A study of Meharg et al. (2008) reported the difference in As concentration and localization in brown and white rice. Higher As concentrations were determined in brown rice. The results of S-XRF analyses showed that As was, particularly, localized at the surface between pericarp and aleurone layer of brown rice (Figure 2 (a)). In contrast to brown rice, dispersion of As throughout the rice grain was found in white rice (Figure 2(b)). In case of Cd, similar Cd distribution patterns were identified in both types of rice (Figure 3 and Figure 4). Cd, generally, localized in the endosperm in both brown and white rice. Meanwhile, Zn was found to be distributed in the similar manner as As in which it commonly localized between pericarp and aleurone layer. According to the earlier findings, Meharg et al. (2008) concluded that rice polishing process could not affect the distribution of Cd in rice grain instead it affected the distribution of As and Zn in rice grain.

In addition, concentrations of metals found in parts of rice grain were reported by Lombi et al. (2009). The hulk was the part that contained the highest As concentration. The decreasing levels were found in bran and endosperm, respectively. In addition, highest Zn concentration was mainly found in the central part of the embryo. Levels of Zn in rice were found to be decreased from the outer parts to inner part of the endosperm. Therefore, it was conclude that the removal of the pericarp and aleurone layer can reduce the Zn content in rice grain (Figure 3 and Figure 4).

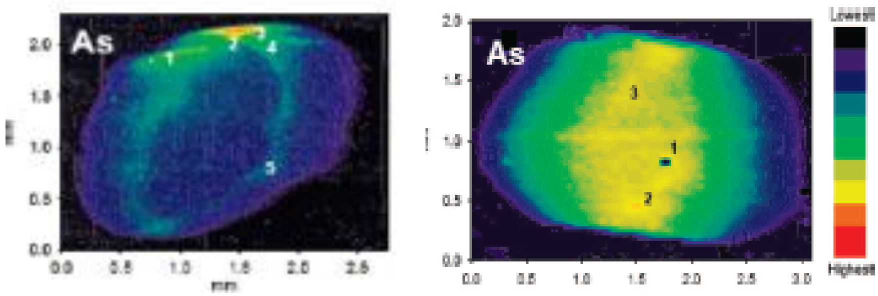

Figure 2 S-XRF elemental maps of As in (a) brown and (b) white rice

Source: Meharg et al. (2008)

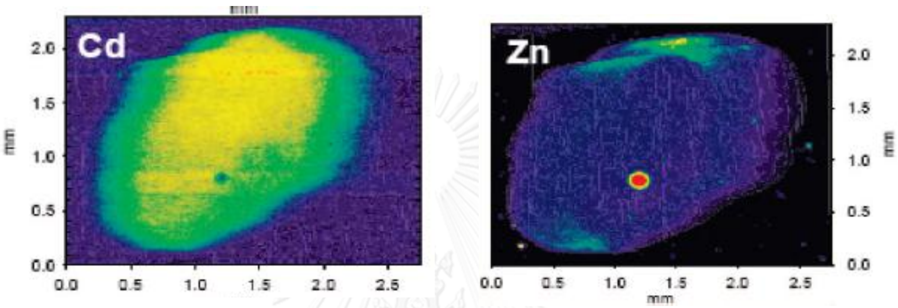

Figure 3 S-XRF elemental maps of Cd and Zn in brown rice

Source: Meharg et al. (2008)

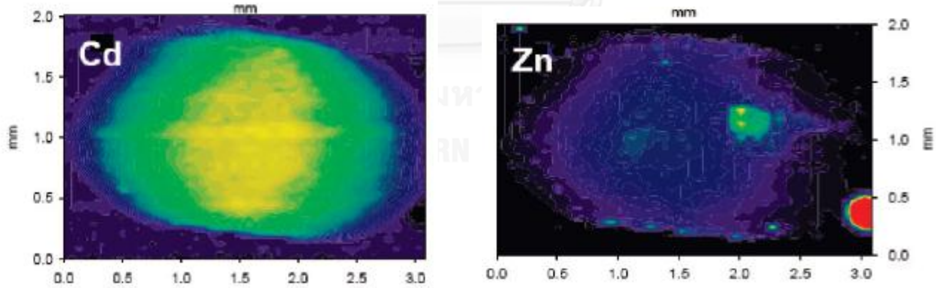

Figure 4 S-XRF elemental maps of Cd and Zn in white rice

Source: Meharg et al. (2008)

2.4 Health risk assessment of rice consumption

Risk assessment is a process which is used to calculate or estimate the risk of population who expose to particular agents (EFSA, 2010). Theoretically, there are four main steps of risk assessment (Figure 5) as following (IPCS, 2004);

1. Hazard identification

This step is used to identify the type and nature of adverse health effects of a chemical.

2. Hazard characterization

This step is used to analyze the relationship between total amount of an agent administered to, taken up by, or absorbed by an organism, system, or population and the changes developed in that organism, system, or population in reaction to that agent.

3. Exposure assessment

This step is used to evaluate the concentration or amount of an agent which attains a target population.

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4. Risk characterization

This final step is used to gather all of information and advice for decision-making.

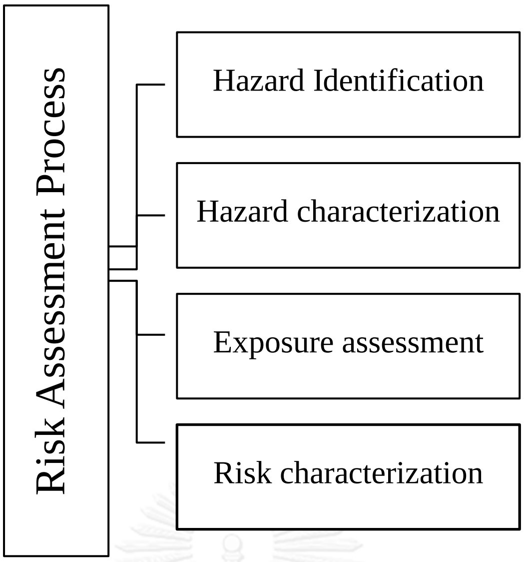

Figure 5 Four steps of risk assessment

In principle, hazard can be identified based on the status and quality of the environment which could cause the negative health effects after exposure. In this study, hazards of heavy metal contamination in different environmental media were reported in many parts of Thailand. For example, As groundwater contamination in Ron Phibun, Nakorn Si Thammarat was found to be ranged from 1.25 to 5,114 µg L^{-1}. About 70% of groundwater wells were found with As concentration exceeded WHO drinking water standard (10 µg L^{-1}) (Kim et al., 2011). The contaminated groundwater was also sometimes used for agricultural purpose. Therefore, As was found to be contaminated in several edible plants grown in that area as well. In addition, Cd contamination in cultivated rice was also found in Mae Tao, Tak. Unhusked rice containing Cd concentrations (0.04 to 1.75 mg kg^{-1}) higher than CODEX standard was also reported (Sriprachote et al., 2012). Therefore, the critical health effects of Cd exposure as shown in Table 2 were also diagnosed in population who live in the contaminated area.

For the hazard characterization, the second step of risk assessment, the information which is necessary for hazard characterization can be obtained from the Integrated Risk Information System (IRIS) of the U.S. Environmental Protection Agency (U.S. EPA). Additionally, IRIS also established the reference doses (RfD) of chemicals (Table 2) for the overall assessment of health risks.

Basically, human health risk can be assessed using the exposure rate and the reference dose (RfD) of chemical intake. It is strongly believed that when the intake rate of particular chemical is higher than the RfD, the critical effects usually occur. Adverse health impacts of metals and their RfD values are summarized in Table 2. As clearly shown in Table 2, only As is classified as carcinogen.

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Table 2 Chronic human health effects and oral reference dose (RfD) of metals

Metal	Critical effects	Carcinogenic classification	RfD ($mg\ kg^{-1} day^{-1}$)	Slope factor ($(mg\ kg^{-1} day^{-1})^{-1}$)
Inorganic As	Hyperpigmentation, keratosis, and possible vascular complications	Human carcinogen	3×10^{-4}	1.5
Cd	Significant proteinuria	Probable human carcinogen	1×10^{-3}	-
Cr (III)	No effects observed	Not classified as human carcinogen	1.5	-
Cr (VI)	No reports on its effects	Not classified as human carcinogen	3×10^{-3}	-
Ni (soluble salt)	Decreased body and organ weights	-	2×10^{-2}	-
Se	Clinical selenosis	Not classified as human carcinogen	5×10^{-3}	-
Zn	Decreases in erythrocyte Cu, Zn-superoxide dismutase (ESOD) activity	-	0.3	-

Note: “-” means no data.

Exposure rate of ingestion can be calculated using Eq. 1

$$\text{Exposure rate} = [C \times IR \times ED] / [BW \times AT] \quad \text{(Eq. 1)}$$

Where;

C = Concentration of chemical per mass of food (mg kg^{-1})

IR = Mass of food in contact with the body (kg $year^{-1}$)

ED = Period of time the person is in contact with chemical (year)

BW = Body weight over the averaging time (AT) (kg)

AT (Averaging time) = Period of exposure (day)

Once exposure rate is calculated, both non-carcinogenic and carcinogenic health risks can be calculated using Eq. 2 and Eq. 3, respectively. This step of calculation can be used to evaluate the overall risks related to the ingestion exposure.

$$HQ = \text{Exposure rate} / RfD \quad \text{(Eq.2)}$$

Where;

Exposure rate = Estimated chemical intake

RfD = Oral reference dose of chemical

$$AELCR = [\text{Exposure rate} \times SF] / DL \times 365 \quad \text{(Eq.3)}$$

Where;

AELCR = Annual excess lifetime cancer risk

SF = Cancer slope factor ((mg $kg^{-1}day^{-1})^{-1}$)

DL = Average human longevity (year)

365, a constant value, is the average number of days per year

In general, the result obtained from risk characterization, hazard quotient (HQ), can be compared with the number of 1. If HQ value is lower than 1, it indicates no apparent risk from the chemical over a lifetime of exposure. Whereas, HQ value is higher than 1 indicates the toxic adverse effects to human cause by ingestion of the chemical. The HQ values are normally additive number that can explain which one is a larger number. It is not the multiplicative number. Therefore, a HQ value of 10 is not ten-fold greater than HQ of 1. Meanwhile, the annual lifetime excess cancer risk (ALECR) is a value indicating the excess probability of cancer development caused by carcinogenic chemicals exposure over a lifetime. That is, for example, ALECR of 3×10^{-6} indicates the probability of 3 of 1,000,000 people that may be subjected to cancer effects.

Presently, there are several studies estimated health risk of rice consumption in many areas of the world. Ke et al. (2015), for example, reported about Cd contamination in rice of China in which HQ values varied between 1.5 and 7.8. Other study also reported health risk of metals exposure through rice consumption especially in Fuzhou of China. HQ values of heavy metals (Pb, Cd, Cr, Cu, As, and Ni) exposure were lower than 1 for non-carcinogenic risk. On the other hand, carcinogenic risk from As exposure was about 3.5×10^{-4} which was higher than an acceptable range of 10^{-6} to 10^{-4} (Fu et al., 2015). Though, HQ can represent the overall risks of chemical exposure, more accurate results can be obtained by using the fraction of heavy metal that is transported and absorbed to human body. Therefore, bioavailable concentrations of metals in food can be used to facilitate the idea of the portion of metals which is digested, absorbed, transported, and eliminated by the gastrointestinal system (Romarís–Hortas et al., 2011).

2.5 Bioavailability of heavy metal in rice

Food ingestion is the main route of human exposure to heavy metals. Some of certain amounts, bioavailable fraction, of metals are readily absorbed to the body. Therefore, the term of bioavailability is usually used to explain the situation when the proportion of the contaminated food is taken to the human body and reached the organism system. Another term that involves with availability of heavy metal is bioaccessibility which is used to describe the contaminant that is released from its matrix during the process of digestion (Versantvoort et al., 2004). Presently, there are two methods, *in-vivo* and *in-vitro*, which are normally used to estimate bioavailable concentration of metals to human body. *In-vivo* method uses animal models to estimate the uptake concentration of contaminant to human body. For example, a group of researchers used in vivo swine model for determination of As bioavailability in vegetables. Briefly, the As concentration in blood plasma of swine was monitored. It was found that about 50% to 100% of the As concentration was absorbed. Even though, the accurate results are obtained from this process, expensive analytical cost and complexity of animal experiments are the main constraints. Therefore, *in-vitro* method is introduced for the calculation of the bioavailable concentration in human (Juhasz et al., 2006). Though, the detail of digestion method is different depending on each study, there are, in principle, three main steps of the digestion involved including oral (mouth) digestion, gastric (stomach) digestion and intestinal (small intestine) digestion (Hur et al., 2011). These three digestion steps are the main human food digestion and absorption (Omar et al., 2013). Step of digestion, function and enzyme involved are summarized in Table 3.

Table 3 A summary of human digestion system

Steps of digestion	Digestive enzyme	Function
Mouth	Salivary amylase	Carbohydrate digestion
Stomach	Pepsin	Protein digestion
Small intestine	Lipase	Fat droplet digestion
	Peptidases	Breaking down peptide into amino acid
	Bile salt	Breaking down fat into small droplets

The *in-vitro* digestion method was used to analyze bioavailable concentration of heavy metals in several foodstuffs, for example, seaweed (Romarís–Hortas et al., 2011), seafood (Moreda–Piñeiro et al., 2012), and rice (Omar et al., 2015).

Several *in-vitro* digestion models were suggested by several researchers in order to obtain the most accurate bioavailable concentration. For example, the Physiologically Based Extraction Test (PBET) and Method E DIN 19738 used two digestion steps, gastric phase and small intestine phase, with different digestion time to estimate bioavailable concentration. Meanwhile, the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) was used to check bioavailable metal in the gastrointestinal system of young children. The TNO intestinal model (TIM), a dynamic model, with the continuous addition of enzyme and changing pH, was also introduced. However, the *in-vitro* digestion model that is believed to be the most appropriate digestion model for rice is RIVM model (Van de Wiele et al., 2007;Omar et al., 2013).

In particular, the *in-vitro* digestion (artificial gastrointestinal fluid) method with high total As contained in rice was published by He et al. (2012). This study found that the percentages of bioavailable of As were ranged from 53% to 102%.

Moreover, the *in-vitro* digestion model was used to extract the bioaccessible fraction of Cd in rice. The uncooked rice cultivated in mining and non-mining areas were determined with 52.49 μg kg^{-1} and 7.93 μg kg^{-1} of bioaccessible Cd, respectively. Regarding to total Cd concentration, the bioaccessibility of Cd in rice was lower than 17%.

The majority of researches commonly use total concentrations of heavy metals as the estimation of heavy metal ingestion by human through rice consumption. Nonetheless, this is not the actual concentration of metals that human can be exposed. Instead, the bioavailability is used for calculation of the accurate concentration of contaminant that can get into the human body. Omar et al. (2015), as an example, studied the bioavailability of Cd, Cu, Cr, Co, Al, Fe, Pb, As, and Zn in rice and found that Zn and As concentrations were the highest and lowest bioavailable metals in cooked rice, respectively. The levels of HQ for mixtures of metals exposure in adult were higher than 1, an acceptable level of HQ (Omar et al., 2015).

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CHAPTER III

METHODOLOGY

3.1 Sample collection

A total of 97 rice samples regarding the sample estimation recommended by Israel (2013) were collected from eight representative local markets in Bangkok (Figure 5). Regarding to the amount of rice consumption, types of rice collected were white jasmine, brown (non-polished) jasmine, white and glutinous rice (Figure 6). About 300 grams of each rice sample were collected in a clean zip lock bag. Then, they were kept in a refrigerator at 4°C until further sample preparation. During sample collection, information on areas of rice cultivation was also recorded. Rice samples collected in this study were mainly cultivated in 3 regions of the country including central, north eastern, and northern parts.

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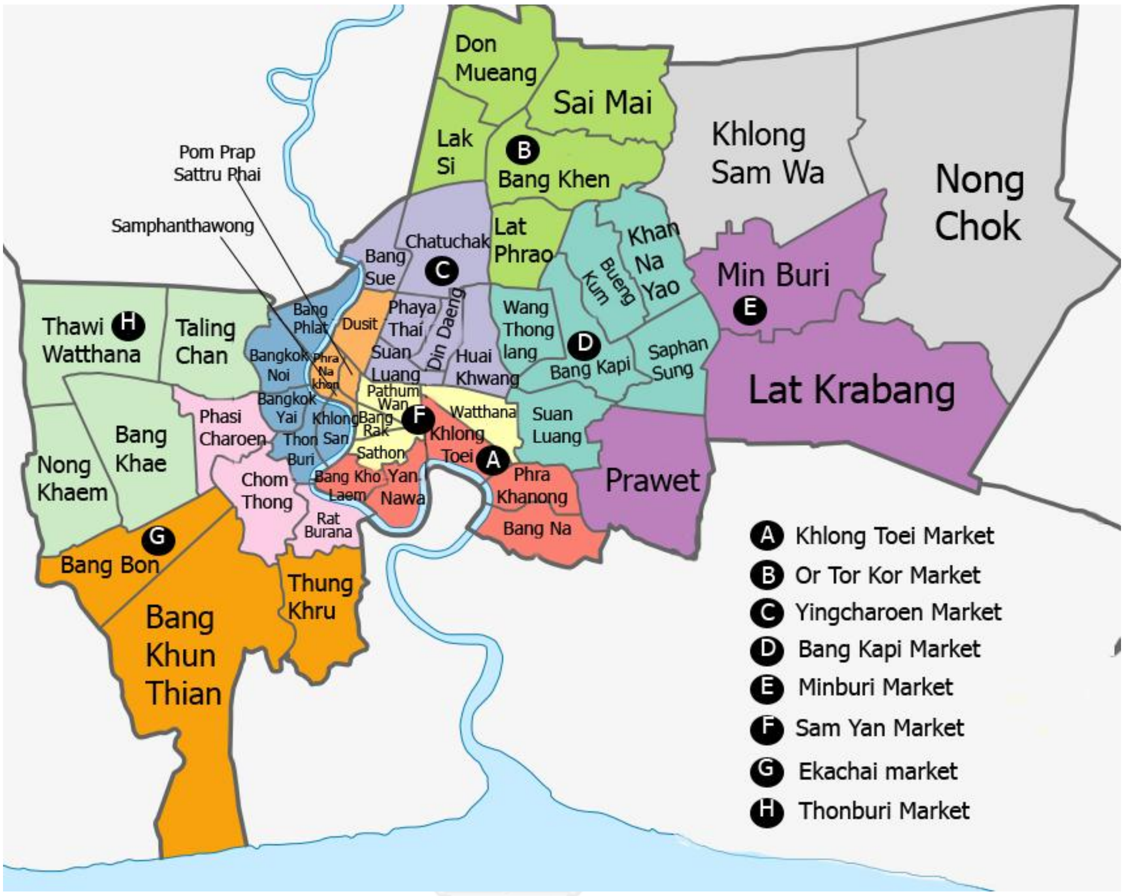

Figure 6 A map showing locations of market for rice sample collection
Source: http://thehistoryofsiam.blogspot.com/2013/01/blog-post_12.html
(Accessed on January 3, 2016)

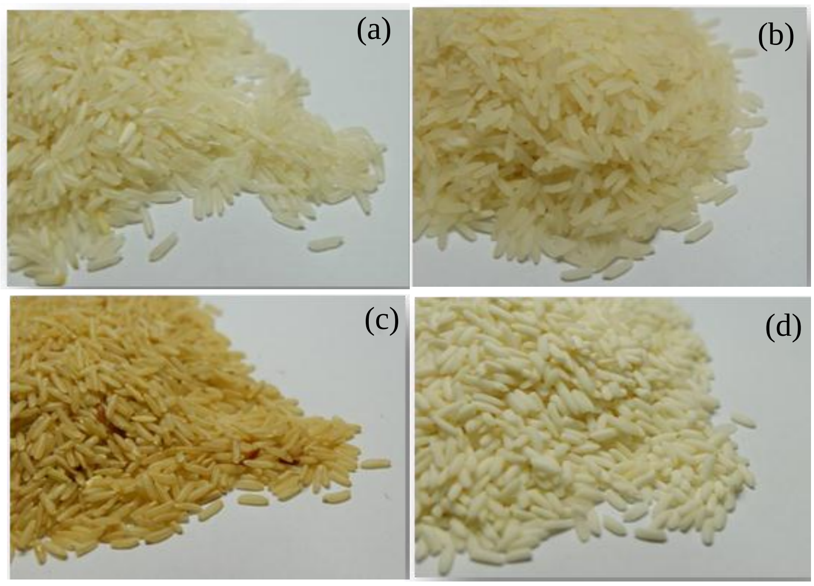

Figure 7 Pictures demonstrating collected samples
(a) white jasmine, (b) white, (c) brown jasmine, and (d) glutinous rice

3.2 Sample preparation and digestion for total metal concentrations

All rice samples were ground by a mortar and a pestle and passed through a 40 mesh sieve. Then, all samples were dried at 80 °C in an oven until a constant weight was obtained. Next, samples were sealed in a clean plastic tube and stored in a desiccator until analyses.

For sample digestion for total metal concentration, an acid digestion method following Phan et al. (2013) was conducted. In brief, about 0.10 g of rice sample was weighed into a 15 mL polyethylene tube. Then, 1 mL of concentrated nitric acid (superpure grade for trace analysis) was added into the tube. After that, the tube was capped and left in a hood at room temperature for 48 hours. Next, 9 mL of deionized water was added into a tube containing acid digestion solution. Finally, the final solution was filtrated through a 0.45 µm syringe filter to obtain a final solution for the analyses of total metal concentrations. These solutions were kept at 4 °C until analyses.

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3.3 Digestion for bioavailable metal concentrations

The bioavailability of heavy metal in rice was studied using the *in-vitro* digestion method proposed by Versantvoort et al. (2004). This digestion method can be divided into three types of digestion system, including 1) the digestion in mouth by saliva, 2) the digestion in stomach by gastric juice, and 3) the digestion in small intestine by duodenal juice and bile salt. To conduct those digestion systems, the following steps were conducted (Table 4).

Briefly, about 4.5 grams of rice sample was firstly incubated for 5 minutes with 6 mL of saliva. Then, the sample was left for another 2 hours after 12 mL of gastric juice was added. Next, it was left for another 2 hours after 12 mL of duodenal juice, 6 mL of bile salt, and 2 mL of HCO^{3-} were added. All digestive systems were performed at 37 °C with an orbital shaking. Afterward, the solutions were centrifuged for 5 minutes at 3000 rpm, to enable complete separation of the supernatant and the precipitate phase. Finally, the supernatant solutions were kept at 4°C until analyses.

Table 4 An *in-vitro* digestion method for the analysis of bioavailable metal concentrations

Digestion step	Digestive juice	Volume of digestive juice (mL)	pH	Time
Mouth	Saliva	6	6.8	5 minutes
Stomach	Gastric juice	12	2-3	2 hours
Small intestine	Duodenal juice	12		
	Bile salt	6	6.5-7	2 hours
	HCO^{3-}	2		

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All digestive juices used in this experiment were freshly prepared. The preparation methods of each digestive juice are summarized as following (Mandak and Nyström, 2012);

Stimulate Saliva

Saliva was prepared from 1 mL of KCl (89.6 g L^{-1}), 1 mL of NaH_2PO_4 (88.8 g L^{-1}), 1 mL of Na_2HPO_4 (57 g L^{-1}), 0.17 mL of NaCl (175.3 g L^{-1}), 0.18 mL of inorganic NaOH (40 g L^{-1}) and 0.8 mL of urea (25 g L^{-1}). Inorganic and organic solutions of saliva were augmented to 50 mL with distilled water. Finally, 14.5 mg of α-amylase, 1.5 mg of uric acid, and 5 mg of mucin was added to saliva.

Stimulate gastric juice

Gastric juice was prepared from 1.57 mL of NaCl (175.3 g L^{-1}), 0.3 mL of NaH_2PO_4 (88.8 g L^{-1}), 0.92 mL of KCl (89.6 g L^{-1}), 1.8 mL of $CaCl_2•2H_2O$ (22.2 g L^{-1}), 1 mL of NH_4Cl(30.6 g L^{-1}), 0.83 mL of inorganic HCl (37% g g^{-1}), 1 mL of glucose (65 g L^{-1}), 1 mL of glucuronic acid (2 g L^{-1}), 0.34 mL of urea (25 g L^{-1}), and 1 mL of organic glucosamine hydrochloride (33 g L^{-1}). Inorganic and organic solutions of gastric juice were augmented to 50 mL with distilled water. Lastly, 0.1 g of bovine serum albumin (BSA), 0.1 g of pepsin, and 0.3 g of mucin was added to gastric juice.

Stimulate duodenal juice

Duodenal juice was prepared from 8 mL of NaCl (175.3 g L^{-1}), 8 mL of $NaHCO_3$ (84.7 g L^{-1}), 2 mL of KH_2PO_4 (8 g L^{-1}), 1.26 mL of KCl (89.6 g L^{-1}), 2 mL of $MgCl_2$ (5 g L^{-1}), 36 µL of inorganic HCl (37% g g^{-1}) and 0.8 mL of organic urea (25 g L^{-1}). Inorganic and organic solutions of duodenal juice were augmented to 100 mL. Finally, 1.8 mL of $CaCl_2•2H_2O$ (22.2 g L^{-1}), 0.2 g of BSA, 0.6 g of pancreatin, and 0.1 g of lipase was added to duodenal juice.

Stimulate bile salt

Bile was prepared from 3 mL of NaCl (175.3 g L^{-1}), 6.83 mL of $NaHCO_3$ (84.7 g L^{-1}), 0.42 mL of KCl (89.6 g L^{-1}), 20 µL of inorganic HCl (37% g g^{-1}) and 1 mL of organic urea (25 g L^{-1}). Inorganic and organic solutions of bile were augmented to 50 mL with distilled water. Then, 1 mL of $CaCl_2•2H_2O$ (22.2 g L^{-1}), 0.18 g of BSA, and 0.6 g of bile salt was added to bile.

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3.4 Sample analyses

All digested solution for both total and bioavailable concentrations were analyzed by an inductively coupled plasma-mass spectrometry (ICP - MS) (Agilent 7500c, Tokyo, Japan) for As and Cd and an inductively coupled plasma-optical emission spectrometry (ICP - OES) (PerkinElmer Optima 4300DV, Waltham, MA) for Zn. The standard reference materials (SRM) of rice flour (NIST SRM 1568a) as well as blank samples were treated with the same manner as sample digestion to verify the accuracy of digestion method. In addition, the SRM of trace element in water (NIST SRM 1643e) was used to ensure the accuracy and precision of the instrumental analyses. The acceptable ranges (±15%) of recovery of all three elements of interest for both digestion method and instrumental analyses were obtained.

3.5 Health risk assessment

The exposure rate of each metal through rice consumption was calculated using Eq.4

$$\text{Exposure rate} = [C \times IR \times ED] / [BW \times AT] \qquad \text{(Eq.4)}$$

Where;

C = Concentration of metal per mass of rice (mg kg^{-1})

IR = Mass of rice in contact with the body (kg $year^{-1}$)

ED = Period of time the person is in contact with metal (year)

BW = Body weight over the averaging time (AT) (kg)

AT (Averaging time) = Period of exposure (day)

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Once the exposure rate of each metal was calculated, the hazard quotient (Eq.5) of each particular metal was estimated to assess the non-carcinogenic risk.

$$HQ = \text{Exposure rate} / RfD \qquad \text{(Eq.5)}$$

Exposure rate = Estimated metal intake

RfD = Oral reference dose

Moreover, hazard index (HI) was also calculated in order to indicate non-carcinogenic risk of all metals exposure through rice consumption. In this study, HI was calculated as a summation of all HQ of metals of interests as shown in Eq.6.

$$HI = \sum HQ \qquad \text{(Eq. 6)}$$

Where;

HI = Hazard index

HQ = Hazard quotient of each metal

Meanwhile, the carcinogenic risk was assessed using Eq.7

$$AELCR = [\text{Exposure rate} \times SF] / DL \times 365 \qquad \text{(Eq.7)}$$

Where;

AELCR = Annual excess lifetime cancer risk

SF = Cancer slope factor ((mg $kg^{-1}day^{-1})^{-1}$)

DL = Average human longevity (year)

365, a constant value, is the average number of days per year

It should be noted that, in this study, the ingestion rate of rice in adult Thai population of 0.141 kg day^{-1} and the average body weight of adult Thai population of 59.3 kg were used (Bureau of Product Standards and Quality Systems, 2006). The average Thai life expectancy is 75 years (WHO, 2012).

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3.6 Statistical analyses

All statistical analyses were performed using the IBM SPSS Statistical Package Version 22. Normality was confirmed by the Kolmogonov-Sminov test ($n > 50$) for all rice samples and Shapio-Wilk test ($n \leq 50$) for each types of rice. Then, the non-normal distribution data set was transformed in order to obtain normal distribution. After that the transformed data was used as new data set. One sample t-test was applied to compare the mean values of each element. The correlations between elemental concentrations were measured by a Pearson correlation coefficient (r). One-way ANOVA and post hoc tests were used in order to determine whether there are any significant differences between the means of metal concentrations in each type of rice.

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CHAPTER IV

RESULTS AND DISCUSSION

4.1 Total concentrations of As, Cd, and Zn in rice grain

To ensure the accuracy and precision of both acid digestion and analytical methods, the standard reference material (SRM) 1568a (rice flour) and 1643e (trace elements in water) were used, respectively. The accuracy of acid digestion method is shown in Table 5. Meanwhile, the precision of the analytical methods is shown in Table 6. As the percentage recovery of both digestion and analytical method of all metal of interests were within ± 15% of the certified values with an exception of Zn recovery from the digestion method, it can be concluded that all results in this present study were accurately obtained.

Table 5 Recovery rate of metal of interests obtained from the acid digestion.

Element	Certified values (mg kg^{-1})	Experiment values (mg kg^{-1})	Recovery rate (%)
As	0.285	0.317	111.3
Cd	0.022	0.019	86.7
Zn	19.4	17.6	90.7

Table 6 Recovery rate of instrumental analysis

Element	Certified values (µg L^{-1})	Experiment values (µg L^{-1})	Differential (%)
As	9.0675	9.7580	7.6
Cd	0.9852	1.0400	5.6
Zn	11.8	16.0	35.9

4.1.1 As concentrations in rice grain

Total As concentrations of all samples are summarized in Table 7. The concentrations varied from 0.0835 mg kg^{-1} to 0.4893 mg kg^{-1}. The average concentration of As in all rice samples was 0.2049 mg kg^{-1}. Regarding types of rice collected, the average concentration of 0.2033 mg kg^{-1}, 0.1697 mg kg^{-1}, 0.1480 mg kg^{-1}, and 0.3290 mg kg^{-1} were found in white jasmine, white, glutinous, and brown jasmine rice, respectively. Interestingly, the highest average level of total As (0.3290 mg kg^{-1}) were found in brown (non-polished) jasmine rice. While, the lowest average level of 0.1480 mg kg^{-1} was detected in glutinous rice. Results of statistical analyses also presented significant higher As concentrations in brown jasmine rice than other types of rice ($p < 0.01$). However, there were no significant differences ($p > 0.01$) in total As concentrations in the same types of rice cultivated in different areas as well as collected from different markets.

When compare total As concentrations in all rice samples to the level of As in rice regulated by the Food and Drug Administration (FDA) of Thailand of 2 mg kg^{-1}, levels of As in all samples were well within the standard. Results of this study were also compared to other studies. For example, Nookabkaew et al. (2013) reported the average ± SD values of As concentrations contained in white rice (0.1395± 0.0059 mg kg^{-1}), brown rice (0.2390 ± 0.0158 mg kg^{-1}), and white glutinous rice (0.0935 ± 0.0048 mg kg^{-1}) which were all lower than the levels of As in rice found in this study. Comparing to the levels of As in exported rice from Thailand which were reported by Meharg et al. (2009) and Rowell et al. (2014), ranges of total As in rice in this study were in the ranges of As concentrations reported by those research groups. Levels of As in rice reported by Meharg et al. (2009) and Rowell et al. (2014) were 0.0010 –

0.3900 mg kg^{-1} and 0.0748 – 0.2440 mg kg^{-1}, respectively. However, the average concentration of total As found this present study was higher than the average As concentrations reported by those two studies by about 1.5 times.

Recently, there are several studies reported about different As concentrations in rice of many countries. For example, a study of Tattibayeva et al. (2016), the most recent study, reported the total As concentration in polished Kazakh rice (0.01 mg kg^{-1}), polished Spanish rice (0.28 mg kg^{-1}), polished Portuguese rice (0.36 mg kg^{-1}), and non-polished Kazakh rice (0.24 mg kg^{-1}). It was obviously that the total As concentrations of Kazakh rice was lower than rice of other countries. In 2015, Huang et al., as an example, determined total As concentrations in 653 white rice samples of Zhejiang, China and found the maximum level of 0.665 mg kg^{-1}. The average concentration was 0.1265 mg kg^{-1}. Another study published in 2016 reported the As levels of rice grown in acid mine drainage affected areas in Guangdong province of China. Concentration of As in rice (0.51 mg kg^{-1}) was around 2.5 times higher than allowable maximum concentration. In addition, comparing to the level of As in rice cultivated in an uncontaminated area in the same province, rice grown in As contaminated areas has about 5 times higher in concentrations than rice grown in the uncontaminated area (Liao et al., 2016). Therefore, it can be concluded that the accumulation of As in rice depends on several factors including concentration of As in irrigated water, cultivated soil, accumulation capacity, and life span of plants etc.

As it was mentioned in the part of literature review, the total As concentration can be further divided into inorganic and organic As in which inorganic As is more toxic than organic As. Therefore, concentrations of inorganic As in rice of this present study were then estimated based on the percentages of inorganic As in various types of rice reported by previous study. Nookabkaew et al. (2013) determined As speciation and found about 63.2% and 53.6% of inorganic As in white and brown Thai rice, respectively. The estimated inorganic As concentrations in rice of this study are summarized in Table 8**.** Ranges of inorganic As contained in all types of rice (0.094 mg kg^{-1} to 0.1763 mg kg^{-1}) were not exceed the Codex allowable limit of inorganic As of 0.2 mg kg^{-1}. Results of statistical analyses indicated similar results to total As concentrations in which significant higher inorganic As concentrations were found in brown jasmine rice than other types of rice ($p < 0.01$).

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Table 7 Concentrations of As, Cd, and Zn in different types of rice

Rice types	Concentration (mg kg^{-1})		
	As	Cd	Zn
White jasmine (n=32)			
Minimum	0.0878	0.0040	14.51
Maximum	0.2949	0.0330	20.27
Average	0.2033	0.0166	17.58
Median	0.2120	0.0181	17.64
SE	0.0081	0.0012	0.26
White (n=31)			
Minimum	0.0835	0.0066	11.95
Maximum	0.2650	0.0263	16.47
Average	0.1697	0.0144	13.97
Median	0.1730	0.0138	13.90
SE	0.0085	0.0009	0.23
Glutinous (n=17)			
Minimum	0.0937	0.0170	17.43
Maximum	0.1742	0.0617	21.85
Average	0.1480	0.0365	19.20
Median	0.1501	0.0388	18.75
SE	0.0047	0.0033	0.31
Brown jasmine (n=17)			
Minimum	0.1808	0.0070	25.71
Maximum	0.4893	0.0323	45.98
Average	0.3290	0.0139	35.18
Median	0.3253	0.0113	35.25
SE	0.0209	0.0017	1.50
Total (n=97)			
Minimum	0.0835	0.0040	11.95
Maximum	0.4893	0.0617	45.98
Average	0.2049	0.0189	19.79
Median	0.1965	0.0165	17.53
SE	0.0081	0.0012	0.80

Note: SE is standard error

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Table 8 Estimated inorganic As in rice

Rice types	Total As (mg kg^{-1})				Inorganic As (mg kg^{-1})			
	Min	Max	Average	Median	Min	Max	Average	Median
White jasmine	0.0878	0.2949	0.2033	0.2120	0.0555	0.1863	0.1285	0.1340
White	0.0835	0.2650	0.1697	0.1730	0.0527	0.1675	0.1073	0.1093
Glutinous	0.0937	0.1742	0.1480	0.1501	0.0595	0.1106	0.0940	0.0953
Brown jasmine	0.1808	0.4893	0.3290	0.3283	0.0969	0.2623	0.1763	0.1744

4.1.2 Cd concentrations in rice grain

Total Cd concentrations in rice varied from 0.0040 mg kg^{-1} to 0.0617 mg kg^{-1} in which the average value was about 0.0189 mg kg^{-1}. Results of Cd concentrations in all rice types studied are summarized in Table 7. In short, the average concentrations were 0.0166 mg kg^{-1} for white jasmine rice, 0.0138 mg kg^{-1} for white rice, 0.0388 mg kg^{-1}for glutinous rice, and 0.0133 mg kg^{-1}for brown jasmine rice. In addition, statistical analyses indicated that glutinous rice usually contains significant higher concentrations of Cd than other types of rice ($p < 0.01$). Total Cd concentrations in all rice samples of Bangkok were well below the Codex standard of Cd in rice (0.4 mg kg^{-1}). However, significant differences in Cd concentrations in rice either cultivated in the different areas or collected from different markets could not be observed. Yet, it should be noted Thailand does not regulate the maximum Cd level in rice. Therefore, Cd concentrations in rice of this present study were compared to the levels of Cd in Thai rice reported by previous researchers. Total Cd concentrations in rice found in this present study were in good agreement with the range of Cd concentration (ND - 0.016 mg kg^{-1}) in commercial rice sold in Thailand reported by Zwicker et al. (2010). Recently, Naseri et al. (2015) found about 0.44 ± 0.03 mg kg^{-1} total Cd in imported rice from Thailand which was about 23 times higher than the concentrations of Cd found in this study.

When compared to concentrations of Cd in rice of other countries, variation in results was obtained. For example, Indian rice was found with Cd contents of 0.002 mg kg^{-1} to 0.127 mg kg^{-1}. Though, the maximum Cd in Indian rice was about 2 times higher than maximum Cd concentrations found in this study, the average concentrations of Cd in rice of the two studies were similar (Indian rice: 0.019 mg kg^{-1}, this study: 0.0189 mg kg^{-1}). With an exception of Cd level in glutinous rice, levels of Cd in other types of rice in this present study was in the same magnitude (0.03 mg kg^{-1}) of Cd in rice grown in the controlled areas of Iran (Moradi et al., 2016). However, rice grown in the industrial areas in Iran was found with about 3 - 25 times of Cd higher than this study. In contrast, average levels of Cd found in white jasmine, white, and glutinous rice in this study were larger than Cd concentrations in rice cultivated in the industrial areas of Jingsu, China (0.014 mg kg^{-1}) (Cao et al., 2010). As reported by Sebastian and Prasad (2013), Cd uptake and accumulation in rice grain is not only affected by status of elements in soil but also it can be affected by other soil characteristics such as redox potential, pH, and organic matter content.

4.1.3 Zn concentrations in rice grain

Zn is an essential trace element for human body which can be supplied by food source. It is a component of several metalloenzymes which was reported to be important in the regulation of gene expression and intracellular signaling

(Duan et al., 2013). However, high level of Zn can cause toxic effects such as dysfunction of genetic activity as well (Bhat and Gómez-López, 2014). As shown in Table 7, the average Zn concentration in rice of this study was 19.79 mg kg^{-1}. The total Zn concentrations were ranging from 11.95 mg kg^{-1} to 45.98 mg kg^{-1}. There was a significant difference in Zn concentrations regarding to the different types of rice ($p > 0.01$) in which brown jasmine rice was found the highest Zn concentration. However, significant differences in Zn concentrations in rice from different cultivation areas and different market could not be observed ($p > 0.01$). The average concentrations of each types of rice were 17.58 mg kg^{-1}, 13.97 mg kg^{-1}, 19.20 mg kg^{-1}, and 35.18 mg kg^{-1} for white jasmine, white, glutinous, and brown jasmine rice, respectively. As there is no limit of Zn concentration in rice regulated in Thailand, results of this present study were also compared to previous studies. Rowell et al. (2014) reported lower Zn concentration in imported rice from Thailand comparing to this current study. Rowell et al. (2014) found 13.5 mg kg^{-1} as an average and 4.40 mg kg^{-1} - 20.3 mg kg^{-1} as a range of total Zn concentrations in rice. Another study by Rahman et al. (2014) reported the range of Zn concentrations in Thai rice of 14.1 mg kg^{-1} - 22.4 mg kg^{-1} in which the average Zn concentration was 17.8 mg kg^{-1}. These Zn concentrations reported were closed to the concentration of Zn contained in white rice in this study. In addition, Tattibayeva et al. (2016) determined concentrations of Zn in rice samples collected from Kazakhstan and the European community and found no significant different in the average Zn concentrations in white rice (19.98 mg kg^{-1}) and brown rice (20.95 mg kg^{-1}) of Kazakhstan. For white rice of Spain and Portugal, the average concentrations of Zn were 17.36 mg kg^{-1} and 23.68 mg kg^{-1}, respectively. Results reported by Tattibayeva et al. (2016) agree well with the results of this study in terms of both the range of Zn concentrations and non-significant difference in Zn concentrations found in both white and brown rice. In contrast, the average Zn concentration of 10.2 mg kg^{-1} in Nigerian rice (Adedire et al., 2015) was about 1.4 to 3.4 times lower than the average Zn concentrations found in this study. Interestingly, it was found that concentrations of Zn in brown jasmine rice, in particular, in this study were not significantly different from Zn levels (31.98 ± 5.32 mg kg^{-1}) in rice cultivated in mining areas in China (Liao et al., 2016).

4.2 Correlation of heavy metal concentrations in rice grain

Relationship of As, Cd, and Zn concentrations are demonstrated in Figure 7 to Figure 9. Figure 7 shows a positive significant correlation between As and Zn concentrations ($p < 0.01$, $R^2 = 0.573$). On the other hand, there were no significant relationship between As and Cd concentrations ($p = 0.026$) as well as Cd and Zn concentrations ($p = 0.032$) as shown in Figure 8 and Figure 9, respectively. A significant correlation between As and Zn with lower correlation level ($R^2 = 0.041$), compared to this study, was also reported by Rowell et al. (2014). Since As and Zn are found to be localized mainly in the rice bran and endosperm and they are similar in their complexity with the thiol groups (-SH groups) (Meharg et al., 2008), the relationship between these two elements are expected, whereas, Cd usually presented in the endosperm. Therefore, relationship between Cd and As and Zn could not be observed.

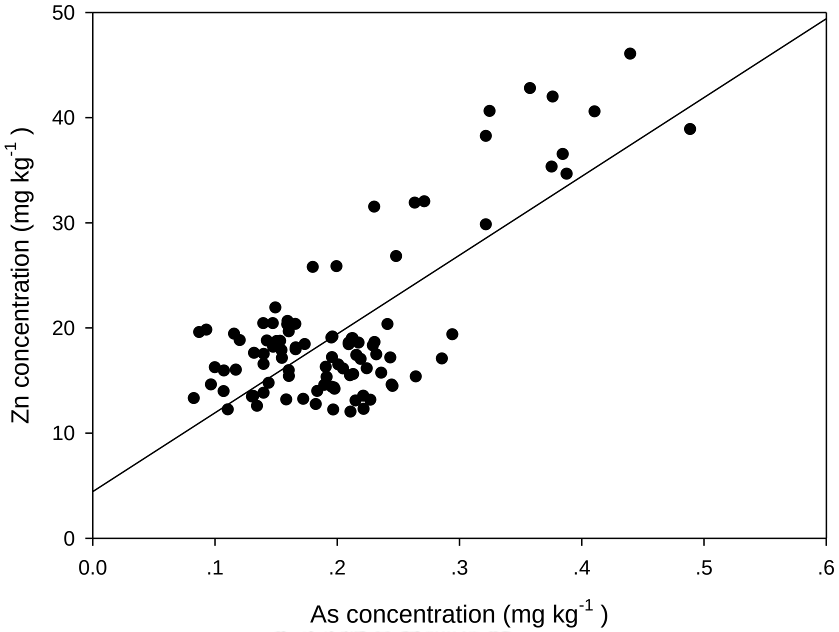

Figure 8 Relationship between As and Zn concentrations in rice

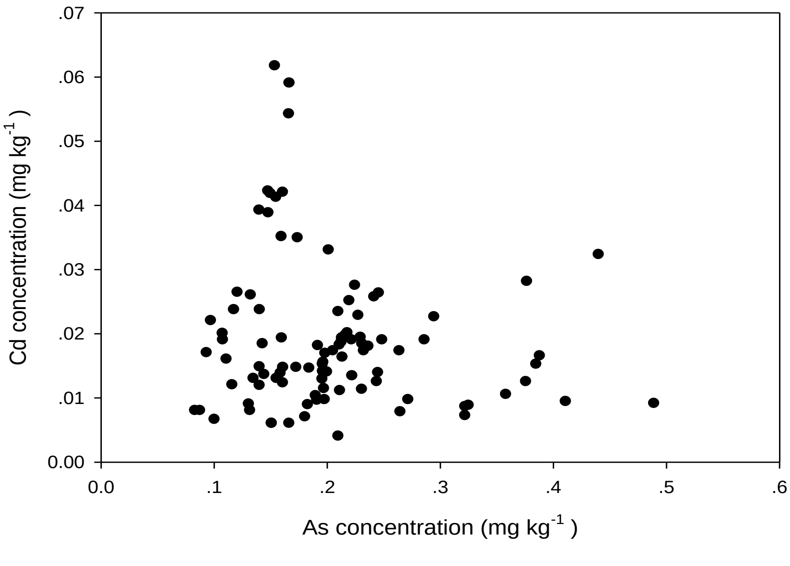

Figure 9 Relationship between As and Cd concentrations in rice

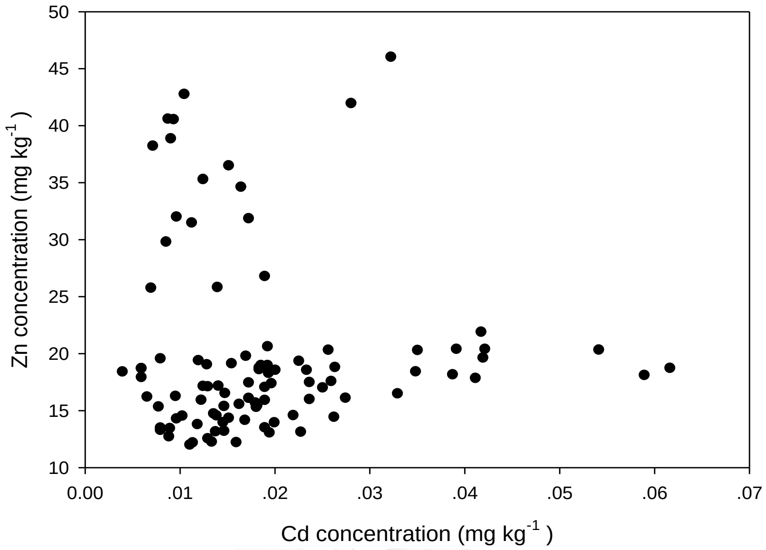

Figure 10 Relationship between Cd and Zn concentrations in rice

4.3 Exposure assessment of As, Cd, and Zn ingestion

The exposure rates of As, Cd, and Zn from rice digestion were calculated using the concentrations of those elements, body weight, and rice consumption rate.

This present study used the average body weight by age and gender of Thai population (Table 9) reported the Bureau of Product Standard and Quality System (2006). According to the national statistics, body weights of Thai adult, adult male and adult female, who are in the age group of 19 to 65 years, were 59.33 kg, 61.89 kg, and 56.68 kg, respectively.

Table 9 Body weight (average ± SD) of Thai population

Age Group	Body weight (kg) (±SD)		
	Male	Female	Total
0 - 3 years	10.21 ± 4.61	9.89 ± 3.98	10.05 ± 4.31
3 - 6 years	17.31 ± 4.61	16.90 ± 4.90	17.10 ± 4.76
6 - 9 years	23.04 ± 6.33	22.56 ± 5.83	22.80 ± 6.09
9 - 16 years	39.67 ± 12.84	39.78 ± 11.56	39.73 ± 12.20
16 - 19 years	56.85 ± 11.64	49.71 ± 8.96	53.23 ± 10.96
19 - 35 years	61.80 ± 10.50	54.71 ± 11.48	58.28 ± 11.56
35 - 65 years	61.98 ± 10.28	58.64 ± 10.61	60.37 ± 10.57
Above 65 years	56.92 ± 10.63	51.89 ± 11.48	54.53 ± 11.32
> 3 years average	55.34 ± 56.63	51.76 ± 53.08	53.54 ± 54.90
Thai capita average	53.04 ± 60.24	49.71 ± 56.45	51.36 ± 58.38

In case of rice consumption, average amounts of raw rice consumed by different age group (Table 10) and gender (Table 11) as reported by the Bureau of Product Standards and Quality Systems (2006) were also used for the exposure rate assessment. As clearly shown in Table 10 and Table 11, the highest rice consumption was found in adult (141.33 g day^{-1}) and male (149.58 g day^{-1}), respectively.

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Table 10 Rice consumption per capita of Thai population by age

Age	Rice consumption per capita (g day^{-1})
Children (3 – 9 years)	88.97
Adolescents (9 – 19 years)	128.58
Adults (19 – 65 years)	141.33
Seniors (above 65 years)	118.66

Table 11 Rice consumption per capita of Thai population above 3 years by gender

Sex	Rice consumption per capita (g day^{-1})
Male	149.58
Female	118.46
All	134.17

With all secondary data obtained, body weight and rice consumption rate shown in Table 12 were used to calculate exposure rate and risks of As, Cd, and Zn exposure through rice ingestion. This present study have divided the exposure rate calculation into four population groups including children (3–9 years), adolescents (9–19 years), adults (19–65 years), and seniors (above 65 years).

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Table 12 Summary of variables used to calculate exposure rate of As, Cd, and Zn through rice ingestion

Category	Population	Variables	
		Body weight (kg)	Consumption per capita (g day^{-1})
Age group	Children (3 – 9 years)	19.95	88.97
	Adolescents (9 – 19 years)	46.48	128.58
	Adults (19 – 65 years)	59.33	141.33
	Seniors (above 65 years)	54.53	118.66
Gender	Male	55.34	149.58
	Female	51.76	118.46

4.3.1 Exposure assessment of As through rice consumption

Daily exposure of As ingestion concerning different types of rice consumption is summarized in Table 13. The average As exposure rates (mg kg^{-1} bw day^{-1}) through daily rice ingestion in children, adolescents, adults, and seniors were 9.14×10^{-4}, 5.67×10^{-4}, 4.88×10^{-4}, and 4.46×10^{-4}, respectively. Among all age groups studied, children expose to the highest levels of As regardless types of rice consumed (Table 12). Levels of daily As exposure rate were children > adolescents > adults > seniors. Though rice consumption rate in children is lower, the exposure rate of As is the highest. Since the exposure assessment is calculated based on the amounts of chemical of interests exposed to individual's body weight, the lower in body weight could resulted in the higher exposure rate. In other word, the exposure rate of As in those who are low in their body weight are higher than the exposure rate of As in those who consume the same amount of rice but they are higher in their body weight. Concerning the same types of rice consumption, results of statistical analyses confirmed that children are the population with significant higher As exposure rate than other groups of population ($p < 0.01$).

Concerning the As exposure through different types of rice consumption, consumption of brown jasmine rice could cause the highest As exposure in all age groups. The levels of As exposure decrease by consuming white jasmine rice, glutinous rice, and white rice, respectively (Table 13). These results mainly cause by the highest average As concentrations found in brown jasmine rice (Table 7). The order of As concentrations and As exposure rates in different types of rice are similar that is brown jasmine > white jasmine > glutinous > white rice. A review on regional As exposure through rice consumption strongly confirmed that the total As concentration in rice should be an issue of concern. This mainly due to the fact that the levels of regional As exposure are in the following order: Asia > South America > Middle East, North America > Europe. Apart from this study, the As exposure rates through rice consumption were reported in countries such as Bangladesh and Kazakhstan. The expose rate of As through rice consumption (2.2×10^{-3} mg kg^{-1} bw day^{-1}) was observed in Bangladeshi adult. This level of exposure rate was higher than the As exposure rate in Thai adult in this study by about 2.5 times. Ahmed et al. (2015) concluded that higher concentrations of As in rice and higher rice consumption rate are the key factors that could cause an elevation in As exposure rate. The As exposure rate of Kazakh adult was reported with the range of 3.14×10^{-5} mg kg^{-1} bw day^{-1} – 2.4×10^{-4} mg kg^{-1} bw day^{-1} (Tattibayeva et al., 2016). The levels of As exposure rate via rice ingestion in Kazakh and Thai adults (this study) were similar.

When compare the As exposure rates among male and female, male generally expose to higher levels of As than female. This is due to the factor that about 1.3

times higher in rice consumption rate of male than female (Table 14). In addition, brown jasmine and white rice consumption may cause the highest and lowest As exposure rates, respectively, in both male and female population.

Regarding to the exposure assessment of inorganic As uptake via rice ingestion. It should be noted that the concentrations of inorganic As in rice were estimated from previous results reported by Nookabkaew et al. (2013). Table 13 shows similar pattern of inorganic As exposure for both age group and gender classifications in which; 1) highest inorganic As exposure (7.86×10^{-4} mg kg^{-1} bw day^{-1}) was found in children who consume brown jasmine rice (Table 13) and 2) male usually expose to higher dose of inorganic As than female who consume the same type of rice (Table 13). Comparing to Cambodian, the higher exposure rate of inorganic As was found (1.3×10^{-3} mg kg^{-1} bw day^{-1} to 1.9×10^{-3} mg kg^{-1} bw day^{-1}) because Cambodian population generally consume more rice and they are lower in their body weight comparing to the Thai population (Gilbert et al., 2015). The exposure rates of inorganic As were also observed in Taiwan. The average of exposure rate of Taiwanese adult (19–65 years) was 6.83×10^{-6} mg kg^{-1} bw day^{-1} for male and 4.96×10^{-6} mg kg^{-1} bw day^{-1} for female (Chen et al., 2005). Although, the exposure rate of this present study was higher than results of Chen et al. (2005), the similar patterns of exposure were observed. The exposure rate of inorganic As in male was higher than female. More importantly, young children were expected to expose to the strongest concentration of inorganic As.

4.3.2 Exposure assessment of Cd through rice consumption

Table 13 showed the summary of daily exposure of Cd in different types of rice consumption. The average Cd exposure rates (mg kg^{-1} bw day^{-1}) in children, adolescents, adults, and seniors were 8.44×10^{-5}, 5.24×10^{-5}, 4.51×10^{-5}, and 4.12×10^{-5}, respectively. The exposure rate of Cd was very much alike the As exposure rate, children exposed to the highest levels of Cd among other age group who consumed the same types of rice. For statistical analyses, the exposure rate of Cd in children was significantly higher than the others ($p < 0.01$). Therefore, the order of Cd exposure rate were children > adolescents > adults > seniors. The exposure rates of Cd found in this study were found to be lower than those HQ values of Cd exposure in Chinese population reported by Zhang et al. (2016) and Yuan et al. (2014). Both studies reported the HQ values of 3.1 x 10^{-4} mg kg^{-1} bw $day^{-1}$and 4.5 x 10^{-4} mg kg^{-1} bw day^{-1} which were higher than this study.

In the case of the same age group, it was found that the daily exposure of Cd through glutinous rice consumption was the highest among the other rice types. The levels of Cd exposure in population who consumed different types of rice were glutinous > brown jasmine > white jasmine = white rice. In comparison to the exposure rate of domestic rice in Shiraz, Iran, a slightly higher in exposure of Cd of 6.3 x 10^{-4} mg kg^{-1} bw day^{-1} was found especially in Thai adults who consumed glutinous rice (Naseri et al., 2015). Another study in Iran reported the higher Cd exposure rate in population who consumed rice cultivated in industrial area (4.7×10^{-4} mg kg^{-1} bw $day^{-1}$to 5.3×10^{-4} mg kg^{-1} bw day^{-1}) (Moradi et al., 2016). While, the exposure rate of those population in non-industrial area (9.0×10^{-5} mg kg^{-1}

bw day^{-1}) was almost equal to the Cd exposure rate of Thai rice consumption found in this study.

Regarding gender classification, the Cd exposure rate was compared between male and female as shown in Table 14. Cd exposure rate in male was higher than female around 1.2 times due to the higher rice consumption rate. Moreover, glutinous rice consumption could contribute to the highest Cd exposure rates in both genders.

4.3.3 Exposure assessment of Zn through rice consumption

The average Zn exposure rates through daily rice ingestion in children, adolescents, adults, and seniors were 0.088, 0.055, 0.047 and 0.043 mg kg^{-1} bw day^{-1}, respectively. Daily exposure of Zn ingestion in different types of rice consumption is summarized in Table 13. The exposure rate of Zn was in the same manner with the As exposure rate due to the correlation between As concentrations and Zn concentrations. There was a significant difference in exposure rate of all age groups who consumed same rice type ($p < 0.01$). In addition, children were still the population that exposed to Zn in the highest levels. Concerning the age group, the exposure rates of Zn from each types of rice consumption were found to be significantly different in which the highest Zn exposure rate in population who consumed different rice types were found in brown jasmine rice.

A study of Tattibayeva et al. (2016) reported the Zn exposure rate of Kazakh, Spanish, and Portuguese of 6.1×10^{-3} mg kg^{-1} bw day^{-1}, 2.9×10^{-3} mg kg^{-1} bw day^{-1}, and 1.6×10^{-2} mg kg^{-1} bw day^{-1}, respectively. It was clearly found that exposure rate of Portuguese adults were the highest due to the high rice consumption rate (46.56 g day^{-1}) comparing to other nationalities. However, the exposure rates of Zn for those three countries were still lower than the exposure rate of Zn found in this study. In case of Indian Zn exposure, the average daily intake in adult of 0.0766 mg kg^{-1} bw day^{-1}. (Kumar et al., 2016) was in the same range of Thai's study.

When compare Zn exposure rates between male and female population, male generally expose to higher levels of Zn than female (Table 14) as similar as the exposure rates of As and Cd reported earlier. In addition, brown jasmine and white rice consumption may cause the highest and lowest As exposure rates, respectively, in both male and female population.

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Table 13 Daily exposure (mg kg^{-1} bw day^{-1}) of As, Cd, and Zn through different types of rice ingestion by age

Element	Rice type	Age group			
		Children	Adolescents	Adults	Seniors
As					
	White jasmine	8.31×10^{-4}	5.15×10^{-4}	4.44×10^{-4}	4.05×10^{-4}
	White	6.33×10^{-4}	3.92×10^{-4}	3.38×10^{-4}	3.09×10^{-4}
	Glutinous	6.60×10^{-4}	4.09×10^{-4}	3.53×10^{-4}	3.22×10^{-4}
	Brown jasmine	1.47×10^{-3}	9.10×10^{-4}	7.84×10^{-4}	7.16×10^{-4}
Inorganic As					
	White jasmine	5.73×10^{-4}	3.55×10^{-4}	3.06×10^{-4}	2.80×10^{-4}
	White	4.78×10^{-4}	2.97×10^{-4}	2.55×10^{-4}	2.33×10^{-4}
	Glutinous	4.19×10^{-4}	2.60×10^{-4}	2.24×10^{-4}	2.05×10^{-4}
	Brown jasmine	7.86×10^{-4}	4.88×10^{-4}	4.20×10^{-4}	3.84×10^{-4}
Cd					
	White jasmine	6.32×10^{-5}	3.92×10^{-5}	3.38×10^{-5}	3.08×10^{-5}
	White	6.33×10^{-5}	3.93×10^{-5}	3.38×10^{-5}	3.09×10^{-5}
	Glutinous	1.63×10^{-4}	1.01×10^{-4}	8.70×10^{-5}	7.95×10^{-5}
	Brown jasmine	6.21×10^{-5}	3.85×10^{-5}	3.32×10^{-5}	3.03×10^{-5}
Zn					
	White jasmine	7.63×10^{-2}	4.74×10^{-2}	4.08×10^{-2}	3.72×10^{-2}
	White	6.38×10^{-2}	3.96×10^{-2}	3.41×10^{-2}	3.11×10^{-2}
	Glutinous	8.56×10^{-2}	5.31×10^{-2}	4.57×10^{-2}	4.18×10^{-2}
	Brown jasmine	1.57×10^{-1}	9.73×10^{-2}	8.38×10^{-2}	7.66×10^{-2}

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Table 14 Daily exposure (mg kg^{-1} bw day^{-1}) of As, Cd, and Zn through different types of rice ingestion by gender

Element	Rice type	Gender	
		Male (above 3 years)	Female (above 3 years)
As			
	White jasmine	5.03×10^{-4}	4.26×10^{-4}
	White	3.83×10^{-4}	3.25×10^{-4}
	Glutinous	4.00×10^{-4}	3.39×10^{-4}
	Brown jasmine	8.89×10^{-4}	7.53×10^{-4}
Inorganic As			
	White jasmine	3.47×10^{-4}	2.94×10^{-4}
	White	2.90×10^{-4}	2.46×10^{-4}
	Glutinous	2.54×10^{-4}	2.15×10^{-4}
	Brown jasmine	4.77×10^{-4}	4.03×10^{-4}
Cd			
	White jasmine	3.83×10^{-5}	3.24×10^{-5}
	White	3.84×10^{-5}	3.25×10^{-5}
	Glutinous	9.87×10^{-5}	8.36×10^{-5}
	Brown jasmine	3.76×10^{-5}	3.19×10^{-5}
Zn			
	White jasmine	4.63×10^{-2}	3.92×10^{-2}
	White	3.87×10^{-2}	3.28×10^{-2}
	Glutinous	5.19×10^{-2}	4.39×10^{-2}
	Brown jasmine	9.51×10^{-2}	8.05×10^{-2}

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Once the daily exposure of all metal of interest was obtained, the weekly exposure of each metal was calculated and compared to the provisional tolerable weekly intake (PTWI). WHO has used this PTWI as a recommended maximum intake of chemical in adult. PTWI of 1.5×10^{-2}, 2.5×10^{-3}, and 7 mg kg^{-1} $week^{-1}$ are recommended for inorganic As (FAO/WHO, 2010), Cd (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2011), and Zn (FAO/WHO, 1982) exposure, respectively. Though, PTWI of As was withdrawn since 2010, the most recent research of Tattibayava et al. (2016) still used this number as the guideline for exposure assessment. The highest of inorganic As exposure rate in adult was found in brown jasmine rice of 2.94×10^{-3} mg kg^{-1} $week^{-1}$. It was lower than the PTWI of As. Next, Cd exposure rate of 6.0×10^{-4} mg kg^{-1} $week^{-1}$ was the highest value in adults which was smaller than the PTWI of Cd around 4 times. Last, the exposure rate of Zn, the largest level was 0.56 mg kg^{-1} $week^{-1}$ which was much lower than PTWI.

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4.4 Health risk assessment of As, Cd, and Zn exposure through rice consumption

4.4.1 Non-carcinogenic risk assessment

In this recent study, the hazard quotient (HQ) was used as an index of non-carcinogenic risk. In general, HQ value obtained is compared to the threshold value of 1. The greater HQ value than 1 generally indicates the more adverse non-carcinogenic effects in human caused by the particular metal.

Non-carcinogenic risk of As exposure by age group and gender are shown in Figure 11 and Figure 12, respectively. Non-carcinogenic risks indicating by HQ values ranked as the following order: children > adolescents > adults > seniors. The HQ of children ranging from 2.11 to 4.89 of all types of rice consumption was the largest among all age groups. The HQ values of As in children were significantly higher than the others for all rice types ($p < 0.01$) however, there were significant difference in HQ values of As in adolescents and adults ($p > 0.01$). Moreover, the HQ values of As in brown jasmine rice consumption in all age groups were significantly higher than the HQ values of the other types of rice ($p < 0.01$). Interestingly, HQ values of As exposure via all types of rice consumption in all age groups exceeded the threshold limit (HQ > 1). This indicates that all population may experience significant non-carcinogenic health effects. Due to the highest number of the exposure rate in brown jasmine rice, the HQ of total As exposure from brown jasmine rice consumption showed the highest level as well. In case of gender, the results showed similar outcomes to the age groups in which the HQ values of brown jasmine rice was the highest and white rice was the lowest. In addition, HQ values of all types of rice ingestion were higher in male than female.

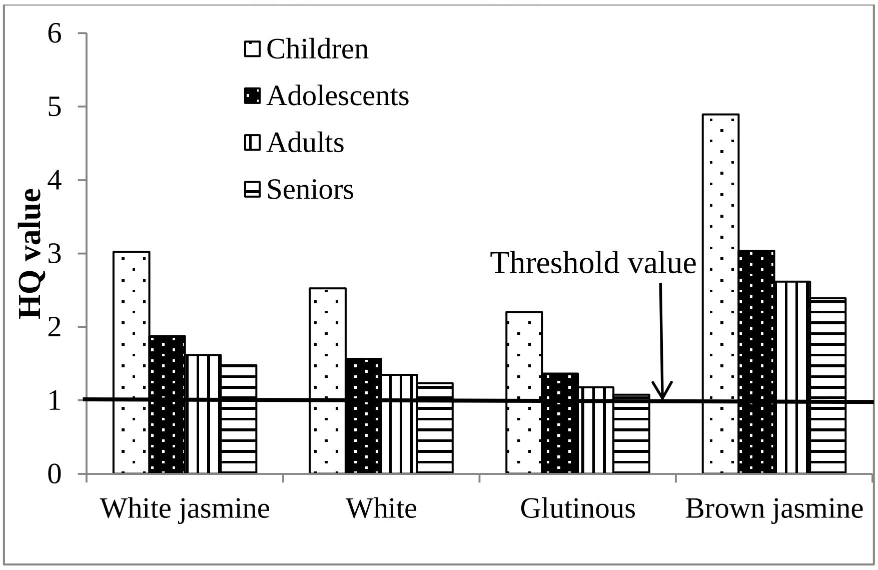

Figure 11 Non-carcinogenic risk of total As exposure by age

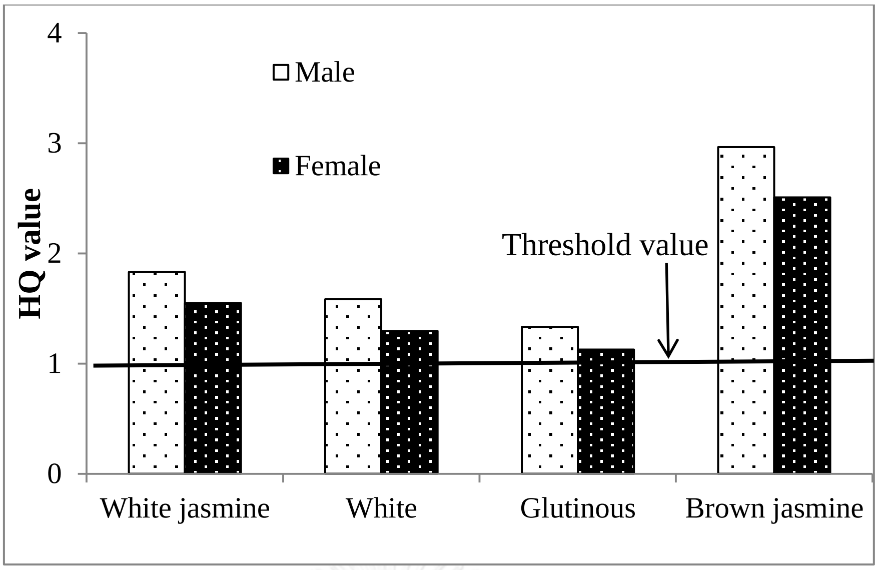

Figure 12 Non-carcinogenic risk of total As exposure by gender

The estimated HQs of inorganic As ingestion are shown in Figure 13 and Figure 14 for age groups and gender groups, respectively. Even, the HQ value of inorganic As were lower than the HQ values of total As exposure, they still showed the HQ value exceeding the threshold limit of 1 especially, in brown jasmine rice and white jasmine rice for almost age groups (except senior group in white jasmine rice). This result clearly demonstrated that more attention should be paid to the rice consumption in children as HQ values As exposure were higher than the threshold by almost 2 to 3 times (Figure 13). For gender groups, the HQ values of brown jasmine rice consumption in both male and female were above the threshold limit. With an exception of white jasmine rice consumption in male, HQ values of white jasmine, white, and glutinous rice consumption were lower than 1.

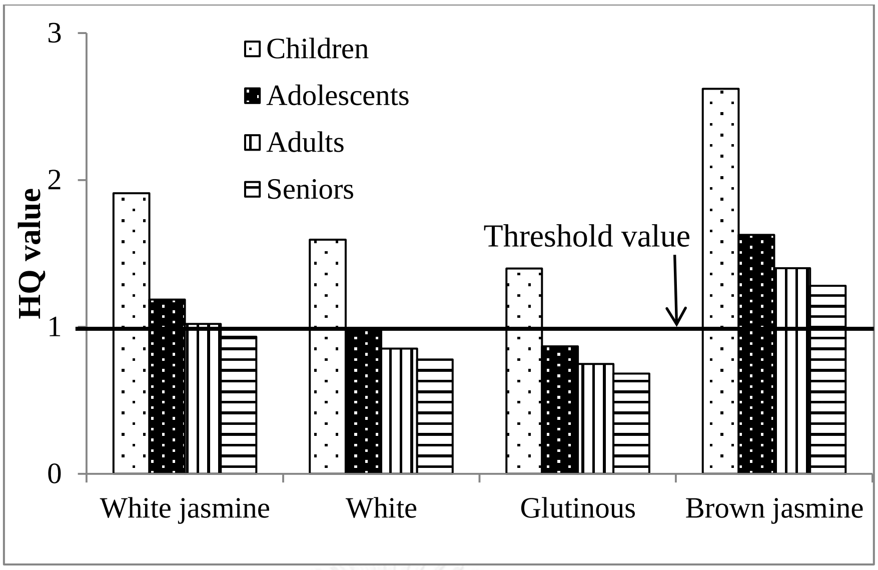

Figure 13 Non-carcinogenic risks of inorganic As exposure by age

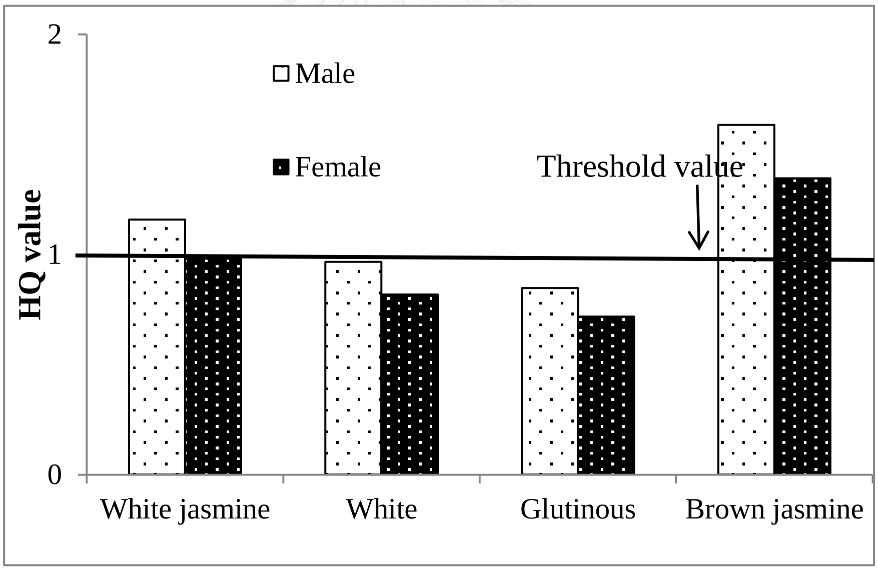

Figure 14 Non-carcinogenic risks of inorganic As exposure by gender

When compared the results of this present study to other studies, it was found that the HQ values of As exposure through rice consumption in this study were approximately 1 to 16 times higher than HQ values of As exposure in Chinese population in all age and gender. Huang et al. (2015) and Fu et al. (2015) reported the HQ values of 0.060 - 0.144 and 0.8 in Chinese population and concluded that the low As concentrations in rice and low rice consumption are the main factors affecting to lower HQ values. However, HQ values of rice grown around the mining areas and As contaminated areas in Bangladesh and China (Ahmed et al., 2015;Liao et al., 2016) of 7.42 and 22.4, respectively, were relatively higher than the HQ values of As exposure found in this study.

For Cd risk assessment, the HQ values of all age and gender groups are shown in Figure 15 and Figure 16. Both figures depicted the HQ values smaller than one. HQ values of all rice types consumption in different age groups ranging from 0.03 to 0.16. Even, the Cd's HQ values were not exceed the threshold limit, the HQ value in children still showed significantly higher in value than the HQ values of the other age groups ($p < 0.01$). Moreover, there was a significant higher in HQ value of all age who consumed glutinous rice comparing to the consumption of the other rice types ($p < 0.01$). Similarly, HQ values of all rice types consumption in male and female were also lower than 1. Hence, no potential non-carcinogenic health impacts from Cd exposure through rice consumption are expected to be developed in all Bangkok population.

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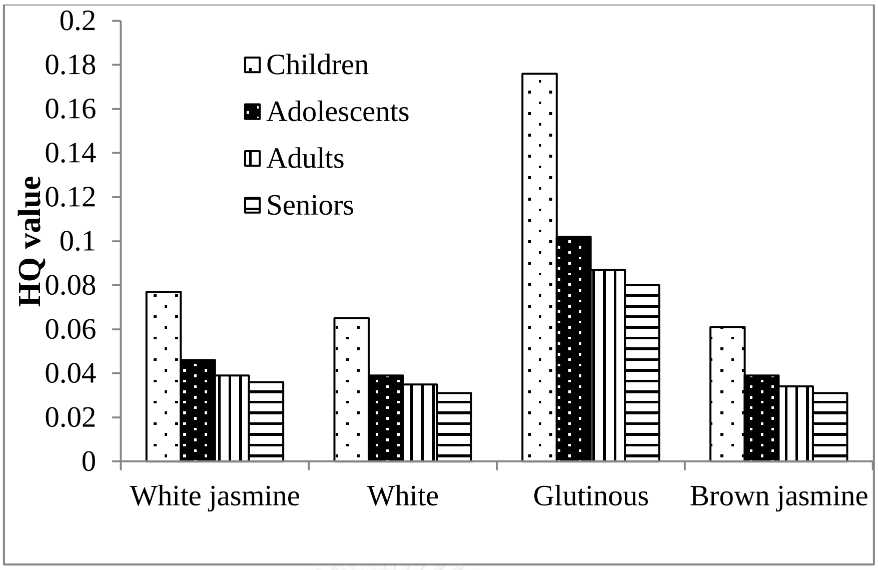

Figure 15 Non-carcinogenic risks of Cd exposure by age

Figure 16 Non-carcinogenic risks of Cd exposure by gender

Non-carcinogenic risks of Zn exposure by age and gender are illustrated in Figure 17 and Figure 18, respectively. Similar to the results of non-carcinogenic risks of Cd exposure, HQ values of Zn exposure were lower than 1. Therefore, it can be concluded that there is no potential non-carcinogenic risk associated with the intake of Zn via rice consumption. In addition, the HQ values of Zn were not significantly different in both age groups and rice types.

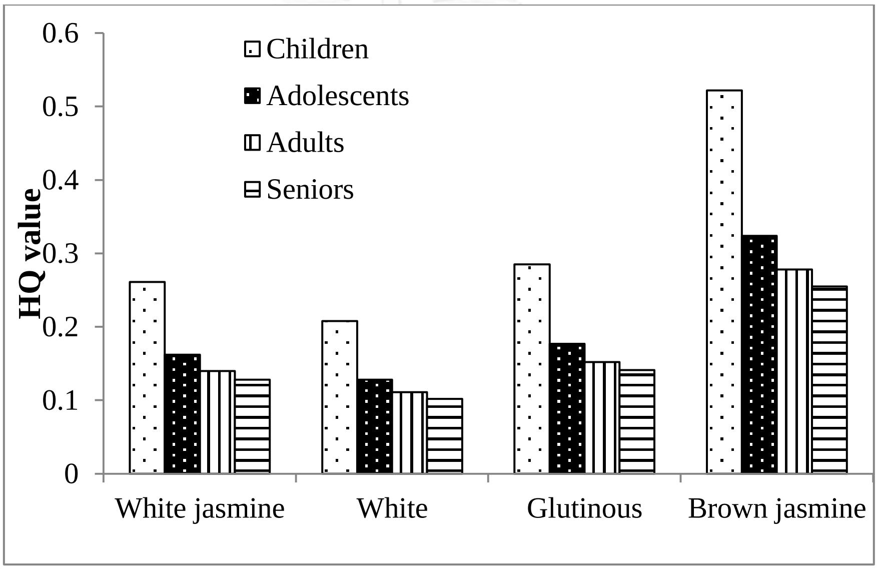

Figure 17 Non-carcinogenic risks of Zn exposure by age group

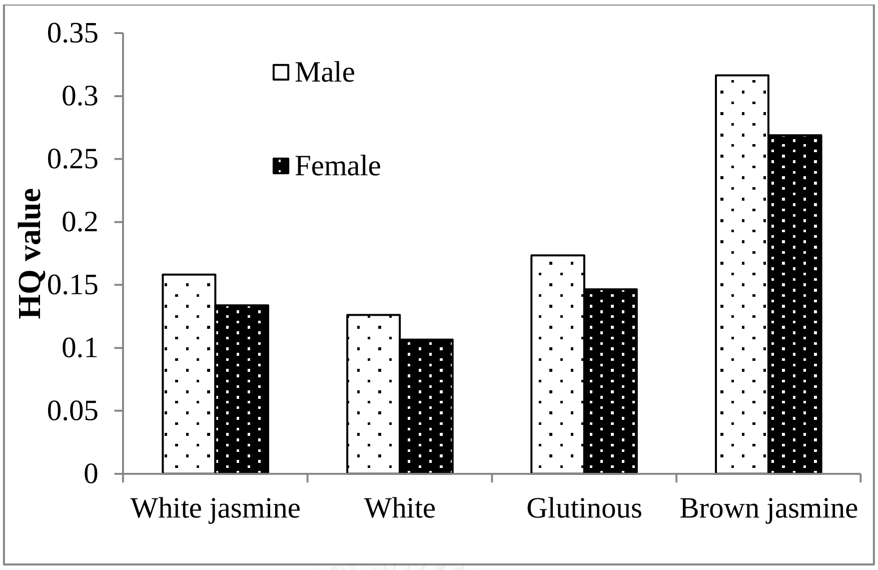

Figure 18 Non-carcinogenic risk of Zn exposure by gender

Lastly, HI values indicating the overall non-carcinogenic risks of As, Cd, and Zn through rice consumption by age and gender are summarized in Table 15 and Table 16, respectively. The HQs ranked in order of As > Zn > Cd, indicating that As has contributed the most of non-carcinogenic risk to human body. The contribution of As to the overall non-carcinogenic risk (HI) was approximately 90%. Interestingly, the highest HI values were found mostly in children regardless types of rice. Therefore, it can be concluded that children are the group with the highest risk to experience the non-carcinogenic health effects of heavy metals through rice consumption.

Table 15 Overall non-carcinogenic risk of As, Cd, and Zn exposure via rice consumption by age

Rice types		White jasmine	White	Glutinous	Brown jasmine
Children (3 - 9 years)	Min	1.63	1.49	1.95	3.11
	Max	4.75	4.2	3.02	7.91
	Average	3.36	2.79	2.66	5.47
	SD	0.7	0.7	0.28	1.37
Adolescents (9-19 years)	Min	1.01	0.91	1.09	1.92
	Max	2.96	2.61	1.87	4.9
	Average	2.08	1.73	1.64	3.4
	SD	0.43	0.44	0.2	0.85
Adults (19-65 years)	Min	0.87	0.79	0.94	1.66
	Max	2.55	2.24	1.61	4.22
	Average	1.79	1.49	1.42	2.93
	SD	0.37	0.38	0.18	0.73
Senior (> 65 years)	Min	0.8	0.72	0.86	1.51
	Max	2.33	2.05	1.47	3.85
	Average	1.64	1.36	1.29	2.67
	SD	0.34	0.34	0.16	0.67

Table 16 Overall non-carcinogenic risk of As, Cd, and Zn exposure via rice consumption by gender

Rice types		White jasmine	White	Glutinous	Brown jasmine
Male	Min	0.99	0.89	1.07	1.88
	Max	2.89	2.55	1.83	4.78
	Average	2.04	1.69	1.61	3.32
	SD	0.42	0.43	0.2	0.83
Female	Min	0.84	0.76	0.9	1.59
	Max	2.45	2.15	1.55	4.05
	Average	1.72	1.43	1.36	2.81
	SD	0.36	0.36	0.17	0.7

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4.4.2 Carcinogenic risk assessment

Table 17 summarized carcinogenic risks of rice consumption. Since As is the only element that is classified as human carcinogenic substance, the AELCR is calculated based on As exposure. The AELCR values of all age groups and gender in all rice types consumption were in the magnitude of 10^{-8}. This indicates that the level of carcinogenic risk was lower than the acceptable cancer risk level of 10^{-4} and the threshold level of 10^{-6}. Therefore, it can be concluded that the cancer risk of As exposure through rice consumption in this present study is acceptable. The possibility of cancer to be developed is considered to be as low as less than 1 in 100 million people.

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Table 17 Carcinogenic risk developed from rice consumption

Element	Rice type		White jasmine	White	Glutinous	Brown jasmine
As	Age group	3 – 9 years	4.55×10^{-8}	3.47×10^{-8}	3.62×10^{-8}	8.04×10^{-8}
		9 – 19 years	2.82×10^{-8}	2.15×10^{-8}	2.24×10^{-8}	4.99×10^{-8}
		19 – 65 years	2.43×10^{-8}	1.85×10^{-8}	1.93×10^{-8}	4.29×10^{-8}
		> 65 years	2.22×10^{-8}	1.69×10^{-8}	1.76×10^{-8}	3.92×10^{-8}
	Gender	Male	2.76×10^{-8}	2.10×10^{-8}	2.19×10^{-8}	4.87×10^{-8}
		Female	2.34×10^{-8}	1.78×10^{-8}	1.86×10^{-8}	4.13×10^{-8}
Inorganic As	Age group	3 – 9 years	3.14×10^{-8}	2.62×10^{-8}	2.30×10^{-8}	4.31×10^{-8}
		9 – 19 years	1.95×10^{-8}	1.63×10^{-8}	1.42×10^{-8}	2.67×10^{-8}
		19 – 65 years	1.68×10^{-8}	1.40×10^{-8}	1.23×10^{-8}	2.30×10^{-8}
		> 65 years	1.53×10^{-8}	1.28×10^{-8}	1.12×10^{-8}	2.10×10^{-8}
	Gender	Male	1.90×10^{-8}	1.59×10^{-8}	1.39×10^{-8}	2.61×10^{-8}
		Female	2.34×10^{-8}	1.78×10^{-8}	1.86×10^{-8}	4.13×10^{-8}

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4.5 Bioavailable concentrations of As in rice grain

As mentioned earlier, the risk assessment using total concentrations of heavy metals in rice was used to analyze and calculate daily intake of metals by many groups of researchers since the analytical method of total concentrations is relatively simple and cheap to performs and provide quick results. However, this method hypothesize that 100% of metals are available and readily absorbed to human. In fact, total concentrations could not provide accurate risk information because not all concentrations are bioavailable. Thus, the bioavailable concentrations should be performed to provide a more accurate data for risk calculation and assessment of the daily exposure to metals. Due to its simple practices and high efficiency, the *in vitro* digestion was chosen to analyze the bioavailable concentration (Versantvoort et al., 2004). This study also focused on bioavailable As concentration because it is the most toxic substance among all metals of interests. In addition, some samples especially jasmine rice were determined with total As exceeding the FDA standard (> 0.2 mg kg^{-1}). It was reported earlier that more than 50% of As is usually bioavailable and absorbed to the digestive tracts (He et al., 2012).

Because of those reasons, the bioavailable As concentrations were studied in this study. It should be noted that only white and brown jasmine rice samples with high total As concentrations were determined for As bioavailability. Table 17 summarizes the average bioavailable As concentrations and their bioavailability in rice samples. It was found that bioavailable concentrations in brown jasmine rice were higher than white jasmine rice about 3.5 times. The reason behind this finding is as similar as the reason why higher total As concentration was found in brown jasmine rice than white jasmine rice. Rice milling, a process of removing the husk and bran layer to produce white rice, could reduce the As contents in white rice. As it was reviewed earlier in the literature review, As generally localizes at the surface between pericarp and aleurone layer of brown rice.

The percentage of bioavailable As to total concentrations of As was found to be ranging from 8.7 to 23.0 in white jasmine rice and 29.4 to 37.6 in brown jasmine rice as shown in Table 1**7.** Comparing to the result of bioavailable As reported in other study, the lower bioavailable As was reported. Omar et al. (2015), as an sample, found about 0.014 - 0.017 mg kg^{-1} of bioavailable As in cooked rice. Furthermore, about 33% of bioavailable of As was determined in cooked rice. The bioavailability As in brown jasmine rice in this present study was in good agreement with the results reported by Juhasz et al. (2006). However, white jasmine rice in this study contained about 1–3 times of bioavailable As higher than the previous report.

In case of Cd bioavailable in rice samples, 17% of total Cd was estimated to be available to human after ingestion (Yang et al., 2012). Since low concentrations of total Cd and low human health risks were determined in this study, the bioavailability of Cd was not determined.

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Table 18 Concentrations and percentage of bioavailable As in rice

No.	Rice type	Origin	Total concentration ($mg\ kg^{-1}$)	Bioavailable concentration ($mg\ kg^{-1}$)	Percentage of bioavailability
1	White jasmine	Yasotorn	0.2419	0.0305	12.6
2	White jasmine	Surin	0.2442	0.0400	16.4
3	White jasmine	Chachengsao	0.2864	0.0505	17.6
4	White jasmine	Yasotorn	0.2452	0.0563	23.0
5	White jasmine	Surin	0.2949	0.0256	8.7
6	Brown jasmine	Yasotorn	0.3851	0.1141	29.6
7	Brown jasmine	Yasotorn	0.4403	0.1294	29.4
8	Brown jasmine	Yasotorn	0.4112	0.1499	36.5
9	Brown jasmine	N/A	0.4893	0.1629	33.3
10	Brown jasmine	Yasotorn	0.3883	0.1461	37.6

Note: N/A means data is not available

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CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This present study studied the As, Cd, and Zn contamination in sold rice in Bangkok. The total concentrations of metals and metal ingestion rates were determined. Both non-carcinogenic and carcinogenic risks were calculated. Health risk assessment was calculated to indicate potential risks of rice consumption. Furthermore, bioavailable concentrations were determined in order to accurately estimate the fractions of metals that are absorbed to human body. Key results and important findings found in this present study were listed as follow.

1. Total As concentrations in each type of rice were in the order of brown jasmine > white jasmine > white > glutinous rice. In addition, total As concentrations in brown jasmine rice were significantly higher than the others. About 45 % of all rice samples contained As higher than the FDA standard of 0.2 mg kg^{-1}.
2. Total Cd concentrations in each type of rice were in the order of glutinous > brown jasmine > white jasmine = white rice. Glutinous rice was found with significant Cd concentrations higher than the others.
3. Total Zn concentrations were significantly different regarding types of rice in which brown jasmine rice contained the highest level of Zn.
4. A moderately strong positive correlation (R^2 = 0.573) was found between total As and total Zn concentrations.

5. HQ values of As exceeded the threshold level (> 1) by about 2 – 3 times, while, the HQ values of Cd and Zn were much lower than the limit. Therefore, non-carcinogenic impacts of As are expected to be developed as a result of rice consumption.
6. The HI gross indices of As, Cd, and Zn exposure through rice ingestion were found with the main contribution from As exposure (> 90% contribution).
7. Children are the group of population who has greater possibility to experience the adverse non-carcinogenic health effects than the other age group (adolescents, adults, and seniors).
8. Carcinogenic effects caused by As exposure through rice consumption on a daily basis was in magnitude of 10^{-8} which is in the acceptable range of 10^{-6} – 10^{-4}. Hence, there is a low possibility of cancer to be developed. In addition, the prevalence of cancer caused by As exposure is only 1 in 100 million people.
9. Approximately 15.7% and 33.3% of total As were determined as bioavailable As in white jasmine and brown jasmine, respectively.

Based on the above finding, the awareness on public health impacts from metal contamination in rice, especially As, should be raised. Therefore, simple and practical recommendations should be introduced in order to reduce As exposure through rice consumption. For instance, several studies reported that washing of rice before cooking can decrease As by about 10%. Moreover, cooking process in which rice is cooked with excess water (a traditional method), can also reduce total and inorganic As concentrations by about 35% and 45%, respectively. Whereas the modern cooking method could not reduce As content in rice (Raab et al., 2009).

5.2 Recommendations for further study

In order to obtain the more accurate risk information and for better understanding of metal contamination in rice, following recommendations should be concerned in the future study.

1. Sample size of each type of rice studied should be increased to assure the representativeness of sample.
2. The study area should be designed to cover the whole country for the complete risk assessment of rice consumption in Thailand.
3. Rice samples should be collected from the contaminated areas, for example, Cd contaminated area in Tak or lead (Pb) contaminated area in Kanchanaburi to compare the differences in concentrations of metals in rice grown in contaminated and non-contaminated areas to classified As into organic and inorganic As, the more toxic species.
4. Localization of heavy metal in rice grain should be identified in order to explain the accumulation pattern of metals in the grain.
5. The bioavailability of metal in raw rice and cooked rice should be compared to confirm whether the cooking methods could have some effects on bioavailability of metal in food.

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APPENDIX

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Table 1 As, Cd, and Zn concentration with market and origin of rice

Market	Origin	Total concentration (mg kg^{-1})					
		As		Cd		Zn	
		R1	R2	R1	R2	R1	R2
White jasmine rice							
Klongtoei	Surin	0.207	0.186	0.014	0.014	17.05	17.20
Klongtoei	Phatumtani	0.205	0.235	0.022	0.028	16.53	17.40
Klongtoei	Yasotorn	0.215	0.206	0.023	0.024	17.49	19.53
MOF	Surin	0.189	0.205	0.015	0.016	19.50	18.66
MOF	Yasotorn	0.252	0.231	0.030	0.022	21.32	19.22
MOF	Cheingrai	0.198	0.194	0.013	0.013	18.30	19.68
Yingcharen	Surin	0.207	0.219	0.018	0.020	18.97	18.87
Yingcharen	Surin	0.225	0.235	0.019	0.020	18.40	18.07
Yingcharen	Yasotorn	0.228	0.208	0.020	0.020	18.03	18.99
Bangkapi	Cheingrai	0.229	0.233	0.017	0.020	17.84	19.28
Bangkapi	Cheingrai	0.209	0.217	0.019	0.019	19.43	18.43
Bangkapi	Surin	0.222	0.211	0.021	0.018	19.33	15.33
Minburi	Surin	0.228	0.221	0.027	0.028	14.98	17.13
Minburi	Surin	0.235	0.254	0.011	0.014	17.33	16.87
Minburi	Chachengsao	0.274	0.299	0.013	0.025	16.34	17.66
Samyan	Yasotorn	0.251	0.239	0.015	0.013	14.34	14.69
Samyan	Surin	0.179	0.206	0.017	0.019	15.74	14.77
Samyan	Cheingrai	0.211	0.192	0.035	0.031	16.55	16.33
Eakachai	Yasotorn	0.233	0.240	0.018	0.018	16.08	15.21
Eakachai	Surin	0.352	0.238	0.026	0.020	23.94	14.66
Eakachai	Surin	0.200	0.222	0.017	0.019	15.34	15.46
Sanamluang2	Ubonratchatani	0.216	0.212	0.015	0.018	15.20	15.84
Sanamluang2	Surin	0.187	0.196	0.010	0.009	16.22	16.20
Buddhamonthon	N/A	0.232	0.234	0.017	0.018	17.45	17.36
White rice							
Klongtoei	Surin	0.228	0.216	0.019	0.019	13.52	13.41
Klongtoei	Chainat	0.253	0.277	0.008	0.008	15.99	14.60
Klongtoei	N/A	0.226	0.218	0.014	0.013	12.26	12.15
MOF	Nakornsawan	0.172	0.174	0.014	0.015	13.34	12.97
MOF	Nakornsawan	0.198	0.197	0.010	0.013	11.96	12.31
MOF	Pijitr	0.209	0.214	0.009	0.013	11.64	12.26
Yingcharen	Pijitr	0.140	0.141	0.012	0.012	14.23	13.25
Yingcharen	Pijitr	0.239	0.253	0.026	0.027	14.59	14.20
Yingcharen	Nakornsawan	0.229	0.227	0.025	0.020	13.39	12.77
Bangkapi	Nakornsawan	0.189	0.207	0.010	0.009	13.89	14.58

Note: N/A means data is not available.
R1 and R2 are duplicate analyses results.

Table 1 As, Cd, and Zn concentration with market and origin of rice (*Cont.*)

Market	Origin	Total concentration (mg kg^{-1})					
		As		Cd		Zn	
		R1	R2	R1	R2	R1	R2
White rice							
Bangkapi	Pijitr	0.182	0.184	0.009	0.008	12.75	12.60
Bangkapi	Pijitr	0.164	0.154	0.014	0.013	13.03	13.19
Minburi	Pijitr	0.205	0.206	0.016	0.018	15.84	16.27
Minburi	Saraburi	0.199	0.198	0.016	0.018	14.37	13.87
Minburi	Nontaburi	0.185	0.184	0.014	0.015	14.20	13.63
Samyan	Saraburi	0.207	0.186	0.017	0.014	13.80	14.81
Samyan	N/A	0.157	0.166	0.014	0.015	14.97	15.69
Samyan	N/A	0.144	0.146	0.014	0.013	14.60	14.76
Eakachai	Saraburi	0.219	0.213	0.017	0.023	12.94	13.09
Eakachai	Chainat	0.162	0.161	0.015	0.010	16.42	15.34
Eakachai	Chainat	0.190	0.191	0.009	0.012	14.45	14.53
Sanamluang2	Chainat	0.100	0.102	0.006	0.007	15.52	16.80
Buddhamonthon	N/A	0.138	0.143	0.014	0.015	15.95	17.00
Buddhamonthon	Nakornratchasima	0.119	0.117	0.015	0.032	16.68	15.20
Glutinous rice							
Klongtoei	Udontani	0.132	0.133	0.027	0.025	17.38	17.68
Klongtoei	N/A	0.163	0.145	0.064	0.06	19.20	18.16
MOF	Cheingrai	0.147	0.149	0.041	0.043	19.76	20.93
MOF	Cheingrai	0.154	0.146	0.042	0.042	22.44	21.26
Yingcharen	Cheingrai	0.141	0.145	0.021	0.016	19.91	17.51
Yingcharen	Ubonratchatani	0.141	0.141	0.024	0.023	17.40	17.46
Bangkapi	Cheingrai	0.167	0.166	0.054	0.055	20.93	19.62
Bangkapi	Cheingrai	0.140	0.140	0.041	0.038	20.08	20.61
Minburi	Udontani	0.158	0.162	0.019	0.02	20.32	20.82
Minburi	Cheingmai	0.158	0.161	0.043	0.027	21.14	19.34
Samyan	Udontani	0.160	0.162	0.037	0.047	16.75	22.41
Samyan	Cheingrai	0.178	0.170	0.036	0.034	18.01	18.73
Eakachai	Cheingrai	0.157	0.153	0.041	0.042	17.81	17.80
Eakachai	Payao	0.145	0.152	0.038	0.04	18.43	17.79
Sanamluang2	Ubonratchatani	0.168	0.166	0.057	0.061	18.21	17.89
Buddhamonthon	Udontani	0.122	0.120	0.027	0.026	18.78	18.73
Brown jasmine rice							
Klongtoei	Burirum	0.382	0.388	0.016	0.015	35.91	37.00
Klongtoei	N/A	0.357	0.360	0.009	0.012	41.94	43.48

Note: N/A means data is not available.
R1 and R2 are duplicate analyses results.

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Table 1 As, Cd, and Zn concentration with market and origin of rice (*Cont.*)

Market	Origin	Total concentration (mg kg^{-1})					
		As		Cd		Zn	
		R1	R2	R1	R2	R1	R2
Brown jasmine rice							
MOF	Yasotorn	0.376	0.377	0.029	0.027	41.55	42.27
MOF	Yasotorn	0.385	0.496	0.028	0.037	41.16	50.79
Yingcharen	Yasotorn	0.402	0.421	0.009	0.01	40.27	40.72
Yingcharen	Surin	0.377	0.375	0.012	0.013	34.20	36.31
Bangkapi	Nakornratchasima	0.327	0.323	0.008	0.009	39.56	41.51
Bangkapi	N/A	0.495	0.484	0.009	0.009	38.97	38.66
Minburi	Surin	0.322	0.322	0.007	0.007	38.14	38.20
Minburi	Surin	0.172	0.356	0.009	0.025	31.24	32.38
Samyan	Yasotorn	0.356	0.421	0.025	0.008	34.49	34.65
Samyan	Payao	0.387	0.258	0.009	0.008	29.24	30.28
Eakachai	Surin	0.252	0.292	0.008	0.011	32.00	31.90
Sanamluang2	Srisaket	0.283	0.179	0.013	0.01	31.34	31.51

Note: N/A means data is not available
R1 and R2 are duplicate analyses results.

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