Health Risk Assessment of Burmese Related to Consumption of Heavy Metals Contaminated Fish from Local Market in Bangkok, Thailand

Miss Myat Myitzu

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การประเมินความเสี่ยงผลกระทบต่อสุขภาพของชาวเมียนมา จากการบริโภคปลาที่ปนเปื้อนโลหะหนักจากตลาดท้องถิ่น ในกรุงเทพมหานคร ประเทศไทย

น.ส.เมียท มิตซึ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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By	Miss Myat Myitzu
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Thesis Advisor	Dr. POKKATE WONGSASULUK

Accepted by the GRADUATE SCHOOL, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

.... Dean of the GRADUATE SCHOOL
(Associate Professor Dr. YOOTTHANA CHUPPUNNARAT)

THESIS COMMITTEE

.... Chairman
(Professor Dr. SRILERT CHOTPANTARAT)

.... Thesis Advisor
(Dr. POKKATE WONGSASULUK)

.... Examiner
(Assistant Professor Dr. PENRADEE CHANPIWAT)

.... Examiner
(Assistant Professor Dr. VORAPOT KANOKKANTAPONG)

.... External Examiner
(Professor Dr. RATANA SOMRONGTHONG)

เมียท มิตซึ : การประเมินความเสี่ยงผลกระทบต่อสุขภาพของชาวเมียนมา
จากการบริโภคปลาที่ปนเปื้อนโลหะหนักจากตลาดท้องถิ่น ในกรุงเทพมหานคร ประเทศไทย. (Health Risk Assessment of Burmese Related to Consumption of Heavy Metals Contaminated Fish from Local Market in Bangkok, Thailand) อ.ที่ปรึกษาหลัก : ปกเกศ วงศาสุลักษณ์

การรับสัมผัสโลหะหนัก จากการบริโภคปลาที่ปนเปื้อนโลหะหนัก อาจนำไปสู่ปัญหาสุขภาพที่ร้ายแรงได้ การศึกษานี้เป็นการศึกษาแบบภาคตัดขวาง ที่ดำเนินการในช่วงเดือนกุมภาพันธ์และมีนาคม 2565 ในกรุงเทพมหานคร ประเทศไทย วัตถุประสงค์ของการศึกษานี้ คือ 1) เพื่อหาความเข้มข้นของโลหะหนักที่ปนเปื้อนในปลา จากตลาดในประเทศไทย 2) เพื่อประเมินความเสี่ยงผลกระทบต่อสุขภาพจากการบริโภคปลา การสัมภาษณ์และแบบสอบถามออนไลน์ถูกนำมาใช้เพื่อรวบรวมข้อมูลส่วนบุคคลของชาวพม่า 400 คนที่อาศัยอยู่ในกรุงเทพฯ การวิเคราะห์ปริมาณสารหนู แคดเมียม โครเมียม ตะกั่ว และปรอท จะใช้ ICP-MS ตรวจ จากตัวอย่างปลา 4 ชนิด ได้แก่ ปลานิล ปลาดุก ปลาแมคเคอเรล และปลากะพงขาว ผลการศึกษาในปลานิล พบว่า ความเข้มข้นเฉลี่ยของ สารหนู แคดเมียม โครเมียม ตะกั่ว และปรอท เท่ากับ 0.092 ±0.0075 มก./กก. 0.008 มก./กก. 0.015 ± 0.008 มก./กก. 0.004 มก./กก. และ 0.028 ± 0.007 มก. /กก. ตามลำดับ สำหรับปลาดุก พบว่า ความเข้มข้นเฉลี่ยของ สารหนู แคดเมียม โครเมียม ตะกั่ว และปรอท มีค่า0.012 ± 0.0035 มก./กก. 0.008 มก./กก. 0.011 ± 0.008 มก./กก. 0.004 มก./กก. และ 0.029 ± 0.011 มก./กก. ตามลำดับ ความเข้มข้นเฉลี่ยของสารหนู แคดเมียม โครเมียม ตะกั่ว และปรอท ในปลาแมคเคอเรล คือ 0.449 ± 0.052 มก./กก., 0.03 ± 0.009 มก./กก., 0.0153 ± 0.0081 มก./กก., 0.027 ± 0.0066 มก./กก. และ 0.02 ตามลำดับ สำหรับปลากะพงขาว พบความเข้มข้นเฉลี่ยของ สารหนู แคดเมียม โครเมียม ตะกั่ว และปรอท เท่ากับ 0.283 ± 0.1624 มก./กก., 0.008 มก./กก., 0.006 มก./กก., 0.020 มก./กก., 0.022 ± 0.0021 มก./กก. ตามลำดับ ความเข้มข้นทั้งหมดไม่เกินค่ามาตรฐาน สำหรับผลการประเมินความเสี่ยง พบว่า ค่า Hazard Index (HI) จากการบริโภคปลานิล ปลาดุก ปลาแมคเคอเรล และปลากะพงขาวมีค่าเท่ากับ 0.552+0.393, 0.115+0.086, 1.218+0.588 และ 1.138+0.602 ตามลำดับ ทั้งปลาแมคเคอเรลและปลากะพงขาวมีค่าเกินระดับความเสี่ยงที่ยอมรับได้ สำหรับ Total Cancer Risk (TCR) พบว่าในปลานิลมีค่าเท่ากับ $8.49x10^{-5}+8.17x10^{-5}$ ปลาดุก $1.57+10^{-5}+1.53x10^{-5}$ ปลาแมคเคอเรล $1.61x10^{-4}+1.17x10^{-4}$ และปลากะพงขาว $1.39x10^{-4}+1.15x10^{-4}$ จากค่า TCR ของปลาทั้ง 4 ชนิด พบว่ามีความเสี่ยงต่อการเกิดมะเร็ง จากการบริโภคปลาในระยะยาว นอกจากนี้ สำหรับผู้ที่บริโภคปลาทั้ง 4 ชนิดเป็นประจำ พบค่าเฉลี่ย HI และ TCR คือ 2.795+1.108 และ $3.58x10^{-4}+2.22x10^{-4}$ ผลการศึกษานี้ ชี้ให้เห็นว่า การบริโภคปลาแมคเคอเรลและปลากะพงขาว ควรคำนึงถึงปริมาณและความถี่ในการบริโภค หากบริโภคในปริมาณมากและต่อเนื่อง อาจทำให้เกิดผลกระทบต่อสุขภาพได้ในระยะยาว

สาขาวิชา	การจัดการสารอันตรายและสิ่งแวดล้อม	ลายมือชื่อนิสิต ....
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Heavy metals contamination in human through the ingestion of contaminated fish may lead to serious health problems. This study was a cross sectional study conducted during February and March 2022 in Bangkok, Thailand. The objectives of this study were 1) to find the concentration of heavy metals contaminated in fish from local market in Thailand 2) to find the cancer risk and non-cancer risk of heavy metals from fish consumption. Face-to-face interview and online questionnaire were used to collect the personal information of 400 Burmese living in Bangkok. As, Cd, Cr, Pb, and Hg were analyzed by ICP-MS from four fish species: nile tilapia, catfish, mackerel, seabass. The concentration of heavy metals in nile Tilapia found average As, Cd, Cr, Pb, and Hg were 0.092 ±0.0075 mg/kg, 0.008 mg/kg, 0.015 ± 0.008 mg/kg, 0.004 mg/kg, and 0.028 ± 0.007 mg/kg, respectively. For catfish, found average As, Cd, Cr, Pb, and Hg were 0.012 ± 0.0035 mg/kg, 0.008 mg/kg, 0.011 ± 0.008 mg/kg, 0.004 mg/kg, and 0.029 ± 0.011 mg/kg, respectively. The average As, Cd, Cr, Pb, and Hg in Mackerel were 0.449 ± 0.052 mg/kg, 0.03 ± 0.009 mg/kg, 0.0153 ± 0.0081 mg/kg, 0.027 ± 0.0066 mg/kg, and 0.02, respectively. For seabass, found average As, Cd, Cr, Pb, and Hg were 0.283 ± 0.1624 mg/kg, 0.008 mg/kg, 0.006 mg/kg, 0.020 mg/kg, 0.022 ± 0.0021 mg/kg, respectively. All concentrations were not exceeded Thai and International Standards. For non-cancer risk result, the Hazard Index (HI) through the consumption of nile tilapia, catfish, mackerel and seabass were 0.552 + 0.393, 0.115 + 0.086, 1.218 ± 0.588, and 1.138 ± 0.602 respectively. Both mackerel and seabass were exceeded the acceptable risk level. For the total cancer risk (TCR), nile Tilapia was $8.49 \times 10^{-5} + 8.17 \times 10^{-5}$, catfish was $1.57 \times 10^{-5} + 1.53 \times 10^{-5}$, Mackerel was $1.61 \times 10^{-4} + 1.17 \times 10^{-4}$, and seabass was $1.39 \times 10^{-4} + 1.15 \times 10^{-4}$. The TCR from all fish species were above the acceptable level; hence, there may have cancer risk through the long-term consumption of all targeted fish species from this research. Furthermore, for people who consume all four targeted fish species, the mean HI and TCR were 2.795 + 1.108 and $3.58 \times 10^{-4} + 2.22 \times 10^{-4}$. According to the risk results, this study suggests that mackerel and seabass should be concerned amount and frequency of consumption due to they may cause chronic and acute effects from long term consumption.

Field of Study:	Hazardous Substance and Environmental Management	Student's Signature ....
Academic Year:	2021	Advisor's Signature ....

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ABBREVIATION AND ACRONYM

ADI	Average Daily Dose
As	Arsenic
AT	Averaging Time
BMI	Body Mass Index
BW	Body Weight
CALEPA	California Environmental Protection Agency, U.S
Cd	Cadmium
CDI	Chronic Daily Intake
Cr	Chromium
CR	Cancer Risk
Cu	Copper
DOEA	Department of Environmental Affairs
DOF	Department of Fishery
EC	The commission of the European Committees
ED	Exposure Duration
EF	Exposure Frequency
FAO	Food and Agriculture Organization
Fe	Iron
Hg	Mercury
HI	Hazard Index
HQ	Hazard Quotient
IARC	International Agency for Research on Cancer
ICPMS	Inductively Coupled Plasma Mass Spectroscopy
IR	Ingestion Rate
IRIS	Integrated Risk Information System
JECFA	Joint FAO/WHO Expert Committee on Food Additive online database
LOD	Limit of Detection
LOQ	Limit of Quantitation
MeHg	Methylmercury

Mg	Magnisium
Mn	Manganese
Na	Sodium
P	Potassium
Pb	Lead
RfD	Reference Dose
SF	Slope Factor
TCR	Total Cancer Risk
USDOE	U.S. Department of Energy;
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
Zn	Zinc

CHAPTER I

1. Introduction

Heavy metals are chemical elements with relatively large densities, atomic numbers, and hazardous or dangerous properties at specific concentrations. There are essential elements such as zinc, iron, and nickel; however, heavy metals such as mercury, lead, and cadmium were non-essential elements, and they are toxic even at low concentrations. Heavy metals can be existed naturally in the environment; however, anthropogenic heavy metals release leads to significant effects to environment including marine biodiversity. Human can get harmful effects of heavy metals from different sources, and consumption of aquatic animals is one of the exposures of heavy metals toxicity.

1.1 Heavy Metals Problems

Environmental pollution is the world's common problem that leads to various diseases and even premature death. According to the World Bank, over 9 millions of people are dying in their early ages because of directly or indirectly effects of air, water, and soil pollution. This amount is around 16% of all deaths worldwide (Bank). Therefore, environmental pollution becomes critical concern because the impacts on human health are unacceptable. Among the environmental pollution, anthropogenic heavy metals pollution sources are repeatedly released into the environment and give serious toxic threat to the food chain because of bioaccumulation and biomagnification processes (Alturiqi & Albedair, 2012).

The significant water pollution related with heavy metal is that they can dissolve in water, and accumulate in the aquatic animals such as shellfish, fish, and invertebrates. Although some kinds of heavy metals (such as Co, Fe, Cu, Zn, Mn, Se, Mo, Cr, Ca, Mg, S, P, and Na) are necessary at low concentrations for living organisms, non-essential elements (As, Pb, Ca, Ni, and Hg) can give harmful effect even in a small amount of concentration (Fang et al., 2014; Järup, 2003). Once heavy metals are released into river, lake or ocean, the aquatic living things absorb these heavy metals and the whole food chain is ruined by them through bioaccumulation process. Then, human can be toxic through seafood and fish consumption. Therefore, heavy metal contamination in aquatic animals and other food products has been concerned for checking those toxic effects to human health (Farkas et al., 2003).

1.1.1 Heavy Metals Problems in Thailand

As one of the developing countries, Thailand has been processing in urbanization and industrialization sectors since around 1990. The environmental resources have been utilized in order to achieve economic growth and industrial development. Being weak in hazardous waste treatment and waste disposal system in the past, rivers, oceans, drainage system and landfills are the places where all the waste and wastewater directly go in. Additionally, the leachate from landfills and wastewater from treatment plants containing heavy metals leak into water system through groundwater (Pansuwan, 2013); therefore, heavy metal pollution is quite serious problem in Thailand. According to (Wongsasuluk et al., 2018) in Ubon Ratchathani province, Thailand, urine samples were used as biomarkers from who use groundwater as a drinking water source resulted that the health risks related with As, Cd, Pb, and Hg were higher than those from who didn't use the groundwater for drinking.

Thailand is situated on the Indochina peninsula in Southeast Asia, and is bordered on the west and east by the Andaman Sea and the Gulf of Thailand. According to FAO 2018, Thailand occupies land area of 514,000-km^2 and 319,750 km^2 of total water area with 2,624 km continental coastline (Nakai, 2018). The two vital rivers in Thailand are Mekong and Chao Phraya River along with many river basins. Moreover, Thailand occupies much area of lakes (300.000 ha) and reservoirs (255,000 ha) (Bank, 2011). Therefore, it is undeniable that the fish/ seafood production is one of the main sectors for country's consumption. The main protein source for Thai people, especially those living in the coastal area, comes from fish. According to FAO 2016 data, consumption of fish was 33.73 kg/capita/year higher than other meats consumption such as chicken, pork and beef (Officer, 2016).

According to Tanee (2013), heavy metals (Zn, Fe, Cu, Pb) were detected in seven fish samples collected from the Chi River which is located in the Maha Sarakham Province of Thailand. The concentration of Pb in fish was above the acceptable level; however, the remaining elements (Zn, Cu and Fe) were within the acceptable concentration (Tanee et al., 2013).

Heavy metals are toxic in human body and leading to cancer in the lungs, skin and urinary tracts such as cardiovascular disease, diabetes and neurotoxicity. Among the exposure partways, heavy metal poisoning via ingestion is significantly important because people can get 100% of heavy metals contaminated in the fish by oral way; furthermore, happens bioaccumulation. Therefore, investigating the heavy metal content in fish and assessing health risk by eating contaminated fishes are required urgently to examine.

1.2 Sources of Heavy Metal Contamination in Fish

Pollution in water, a common greatest problem worldwide, inextricably links to human activities such as urbanization, industrialization, and modernization of the world. Among them, industrial activity is the significant source of all environmental pollutions related with heavy metals (Ali et al., 2021). Heavy metal pollution in water is very hazardous for both aquatic ecosystem and human health because of their toxicity, bioaccumulation, and long persistence.

Toxic contaminants go into various kinds of water sources: freshwater sources such as lakes, rivers; and seawater sources such as oceans.

1.2.1 Seawater Pollution

Heavy metals in the ocean can be detected through natural processes or human activities. Industrial waste discharge, agriculture, coastal drilling, and construction are the significant processes for heavy metal pollution in seawater. When non-essential elements release into marine environment, it can cause serious problems to marine living things, because of their non-biodegradable, long lasting and bioaccumulation properties (Shah, 2021).

Oil spills are another dominant source of heavy metal pollution in marine environment because crude oil includes hydrocarbon and non- hydrocarbon compounds including heavy metals. The prominent source of oil spills are offshore oil and gas drilling, extraction processes, pipeline leakage and accidental oil spill in oceans. Depending on the type and amount of oil spilled, the effect in water may be different (UKELA). Oil pollution in water affects negatively to marine wildlife by suffocating the aquatic animals in water and poisoning the fishes with heavy metal content in oil. Moreover, oil pollution in water damages the marine plants by blocking the sunlight that is essential for photosynthesis process.

1.2.2 Freshwater Pollution

Industrial sector is the main source of water pollution around the world. Heavy metals released from industrial discharge are very harmful not only for human health but also for the plants and animals. Huge amount of freshwater is used for industrial facilities, and the waste from the factory is discharged directly into rivers and lakes, especially in developing countries (SDWF). The common contaminants from industrial areas are asbestos, lead, mercury, cadmium, chromium, zinc, oils, nitrates, sulphur, phosphates and petrochemicals (Jaishankar et al., 2014). The presence of these heavy metals in water negatively affect to human, plants, aquatic animals, and water body in either a direct or indirect way. The entire ecosystem might be in danger when these chemicals or heavy metals get over limitation (Masindi & Muedi, 2018). In developing countries, sewage and municipal waste are also the major problems of various water pollution due to improper sewage system or lack of wastewater treatment plants (Edokpayi et al., 2017). Heavy metals also contain in sewage and municipal wastewater. In some areas, there are still directly discharges of domestic and wastewater effluents into water sources, and it leads to serious problem in water quality (UNEP). Moreover, urbanization is also one of the heavy metal pollution sources.

1.3 Health Effects of Heavy Metals

The water supply gets polluted by heavy metal from various kinds of pollution sources. The surplus amount of heavy metals are poisonous to ecosystem since they can enter into human bodies from the food chain through a process called bioaccumulation. The heavy metal pollution has negative impacts to the biota, and it may even lead to death of living organisms. The people suffer heavy metal toxicity from three basic exposures pathways: inhalation, injection and skin contact. Heavy metals (namely lead (Pb), arsenic (As), Cadmium (Cd), mercury (Hg), etc.,) are very toxic to human health even in little amount (Fang et al., 2014). Here are environmental and health effects of some common heavy metals released from different sources.

Lead (Pb): Depending on the amount and duration of exposure, the effects of lead on environment and human can be varied. Phytoplankton, the source of oxygen production for aquatic animals, also get polluted by lead. Pb is one of the dangerous chemicals because it can enter into the entire food chains through the bioaccumulation in individual organisms. Mostly, people receive lead from injection like from food and drinking water. Humans can get toxic effects from excess quantity of lead contamination, and it may lead to serious health problems such as the synthesis of hemoglobin (Hb), disease on excretory organ, a rise in blood pressure, gastrointestinal tract (GIT), brain damage, declined fertility of men, and Miscarriages and subtle abortions. Infants and children can be more effected by lead poison than the adult.

Mercury (Hg) and Methylmercury (MeHg): According to WHO, mercury includes in the list of 10 most toxic chemicals for human health (Bolger & Schwetz, 2002). When the environment gets polluted by mercury, it can be transformed into methylmercury by bacteria (Hamdy & Noyes, 1975). Methylmercury bioaccumulates in aquatic organisms such as fish and shellfish. Although people can receive mercury in elemental form under different exposures, injection through eating fish and shellfish contaminated with methylmercury is the significant way (Hong et al., 2012). Both mercury and methylmercury have toxic effects to human health. People can suffer corrosiveness to the skin and eyes through dermal exposure, and gastrointestinal tract failure and kidney toxicity through injection of contaminated water and food. People can get neurological disorders from different mercury compounds by all kinds of exposure, inhalation, ingestion or dermal exposure. Unborn infants can suffer the failure in brains and nervous system by methylmercury exposure if their mothers eat the contaminated fish and shellfish. Even the babies can be exposed to methylmercury through breastfeeding milk. The children can get the negative impacts such as cognitive thinking, attention, language learning, and visual spatial skills after exposing to methylmercury (Grandjean et al., 1994).

Arsenic (As): Arsenic is commonly used in some industrial processes such as glass manufacturing, textile factory, pulp and paper process, metal adhesives, and wood preservation. Drinking and using contaminated water as domestic water supply, irrigation and eating contaminated food are the main sources of exposure to people. As soon as exposed to acute arsenic, people may vomit, pain in abdominal and diarrhea as quick symptoms. In extreme cases, death can be followed after these symptoms (Epa, 1998). (Wongsasuluk et al., 2018) revealed that drinking water is one of the main reasons for contributing factors to heavy metals in urine. In compliance with WHO investigation, long term exposure to arsenic may cause chronic effect from drinking water and eating food contaminated with arsenic. The most prominent health effects from suffering arsenic pollution are skin lesions and skin cancer (Abernathy et al., 2003). However, the first symptoms start with hard skin on hands and feet, and pigmentation changes. Long term effects of exposing arsenic may lead to the following adverse health effects: Pulmonary disease, cardiovascular disease, adverse pregnancy outcomes and infant mortality, and failure in cognitive development, intelligence and memory (Hong et al., 2014).

Cadmium (Cd): mostly come from production of zinc as a by-product, and it is very harmful to human health, especially from occupational environment. Once human get polluted by cadmium, it accumulates and persistent in the body lifetime. Cadmium is very harmful to renal, and it can cause bone demineralization (Järup, 2002).

Table 1-1: Toxicity effects of some heavy metals from ingestion pathway

Elements	Target Organ	Toxic condition	Disease
Pb	Central nervous system, liver	Acute	Appetite loss, headache, abdominal pain, weakness, insomnia, and hallucinations
		Chronic	congenital malformation, mental defect, autistic spectrum disorder, mental illness, immobility, weight loss, hyperactivity, renal damage, coma
Hg	Kidney, brain, developing fetus	Acute	depression, headache, weakness, memory loss, hair fall
		Chronic	weakness in muscles of hands, memory problem, and insomnia
As	Stomach, liver, colon, kidney	Acute	Nausea, vomiting, destruction gastrointestinal tissue and heartbeat abnormalities
		Chronic	cancer, serious effect to main organs, and death
Cd	Stomach, bones	Acute	vomiting and diarrhea
		Chronic	Osteoporosis
Cr		Acute	irritates the mucosal tissue of the gastrointestinal tract
		Chronic	hematological effects, central nervous system

According to the research related with heavy metals in fish from Saudi Arabian markets, heavy metals order was Fe > Zn > Mn > Pb > Cu > Cd > Hg in three fish species (Blackspot emperors, groupers, sardines). Lead and Cadmium content in fish samples exceeded the permissible level of EC (2001) and FAO (1983), 0.4 mg/kg and 0.5 mg/kg respectively. Although mercury level was below the acceptable limit in the edible portion, all studied fish species were detected by Hg (Alturiqi & Albedair, 2012).

High content of cadmium (Cd) was found in the muscles of all 25 fish samples of freshwater fish. The researcher purchased the samples from Gawwein fish landing where is the only site for distributing fishes to markets, Mandalay, Myanmar. This research highlight the Cd content in fish from Ayeyarwaddy River, and the human health risk related to consumption of fish (Mar, 2020).

People can get heavy metal content in fish by consuming and it may lead to health problems. Therefore, examining heavy metal concentration in fish and assessing health risk related with those pollution become indispensable research all over the world.

1.4 Research Questions

- What are the heavy metals concentration (As, Cd, Cr, Pb, and Hg) contaminated in fish from a local market in Thailand?
- Are there any cancer risk and non-cancer risk of heavy metals from fish consumption?

1.5 Research Objectives

- To find the concentration of heavy metals (As, Cd, Cr, Pb, and Hg) contaminated in fish from local market in Thailand.
- To find the cancer risk and non-cancer risk of Burmese from heavy metals (As, Cd, Cr, Pb, and Hg) through the consumption of fish.

1.6 Hypothesis

- Heavy metals contamination in fish from Thailand local market are beyond Thailand and some international standards.
- There are cancer risk and non-cancer risk of heavy metals from fish consumption.

1.7 Scope of Study

Study Area

- The present study was explored in Bangkok, Thailand to study health risk assessment related to heavy metals contamination in fish.

Subjects

- The subjects of the study were Burmese people who are who are currently living in Bangkok, Thailand.
- Interview questionnaire was constructed, and the adult (all genders) between 18 - 60 years old who have been living at the proposed research area for at least six months were interviewed by using convenience sampling method for individual food frequency surveying to examine potential risk factors.
- Sample collection and interviewing started in February-March 2022.

Sampling Method

- Fish samples were purchased from Petchaburi Soi 10 local market to examine heavy metal concentrations and to assess cancer risk and non-cancer risk to human.
- Fish muscle was used in this study.

Ethic

- Then, this study was submitted to the Research Ethics Review Committee for Research Involving Human Research Participants, Group 1, Chulalongkorn University.

1.8 Expected Outcome

- The concentration of heavy metals contaminated in fish from local market.
- The cancer risk and non-cancer risk relate to heavy metals contaminated in fish from local market.

CHAPTER II

2. Literature Review

The present study was about finding out heavy metals concentration in fish and human health risk related with the heavy metal through the consumption of fish. Therefore, literature review was done related with heavy metal pollution in water, heavy metals accumulation in fish and the related health risks.

2.1 Heavy Metals Accumulation in Fish

Heavy metals pollution is the global problem because they can enter food chains through the aquatic environment. The consumption of fish in the Southeast Asian Countries ranges between 6.1- 15 kg per each person yearly; therefore, plays as a the significant diet (FAO, 2014). Fishes are rich in proteins, vitamins and minerals needed for human, and also include unsaturated fatty acids that is benefit for intellectual system (Medeiros et al., 2012). However, the previous research revealed that the people can get harmful effects by eating fish from both local market and aquaculture. The heavy metal pollution problem is increasing because metals can be transported and accumulated in the food chain, and very persistent in the environment (Isangedighi & David, 2019). The researcher emphasized on consequential health effects of As, Cu and Zn by eating contaminated fish in daily life (Chanpiwat et al., 2016). Moreover, Cheng (2013) showed that the prominent negative impacts of heavy metal contamination in fish specifically from large fish farm (Cheng et al., 2013).

The more sensitivity of seawater (marine) fish to water pollution than freshwater fish has been indicated by Scheier et al. (Scheier et al., 1979). Several research have already done to certify heavy metals concentration in ocean animals under various environmental circumstances (Filazi et al., 2003).

Mokarram's (2021) conducted a study on heavy metal content on marine organisms and water quality contamination from petrochemical industry. The study was conducted in Persian Gulf, Asaloyeh, Iran to find out the subsequence effects of Cr, Fe, Pb, Cd, Se, Zn, Cu, and Ni in five fish species (*Scomberomorus guttatus*, *Lethrinus nebulosus*, *Brachirus orientalis*, *Pomadasys* kaakan, *and Scomberomorus commerson)*. A total of 42 samples were analyzed by collecting 3 samples per each species. The average heavy metals (Pb, Cd, Cu, Zn, Fe, Se, and Ni) content in S. guttatus were 1.56 ± 0.90, 2.05 ± 1.16, 0.41 ± 0.12, 6.98 ± 0.91, 36.6 ± 0.82, 3.14 ± 0.44, and 0.109 ± 0.01 mg/kg, respectively. According to the result, Ni, Pb, Cd, and Se concentration were above the maximum allowable concentration of WHO (Pb = 1.5, Cd = 0.2, Cu = 10, Zn = 40, Se = 0.05, and Ni = 0.05 mg/kg). The selected heavy metal contamination in second species, B. orientalis, were 3.98 ± 0.52, 2.11 ± 0.21, 2.72 ± 0.43, 0.03 ± 0.01, 13.71 ± 0.04, 53.77 ± 9.90, 9.68 ± 0.39 mg/kg, whereas Pb, Cu, Zn, Fe, Se, and Ni were beyond the standard. Moreover, Pb and Fe concentration values of P. kaakan were also above the threshold limit. The corresponding mean concentration in L. nebulosus were 0.1 ± 0.014, 1.41 ± 0.11, 18.10 ± 0.89, 5.68 ± 0.52, 0.44 ± 0.39, 2.56 ± 0.47, and 0.82 ± 0.12, while those concentration in S. commerson were 0.14 ± 0.07, 3.49 ± 0.15, 28.94 ± 0.66, 9.37 ± 0.36, 0.3 ± 0.32, 0.57 ± 0.22, and 0.32 ± 0.10, respectively. The concentration of Fe, Cd, Pb, and Ni in L. nebulosus, and Se, Fe, Cd and Pb in S. commerson were above the WHO standard. In that study, it is noticeable that the concentration of most targeted heavy metals in all selected fish species were above the acceptable level in fish(Mokarram et al., 2021).

A study of heavy metals bioaccumulation in selected cultured fish and human health risk assessment Mymensingh Sadar Upazila, Bangladesh was conducted by Ghosh (2021). Five fish species (Pangasius pangasius, Oreochromis niloticus, Heteropneustes fossilis, Anabustes tudineus, Clarias batrachus) were targeted and three samples from each species were collected from five divergent sampling spots. Then, the mean concentration of Fe, Cu, Cr, Co, As, Zn, Hg, and Pb in all fish species were analyzed by using the Metal Pollution Index (MPI). All the targeted heavy metals were detected in all fish species with the exception of cobalt was not found in P. pangasius, and in O.niloticusfish species. However, the average concentration of cobalt (Co) in remaining fish species were beyond the permissible limit of 0.01 μg/g. Iron concentration in C.batrachus species was 104.55 ±6.13 μg/g, while the maximum allowable value of FAO and WHO is 100 μg/g. Highest level of mercury content (1.07 ±0.13 μg/g) was found in P. pangasius and it was above the permissible limit of 0.5. Lead (Pb) and arsenic (As) were detected in all fish species; however, the mean concentration in all fish species were within the range of acceptable limit. The average Cr content in all fish species was in the range of 2.62 to 6.73 μg/g, while the safety limit for Cr is 0.15 (Ghosh et al., 2021).

Moreover, recent research in Thailand was conducted to observe the Co, Pb, Mo, Sr, Cr, Zn, As, Ni, Cd, Ba and Cu content in fish species (both seawater fish and freshwater fish) and sediment. Fish and sediment samples were collected from 10 different areas: Khao Hin Sorn, Khon Kaen, Klong Dan, Loei, Map Ta Phut, Rayong IRPC industrial zone, Praeksa, Samut Sakhon, Tha Tum, Klong Dan, Chanthaburi, and Thap Lan National Park and Klong Yang Canal. Standard operating procedures (SOP) was used to find out heavy metal concentration in fish muscles. The analysis result of fish from Chanthaburi area showed that Hg concentration in *Neolissochillus stracheyi* (masheer barb) exceeded 16 times more than Thai legal standards of freshwater fish. In Khao Hin Sorn area, Hg and MeHg in Snakehead fish reached the safety limit in tissue of fish. Similarly, methylmercury concentration in *Channa micropeltes* (giant snakehead fish) and *Hampala macrolepidota* (hampala barb) collected from Khon Kean reached USEPA standard for daily MeHg consumption. For Klong Dan area, although heavy metal concentration in sediment samples were significantly higher than the acceptable level, those in fish were within the safety limit. According to USEPA standard, eating only 27.3 g of *Oxyeleotris marmorata* (Marble Goby) collected from Loei area would harm the human health because of exceeding two times more than the allowable daily intake of MeHg. Hg and MeHg concentration were significantly high in carnivorous type of fish (such as Goldsilk Seabream, Javelin Grunter, Needle fish, Snakehead fish) collected from Map Ta Phut Town, ranging between 0.042- 1.027 mg kg^{-1} for THg and 0.016- 0.998 mg kg^{-1} for MeHg, respectively. In Prachinburi area, *Channa striata* (snakehead fish) was detected Hg and MeHg by 0.561 mg kg^{-1} and 0.449 mg kg^{-1}, respectively. Moreover, Hg and MeHg concentration in all fish samples (snakehead fish, marble goby, nile tilapia) were beyond the Thai standard for food and USEPA daily intake standard as well. Likewise, THg in threadfin, Asiatic glassfish, and mullet were higher than the maximum allowable limit of Thai standard for food. In this study, mercury and methylmercury were significantly higher than other heavy metals, and leading to human health problems (Tremlová, 2017).

Table *2-1*shows the overview of heavy metals content in fish both from Thailand and international based on recent research results.

Table 2-1: Thailand and International overview of heavy metal content in fish

HM	Fish species	Conc. (mg/kg)	Study area	Safety limit(mg /kg)	Ref.
THAILAND					
Cd	Monopterus albus	6.70 ± 1.26	Mae Sot district, Tak Province	1[a]	(Wahid et al., 2017)
Cd	Sillago sihama	0.33	Samut Sakhon	1[a]	(Tremlová, 2017)
Cd	C. cirrhosus	0.09 ± 0.04	Phu Sang Mountain in Chiang Khan District of Loei province	1[a]	(Sutee Chowrong, 2020)
Cr	C. cirrhosus	0.32 +/- 0.47	Phu Sang Mountain in Chiang Khan District of Loei province	-	(Sutee Chowrong, 2020)
Cr	Catfish	0.72 ± 0.12	Khon KaenProvince	-	(Neeratanaphan et al., 2020)
As	Sillago sihama	0.680	Samut Sakhon	2[b]	(Tremlová, 2017)
As	C. cirrhosus	0.09 ± 0.06	Phu Sang Mountain in Chiang Khan District, Loei province	2[b]	(Sutee Chowrong, 2020)
Fe	C. cirrhosus	41.12 ± 7.08	Phu Sang Mountain in Chiang Khan District of Loei province		(Sutee Chowrong, 2020)
Hg	Snakehead Fish	0.067 ± 0.526	Shalongwaeng Canal	0.5[a]	(IPEN, 2013)
Hg	Fish	0.005 ± 0.840	Erawan (Offshore)	0.5[a]	(Menasveta & Piyatiratitivorakul, 2008)
Hg, MeHg	Lutjanus johnii	0.103, 0.096	Map Ta Phut	0.5[a]	(Tremlová, 2017)
Hg, MeHg	Belonidae	1.027, 0.988	Map Ta Phut	0.5[a] 0.2[b]	(Tremlová, 2017)
Hg, MeHg	Sillago sihama	0.012, <0.015 and 0.60	Samut Sakhon	0.5[a] 0.2[b]	(Tremlová, 2017)
Pb	Sillago sihama	0.11	Samut Sakhon	0.3[b]	(Tremlová, 2017)
Cr	T	2.18 ± 2.79	Mae Klong River, Thailand		(Mazed, 2019)

HM	Fish species	Conc. (mg/kg)	Study area	Safety limit(mg /kg)	Ref.
Pb		8.41 ± 4.09	Mae Klong river, Thailand	0.3[b]	(Mazed, 2019)
INTERNATIONAL					
Pb,	*S. guttatus*	1.56 ± 0.90	Persian Gulf, Iran	0.3[b]	(Mokarram et al., 2021)
Cd	*S. guttatus*	2.05 ± 1.16,	Persian Gulf, Iran	1[a]	(Mokarram et al., 2021)
Zn	*S. guttatus*	6.98 ± 0.91	Persian Gulf, Iran	-	(Mokarram et al., 2021)
Se	*S. guttatus*	3.14 ± 0.44	Persian Gulf, Iran	-	(Mokarram et al., 2021)
Pb	*B. orientalis*	3.98 ± 0.52	Persian Gulf, Iran	0.3[b]	(Mokarram et al., 2021)
Cd	*B. orientalis*	2.11 ± 0.21	Persian Gulf, Iran	1[a]	(Mokarram et al., 2021)
Cu	*B. orientalis*	2.72 ± 0.43	Persian Gulf, Iran	-	(Mokarram et al., 2021)
Zn	*B. orientalis*	0.03 ± 0.01	Persian Gulf, Iran	-	(Mokarram et al., 2021)
Fe	*B. orientalis*	13.71 ± 0.04	Persian Gulf, Iran	-	(Mokarram et al., 2021)
Se	*B. orientalis*	53.77 ± 9.90	Persian Gulf, Iran	-	(Mokarram et al., 2021)
Ni	*B. orientalis*	9.68 ± 0.39	Persian Gulf, Iran	-	(Mokarram et al., 2021)
Cu	*L. nebulosus*	18.10 ± 0.89	Persian Gulf, Iran	-	(Mokarram et al., 2021)
Cd	*S. commerson*	3.49 ± 0.15	Persian Gulf, Iran	1[a]	(Mokarram et al., 2021)
Cu	*S. commerson*	28.94 ± 0.66	Persian Gulf, Iran	1[a]	(Mokarram et al., 2021)
Cu	*P. kaakan*	6.24 ± 1.20	Persian Gulf, Iran	-	(Mokarram et al., 2021)
Fe	*P. kaakan*	7.62 ± 0.03	Persian Gulf, Iran	-	(Mokarram et al., 2021)
Se	*P. kaakan*	25.75 ± 2.71	Persian Gulf, Iran	-	(Mokarram et al., 2021)
Fe	*E. encrasicolus*	7.13 ± 0.19	Malatya, Turkey	-	(Ayhan & Yaman, 2021)
Zn	*S.aurata*	11.00 ± 0.28	Malatya, Turkey	-	(Ayhan & Yaman, 2021)
Hg	Pangasius pangasius	1.07	Mymenshing Sadar Upazil, Bangladesh	0.5[a]	(Ghosh et al., 2021)
Cu	Anabustestu dineus	32.88	Mymenshing Sadar Upazil, Bangladesh	-	(Ghosh et al., 2021)

HM	Fish species	Conc. (mg/kg)	Study area	Safety limit(mg /kg)	Ref.
Zn	Clariasbatra chus	96.56	Mymenshing Sadar Upazil, Bangladesh	-	(Ghosh et al., 2021)

Notes: [a] Thai Ministerial Notification No. 98 of B.E. 2529/1986 and No. 273 of B.E. 2546/2003, [b] US EPA Fact Sheet No. 823-R-01- 001/2001.

Heavy metal contents of fishes in some study areas exceeded the maximum allowable level of some international or Thailand guidelines (comparing Table 1-2 and Table 2-2).

Table 2-2: International standards for maximum allowable levels of some heavy metals in fish and seafood

Reference to	**Foodstuffs**	**Pb**	**Hg**	**MeHg**	**Cd**	**As**
		Maximum Level (Mg/kg)				
JECFA	Fish	0.3	NA	0.5	-	-
"Commission Regulation (EC) No. 1881/2006"	"For selected fishery products: *Lophius spp., Anarhichas lupus, Sarda sarda, Anguilla spp., Hoplostethus spp., Coryphaenoides rupestris, Hippoglossus hippoglossus, Genypterus capensis, Makaira spp., Lepidorhombus spp., Mullus spp., Genypterus blacodes, Esox ivipa, Orcynopsis unicolor, Trisopterus minutus, Centroscymnes coelolepis, Raja spp., Sebastes marinus, S. mentella, S. iviparous, Istiophorus platypterus, Lepidopus caudatus, Aphanopus carbo, Pagellus spp., Carcharodon spp., Lepidocybium flavobrunneum, Ruvettus pretiosus, Gempylus serpens, Acipenser spp., Xiphias gladius, Thunnus, Euthynnus, Katsuwonus pelamis*."	0.3	1.0	-	-	-
	Fishery products excluding the above species	-	0.5	-	-	-
	Muscle meat of fish	0.3	-	-	0.05	-
"US EPA Fact Sheet No. 823-R-01- 001/2001"	Fish and Seafood	NA	0.3 (THg)	0.2	-	-
"Health Canada 2007: Human Health Risk Assessment"	Fishery products	-	0.2 (THg)	-	-	-
"Thai Ministerial Notification No. 98 of B.E. 2529/1986 and No. 273 of B.E. 2546/2003"	Fish and seafood	0.3	0.5	-	1	2 (inorganic
"Australia New Zealand Food Standards Code - Standard 1.4.1 - Contaminants and Natural Toxicants"	Fish, fish products and molluscs	0.5	0.5	-	2	2

2.2 Fish Consumption in Thailand and Myanmar

According to Food and Agriculture Organization (FAO, 2006), the following fishes are the major fish species and the most produced fish species in Myanmar (FAO).

Table 2-3: Fish species widely consumed in Myanmar

Common Name	**Scientific Name**
Roho	*Labeo rohita*
Catla	*Catla catla*
Catfish	*Clarias macrocephalus*
Common carp	*Cyprinus carpio*
Grass carp	*Ctenopharyngodon idellus*
Tilapia	*Oreochromis niloticus*
Striped catfish	*Pangasius sutchi*
Philippine catfish	*Clarias batrachus*
Snakehead fish	*Channa spp.*
Mrigal	*Cirrhinus mrigala*
Silver carp	*Hypophthalmichthys molitrix*
Snakeskin gourami	*Trichogaster*
Spiny eel	*Mastacembelus*

Fish species, including freshwater fish and marine fish, mostly consumed by Thai people are shown in Table 2-5.

Table 2-4: Fish species widely consumed in Thailand

Common name[1]	Local name[1]	Scientific name[1]
Freshwater fish		
Common silver barb	Pla Ta-pien	*Puntius gonionotus*
Nile tilapia	Pla Nil	*Oreochromis niloticus*
Spotted featherback	Pla Graai	*Notopterus chitala*
Snake skin gourami	Pla Sa-lid	*Trichogaster pectoralis*
Striped catfish	Pla Sa-waai	*Pangasius sutchi*
Striped snake-head fish	Pla Chon	*Channa striatus*
Swamp eel	Pla Lai	*Fluta alba*
Walking catfish	Pla Duk-oui	*Clarias macrocephalus*
Marine fish		
Black-banded trevally	Pla Samlee	*Seriolima nigrofasciata*
Black pomfret	Pla Jalamet-dum	*Parastromateus niger*
Silver pomfret	Pla Jalamet-khao	*Pampus argenteus*
Grouper	Pla Gow	*Epinephelus sexfasciatus*
Giant seaperch	Pla Ga-pong khao	*Lates calcarifer*
Malabar red snapper	Pla Ga-pong dang	*Lutjanus malabaricus*
Short-bodied mackerel	Pla Tu-sod	*Rastrelliger brachysoma*
Short-bodied mackerel, steamed	Pla Tu	*Rastrelliger brachysoma*
Spanish mackerel	Pla In-see	*Scomberomorus commerson*

As closed neighboring countries, some fish species popular (such as eel, catfish, tilapia, and snakehead fish, etc.) in Thailand and Myanmar are the same. For the proposed research study, 4 fish species was chosen based on pilot study to the subject’s group, Burmese people who are currently living in Bangkok, in order to evaluate heavy metal concentration in fish. As confirming to the pilot survey, *Oreochromis niloticus*, *Clarias batrachus,* *Rastrelliger brachysoma*, and *Lates calcarifer* are most frequently eat fish species by Burmese people.

According to the literature review, mercury in snakehead fish (*Channa striata)*, and nile tilapia (*Oreochromis niloticus*) from Prachinburi area, Thailand was beyond the Thai standard for food and USEPA daily intake standard (Tremlová, 2017). As reported by a study in Bangladesh , Fe, Cu, Cr, Co, As, Zn, Hg, and Pb concentration were found in the muscle tissue of *Clarias batrachus*, whereas Co and Fe concentration were higher than the maximum allowable value of FAO and WHO (Ghosh et al., 2021).

In both Thailand and other countries, the most common heavy metals that can be accumulated in fishes are Cadmium (Cd), Chromium (Cr), Copper (Cu), Zinc (Zn), Mercury (Hg), Arsenic (As), Lead (Pb), and Selenium (Se). However, the concentration level also depends on species, habitat area, biological habitat, age, gender, body weight and physiological conditions of the fishes (Benzer et al., 2013; Has-Schön et al., 2006; Thakur & Mhatre, 2015). Tremlova (2017) revealed that significant heavy metals contamination in fish were Hg, MeHg, As and following by Fe, Cu, Cr, Zn, and Pb. Therefore, the study was conducted heavy metal (Pb, Cr, As, Cd and Hg) concentration in *Oreochromis niloticus*, *Clarias batrachus,* *Rastrelliger brachysoma*, and *Lates calcarifer* fish species and health risk assessment related with these selected heavy metals contamination in fish.

2.3 Heavy Metal Problems to Human Health

Some heavy metals create several health risks in animals and human through the food chain, leading to chronic health effects. The toxic level in human can be varied according to the intake amount, the exposure pathway and the duration of exposure (acute or chronic) (Jaishankar et al., 2014). According to Di Simplicio (1990) and Patrick (2013), mitochondrial syndrome, lipid peroxidation, and accumulation of neurotoxic molecules are the negative impacts of mercury (Hg) toxicity in the body, and leading to Alzheimer's disease, depression and skin diseases, etc. (Di Simplicio et al., 1990), (Patrick, 2013).When people get contact to arsenic (As) and lead (Pb), it particularly cause respiratory problem and nervous system disorder (Mathew et al., 2019). Entering lead (Pb) into human body can harm to nervous system, cardiovascular system, brain, red blood cells (Rao et al., 2014) and even leukemia (Maiti & Banerjee, 2012). Though zinc (Zn) is an essential elements for the metabolism of the fish (Irerhievwie & Akpoghelie, 2015; Tapia et al., 2012), large amount of accumulation is harmful and can lead to reproductive system failure and less growth rate (Irerhievwie & Akpoghelie, 2015). Subsequently, people who consume the fish can get toxicity gradually through bioaccumulation process (Singer, 2013). Nausea, exhausting and weak in immune system are the health effect of Zn poisoned in human body (Ugokwe & Awobode, 2015).

Human health risk of heavy metals by eating contaminated fish is quite serious, and fish still remains as a primary diet; therefore, observing heavy metal concentration in fishes and assessing health risk by eating fish should be promoted.

Table 2-5: Summaries of sources and symptoms of heavy metal exposure

Heavy Metals	**Source**	**Main toxic form**	**Symptoms and disease**	**References**
Mercury	mining, smelting, waste incineration, coal combustion, barreries	methyl mercury, Hg^{2+}	Mental deficiency, heart rate or blood pressure issues, depression, memory disorder, skin diseases	(Carmona et al., 2008), (Patrick, 2013), (Di Simplicio et al., 1990)
Arsenic	rock weathering, mining and smelting, pigments, drugs, pesticides, fertilisers	arsenites, arsenates, and arsenolipids	Dermal lesions, problem in glucose metabolism, cardiovascular diseases, cancer, nervous disease	(Gentry et al., 2010), (Hughes et al., 2011), (Lin et al., 1999)
Lead	paints, pesticides, gasoline, mining, fossil fuel burning, cosmetics	Pb^{2+}	Abnormal function of CNS, kidney and cardiovascular problems	(Mathew et al., 2019), (Ruden et al., 2009), (Tang et al., 1994)
Cadmium	weathering, pigments, volcanic eruptions, barreries, pesticides	Cd^{2+}	Bone disease, kidney stone, itai-itai disease, carcinogen	(Park et al., 2002), (Rizki et al., 2004), (Fahmy & Aly, 2000)
Chromium	electrochemical	Cr^{6+}	ulcers, inhalation	(Ducros,

	industry, leather processing, chemical plants, sewage, paper production		problem, liver damage	1992), ((Ducros, 1992), (McKenna et al., 2001)
Zinc	Mining, steel production, coal burning, burning of waste	-	stomach cramps, nausea, and vomiting, anemia, damage the pancreas	(Roney, 2005)

2.4 Exposure Pathways

There are three basic exposure pathways to heavy metals for human: inhalation, ingestion and dermal contact. Among these three different exposure pathways, food chain is significant for increasing heavy metal risk to human health (MacIntosh et al., 1996). Moreover, the researchers revealed that heavy metal contamination through eating the food was the main contributor for health risk (Handy, 1996; Zhang et al., 2019). Ingestion pathway is important because the consumers can get the whole quantity of heavy metals contaminated in fishes by eating them. Many researchers have already confirmed that eating fish contaminated with much amount of heavy metals (such as Cd, Pb, Ar, Hg, etc) associated to serious health issues (Burger & Gochfeld, 2005); (Andreji et al., 2006), (Falcó et al., 2006), (Has-Schön et al., 2006). For the general population who have no direct exposure with heavy metals pollution sources, diet is the main pathway for receiving high content in their body (Castro-González & Méndez-Armenta, 2008).

2.5 Health Risk Assessment

A health risk assessment is the process to assists people in identifying and understanding their health risks as by exposing to contaminated environment well as tracking their health through time (USEPA). The recent research about health risk assessment pointed out the health effects of heavy metal contaminated in fish through ingestion pathway.

Ghosh (2021) conducted a study of heavy metals bioaccumulation in selected cultured fish and human health risk assessment Mymensingh Sadar Upazila, Bangladesh. Five fish species (*Pangasius pangasius*, *Oreochromis niloticus*, *Heteropneustes fossilis*, *Anabustes tudineus, Clarias batrachus)* were targeted and three samples from each species were collected from five divergent sampling spots. Then, the mean concentration of Fe, Cr, Hg, Cu, Co, Zn, Pb, and As in all fish species were analyzed by using the Metal Pollution Index (MPI). After that, cancer risk and non-cancer risk for adult and children, who were exposed to heavy metal contamination by ingestion pathway, was calculated. The researcher used the average body weights for children and adult based on the previous research that has been done in that study area. Fish consumption data was got from Department of Fisheries (DOF). For both adult and children, health risk index (HRI) value for mercury in all fish species was the highest; however, HRI for Pb was the lowest. Target Risk for Pb in all fish species was presented ranging from 1.33×10^{-4} to 1.93×10^{-4}, while the acceptable level of USEPA for cancer risk range from 10^{-6} to 10^{-4} (USEPA, 2013). The targeted hazard quotient (THQ) for the metals (Cu, Fe, Pb, Zn, Cr, As, and Co) were acceptable (less than 1), while THQ for Hg in *P. pangasius* fish species was 2. Therefore, the result showed that consumption of fish contaminated with heavy metal can cause cancerogenic and non-cancerogenic effects in human (Ghosh et al., 2021).

Mokarram's (2021) found out the effects of heavy metal content on marine organisms and water quality contamination from petrochemical industry. The study was conducted in Persian Gulf to find out the subsequence effects of Cr, Pb, Fe, Ni, Cd, Zn, Se, and Cu in five fish species (*Scomberomorus guttatus*, *Lethrinus nebulosus*, *Brachirus orientalis*, *Pomadasys* kaakan, *and Scomberomorus commerson)*. A total of 42 samples were analyzed by collecting 3 samples per each species. The researcher calculated Heavy metal pollution index (HMPI) and non-carcinogenic hazard quotient (NHQI) to find out pollution level in water and fish muscles. For P. kaakan and B. orientalis species, NHQI values were 1.036 and 1.046 respectively. The values are slightly higher than the acceptable limit 1, and they indicate that eating these fish species can cause serious effects to human health. The hazard index (HI) in *S. guttatus*, *P. kaakan* and *S. commerson* species for Se, Cd, Pb, Cu, Ni, Zn, and Fe were also found higher than 1. Therefore, the result indicated that the consumption of marine fish species can lead to potential health risk in human. In that study, the researcher pointed out the need of wastewater treatment plants for removing hazardous elements before discharging into receiving water (Mokarram et al., 2021).

A study on heavy metals concentration such as Mn, Zn, Cu, As Fe, Ni, Cd, Pb and health risk assessment in consumable fishes species were observed by Zhu (2015) in Nansi Lake, China.. A total of 288 samples for 9 fish species (*Ophiocephalus argus*, *Carassius auratus*, *Pseudobagrus fulvidraco*, *Parabramis pekinensis*, *Atractoscion nobilis*, *Ctenopharyngodon idellus*, *Scomberomorus niphonius*, *Silurus asotus*, *and Cyprinus carpio*) were collected from 4 different lakes. Edible part of fish was used to assess health risk related with the selected heavy metals on wet weight basis. The subjects of this research targeted to two different groups: general population and fishermen group. Total 1450 adults were participated in questionnaire survey. The HQ of each heavy metal for both general population and fishermen were below the maximum allowable value. Then, HI values for general population were ranged from 0.480 to 0.679, indicating that no adverse health effect cannot cause by consuming fish. However, HI values for fishermen were higher than 1, ranging from 1.165 to 1.742. The major components leading potential health risk of non-carcinogenic consequences for the general population and fisherman include arsenic, lead, and cadmium (Zhu et al., 2015).

The literature reviews showed that both essential heavy metals and non-essential heavy metals (such as Fe, Cu, Hg, As, Zn, Mn, Pb, Cr, Cd, etc.,) can be accumulated in various kind of aquatic animals; therefore, this research focused on the most toxic five non-essential heavy metals (As, Cd, Cr, Pb, and Hg) that were also commonly found in fish of the previous studies.

CHAPTER III

3. Research Methodology

First and foremost, pilot study was done in order to evaluate the possibility for continuing this research. Then, the study was a cross sectional design, and both face to face interview and online google form were used to conduct the questionnaire survey to find out the required information such as body weight, rate, frequency and fish consumption quantity of Burmese who live in Bangkok, Thailand. Moreover, cancer risk and non-cancer risk related with heavy metals ingestion were observed by using the heavy metal concentration in fish and the field survey data.

3.1 Pilot Study

A pilot study was conducted to the subject group, Myanmar people, who are currently living in Thanon Petchaburi, Ratchathewi District, Bangkok, Thailand. Ratchathewi District was chosen as a pilot study area because it is not only one of the places where many Bumese people are residing within Bangkok but also the downtown area.

In this study, a short questionnaire was created that included questions on fish consumption (fish species they eat the most, and the market where they obtain fish) and 32 participants responded. According to the Browne (1995), the sample size for the pilot study is cited as "at least 30 subjects or greater to estimate a parameter" (Browne, 1995). Therefore, 32 participants were interviewed during the pilot survey. The questionnaire for pilot study related with fish species was based on the types of freshwater and marine fishes that are commonly found in Ratchathewi District, Bangkok, Thailand are shown in Table *2-4*. (Puwastien et al., 1999).

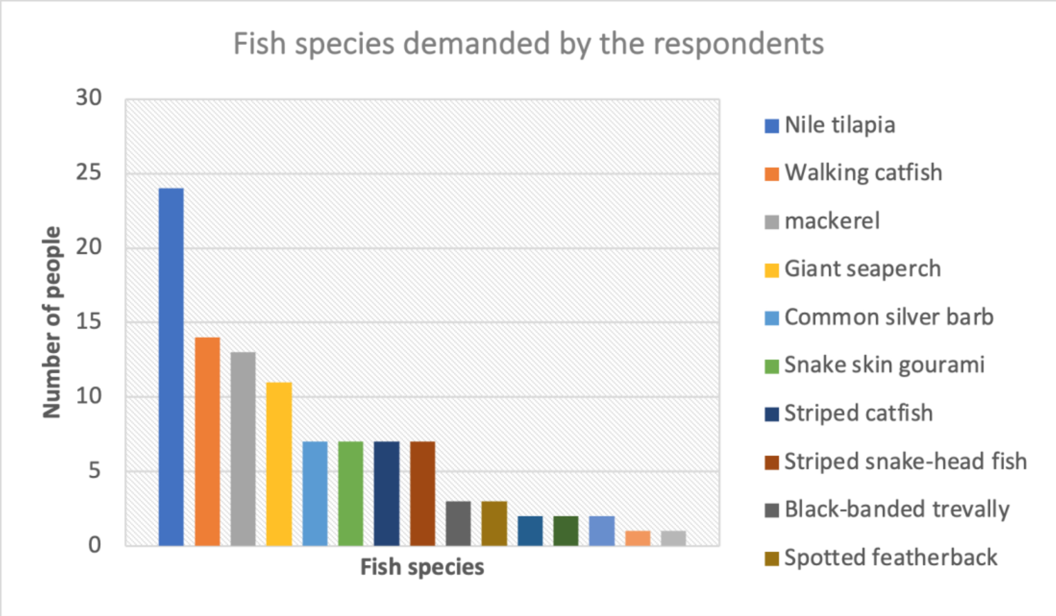


Figure 3-1: Fish species demanded by Burmese according to pilot study

The surveying also included about fish species that Myanmar people frequently eat. According to the data analysis, nile tilapia, walking catfish, mackerel, common silver barb, snakeskin gourami, black-banded trevally, striped catfish, striped snakehead fish, black pomfret, seaperch (seabass), silver pomfret, grouper, spotted featherback, spanish mackerel, Malabar red snapper are fish species normally eat by the participants. nile tilapia has notably demanded by the respondents and followed by walking catfish, mackerel, seaperch (seabass), common silver barb, etc.

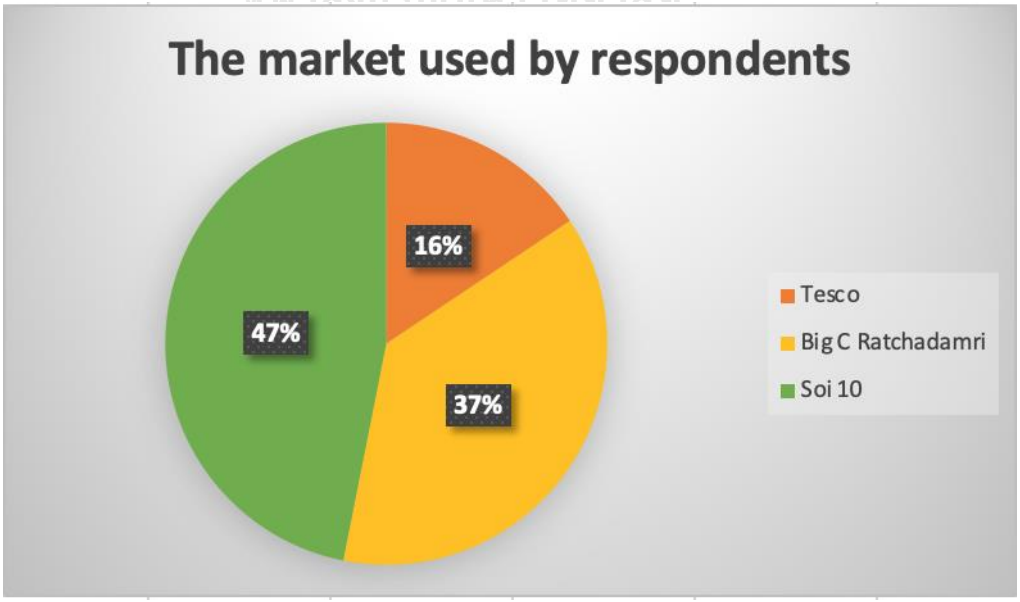


Figure 3-2: The percentages of the markets where people go for buying fishes

From the total study population of 32, the most frequently go markets were Big C (Ratchadamri) and soi 10 local market, while the percentage of going Tesco Rama1 was the lowest: 16

3.2 STUDY AREA

According to pilot survey result on questioning about the place for buying fish, 47% of the respondents answered for soi 10 market out of three markets around Petchaburi road. It is a small market where vegetables, meat, fish, seafood, groceries, and some street food can be purchased. A feasibility study was conducted in the market to find out whether all targeted fish species can be collected from that study area. All selected four fish species (*Oreochromis niloticus*, *Clarias batrachus*, *Rastrelliger brachysoma*, and *Lates calcarifer*) were found in the market. Three fish shops open daily: two opens from morning to noon (around 1 pm), but another fish shop starts open from 1 pm until evening.

Heavy metals contamination in fish were analyzed, and human health risk by consuming fish was calculated. Health risk assessment was done to figure out the sectors to answer, "the contamination of heavy metals to human health by consuming fishes and what types of fish can deteriorate human health more".

Figure 3-3: Fish shops and situation of soi 10 market

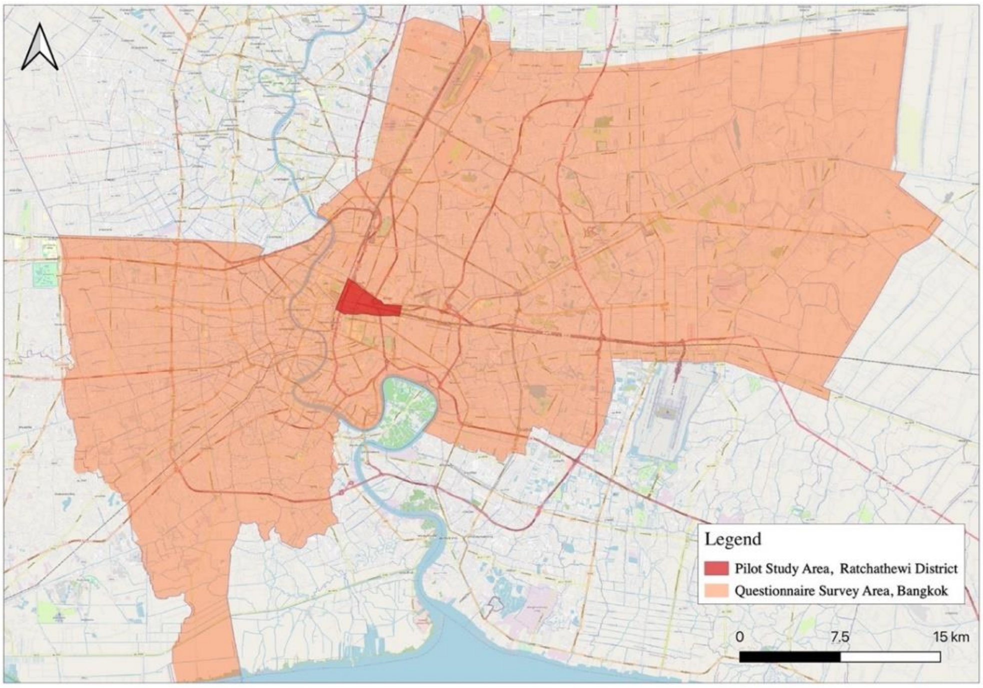


Figure 3-4: Map showing location of pilot study area

3.2 Subjects

The subjects in this study were Burmese people who are currently living around in Bangkok to examine health risk for people who eat fish contaminated with heavy metals. Therefore, individual interviewing for eating fish and some information about interviewee were performed during February and March 2022, and convenience sampling method was used. Both online survey and face to face interview for 400 participants was conducted: 278 participants from online and 122 participants from face-to-face interview. For the question about fish species that the participants eat the most, fish pictures were prepared for their better understanding. In the questionnaire, there are two parts: part A, interviewee information and part B, food frequency survey. There were four questions in interviewee information part and thirteen questions in food frequency survey part. The estimated time for both parts was around 5 minutes. Online surveying was conducted by using Google forms platform and the link was sent to the respondents via email or social media applications (such as Line, Facebook messenger, Instagram, etc.). The information consent form was also sent to make sure that all the respondents understand about the research before taking part in surveying.

A screening form that included inclusion/ exclusion criteria was prepared not to waste the time of the interviewee if they are not relevant with the research criteria. Information related directly to participants was kept confidential and the detail of the personal information such as name and address didn't even included in the questionnaire. Results of the study were reported as pictures, charts or graphs. Participation to the study was voluntary.

The detail information about questions is shown in questionnaire form in the Appendix V (English version) and Appendix VI (Myanmar version).

Figure 3-5: Questionnaire surveying (face to face interview)

3.2.1 Reliability test

To test the internal consistency of the food frequency survey questionnaire, the reliability test using the Cronbach's alpha was calculated using excel. The sample size was 30 people who are currently living in Bangkok. The raw data was described in the following table.

Reliability Test: Cronbach's Alpha

Cronbach's Alpha formula

$$\alpha = \frac{K}{K-1}\left[1 - \frac{\sum s^2 y}{s^2 x}\right]$$

where,

K. = the number of test items
$\sum s^2 y$ = the sum of the item variance
$s^2 x$ = variance of the total score

Cronbach's Alpha	Internal Consistency
1	Excellent
0.90> α >0.80	Good
0.80> α >0.70	Acceptable
0.70> α >0.60	Questionable
0.60> α >0.50	Poor
0.50> α	Unacceptable

Table 3-1: Internal consistency result by using Cronhach's alpha measurement tool

Variables	**Description**	**Values**	**Internal Consistency**
K	the number of test items	20	Acceptable
$\sum S^2 y$	the sum of the item variance	13.3522222	
$S^2 x$	variance of the total score	49.01	
$\propto$		0.76585397	

3.2.2 Sample size calculation

Bangkok occupied total 10.539 million as of 2020 population data. According to 2010 population and housing census, 2% of people was from Myanmar (NSO, 2010).

Therefore, 210,780 of Burmese people have been residing in Bangkok, and Yamane's Formula was used to calculate sample size.

$$n = \frac{N}{1 + N(e)^2}$$

Where, n= sample size

N= population size

e = Level of precision (5%)

Calculation: N = 210780

e = 0.05

$$n = \frac{210780}{1 + 210780(0.05)^2}$$

n= 399 ≈ 400

Total of 400 Burmese people were randomly selected for individual sampling by considering the following inclusion and exclusion criteria.

3.2.3 Inclusion criteria

- Burmese who have been residing in Bangkok, Thailand at least six months.
- Adult male and female (Age range 18-60 years old)
- Can speak and read Burmese.

3.2.4 Exclusion criteria

At the time of individual surveying, the people who are considered as obtaining the following situation were excluded.

- whoever having severe disease (such as heart disease, cancer, and other chronic diseases), unhealthy, bed-ridden or psychological problem were not be considered.
- whoever having fish allergy.
- Whoever under diet program and cannot usually eat fish.

3.2.5 Data Collection under COVID-19 Pandemic

In order to reduce the risk of COVID-19 spread, online google survey form was used to interview Burmese in Bangkok as many as possible. However, face to face interview was also detected. During face-to-face interview, the following ways were obeyed throughout the surveying.

- Maintaining a safe distance from interviewee
- Keeping the mask on all the time (both interviewer and interviewee)
- Sanitize all the documents before delivering to interviewee and collecting from them
- Bringing alcohol sanitizer/ hand gel throughout the interviewing

3.3 Fish Sample Collection

Fish sample collection was performed in April 2022 at a fresh market located on Petchaburi soi 10, Ratchathewi, Bangkok, Thailand for the purpose of observing heavy metal contamination in them. The fish species were selected based on the result of pilot survey that has been done within the proposed study area. The top ranking of fish species that are mostly eaten by Myanmar people is in the order of nile tilapia> walking catfish> mackerel > seabass. Among them, nile tilapia and walking catfish are freshwater fishes and the remaining species (mackerel and seabass) are marine fish species.

According to feasibility survey, there are three fish shops at soi 10 market and collected fish samples from these three fish shops. This research targeted to four fish species: three fishes per species were collected from each fish shop. Therefore, a total of 36 fishes were collected from three fish shops and immediately deep-freezed, kept in polystyrene boxes to keep the freshness before digestion. The detail information including the number of samples and heavy metals that were analyzed are described in the Table *3-3*. And, Table *3-2* illustrates the pictures of the fish along with their scientific names and common names.

Table 3-2: Table showing figures of selected fish species

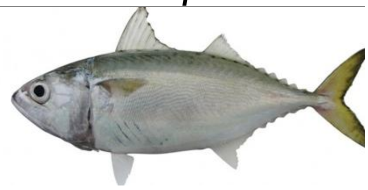
No	*Freshwater fish*	*No*	*Marine fish*
1		*3*	
	Oreochromis niloticus (nile tilapia)		*Rastrelliger brachysoma (mackerel)*
2		*4*	
	Clarias batrachus (catfish)		*Lates calcarifer (seabass)*

Table 3-3: Targeted heavy metals and number of fish samples

No	**Fish**	**Number of samples**			**Elements**				
		Shop (A)	**Shop (B)**	**Shop (C)**	**Hg**	**Pb**	**As**	**Cd**	**Cr**
Freshwater Fish									
1	nile Tilapia	3	3	3	✓	✓	✓	✓	✓
2	catfish	3	3	3	✓	✓	✓	✓	✓
Seawater Fish									
3	mackerel	3	3	3	✓	✓	✓	✓	✓
4	seabass	3	3	3	✓	✓	✓	✓	✓
Total Samples		**36**							

3.4 Fish Samples Analysis

In the laboratory, the fish samples were kept at room temperature, and skin removing, tissue isolation were done by using polyethylene cutter to avoid metal contamination. All the fish samples were analyzed in triplicate and the average concentration was used as heavy metal concentration. The remaining parts such as head, gill, fins, tail and bones didn’t use for this research.

All fish samples were weighted 20 gram of epaxial muscle for analysis. 20 grams of fish muscles for each species were washed with distilled water, dried with filter paper, and stored in a freezer at -20°C till further analysis.

For preparing to get dry weight, the samples were placed into porcelain crucibles that are pre-rinsed with 20% nitric acid. The crucible was heated in muffle furnace 50℃ per hour to 450℃ for 8 hours or overnight until no weight loss. Porcelain mortar and pestle were used to ground into fine powder (Ruden et al., 2009).

0.5 grams ash samples were put in a screw cap polypropylene sample tube. Then, nitric acid (3 ml), hydrogen peroxide (2 ml) and Milli-Q water (3 ml) were added. The sample tube's cap was tightened and cased in an airtight plastic box to prevent acid fumes during digestion process. The liquid samples were filtered by using 0.45 μ membrane filter. Then, the samples were kept in 60 g volumetric flasks and diluted with deionized water to make up final volume of 50 g sample solution (Jarapala et al., 2014) (EPA, 1996).

An analytical method called inductively coupled plasma mass spectrometry (ICP-MS), introduced by Houk et al. in 1980 in the USA, can be used to analyze heavy metal concentration in biological samples. ICPMS has developed into one of the most crucial methods for detecting more than 70% of the elements in the periodic table throughout the following decades, and it can also measure the accurate content of heavy metals. Therefore, Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) was used to analyze mercury (Hg), lead (Pb), arsenic (As), cadmium (Cd), and chromium (Cr) as soon as after digestion.

3.5 Quality Control/ Quality Assurance

All the samples were digested in triplicate and heavy metal contents were presented as an average (Djedjibegovic et al., 2020). In-house method TE-CH-035 was used based on AOAC (2019), 2015.01 was used to analyze arsenic (As) and chromium (Cr). For cadmium (Cd), lead (Pb), mercury (Hg), in-house method TE-CH-035 based on Analyst, 1994, vol119, p.1683-1686 was applied. Limit of detection (LOD) for arsenic, cadmium, chromium, lead, and mercury were 0.010, 0.003, 0.005, 0.006, and 0.004 respectively. Precautionary measures were taken to prevent possible contamination of the samples. Prior to usage, all laboratory equipment were soaked in 2 M HNO3 for two days and then washed multiple times with distilled and deionized water (Jarapala et al., 2014).

Table 3-4: QA/QC result of analytical method for heavy metal concentrations

Element	LOD (mg/kg)	%Recovery	%RSD
As	0.01	98	1.17
Cd	0.003	86.3	2.19
Cr	0.005	88.6	0.99
Pb	0.006	86.5	2.01
Hg	0.004	90.8	1.60

LOD= Limit of detection, RSD= Relative Standard Deviation

3.6 Data Analysis

Heavy metal concentrations data were analyzed by excel (version 16.61.1) to get mean, median, standard deviation, minimum and maximum values. The mean concentrations of heavy metals were used in the risk calculation part. Descriptive analysis was used in this research. Microsoft excel was used to create graphs, and charts.

3.7 Human Health Risk Assessment

Health risk assessment model has been widely used to assess carcinogenic and non-carcinogenic risk in human. The four steps are needed to follow for health risk assessment of heavy metals contaminated in fishes: (a) hazard identification; (b) examining the dose; (c) determining the exposure pathways; (c) assessing the exposure risks. Heavy metals contaminated in fish can enter the human bodies through direct ingestion.

(a) **Hazard Identification:** Hazard identification is the process of evaluating whether the exposure can be related to the occurrence of undesirable health consequences such as cancer and non-cancer diseases. The aim of this step is to define the quality and weight of evidence supporting the forms of adverse health effects that may be produced by exposure to the agent, as well as to identify the types of adverse health effects.

(b) **Dose-Response Assessment:** Dose response relationship shows that how the exposure of an agent (the dose) can get the negative impacts on health (the response).

The reference dose (RfD), consideration of oral exposure on a daily basis, is the dose (oral) derived from the NOAEL and LOAEL with uncertainty factors (Ufs).

Table 3-5: Reference doses and slope factors of heavy metals

Elements	Classification by IARC [e]	RfD (mg/kg*day)	Ref.		Ref.	SF (mg/kg*day)$^{-1}$	References
As	Group I	$3*10^{-4}$	IRIS[a]	3.00E´04	(Kamunda et al., 2016)	1.5	IRIS[a],
Cd	Group I	$1*10^{-3}$	IRIS[a]	5.00E´04	(Kamunda et al., 2016)	0.38	(USDOE[b], 2011)
Cr	Group I	1.5	IRIS[a]	3.00E´03	(Kamunda et al., 2016)	0.5	CALEPA[c]
Pb	Group II (B)	$3.6*10^{-3}$	IRIS[a]	3.60E´03	(Kamunda et al., 2016)	8×10^{-3}	(DOEA[d], 2010)
Hg	Group III	$1*10^{-4}$	IRIS[a]	3.00E´04	(Kamunda et al., 2016)	-	-

Notes: Note: [a] Integrated Risk Information System, U.S. EPA; [b] U.S. Department of Energy; [c] California Environmental Protection Agency, U.S, [d] Department of Environmental Affairs. [e] International Agency for Research on Cancer

Table 3-6: Classification of heavy metals carcinogenicity by IARC

Item	Classified by IARC	
As	Group I	"Carcinogenic to humans"
Cd	Group I	"Carcinogenic to humans"
Cr	Group I	"Carcinogenic to humans"
Pb	Group II B	"Probably Carcinogenic to humans"
Hg	Group III	"Not classifiable as to its carcinogenicity to humans"

(c) Exposure Assessment: This research focuses on health risk assessment through consuming the fishes contaminated with heavy metals. Exposure assessment is the measuring the level of human exposure to a contaminant in the environment.

Average Daily Intake (ADI) for non-cancer risk,

$$ADI = \frac{Cs \times IR \times FI \times EF \times ED}{BW \times AT} \quad \text{equation (1)}$$

Where, C_s = the concentration of heavy metal (mg/kg)

EF = the exposure frequency (meals/year)

FI= Fraction ingestion from contaminated source (unitless)

ED = the exposure duration (years)

BW = the body weight (kg)

AT = the average time (days)

IR = the ingestion rate (kg/meal)

The averaging time, non- carcinogen (ED x 365 days/year)

Chronic Daily Intake (CDI) for cancer risk,

$$CDI = \frac{Cs \times IR \times FI \times EF \times ED}{BW \times AT} \quad \text{equation (2)}$$

Where, C_s = the concentration of heavy metal (mg/kg)

EF = the exposure frequency (meals/year)

FI= Fraction ingestion from contaminated source (unitless)

ED = the exposure duration (years)

BW = the body weight (kg)

AT = the average time (days)

IR = the ingestion rate (kg/meal)

The averaging time for cancer effects, equal to the life expectancy time (70 x 365 = 25,550 days)

The body weight of each participant from individual surveying was used in calculating intake. Similarly, the values of EF, ED, BW, AT and IR were calculated based on the information during individual surveying to Burmese people. The value of the concentration of heavy metals got from fish sample analysis were used in calculations.

(d) **Risk Characterization:** In risk characterization step, the critical findings obtained from dose-response assessment and exposure assessment are needed to be combined (USEPA).

$$\boxed{Risk = Hazard \times Exposure} \qquad \text{equation (3)}$$

For non-cancer endpoint,

Based on USEPA protocols non-cancer endpoint risks are characterized as the hazard Quotient (HQ) (USEPA).

$$\boxed{HQ = \frac{ADI}{RfD}} \qquad \text{equation (4)}$$

ADI= Acceptable Daily Intake, RfD= Reference Dose

If HQ > 1, Risk of Adverse Health Effect

If HQ ≤ 1, No Adverse Health Effect Anticipated

For cancer Endpoint,

The cancer risk (CR) was calculated by multiplying the average daily intake over a lifetime with a cancer slope factor (SF) (USEPA).

$$\boxed{CR = CDI \times SF} \qquad \text{equation (5)}$$

Chronic Daily Intake is averaged over 70 years.

Typically, an acceptable risk is defined as < 1 x 10^{-6}. However, the values, 10^{-4} to 10^{-5} may be acceptable.

Aggregate risks for multiple substances

Carcinogenic effects

$$Risk_T = \sum_i Risk \qquad \text{equation (6)}$$

$Risk_T$ = Total cancer risk

$Risk_i$ = The risk estimated for the i substance

Non-carcinogenic effects

The hazard index (HI) was calculated to get the potential risk of negative health risk from a mixture of heavy metals (As, Cd, Cr, Pb, and Hg) in fish. HI was calculated by the sum of HQ for each heavy metal.

$$Hazard\ Index\ (HI) = \sum HQ \qquad \text{equation (7)}$$

If HI < 1, it is acceptable for cumulative exposure and chronic risks cannot be happened, whereas non-cancer risks are likely to occur in case HI =1 or HI>1.

Synergistic effect of heavy metals can be occurred when individual heavy metal interacts or cooperate. Therefore, it's needed to calculate the aggregate risk.

Degree of risk

For non cancer risk, if HQ or HI is equal or higher than 1, there is risk by eating fish in long term. The HQ or HI value lower than 1 means acceptable. For the cancer risk, the value 1 x 10^{-6} is acceptable, and it means there is only one person in a million having the cancer risk.

3.8 Research Ethic

The research instruments/tools (questionnaire), research proposal along with required documents were submitted to the Research Ethics Review Committee for Research Involving Human Research Participants, Group 1, Chulalongkorn University. The committee has approved on 2nd March 2022 and will be expired on 1st March 2023. The research ethic approved with a Certificate of Approval (COA) No. 054/65.

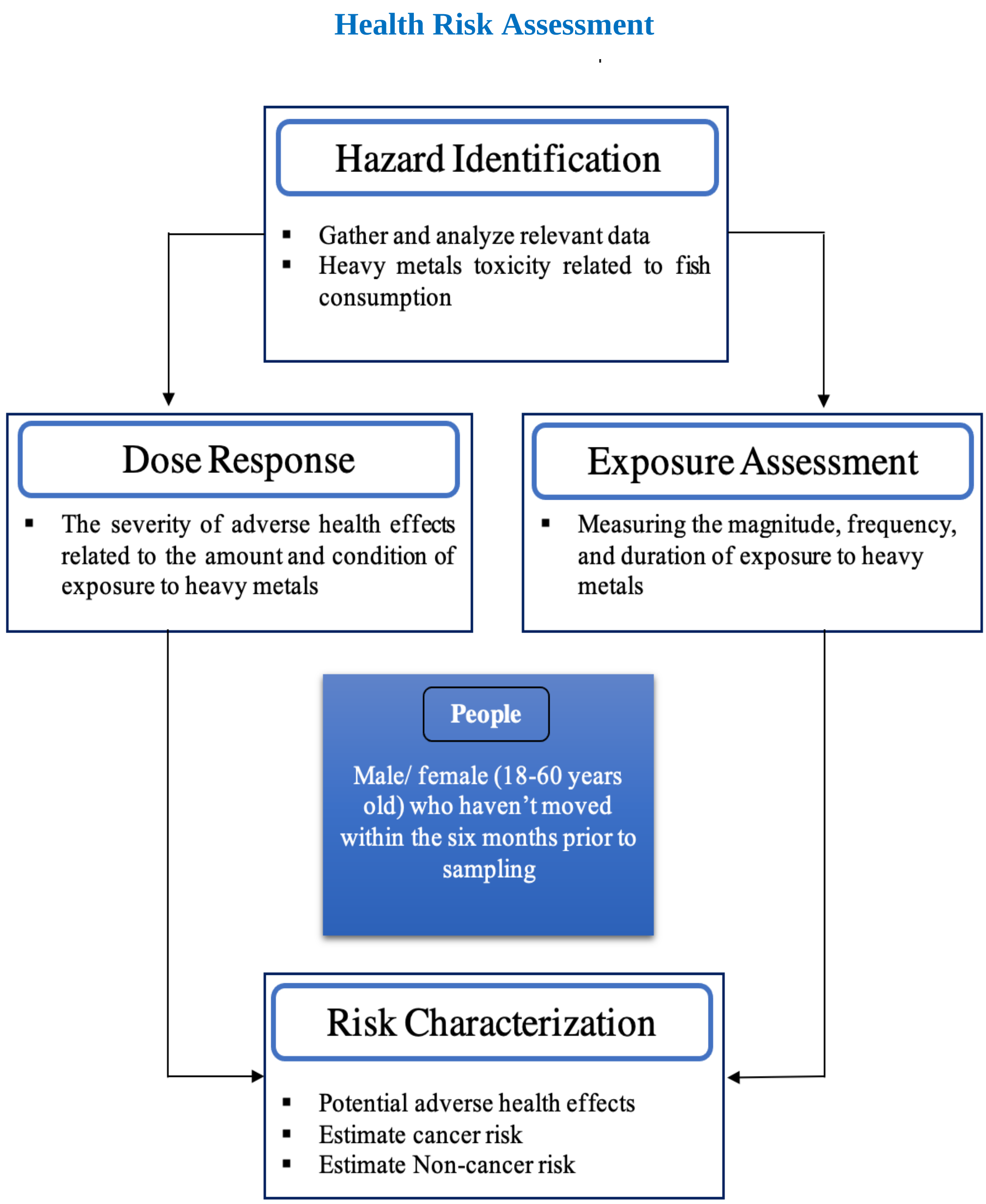


Figure 3-6: Flow chart of health risk assessment process

CHAPTER IV

4. Results

This study was a cross sectional study performed in Bangkok, Thailand to find out heavy metals concentration in fish and to examine the health risk related with the consumption of fish. The questionnaire was used to observe the necessary personal information and fish consumptions such as frequency and amount of fish per meal. Both face to face and online google form were used to conduct the survey. This chapter provides the results of heavy metals concentration in fish and human health risk (carcinogenic and non-carcinogenic) through eating of fish from a local market of Bangkok.

4.1 Heavy Metals Concentration in Fish

The concentration of mercury (Hg) in nile tilapia and catfish, and lead (Pb) in seabass were lower than the limit of detection. Hg concentration in mackerel and seabass were 0.028 mg/kg and 0.02 mg/kg, and lead (Pb) in tilapia, catfish and mackerel were the same, < 0.02 mg/kg. Cr concentration in tilapia, catfish, mackerel and seabass were 0.02 mg/kg, 0.022 mg/kg, < 0.02 mg/kg, and 0.024 mg/kg; therefore, Cr contents in four targeted fish species from shop-1 were not much different. Similarly, cadmium (Cd) in three fish species (tilapia, catfish, and seabass) were the same (< 0.008 mg/kg), and 0.028 mg/kg in mackerel. The arsenic (As) concentration in nile tilapia, mackerel and seabass were 0.099 mg/kg, 0.507 mg/kg, and 0.196 mg/kg, respectively. As was not found in catfish.

From the fish shop-2, arsenic (As) concentrations were 0.093 mg/kg in tilapia, 0.432 mg/kg in mackerel, 0.182 mg/kg in seabass, and it was lower than the limit of detection (LOD). Cadmium (Cd) concentration in tilapia, catfish, and seabass were the same (< 0.008 mg/kg), and Cd content in mackerel was 0.039 mg/kg. Lead (Pb) concentrations in all fish species from fish shop 2 were lower than LOD. Likewise, mercury in nile tilapia and catfish were lower than LOD; however, 0.033 mg/kg and less than 0.02 mg/kg of Hg were found in mackerel and seabass. Then, chromium (Cr) in tilapia, catfish, mackerel and seabass were 0.033 mg/kg, 0.042 mg/kg, < 0.02 mg/kg, and 0.023 mg/kg, respectively.

From the fish shop-3, arsenic (As) concentrations were 0.084 mg/kg, 0.016 mg/kg, 0.408 mg/kg, 0.47 mg/kg in tilapia, catfish, mackerel, and seabass. Cadmium (Cd) content was less than 0.008 mg/kg in tilapia, cadmium, and seabass; however, Cd in mackerel is slightly higher than the other three fish species (0.022 mg/kg). Lead (Pb) in tilapia and mackerel were lower than 0.02 mg/kg, and it was less than 0.006 mg/kg in catfish and seabass. Similarly, Hg concentration in mackerel and seabass were lower than 0.02 mg/kg, and it was not found in tilapia and catfish. Chromium (Cr) contents in mackerel and seabass were < 0.02 mg/kg, and in tilapia and catfish were 0.032 mg/kg and 0.024 mg/kg.

Table 4-1: Mean heavy metals concentrations in fish species

Items		**Nile Tilapia**	**Catfish**	**Mackerel**	**Seabass**
As	Mean ± SD	0.092 ±0.0075	0.012 ± 0.0035	0.449 ± 0.052	0.283 ± 0.1624
Cd		0.008	0.008	0.03 ± 0.009	0.008
Pb		0.015 ± 0.008	0.011 ± 0.008	0.0153 ± 0.0081	0.006
Hg		0.004	0.004	0.027 ± 0.0066	0.020
Cr		0.028 ± 0.007	0.029 ± 0.011	0.02 ± 0	0.022 ± 0.0021

Heavy metal mean concentration ± standard deviation for each fish species is described in Table *4-1* and Figure *4-1*. As concentration was remarkably higher in mackerel (0.449 ± 0.052 mg/kg) than other three fish species (0.092 ± 0.0075 in tilapia, 0.012 ± 0.0035 mg/kg in catfish, and 0.283 ± 0.1624 mg/kg in seabass). Moreover, heavy metals (As, Cd, Pb, and Hg except Cd) concentrations were the highest in mackerel than other three fish species. Chromium (Cr) content was the highest in catfish although As, Cd, Pb, Hg were found the lowest in catfish.

Heavy metals concentration order in nile tilapia was As > Cr > Pb > Cd > Hg, therefore As was found highest amount in tilapia. Likewise, the concentration order in remaining fish species were Cr > Cd> Pb > As > Hg for catfish, As > Cd > Hg > Cr > Pb in mackerel, and As > Cr > Hg > Cd > Pb for seabass, respectively.

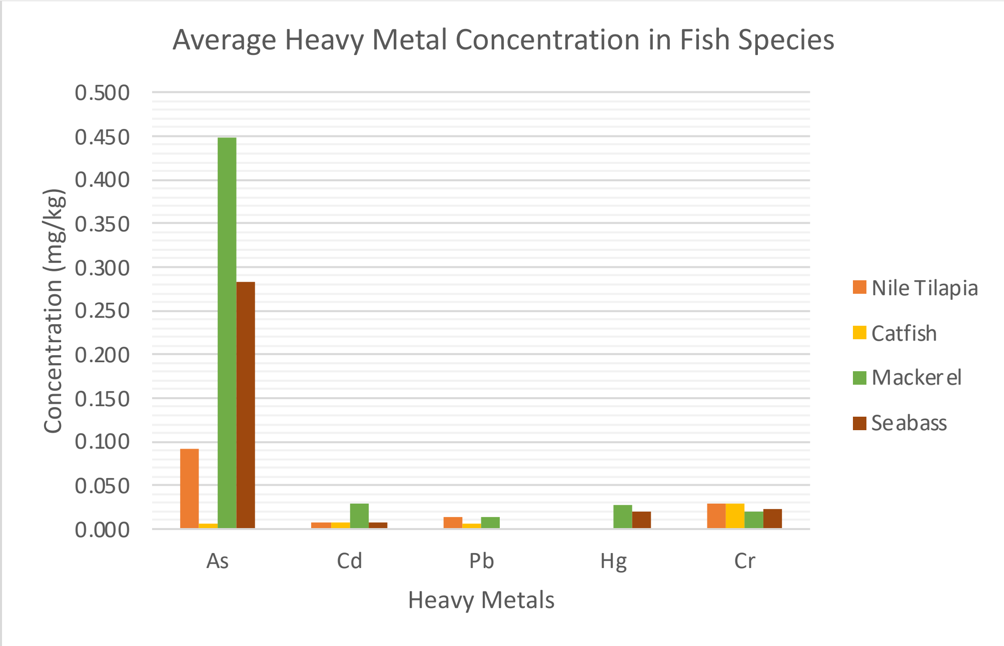


Figure 4-1: Concentration of heavy metals in fish species

4.2 Personal Information of Participants

According to surveying, a total of 400 Burmese (245 female and 155 male) were interviewed who were currently residing in Bangkok, Thailand. 22% of more female were participated than male. Both face to face interview and online questionnaire were used to collect personal information and fish consumption.

The age of all participants was ranged from 18 to 60 years old, and the average age ± standard deviation was 37 ± 12 years. For 155 male participants and 245 female participants, mean age ± SD were 38 ±12 and 36 ±12 years, respectively. When the participants' ages were divided into range 18-24, 25-34, 35-44, 45-54, and 55-60 years old, the age range between 25-34 was the highest number that participated (31%) in surveying and followed by 35-44 (23%), 45-54 (18%), 18-24 (16%), and 55-60 (12%) age ranges. In numbers, 66, 122, 92, 71 and 49 participants were in age range 18-24, 25-34, 35-44, 45-54, and 55-60 years old, respectively.

The minimum and maximum body weight of the participants were 40 to 92 kilograms with the average weight of 60.6 kg. Average height was 162 cm with a range of 140 to 184 cm. For only male participants, mean body weight and height ± standard deviation were 63.27 ± 9.93 kg and 163.87 ± 9.79 cm, whereas the range were 45 to 92 kg and 150 to 184 cm. For female participants, mean body weight and height ±

standard deviation were 58.91 ± 9.31 kg and 160.84 ± 8.98 cm. Body weight and height of female participants ranged from 40 to 85 kg and 140 to 183 cm. Average BMI of the participants was 23.11 ± 3.46 Kg/m^2, with the range of 18.13 to 32.99 Kg/ $m^{2.}$

Table 4-2: Characteristics of participants

Characteristics	**N**	**Percentage**
Sex		
Male	155	39%
Female	245	61%
Age Range		
18-24	66	17%
25-34	122	31%
35-44	92	23%
45-54	71	18%
55-60	49	12%
Characteristics	**Category**	**Values**
Age (years)	Mean	37.21
	SD	12.12
	Median	35.00
	Minimum	18.00
	Maximum	60.00
Body Weight (Kg)	Mean	60.61
	SD	9.77
	Median	60.00
	Minimum	40.00
	Maximum	92.00
Height (cm)	Mean	162.01
	SD	9.41
	Median	160.00
	Minimum	140.00
	Maximum	184.00
BMI (kg/m2)	Mean	23.11
	SD	3.46
	Median	22.36

Minimum	18.13
Maximum	32.99

n= number of participants, SD= standard deviation

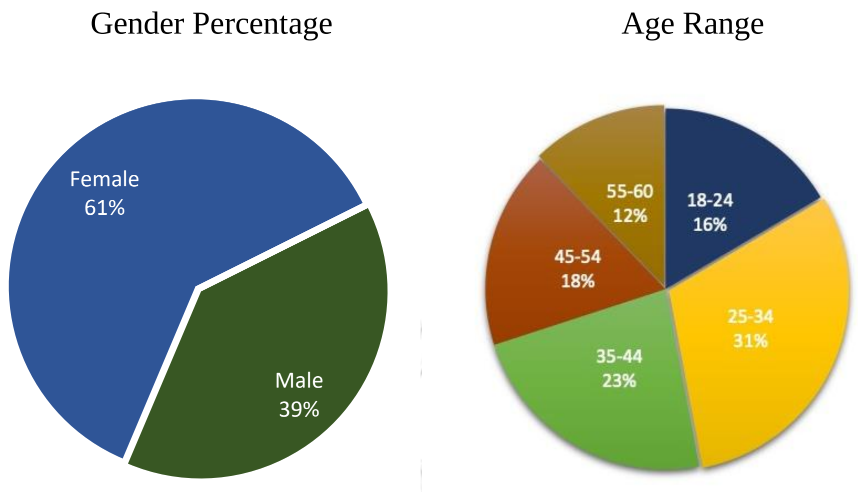


Figure 4-2: Gender percentage and age range of respondents

4.3 Exposure Assessment

By using food frequency survey form, four fish species [*Oreochromis niloticus* (nile tilapia), *Clarias batrachus* (catfish), *Rastrelliger brachysoma* (mackerel), and *Lates calcarifer* (seabass)] were included to evaluate whether the participants eat these fish species or not. According to survey result, *Oreochromis niloticus* (nile tilapia) was the fish species the most consumed by Burmese. 375 participants out of 400 answered “YES” for the question “do you eat nile tilapia?”. Among 400 participants, the number of participants who eat catfish, seabass, mackerel were 366, 343 and 316, respectively. It is found out that mackerel was the fish species least preferred by Burmese.

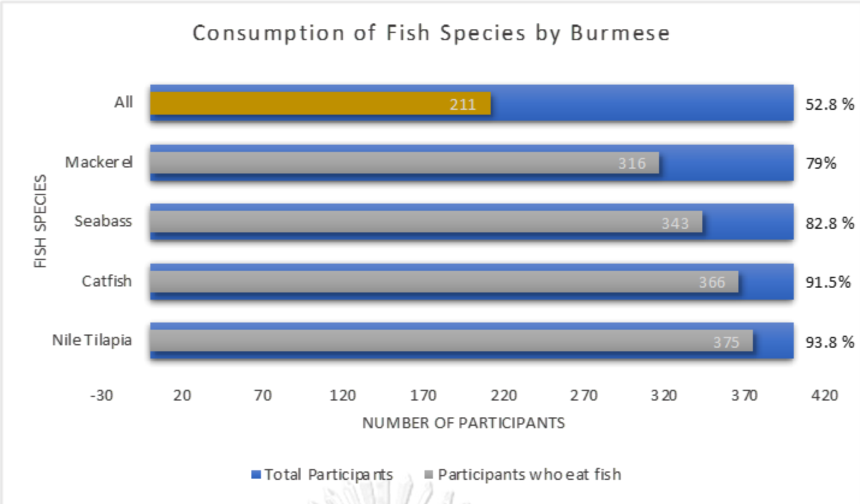


Figure 4-3: Consumption of fish species by Burmese

Then, the ingestion rate for each species (nile tilapia, catfish, mackerel, seabass) were evaluated. Mean ± standard deviation of nile tilapia by Burmese was 0.35 ± 0.16 kg/day, and the minimum and maximum consumption rate were 0.03 kg/day and 0.6 kg/day. Similarly, the ingestion rate of catfish mackerel, and seabass were 0.28 ± 0.13 kg/day, 0.17 ± 0.10 kg/day, and 0.25 ± 0.11 kg/day, respectively. The minimum and maximum ingestion rate were 0.05 g/day, 0.50 kg/day for catfish, 0.04 kg/day, 0.5 kg/day for mackerel, and 0.1 kg/day, 0.5 kg/day for seabass. The maximum ingestion rate of nile tilapia was the highest and catfish, mackerel and seabass ingestion rate were the same (0.5 kg/day).

Exposure frequencies were not much different for four targeted fish species by Burmese: 94.69 ± 44.24 day/year for nile tilapia, 93.61 ± 45.94 day/ year for catfish, 95.82 ± 43.86 day/year for mackerel, and 92.53 ± 45.47 day/year for seabass.

Table 4-3: Ingestion rate and exposure frequency for each species by Burmese

	IR (kg/day)	**EF (day/year)**	**ED (years)**
		Nile Tilapia	
Mean	0.35	94.69	23.43
Median	0.35	104.28	21.00
SD	0.16	44.24	11.20
Min	0.03	26.07	1.00
Max	0.60	156.42	56.00
		Catfish	
Mean	0.28	93.61	23.53

Median	0.25	104.28	21.00
SD	0.13	45.94	11.41
Min	0.05	26.07	0.33
Max	0.50	208.56	57.00
		Mackerel	
Mean	0.17	95.82	23.28
Median	0.15	104.28	20.00
SD	0.10	43.86	12.03
Min	0.04	26.07	1.00
Max	0.50	156.42	59.00
		Seabass	
Mean	0.25	92.53	22.17
Median	0.25	104.28	20.00
SD	0.11	45.47	16.21
Min	0.10	26.07	1.00
Max	0.50	156.42	200.00

IR= ingestion rate (kg/day), EF= exposure frequency (day/year), ED= exposure duration (years), SD= standard deviation, Min= minimum, Max= maximum

4.4 Non-Cancer Risk Assessment

To evaluate non cancer risk, average daily intake (ADI) was calculated by inserting heavy metal concentration, ingestion rate (IR), exposure frequency (EF), exposure duration (ED), body weight (BW), and averaging time of each participant according to the equation (1). The ADI was calculated for each fish species for the reason that IR, EF, and ED were different depending on fish species. Then, hazard quotient (HQ) was calculated for the heavy metals (As, Cd, Cr, Pb, Hg) in tilapia, catfish, mackerel and seabass for each of the participants by using equation (4). To find out the sum of non-cancer risks of As, Cd, Cr, Pb and Hg in fish, hazard index (HI) was evaluated according to the equation (7).

Table 4-4: Average daily intake (ADI) and hazard index (HI) of heavy metals by consuming targeted fish species

Element	Category	Mean	Median	SD	Min	Max
			Tilapia			
As	ADI	1.42×10^{-4}	1.17×10^{-4}	1.01×10^{-4}	5.01×10^{-6}	4.82×10^{-4}
	HQ	4.72×10^{-1}	3.89×10^{-1}	3.36×10^{-1}	1.67×10^{-2}	1.61
Cd	ADI	1.23×10^{-5}	1.01×10^{-5}	8.77×10^{-6}	4.36×10^{-7}	4.19×10^{-5}
	HQ	1.23×10^{-2}	1.01×10^{-2}	8.77×10^{-3}	4.36×10^{-4}	4.19×10^{-2}

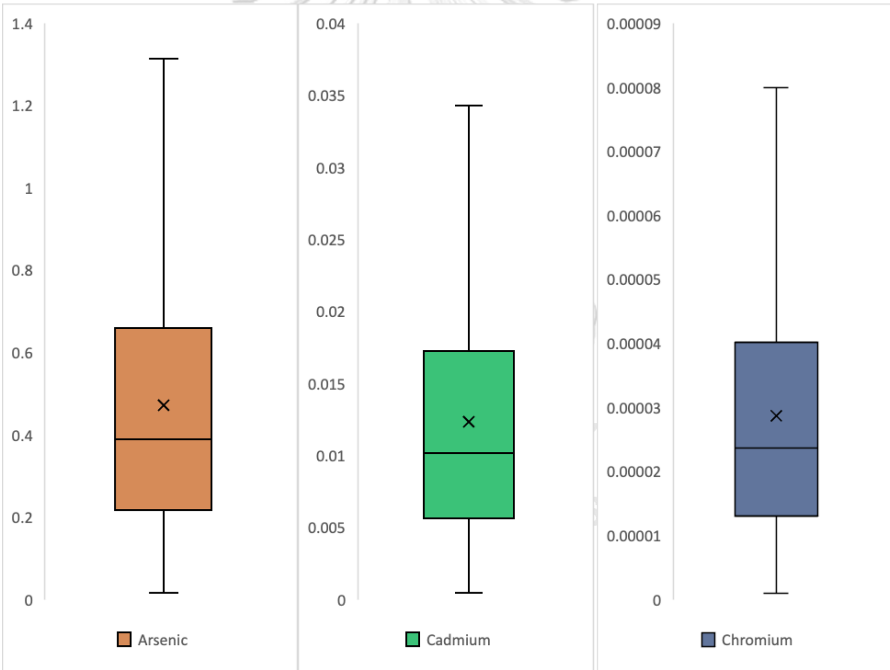
Cr	ADI	4.31×10^{-5}	3.55×10^{-5}	3.07×10^{-5}	1.53×10^{-6}	1.47×10^{-4}
	HQ	2.87×10^{-5}	2.37×10^{-5}	2.05×10^{-5}	1.02×10^{-6}	9.78×10^{-5}
Pb	ADI	2.31×10^{-5}	1.90×10^{-5}	1.64×10^{-5}	8.17×10^{-7}	7.86×10^{-5}
	HQ	6.41×10^{-3}	5.28×10^{-3}	4.57×10^{-3}	2.27×10^{-4}	2.18×10^{-2}
Hg	ADI	6.15×10^{-6}	5.07×10^{-6}	4.38×10^{-6}	2.18×10^{-7}	2.10×10^{-5}
	HQ	6.15×10^{-2}	5.07×10^{-5}	4.38×10^{-2}	2.18×10^{-3}	2.10×10^{-1}
HI		0.55	0.45	0.39	0.02	1.88
			Catfish			
As	ADI	1.51×10^{-5}	1.14×10^{-5}	1.13×10^{-5}	5.04×10^{-7}	5.59×10^{-5}
	HQ	5.03×10^{-2}	3.81×10^{-2}	3.76×10^{-2}	1.68×10^{-3}	1.86×10^{-1}
Cd	ADI	1.01×10^{-5}	7.62×10^{-6}	7.52×10^{-6}	3.36×10^{-7}	3.73×10^{-5}
	HQ	1.01×10^{-2}	7.62×10^{-3}	7.52×10^{-3}	3.36×10^{-4}	3.73×10^{-2}
Cr	ADI	3.64×10^{-5}	2.76×10^{-5}	2.73×10^{-5}	1.22×10^{-6}	1.35×10^{-4}
	HQ	2.43×10^{-5}	1.84×10^{-5}	1.82×10^{-5}	8.12×10^{-7}	9.01×10^{-5}
Pb	ADI	1.38×10^{-5}	1.05×10^{-5}	1.03×10^{-5}	4.62×10^{-7}	5.12×10^{-5}
	HQ	3.84×10^{-3}	2.91×10^{-3}	2.87×10^{-3}	1.28×10^{-4}	1.42×10^{-2}
Hg	ADI	5.03×10^{-6}	3.81×10^{-6}	3.76×10^{-6}	1.68×10^{-7}	1.86×10^{-5}
	HQ	5.03×10^{-2}	3.81×10^{-2}	3.76×10^{-2}	1.68×10^{-3}	1.86×10^{-1}
HI		0.11	0.09	0.09	0	0.42
			Mackerel			
As	ADI	3.04×10^{-4}	3.00×10^{-4}	1.47×10^{-4}	1.63×10^{-5}	5.83×10^{-4}
	HQ	1.01	1.00	4.89×10^{-1}	5.42×10^{-2}	1.94
Cd	ADI	2.03×10^{-5}	2.00×10^{-5}	9.79×10^{-6}	1.09×10^{-6}	3.90×10^{-5}
	HQ	2.03×10^{-2}	2.00×10^{-2}	9.79×10^{-3}	1.09×10^{-3}	3.90×10^{-2}
Cr	ADI	1.35×10^{-5}	1.34×10^{-5}	6.53×10^{-6}	7.25×10^{-7}	2.60×10^{-5}
	HQ	9.02×10^{-6}	8.91×10^{-6}	4.35×10^{-6}	4.83×10^{-7}	1.73×10^{-5}
Pb	ADI	1.03×10^{-5}	1.02×10^{-5}	4.99×10^{-6}	5.54×10^{-7}	1.99×10^{-5}
	HQ	2.87×10^{-3}	2.84×10^{-3}	1.39×10^{-3}	1.54×10^{-4}	5.52×10^{-3}
Hg	ADI	1.83×10^{-5}	1.80×10^{-5}	8.81×10^{-6}	9.78×10^{-7}	3.51×10^{-5}
	HQ	1.83×10^{-1}	1.80×10^{-1}	8.81×10^{-2}	9.78×10^{-3}	3.51×10^{-1}
HI		1.22	1.2	0.59	0.07	2.34
			Seabass			
As	ADI	2.81×10^{-4}	2.64×10^{-4}	1.47×10^{-4}	2.81×10^{-5}	5.88×10^{-4}
	HQ	9.37×10^{-1}	8.79×10^{-1}	4.91×10^{-1}	9.36×10^{-2}	1.96
Cd	ADI	7.95×10^{-6}	7.45×10^{-6}	4.16×10^{-6}	7.94×10^{-7}	1.66×10^{-5}
	HQ	7.95×10^{-3}	7.45×10^{-3}	4.16×10^{-3}	7.94×10^{-4}	1.66×10^{-2}
Cr	ADI	2.19×10^{-5}	2.05×10^{-5}	1.14×10^{-5}	2.18×10^{-6}	4.57×10^{-5}
	HQ	1.46×10^{-5}	1.37×10^{-5}	7.63×10^{-6}	1.45×10^{-6}	3.05×10^{-5}
Pb	ADI	5.96×10^{-6}	5.59×10^{-6}	3.12×10^{-6}	5.95×10^{-7}	1.25×10^{-5}
	HQ	1.66×10^{-3}	1.55×10^{-3}	8.67×10^{-4}	1.65×10^{-4}	3.46×10^{-3}

Hg	ADI	1.99×10^{-5}	1.86×10^{-5}	1.04×10^{-5}	1.98×10^{-6}	4.16×10^{-5}
	HQ	1.99×10^{-1}	1.86×10^{-1}	1.04×10^{-1}	1.98×10^{-2}	4.16×10^{-1}
HI		1.13	1.06	0.6	0.11	2.47

ADI= average daily intake (mg/kg/day), SD= standard deviation, Min= minimum, Max= maximum

4.4.1 Non-Cancer Risk of Nile Tilapia

The mean hazard quotient (HQ), median, minimum and maximum for tilapia, catfish, mackerel, and seabass were showed in Figure *4-4*, Figure *4-6*, Figure *4-8*, and Figure *4-10*, respectively. The mean hazard quotient ± standard deviation of As, Cd, Cr, Pb, Hg for nile tilapia were $4.72 \times 10^{-1} \pm 3.36 \times 10^{-1}$, $1.23 \times 10^{-2} \pm 8.77 \times 10^{-3}$, $2.87 \times 10^{-5} \pm 2.05 \times 10^{-5}$, $6.41 \times 10^{-3} \pm 4.57 \times 10^{-3}$, and $6.15 \times 10^{-2} \pm 4.38 \times 10^{-2}$; therefore the mean hazard quotient for heavy metals in nile tilapia were lower than USEPA standard hazard quotient of 1.



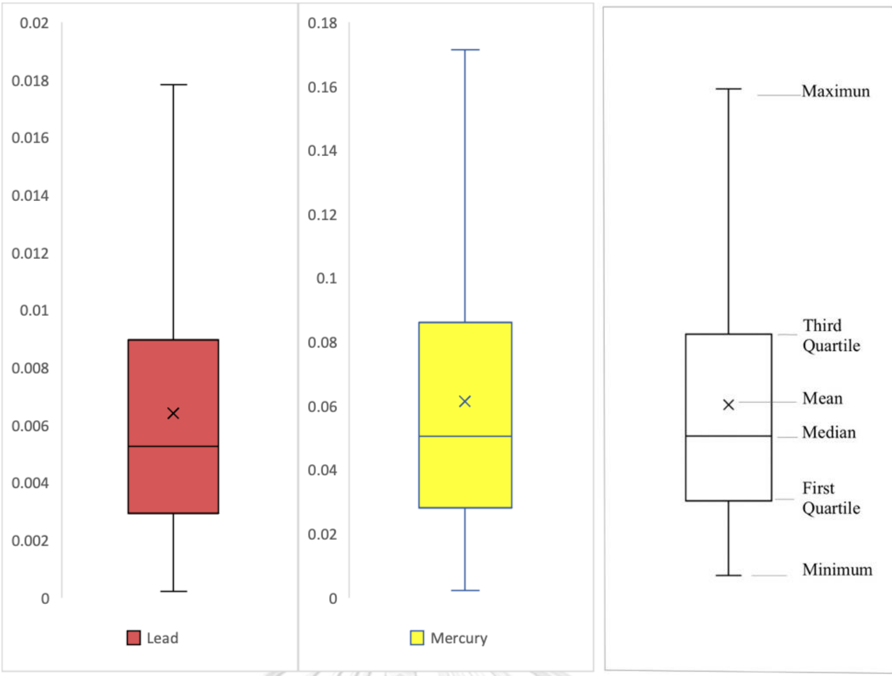


Figure 4-4: Hazard quotient (HQ) of heavy metals in Tilapia

4.4.2 Cumulative Non-Cancer Risk of Nile Tilapia

Then, the cumulative hazard index (HI) for nile tilapia was evaluated by summing up the HQ of all heavy metals for each participant and the mean value was 5.52 x 10^{-1} ± 3.93 x 10^{-1}. The mean HI value was under USEPA standard; however, the chart for each participant (Figure *3-1*Figure *4-5*) showed that some participants were above the safety limit. Therefore, the result means that some people who eat nile tilapia were in non-carcinogenic health risk.

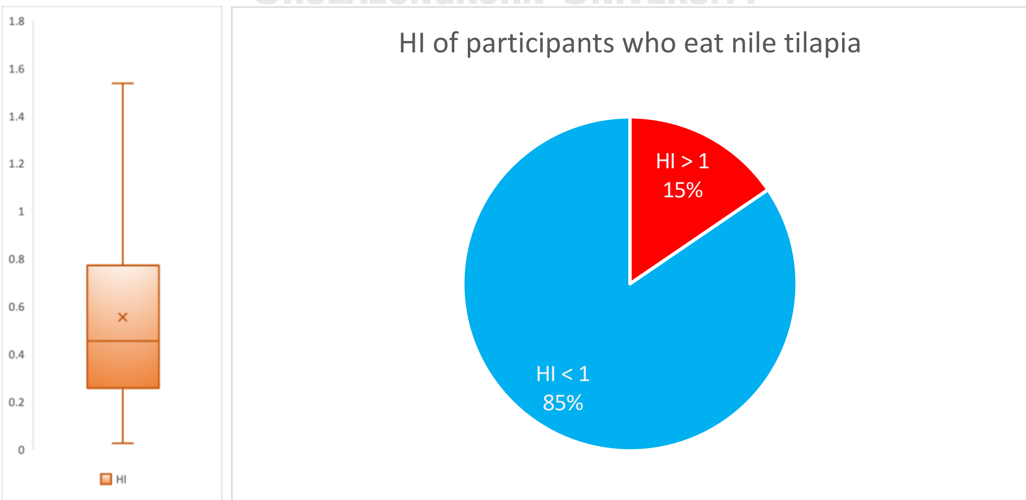


Figure 4-5: Hazard index (HI) of Tilapia

4.4.3 Non-Cancer Risk Assessment of Catfish

The mean hazard quotient (HQ) of catfish for As, Cd, Cr, Pb, and Hg are illustrated in Figure *4-6*, and the values were 5.03 x 10^{-2} ± 3.76 x 10^{-2}, 1.01 x10^{-2} ± 7.52 x 10^{-3}, 2.43 x 10^{-5} ± 1.82 x 10^{-5}, 3.84 x 10^{-3} ± 2.87 x 10^{-3}, and 5.03 x 10^{-2} ± 3.76 x 10^{-2}, respectively. The mean HQ for all targeted heavy metals were lower than 1. Therefore, there might not have non carcinogenic health effects by consuming catfish.

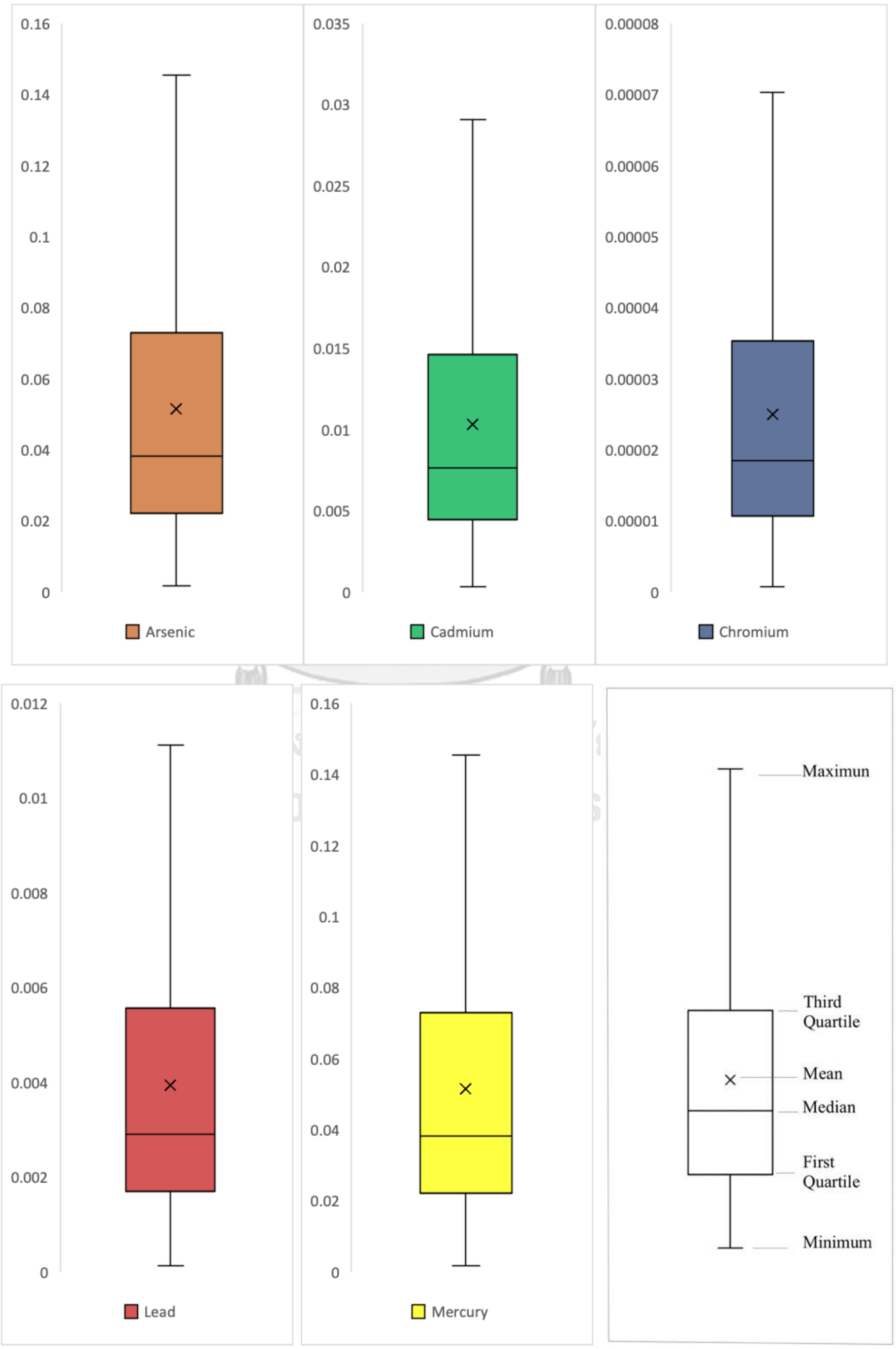


Figure 4-6: Hazard quotient (HQ) of heavy metals in Catfish

4.4.4 Cumulative Non-Cancer Risk of Catfish

The hazard index (HI) ± standard deviation for consuming catfish was lower than 1 ($1.14 \times 10^{-1} \pm 8.57 \times 10^{-2}$). The HI values for each participant were also under the USEPA safety limit of 1. Therefore, it is found out that the consumption of catfish has no carcinogenic effects.

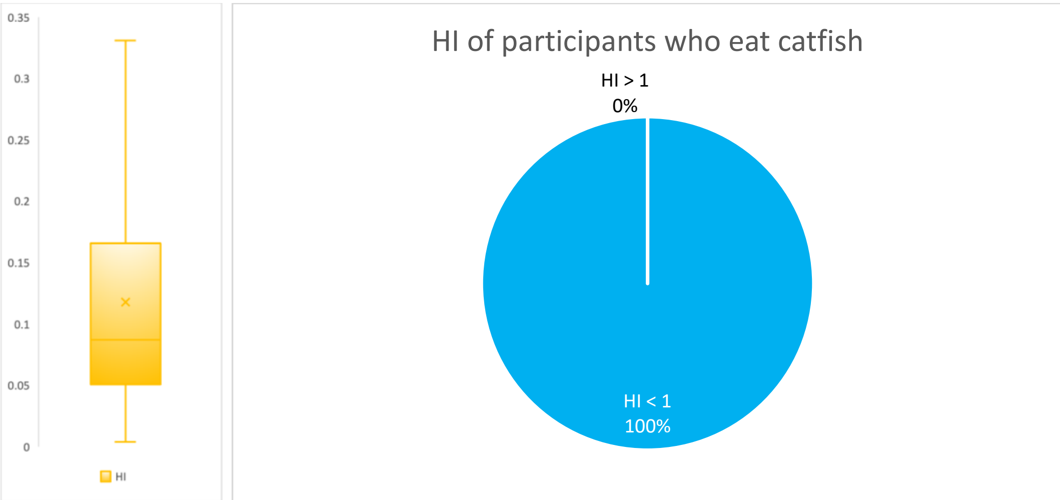


Figure 4-7: Hazard index (HI) of Catfish

4.4.5 Non-Cancer Risk of Mackerel

The hazard quotients (HQ) of As, Cd, Cr, Pb, Hg contaminated in mackerel were evaluated, and the means ± standard deviations were $1.01 \pm 4.89 \times 10^{-1}$, $2.03 \times 10^{-2} \pm 9.79 \times 10^{-3}$, $9.02 \times 10^{-6} \pm 4.35 \times 10^{-6}$, $2.87 \times 10^{-3} \pm 1.39 \times 10^{-3}$, and $1.83 \times 10^{-1} \pm 8.81 \times 10^{-2}$. The hazard risk of chromium by eating mackerel was the lowest ($9.02 \times 10^{-6} \pm 4.35 \times 10^{-6}$), and the risk of Cd, Cr, Pb, Hg were lower than USEPA hazard quotient of 1. However, HQ of arsenic (AS) in mackerel was slightly higher than 1. Therefore, there may have acute non-carcinogenic health effects such as Nausea, vomiting, destruction gastrointestinal tissue and heartbeat abnormalities. The hazard quotient (HQ) for each heavy metal evaluated for mackerel were shown in Figure *4-8*.

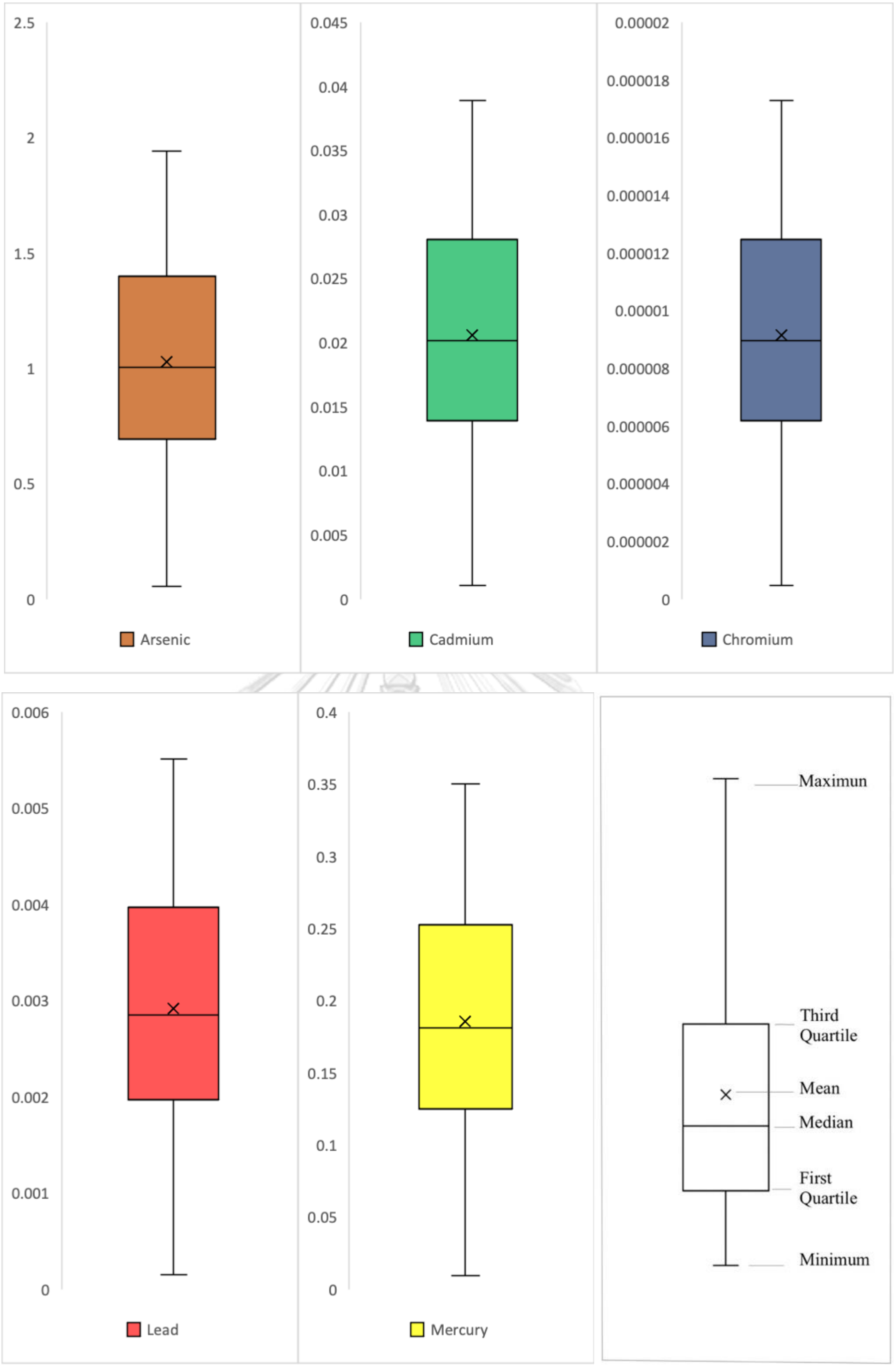


Figure 4-8: Hazard quotient (HQ) of heavy metals in mackerel

4.4.6 Cumulative Non-Cancer Risk of Mackerel

Then, the cumulative hazard index (HI) was calculated for each participant. The mean HI was 1.22 ± 0.59; therefore, the HI value was above USEPA standard and may have cumulative health risk effects of As, Cd, Cr, Pb, and Hg. Furthermore, the HI of 66% of participants were above USEPA standard 1. The result means although 34% of participants were safe from cumulative health risk of heavy metals by eating mackerel, 66% may have accumulating health hazard.

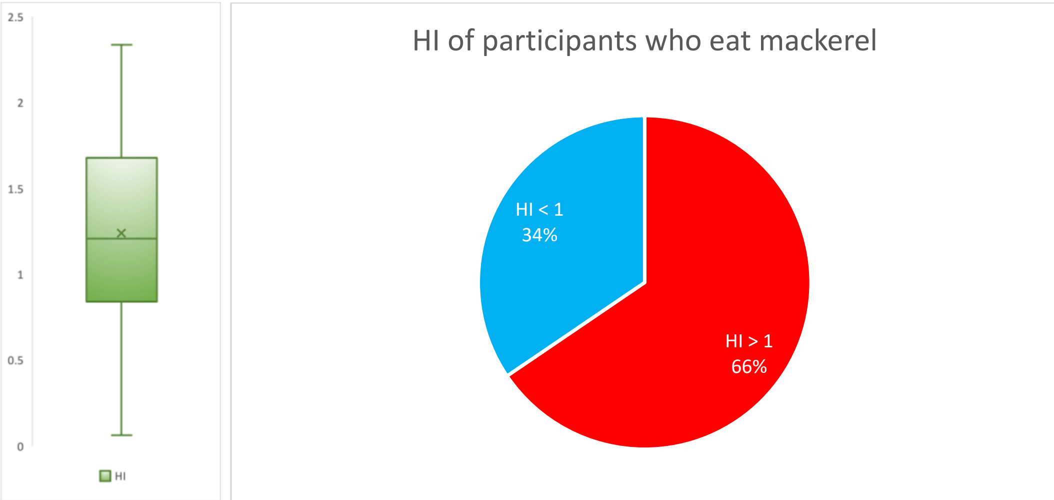


Figure 4-9: Hazard index (HI) of Mackerel

4.4.7 Non-Cancer Risk of Seabass

Finally, the HQ values through consuming seabass were 9.29 x 10^{-1} ± 4.95 x 10^{-1} for arsenic, 7.88 x 10^{-3} ± 4.19 x 10^{-3} for cadmium, 1.44 x 10^{-5} ± 7.69 x 10^{-6} for chromium, 1.64 x 10^{-3} ± 8.74 x 10^{-4} for lead, and 1.97 x 10^{-1} ± 1.05 x 10^{-1} for mercury. HQ for all targeted heavy metals were below USEPA hazard quotient 1. However, HQ of arsenic was 9.29 x 10^{-1}, and it was marginally to reach the standard value 1. The mean, median, minimum, and maximum of HQ for each heavy metal were described in Figure *4-10*.

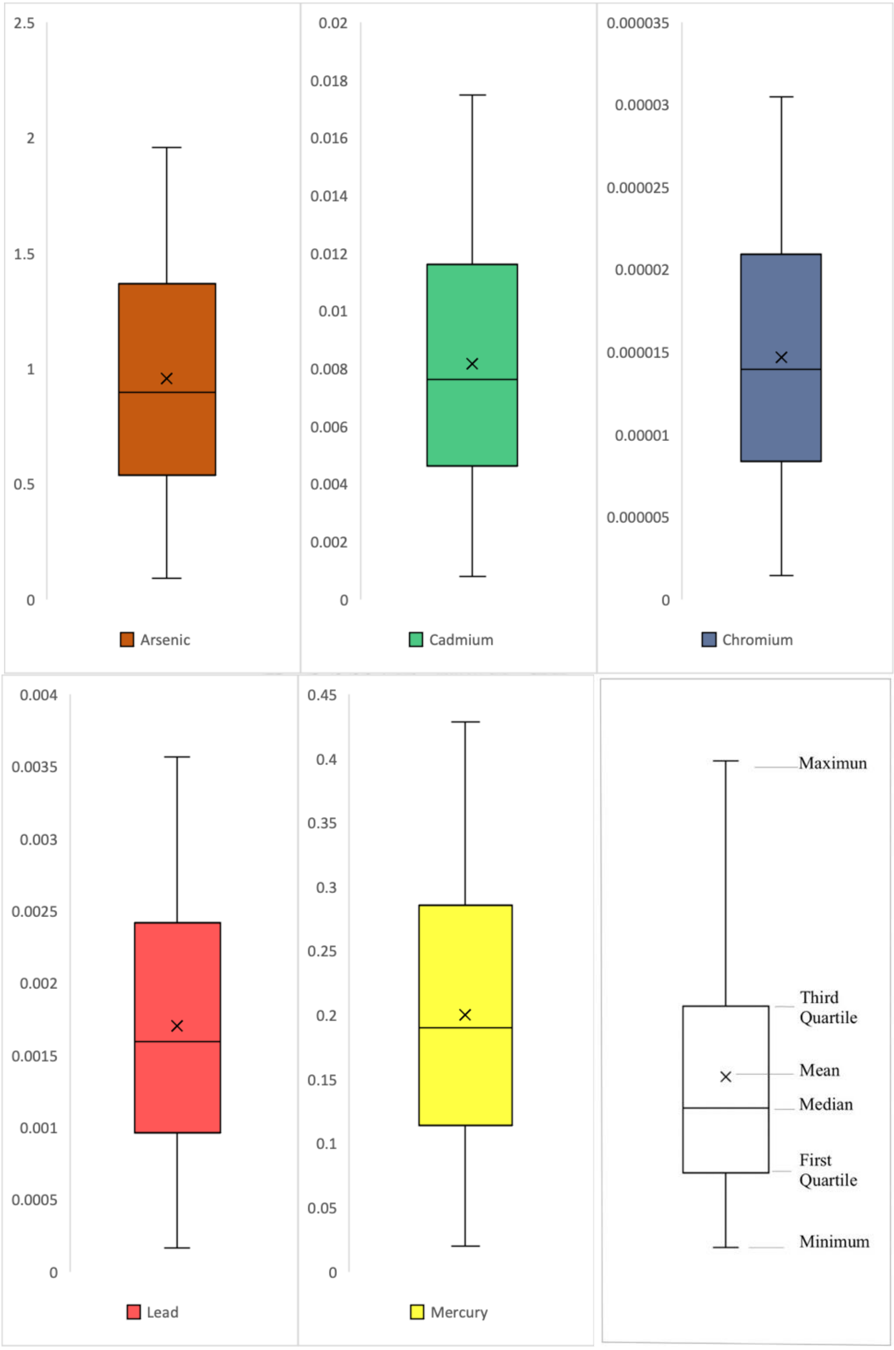


Figure 4-10: Hazard quotient (HQ) of heavy metals in seabass

4.4.8 Cumulative Non-Cancer Risk of Seabass

The cumulative health risk was calculated and the mean hazard index (HI) ± standard deviation was 1.14 ± 0.60, and mean HI was slightly above 1. Therefore, there may have cumulative toxic effects of heavy metals (As, Cd, Cr, Pb, and Hg) by eating seabass. When HI for each participant was evaluated, HI of 188 participants out of 343 participants who eat seabass were above USEPA standard. Hence, more than

50% of participants (55%) may suffer non carcinogenic health effects from consumption of seabass. The mean hazard index (HI) and HI for each participant were described with the following Figure 4-11.

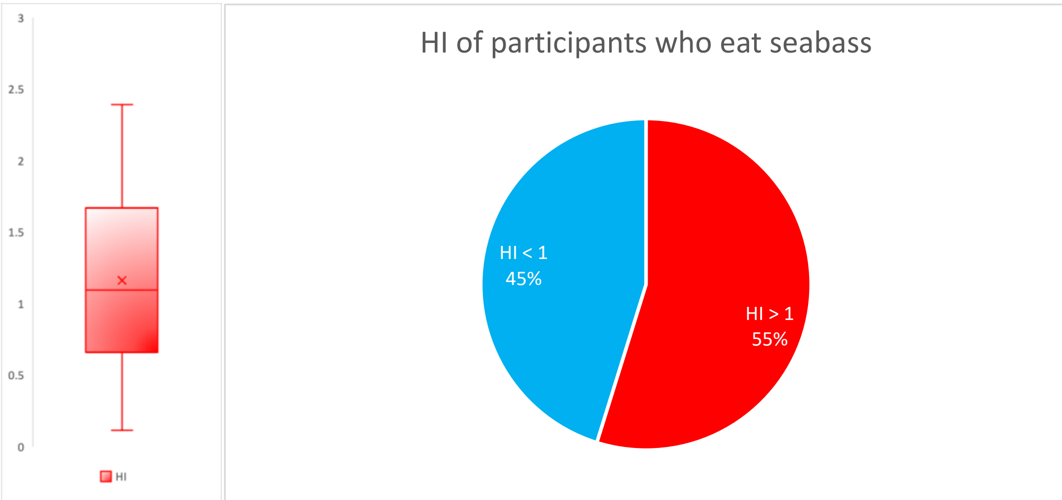


Figure 4-11: Hazard index (HI) of Seabass

4.5 Cancer Risk Assessment

To find out the carcinogenic risk, chronic daily intake (CDI) was calculated by inserting heavy metal concentration, ingestion rate (IR), exposure frequency (EF), exposure duration (ED), body weight (BW), and averaging time (AT) of participants by using equation (2) for heavy metals (As, Cd, Cr, and Pb) in nile tilapia, catfish, mackerel and seabass. Hg was not considered in calculating cancer risk by ingestion because Hg is categorized by US EPA Integrated Risk Information System (IRIS) as D (Not classifiable as to human carcinogenicity) (Usepa, 2011). USEPA formula equation (5) was used to evaluate cancer risk by oral exposure. Then, each CDI value for As, Cd, Cr, and Pb were multiplied by the slope factor of relevant heavy metal to find cancer risk of participants. Afterwards, the total cancer risk (TCR) was calculated by summing up the cancer risk of As, Cd, Cr, and Pb with the equation equation (6). Chronic daily intake (CDI) and cancer risk for As, Cd, Cr, and Pb of fish species (nile tilapia, catfish, mackerel, and seabass) are described in the following Table 4-6.

Table 4-5: Chronic Daily Intake (CDI) and total cancer risk of heavy metals by consuming targeted fish species

Elements	Category	Mean	Median	SD	Min	Max
			Nile Tilapia			
As	CDI	5.04×10^{-5}	3.68×10^{-5}	4.84×10^{-5}	2.72×10^{-7}	3.15×10^{-4}
	CR	7.55×10^{-5}	5.52×10^{-5}	7.26×10^{-5}	4.08×10^{-7}	4.73×10^{-4}
Cd	CDI	4.38×10^{-6}	3.20×10^{-6}	4.21×10^{-6}	2.37×10^{-8}	2.74×10^{-5}
	CR	1.66×10^{-6}	1.22×10^{-6}	1.60×10^{-6}	8.99×10^{-9}	1.04×10^{-5}
Cr	CDI	1.53×10^{-5}	1.12×10^{-5}	1.47×10^{-5}	8.28×10^{-8}	9.60×10^{-5}
	CR	7.66×10^{-6}	5.60×10^{-6}	7.37×10^{-6}	4.14×10^{-8}	4.80×10^{-5}
Pb	CDI	8.21×10^{-6}	6.00×10^{-6}	7.89×10^{-6}	4.44×10^{-8}	5.14×10^{-5}
	CR	6.98×10^{-8}	5.10×10^{-8}	6.71×10^{-8}	3.77×10^{-10}	4.37×10^{-7}
TCR		8.49×10^{-5}	6.20×10^{-5}	8.17×10^{-5}	4.59×10^{-7}	5.32×10^{-4}
			Catfish			
As	CDI	5.29×10^{-6}	3.58×10^{-6}	5.14×10^{-6}	2.50×10^{-8}	2.70×10^{-5}
	CR	7.94×10^{-6}	5.37×10^{-6}	7.72×10^{-6}	3.74×10^{-8}	4.05×10^{-5}
Cd	CDI	3.53×10^{-6}	2.39×10^{-6}	3.43×10^{-6}	1.66×10^{-8}	1.80×10^{-5}
	CR	1.34×10^{-6}	9.07×10^{-7}	1.30×10^{-6}	6.32×10^{-8}	6.84×10^{-6}
Cr	CDI	1.28×10^{-5}	8.66×10^{-6}	1.24×10^{-5}	6.03×10^{-8}	6.52×10^{-5}
	CR	6.39×10^{-6}	4.33×10^{-6}	6.21×10^{-6}	3.02×10^{-8}	3.26×10^{-5}
Pb	CDI	4.85×10^{-6}	3.28×10^{-6}	4.71×10^{-6}	2.29×10^{-8}	2.47×10^{-5}
	CR	4.12×10^{-8}	2.79×10^{-8}	4.01×10^{-8}	1.95×10^{-10}	2.10×10^{-7}
TCR		1.57×10^{-5}	1.06×10^{-5}	1.53×10^{-5}	7.41×10^{-8}	8.01×10^{-5}
			Mackerel			
As	CDI	1.04×10^{-4}	9.25×10^{-5}	7.59×10^{-5}	9.30×10^{-7}	4.37×10^{-4}
	CR	1.56×10^{-4}	1.39×10^{-4}	1.14×10^{-4}	1.39×10^{-6}	6.56×10^{-4}
Cd	CDI	6.97×10^{-6}	6.18×10^{-6}	5.07×10^{-6}	6.21×10^{-8}	2.92×10^{-5}
	CR	2.65×10^{-6}	2.35×10^{-6}	1.93×10^{-6}	2.36×10^{-8}	1.11×10^{-5}
Cr	CDI	4.64×10^{-6}	4.12×10^{-6}	3.38×10^{-6}	4.14×10^{-8}	1.95×10^{-5}
	CR	2.32×10^{-6}	2.06×10^{-6}	1.69×10^{-6}	2.07×10^{-8}	9.73×10^{-6}
Pb	CDI	3.55×10^{-6}	3.15×10^{-6}	2.59×10^{-6}	3.17×10^{-8}	1.49×10^{-5}
	CR	3.02×10^{-8}	2.68×10^{-8}	2.20×10^{-8}	2.69×10^{-10}	1.27×10^{-7}
TCR		1.61×10^{-4}	1.43×10^{-4}	1.17×10^{-4}	1.44×10^{-6}	6.77×10^{-4}
			Seabass			
As	CDI	8.97×10^{-5}	7.32×10^{-5}	7.41×10^{-5}	8.49×10^{-7}	4.31×10^{-4}
	CR	1.35×10^{-4}	1.10×10^{-4}	1.11×10^{-4}	1.27×10^{-6}	6.46×10^{-4}
Cd	CDI	2.53×10^{-6}	2.07×10^{-6}	2.09×10^{-6}	2.40×10^{-8}	1.22×10^{-5}
	CR	9.63×10^{-7}	7.86×10^{-7}	7.96×10^{-7}	9.12×10^{-9}	4.63×10^{-6}
Cr	CDI	6.97×10^{-6}	5.69×10^{-6}	5.76×10^{-6}	6.60×10^{-8}	3.35×10^{-5}
	CR	3.49×10^{-6}	2.85×10^{-6}	2.88×10^{-6}	3.30×10^{-8}	1.67×10^{-5}

Elements	Category	Mean	Median	SD	Min	Max
Pb	CDI	1.90 x 10^{-6}	1.55 x 10^{-6}	1.57 x 10^{-6}	1.80 x 10^{-8}	9.13 x 10^{-6}
	CR	1.62 x 10^{-8}	1.32 x 10^{-8}	1.33 x 10^{-8}	1.53 x 10^{-10}	7.76 x 10^{-8}
TCR		1.39 x 10^{-4}	1.13 x 10^{-4}	1.15 x 10^{-4}	1.32 x 10^{-6}	6.67 x 10^{-4}

CDI= chronic daily intake (mg/kg/day), SD= standard deviation, Min= minimum, Max= maximum

4.5.1 Cancer Risk of Nile Tilapia

Figure 4-12 represents the potential cancer risk of As, Cd, Cr and Pb through the consumption of nile tilapia. The mean cancer risk ± standard deviation of As, Cd, Cr, and Pb were 7.55 x 10^{-5} ± 7.26 x 10^{-5}, 1.66 x 10^{-6} ± 1.6 x 10^{-6}, 7.66 x 10^{-6} ± 7.37 x 1^{-6}, and 6.98 x 10^{-8} ± 6.71 x 10^{-8}, respectively, while USEPA standard for cancer risk is between 1 x 10^{-4} to 1 x 10^{-6}. Mean cancer risk of Pb through eating nile tilapia is (6.98 x 10^{-8} ± 6.71 x 10^{-8}) lower than USEPA standard 1 x 1^{-6}. However, the mean cancer risk of three remaining heavy metals (As, Cd, and Cr) are higher than USEPA standard. Therefore, long term consumption of nile tilapia may have potential carcinogenic health effects in human.

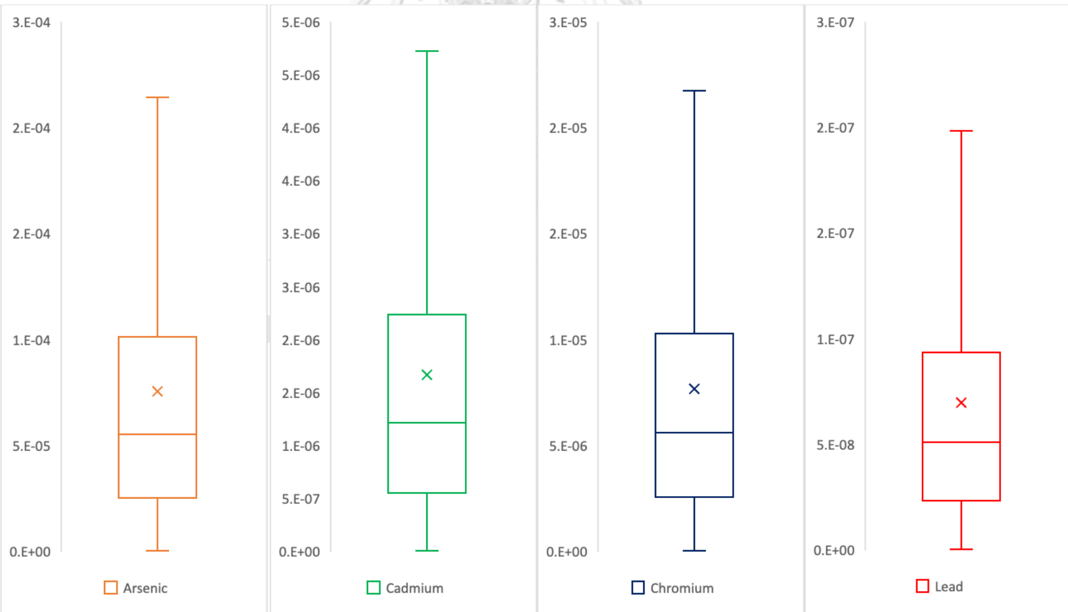


Figure 4-12: Potential cancer risk of heavy metals by consuming nile tilapia

4.5.2 Total Cancer Risk of Nile Tilapia

Then, the total cancer risk was evaluated by summing up all the potential cancer risk of heavy metals (As, Cd, Cr, and Pb) in nile tilapia. The mean total cancer risk was resulted as 8.49 x 10^{-5} ± 8.17 x 10^{-5}; therefore, the cumulative total cancer risk was over USEPA safety limit. When the total cancer risk was evaluated for each participant, 99% of participants who eat nile tilapia may have carcinogenic health risk by consuming long term.

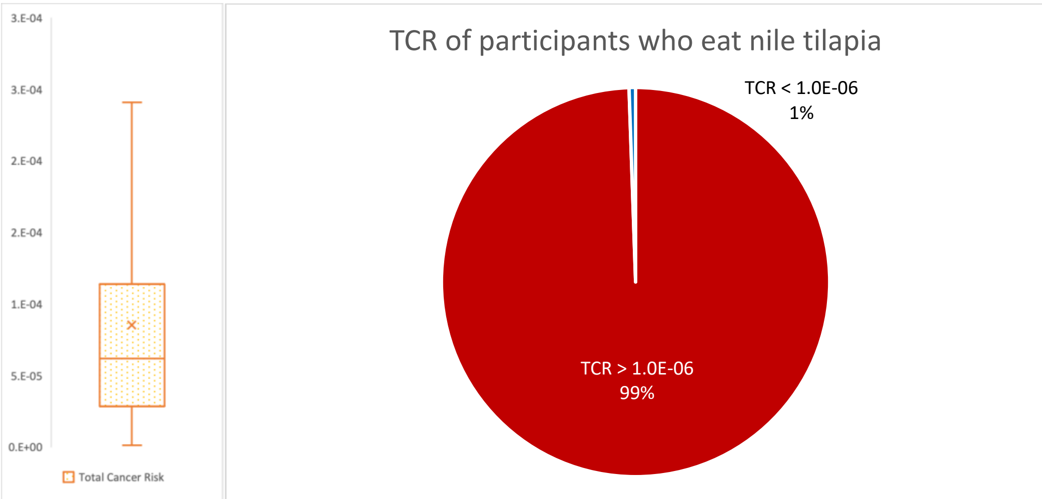


Figure 4-13: Total cancer risk of nile tilapia

4.5.3 Cancer Risk of Catfish

In case of consuming catfish, the cancer risk for heavy metals were evaluated, and the means ± standard deviation were $7.94 \times 10^{-6} \pm 7.72 \times 10^{-6}$ for As, $1.34 \times 10^{-6} \pm 1.3 \times 10^{-6}$ for Cd, $6.39 \times 10^{-6} \pm 6.21 \times 10^{-6}$ for Cr, and $4.12 \times 10^{-8} \pm 4.01 \times 10^{-8}$ for Pb. The mean cancer risk for lead was under the USEPA safety limit (1×10^{-6}), but the cancer risk of As, Cd, Cr through the consumption of catfish were slightly higher than the limit. Therefore, people who eat contaminated catfish may suffer the toxic carcinogenic effects of As, Cd, and Cr in long term.

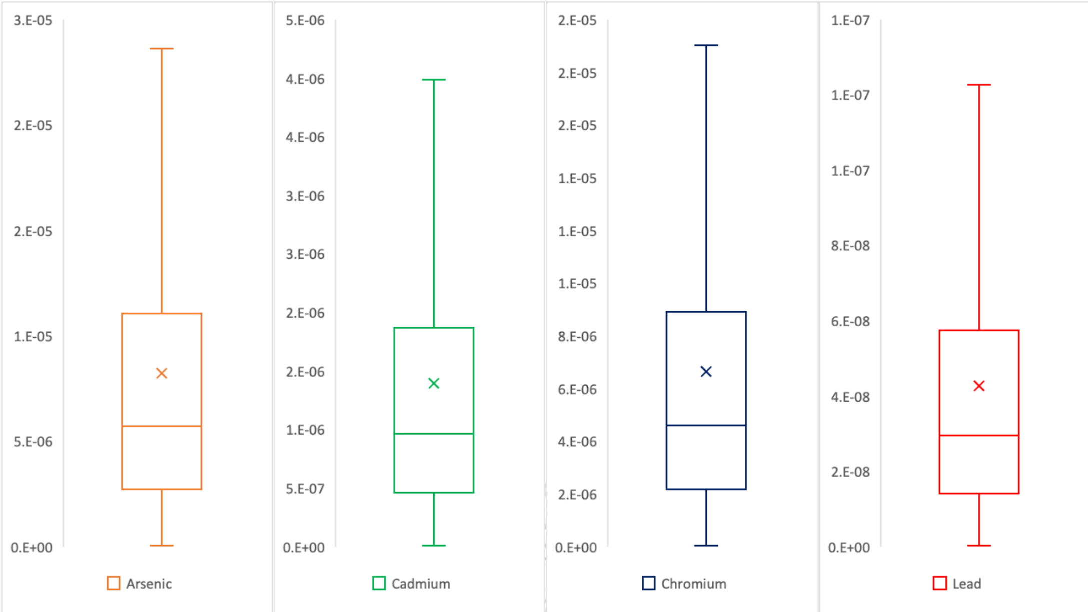


Figure 4-14: Potential cancer risk of heavy metals by consuming catfish

4.5.4 Total Cancer Risk of Catfish

Figure *4-15* shows that the mean total cancer risk for four cancer toxic heavy metals (As, Cd, Cr, and Pb) and the percentage of participants who are under potential cancer risk. The mean total cancer risk of consuming catfish was 1.57 x 10^{-5} ± 1.53 x 10^{-5}, while the USEPA safety limit is 1 x 10^{-4} to 1 x 10^{-6}. Hence, there may have potential cancer risk by eating catfish for lifetime. However, the total cancer risk for each participant shows that 46% of participants were safe from cancer hazard and 54% were above the standard.

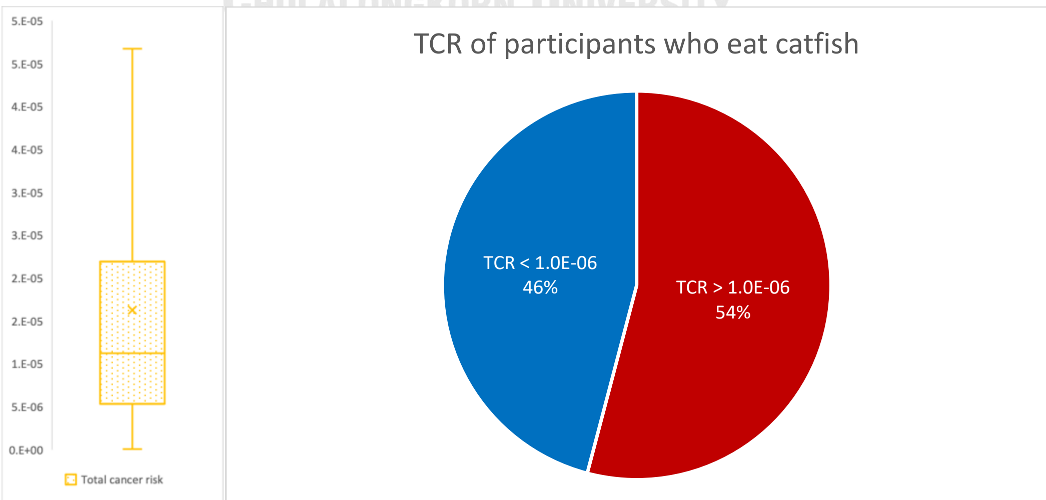


Figure 4-15: Total cancer risk of catfish

4.5.5 Cancer Risk of Mackerel

The evaluation of cancer risk for As, Cd, Cr, and Pb through eating mackerel is shown in Figure *4-16*. The mean cancer risk of the participants who eat mackerel were 1.5 x 10^{-4} ± 1.14 x 10^{-4} for As, 2.65 x 10^{-6} ± 1.93 x 10^{-6} for Cd, 2.32 x 10^{-6} ± 1.69 x 10^{-6} for Cr, and 3.02 x 10^{-8} ± 2.2 x 10^{-8} for Pb. Cancer risk of Pb by eating mackerel was the lowest and it was below the USEPA cancer risk safety limit. The cancer risk of remaining three heavy metals (As, Cd, Cr) were above the standard; therefore, there may have chronic health effects of As, Cd and Cr by eating mackerel in long term.

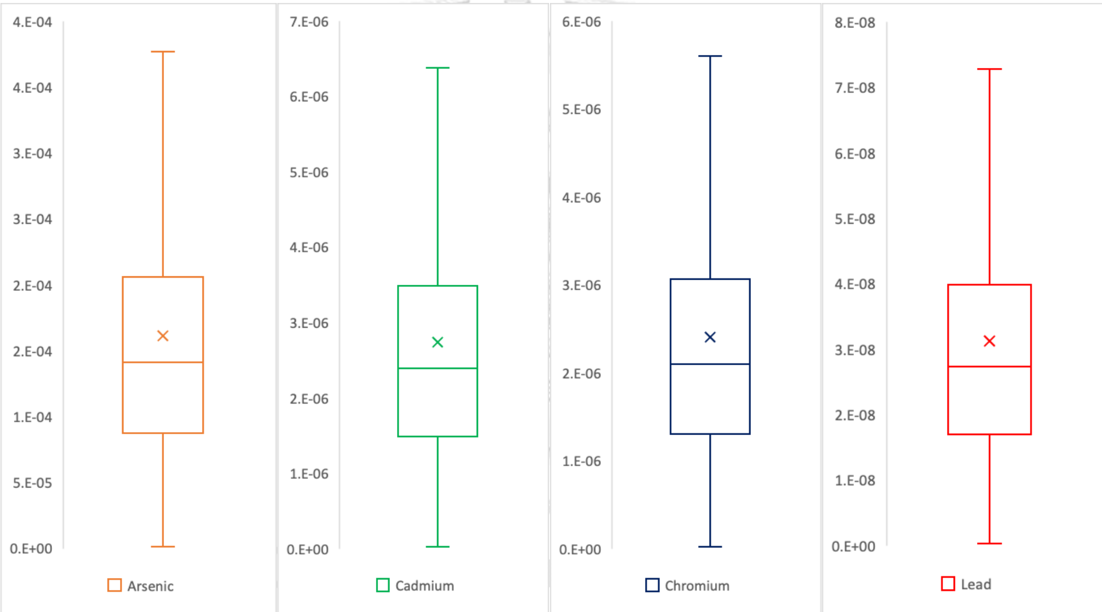


Figure 4-16: Potential cancer risk of heavy metals by consuming mackerel

4.5.6 Total Cancer Risk of Mackerel

Then, the total cancer risk for four carcinogenic toxic heavy metals in mackerel was calculated, and the mean value was 1.61 x 10^{-4} ± 1.17 x 10^{-4}. The minimum and maximum total cancer risk of participants were 1.14 x 10^{-6} and 6.77 x 10^{-4}; therefore, all participants were above the USEPA standard of 1 x 10^{-4} to 1 x 10^{-6}. The result shows that eating mackerel for lifetime may develop cumulative cancer risk of As, Cd, Cr and Pb.

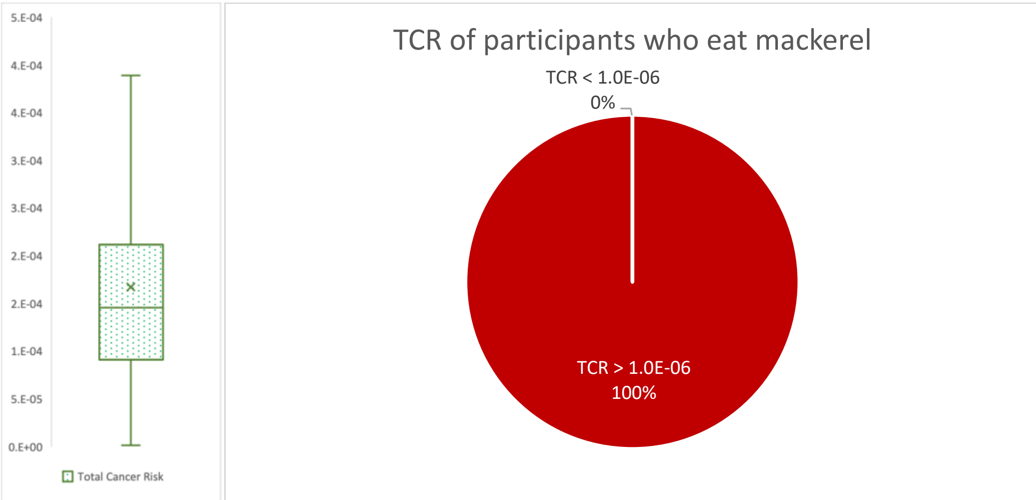


Figure 4-17: Total cancer risk of mackerel

4.5.7 Cancer Risk of Seabass

Finally, the carcinogenic risks for As, Cd, Cr, and Pb contents in seabass were calculated and the mean values were 1.35 x 10^{-4} ± 1.11 x 10^{-4}, 9.63 x 10^{-7} ± 7.96 x 10^{-7}, 3.49 x 10^{-6} ± 2.88 x 10^{-6}, and 1.62 x 10^{-8} ± 1.33 x 10^{-8}, respectively. The cancer risk by Cd and Pb in seabass were below the USEPA standard, but As and Cr cancer risk values were more than 1 x 10^{-6}. Therefore, the carcinogenic risk of arsenic and chromium may occur by consuming contaminated seabass for long term.

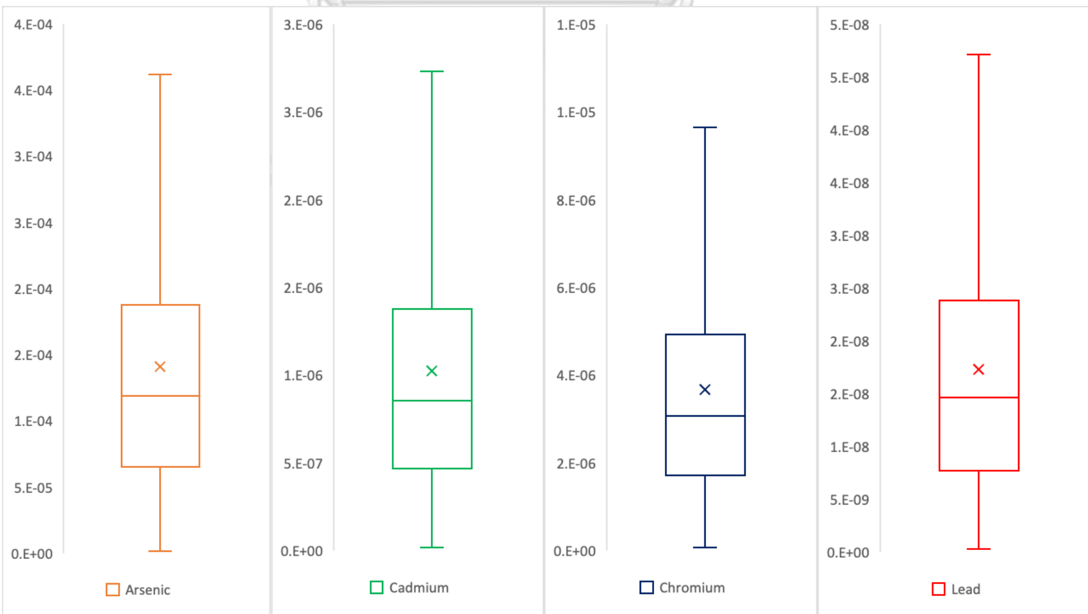


Figure 4-18: Potential cancer risk of heavy metals by consuming seabass

4.5.8 Total Cancer Risk of Seabass

Then, the cumulative cancer risk of arsenic, cadmium, chromium, and lead were evaluated, and the mean ± standard deviation was resulted as $1.40 \times 10^{-4} \pm 1.15 \times 10^{-4}$. Total carcinogenic risk was calculated for each participant and the minimum and maximum cancer risk were 1.32×10^{-6} and 6.67×10^{-4}. The result shows that all participants who eat seabass may have total cancer risk since the TCRs of all participants were above the USEPA safety limit of 1×10^{-6}.

Figure 4-19: Total cancer risk of seabass

4.6 Risk Level in People who Eat All Fish Species

Aggregate non-cancer risk and total cancer risk were evaluated for the people who eat all fish targeted species in this study. Total non-cancer risk (HI) for these people was calculated by summing up all hazard quotient (HQ) of five heavy metals (As, Cd, Cr, Pb, and Hg) in four fish species (nile tilapia, catfish, mackerel, and seabass). Similarly, total cancer risk (TCR) was quantified by summarizing cancer risk (CR) of five heavy metals (As, Cd, Cr, Pb, and Hg) in four fish species (nile tilapia, catfish, mackerel, and seabass).

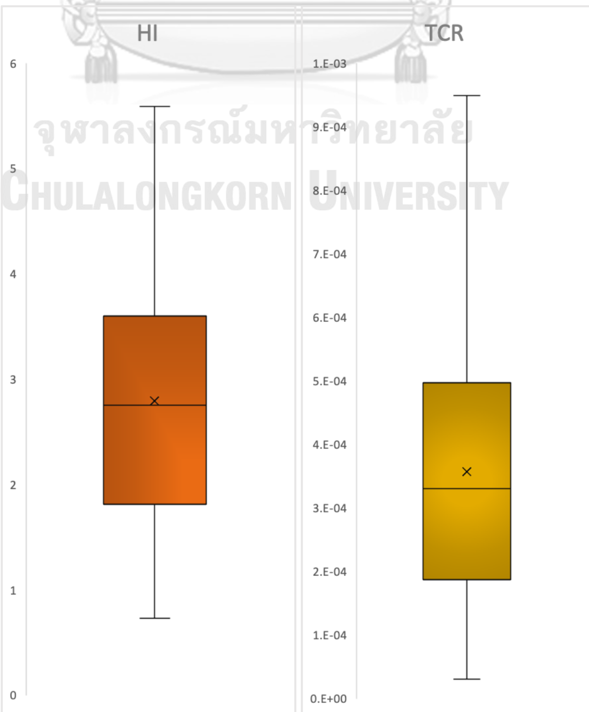
Based on the questionnaire surveying, there were 211 participants who eat all the targeted fish species in this study. Mean hazard index (HI) and total cancer risk (TCR) of the participants who eat all fish species was 2.7945 ± 1.1082 with the range of (0.7323 to 5.5933), and $3.58 \times 10^{-4} \pm 2.22 \times 10^{-4}$ with the range of (3.07×10^{-5} to 1.13×10^{-3}).

Table 4 6 shows mean, median, standard deviation, minimum and maximum values of 211 participants who eat all four fish species.

Table 4-6: Hazard Index (HI) and Total Cancer Risk (TCR) of the participants who eat all four targeted fish species (a) HI (b) TCR

Category	**Mean**	**SD**	**Median**	**Min**	**Max**
HI (nile tilapia)	0.4663	0.3696	0.3533	0.0195	1.8796
HI (catfish)	0.1002	0.0786	0.0712	0.0038	0.3682
HI (mackerel)	1.1326	0.6110	1.0909	0.0652	2.3387
HI (seabass)	1.0954	0.5905	0.9882	0.1144	2.3957
HI (Total)	2.7945	1.1082	2.7524	0.7323	5.5933
TCR (nile tilapia)	6.41×10^{-5}	6.68×10^{-5}	4.35×10^{-5}	4.59×10^{-7}	3.52×10^{-4}
TCR (catfish)	1.28×10^{-5}	1.37×10^{-5}	8.13×10^{-6}	7.41×10^{-8}	7.32×10^{-5}
TCR (mackerel)	1.51×10^{-4}	1.26×10^{-4}	1.33×10^{-4}	1.44×10^{-6}	6.77×10^{-4}
TCR (seabass)	1.30×10^{-4}	1.14×10^{-4}	1.03×10^{-4}	1.32×10^{-6}	6.67×10^{-4}
TCR (Total)	3.58×10^{-4}	2.22×10^{-4}	3.31×10^{-4}	3.07×10^{-5}	1.13×10^{-3}

Figure 4-20 establishes cumulative non-cancer risk (HI) and total cancer risk (TCR) of the participants who eat all four targeted fish species.



(a) (b)

Figure 4-20: Hazard Index (HI) and Total Cancer Risk (TCR) of the participants who eat all four targeted fish species (a) HI (b) TCR

CHAPTER V

5. Discussion

This part provides the detail discussion for the results of heavy metals concentrations, personal information, consumption of fish, and health risk assessment including cancer and non-cancer risk. Compare and contrast with international standards and other studies' results are also describe in this chapter.

5.1 Heavy Metals Concentration in Fish

In order to compare heavy metals concentration resulted from this study with the international maximum allowable concentration in fish, Table *5-1* expresses mean ± standard deviation of heavy metal concentration in four fish species with the international standards: Thai standard of contamination in food, the commission of the European Committees standard (EC), Joint FAO/WHO Expert Committee on Food Additive online database (JICFA), and Ministry of Health of the People's Republic of China standard.

Arsenic concentration in four fish species (nile tilapia, catfish, mackerel, and seabass) of this study were 0.0920 ± 0.0075 mg/kg, 0.012 ± 0.0035 mg/kg, 0.449 ± 0.052 mg/kg, and 0.283 ± 0.1624 mg/kg, whereas the safety limit of Thai standard is 2 mg/kg. As contents in all targeted fish species were quite lower than the Thai standard of contamination in food.

The standard concentration of cadmium (Cd) in fish of Thai, European Commission Regulation (EC), and China varied depending on the country's maximum allowable limit, 1 mg/kg, 0.05 mg/kg, and 0.1 mg/kg respectively. The mean ± SD concentration in nile tilapia, catfish, mackerel, and seabass were 0.0080 mg/kg, 0.0080 mg/kg, 0.03 ± 0.009 mg/kg, and 0.0080 mg/kg, and concentration in all fish species were under the maximum allowable limit of Thai, EC and China's standards.

Lead (Pb) concentration found in nile tilapia, catfish, mackerel, and seabass were 0.015 ± 0.008 mg/kg, 0.011 ± 0.008 mg/kg, 0.0153 ± 0.0081 mg/kg, and 0.0060 mg/kg, while the standard concentration in fish of Thai, EC (2005) were 0.3 mg/kg, 0.2 mg/kg, and JICFA, and China maximum allowable limit are the same (0.5 mg/kg)

(China, 2005) (Organization, 2017). Therefore, the concentrations of Pb in fish of the present study were not higher than the acceptable limit.

nile tilapia, catfish, mackerel, and seabass were analyzed to find Hg detection and the concentrations were 0.0040 mg/kg, 0.0040 mg/kg, 0.027 ± 0.0066 mg/kg, and 0.0200 mg/ kg, respectively. The safety standard of Hg concentration in fish of Thai, EC and JIFCA are 1 mg/kg, 0.5 mg/kg, and 0.5 mg/kg; therefore, Hg contents were in acceptable range of international standards.

Similarly, chromium (Cr) concentration was 0.028 ± 0.0072 mg/kg in nile tilapia, 0.029 ± 0.011 mg/kg in catfish, 0.0200 mg/kg in mackerel, and 0.022 ± 0.0021 mg/kg in seabass. China's maximum level for Cr contamination in fish is 2mg/kg; therefore, Cr concentrations in fish species were also in acceptable limit.

Therefore, all targeted heavy metals in this study (As, Cd, Cr, Pb, and Hg) were detected in all fish species nile tilapia (*Oreochromis niloticus)*, catfish *(Clarias batrachus)*, mackerel (*Rastrelliger brachysoma) and* seabass (*Lates calcarifer)*, and the concentrations were within the safety levels of international standards.

Table 5-1: Comparison of heavy metals concentration in fish species with international standards

Reference to	Concentration (mg/kg)					Foodstuffs/ Species	
	As	**Cd**	**Pb**	**Hg**	**Cr**		
JECFA[3]	-	-	0.5	0.5	-	Fish muscle	
EC[2] (2005)	-	0.05	0.2	1	-	Fish muscle	
EU	-	0.5-1	0.3			Fish muscle	
Thai[1]	2	1	0.3	1	-	Fish muscle	
Australia/ New Zealand	2	2	0.5	0.5		Fish, fish products and molluscs	
China[4]	-	0.1	0.5	-	2	Fish muscle	
Present Study	0.0920 ± 0.0075	0.008	0.015 ± 0.008	0.004	0.028 ± 0.0072	*Oreochromis niloticus*	Nile Tilapia
	0.012 ± 0.0035	0.008	0.011 ± 0.008	0.004	0.029 ± 0.011	*Clarias batrachus*	Catfish
	0.449 ± 0.052	0.03 ± 0.009	0.0153 ± 0.0081	0.027 ± 0.0066	0.02	*Rastrelliger brachysoma*	Mackerel
	0.283 ± 0.1624	0.008	0.006	0.02	0.022 ± 0.0021	*Lates calcarifer*	Seabass

Note: [1] Standard of contaminants in food (No. 2), Notification of the Ministry of Public Health (No. 273) B.E. 2546 (2003), [2] The commission of the European Committies, Commission regulation (EC) No 78/2005 of 19 January 2005, [3] Joint FAO/WHO Expert Committee on Food Additive online database, [4] Ministry of Health of the People's Republic of China.

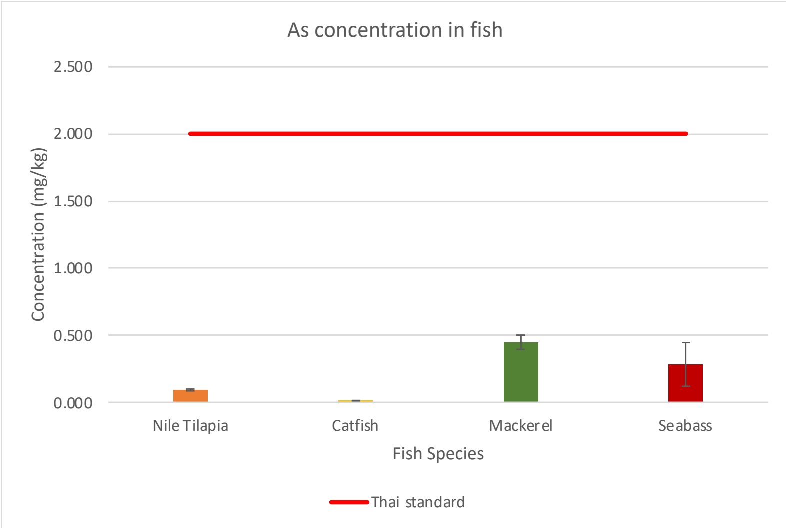


Figure 5-1: Comparison of mean As content in fish with standard

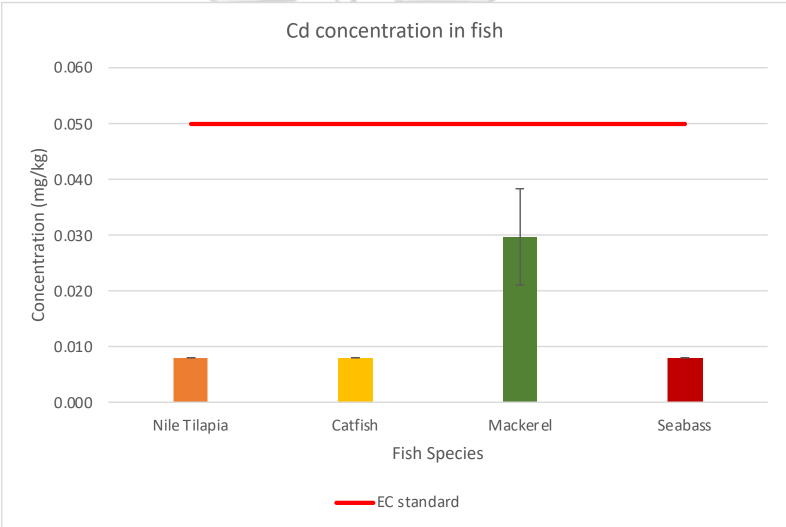


Figure 5-2: Comparison of mean Cd content in fish with standard

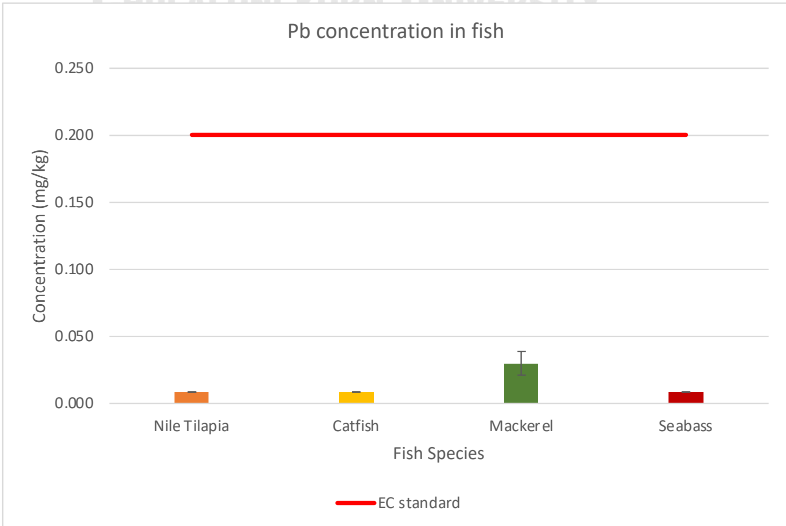


Figure 5-3: Comparison of Pb content in fish with standard

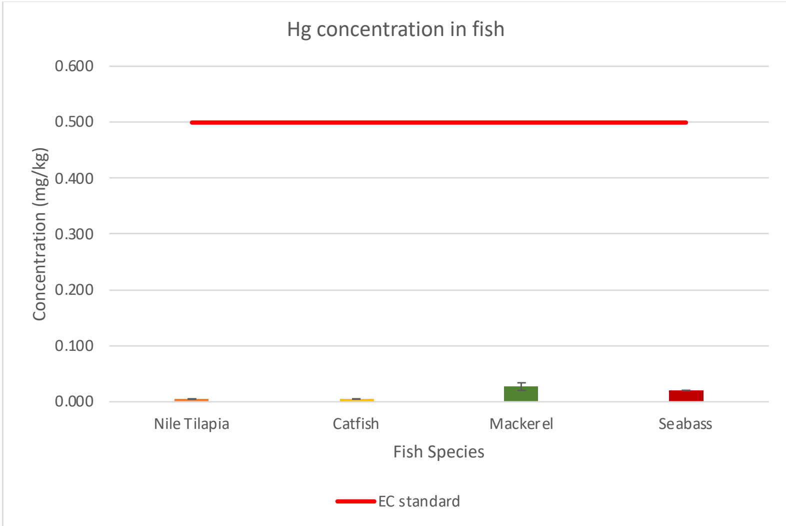


Figure 5-4: Comparison of Hg content in fish with standard

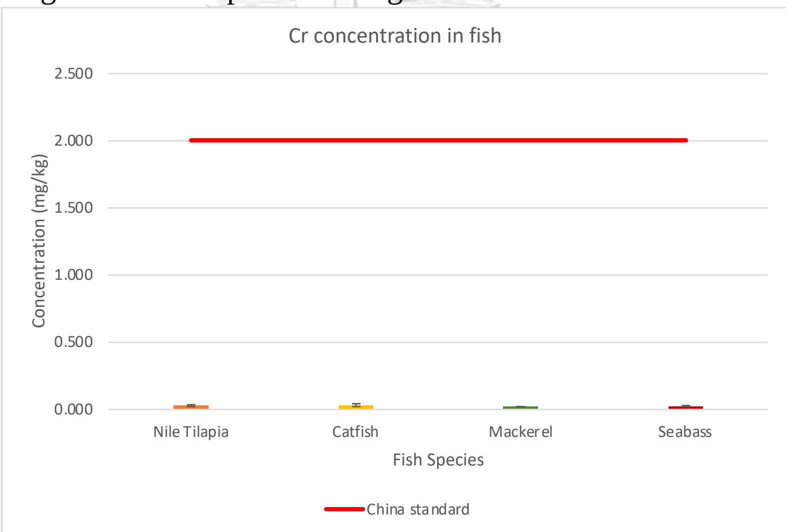


Figure 5-5: Comparison of Cr content in fish with standard

Table 5-1 shows heavy metals concentration in various kind of fish species in several countries. In Iran, Cd in Spanish mackerel and Orange-spotted spinefoot and Cd, Pb concentrations in Orange-spotted spinefoot from the previous research (Mokarram et al., 2021) were higher than those in all fish species of the present study for the reason that the fish samples were collected from the Persian Gulf where it is closed to the petrochemical oil and gas plants.

Hg in Pangas catfish from Bangladesh by (Ghosh et al., 2021) was also higher than the present study. According to (Tremlová, 2017) in Thailand, As, Pb, Hg in silver whiting were 0.09 ± 0.06 mg/kg, 0.11 mg/kg, 0.012 mg/kg, and Pb and Hg concentrations were higher than the heavy metals concentration in all targeted fish species of the present study. However, As concentration of that previous study was almost the same with the mean concentration As in nile tilapia of the present study. Mean As concentration in catfish and mackerel were 0.012 ± 0.0035 mg/kg, and 0.449 ± 0.052 mg/kg; therefore, these concentrations were low when they compared to (Tremlová, 2017) study. However, mean As concentration in seabass of this study was higher than the other fish species (nile tilapia, catifish, mackerel) and silver whiting of (Tremlová, 2017)study.

Another previous study in Thailand studied mrigal carp, and Cd and Cr concentrations were 0.09 ± 0.04 mg/kg and 0.32 + 0.47 mg/kg, while Cd and Cr contents of the present study in all targeted fish species were lower than (Sutee Chowrong, 2020). Mrigal carp is detritivores that eat dead plants and organisms, fungus, insects and sediments. Feeding habitat might also affect the concentration of heavy metals in aquatic species.

Research related with heavy metals concentration in fish from Myanmar found Cd 0.073 +0.007 mg/kg in snakehead fish and Cd 0.083 +0.009 in nile tilapia. Both Cd contents in two fish species were higher than the Cd concentrations of the present study. When Cd concentration in nile tilapia from Myanmar and present study were compared, it is found out that tilapia from Myanmar has higher Cd concentration.

(Rodriguez-Mendivil et al., 2019) examined Cd, Pb, Hg and Cr in yellowfin tuna in Mexico and resulted as 0.0019 ± 0.0001 mg/kg, 0.116 ± 0.026 mg/kg, 0.102 ± 0.020 mg/kg, and 0.377 ± 0.161 mg/kg. Heavy metals (Pb, Hg and Cr) of the present study except As concentrations were notably lower than the concentrations of yellowfin tuna from Mexico.

Apart from fish species, heavy metals can also be accumulated in other aquatic animals such as shrimps, shells, and snails. The research in Bangladesh (2015) conducted As, Cd, Pb and Cr concentrations in horn snail and freshwater prawn. The mean concentrations in freshwater prawn were 1.19 ± 0.04 mg/kg for As, 1.51 ± 0.04 mg/kg for Cd, 0.51 ± 0.01 mg/kg for Pb and 1.59 ± 0.93 mg/kg for Cr. The concentrations were quite higher than the mean concentrations of these heavy metals in all fish species of the present study. Then, the concentrations of As, Cd, Pb and Cr for horn snail were 1.02 ± 0.03 mg/kg, 0.05 ± 0.00 mg/kg, 4.55 ± 0.11 mg/kg, and 16.05 ± 1.48 mg/kg, respectively (Ahmed et al., 2015). Pb and Cr concentrations were remarkably higher when compared to the concentrations of all fish species shown in Table 5-2.

Table 5-2: Heavy metals concentration in present study, other studies and their feeding habitats

Reference to	Concentration (mg/kg)					Species	Feeding Habits
	As	**Cd**	**Pb**	**Hg**	**Cr**		
Present Study	0.0920 ± 0.0075	0.008	0.015 ± 0.008	0.004	0.028 ± 0.0072	Nile Tilapia	Omnivorous
	0.012 ± 0.0035	0.008	0.011 ± 0.008	0.004	0.029 ± 0.011	Catfish	Carnivore
	0.449 ± 0.052	0.03 ± 0.009	0.0153 ± 0.0081	0.027 ± 0.0066	0.02	Mackerel	Carnivore
	0.283 ± 0.1624	0.008	0.006	0.02	0.022 ± 0.0021	Seabass	Carnivore
Bangladesh **(Ghosh et al., 2021)**	-	-	-	1.07	-	Pangas catfish	Herbivore/ Omnivore
Iran **(Mokarram et al., 2021)**	-	3.49 ± 0.15	-	-	-	Spanish Mackerel	Carnivore
Iran (Mokarram et al., 2021)	-	2.05 ± 1.16,	1.56 ± 0.90	-	-	Orange-spotted spinefoot	Herbivore/ Omnivore
Thai **(Tremlová, 2017)**	0.09 ± 0.06	-	0.11	0.012,	-	silver whiting	
Thai **(Tremlová, 2017)**	-	-	-	1.027	-	Needlefish	Carnivore
Thai **(Sutee Chowrong, 2020)**	-	0.09 ± 0.04	-	-	0.32 ± 0.47	Mrigal carp	Detritivore
Myanmar **(Mar, 2020)**	-	0.073 ±0.007	-	-	-	Snakehead Fish	Carnivore
Myanmar **(Mar, 2020)**		0.083 ±0.009	-	-	-	Nile Tilapia	Omnivore
Bangladesh	1.19 ± 0.04	1.51 ± 0.04	0.51 ± 0.01		1.59 ± 0.93	freshwater	Omnivore

(Ahmed et al., 2015)						prawn	
Bangladesh **(Ahmed et al., 2015)**	1.02 ± 0.03	0.05 ± 0.00	4.55 ± 0.11		16.05 ± 1.48b	horn snails	Detritivore
Mexico **(Rodriguez-Mendivil et al., 2019)**	-	0.0019 ± 0.0001	0.116 ± 0.026	0.102 ± 0.020	0.377 ± 0.161	yellowfin tuna	Carnivore

Regarding to the bioaccumulation process, many factors such as physical-chemical qualities of the contaminated heavy metals, environmental factors, types of the exposed organism, and the organism's food chain depend on the quantity of the metal content in affected organism (Carvalho et al., 2005).

The research in China (Li et al., 2015) revealed that As, Cd, Pb and Hg were accumulated more in carnivorous species, followed by the omnivorous species and the filter-feeding species. (Yousafzai et al., 2010) showed that higher heavy metals contents were found in omnivorous fish than carnivorous fish species. Higher heavy metal accumulation in omnivorous fish species than carnivorous fish species has also been reported by (Štrbac et al., 2014) in Tisza River, Serbia.

In the present study, nile tilapia and catfish are freshwater fish, and mackerel and seabass are marine fish. As the information shown in Table 5-2, catfish, mackerel and seabass are carnivorous fish species, and nile tilapia is omnivore. According to the present study, As, Cd, Pb, and Hg were highest in the mackerel fish, and As, Cd and Hg were the second highest in seabass, while Cr was the highest in catfish, followed by tilapia, seabass and mackerel. Both mackerel and seabass are the marine carnivorous fish species; therefore, the marine carnivorous fish species of the present study have been accumulated for As, Cd and Hg than the freshwater carnivorous (catfish) or freshwater omnivorous fish (nile tilapia) species. When comparing within the freshwater fish, omnivorous nile tilapia was found higher concentration of As, Cd, Pb and Hg than the carnivorous catfish. Thus, the present study supports the assumption of (Štrbac et al., 2014) and (Yousafzai et al., 2010) while comparing the heavy metals quantities in freshwater fish.

Table 5-3: Order of fish species based on heavy metal concentration level

Elements	Order of fish species for heavy metal concentration
As	mackerel > seabass > nile tilapia > catfish
Cd	mackerel > seabass ≥ nile tilapia ≥ catfish
Cr	catfish > nile tilapia > seabass > mackerel
Pb	mackerel > nile tilapia > catfish > seabass
Hg	mackerel > seabass > nile tilapia ≥ catfish

One of the possible reasons of high heavy metals concentration in marine fish is that the marine water sources are where all the pollutants end up from both point sources and non-point sources. According to (Simachaya, 2000), the key point sources of bad quality at marine coastal areas of Thailand caused are industrial/ domestic wastewater discharge, sewage from boat, and development activities near the shore line for the tourism. However, there are many pointless sources of pollution in marine water such as urban runoff, agrochemicals from agricultural lands, residential areas, nutrients from livestock, pet wastes, etc,.

Additionally, the two-marine fish in the present study are carnivores. Heavy metals are non-biodegradable, and they accumulate in fish as per bioaccumulation/ biomagnification process; therefore the predator fish that are at the top of the food chain get higher heavy metals content.

5.2 Personal Information of Participants

The surveying about the personal information of the present study (total 400 participants) revealed that the average age $\pm$ SD was 37.21 $\pm$ 12.12 years old. When the participants were divided with the age range, 25-34 years old age (31% of all participants) range was the most participated in this research, and followed by 35-44 years old (23%), 45-54 years old (18%), 18-24 years old (17%), and 55-60 years old (12%). Mean body weight was 60.61 $\pm$ 9.77 Kg.

When body mass index (BMI) was evaluated based on body weight and height, mean BMI ± standard deviation was 23.11 ± 3.46 kg/m^2, with range of 18.13 to 32.98. According to World Health Organization (WHO) and Asian-Pacific guidelines, BMI falls into four categories described in Table *5-4*.

Table 5-4: Nutritional Status according to Who and Asian-Pacific guidelines

Categories	**WHO BMI (kg/m^2)**	**Asian-Pacific (kg/m^2)**
Underweight	Below 18.5	Below 18.5
Normal weight	18.5 - 24.9	18.5 - 22.9
Pre-obesity	25.0 - 29.9	23.0 - 24.9
Obesity	$\geq$ 30.0	$\geq$ 25

When BMI of 400 participants were compared with WHO BMI standard, 281 participants were in normal BMI range (18.5 – 24.9 kg/m^2), 89 participants were in pre-obesity (25.0 – 29.9 kg/m^2), and 20 participants fell in obesity range (above 30.0

kg/m^2), whereas BMI of 10 people were below 18.5 kg/m^2 and regarded as underweight. However, minimum BMI was 18.13 kg/m^2; therefore, 10 people who fell in underweight category were only slightly lower than the normal BMI of 18.5 – 24.9 kg/m^2. In age range 55-60, there was no one who is underweight; 35 participants got normal BMI, 10 participants were pre-obesity and 4 were in obesity range. The age range 25 – 34 years old has the highest participants, and also has the highest number of people for normal BMI, pre-obesity and obesity range, 90, 23, and 6, respectively. The detail information about BMI of participants and their age range was described in Table *5-5* and Figure *5-6*.

Table 5-5: Age range and body mass index (BMI) of the respondents

Age Range	Underweight	Normal	Pre-obesity	Obesity	Total
18-24	1	50	13	2	66
25-34	3	90	23	6	122
35-44	2	66	20	4	92
45-54	4	40	23	4	71
55-60	0	35	10	4	49
Total	10	281	89	20	400

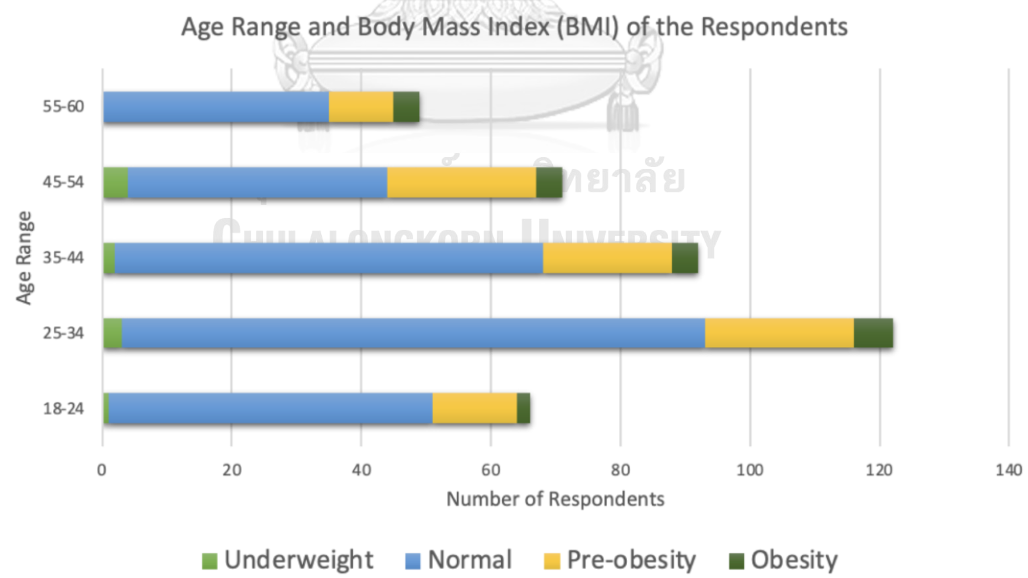
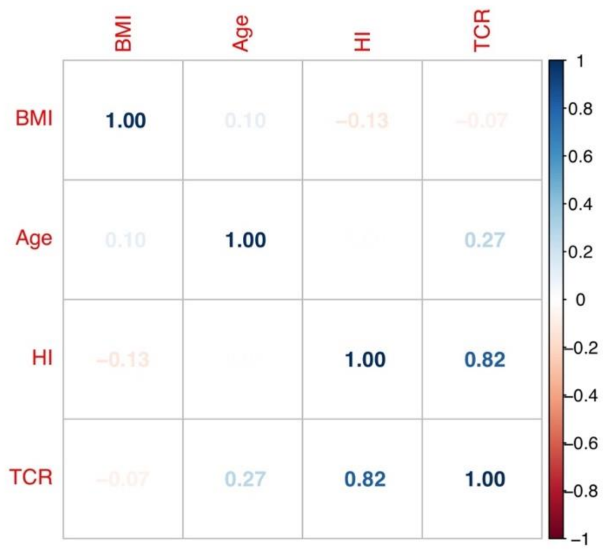


Figure 5-6: Body mass index (BMI) of the respondents based on age range



(-) = negative correlation between two variables
(+) = positive correlation between two variables

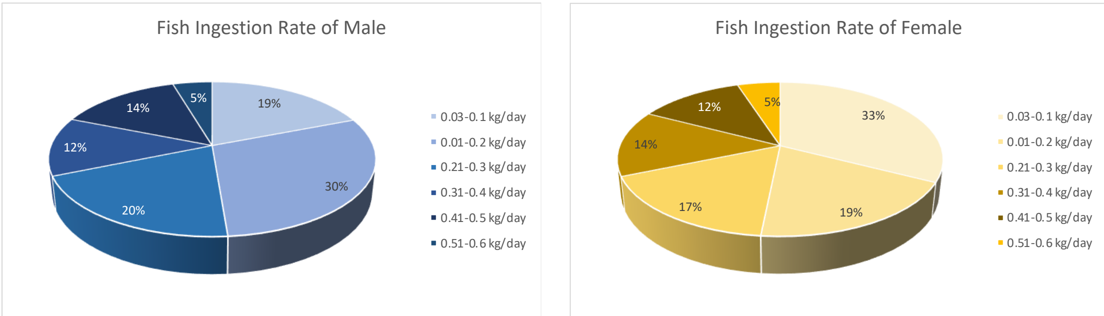
Figure 5-7: The correlation matrix showing relationship between BMI, Age, HI and TCR

The correlation matrix was evaluated by using R studio software (version 2022.02.3 Build 492). Figure *5-7* shows that the relationship between body mass index, age and the cumulative risk of heavy metal ingestion including cancer and non-cancer risks. BMI and non-carcinogenic risk and carcinogenic risk were inversely correlated, -0.13 and -0.07. Therefore, people who have low BMI may get the higher risk, especially non-carcinogenic risk.

With regard to the correlation between age and risk among the adult (18-60 years old), it was found out that the correlation is negligible for the non-cancer risk; however, there was positive relationship between age and cumulative cancer risk for the reason that cancer risk relates to the long-term exposure.

5.3 Exposure Assessment

The survey result of 400 participants showed that *Oreochromis niloticus* (nile tilapia) was the most favorite fish species out of four fish species: [*Oreochromis niloticus* (nile tilapia), *Clarias batrachus* (catfish), *Rastrelliger brachysoma* (mackerel), and *Lates calcarifer* (seabass)]. Nile Tilapia is also one of widely consumed fish species in Thailand (Table 2-5). The ingestion rate based on four fish species were 0.35 ± 0.16 kg/day for nile tilapia, 0.28 ± 0.13 kg/day for catfish, 0.17 ± 0.10 kg/day for mackerel, and 0.25 ± 0.11 kg/day for seabass.



(a) Male (b) Female

Figure 5-8: Fish ingestion rate of male and female

When the ingestion rates were compared with the gender, the minimum amount of the ingestion rate (0.01-0.1 kg/day) was consumed by 19% of the male, while that amount of ingestion rate was taken by 33% of the female, the highest percentage out of remaining ingestion rate range. However, 0.01 to 0.2 kg/day of fish was consumed by 30% of the male. Only 5% of both male and female consume the highest amount 0.51-0.6 kg per day. The remaining ingestion rate rages, 0.21-0.3 kg/day, 0.31-0.4 kg/day, 0.41-0.5 kg/day, were not much different depending on gender: 20%, 12% 14% for male, and 17%, 14%, 12% for female respectively.

For nutrition and protein database of FAO 2002, the ASEAN people rely largely on fish. Seafood accounted for roughly 38% of animal protein in the region's diet in 2011, followed by meat (33%), milk (20%), eggs (6%), and animal fats and organ meats (3%) (Food & Organization, 2002). According to (Chan et al., 2017), in comparison to countries, Myanmar is the second highest per capita fish consumption (55.3 kg/person/year) after Malasia (58.1 kg/person/year).

However, the fish consumption of 400 responds of the present study showed that the mean consumption was 28.58 $\pm$ 13.13 kg/person/year with the range of 10.05 to 90.85 kg/person/year. Therefore, it shows that the mean fish consumption of Burmese of the present study (28.58 $\pm$ 13.13 kg/person/year) is lower than the fish consumption of Burmese referenced by “Fish to 2050 in the ASEAN Region” report (Chan et al., 2017).

Fish consumption in Thailand according to (Chan et al., 2017) was 25 kg/person/year, and the fish consumption of the Burmese who live in Bangkok is 28.58 kg/person/year. Therefore, the consumption of fish in Thailand and the consumption resulted by the present were not much different, and it can be said that consumption rate also relies not only on culture and religion of the consumers but also on the location where they live in.

Table 5-6: Fish consumption rate of ASEAN countries and present study

Countries	Fish consumption (kg/person/year)	Fish consumption of Present Study (kg/person/year)	
Cambodia	35.5		
Indonesia	28.9		
Laos	16.6	Mean	28.58
Malasia	58.1	SD	13.13
Myanmar	55.3	Median	26.10
Philippines	32.7	Min	10.05
Thailand	25	Max	90.85
Vietnam	33.3		
Asean Region	33.5		
World	18.9		

5.4 Non-Cancer Risk Assessment

For non-cancer risk assessment of the present study, consuming catfish is safe both for acute health effects for each targeted heavy metals and cumulative effects of five heavy metals. Generally, nile tilapia is safe for the individual heavy metals (As, Cd, Cr, Pb, Hg) non-cancer effects and cumulative of these heavy metal effects; however, cumulative non-cancer effects of these five heavy metals can be found on some participants based on their ingestion rate, body weight, exposure duration etc. Individual non-cancer risk of As can be occurred by eating mackerel fish, but no individual effects of heavy metals cannot be found by eating seabass. Cumulative non-cancer risk effects can be suffered through the eating of both mackerel and seabass in long term.

Previous research in Machilipatnam Coast, Andhra Pradesh, India showed that non carcinogenic risk of Zn, Pb, Ni, Cu, and Hg through the consumption of marine fish's muscle, namely *Liza macrolepis*, were 17.9, 7.3, 5.3, 17.2, 1.08 and all the values were above the USEPA hazard quotient safety value, while HQ of Cd was 0.4 (Krishna et al., 2014). The study was focused on both essential and non-essential heavy metals, and it showed that non-cancer risks of essential heavy metals were comparatively higher than the non-essential heavy metals' risk. However, when comparing with the present study, non-cancer risks of essential heavy metals in the marine fish were still lower than that of essential heavy metals in the marine fish of (Krishna et al., 2014).

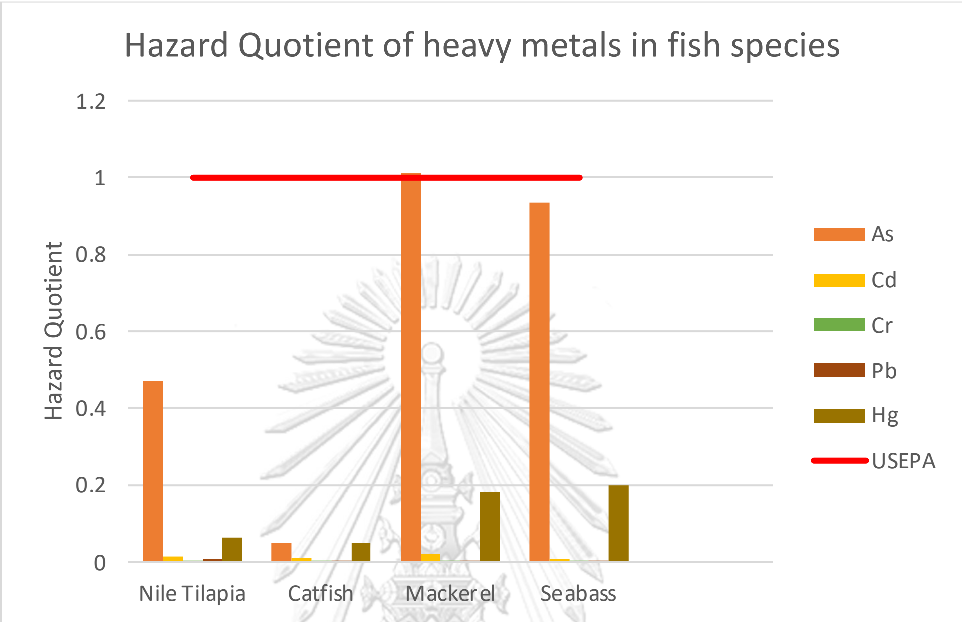


Figure 5-9: Hazard Quotient (HQ) of heavy metals in fish species

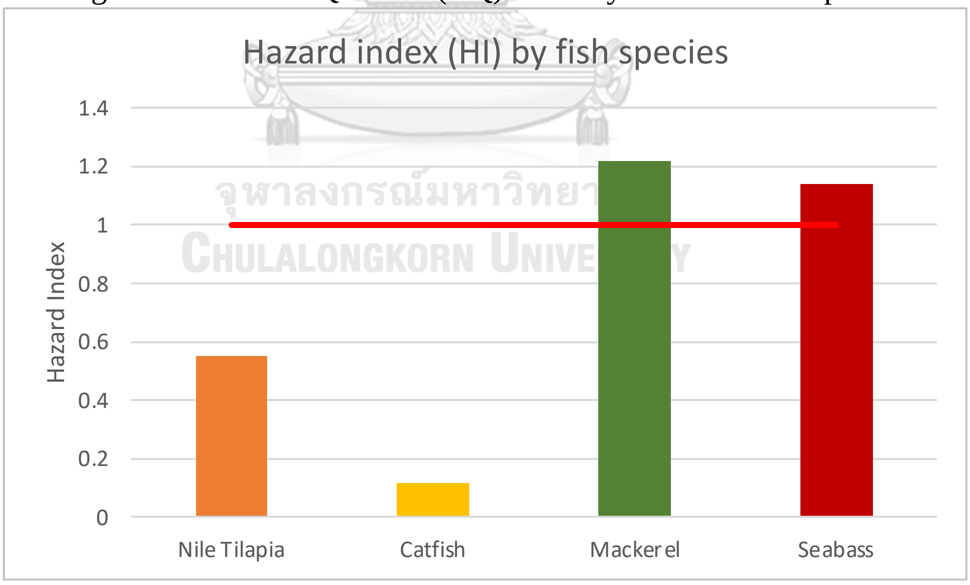


Figure 5-10: Hazard index (HI) by fish species

5.5 Cancer Risk Assessment

For cancer risk assessment of the present study, the cancer risk of Pb cannot be found in all fish species (nile tilapia, catfish, mackerel, and seabass). However, cancer risk of As, Cd, and Cr can be found by eating nile tilapia, catfish, and mackerel in long term. Then, Cd and Pb cancer risk might be suffered in people who consume seabass in long term. For the total cancer risk, all fish species of the present study have the aggregated cancer risk of As, Cd, Cr, and Pb through the lifelong consumption. Although the concentrations of heavy metals were not higher than the international standard, aggregated cancer might be occurred in all fish species for the reason that cancer risk was calculated for long term.

In China, (Zhong et al., 2018) conducted non-carcinogenic health risk assessment of eight heavy metals (Cu, Cr, Zn, Pb, As, Cd, Mn and Ni) through he consumption of wild freshwater fish and farm freshwater fish. It was found out that heavy metals contents in wild fish were much higher than those of farmed fish. The average carcinogenic risk of As in both farmed and wild fish were ranged 5.11×10^{-6}–1.95×10^{-4} for adults and 2.71×10^{-6}–1.04×10^{-4} for the children. Comparing to USEPA, the results were higher than 1×10^{-6}. Cancer risk of As in the present study was also the highest risk; therefore, the pollution sources of As should be controlled.

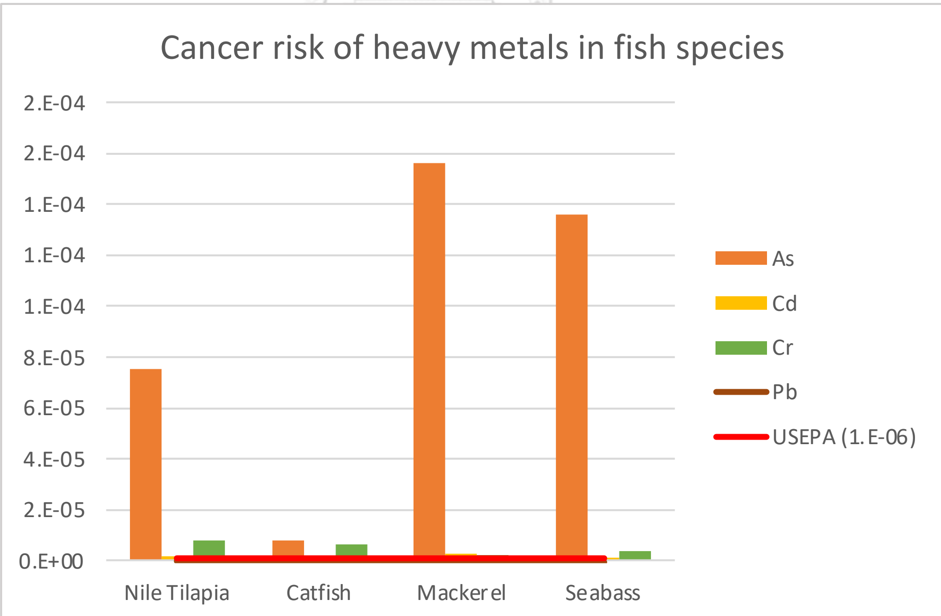


Figure 5-11: Cancer risk of heavy metals in fish species

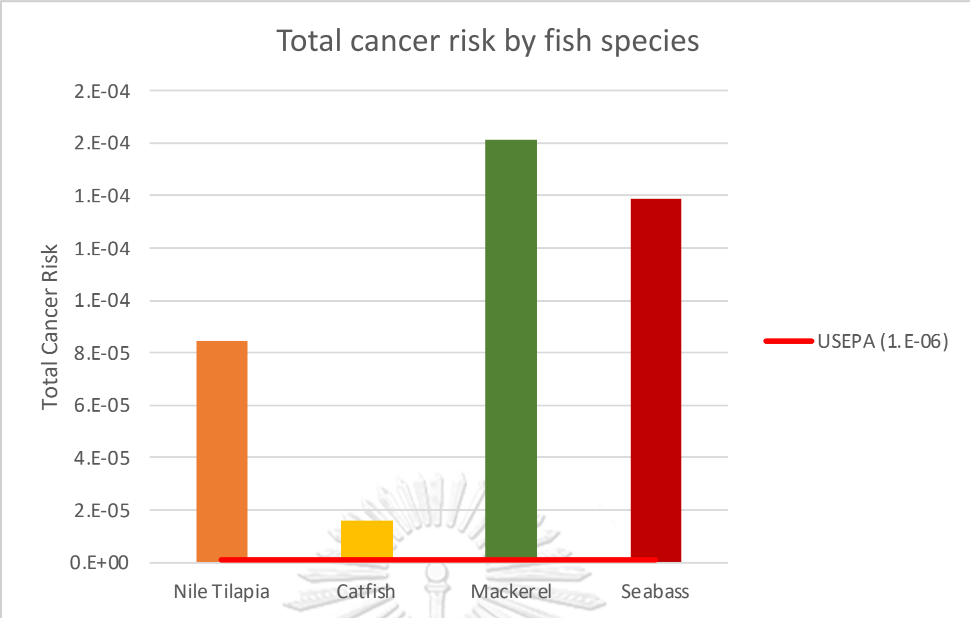


Figure 5-12: Total cancer risk by fish species

5.6 Risk Level in People who Eat All Fish Species

The questionnaire survey examines that there are 211 participants out of 400 who eat all targeted fish species (nile tilapia, catfish, mackerel, and seabass). Non-carcinogenic and carcinogenic risk were calculated for 211 participants and the mean$\pm$ standard deviation of total HI and TCR (of 5 heavy metals in four fish species) were 2.7945 $\pm$ 1.1082 with the range of (0.7323 to 5.5933), and 3.58 x 10^{-4} $\pm$ 2.22 x 10^{-4} with the range of (3.07 x 10^{-5} to 1.13 x 10^{-3}). Therefore, HI level is almost three times higher than the USEPA non-cancer hazard safety limit, 1. Likewise, total cancer risk was 3.58 x 10^{-4} $\pm$ 2.22 x 10^{-4}; therefore, the value was above the safety limit of 1 x 10^{-6}. It means that cancer risk can be occurred one in 35,000 people through the consumption of these fish species, while the USEPA specification for cancer risk is one in a million (1 x 10^{-6}).

Therefore, high synergistic effects can be found in people who eat various types of heavy metals contaminated fish species even though the concentration of individual heavy metal are lower than the international standards of heavy metals content in muscles of fish.

CHAPTER VI

6. Conclusion and recommendation

This chapter describes three main sub-parts: the conclusion of the whole study, some limitations, and the recommendations to personal, community level, policy makers and the future researchers.

6.1 Conclusion

This study was conducted to investigate the concentration of heavy metals (As, Cd, Cr, Pb, and Hg) in four fish species (nile tilapia, catfish, mackerel, and seabass) from the market of Bangkok, Thailand, and to assess the carcinogenic and non-carcinogenic health risk of Burmese. The questionnaire was developed in order to find out the necessary information for health risk assessment such as personal information to find out gender, age, body weight, and height, and food frequency survey to find out the frequency, amount and duration of eating fish. The carcinogenic and non-carcinogenic health risk were evaluated based on the ingestion of fish.

6.1.1 Heavy Metals Concentration in Fish

Five heavy metals contents in the muscle of four fish species were analyzed and the mean targeted heavy metal concentrations in mackerel with the exception of chromium were higher than other species: 0.449 ± 0.052 mg/kg for As, 0.03 ± 0.009 mg/kg for Cd, 0.0153 ± 0.0081 mg/kg for Pb, and 0.027 ± 0.0066 mg/kg for Hg. However, all heavy metals contents in all fish species' muscles were lower than the international standards.

6.1.2 Personal Information of Participants

The study subject was Burmese people who have been living in Bangkok, Thailand at least 6 months with the age between 18-60 years old. Both online and face to face interview was performed to 400 Burmese. 245 females and 155 males were participated and the average age ± standard deviation was 37 ± 12 years old. The age range between 25-34 was the highest number that took part in surveying, it was 31% of total participants and followed by 35-44 (23%), 45-54 (18%), 18-24 (16%), and 55-

60 (12%) age ranges. The average BMI of the participants was 23.11 ± 3.46 kg/m^2, with range of 18.13 to 32.98.

6.1.3 Exposure Assessment

Based on the survey results, nile tilapia was the most consumed by Burmese people, 375 out of 400 participants (93.8 %), and then followed by catfish (91.5 %), seabass (82.8 %), mackerel (79 %). There were 211 participants (52.8 %) who eat all targeted fish species of the present study. The mean ingestion rate (IR) for nile tilapia, catfish, mackerel, and seabass were 0.35 ± 0.16 kg/day, 0.28 ± 0.13 kg/day, 0.17 ± 0.1 kg/day, and 0.25 ± 0.11 kg/day, respectively. The mean exposure frequency (EF) for nile tilapia, catfish, mackerel, and seabass were 94.69 ± 44.24 day/year, 93.61 ± 45.94 day/year, 95.82 ± 43.86 day/year, and 92.53 ± 45.47 day/year, respectively. The mean exposure duration for nile tilapia, catfish, mackerel, and seabass were 23.43 ± 11.2 years, 23.53 ± 11.41 years, 23.28 ± 12.03 years, and 22.17 ± 16.21 years, respectively.

6.1.4 Non-Cancer Risk Assessment

Non-carcinogenic health risks of all targeted heavy metals in nile tilapia, catfish, mackerel (except As), and seabass were under the USEPA hazard quotient of 1. Non cancer risk of As in mackerel was 1.01 ± 4.89 x 10^{-1}, and it was slightly over the safety limit. Therefore, there might generally have no adverse non-carcinogenic health effects of As, Cd, Cr, Pb, and Hg by eating nile tilapia, catfish, seabass. But, eating mackerel may have the acute effects of arsenic such as nausea, vomiting, destruction gastrointestinal tissue and heartbeat abnormalities. Then, the mean cumulative hazard index (HI) of nile tilapia and catfish were lower than 1, and the mean HI of mackerel and seabass were 1.22 ± 0.59 and 1.14 ± 0.60. Hence, consuming mackerel and seabass may have cumulative non carcinogenic health effects. Although the mean HI of nile tilapia was in acceptable range, the calculation of HI for each participants showed that 15% of the participants who eat nile tilapia were above the USEPA hazard index of 1.

6.1.5 Cancer-Risk Assessment

The carcinogenic health risks of Cd and Cr of all analyzed fish species (nile tilapia, catfish, mackerel, and seabass) were above USEPA acceptable range of 1×10^{-4} to 1×10^{-6}. The cancer risks caused by As in three fish species (nile tilapia, catfish, and mackerel) were also beyond the safety range. Therefore, the carcinogenic health effects of As, Cd, and Cr can be occurred by eating nile tilapia, catfish and mackerel and Cd and Cr carcinogenic effects by eating seabass. Cancer risks of lead (Pb) in all fish species were under the safety range, and the participants might not suffer cancer risk caused by Pb. Then, the total cancer risks of all fish species were $8.49 \times 10^{-5} \pm 8.17 \times 10^{-5}$ (nile tilapia), $1.57 \times 10^{-5} \pm 1.53 \times 10^{-5}$ (catfish), $1.61 \times 10^{-4} \pm 1.17 \times 10^{-4}$ (mackerel), and $1.40 \times 10^{-4} \pm 1.15 \times 10^{-4}$ (seabass). The mean total cancer risks (TCR) of all targeted fish species were above the USEPA safety limit. Therefore, the participants might have cancer risk through the consumption of nile tilapia, catfish, mackerel and seabass in their lifetime.

6.1.6 Risk Level in People who Eat All Fish Species

Afterwards, both carcinogenic and non-carcinogenic risk level were calculated for the participants who consume all four targeted fish species of this research. There were 211 participants who eat all fish species and the mean± standard deviation of total HI and TCR were 2.7945 ± 1.1082 and $3.58 \times 10^{-4} \pm 2.22 \times 10^{-4}$. Thus, the results show that the risk levels were high in people who eat more than one fish species because of aggregate risk of heavy metals from each of the fish species.

6.2 Limitations

- This study only focused on five heavy metals (As, Cd, Cr, Pb, and Hg) in the muscle of the four fish species (nile tilapia, catfish, mackerel, and seabass).
- The fish samples are collected only from one local fresh market that is located at Soi 10, Phetchaburi, Bangkok, Thailand.
- The subject was only Burmese people who live in Bangkok, Thailand.
- With regards to questionnaire surveying, 69.5 % was participated by online surveying, and therefore there may have limitation for answering about the fish consumption.

- Only oral exposure was examined for cancer and non-cancer risk assessment.

6.3 Recommendations

This research would like to recommend in four sectors as followings_

6.3.1 Recommendation to Personal Level

- Fish consumer should concern consumption of mackerel and seabass because both non-cancer risk and cancer risk found in these fish species were higher than safety limit.
- Apart from eating fish, people should also concern the aggregate risk by eating other aquatic animals such as shrimp, crab, mussel, etc. and smoking cigarette.
- This study recommends the subjects participated in questionnaire surveying to decrease the amount and frequency of eating fish (consumption per week) especially mackerel and seabass.

6.3.2 Recommendation to Community Level

- Primary health care should have health promotion on heavy metals exposure from eating fish, health check-up regularly, etc. to prevent risk.

6.3.3 Recommendation to Government Level

- Government should concern about the fish farm activities for the reason that heavy metals can also be accumulated in fish from the fish feed and water supply source.
- The representative authorities should monitor wastewater release/ leak from industrial, domestic and landfill sites.
- Policy makers should lower the safety concentration in foodstuffs based on the local consumption rate.

6.3.4 Recommendation for Further Researchers

- This study can be used as a baseline information for human health risk assessment through the consumption of fish.
- This study can be used as reference in other studies such as waste management and heavy metal pollution in water sources.

- Other information such as weight, height, fish consumption rate and frequency of Burmese can be used as secondary data in further studies.
- Future research should study other metals because of syngenetic effects, including essential heavy metals such as Zn, Fe, Cu, Ni, Se, etc. since essential heavy metals may also be toxic at high concentrations.
- Further studies should be done other fish species that are popular among the fish consumers.

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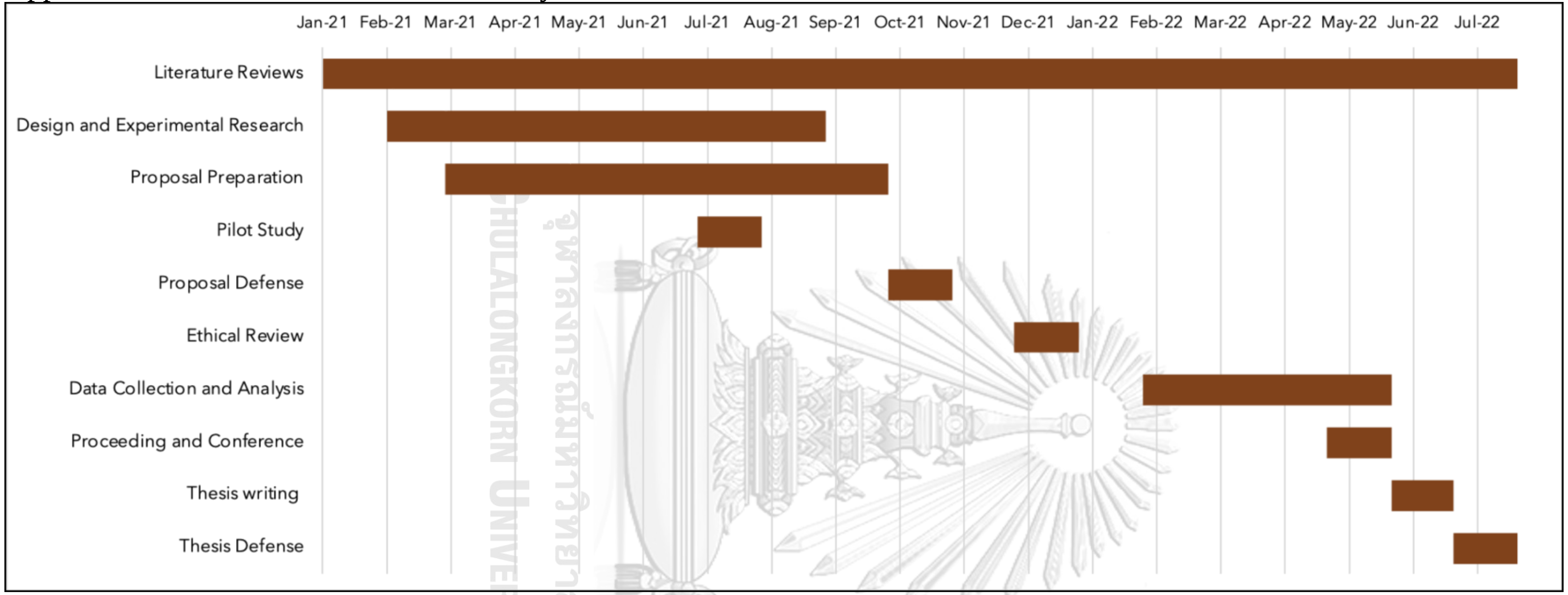
APPENDIX

Appendix I: Timeline for the research study



Appendix II: Pilot questionnaire survey form

This study is being conducted by Chulalongkorn University student as part of research. This study is intended for educational purpose only, and not for any other purpose. This questionnaire will take you around 3 minutes. It will be completely anonymous.

No	Which market do you mostly go for fish?	Please rank the fish species you eat the most																
		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	Others
1																		
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3																		
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Appendix III: Pilot questionnaire survey form

Item		Name	Picture
a	Local Name	Pla Ta-pien	
	Common Name	Common silver barb	
b	Local Name	Pla Nil	
	Common Name	Nile tilapia	
c	Local Name	Pla Graai	
	Common Name	Spotted featherback	
d	Local Name	Pla Sa-lid	
	Common Name	Snake skin gourami	
e	Local Name	Pla Sa-waai	
	Common Name	Striped catfish	
f	Local Name	Pla Chon	
	Common Name	Striped snake-head fish	
g	Local Name	Pla Lai	
	Common Name	Swamp eel	
h	Local Name	Pla Duk-oui	
	Common Name	Walking catfish	

(1)

Item		Name	Picture
i	Local Name	Pla Samlee	
	Common Name	Black-banded trevally	
j	Local Name	Pla Jalamet-dum	
	Common Name	Black pomfret	
k	Local Name	Pla Jalamet-khao	
	Common Name	Silver pomfret	
l	Local Name	Pla Gow	
	Common Name	Grouper	
m	Local Name	Pla Ga-pong khao	
	Common Name	Giant seaperch	
n	Local Name	Pla Ga-pong dang	
	Common Name	Malabar red snapper	
o	Local Name	Pla Tu-sod	
	Common Name	Short-bodied mackerel	
p	Local Name	Pla In-see	
	Common Name	Spanish mackerel	

Appendix IV: Market and fish species responded by the participants

No	Market	Most frequently eat fish species				
		1	2	3	4	5
1	Tesco Rama I	Nile tilapia	Giant seaperch	Striped catfish	Spanish mackerel	
2	Big C (Ratchadamri)	Snake skin gourami	Walking catfish	mackerel	Giant seaperch	
3	Tesco Rama I	Nile tilapia	Striped catfish			
4	Big C (Ratchadamri)	Nile tilapia	Walking catfish	mackerel		
5	Big C (Ratchadamri)	Walking catfish	Nile tilapia	mackerel		
6	Big C (Ratchadamri)	Black pomfret	Silver pomfret	Giant seaperch	mackerel	Spanish mackerel
7	Big C	Walking catfish	Nile tilapia	mackerel		
8	Soi 10	Nile tilapia	Striped snake-head fish	mackerel		
9	Soi 10	Common silver barb	Striped catfish	Giant seaperch	Nile tilapia	Spotted featherback
10	Soi 10	Walking catfish	Nile tilapia	Common silver barb		
11	Soi 10	Nile tilapia	Black-banded trevally	Common silver barb		
12	Big C and Soi 10	Nile tilapia	Walking catfish			
13	Big C and Tesco	mackerel	Giant seaperch	Grouper	Spotted featherback	Snake skin gourami
14	Soi 10 and Big C	Common silver barb	Nile tilapia	Spotted featherback	Striped snake-head fish	Walking catfish
15	Big C (Ratchadamri)	Nile tilapia	Walking catfish	Striped catfish	Malabar red snapper	
16	Soi 10	Black-banded trevally	Walking catfish	Nile tilapia		
17	Big C (Ratchadamri)	mackerel	Snake skin gourami	Grouper		
18	Chareonphol Morning Mar	Nile tilapia	Striped snake-head fish	Walking catfish		
19	Soi 10	Nile tilapia	Striped catfish			
20	Chareonphol Morning Mar	Nile tilapia	Giant seaperch	Common silver barb	Striped snake-head fish	
21	Soi 10	Nile tilapia	Snake skin gourami	Striped snake-head fish		
22	Big C	Striped catfish	Striped snake-head fish	mackerel		
23	Tesco Rama I	Nile tilapia	Giant seaperch	Silver pomfret		
24	Soi 10	Snake skin gourami	Snake skin gourami	Walking catfish		
25	Soi 10	Nile tilapia	Striped catfish	Walking catfish		
26	Soi 10	Nile tilapia	mackerel			
27	Big C (Ratchadamri)	Walking catfish	Common silver barb	Giant seaperch		
28	Big C (Ratchadamri)	Striped snake-head fisl	Nile tilapia	Giant seaperch		
29	Big C (Ratchadamri)	Nile tilapia	Snake skin gourami	Walking catfish	mackerel	
30	Soi 10	Common silver barb	mackerel	Giant seaperch		
31	Soi 10	mackerel	Nile tilapia			
32	Big C (Ratchadamri)	Black-banded trevally	Nile tilapia	Giant seaperch		

Appendix V: Item-Objective Congruence (IOC) Test Result

Item- Objective Congruence (IOC)

This questionnaire is a part of master's degree thesis research of International Program in Hazardous Substance and Environmental management, the title "Health Risk Assessment of Burmese Related to Consumption of Heavy Metals Contaminated Fish from Local Market in Bangkok, Thailand".

Researcher name: Myat Myitzu student ID- 6288524220

Examiners

Expert 1: Dr.Pokkate Wongsasuluk, the instructor from College of Public Health Sciences, Chulalongkorn University.

Expert 2: Srilert Chotpantarat, Deputy Director of Environmental Research Institute (ERIC), Geology Department, Faculty of Science Chulalongkorn University, Thailand

Expert 3: Myat Thandar Oo, Technical Associate, Advancing Life And Regenerating Motherland (ALARM), Yangon, Myanmar.

From three experts, the average IOC score of this questionnaire was 1.
This questionnaire is divided into two parts: part 1 personal information and part 2 fish consumption. Please insert ✓ in the table as following:

- The score = +1, if the expert is sure that this item really measured the attribute.
- The score = 0, if the expert is not sure that the item does measure or does not measure the expected attribute.
- The score = –1, if the expert is sure that this item does not measure the attribute.

The Item-objective congruence (IOC) test was done to evaluate the items of questionnaire based on the score range from -1 to +1. According to the IOC test, the average scores for all questions were 1. Therefore, the values were above 0.5, and all items in the questionnaire were reserved.

No	Items	IOC score			Average Score	Comments
		Aj Srilert	Aj. Pokkate	Myat Thandar		
A	Interviewee Information					
A-1	Gender	+1	+1	+1	1	

Item- Objective Congruence (IOC)

	☐ Male ☐ Female					
A-2	How old are you? ……..……. years	+1	+1	+1	1	
A-3	What is your body weight? ……………Kg	+1	+1	+1	1	
A-4	What is your height? …………….cm	+1	+1	+1	1	

B		Food Frequency Survey				
B-1	Among the fish species below, which fish species do you eat? (You can answer more than one.) **Freshwater fish** **Marine fish** ☐  ☐  Nile Tilapia (*Oreochromis niloticus*) Mackerel (*Rastrelliger brachysoma*) ☐  ☐  Catfish (*Clarias batrachus*) Giant Seaperch (*Lates calcarifer*)	+1	+1	+1	1	

Item- Objective Congruence (IOC)

B-2	How often do you eat Nile Tilapia? ☐ 1 meal per day ☐ 2 meal per day ☐ 3 meal per day How many days per week?...........days Or how many days per month?...............days	+1	+1	+1	1	
B-3	How often do you eat catfish? ☐ 1 meal per day ☐ 2 meal per day ☐ 3 meal per day How many days per week?...........days Or how many days per month?...............days	+1	+1	+1	1	
B-4	How often do you eat mackerel? ☐ 1 meal per day ☐ 2 meal per day ☐ 3 meal per day How many days per week?...........days Or how many days per month?...............days	+1	+1	+1	1	
B-5	How often do you eat giant seaperch? ☐ 1 meal per day ☐ 2 meal per day ☐ 3 meal per day How many days per week?...........days Or how many days per month?...............days	+1	+1	+1	1	

Item- Objective Congruence (IOC)

B-6	How many grams do you eat Nile Tilapia per meal? ……..…grams 1 Nile Tilapia is about 500 grams	+1	+1	+1	1	
B-7	How many grams do you eat catfish per meal? ……..…grams 1 catfish is about 250 grams	+1	+1	+1	1	
B-8	How many grams do you eat mackerel per meal? ……..…grams 1 mackerel is about 70 grams	+1	+1	+1	1	
B-9	How many grams do you eat giant seaperch per meal? ……..…grams 1 giant seaperch is about 700 grams	+1	+1	+1	1	
B-10	How long have you eaten for Nile Tilapia? …………years or …….... months	+1	+1	+1	1	
B-11	How long have you eaten for catfish? …………years or …….... months	+1	+1	+1	1	
B-12	How long have you eaten for mackerel? …………years or …….... months	+1	+1	+1	1	
B-13	How long have you eaten for giant seaperch? …………years or …….... months	+1	+1	+1	1	

Appendix VI: Certificate of Ethical Approval

The Research Ethics Review Committee for Reseach Involving Human Research Participants, Group I, Chulalongkorn University
Chamchuri 1 Building, 2nd Floor, 254 Phayathai Road, Pathumwan, Bangkok 10330 Thailand
Telephone: 02-218-3202, 02-218-3049 Email: eccu@chula.ac.th

COA No. 054/65

Certificate of Approval

Study Title No. 650006 : HEALTH RISK ASSESSMENT OF BURMESE RELATED TO CONSUMPTION OF HEAVY METALS CONTAMINATED FISH FROM LOCAL MARKET IN BANGKOK, THAILAND

Principal Investigator : Ms. Myat Myitzu

Place of Proposed Study/institution : Graduate School, Chulalongkorn University

The Research Ethics Review Committee for Research Involving Human Research Participants, Group I, Chulalongkorn University, Thailand, has approved constituted in accordance with Belmont Report 1979, Declaration of Helsinki 2013, Council for International Organizations of Medical Sciences (CIOM) 2016, Standards of Research Ethics Committee (SREC) 2017, and National Policy and guidelines for Human Research 2015.

Signature Prida Tasanapradit	Signature Raveenan Mingpakanee
(Associate Prof. Prida Tasanapradit)	(Assistant Prof. Dr. Raveenan Mingpakanee)
Chairman	Secretary
Date of Approval : 2 March 2022	**Approval Expire date :** 1 March 2023

The approval documents including:

1. Participant Information Sheet and Consent Form
2. Research proposal
3. Researcher
4. Research instruments/tools

<u>Conditions</u>

The approved investigator must comply with the following conditions:

1. It's unethical to collect data of research participants before the project has been approved by the committee.

2. The research/project activities must end on the approval expired date. To renew the approval, it can be applied one month prior to the expired date with submission of progress report.

3. Strictly conduct the research/project activities as written in the proposal.

4. Using only the documents that bearing the RECCU's seal of approval: research tools, information sheet, consent form, invitation letter for research participation (if applicable).

5. Report to the RECCU for any serious adverse events within 5 working days.

6. Report to the RECCU for any amendment of the research project prior to conduct the research activities.

7. Report to the RECCU for termination of the research project within 2 weeks with reasons.

8. Final report (AF 01-15) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project.

9. Research project with several phases; approval will be approved phase by phase, progress report and relevant documents for the next phase must be submitted for review.

10. The committee reserves the right to site visit to follow up how the research project being conducted.

11. For external research proposal the dean or head of department oversees how the research being conducted

The Research Ethics Review Committee for Research Involving Human Research Participants Group I

Study Title No. 650006
Date of Approval 02 Mar 2022
Approval Expire date 01 Mar 2023

Digital Certificate

Appendix VII: Questionnaire form (English)

Pre-Questionnaire Screening Form

A student, who is pursuing master's degree at Chulalongkorn University. is doing research with the title "Health Risk Assessment of Burmese Related to Consumption of Heavy Metals Contaminated Fish from Local Market in Bangkok, Thailand". To take part in this research as a participant, you are kindly requested to fill out this screening form. This information will remain private and confidential and will not be used for any other purposes.

Please check the box of each question YES (or) NO.

	YES	NO	Additional Information
Can you read or write Myanmar language?	☐	☐	If no, the interviewer will help you to answer.
Are you Burmese?	☐	☐	If no, you can stop here.
Is your age between 18 and 60 years old?	☐	☐	If no, you can stop here.
Are you having severe disease (such as heart disease, cancer, and other chronic diseases), unhealthy, bed-ridden or psychological problem? (if you are not able to answer the questions for 5 minutes)	☐	☐	If yes, you can stop here.
Do you have allergies to fish?	☐	☐	If yes, you can stop here.
Do you eat fish?	☐	☐	If no, you can stop here.
Is your residing time in Bangkok over 6 months?	☐	☐	If no, you can stop here.

The Research Ethics Review Committee for Research Involving Human Research Participants
Group 1

Project Number 650006
Date of approval 02 Mar 2022
Expire date 01 Mar 2023

QUESTIONNAIRE FOR FOOD FREQUENCY SURVEY

This study is being conducted by Chulalongkorn University student as part of research. This study is intended for educational purpose only, and not for any other purpose. This questionnaire will take you around 5 minutes. It will be completely anonymous.

NO ____   Date: _______________

Part A Interviewee Information

A.1. Gender

☐ Male

☐ Female

A.2. Age _____________________ Years

A.3. Body weight _____________________ Kg

A.4. Height _____________________ cm

Part B Food Frequency Survey

B.1. Among the fish species below, which fish species do you eat? (You can answer more than one.)

Freshwater fish	**Marine fish**
☐ 	☐ 
Nile Tilapia (*Oreochromis niloticus*)	Mackerel (*Rastrelliger brachysoma*)
☐ 	☐ 
Catfish (*Clarias batrachus*)	Giant Seaperch (*Lates calcarifer*)

Project Number 650006
Date of approval 02 Mar 2022
Expire date 01 Mar 2023

QUESTIONNAIRE FOR FOOD FREQUENCY SURVEY

B.2. How often do you eat Nile Tilapia?

☐ 1 meal per day ☐ 2 meal per day ☐ 3 meal per day

How many days per week?...........days

Or how many days per month?...............days

B.3. How often do you eat catfish?

☐ 1 meal per day ☐ 2 meal per day ☐ 3 meal per day

How many days per week?...........days

Or how many days per month?...............days

B.4. How often do you eat mackerel?

☐ 1 meal per day ☐ 2 meal per day ☐ 3 meal per day

How many days per week?...........days

Or how many days per month?...............days

B.5. How often do you eat giant seaperch?

☐ 1 meal per day ☐ 2 meal per day ☐ 3 meal per day

How many days per week?...........days

Or how many days per month?...............days

B.6. How many grams do you eat Nile Tilapia per meal? ……..…grams
1 Nile Tilapia is about 500 grams

B.7. How many grams do you eat catfish per meal? ………grams
1 catfish is about 250 grams

QUESTIONNAIRE FOR FOOD FREQUENCY SURVEY

B.8. How many grams do you eat mackerel per meal? ……..…grams
1 mackerel is about 70 grams

B.9. How many grams do you eat giant seaperch per meal? ……..…grams
1 giant seaperch is about 700 grams

B.10. How long have you eaten for Nile Tilapia?
…………years or ……... months

B.11. How long have you eaten for catfish?
…………years or ……... months

B.12. How long have you eaten for mackerel?
…………years or ……... months

B.13. How long have you eaten for giant seaperch?
…………years or ……... months

Your participation is very important for this research and thank you for your kind cooperation.

Appendix VIII: Questionnaire (Myanmar)

Pre-Questionnaire Screening Form

ချူလာလောင်ကွန်းတက္ကသိုလ်တွင် မဟာသိပ္ပံတက်ရောက်နေသော ကျောင်းသူတစ်ယောက်သည် “ထိုင်းနိုင်ငံ ဘန်ကောက်မြို့ ပုံကျဈေးမှ သတ္တုများပါဝင်နေသည့် ငါးများစားသုံးသည့် မြန်မာလူများ၏ ကျန်းမာရေးထိခိုက်မှုအခြေအနေများအား လေ့လာခြင်း” ခေါင်းစဉ်ဖြင့် သုတေသနဆောင်ရွက်လျက်ရှိပါသည်။ ဤသုတေသနတွင် ပါဝင်ရန်အတွက် အောက်ပါ စီစစ်ခြင်းပုံစံလွှာကို ဖြေဆိုပေးရန် မေတ္တာရပ်ခံပါသည်။ မေးမြန်းရရှိသော အချက်အလက်များကို လျှို့ဝှက်ထားမည်ဖြစ်ပြီး အခြားရည်ရွယ်ချက်များအတွက် အသုံးပြုသွားမည်မဟုတ်ပါ။

ကျေးဇူးပြု၍ အောက်ပါတို့ကို အမှန်ခြစ်ပေးပါ။

မေးခွန်းများ	ဟုတ်ပါသည်	မဟုတ်ပါ	မှတ်ချက်
မြန်မာဘာသာကို ပြောတတ် (သို့) ဖတ်တတ်ပါသလား။	☐	☐	မဖတ်တတ်လျှင် မေးခွန်းမေးမြန်းသူမှ ကူညီပေးပါမည်။
မြန်မာလူမျိုးဟုတ်ပါသလား။	☐	☐	မဟုတ်လျှင် ဆက်လက်ဖြေဆိုရန် မလိုပါ။
သင်၏အသက်သည် ၁၆နှစ်နှင့် ၆၀ နှစ်ကြား ဟုတ်ပါသလား။	☐	☐	မဟုတ်လျှင် ဆက်လက်ဖြေဆိုရန် မလိုပါ။
သင်သည် နှလုံးရောဂါ၊ ကင်ဆာ၊ နှင့် အခြားပြင်းထန်သော ကျန်းမာရေးအခြေအနေရှိပြီး အိပ်ယာထဲတွင် လှဲနေရသူတစ်ယောက် (သို့) စိတ်ကျန်းမာရေးမကောင်းသူ တစ်ယောက်လား။ (ကျန်းမာရေးအရ ၅ မိနစ်ခန့် အချိန်ပေးဖြေကြားရန် အဆင်မပြေပါက ဤနေရာတွင် ရပ်တန့်နိုင်ပါသည်။)	☐	☐	ဟုတ်လျှင် ဆက်လက်ဖြေဆိုရန် မလိုပါ။
ငါးစားလျှင် ခါတ်မတည့်ခြင်းမျိုး ရှိပါသလား။	☐	☐	ဟုတ်လျှင် ဆက်လက်ဖြေဆိုရန် မလိုပါ။
ငါးစားပါသလား။	☐	☐	မဟုတ်လျှင် ဆက်လက်ဖြေဆိုရန် မလိုပါ။
ဘန်ကောက်တွင် နေထိုင်နေသည်မှာ ၆ လအထက် ရှိပါသလား။	☐	☐	မဟုတ်လျှင် ဆက်လက်ဖြေဆိုရန် မလိုပါ။

QUESTIONNAIRE FOR FOOD FREQUENCY SURVEY

ဤလေ့လာမှုကို ချူလာလောင်ကွန်းတက္ကသိုလ် ကျောင်းသူတစ်ဦးမှ ဆောင်ရွက်ခြင်းဖြစ်ပြီး အခြားရည်ရွယ်ချက်များအတွက် ဆောင်ရွက်ခြင်းမဟုတ်ပဲ ပညာရေးအတွက်သာ ဆောင်ရွက်ခြင်းဖြစ်ပါသည်။ မေးခွန်းမေးမြန်းရန်ကြာချိန်မှာ ၅ မိနစ်ခန့်ဖြစ်ပြီး အချက်အလက်များကို ဖြေဆိုသူ၏အမည်များဖြင့် ဖော်ပြမည်မဟုတ်ပါ။

အမှတ်စဉ် ___ နေ့ရက် ___________

အပိုင်း (က) ဖြေဆိုသူ၏အချက်အလက်

က-၁ ကျား/မ

☐ ကျား ☐ မ

က-၂ အသက် _________________ နှစ်

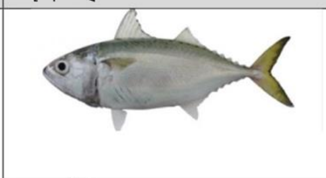
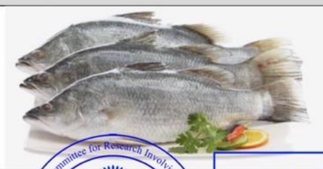
က-၃ ကိုယ်အလေးချိန် _________________ ကီလိုဂရမ်

က-၄ အရပ်အမြင့် _________________ စင်တီမီတာ

အပိုင်း (ခ) အစားအသောက် စားသုံးမှုပုံစံ

ခ-၁ အောက်ပါငါးအမျိုးအစားများအနက် မည်သည့်ငါးမျိုးစိတ်ကို အစားများပါသလဲ။

(တစ်မျိုးထက်ပို၍ ဖြေဆိုနိုင်ပါသည်။)

စဉ်	ရေချိုငါးများ	စဉ်	ရေငန်ငါးများ
၁	 တီလားပီးယားငါး	၃	 မကရယ်ငါး
၂	 ငါးခူ	၄	 ကကတစ်ငါး

QUESTIONNAIRE FOR FOOD FREQUENCY SURVEY

ခ-၂ တီလားပီးယားငါး စားသုံးမှုကြိမ်နှုန်း

☐ တစ်ရက်လျှင် ၁ နပ်　　☐ တစ်ရက်လျှင် ၂ နပ်

☐ တစ်ရက်လျှင် ၃ နပ်

တစ်ပတ်လျှင် ဘယ်နရက်စားသုံးပါသလဲ။ _______________ ရက်

(သို့မဟုတ်)

တစ်လလျှင် ဘယ်နရက်စားသုံးပါသလဲ။ _______________ ရက်

ခ-၃ ငါးခူ စားသုံးမှုကြိမ်နှုန်း

☐ တစ်ရက်လျှင် ၁ နပ်　　☐ တစ်ရက်လျှင် ၂ နပ်

☐ တစ်ရက်လျှင် ၃ နပ်

တစ်ပတ်လျှင် ဘယ်နရက်စားသုံးပါသလဲ။ _______________ ရက်

(သို့မဟုတ်)

တစ်လလျှင် ဘယ်နရက်စားသုံးပါသလဲ။ _______________ ရက်

ခ-၄ မကရယ်ငါး စားသုံးမှုကြိမ်နှုန်း

☐ တစ်ရက်လျှင် ၁ နပ်　　☐ တစ်ရက်လျှင် ၂ နပ်

☐ တစ်ရက်လျှင် ၃ နပ်

တစ်ပတ်လျှင် ဘယ်နရက်စားသုံးပါသလဲ။ _______________ ရက်

(သို့မဟုတ်)

တစ်လလျှင် ဘယ်နရက်စားသုံးပါသလဲ။ _______________ ရက်

ခ-၅ ကကတစ်ငါး စားသုံးမှုကြိမ်နှုန်း

☐ တစ်ရက်လျှင် ၁ နပ်　　☐ တစ်ရက်လျှင် ၂ နပ်

☐ တစ်ရက်လျှင် ၃ နပ်

တစ်ပတ်လျှင် ဘယ်နရက်စားသုံးပါသလဲ။ _______________ ရက်

(သို့မဟုတ်)

တစ်လလျှင် ဘယ်နရက်စားသုံးပါသလဲ။ _______________ ရက်

ခ-၆ ထမင်းတနပ်လျှင် တီလားပီးယားငါးဘယ်လောက်များများ စားပါသလဲ။ (ဂရမ်)

(တီလားပီးယားငါးတစ်ကောင်သည် ခန့်မှန်းခြေ ၅၀၀ ဂရမ်ခန့်ရှိပါသည်။)

_______________ ဂရမ်

ခ-၇ ထမင်းတနပ်လျှင် ငါးခူဘယ်လောက်များများ စားပါသလဲ။ (ဂရမ်)

(ငါးခူတစ်ကောင်သည် ခန့်မှန်းခြေ ၂၅၀ ဂရမ်ခန့်ရှိပါသည်။)

_______________ ဂရမ်

ခ-၈ ထမင်းတနပ်လျှင် မကရယ်ငါး ဘယ်လောက်များများ စားပါသလဲ။ (ဂရမ်)

(တမကရယ်ငါးတစ်ကောင်သည် ခန့်မှန်းခြေ ၇၀ ဂရမ်ခန့်ရှိပါသည်။)

_______________ ဂရမ်

ခ-၉ ထမင်းတနပ်လျှင် ကကတစ်ငါးဘယ်လောက်များများ စားပါသလဲ။ (ဂရမ်)

(ကကတစ်ငါးတစ်ကောင်သည် ခန့်မှန်းခြေ ၇၀၀ ဂရမ်ခန့်ရှိပါသည်။)

_______________ ဂရမ်

ခ-၁၀ တီလားပီးယားငါး စားတာဘယ်လောက်ကြာပြီလဲ။

_______________ နှစ် (သို့မဟုတ်) _______________ လ

ခ-၁၁ ငါးခူ စားတာဘယ်လောက်ကြာပြီလဲ။

_______________ နှစ် (သို့မဟုတ်) _______________ လ

ခ-၁၂ မကရယ်ငါး စားတာဘယ်လောက်ကြာပြီလဲ။

_______________ နှစ် (သို့မဟုတ်) _______________ လ

ခ-၁၃ ကကတစ်ငါး စားတာဘယ်လောက်ကြာပြီလဲ။

_______________ နှစ် (သို့မဟုတ်) _______________ လ

အချိန်ယူဖြေကြားပေးသည့်အတွက် အထူးကျေးဇူးတင်ရှိပါသည်။

Appendix IX: Research Participant Information Sheet and Consent Form (English)

AF 03-06

Research Participant Information Sheet and Consent Form

Title of research project: Health Risk Assessment of Burmese Related to Consumption of Heavy Metals Contaminated Fish from Local Market in Bangkok, Thailand

Principal researcher's name Myat Myitzu **Position** Master Degree Student

Home address House No. 138/38 Studio Zone Condo (Room No. 1910) Ladprao Road Soi Ladprao 102, Plubpla Wangthonglang, Bangkok 10310

Cell phone: 0814239082 **E-mail:** myitzu.yy@gmail.com

You are being invited to take part in a research project. Before you decide to participate it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and do not hesitate to ask if anything is unclear or if you would like more information.

Contents:

1. This research is about calculating health risk for human who eat fish contaminated with heavy metals. Therefore, individual interviewing for eating fish and some information about interviewee will be included.
2. Both online google survey form and face to face interview will be used during surveying. For the participants who cannot read and write, if they have time for 5 minutes and they are willing to participate for my research question, I will ask the questions and will note their answers on the questionnaire form.
   According to my exclusion criteria, I will exclude for the vulnerable group e.g. psychosis, prisoner, mental retarded, person under eighteen years old, pregnant woman, dementia, disabled, minority, conscription, very sick person, refugee, etc.
3. Details of participant.
   - 399 participants for this research project.
   - Only Burmese people_
     Inclusion Criteria
       - Who has not been moved within six months from Bangkok
       - All genders between 18 to 60 years age range, and
       - Who can speak and read Burmese

     Exclusion Criteria
       - People who has severe diseases (such as heart disease, cancer, and other chronic diseases), and mentally unhealthy.

- People who cannot eat fish and seafoods

- This study needs around 399 participants.

4. Details of screening process of inclusion/exclusion criteria or qualifications.
   - I prepared a screening form that include inclusion/ exclusion criteria for the individual surveying, and every participant is needed to pass that screening form before questioning.
   - I will ask some short questions by using the screening form after making introduction about my research at most 2 minutes. If the participants don't pass the screening, I will express my appreciation for their valuable time and will explain why they need to pass the screening test for the main questionnaire form.
5. Procedure upon participants:
   - I will do both online survey and face to face interview for 399 participants. For the question about fish species, they eat the most, fish pictures will be prepared for their better understanding. *Online surveying will be conducted by using Google forms platform and the link will be sent to the respondents via email and social media applications (such as Line, Facebook messenger, Instagram, etc.). The information consent form will also be sent to collect their e-signature on it to make sure that all the respondents understand about the research before taking part in surveying.*
   - In the questionnaire, there are two parts: part A, interviewee information and part B, food frequency survey. There are four questions in interviewee information part and thirteen questions in food frequency survey part. The estimated time for both parts will be around 5 minutes.
6. There is minimal risk to participate this study such as time loss for interview".
7. This research study will find out the health effects of heavy metals by eating fishes. The result from this finding may lead to public awareness for not only Myanmar people but also Thai people who eat fish.
8. Information related directly to you will be kept **confidential** and the detail of the personal information such as name and address will not be literally described**.** Results of the study will be reported as picture, charts or graphs. Any information which could be able to identify you will not appear in the report.

The Research Ethics Review Committee for Research Involving Human Research Participants Group I

Project Number 650006
Date of approval 02 Mar 2022
Expire date 01 Mar 2023

9. After research project is completed, the questionnaire form that include the personal data will be destroyed and the data I collected from individual surveying will be only for this research purpose.
10. The participation in this study will be completely voluntary and there will have no compensation for participation.
11. Participation to the study is **voluntary** and participant has the **right to deny** and/or **withdraw** from the study at any time, no need to give any reason, and there will be no bad impact upon that participant.
12. If you have any question or would like to obtain more information, the researcher can be reached at all time. If the researcher has new information regarding benefit on risk/harm, participants will be informed as soon as possible.
13. If researcher does not perform upon participants as indicated in the participant information sheet and consent form, participants can report the incident to the Research Ethics Review Committee for Research Involving Human Research Participants, Group I, Chulalongkorn University (RECCU) Jamjuree 1 Bldg., 254 Phyathai Rd., Patumwan district, Bangkok 10330, Thailand, Tel./Fax. 0-2218-3202, 0-2218-3049 E-mail: eccu@chula.ac.th"

I have been explained by researcher and understand all the details provided. And I voluntarily signed my name to enroll in this project and receive a copy of this document.

Sign..	Sign..
(..)	(..)
Principal investigator	Research participant
Date......../............./....................	Date......../............./....................

Sign..

(..)

Witness

Date......../............./....................

The Research Ethics Review Committee for Research Involving Human Research Participants Group I

Project Number 650006

Date of approval 02 Mar 2022

Expire date 01 Mar 2023

Appendix X: Research Participant Information Sheet and Consent Form (English)

Research Participant Information Sheet (Myanmar Version)

သုတေသနခေါင်းစဉ် - ထိုင်းနိုင်ငံ ဘန်ကောက်မြို့ ပုံကျစျေးမှ သတ္တုများပါဝင်နေသည့် ငါးများစားသုံးသည့် မြန်မာလူများ၏ ကျန်းမာရေးထိခိုက်မှုအခြေအနေများအား လေ့လာသော သုတေသန။
သုတေသနခေါင်းဆောင်အမည် - မြတ်မဥ္စူ
ရာထူး - မဟာသိပ္ပံကျောင်းသူ၊ ဘေးအန္တရာယ်ရှိပစ္စည်းနှင့် ပတ်ဝန်းကျင်စီမံခန့်ခွဲမှုပရိုဂရမ်၊ ချူလာလောင်ကွန်းတက္ကသိုလ်။
လက်ရှိနေရပ်လိပ်စာ- အိမ်အမှတ် ၁၃၈/၃၈၊ စတူဒီယိုဇုံကွန်ဒို၊ အခန်းနံပါတ် ၁၉၁၀၊ လက်ပရိုလမ်း- ၁၀၂၊ ပတ်ပလာ ဝမ်သုန်လန်, ဘန်ကောက် ၁၀၃၁၀။
လက်ကိုင်ဖုန်းနံပါတ် - ဝ၈၁၄၂၃၉ဝ၈၂၊ **အီးမေးလ်** - myitzu.yy@gmail.com

ဤသုတေသနတွင် ပါဝင်ရန် သင့်အား ကမ်းလှမ်းဖိတ်ခေါ်ပါတယ်။ ပါဝင်ဖို့ မဆုံးဖြတ်ခင် ဘာအတွက်ကြောင့် ဤသုတေသနကိုလုပ်ရကြောင်း၊ ဘယ်လိုအစီအစဉ်တွေ ပါဝင်သွားမှာဖြစ်ကြောင်း စတာတွေကို သင်သိရှိနားလည်ထားရန် အရေးကြီးပါတယ်။ အောက်ပါ သတင်းအချက်အလက် အကြောင်းအရာများကို အချိန်ပေးပြီး ဂရုတစိုက်ဖတ်စေချင်ပါတယ်။ အကယ်၍ မရှင်းလင်းတာ၊ နားမလည်တာရှိလျှင်၊ တခြားအချက်အလက်တွေပိုသိချင်လျှင် မေးမြန်းဖို့ အားမနာပါနှင့်။

၁။ ဤသုတေသနသည် သတ္တုများပါဝင်နေသော ငါးများစားသုံးသည့် မြန်မာလူများ၏ ကျန်းမာရေး ထိခိုက်မှု အခြေအနေများအား လေ့လာသော သုတေသနဖြစ်ပါသည်။ ထို့ကြောင့် ငါးစားသုံးမှုအကြောင်း မေးမြန်းခြင်းနှင့် ဖြေဆိုသူ၏ တချို့အချက်အလက် မေးမြန်းခြင်းများ ပါဝင်ပါသည်။

၂။ အွန်လိုင်းနှင့် မျက်နှာချင်းဆိုင် မေးမြန်းခြင်း နည်းလမ်း နှစ်မျိုးလုံးသုံး၍ ဆောင်ရွက်သွားပါမည်။ မြန်မာစာမရေးတတ် မဖတ်တတ်သော ဖြေဆိုသူများရှိပါက အချိန် ၅ မိနစ်ခန့်ပေးနိုင်ပြီး ဤသုတေသနတွင် ပါဝင်လိုစိတ်ရှိပါက မေးခွန်းများကိုမေးပေးပြီး ၄င်းတို့၏အဖြေများကို မေးခွန်းလွှာပေါ်တွင် မှတ်သားပေးသွားပါမည်။

ပါဝင်ရန်အကျုံးမဝင်သည့်အချက်များအရ စိတ်အခြေအနေပုံမှန်မဟုတ်သူများ၊ ထောင်သားများ၊ ၁၈ နှစ်အောက်သူများ၊ ကိုယ်ဝန်သည်များ၊ မှတ်ဉာဏ်ပျောက်နေသူများ၊ လူနည်းစုလူများများ၊ စစ်မှုထမ်းများ၊ နာမကျန်းသူများ၊ ဒုက္ခသည်များ ပါဝင်မည်မဟုတ်ပါ။

၃။ ဖြေဆိုသူများ၏အသေးစိတ်အချက်များ
ဤသုတေသနအတွက် ၃၉၉ ယောက်ကိုမေးမြန်းသွားပါမည်။
ဤသုတေသနအတွက် မြန်မာလူများများကိုသာ မေးမြန်းသွားမည်ဖြစ်ပါသည်။
<u>ပါဝင်ရန်အကျုံးဝင်သည့်အချက်များ</u>

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- ထိုင်းနိုင်ငံ၊ ဘန်ကောက်မြို့တွင် အနည်းဆုံး ၆ လ နေထိုင်ဖူးသူဖြစ်ရပါမည်။
- အသက် ၁၈ နှစ်နှင့် ၆၀ နှစ်ကြား ကျား/မ မရွေး မေးမြန်းသွားမည်ဖြစ်ပါသည်။
- မြန်မာစာ ရေးတတ်၊ ဖတ်တတ်သော သူများပါဝင်ပါမည်။

<u>ပါဝင်ရန်အကျုံးမဝင်သည့်အချက်များ</u>

- ကျန်းမာရေးမကောင်းသူ (နှလုံးရောဂါ၊ ကင်ဆာနှင့် အခြားပြင်းထန်သော ရောဂါခံစားနေရသူ) (သို့မဟုတ်) စိတ်ပိုင်းဆိုင်ရာအခြေအနေပုံမှန်မဟုတ်သောသူများ
- ငါးမစားသူများ

ဤသုတေသနတွင် ခန့်မှန်းခြေ ၃၉၉ ဦးကိုမေးမြန်းသွားပါမည်။

၄။ တစ်ဦးချင်းမေးမြန်းမှုများအတွက် ဖြေဆိုရန် အကျုံးဝင်သည့်အချက်များ/ အကျုံးမဝင်သည့် အချက်များ ပါဝင်သော စိစစ်မည့်ပုံစံလွှာကို ပြင်ဆင်ထားပြီး မေးခွန်းများမမေးခင်တွင် ၄င်းစိစစ်ပုံစံလွှာပါ အချက်အလက်များနှင့် ကိုက်ညီရန်လိုအပ်ပါသည်။

ဤသုတေသနအကြောင်း ရှေးဦးစွာမိတ်ဆက်ပြီးပါက စိစစ်ပုံစံလွှာကိုအသုံးပြု၍ မေးခွန်းတိုများကို မေးမြန်းသွားမည်ဖြစ်ပါသည်။ အကယ်၍ ၄င်းစိစစ်ပုံစံလွှာအချက်အလက်များနှင့် မကိုက်ညီပါက ထိုသူများကို အချိန်ပေးဖြေကြားမှုအတွက် ကျေးဇူးတင်ကြောင်းပြောပြီး မေးခွန်းဆက်လက်မေးမြန်းမည်မဟုတ်ပါ။ မေးခွန်းဖြေကြားရန် ဘာကြောင့် စိစစ်ပုံစံလွှာအချက်များနှင့်ကိုက်ညီရန်လိုအပ်ကြောင်းကိုလည်း ရှင်းပြသွားမည် ဖြစ်ပါသည်။

၅။ သုတေသနတွင် ပါဝင်ဖြေဆိုသူများနှင့်ပတ်သက်၍ ဆောင်ရွက်သွားမည့်လုပ်ငန်းများ

ဤသုတေသနတွင် လူဦးရေ *၃၉၉* အတွက် မျက်နှာချင်းဆိုင်မေးမြန်းမှုများကိုဆောင်ရွက်သွားပါမည်။ အများဆုံး စားသုံးသည့် ငါးမျိုးစိတ်အကြောင်းမေးမြန်းသည့်မေးခွန်းနှင့်ပတ်သက်၍ နားလည်လွယ်စေရန် ငါးပုံများကို ပြင်ဆင်သွားမည်ဖြစ်ပါသည်။ *အွန်လိုင်းမှတဆင့် မေးမြန်းခြင်းကို google form အသုံးပြု၍ မေးမြန်းသွားမည်ဖြစ်ပြီး link ကို အီးမေးလ်နှင့် လူမှုမီဒီယာများ (Line, facebook messenger, Instagram, စသည်ဖြင့်) မှတဆင့် ပေးပို့သွားပါမည်။ အွန်လိုင်းမှ ဖြေဆိုသူများကိုလည်း ဤသုတေသနအကြောင်းသေချာစွာ နားလည်ရန်နှင့် ပါဝင်ရသည့်အကြောင်းအရင်းကို သေချာစွာ နားလည်စေရန် information consent ဖောင်ကို ပေးပို့ကာ e-signature များရယူသွားပါမည်။*

မေးခွန်းလွှာတွင် အပိုင်း (က) နှင့် အပိုင်း (ခ) နှစ်ပိုင်းရှိပါသည်။ အပိုင်း (က) သည် ဖြေဆိုသူများ၏ အချက်အလက်များဖြစ်ပြီး မေးခွန်း (၄) ခုပါဝင်ပါသည်။ အပိုင်း (ခ) သည် အစားအသောက်စားသုံးမှုအကြိမ်ရေ စစ်တမ်းဖြစ်ပြီး မေးခွန်း (၁၃) ခုပါဝင်ပါသည်။ အပိုင်း (က) နှင့် (ခ) နှစ်ပိုင်းလုံးအတွက် မေးမြန်းရန် ကြာမြင့်ချိန်မှာ (၅) မိနစ်ခန့်ဖြစ်ပါသည်။

၆။ ဤသုတေသနတွင် ပါဝင်ဖြေဆိုခြင်းသည် မည်သည့်ဆိုးကျိုး တစုံတရာမျှ မရှိစေရုံသာမက အချိန်များစွာလည်း ပေးစရာမလိုအပ်ပါ။

၇။ ဤသုတေသနသည် သတ္တုများပါဝင်သော ငါးစားသုံးခြင်းဖြင့် ကျန်းမာရေးထိခိုက်မှုများကို ဖော်ထုတ်နိုင်မည်ဖြစ်ပါသည်။ ဤသုတေသနရလဒ်များအရ ငါးစားသုံးသော မြန်မာလူမျိုးများသာမက ထိုင်းလူများများအတွက်ပါ လူထုအသိပညာပေးလုပ်ငန်းများအထိ ဆောင်ရွက်သွားနိုင်ပါသည်။

၈။ သင်နှင့်ပတ်သက်သောအချက်အလက်များကို လျှို့ဝှက်ထားမည်ဖြစ်ပြီး အမည်၊ လိပ်စာကဲ့သို့သော ကိုယ်ရေးကိုယ်တာနှင့် သက်ဆိုင်သော အချက်အလက်များကို လုံးဝ ဖော်ပြသွားမည်မဟုတ်ပါ။ ဤလေ့လာမှုမှ ရရှိလာသော ရလာဒ်များကို ပုံ၊ ဇယား (သို့) ဂရပ်များဖြင့် ဖော်ပြသွားပါမည်။ ဖြေဆိုသူ မည်သူမည်ဝါ သိသာစေသော အချက်အလက်များကို ဖော်ပြသွားမည်မဟုတ်ပါ။

၉။ သုတေသနလုပ်ငန်းပြီးဆုံးသွားပါက ဖြေဆိုသူများ၏ ကိုယ်ရေးအချက်အလက်များပါဝင်သော မေးခွန်းအဖြေလွှာများကို ဖျက်ဆီးသွားမည်ဖြစ်ပြီး ကောက်ယူထားသော အချက်အလက်များသည်လည်း ဤသုတေသနအတွက်သီးသန့် အသုံးပြုရန်အတွက်သာ ဖြစ်ပါသည်။

၁၀။ ဤသုတေသနတွင်ပါဝင်သော ဖြေဆိုသူတစ်ယောက်ချင်းစီသည် မိမိတို့၏ သဘောဆန္ဒအရပါဝင်သည် ဖြစ်ပြီး ပါဝင်ဖြေဆိုမှုအတွက် တစုံတရာ ရရှိမည်မဟုတ်ပါ။

၁၁။ ဤသုတေသနတွင်ပါဝင်သော ဖြေဆိုသူတစ်ယောက်ချင်းစီသည် မိမိတို့သဘောဆန္ဒအရပါဝင်နိုင်ပြီး အကြောင်းပြချက် ပေးစရာမလိုအပ်ပဲ ဖြေဆိုခြင်းမှ အချိန်မရွေးနှုတ်ထွက်ခွင့် ရှိပါသည်။ ထိုသို့ ဆောင်ရွက်ခြင်းအတွက် ထိခိုက်မှုတစုံတရာ မရှိပါ။

၁၂။ မေးမြန်းလိုသည်၊ သိရှိလိုသည်များရှိပါက သုတေသနခေါင်းဆောင်အား အချိန်မရွေး ဆက်သွယ်နိုင်ပါသည်။ အကယ်၍ သုတေသနနှင့်ပတ်သက်ပြီး ကောင်းကျိုး/ ဆိုးကျိုးများ ရှိလာပါက ပါဝင်ဖြေဆိုသူများကို အချိန်နှင့်တပြေးညီ အသိပေးသွားမည်ဖြစ်ပါသည်။
သုတေသနခေါင်းဆောင်

၁၃။ အကယ်၍ သုတေသနခေါင်းဆောင်သည် ဤသတင်းအချက်စာလွှာပါ အချက်များအတိုင်း ဆောင်ရွက်မှုမရှိပါက ချူလာလောင်ကွန်းတက္ကသိုလ်၊ ကျန်းမာရေးသိပ္ပံအဖွဲ့၏ လူပုဂ္ဂိုလ်များအပေါ် သုတေသနပြုခြင်း ကျင့်ဝတ်ဆိုင်ရာ ဘုတ်အဖွဲ့ထံ ချက်ချင်းသတင်းပို့ တင်ပြနိုင်ပါသည်။ ဘုတ်အဖွဲ့၏ ဆက်သွယ်ရန် လိပ်စာမှာ ချမ်ချူရီ အဆောက်အဦး (၁)၊ ဒုတိယထပ်၊ အမှတ် (၂၅၄)၊ ပရာထိုင်းလမ်းမ၊ ပတ်သူဝမ်၊ ဘတ်ကောက် ၁၀၃၃၀၊ ထိုင်းနိုင်ငံ ဖြစ်ပါသည်။ တယ်လီဖုန်း၊ ဖက်စ်နံပတ် ၀၂၂၁၈၃၀၂၊ အီးမေးလ် eccu@chula.ac.th သို့ ဆက်သွယ်သတင်းပို့တင်ပြနိုင်ပါသည်။

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အထက်ဖော်ပြပါအကြောင်းအရာအသေးစိတ်ကို နားလည်သိရှိပြီးဖြစ်ပါသည်။ ကျွန်ုပ်သည် ဤလေ့လာမှုတွင် မိမိသဘောအလျောက်ပါဝင်ခြင်းဖြစ်ပါသည်။

လက်မှတ်...
(...)
သုတေသနဆောင်ရွက်သူ
နေ့ရက် ..../............./....................

လက်မှတ်...
(...)
သုတေသနတွင်ပါဝင်ဖြေဆိုသူ
နေ့ရက် ..../............./....................

လက်မှတ် ....
(...)
မျက်မြင်သက်သေ
နေ့ရက် ..../............./....................

Appendix XI: Concentration of heavy metals in fish species from three fish shops

Fish Species	**Heavy Metal**	**Concentration (mg/kg)**	**LOD**
SHOP-1			
nile tilapia			
	Arsenic (As)	0.099	0.01
	Cadmium (Cd)	0.008	0.003
	Lead (Pb)	0.02	0.006
	Mercury (Hg)	Not Detected (<0.004)	0.004
	Chromium (Cr)	0.02	0.005
Catfish			
	Arsenic (As)	Not Detected (<0.01)	0.01
	Cadmium (Cd)	0.008	0.003
	Lead (Pb)	0.02	0.006
	Mercury (Hg)	Not Detected (<0.004)	0.004
	Chromium (Cr)	0.022	0.005
Mackerel			
	Arsenic (As)	0.507	0.01
	Cadmium (Cd)	0.028	0.003
	Lead (Pb)	0.02	0.006
	Mercury (Hg)	0.028	0.004
	Chromium (Cr)	0.02	0.005
Seabass			
	Arsenic (As)	0.196	0.01
	Cadmium (Cd)	0.008	0.003
	Lead (Pb)	Not Detected (<0.006)	0.006
	Mercury (Hg)	0.02	0.004
	Chromium (Cr)	0.024	0.005
SHOP-2			
nile tilapia			
	Arsenic (As)	0.093	0.01
	Cadmium (Cd)	0.008	0.003
	Lead (Pb)	Not Detected (<0.006)	0.006
	Mercury (Hg)	Not Detected (<0.004)	0.004
	Chromium (Cr)	0.033	0.005
Catfish			
	Arsenic (As)	Not Detected (<0.01)	0.01
	Cadmium (Cd)	0.008	0.003
	Lead (Pb)	Not Detected (<0.006)	0.006
	Mercury (Hg)	Not Detected (<0.004)	0.004
	Chromium (Cr)	0.042	0.005

Fish Species	Heavy Metal	Concentration (mg/kg)	LOD
Mackerel			
	Arsenic (As)	0.432	0.01
	Cadmium (Cd)	0.039	0.003
	Lead (Pb)	Not Detected (<0.006)	0.006
	Mercury (Hg)	0.033	0.004
	Chromium (Cr)	0.02	0.005
Seabass			
	Arsenic (As)	0.182	0.01
	Cadmium (Cd)	0.008	0.003
	Lead (Pb)	Not Detected (<0.006)	0.006
	Mercury (Hg)	0.02	0.004
	Chromium (Cr)	0.023	0.005
SHOP-3			
nile tilapia			
	Arsenic (As)	0.084	0.01
	Cadmium (Cd)	0.008	0.003
	Lead (Pb)	0.02	0.006
	Mercury (Hg)	Not Detected (<0.004)	0.004
	Chromium (Cr)	0.032	0.005
Catfish			
	Arsenic (As)	0.016	0.01
	Cadmium (Cd)	0.008	0.003
	Lead (Pb)	Not Detected (<0.006)	0.006
	Mercury (Hg)	Not Detected (<0.004)	0.004
	Chromium (Cr)	0.024	0.005
Mackerel			
	Arsenic (As)	0.408	0.01
	Cadmium (Cd)	0.022	0.003
	Lead (Pb)	0.02	0.006
	Mercury (Hg)	0.02	0.004
	Chromium (Cr)	0.02	0.005
Seabass			
	Arsenic (As)	0.47	0.01
	Cadmium (Cd)	0.008	0.003
	Lead (Pb)	Not Detected (<0.006)	0.006
	Mercury (Hg)	0.02	0.004
	Chromium (Cr)	0.02	0.005

Appendix XII: Average Daily Intake (ADI) calculation Example

Average daily dose formula:

$$ADI = \frac{Cs \times IR \times FI \times EF \times ED}{BW \times AT}$$

Where,

C_s = the concentration of heavy metal (mg/kg)
EF = the exposure frequency (meals/year)
FI = Fraction ingestion from contaminated source (unitless)
ED = the exposure duration (years)
BW = the body weight (kg)
AT = the average time (days)
IR = the ingestion rate (kg/meal)

The averaging time, non- carcinogen (ED x 365 days/year)

<u>Example calculation for the participant 1:</u>

C_s = 0.092 mg/kg (As concentration from heavy metal analysis)
EF = 52.14 day/year
FI = 1
ED = 1
BW = 51
AT = 365 days
IR = 0.2 kg/day

By inserting the numbers in the above formula,

$$ADI_{As1} = \frac{0.092 \times 0.2 \times 1 \times 52.14 \times 1}{51 \times 365}$$

Then, the result of average daily intake (ADI) for the As through the consumption of nile tilapia was 5.15 x 10^{-5} mg/kg/day .

Appendix XIII: Quality control/ Quality Assurance of Heavy Metals Analysis

Sample	Weight (g)	Chromium (Cr)		Arsenic (As)		Cadmium (Cd)		Mercury (Hg)		Lead (Pb)	
		Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)
001A	1.0057	0.8010	0.0199	3.9990	0.0994	0.2732	0.0068	0.0000	0.0000	0.3758	0.0093
001B	1.0024	0.8038	0.0200	3.9530	0.0986	0.2773	0.0069	0.0000	0.0000	0.3659	0.0091
	Average	0.8024	0.0200	3.9760	0.0990	0.2753	0.0070	0.0000	0.0000	0.3709	0.0090
			0.7		0.8		1.8		0		2.3

Fortified Sample	Weight (g)	Chromium (Cr)		Arsenic (As)		Cadmium (Cd)		Mercury (Hg)		Lead (Pb)	
		Conc. (µg/l)	%Recovery	Conc. (µg/l)	%Recovery	Conc. (µg/l)	%Recovery	Conc. (µg/l)	%Recovery	Conc. (µg/l)	%Recovery
%Recovery A	1.0014	9.6810	89.6200	13.7100	98.1000	8.8510	86.1400	9.0680	90.6800	8.9540	86.3400
%Recovery B	1.0080	9.4670	87.6400	13.6900	97.9500	8.8800	86.4200	9.1010	91.0100	8.9960	86.7400
	Average	9.5740	88.6300	13.7000	98.0250	8.8655	86.2800	9.0845	90.8450	8.9750	86.5400
	%RPD	2.2		0.1		0.3		0.4		0.5	

Sample	Weight (g)	Chromium (Cr)		Arsenic (As)		Cadmium (Cd)		Mercury (Hg)		Lead (Pb)	
		Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)
002	1.0014	0.8680	0.0220	0.2737	0.0070	0.2696	0.0070	0.0000	0.0000	0.3248	0.0080
003	1.0056	0.6283	0.0160	20.4100	0.5070	1.1280	0.0280	1.1260	0.0280	0.6787	0.0170
004	1.0010	0.9678	0.0240	7.8560	0.1960	0.2732	0.0070	0.2000	0.0050	0.1419	0.0040
005	1.0077	1.3480	0.0330	3.7510	0.0930	0.2782	0.0070	0.0000	0.0000	0.1634	0.0040
006	1.0061	1.6730	0.0420	0.3500	0.0090	0.2623	0.0070	0.0000	0.0000	0.1425	0.0040
007	1.0081	0.2546	0.0060	17.4400	0.4320	1.5790	0.0390	1.3210	0.0330	0.1140	0.0030
008	1.0010	0.9122	0.0230	7.2750	0.1820	0.2696	0.0070	0.2832	0.0070	0.1951	0.0050
009	1.0028	1.2840	0.0320	3.3710	0.0840	0.2679	0.0070	0.0000	0.0000	0.2562	0.0060
010	1.0036	0.9470	0.0240	0.6267	0.0160	0.2856	0.0070	0.0000	0.0000	0.1503	0.0040

Sample	Weight (g)	Chromium (Cr)		Arsenic (As)		Cadmium (Cd)		Mercury (Hg)		Lead (Pb)	
		Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)
011A	1.0017	0.6860	0.0171	16.7000	0.4168	0.9142	0.0228	0.2925	0.0073	0.3054	0.0076
011B	1.0059	0.6231	0.0155	16.0500	0.3989	0.8847	0.0220	0.2755	0.0068	0.2882	0.0072
	Average	0.6546	0.0163	16.3750	0.4079	0.8995	0.0224	0.2840	0.0071	0.2968	0.0074
Sample	Weight (g)	Chromium (Cr)		Arsenic (As)		Cadmium (Cd)		Mercury (Hg)		Lead (Pb)	
		Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)	Conc. (µg/l)	Conc.(mg/kg)
012	1.0011	0.5541	0.0140	18.8300	0.4700	0.2666	0.0070	0.4513	0.0110	0.0992	0.0020

VITA

NAME	Myat Myitzu
DATE OF BIRTH	28 July 1992
PLACE OF BIRTH	Kawlin, Sagaing Division, Myanmar
INSTITUTIONS ATTENDED	University of Forestry (Bachelor Degree)
HOME ADDRESS	No-306, 19th street, Kawlin Township, Sagaing Division, Myanmar