UNIVERSITI MALAYSIA KELANTAN

Heavy Metal Determination and Health Risk Assessment of Selected Canned Sardine Available in Malaysian Market

Nurul 'Ain Binti Abd Rahim

F18A0201

A thesis submitted in the fulfilment of requirements for the degree of Bachelor of Applied Science (Food Security) with Honours

Faculty of Agro Based Industry

University Malaysia Kelantan

2022

THESIS DECLARATION

I declare that this thesis entitled “Heavy Metal Determination and Health Risk Assessment of Selected Canned Sardine Available in Malaysian Market” is the results of my own research except as cited in the references.

Signature :

Student’s name : NURUL ‘AIN BT ABD RAHIM

Matric number : F18A0201

Date :

Verified by :

Signature :

Supervisor’s name : DR KRISHNA VENI VELOO

Stamp :

Date :

ACKNOWLEDGEMENT

Praise be to Allah, the most Gracious and the most Merciful. The almighty, who have blessed me with numerous blessings, knowledge and opportunities for me to finally finished and completed my thesis. I am much grateful to the almighty for His kindness, graces and love throughout the journey of my thesis writing and He who are the most Passionate have providing me this opportunity to be able to keep me stepped with strength and smooth in this manner.

First and foremost, the highest appreciation goes to my supervisor, Dr Krishna Veni Veloo as I am deeply grateful for her invaluable advice, continuous support, and patience along the journey of my final year project. I would like to express my sincere gratitude for her excellent supervision and mentorship that have contribute the most to the idea and further progress of my academic research. Her immense knowledge and bountiful experience have always encouraged me in finishing my thesis as well as in my daily life.

Finally, I would like to offer my special thanks to my close friends in UMK Jeli and the members of my research teammates, Nurin Syuhadah, Nurhafizah and Ng Yi Jie for their assistance, kind help, cooperation and support since day one of our collaboration until the end of the journey. My sincere gratitude is also extended to my parents Mr Abd. Rahim and Mrs Siti Eshah as well as to my sister, Nurul Izzati for their unwavering support and belief in me to further my study to this level. It would have been difficult and tough for me to stand firm and finish my studies without their tremendous understanding, encouragement and reassurance throughout the years.

Heavy Metal Determination and Health Risk Assessment of Selected Canned Sardine from Malaysia Market

ABSTRACT

Canned sardine is one of the most common canned food products due to its conveniency and is affordable among the local people. However, despite of having a good taste and higher nutritive value, canned sardine may also contain chemical contaminants such as heavy metals which resulted from the pollution of water bodies as well as the usage of unsuitable packaging materials. The purpose of this study is to determine the concentration of heavy metal and health risk assessment of canned sardines that are purchased from selected market in Malaysia. In this study, the heavy metal residues from the selected samples are extracted using acid digestion method and the concentration is determined by using Atomic Absorption Spectrometer (AAS). The health risk assessment of local people caused by the consumption of canned sardine is also evaluated by estimated daily intake (EDI), estimated weekly intake (EWI), target hazard quotient (THQ) and Hazard Index (HI). The average mean concentration of the heavy metals in canned sardine is found to be highest for Zn with 1.0464 mg/kg, followed by Pb with 0.1108 mg/kg, Cu with 0.0932 mg/kg and Cd with 0.0156 mg/kg, which is the lowest. Therefore, the ranking of heavy metals accomplished were Zn > Pb > Cu > Cd. In health risk assessment, the highest average value of THQ in canned sardine are in Zn which is 7.65×10^{-4}, followed by THQ of Pb with 6.06×10^{-3}, Cd is 3.42×10^{-4} while, Cu is 3.08×10^{-2}. With the HI value for all five different samples of canned sardine are less than 1 which indicates that no cancer risk present for the canned sardine sample, where HI is 0.03. Thus, all five samples of canned sardine of different brands are safe for human consumption. This study is significant to provide information about the heavy metal residues that may accumulated in canned food products as well as spread awareness and educate consumers on the food safety.

Keywords: Canned food, heavy metals, pollution, health risk, atomic absorption spectrometer

Determinasi Logam Berat dan Penilaian Risiko Kesihatan terhadap Sardin Dalam Tin di Pasaran Malaysia

ABSTRAK

Ikan sardin dalam tin merupakan salah satu produk makanan dalam tin ruji kerana ianya mudah dan mampu dimiliki oleh penduduk tempatan. Walau bagaimanapun, walaupun mempunyai rasa yang enak dan nilai khasiat yang tinggi, sardin dalam tin juga mungkin mengandungi pencemaran kimia seperti logam berat yang terhasil daripada pencemaran air serta penggunaan bahan pembungkus yang tidak sesuai. Tujuan kajian ini adalah untuk menentukan kepekatan logam berat dan penilaian risiko kesihatan terhadap sardin dalam tin yang dibeli dari pasaran terpilih di Malaysia. Dalam kajian ini, sisa logam berat daripada sampel terpilih diekstrak menggunakan kaedah penghadaman asid dan kepekatannya ditentukan dengan menggunakan Spektrometer Penyerapan Atom. Penilaian risiko kesihatan penduduk tempatan yang disebabkan oleh pengambilan ikan sardin dalam tin juga dinilai oleh Anggaran Pengambilan Harian, Anggaran Pengambilan Mingguan, Sasaran Target Bahaya dan Indeks Bahaya. Purata kepekatan logam berat sardin dalam tin didapati paling tinggi bagi Zn dengan 1.0464 mg/kg, diikuti oleh Pb dengan 0.1108 mg/kg, Cu dengan 0.0932 mg/kg dan Cd dengan 0.0156 mg/kg, iaitu paling rendah. Oleh itu, kedudukan susunan logam berat yang dicapai ialah Zn > Pb > Cu > Cd. Dalam penilaian risiko kesihatan, nilai purata tertinggi Sasaran Target Bahaya dalam sardin di dalam tin adalah bagi Zn iaitu 7.65×10^{-4}, diikuti oleh Sasaran Target Bahaya Pb dengan 6.06×10^{-3}, Cd ialah 3.42×10^{-4}, manakala Cu ialah 3.08×10^{-2}. Nilai Indeks Bahaya bagi kesemua lima sampel berbeza sardin dalam tin adalah kurang daripada 1 iaitu 0.03, yang menunjukkan bahawa tiada kehadiran risiko kanser untuk sampel sardin dalam tin. Oleh itu, kelima-lima sampel sardin dalam tin dengan jenama berbeza adalah selamat untuk dimakan oleh manusia. Kajian ini adalah penting untuk memberi maklumat tentang sisa logam berat yang mungkin terkumpul dalam produk makanan dalam tin serta untuk menyebarkan kesedaran dan mendidik pengguna tentang keselamatan makanan.

Kata kunci: Produk dalam tin, logam berat, pencemaran, risiko kesihatan, spektrometer penyerapan atom

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS

> More than

< Less than

°C Degree Celsius

+ Addition

- Subtraction

× Multiplication

÷ Division

± Standard error

LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization
WHO	World Health Organization
MFA	Malaysian Food Act
AAS	Atomic Absorption Spectrometer
ROS	Reactive Oxygen Species
Pb	Lead
Cu	Cooper
Zn	Zinc
Cd	Cadmium
mg	Milligram
mg/kg	Miligram per kilogram
MRL	Maximum Residue Limits
mm	Millimetre
L	Litre
mL	Millilitre
mg/L	Milligram per Litre
HNO_3	Concentrated nitric acid
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
$Pb(NO_3)_2$	Lead (II) nitrate

$Cd(NO_3)_2$ Cadmium nitrate

$Zn(NO_3)_2$ Zinc nitrate

$Cu(NO_3)_2)$ Copper nitrate

mg/kg/day Miligram per kilogram per day

mg/kg/week Miligram per kilogram per week

USEPA United States Environmental Protection Agency

JECFA The Joint FAO/WHO Expert Committee on Food Additives

CCFAC Codex Committee on Food Additives and Contaminants

CDC Centres for Disease Control

TDI Tolerable Daily Intake

EDI Estimated Daily Intake

EWI Estimated Weekly Intake

PTWI Provisional Tolerable Weekly Intake

THQ Target Hazard Quotient

HI Hazard Index

CHAPTER 1

INTRODUCTION

1.1 Research Background

Fish is one of the aquatic animals that have been consumed by many people. It is also one of the most significant sources of protein and other essential nutrients for the body. Humans consumed a lot of fish because it contains high protein, trace elements, low saturated fat, essential vitamins and omega-3 fatty acids which are known to aids in good health (Jiali Gu, 2014). Besides, fish especially sardines are well-known for being processed and packed in can as canned sardine is considered easy to prepare and convenient at all costs. Other than having a good taste, canned sardine is also affordable and most common canned food products available in local market.

Canned food product is any of the edible material that has undergo preservation process in a long period of time and then are packed in the airtight container (Shafawati Ismail, 2018). The advantage of having the food being canned, especially sardine will be able to extend its shelf life, does not need to be kept in the freezer or at a low temperature, easy to be transported and distributed as well as reducing food wastage.

However, despite all of the awesomeness of canned product, the contamination of heavy metal residue in the canned products is the issue that people have been talking about for ages. In the last few decades, the attention has been drawn to the study on determining heavy metal levels in processed food especially freshwater environment and marine food product. Canned food products, despite of the nutritive values and having a good taste, they can also contain chemical contaminants which is the main source in the environment and from the technological processing or incorrect packaging (Gra˙zyna Kowalska, 2020).

The source of heavy metal residue in the fish itself may be leads by the polluted water from industrial area, excessive usage of chemicals in agricultural activities which then flowed into the water by surface run-off and as well as domestic waste produced by human. Fish consumption and accumulation of these toxic metals from the polluted aquatic environments are subsequently passed down the food chain to human as a concentrated toxicant carrier (Jiali Gu, 2014). In addition, when the fish like sardine undergo canning process, it once again contaminated by heavy metal elements during processing, packaging, storage, and transportation. The heavy metals such as tin which come from the packaging of the canned food are prone to migration into food depending on the pH of food, temperature of the canned foods, storage time, poor lacquering, corrosion of the can as well as the exposure to air prior to opening of the can (Thakali & Jean, 2021).

Moreover, heavy metals can be classified into two classes which are essential elements and toxic elements. The toxic elements are lead (Pb) and cadmium (Cd), while the essential elements are zinc (Zn) and copper (Cu). Both toxic elements, Cd and Pb can affect the human health negatively even at trace amounts as they are acknowledged to have no functions in biological systems. On top of that, Cu and Zn which are among the essential elements can also lead to toxicity and bring harm to the body if they are being consumed in excess amount. When ingested over a long period of time, toxic elements can be extremely dangerous even at low concentrations, and essential metals can also be toxic if consumed in excess (Morshdy et al., 2013).

Therefore, the health risk caused by the accumulation of heavy metal in canned sardine need to be seriously assessed. It is because, heavy metals are regard as a threat to human health out of the numerous contaminants. The initiation of reactive oxygen species (ROS), which results in oxidative damage and health-related deleterious effects, is thought to be the general mechanism implicated in heavy metal-induced toxicity (Rehman et al., 2017). Therefore, resulting in acute and chronic poisoning symptoms that can contribute to morbidity as well as mortality. Neurological disorders, kidney and skeletal damages, endocrine disruption, carcinogenic effects and cardiovascular disfunction are some of the health risks associated with metal toxicity (P. K. Maurya et al., 2019).

1.2 Problem Statement

The demand of canned sardines in Malaysia market have been increasing due to the changing in eating trend of consumers nowadays which emphasized on the conveniency and affordability of food. This change is even prominent among urban people who lived a stressful lifestyle and heavily depending on external supplies for their living (Shathees Baskaran, 2017). People who are busy working especially those in the professional field tend to choose more convenient food as it could save time, require less energy and easier to prepare. Therefore, canned sardine is a perfect choice of food that is much affordable and convenient, making it ideal for the changing of consumers' eating trend.

Nevertheless, pollution of water bodies as well as processing and packaging of the product are much contribute to the level of heavy metal residues found in canned sardine. It is because, amounts of pollutants from numerous human activities including industrial, agriculture, mining and urbanization are then freely drained into water bodies without any appropriate treatment. Organic materials, nutrients, salinization, oil and grease, suspended sediment, and enteric virus are all directly influencing the water quality in river basins due to the contents of spatial disposal or untreated disposal products (Yamada, 2017). Most of the heavy metals end up settling down in the soil and sediments of water bodies which then greatly affecting the aquatic animals lived there.

Moreover, ingestion of canned food products such as canned sardines are risky to human health as they are exposed to the heavy metal contamination. The essential elements such as Cu and Zn can triggered disease when they are being highly ingested for quite a long time. Cu may have an effect on cardiovascular, gastrointestinal, hepatic, hematological, renal and CNS activity, while Zn can cause nausea, vomiting, chest tightness, coldness, excitement, unconsciousness, coma, and even death due to pulmonary edema and liver harm (P. K. Maurya et al., 2019). In regard of this issue, further study on determination of heavy metal in canned sardine is likely to be focused on as people have been paying more attention to the safety of their food and become aware on the heavy metal contamination in canned food product such as canned sardine.

1.3 Significance of Study

Canned sardine is no stranger to the consumers as it can be easily found on almost every shelf in the markets. Being one of the most familiar among canned food products, canned sardine is no exception to the threat of being contaminated by heavy metal. The presence of metal in canned sardine can be caused largely by water pollution in water bodies and practically during the packaging and processing in the industry. For instance, metallic cans are often used as the packaging which may be the reason of the metallic ions such as tin and iron to migrate into the food (Jeevanaraj et al., 2020).

Through this research, the level of heavy metal concentration in selected canned sardine can be determined by the extraction of the sample and will be further tested using Atomic Absorption spectrometer (AAS). Other than that, the determination of heavy metal residue in canned sardine is worth to be assessed on as it able to increase the consumer awareness towards the risk of hazardous effect on their health due to consumption of canned food product. Along with the rising of consumer awareness towards food safety, the awareness on the health risk caused by consumption of canned food products has to be increased as well.

In addition, other than consumers, this research is also significant and beneficial to the fellow researchers in this field, health workers, authorities as well as policy making in order to provide information and serve as guidance in determination of heavy metal residue in canned sardine together with the respective assessment on the health risk.

1.4 Objective of Study

The objective of this study is to

1. Extract the heavy metal residues from selected canned sardine purchased from local market.
2. Determine the concentration of heavy metal residues in canned sardine using Atomic Absorption Spectrometer (AAS).
3. Evaluate the health risk assessment by the consumption of canned sardine in Malaysia.

1.5 Scope of Study

This research is mainly focused on the extraction of heavy metals such as cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) which present within the selected canned sardine in the local market in Malaysia. The canned sardine comprised of several brands that can be easily purchased in the market were extracted using acid digestion method prior to the analysation of heavy metal residue by Atomic Absorption Spectrometer (AAS).

Besides, the level of heavy metal residues analyzed from the respective canned sardine were compared to the safe level of heavy metal as permitted by World Health Organization (WHO), Food and Agriculture Organization (FAO) and Malaysian Food Act 1983 & Regulation 1985. Furthermore, the assessment of the health risk due to the consumption of canned sardine was determined among Malaysian people.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metal

Heavy metals are naturally occurring elements that have a high atomic weight and have a density five times that of water. They present by natural and anthropogenic pathways, not only in water, but also in soil and air. Naturally, heavy metal sources include metallic minerals as well as parent rocks. While the anthropogenic pathways are through fertilizers and pesticides from agriculture, metallurgy of mining, sewage disposal and energy production in power plant (Nkwunonwo et al., 2020).

In natural waters, heavy metals are mostly toxic even at a very low concentration. For examples, heavy metals such as arsenic (As), nickel (Ni), lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), chromium (Cr) and mercury (Hg) are having a high level of toxicity even their existence are in a trace amount. Heavy metals may arrive in the human body over the most common route which is over the consumption of contaminated food and water. In the body, they are not being metabolised by the body and consequently accumulated in the soft tissues which then lead to toxicity.

Among all the pollutants, heavy metal is proved to be one of the most serious problems due to its toxic nature and the possibility on the accumulation by marine organisms especially fish. The amounts of heavy metals such as As, Hg, Cd, Cr, Cu, Ni, Pb, and Zn in water resources are critically increased and affecting marine organisms ever since many industries are discharging their metal containing effluents into water without being treated beforehand. Marine life is also susceptible to the contamination of heavy metals as they are widely contaminated the ecosystem due to agricultural runoff, industrial, and mining activities (Shafawati Ismail, 2018).

Other than that, Contamination of toxic heavy metals in aquatic ecosystems has increase the concern on the public health. Natural events especially pedological processes such as weathering of rocks are also responsible for the existence of metals in water by surface runoff (P. K. Maurya et al., 2019). The accumulation of heavy metals in the environment will consequently contaminated both food chains and food web of the livings. The trophic transfer of the heavy metal elements into the aquatic food chains as well as food webs poses a potential health threat to the consumers especially humans (Hazrat Ali, 2019). Fish and other marine organisms can accumulate huge amounts of metals in their tissues especially muscles which in turn represented a major source of heavy metals in humans' diet.

2.1.1 Classification of Heavy Metals

Heavy metals are being categorized into two categories which is toxic elements and essential elements. Toxic elements such as lead (Pb), cadmium (Cd) and Nickel (Ni) are the natural trace component in the aquatic environment. However, the accumulation of these toxic elements is increasing due to agricultural activities, industrial as well as mining. The toxic elements that accumulated in the chain of food both at the stage of primary producers and at consumer level are because of the toxic elements are bioaccumulative and do not breakdown in the environment (Ackova, 2018).

The toxic elements do not take part in the biological systems and are not easily metabolized, thus causing harm to the human health even in trace quantities (Ackova, 2018). The other category of heavy metal is the essential elements. For example, Zinc (Zn) and copper (Cu) are vital metals because they play critical roles in biological systems (Shkhaier, 2016). Cu is recognized as a fundamental constituent of metalloenzymes and is needed for catalysis of metabolic responses as well as haemoglobin synthesis.

2.2 Heavy Metals in Canned Sardine

According to Gra˙zyna Kowalska (2020), canned food products are the products being enclosed in metal cans, ensured a long shelf life by the process of pasteurization and airtightness in the packaging, hence provide protection against the access of contaminants and air. While, fish is the main important source of protein in human diet where the global per capita consumption of fish has increased above 20 kg per year (P. K. Maurya et al., 2019). Canned sardines are fishes which being enclosed in a can and are well consumed by Malaysian people especially those who come from working families. Canned sardine is considered quite helpful throughout a long day into their life as it tastes good, convenience, and required less time for cooking and preparing.

The canning process are diverse with the shape and size of the can and the product being canned. Generally, there are principle steps in common practice and the most vital in canning process is the selection and preparation of raw materials which agreed with the quality control standards. Especially in seafood canned products, sardines obtained have high chances of contamination to the toxic metals due to natural runoff, contributory rivers as well as industrial and domestic wastewater. Fish largely accumulate contaminants in their muscle from a polluted aquatic environment (Morshdy et al., 2013).

The presence of heavy metals includes zinc (Zn), Copper (Cu), Lead (Pb) and Cadmium (Cd) in canned sardine has overshadowed its nutritional advantage of having contain significant source of micronutrient that are vital for human diet and polyunsaturated fatty acids. It is because, the effects of ingesting heavy metals are much more severe than the benefits it serves. Fishes in higher trophic level such as sardine, tuna, shark, and swordfish are normally taken up extensive amounts of heavy metals through bioaccumulation along the food web, while fishes and other aquatic species of lower trophic may accumulate heavy metals from the sediment and their source of water and food (Jeevanaraj et al., 2020). In addition, further contamination of heavy metals in canned sardine is happen during the packaging process. The metallic cans that are often used as the packaging of canned sardine may cause the metallic ions such as tin and iron to migrate into the muscle of fish and thus threatened the consumers' health.

In table 2.1, previous study on contamination of the heavy metal among processed seafood conducted by Pravina Jeevanaraj, Aliah Ahmad Foat and Halimah Tholib (2020) in Malaysia stated that all of the heavy metals in canned fishes were observed at the smallest and lowest concentration as compared to the salted/dried seafood, except for cadmium (0.04 vs 0.02 mg/kg, p = 0.105). For Cd and Pb in canned fish, salted Fourfinger threadfin as well as Acetes are significantly lower than dried anchovies and Toli shad. While, Hg was testified to be higher in Fourfinger threadfin than in canned fish, anchovies and Acetes. Other than that, former research by Lima et. al, (2021) pertaining heavy metals in canned sardine samples which being sold in Brazil shows that Cr, Cu, Zn, Pb and As are all present in canned sardine with tomato sauce. At the same time, no traces of Cd being found.

Table 2.1: Summary of previous studies on the concentration of heavy metal residues in canned sardine.

Canned sardine products	The concentrations of heavy metals						Location	References
	Cr	Cd	Zn	Cu	Pb	As		
Canned sardine	-	0.08 ± 0.71	42.73 ± 0.37	2.10 ± 0.60	1.35 ± 1.14	0.88 ± 1.34	Malaysia	(Shafawati Ismail, 2018)
Canned sardine	0.10±0.0245	-	-	2.1±0.367	-	-	Iraq	(Shkhaier, 2016)
Canned sardine	-	-	-	-	0.02±0.01	0.71±0.19	Poland	(Gra˙zyna Kowalska, 2020)
Canned sardine	0.62 ± 0.15	0.18 ± 0.09	11.57 ± 4.62	1.89 ± 0.32	0.05 ± 0.03	-	China	(Jiali Gu, 2014)
Canned sardine	-	0.05 ± 0.00	2.37 ± 0.32	0.22± 0.03	-	-	Egypt	(Morshdy, 2013)
Canned sardine	0.110±0.081	-	0.395 ± 0.201	0.739±1.008	0.011±0.013	2.676±1.044	Brazil	(Lima et al., 2021)
Canned sardine	-	0.04± 0.09	-	-	0.13± 0.04	1.68± 0.03	Malaysia	(Jeevanaraj et al., 2020)

Table 2.2: Summary of maximum residue limits (MRL) of heavy metals concentration (mg/kg) in canned sardine as reported by Malaysian Food Act 1983 & Regulations 1985 and WHO/FAO (2011)

Types of heavy metals	WHO/FAO	MFA
Lead (Pb)	1.50	2.00
Chromium (Cr)	0.03	-
Cadmium (Cd)	0.05	1.00
Copper (Cu)	10.00	30.00
Zinc (Zn)	30.00	100.00
Arsenic (As)	-	2.00

The maximum residue limits (MRL) of heavy metals concentration (mg/kg) of Pb, Cr, Cd, Cu, Zn and As in canned sardine are stated according to World Health Organization (WHO), Food and Agriculture Organization (FAO) as well as Malaysian Food Act 1983 & Regulations 1985 as shown in table 2.2. Humans should pay attention to the permissible limit of heavy metal residue in canned sardine products for a better health while eliminate the health risk due to their toxicity to the body.

2.3 Toxicity of Heavy Metals

Toxicity is the degree of which a substance either a poison or toxin that can cause harm to both animals and humans. Heavy metals mostly are poisonous to the body even at a very low concentration. The mechanism of its toxicity is specific and differ according to the types of heavy metals. It is known to be able to produce free radicals which may cause an oxidative stress and leading to other cellular damages of biological molecules as well as DNA damage which will contributes to neurotoxicity and carcinogenesis (Azeh Engwa et al., 2019). The toxicity of some metals could be acute while other metals could become more chronic and having a delirious health effects to the body after a long-term exposure.

2.3.1 Lead (Pb)

Lead (Pb) is a slightly bluish, bright silvery metal in a dry atmosphere and is mainly exposed by the industrial processes and domestic sources such as gasoline, plumbing pipes, house paint, lead bullets and storage batteries. In water, lead is abundantly present due to the industrial processes and vehicle exhausts in the atmosphere which successfully make their way into the soil composite and finally drifted into the water bodies (Azeh Engwa et al., 2019). Human is highly exposed to lead (Pb) through drinking water or food where fishes and other aquatic species are unconsciously taken up the metals by feeding. High level of lead being accumulated in fish is because of atmospheric deposition and superficial soil erosion that have reached the aquatic system (Jeevanaraj et al., 2020).

The heavy metals accumulate more readily in larger fish through surface action and feeding quantity which is because of the large column feeding nature while smaller size of fish shows lowest accumulations of lead due to their smaller body size (P. K. Maurya et al., 2019). Toxicity of lead which is also known as lead poisoning is mostly linked to the central nervous system and gastrointestinal tract of both adults and children (Azeh Engwa et al., 2019). Acute lead poisoning resulted into the expression of several symptoms such as loss of appetite, headache, vertigo, hallucinations, abdominal pain, exhaustion, hypertension, renal disfunction, and arthritis. Besides, long exposure to lead may resulted into more severe effects of allergies, paralysis, muscular weakness, weight loss, kidney damage as well as brain damage that may lead to coma and may even cause death.

Moreover, toxicity of lead can also cause a birth defects to a developing fetus and badly affect children as they are susceptible to neurotoxic effects because of the contact to the lead. Children, especially those aged 5 and under who are experience this kind of poisoning shows loss of intellectual growth as a result of the loss of intelligence quotient points (Azeh Engwa et al., 2019). According to Centres for Disease Control (CDC) primary in United States, the amount of lead permissible in children's blood is reduce from previously 25 μg/dL to 10 μg/dL and the universal screening on the level of Pb in blood are also suggested for all the children.

Even though lead poisoning is controllable and can be prevented, yet it still worries most people as it will lead to dangerous diseases once affected the organs of the body. Being exposed to a high level of lead can modify the function of central nervous system, disrupt the intracellular second messenger systems and causes edema due to the plasma membrane of the blood brain barrier is moving into the interstitial spaces (Azeh Engwa et al., 2019). The permissible limit of Pb in food is 0.5 μg/g as proposed by FAO and WHO, while 2.0 μg/g as set by FEPA.

2.3.2 Copper (Cu)

In industries, Copper (Cu) is used in the production of cables, copper pipes, copper cookware as well as wires. While in the medical field, Cu is also used in the production of birth control pills and copper intrauterine devices. Cu is a necessary component in the production of certain enzymes and haemoglobin in humans. It is also necessary in human metabolism, however high intakes of this metal are toxic to the cardiovascular, gastrointestinal, renal, hepatic hematological and may resulted in liver and kidney damage.

With various acute effects such as dizziness, headache, nausea, vomiting, abdominal pain and diarrhoea followed shortly after the ingestion, copper intoxication is no exception to badly affected gastrointestinal system with severe gastrointestinal bleeding and gastrointestinal bleeding hepatic (Ouabdesselam et al., 2020). Cu, according to the Codex Committee on Food Additives and Contaminants (CCFAC), presents a high risk to human health when consumed in large quantities. It is because of the high level of Cu in the aquatic environment, free divalent ions of heavy metals are susceptible to absorption by fish gills (P. K. Maurya et al., 2019). Thus, the bioaccumulation of Cu in fish is increases.

Copper is included in a variety of oxidative stress-related enzymes including redox system enzymes (J. Petrovic et al., 2021). Copper ions participation in the formation of reactive oxygen species (ROS) is acknowledge as cuprous (Cu^{1+}) and cupric (Cu^{2+}) which are able to take part in both reduction and oxidation reactions. When biological reductants like ascorbic acid or glutathione are present, Cu^{2+} is reduced to Cu^{+} to catalyse the H_2O_2 decomposition in order to form OH, via the Fenton reaction.

The OH radical is able to react with some biomolecules such as oxidation of bases via oxygen free radicals as well as inducing DNA strand breaks (Azeh Engwa et al., 2019). Besides, copper is able to generate the toxic which known as hydroxyl radicals and superoxide. Both hydroxyl radicals and superoxide are noxious and can cause cellular damage which linked to Wilson's illness and the onset of Alzheimer's dementia prior to excessive amount of exposure to copper (J. Petrovic et al., 2021).

2.3.3 Zinc (Zn)

Zn are present in surface and groundwater. It flows into the environment through a variety of routes including urban runoff, mine drainage, industrial and municipal wastes, and most notably is from the erosion of soil particles that contain Zn (Christos Noulas et al., 2018). Zinc also susceptible to leach into groundwater due to old, galvanized metal pipes and well cribbings that are coated with zinc and can be easily dissolved by acidic waters. The factors that contribute to distinct level of Zn in water are the environmental pollution, as well as soil composition in diverse parts of the world. Besides, the existence of distinct level of Zn in canned food are contribute by the food processing, food accessibility and the traditions of the preparation of food (Christos Noulas et al., 2018).

Zn is one of the essential trace elements that promote good health and proper growth of humans as it plays a key part in a variety of biological processes. Zinc also plays a vital role in gene expression and signal conversion. The requirement of zinc by an adult body is low which is just about 2 to 3 g, only. Therefore, zinc will turn into a harmful substance which then able to generate certain toxic effects when the concentration of zinc surpasses its requirement by the body (Jun Hong et al., 2020).

The excessive or deficiency of zinc in the body can impair cell function and multiplication, thus putting cell survival in jeopardy and causing to sickness (J. Petrovic et al., 2021). The toxicity of zinc (Zn) can lead to coldness, chest tightness, vomiting, nausea, gastrointestinal and immunologic problems, unconscious, coma and even more serious is the death due to liver damage and pulmonary edema. Increased Zn levels can also prevent Cu absorption, resulting in Cu deficient symptoms (Christos Noulas et al., 2018).

According to FAO, the recommended Zn concentration is 30 μg/g (P. K. Maurya et al., 2019). While, the concentration of Zn prescribed by JECFA and Malaysian Food Act and Regulation (1996) is 150 and 100 mg/kg respectively. Since Zn is one of the essential elements, loss of appetite, immunological defects, growth retardation, and skin changes may all result from a lack of it. Other than that, based on the studies that have been conducted by JECFA shows that high level of Zn in dietary intake are responsible for reduction in the activities of several important enzymes in various tissues, decreased concentrations of Fe and Cu absorption as well as anemia (Shafawati Ismail, 2018).

2.3.4 Cadmium (Cd)

One of the heavy metals that can be found in canned sardine is Cadmium (Cd). The high load of Cd is transported into the water by different industrial activities and domestic waste, air and can even be found in different sources (P. K. Maurya et al., 2019). It is because, Cd has multifunction and is frequently used in the industries for the productions of plastics, paints, coatings, pigments alloys as well as an electrode component in batteries. Cd is smelters into fertilizers, groundwater and sewage sludge which remains sedimented in soils for over a decade and eventually taken up by plants. Plants such as fruits, leafy vegetables, grains and cereals that are contaminated with this metal will expose human to its toxicity by the ingestion of the particular foods.

Other than the ingestion of contaminated food, humans are also exposed to the toxicity of Cd through inhalation due to burning of municipal waste. Cadmium (Cd) compounds have countless of health effects to the humans. Cadmium (Cd) is a hazardous and toxic element which may contribute to the damaging functions of renal tubules. Hence, disturbs the metabolism of vitamin D and calcium, increased low-molecular proteins secretion as well as having a neurotoxic and destructive effect to the bone system. Cd can cause bone mineralization to humans which is osteoporosis (skeletal damage).

In Japan, an epidemic of bone fractures which is known as "Itai-itai" disease has been observed with the decreasing of bone density and height in both males and females and the increasing risk of womens' bone fractures (Azeh Engwa et al., 2019). Cadmium intensifies hypertension and cardiovascular diseases, contributes to liver damage, reduces the body resistance, and affects the functioning of the sexual glands (Gra˙zyna Kowalska, 2020).

Furthermore, Cd has been concerned in encouraging DNA damage, DNA methylation, apoptosis and oxidative stress. Cd is highly toxic to kidney as higher accumulation in the proximal tubular cells will eventually lead to renal dysfunction, kidney disease, formation of renal stones, hypercalciuria as well as disturbances in the metabolism of calcium (Azeh Engwa et al., 2019). It also causes to exacerbated which arise due to the inability to excrete cadmium by the kidney where Cd is re-absorbed thus restrict its excretion. In addition, International Agency for Research on Cancer has classified Cd as group 1 carcinogens for humans that is potential for prostate cancer and causing testicular degeneration (Azeh Engwa et al., 2019).

2.3.5 Chromium (Cr)

Chromium (Cr) is also one of the essential minerals that is widely dispersed in human tissues in a variable and extremely low concentrations to aid the metabolism of carbohydrate, lipid, and protein in the body. Chromium and its compounds are being absorbed in human body by the exposure to inhalation routes, oral and dermal. Cr absorption is based on several factors such as oxidation state, particle size, solubility as well as mainly depends on the interaction with biomolecules in lungs. (Chatterjee, 2015).

Chronic exposure to Cr will lead to a toxicity and thus causes skin irritations as well as harmed the kidney, liver, kidney, nerve disorder and circulatory (Sobhanardakani et al., 2018). Cr are comprised of Cr (III) and Cr (VI) respectively and are differ in many ways. Hexavalent chromium is classified as group 1 carcinogen with various complex mechanisms that able to trigger cancer development. Driven multistage carcinogenesis, epigenetic alteration, and genomic instability have all been suggested as potential pathways mechanism for Cr (VI) carcinogenesis (Thelma Pavesi & Josino Costa, 2020).

Cr (VI) is generally believed to be a powerful oxidant by 100 times higher than that of Cr (III). It is because, Cr (VI) has a strong carcinogenic, mutagenic, teratogenic, and effects which is highly believed to be detrimental to organisms (Jun Hong et al., 2020). For example, major mechanisms by which Cr (VI) causes cellular damage is by increasing levels of oxidative stress, DNA adduct formation and breaks of chromosome (Thomas & Max, 2019). While, trivalent chromium is defined as a nonessential metal and is frequently used in nutritional supplementation.

However, it is suggested that extra supplementation is not necessary as humans can absorb enough Cr (III) from diet alone (Thomas & Max, 2019). Besides, there is a significant difference in the transport methods to cells as Cr (III) is known to be less absorbed than Cr (VI). Cr (III) will enter cell by passive diffusion or phagocytosis whereas Cr (VI) present in the cell through non-specific anion which being channel by facilitated diffusion. In red blood cell (RBC), Cr (VI) can easily penetrate into RBC and is converted into Cr (III) which binds to the cellular components (Chatterjee, 2015). Therefore, it stays in RBC is unable to leave the site.

2.3.6 Arsenic (As)

Arsenic (As) is a naturally occurring chemical element in the earth's crust that is widely spread in the environment, particularly in underground water due to agricultural, mining, and industrial runoff. In the periodic system, arsenic (As) is an element that is chemically belonged to Group 15. It is also classes as a metalloid and occur in lot of minerals in the Earth’s crust simultaneously with other metals and sulfur (Nurchi et al., 2020). The case of Arsenic poisoning is often caused by the contaminated underground water, crops that are accidentally irrigated by polluted water as well as seafood and animal products (Othman et al., 2021).

The source of exposure to As metal by human is largely initiated by dietary consumption of contaminated underground water, seafood and animal products as well as contaminated crop. Among the concentration of As found in grain crops and marine organism, the estimated arsenic concentration in marine organism and seafood product marked the higher concentration with an average four to five part per million (ppm)

(Othman et al., 2021). It is because, when it comes to seafood such as fish, shellfish, mussels, scallops and several seaweeds, the organoarsenic compound which is arsenobetaine and arsenosugars. are abundantly found in those marine organisms and are highly responsible for total urinary arsenic levels.

Arsenobetaine is the prevalent arsenical compound in organisms from the upper part of the marine food chain with the concentration of Arsenic up to 100 mg As/kg (Nurchi et al., 2020). In fresh-water organisms, the concentration of As as arsenobetaine, is also present but in much lower concentrations. Lower-ranking organisms have a higher proportion of sulfur-containing molecules that are bonded to sugars and other low-molecular-weight substances (Nurchi et al., 2020).

The toxicity of Arsenic is due to excessive levels of ingestion as well as inhalation of those metal. The result of an Arsenic poisoning continuously showing many acute symptoms of arsenicosis. At first, the skin starts changes to warts and redness, followed by other symptoms such as upper and lower extremity tingling, abnormal heart rhythm as well as gastrointestinal signs which is diarrhoea, nausea and vomiting (Othman et al., 2021). Additionally, long-term exposure or consumption of Arsenic will induce skin darkening, ongoing stomach problem, continuous sore throat, complications of arsenic-related cancers such as bladder, blood, and skin cancer, as well as other health issues corresponding to diabetes, cardiac problem and severe neurotoxicity featuring distal impairment of a symmetrical sensory or motor polyneuropathy (Othman et al., 2021).

These symptoms may appear within five years of intermittent exposure. According to the United States Centers for Disease Control and Prevention (CDC), arsenicosis can be diagnosed by assessing the presence of high amount of Arsenic in the blood and urine sample as well as in the hair and fingernails. For acute exposure cases, patients with arsenicosis are able to be diagnosed mostly by a 24-hour urine test followed by further assessment on the long-term exposure of minimally six months (Othman et al., 2021).

2.4 Impact of Heavy Metals

2.4.1 Impact of Heavy Metals to Humans

Humans are most risky towards the exposure of heavy metal as they may directly interact with heavy metals through occupational exposure at workplace, inhalation of polluted air and most importantly by the consumption of contaminated drinking water, food stuffs and aquatic animals such as fish. The contamination chain of heavy metals that leads to humans starts from the industry to the atmosphere either soil, water and finally in foods which human consumed (Azeh Engwa et al., 2019).

Heavy metals are taken up by humans through several routes which is through gastrointestinal route, inhalation as well as absorbance through the skin. Some heavy metals that are taken up through the gastrointestinal route when eating of the food or when drinking of the water such as cadmium (Cd), manganese (Mg), and arsenic (As). Instead, other heavy metals such as lead (Pb) enter the body during inhalation and being absorbed through the skin (Azeh Engwa et al., 2019).

The Food safety Authority of Ireland has raised the awareness of public health towards the accumulation of trace elements and heavy metals that can be easily found in the tissues of aquatic organisms and hence is transported to human via ingestion of seafood as well as consumption of processed canned seafood. The continuous exposure of heavy metals may lead to various health problems which including cancer while exposure to Zn and Fe in a higher concentration can also becoming toxic to the body even though they are those of essential metals yet, only in trace amount (Jeevanaraj et al., 2020).

Kidney and skeletal damages, cardiovascular disease, neurological disorders, carcinogenic effects and endocrine disruption are all effects of heavy metal on human health. Heavy metals are disrupting their functions by binding with the sulfur which present in enzymes (P. K. Maurya et al., 2019). For example, metals such as Ni, Cd, Cr, Pb and Hg are poisonous metals that have a potential as human cancer-causing agent and can trigger lung and nasal cancers, cardiovascular and kidney diseases, contact dermatitis and lung fibrosis. Some of heavy metals are also have positive impacts on human as they are essential for the biological system and are needed for various physiological and biochemical functions. For example, manganese (Mg), cobalt (Co) and iron (Fe) (P. K. Maurya et al., 2019).

2.4.2 Impact of Heavy Metals to Environment

Industrialization in world nowadays has improved a lot on quality of life, but also has resulted in increasing level of heavy metal in water. Heavy metals are polluting the aquatic environment to the fullest, with the presence of untreated municipal and industrial wastes, together with inputs from the atmosphere are regard as the major sources of fish poisoning. Heavy metals that are illegally discharged into the aquatic environment can bring damage to both aquatic diversity and ecosystems due to their accumulative behaviour and toxicity (Morshdy et al., 2013).

Besides, the distribution of heavy metals in fish, water, and sediments is crucial in determining heavy metal contamination sources in the aquatic environment. Because of their long biological half-life and non-biodegradability, leachate contains heavy metals from dumping sites can pollute groundwater and surface water (P. K. Maurya et al., 2019). By the measure of dissolved oxygen level, the biological activity of the water masses can be measured thus determine the purity of water. Owing to a rise in organic emissions from agricultural runoff, residual fertilisers, and untreated domestic waste, higher levels of Biological Oxygen Demand (BOD) indicate higher water pollution (P. K. Maurya et al., 2019).

Significant impacts of heavy metal to the ecological environment is also because of the illegal activities carried out by human, largely in urban area. Water quality in these areas are most affected by the huge discharge of urban drainage and municipal wastewater into the river basins. The population of people at urban area is rapidly growing from time to time, hence river basins are more likely to be polluted by contaminated water. For example, low dissolved oxygen levels, a wide average annual range in biochemical oxygen demand (BOD) and total coliform levels have all been observed in the rivers of some South Asian countries are typically due to the huge discharge of urban drainage and municipal wastewater and into the river basins (Yamada, 2017).

2.5 Methods of Study in Heavy Metal Residues

Solid samples containing heavy metals residues will be transformed into solutions by an appropriate dissolution method such as Acid digestion methods. The acid digestion method is a significant step in sample preparation because it has a significant impact on the recovery of various analyte contents in highly complex matrices (Uddin et al., 2016). The efficiency of the digestion methods is essential in order to achieve clear background of an optimal sample preparation method.

In this method, Nitric acid is sometimes used as an oxidant reagent, either alone or in combination with another digestion reagents such as hydrogen peroxide or acids, to obtain a consistent analytical method (Uddin et al., 2016). The affordability, accessibility as well as oxidizing capacity of nitric acid is prevalent in this respect. Before being analyse by atomic absorption spectrometer (AAS), most of the samples are dissolved into various acids. The benefits of this acid digestion or wet digestion is that both are effective on inorganic and organic substances. It is because, they able to destroy the matrix of the sample and therefore minimize the interference (Uddin et al., 2016).

2.6 Atomic Absorption Spectrometer (AAS)

In atomic absorption spectrometer (AAS), when an atom absorbs light energy of a certain wavelength, it moves from its "ground state" to its "excited state". In an excited state, the atom will return to the basic energy level by releasing a certain amount of energy in the form of light because of lower stability which caused it to become unstable (Agustina & Tjahjaningsih, 2021). The amount of light absorbed by atoms increases as the number of atoms in the light path increases (Perkin Elmer, 1996). It is necessary to use a narrow-line source which emits the narrow-line spectra of the element of interest in order to provide high intensity and thus make atomic absorption a specific analytical technique since atoms absorb light at a very specific wavelength (Perkin Elmer, 1996). The amounts of metals collected in the samples such as Pb, Cu, Zn, Cd, Fe, Mn and Ni was measured using a Varian AA240 atomic absorption spectrometer (AAS) with a Zeeman background correction system and a graphite furnace (GTA 120).

Other than that, the key sources used in atomic absorption are the electrodeless discharge lamp (EDL) and hollow cathode lamp (HCL) (Perkin Elmer, 1996). HCL is a bright and steady line source for many elements. The HCL that are used for Zn is 213.8 nm, Pb is 283.3 nm, Cu is 324.75 nm, 228.8 nm for Cd and 248.3 nm for Cr, together with slit 0.5 nm. In peak area mood, the atomic signals for Pb, Zn, Cu, Cr and Cd were calculated (P. K. Maurya et al., 2019). The manufacturer of the instrument is responsible to guide and give directions on how to operate the instrument. Atomic Absorption Spectrometer (AAS) is an instrument with high sensitivity, easy, fast lower cost than any other instruments for determination of heavy metal contents. AAS has relatively high sensitivity towards the element content in a sample even with a small concentration.

For testing of the heavy metal content, the sample is placed into a vial which provided in the AAS and then the instrument are runs automatically according to predefined settings. The heating is done in phases by starting with a temperature of 200°C, which is hot enough to evaporate the reagents in the sample. On the other hand, the sample absorption process continues until the ashes of the sample are formed and the reagents are easily ionised at the temperature of 400℃. The ionisation process proceeds accurately at a temperature of 2000℃ to allow an easier reading of the end result (Agustina & Tjahjaningsih, 2021). Throughout each run of the instrument, a triplicate sample was taken which included blank samples as well as recovery, spiked samples. The samples were spiked with different concentration of heavy metal for the recovery in order to determine the validity of the methods for analysis (Ojezele et al., 2021).

2.7 Health Risk Assessment

Health risk assessment is very important to measure the noncarcinogenic and carcinogenic effect on health due to the exposure to chemicals or heavy metals. Estimated daily intake (EDI) of the concentration of metal in the canned sardine is expressed in mg/kg (Obeka & Numbere, 2020). Based on the concentration of heavy metals that were observed in the samples, the estimated weekly ingestion (EWI) rate was determined for the comparison to the tolerable intake rate (Jeevanaraj et al., 2020). Total hazard quotient (THQ) is the ratio of a specific heavy metal's exposure to a specific reference dosage, followed by hazard index (HI) which sums all of HQs for the estimation of the total potential of noncarcinogenic health impacts as a result of ingesting a combination of heavy metals in diet. The THQ has been acknowledged as a convenient parameter for assessing the risk related to consumption of canned sardine contaminated with heavy metal since it indicates the level of risk associated with pollution exposure. An exposed population is determined to be facing health risks if the ratio of THQ obtained is equivalent to one or greater than one (Obeka & Numbere, 2020).

Whereas, lifetime cancer risk (LCR) is evaluated for the estimation of cancer risk according to the possible risks due to the consumption of a specified level of heavy metals (Jeevanaraj et al., 2020). The persons that have been exposed to the amounts of carcinogenic elements for 24 hours a day in 70 years will eventually developed a probability to any type of lifetime cancer risk. On top of that, the accumulation of heavy metals in canned fish products may have a significant impact on consumers' health (P. K. Maurya et al., 2019). Therefore, it is needed for the health risk assessment to be conducted for canned fish that may be originated from the contaminated resources.

Table 2.3: Previous studies on health risk assessment on the consumption of canned sardine

	Cr	Cd	Zn	Cu	Pb	As	References
Total Hazard Quotient (THQ)	-	0.01	-	-	0.04	0.60	(Jeevanara et al., 2020)
	-	0.03	-	-	0.01	0.80	(Gra˙zyna Kowalska, 2020)
Estimated Daily Intake (EDI)	-	0.01	-	-	0.05	0.23	(Gra˙zyna Kowalska, 2020)

CHAPTER 3

METHODOLOGY

3.1 Analytical Instrumentation

The determination of the concentration of heavy metals residue in canned sardine is conducted using an Atomic Absorption Spectrometer (AAS) (Perkin Elmer, 1996). The system is monitored by a computer auto-sampler.

3.2 Chemical and Reagents

In this study, the chemicals and reagents listed in table 3.1 were used and all of the chemicals and reagents involved are of analytical grade.

Table 3.1: List of chemical and reagents

No	Chemical and Reagents
1.	Concentrated nitric acid (HNO_3)
2.	Hydrochloric acid (HCl)
3.	Deionised water
4.	Lead (II) nitrate ($Pb(NO_3)_2$)
5.	Cadmium nitrate ($Cd(NO_3)_2$)
6.	Zinc nitrate ($Zn(NO_3)_2$)
7.	Copper nitrate ($Cu(NO_3)_2$)

3.3. Apparatus

All of the laboratory apparatus that were involved throughout the study are as follows (table 3.2).

Table 3.2: List of Laboratory Apparatus

No	Apparatus
1.	Volumetric flask 50 mL
2.	Ceramic knife
3.	Laboratory fume hood
4.	Digital hot plate
5.	Measuring cylinder 5 mL
6.	Weighing machine
7.	Beaker 50 mL and 100 mL
8.	Glass funnel
9.	Food dehydrator
10.	Lab blender
11.	Polyethylene bag
12.	0.45 mm PTFE filter
13.	Dry mill blender
14.	0.90 mm filter paper
15.	Centrifuge tube 50 mL and 15 mL

3.4 Collection of Samples

The type of canned food products that was purchased are canned sardine. The canned sardines consist of five different brands and all of the canned sardines were purchased from the local market.

3.5 Preparation of Blank Reagent Solution

5 mL concentrated nitric acid (HNO_3) and 5 mL concentrated hydrochloric acid (HCL) were mixed in a 1:1 ratio to make the blank reagent solution (Shafawati Ismail, 2018). The solution was diluted with deionised water in a 50 mL volumetric flask until reach the mark.

3.6 Preparation of Standard Calibration Solution

Stock solution for each and every metals were prepared at the concentration of 1000 mg/L. Dissolved 1.598 g of ($Pb(NO_3)_2$), 2.7441g of ($Cd(N_2O)_6.4H_2O$), 2.744 g of ($Cu(SO)_4.5H_2O$) and 4.397 g of ($Zn(SO)_4.7H_2O$) in deionised water to produce 1000mg/L of each respectively standard solution (Shafawati Ismail, 2018). The standard calibration solution for every metal was prepared at a concentration from 5 µg/L to 100 µg/L. Every heavy metal's stock solution containing sufficient amount of nitric acid (HNO3) and sulphuric acid (H2SO4) was diluted with deionised water.

3.7 Standard Calibration Solution for Pb, Cu, Cd and Zn

3.7.1 Standard Calibration Solution for Pb

To prepare a standard calibration solution for Pb which ranging from 0.000 mg/L to 0.014 mg/L, a fixed volume of the Pb standard stock solution was diluted with 500 μL of 0.5% nitric acid in a 50 mL volumetric flask. Then, further diluted with deionised water up to 50 mL. The standard calibration solution concentration of Pb was set in AAS method (Perkin Elmer, 1996).

3.7.2 Standard Calibration Solution for Cu

To prepare a standard calibration solution for Cu, a fixed volume of the Cu standard stock solution was diluted with 500 μL of 0.5% nitric acid in a 50 mL volumetric flask (Perkin Elmer, 1996). Then, further diluted with deionised water up to 50 mL. The standard calibration solution concentration of Cu was set in AAS method.

3.7.3 Standard Calibration Solution for Cd

To prepare a standard calibration solution for Cd, a fixed volume of the Cd standard stock solution was diluted with 500 μL of 0.5% nitric acid in a 50 mL volumetric flask. Then, further diluted with deionised water up to 50 mL. The standard calibration solution concentration of Cd was set in AAS method.

3.7.4 Standard Calibration Solution for Zn

To prepare a standard calibration solution for Zn which in the range of 0.000 mg/L to 0.468 mg/L, a fixed volume of the Zn standard stock solution was diluted with 500 µL of 0.5% nitric acid in a 50 mL volumetric flask. Then, further diluted with deionised water up to 50 mL. The standard calibration solution concentration of Zn was set in AAS method (Perkin Elmer, 1996).

3.8 Preparation of Sample

Prepare the canned sardines by opening the lid. The samples of canned sardines were thoroughly homogenised by using ceramic knife and lab blender. All of the samples that have been homogenised were dried in a food dehydrator at the temperature of 70ºC for overnight. In order to guarantee the constant weight of the sample, all of the water content in the canned sardine samples were removed once dried (Obeka & Numbere, 2020). After the drying process, the dried sample was grounded into powder form by using dry mill blender. Lastly, the powdered sample was stored at -20℃ in polyethylene bag until the next acid digestion.

3.9 Sample Analysis for Determination of Heavy Metal Residues by Atomic Absorption Spectrometer (AAS)

Standard solutions of lead (Pb), cadmium (Cd), zinc (Zn), and copper (Cu) were prepared from the individuals 1000mg/1 standard in 0.1 N HNO_3 while working standards was prepared from the previous stock solutions (Morshdy et al., 2013). The equipment and apparatus used were washed beforehand with distilled water. Equipment cleaning is vital to avoid contamination, particularly when analysing trace elements or heavy metals (El-Dahman et al., 2019). The method applied in this study is acid digestion method. Approximately 2 g of the powdered sample of canned sardine were weighed on the weighing machine. In the fume hood, 10 mL of digestion mixture which consists of 5 mL nitric acid and 5 mL hydrochloric acid were added to the samples, gradually. First, 5 mL of hydrochloric acid was added to the powdered sample and were digested for 10 minutes (Fong S. et al., 2006). Then, slowly added 5 mL of nitric acid for further digestion until most of the samples were completely dissolve. The solution was heated on the hotplate at 80 °C and the temperature was raised up to 120 °C for about 30 minutes to 1 hour until yellow fume was released (Fong S. et al., 2006). Besides, the samples were slowly evaporated to near dryness (Chukwujindu M. A. et al., 2009).

After heating, the solution was cooled in the fume hood. The digested solution was filtered through 0.90 mm filter paper and glass funnel. The inner walls of the beaker containing the solution was rinsed with deionised water in order to prevent from the loss of the sample (Uddin et al., 2016). The filtrate was syringe filtered with 0.45 mm PTFE filter and then was diluted in 50 mL volumetric flask with sufficient amount of deionised water up to the mark. 1.5 mL of the stock solutions were transferred into 15 mL centrifuge tube and were serial diluted with deionised water into 10^{-1}, 10^{-2},10^{-3} and 10^{-4}. Then, the tubes containing the solutions were kept in the refrigerator prior to the analysation of heavy metal contents by Atomic Absorption Spectrometer (AAS).

The samples containing heavy metals were measured using Atomic Absorption Spectrometer (AAS). Lead (Pb) was measured at 217 nm, cadmium (Cd) was measured at 228.8 nm, and zinc (Zn) was measured at 324.7 nm with hollow cathode lamps. In addition, there was a limit for detection which is 10 µg/kg for Pb, 10 µg/kg for Cu, 1 µg/kg for Cd and 10 µg/kg for Zn. Furthermore, the results obtained were corrected according to the recovery rates which is differ for each metal, the recovery rates of Pb is 97%, 98% for Cd, 96% for Cu and 97% is for Zn respectively (Morshdy et al., 2013).

3.10 Calculation and Evaluation of Result

The concentration (C) of the heavy metals in the sample of canned sardines were calculated by applying the equation (3.1):

$$C = \frac{(a - b) \times v}{m \times 1000}$$

Where,

C = Concentration of the test samples (mg/kg)

a = Concentration of the test solution (mg/L)

b = Average concentration of the blank solution (mg/L)

v = Volume of the test solution (mL)

m = Weight of the test portion

3.11 Survey of Health Risk Assessment

The survey on the health risk assessment of people in Malaysia was conducted by using Google Forms. The survey consists of three sections which is section A, B and C. Section A is on the socio demographic of the respondents, section B is on the analysation of the respondents' knowledge towards heavy metal and the frequency of canned sardine intake within a week and a month time is on the final section which is section C. The respondents participated in the survey are among the local residents in Malaysia.

3.12 Health Risk Assessment

3.12.1 Estimated Daily Intake (EDI)

Estimated daily intake (EDI) (μg/kg body weight) of heavy metals by the consumption of canned sardine were calculated using the following equation (3.2) (Guo J et al., 2016):

$$EDI = \frac{(C \times D)}{BW}$$

Where,

EDI = Estimated daily intake of heavy metal (mg/kg/day)

C = Average concentration of heavy metals (mg/g, fresh weight)

D = Daily canned sardine consumption rate (kg/day/person)

BW = Average body weight

3.12.2 Estimated Weekly Intake (EWI)

Estimated weekly intake (EWI) of heavy metals from the consumption of canned sardine were calculated using the following equation (3.3):

$$EWI = EDI \times 7 \times \frac{x}{7}$$

x = Frequency consumption per week

3.12.3 Target Hazard Quotient (THQ)

The target hazard quotient (THQ) was assessed on the risk related to the consumption of food contaminated with metals. The target hazard quotient (THQ) was calculated by the following equation (3.4) (Gra˙zyna Kowalska, 2020):

$$THQ = \frac{(EF \times ED \times MS \times C)}{(RfD \times BW \times AT)} \times 10^{-3}$$

Where,

EF = Exposure frequency for average consumer

ED = Exposure duration (70 years) correspond to the average human lifespan

MS = Mass of selected dietary ingested in adults.

C = The trace element concentration in canned sardine

RfD = Oral reference dose (Cd 1 x 10^{-3}, Zn 3 x 10^{-1}, Pb 4 x 10^{-3} and Cu 4 x 10^{-4} mg/kg/day)

BW = Average Body weight (kg)

AT = Averaging time for non-carcinogens

3.12.4 Hazard Index (HI)

The hazard index (HI) is the total of the hazard quotients (THQ) for all the heavy metals that was involved in the assessment. The HI was calculated by the application of this formula (3.5):

$$HI = THQ_{Pb} + THQ_{Cu} + THQ_{Zn} + THQ_{Cd}$$

Where,

THQ_{Pb} = Targat Hazard Quotient of Lead, Pb

THQ_{Cu} = Target Hazard Quotient of Cooper, Cu

THQ_{Zn} = Target Hazard Quotient of Zinc, Zn

THQ_{Cd} = Target Hazard Quotient of Cadmium. Cd

RESEARCH FLOW CHART

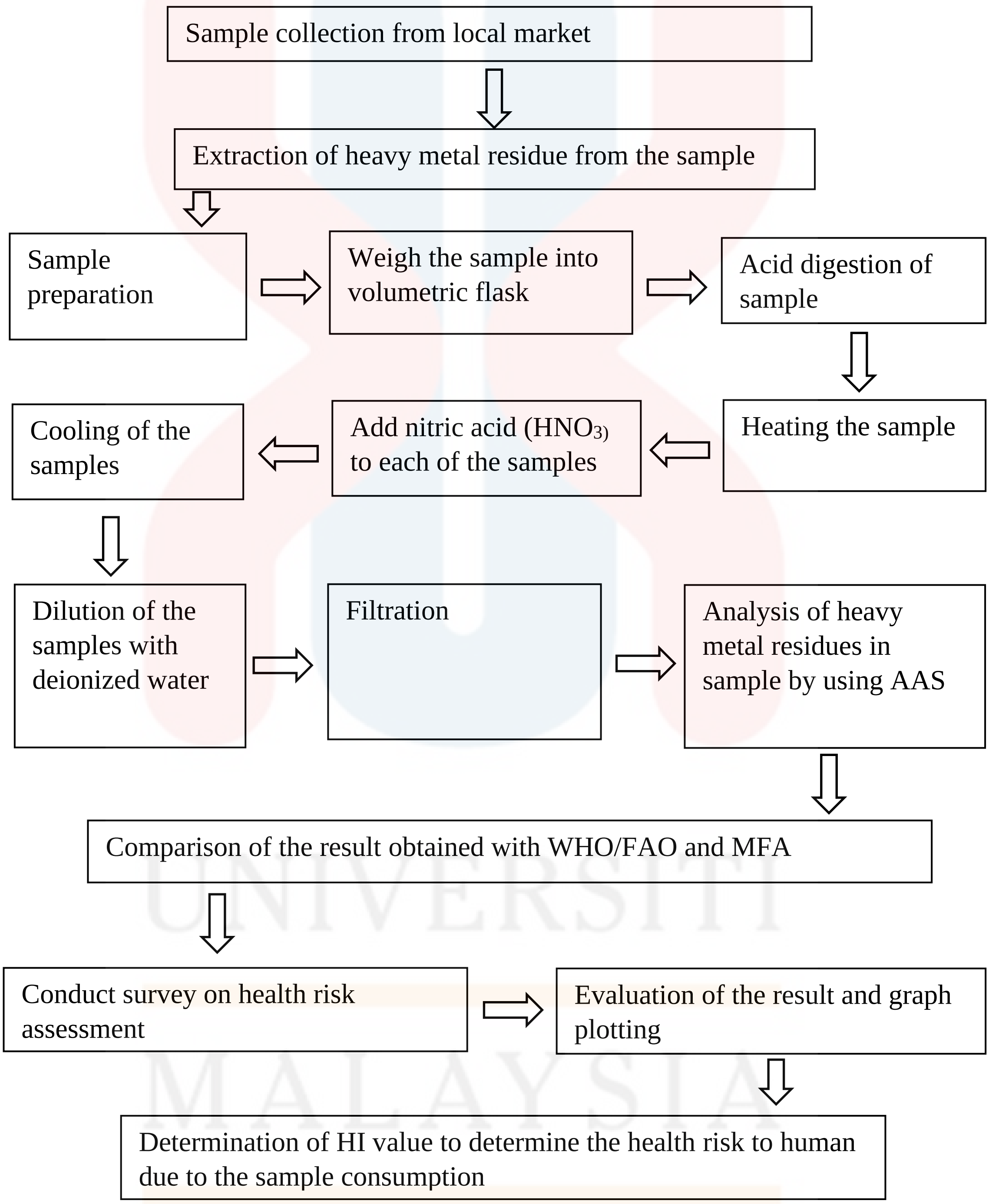


Figure 3.1: Flowchart on the summarize of research activities

CHAPTER 4

RESULT AND DISCUSSION

4.1 Sample Collection

The samples which consist of five different brands of canned sardine were purchased from local market. Such brands of canned sardine are widely sold in the local market and are easily available to the consumers.

4.2 Sample Preparation

The preparation of sample required each lid of the cans to be opened first. By using a ceramic knife and a lab blender, the sardine samples were fully homogenized. The homogenised samples then were dried overnight in a food dehydrator at 70℃. All of the water content in the canned sardine samples was removed once dried in order to ensure a constant weight of the sample (Obeka & Numbere, 2020). The dried material was pulverised into powder form by using a dry mill blender that are readily available at the laboratory. Finally, the powdered sample was placed in a plastic bag and were stored in the refrigerator at about -20°C to maintain its freshness until the next acid digestion.

4.3 Sample Extraction

The acid digestion approach was used in this research. On the weighing machine, approximately 2 g of powdered canned sardine sample were weighed. 10 mL of digestion mixture which consist of 5 mL nitric acid and 5 mL hydrochloric acid was added to the samples. The whole steps were done in the fume hood for precaution and safety. The powdered material was first treated with 5 mL of hydrochloric acid and digested for 10 minutes (Fong S. et al., 2006). Then, slowly add 5 mL of nitric acid until most of the samples are fully dissolved. The solution was heated on a hotplate at 80℃ and then were increased to 120℃. The heating process took up for 30 minutes to one hour, until yellow fumes were produced (Fong S. et al., 2006). Furthermore, the samples were slowly evaporated until they were nearly dry (Chukwujindu M. A. et al., 2009).

The solution was cooled in the fume hood after it had been heated. Using 0.90 mm filter paper and a glass funnel, the digested solution was filtered. To prevent the sample from being lost, the inside walls of the beaker containing the solution were rinsed with deionized water as well (Uddin et al., 2016). Moreover, the filtrate was syringe filtered with 0.45 mm PTFE filter before being diluted in a 50 mL volumetric flask with enough deionized water until reached the desired concentration. 1.5 mL of the stock solutions were transferred to a 15 mL centrifuge tube and serially diluted with deionized water to make 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. The tubes containing the solutions were then chilled before being analysed for heavy metal concentration using an Atomic Absorption Spectrometer (AAS).

4.4 Standard Calibration Curve

A standard calibration curve is used to determine the concentration of a substance in an unknown sample by comparing it to a set of standard samples of a known concentration. Therefore, the plotting of the calibration curve required the absorbances of the solutions to be measured. The calibration line is used to determine the concentration of the unknown metal in the samples being analysed (Ouabdesselam et al., 2020). In this research, the standards solution and blank were run in Atomic Absorption Spectrometer (AAS) in order to determine the concentration of Cd, Cu, Pb and Zn in canned sardine products.

The concentrations of each heavy metal were estimated by interpolation from linear type calibration curves which obtained from the standard solutions of each metal. The measurement of the standard form produces linear regression with correlation coefficient, r for Cd is 0.9983, Cu is 0.9988 whereas 0.9972 for Pb and 0.9992 for Zn. The curve depicted the detector response obtained are considered more accurate with r values closer to 1.00 or $r \geq 0.995$ in general (Rigdon, 2016). The value of $r = 0.999$ implies that rotational concentration and absorbance to have a strong linear relationship (Rewini W. K et al., 2020). The measurement of blank calibration solution and standard calibration curve for heavy metals Cd, Cu, Pb and Zn in canned sardine were shown in the Appendix.

4.5 Metal Concentration in Different Canned Sardine Samples

Table 4.1 shows the concentration of heavy metal residue that are present in different brands of canned sardine being purchased in the local, Malaysia market. By use of Atomic Absorption Spectrometry (AAS) instrument, the concentration values of heavy metal cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) in the sample are immediately determined by the mean values and standard deviation. The values of the same heavy metal residue accumulated in the canned sardines of different brands are compared and shown in table 4.1. The values are measured in the unit of milligram per kilogram (mg/kg) on a wet weight basic.

From the study, it is found that the concentration of heavy metal Cd, Cu, Pb and Zn varies significantly among the different brands of canned sardine purchased from the local market. Between all those metals, zinc, Zn has recorded the highest concentration of metal which is 2.195 mg/kg meanwhile the lowest metal accumulation in canned sardine is cadmium, Cd (0.011 mg/kg). According to table 4.1, the concentration of heavy metal Cd was the highest in canned sardine B1 (0.02 mg/kg); for metal Cu was also the highest in canned sardine B1 (0.187 mg/kg); Pb was highest in canned sardine B1 (0.139 mg/kg); and Zn was in canned sardine B1 (2.195 mg/kg) as well. Besides, the lowest accumulation of cadmium, Cd was initiate in canned sardine B2 (0.011 mg/kg); copper (Cu) and zinc (Zn) were both initiated lesser in canned sardine B2 (0.036 mg/kg; 0.351 mg/kg); and Pb was found initiated lowest in both canned sardine B2 and B5 with the same amount of concentration (0.08 mg/kg).

Moreover, the average concentration of heavy metal cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) that were found in the canned sardine samples were calculated in unit mg/kg. The results of the calculation were as follows; Cd (0.0156 mg/kg), Cu (0.0932 mg/kg), Pb (0.1108 mg/kg) and Zn (1.0464 mg/kg). Therefore, the ranking of heavy metals accomplished were Zn > Pb > Cu > Cd, in the order of decreasing manner. The concentration of heavy metals accumulated in every sample of canned sardine were set side by side and compared to the permissible limits as were regulated by Malaysian Food Act 1983 & Regulations 1985 (MFA) and WHO/FAO (2011). Based on the comparison, the level of concentration of heavy metals Cd, Cu, Pb and Zn in all of the five samples of different canned sardine brands which is B1, B2, B3, B4 and B5 did not exceed the permissible limit stated by Malaysian Food Act 1983 & Regulations 1985 (MFA) and WHO/FAO (2011).

Table 4.1: Level of heavy metal concentration (mg/kg) in canned sardine samples.

Type of canned sardine sample	Concentration of heavy metal, Mean ± SD (mg/kg)			
	Cd	**Cu**	**Pb**	**Zn**
Canned sardine B1	0.02 ± 0.0006	0.187 ± 0.0022	0.139 ± 0.00127	2.195 ± 0.0020
Canned sardine B2	0.011 ± 0.0013	0.036 ± 0.0021	0.08 ± 0.0086	0.351 ± 0.0042
Canned sardine B3	0.017 ± 0.0004	0.119 ± 0.0009	0.138 ± 0.0032	0.729 ± 0.0025
Canned sardine B4	0.016 ± 0.0001	0.081 ± 0.0019	0.117 ± 0.0039	1.43 ± 0.0226
Canned sardine B5	0.014 ± 0.0007	0.043 ± 0.0024	0.08 ± 0.0165	0.527 ± 0.0045
Permissible limit (WHO/FAO)	0.05	10.00	1.50	30.00
Permissible limit (MFA)	1.00	30.00	2.00	100.00

4.6 Result Analysis of Elements

4.6.1 Cadmium (Cd)

Cadmium (Cd) is a hazardous and toxic element that can harm human health by causing osteoporosis, damage renal tubules, high secretion of low-molecular proteins secretion as well as having a neurotoxic and destructive effect to the bone system. In the samples of canned sardine B1, B2, B3, B4 and B5, the concentration of cadmium does not surpass the permissible limit by Malaysian Food Act 1983 & Regulations 1985 (MFA) and WHO/FAO (2011). For MFA (1985), the maximum level of Cd allowed in canned sardine is 1.00 mg/kg, while for WHO/FAO (2011) is 0.05 mg/kg.

According to the study by Shafawati Ismail (2018) in Negeri Sembilan which is one of the states in Malaysia, the concentration of trace metals in local canned sardine and fishes were found to be in between of 0.01 - 1.66 mg/kg for Cd. Besides, the study by Q. Ahmed et al. (2018) came out with ranged from 0.010 to 0.690 mg/kg with an average of 0.183 mg/kg, thus it can be seen that the concentration of Cd in the samples of canned sardine fall between the range. Figure 4.1 shows the concentration of Cd in canned sardine sample are the highest in B1 with the amount of 0.02 mg/kg, followed by B3 with 0.017 mg/kg, B4 with 0.016 mg/kg and B5 with 0.014 mg/kg. Whereas the lowest concentration of Cd is identified in canned sardine B2 with the amount of 0.011 mg/kg.

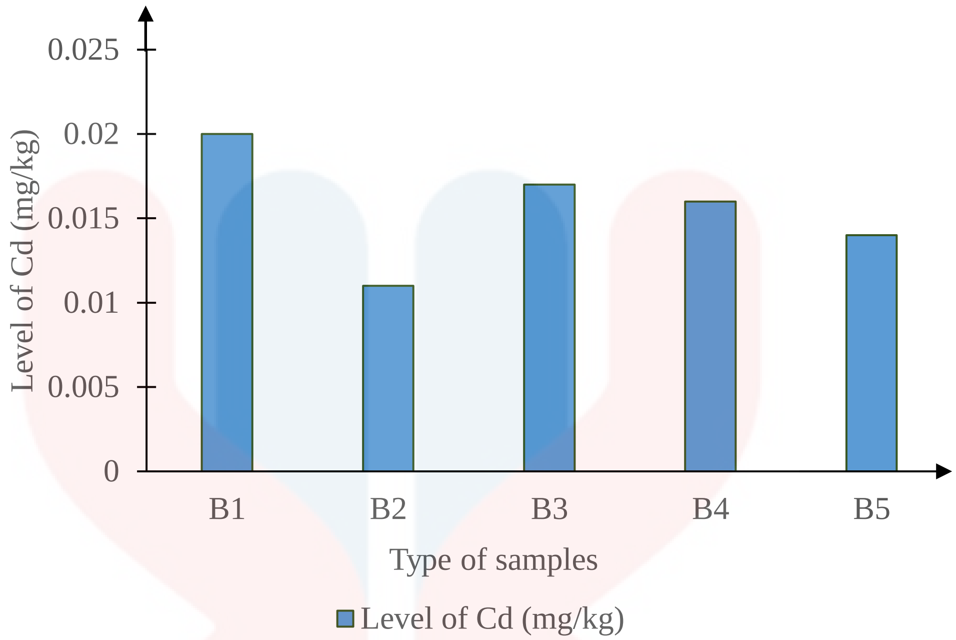


Figure 4.1: Concentration of Cd in canned sardine samples

The concentration of cadmium (Cd) in each sample are low and do not surpass the permissible limit by MFA (1985) and WHO/FAO (2011) as proven in the table 4.2. The greatest mean and standard deviation (SD) were recorded by canned sardine B1 (0.02 ± 0.0006 mg/kg) while the lowest possible mean and SD value were recorded by B2 (0.011 ± 0.0013 mg/kg). B3, B4 and B5 recorded the mean and SD of 0.017 ± 0.0004 mg/kg, 0.016 ± 0.0001 mg/kg and 0.014 ± 0.0007 mg/kg, respectively. According to FAO/WHO regulations, an adult human's acceptable weekly cadmium consumption is 0.4–0.5 mg, with a maximum allowable dose of 60–70 µg per day. The stated maximum permissible quantity of cadmium in sardine is 0.1 mg/kg in accordance with regulation Commission Regulation (EC) 1881 (Gra˙zyna Kowalska, 2020).

Table 4.2: Comparison between level of Cadmium (Cd) in canned sardine with the permissible limit set by regulations

Type of canned sardine brands	**Level of Cd (mg/kg) Mean ± SD**	**Permissible limit of Cd in canned sardine**	
		MFA (1985)	**WHO/FAO (2011)**
B1	0.02 ± 0.0006		
B2	0.011 ± 0.0013		
B3	0.017 ± 0.0004	1.00	0.05
B4	0.016 ± 0.0001		
B5	0.014 ± 0.0007		

Cadmium is placed as seventh most toxic non-essential heavy metal as it is freely plunged into the environment by natural sources such as volcanism and anthropogenic sources. This could be because of the facts that heavy metal absorption and buildup are differs depending on the type of fish, its lifestyle, living habitat, and eating patterns (Jiali Gu, 2014). In marine ecosystems, Cd generally exists in much lower quantities than in open ocean water.

Nonetheless, Cd in coastal and estuarine environments which is the usual habitat of *Sardina pilchardus* exists in higher concentrations as contributed by the intensive industrial discharge, port activity as well as mining activity in the rivers (Tamele, & Vázquez 2020). When the Cd concentrations in canned sardine samples are compared to the previous studies, it can be concluded that even though the Cd concentrations in canned sardine for this study fall within the range, however the concentration level are significantly lower as the cadmium concentrations can barely be identified by an AAS machine. This could be because of the essential metals were willingly taken by the fish at a higher rate than harmful cadmium metals (Jiali Gu, 2014).

4.6.2 Copper (Cu)

In humans, copper is a necessary element as it is required for the production of specific enzymes and haemoglobin. Although it is required for human metabolism, excessive amounts of this metal are hazardous to the cardiovascular, gastrointestinal, renal, hepatic, and haematological systems, and can cause liver and kidney damage. Based on the figure 4.2 below, the concentration of copper (Cu) in five samples of canned sardine which differ by brands are highest in canned sardine B1 with the level of 0.187 mg/kg and the lowest concentration of Cu which is 0.036 mg/kg is found to be in canned sardine B2.

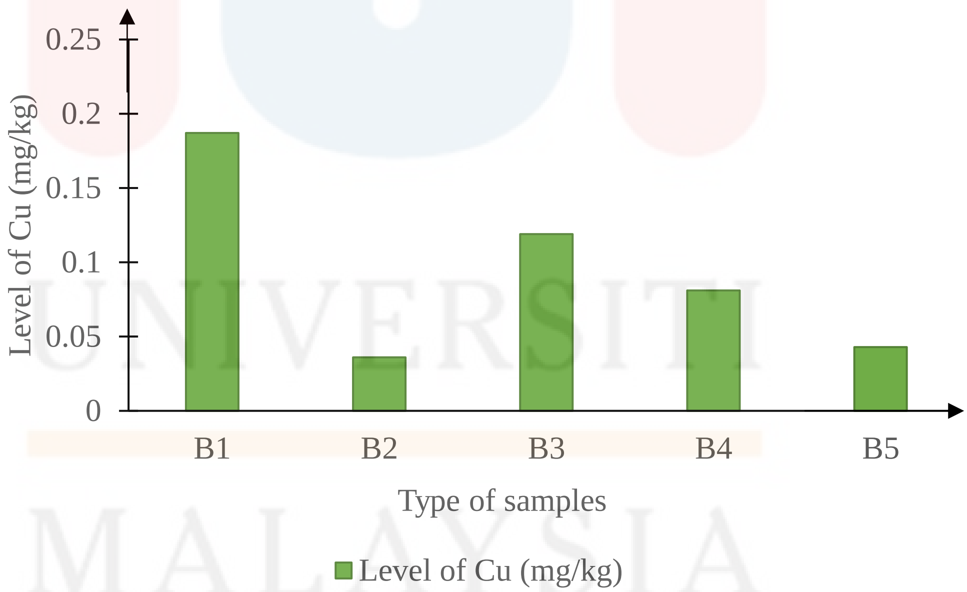


Figure 4.2: Concentration of Cu in canned sardine samples

The mean value and standard deviation of Cu observed in canned sardine are much lower than the maximum limit of Cu as permitted by MFA (1985) and

WHO/FAO (2011), as can be seen in table 4.3. The level of Cu observed in canned sardine B1 is the highest which is 0.187 ± 0.0022 mg/kg. Afterward is 0.119 ± 0.0009 mg/kg being observed in canned sardine B3, 0.081 ± 0.0019 mg/kg is observed in canned sardine B4, 0.043 ± 0.0024 mg/kg was observed in canned sardine B5 and 0.036 ± 0.0021 mg/kg is the least amount of Cu as were observed in canned sardine B2. With the maximum level of Cu regulated by MFA (1985) and WHO/FAO (2011) are 30.00 and 10.00 mg/kg respectively, it can be concluded that the accumulation of Cu in five different brands of canned sardine are all under the maximum permissible level. Low level of copper help to serve as an essential element to the body otherwise high level of copper turned into a toxic element within a series of time.

Table 4.3: Comparison between level of Copper (Cu) in canned sardine with the permissible limit set by regulations

Type of canned sardine brands	Level of Cu (mg/kg) Mean ± SD	Permissible limit of Cu in canned sardine	
		MFA (1985)	WHO/FAO (2011)
B1	0.187 ± 0.0022	30.00	10.00
B2	0.036 ± 0.0021		
B3	0.119 ± 0.0009		
B4	0.081 ± 0.0019		
B5	0.043 ± 0.0024		

Seafood and fishes are well-known for being a good source of dietary copper for a human body. A study conducted by Shkhaier (2016) involved four canned sardines that were originated from different countries. The highest concentration of Cu was 2.1 mg/kg and the lowest reading was 0.7 mg/kg which both of the canned sardine were imported from Morocco. The concentration of Cu in canned sardine which came from China is 1.0 mg/kg, lower than the one from Morocco but higher than that made of Tunisia which is 0.9 mg/kg. As compared to the concentration of Cu calculated in this study, the average concentration of Cu was determined within the accurate range. The results are compatible with the previous research, indicating that Cu levels in five varieties of canned sardines are considerably below the proposed limits.

4.6.3 Lead (Pb)

Lead (Pb) is one of the heavy metals under the category of toxic elements where constant exposure to Pb will affect the hematological system, the central nervous system as well as renal system. Marine traffic contributes largely to the contamination of sea water by releasing huge amounts of pollutants, particularly Pb which come from gasoline, petroleum goods' transfer and boat paints (Mehouel F. et al., 2019). As shown in figure 4.3, cannned sardine B1 came up with the highest concentration of Pb which is 0.139 mg/kg. The concentration of Pb in canned sardine B3 was lower than canned sardine B1 by just one point which is 0.138 mg/kg. Canned sardine B4 is observed to have 0.117 mg/kg of Pb while the concentration of Pb accumulated in both canned sardine B2 and B5 were by 0.08 mg/kg, which is the lowest among others.

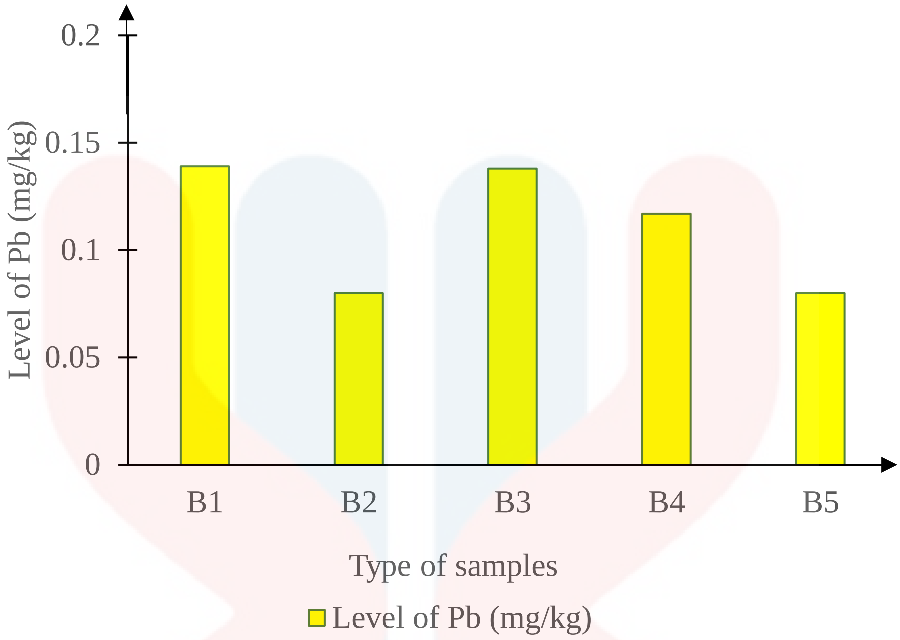


Figure 4.3: Concentration of Pb in canned sardine samples

Table 4.4 clarified that the concentration of Pb in all five brands of canned sardine were not exceeded the maximum limit that is legalized by Malaysian Food Act 1983 & Regulations 1985 (MFA) and WHO/FAO (2011) which is 2.00 and 1.50 mg/kg. It is because, the highest mean and standard deviation (SD) among the five samples of canned sardine was 0.139 ± 0.00127 mg/kg noted in canned sardine B1, followed by 0.138 ± 0.0032 mg/kg; 0.117 ± 0.0039 mg/kg; 0.08 ± 0.0165 mg/kg and 0.08 ± 0.0086 mg/kg which detected in canned sardine B3, B4, B5 and B2. Previously, Morshdy et al. (2013) have conducted a study in Egypt on the heavy metal residues in canned fishes and successfully declared that the mean level of Pb in canned sardine was 0.013 ± 0.0044 mg/kg with the concentration ranged 0.00 – 0.03 mg/kg.

By comparing to the study that have been conducted earlier, mean values of lead (Pb) acquired in this study were more and slightly higher. While, the levels of Pb is found to be within the range as linked to the research by Q. Ahmed et al. (2018) which is 0.13- 1.97mg/kg with an average of 0.835 mg/kg. A possibility of Pb contamination particularly in canned foods is due to poor soldering work. However, in Malaysia, processed foods can employ tinplate as an interior lacquer since it can withstand acid corrosion and it is used as a preservative in processed foods. Metal levels in canned fish may also be controlled by the pH of the canned food, the quality of the lacquer coatings on canned products, oxygen content in the headspace, coating quality, and storage location (Jeevanaraj et. al, 2020).

Table 4.4: Comparison between level of Lead (Pb) in canned sardine with the permissible limit set by regulations

Type of canned sardine brands	**Level of Pb (mg/kg) Mean ± SD**	**Permissible limit of Pb in canned sardine**	
		MFA (1985)	**WHO/FAO (2011)**
B1	0.139 ± 0.00127	2.00	1.50
B2	0.08 ± 0.0086		
B3	0.138 ± 0.0032		
B4	0.117 ± 0.0039		
B5	0.08 ± 0.0165		

These differences arose in findings were justified by the differences in analytical conditions that were simply varied which include matrices, analytical methodology and equipment used. Intrinsic and extrinsic factor were believed to be some of the factors that might be responsible for the results established from the study and those testified by the other researchers to be indifferent and contrast (Mehouel F. et al., 2019). Intrinsic factors involved the size, age, diet and metabolic activity of the living *sardine* *pilchardus* while extrinsic factors were focusing on the processing of canned sardine and its handling during transportation and storage. Bioaccumulation is also influenced by the physical and chemical properties of the trace element in concerned (Mehouel F. et al., 2019).

4.6.4 Zinc (Zn)

Zinc (Zn) are also heavy metals that are classified as a necessary metal alongside with Copper (Cu). Zn is sometimes referred to as the "metal of life" since it is involved in numerous metabolic activities in human metabolism (Popovic et al., 2018). The concentration of Zn detected in five samples of canned sardine are shown in the figure 4.4 below. The level of Zn concentration in canned sardine B2 sample was 0.351 mg/kg indicating as the lowest value among others. B5 and B3 samples were found to have 0.527 mg/kg and 0.729 mg/kg of Zn respectively, which is slightly higher than the B2 sample. Besides, the concentration of Zn which tested in B4 sample was 1.43 mg/kg and followed by canned sardine B1 sample which is greatly contaminated by zinc at the level of 2.195 mg/kg. Even though the concentration of Zn were quite high as compared to other metals determined in this study, yet the concentration values were still complied to the permissible limit allowed by MFA (1985) and WHO/FAO (2011).

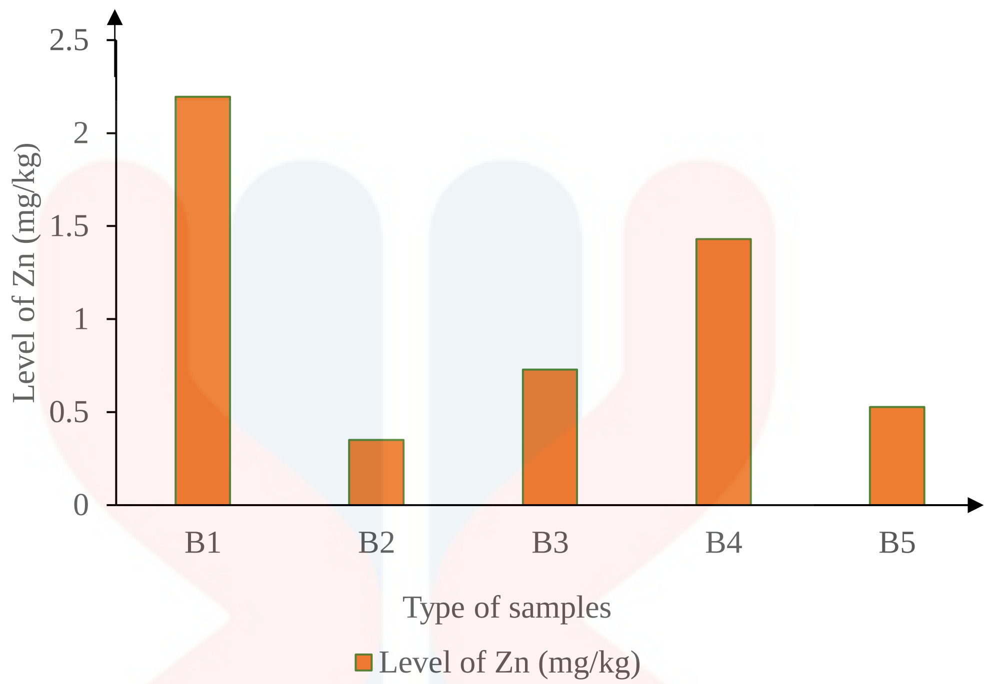


Figure 4.4: Concentration of Zn in canned sardine samples

Furthermore, the comparison between the Zn level in canned sardine and the control limit regulated by MFA (1985) and WHO/FAO (2011) can be seen clearly by referring to the table 4.5. It is shown beyond doubt that the level of Zn in the canned sardine conducted in this study remain within the allowable limit and do not exceed the maximum values which is 100.00 mg/kg and 30.00 mg/kg as stated by MFA (1985) and WHO/FAO (2011). The highest mean and standard deviation (SD) of canned sardine was determine from B1 sample; 2.195 ± 0.0020 mg/kg. Following the B1 sample were canned sardine B4, B3, B5 and B2 with the least mean value and standard deviation; 1.43 ± 0.0226, 0.729 ± 0.0025, 0.527 ± 0.0045 and 0.351 ± 0.0042.

Table 4.5: Comparison between level of Zinc (Zn) in canned sardine with the permissible limit set by regulations

Type of canned sardine brands	Level of Zn (mg/kg) Mean ± SD	Permissible limit of Zn in canned sardine	
		MFA (1985)	WHO/FAO (2011)
B1	2.195 ± 0.0020	100.00	30.00
B2	0.351 ± 0.0042		
B3	0.729 ± 0.0025		
B4	1.43 ± 0.0226		
B5	0.527 ± 0.0045		

In a study on canned fishes which include sardine, tuna and mackerel that take place in Serbia, Popovic et al. (2018) mentioned that the levels of Zn in canned sardines were much higher than canned tuna and mackerel which is 14.053.38 mg/kg, though, they were still placed within the range by National Food Databases (NFD) which is 14-31 mg/kg (Popovic et al., 2018). Zinc is a heavy metal that naturally occurs in water, soil, and rocks as a result of weathering and abrasion of rocks, as well as anthropogenic actions that may have resulted in higher level of zinc accumulated in the fish (Koleleni & Mosha, 2018).

Other than that, the high zinc concentrations studied in canned sardines could be related to the fact that the majority of zinc compounds are water soluble and thus easily absorbed by aquatic species. This indicates that zinc can be used as a good supply of essential elements for human as a whole as zinc is required better experience during lactation, appropriate fetal growth and development as well as prevention for weight loss and stunted growth in children (Koleleni & Mosha, 2018).

4.7 Accumulation of Heavy Metals in Canned Sardine

This study was performed in order to determine the amounts of heavy metals in five different brands of canned sardine purchased from the local market in Malaysia. The purpose of conducting this study was also to determine whether heavy metals were risky and can pose harm to human health if being consumed in large quantities in a human diet. The samples consist of five different brands of canned sardine which is widely sold in the local market and were easily accessible by the community. All of the brands were commonly sold and are known by most of Malaysian society. This research study also contributes to determine the concentration of heavy metals in canned sardines that were readily available in the market as canned sardine is one of the main sources of protein that is convenient, easy to prepare and able to put up with a longer shelf life.

Canned sardines are greatly assimilated to the local dishes as it is normally served as a main dish as well as being used as the main ingredients in the preparation of local food and savory desserts. *Sardina Pilchardus* primarily feeds on phytoplankton, zooplankton, and fish eggs which make them as both primary and secondary consumer. Sardines would be exposed to the contamination of heavy metals through uptake from water and ingestion of filtered food particles and might potentially collect them as well (Uren et al., 2020). Since canned sardine are prone to be contaminated by heavy metal residue, this research is necessary to study the presence of heavy metals in canned sardine product, as well as to determine the amounts of heavy metals observed were exceeded either the permissible values of MFA (1985) or WHO/FAO (1984).

4.8 Survey Conducted

The survey on health risk assessment of canned sardine consumption among people in Malaysia is being conducted. This survey is purposely conducted to determine the health risk assessment of people in Malaysia towards the consumption of canned sardine. All of the questions in this survey are set up as a multiple choices question and are divided into three parts which is part A the socio demographic of the respondents, part B is the knowledge of respondents about heavy metal while the frequency of canned sardine consumption of the respondents is on part C. This survey is conducted in about two months' time through google form and are distributed online by WhatsApp as well as Instagram. The total of 378 respondents aged from 20 years old and below to 51 years old and above which from every state in Malaysia are taking part and contributing their answers in this survey.

4.8.1 Socio Demographic of Respondents

4.8.1.1 Gender of respondents

This survey involved participation from both gender which is male and female. Figure 4.5 shows 64% of respondents are female while 36% of respondents are male. Females are more interested in answering survey that is being circulated on WhatsApp and Instagram than male, thus makes the number of respondents from female are higher as compared to male which is 242 and male is only 136. Other than that, female have higher tendency to take part in a food-based research as they are more concern about the safety of the food that they consumed.

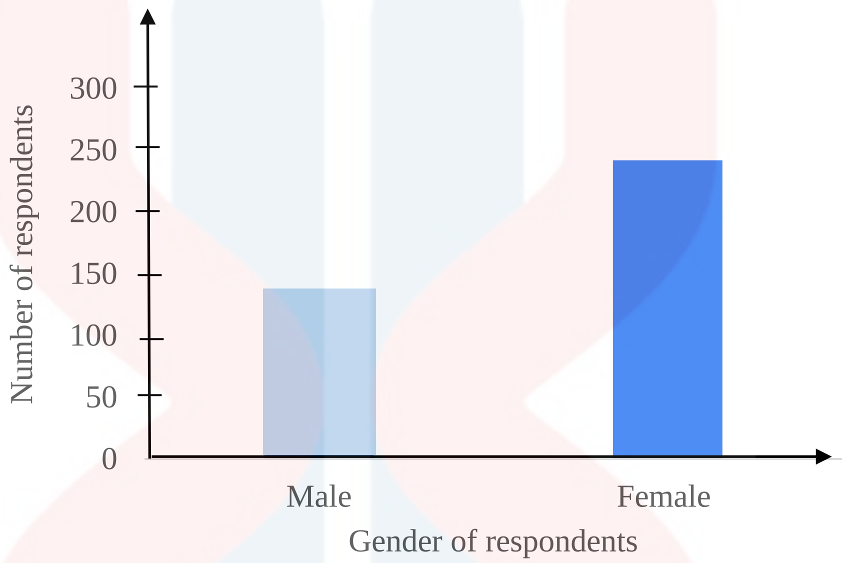


Figure 4.5: Gender of respondents

4.8.1.2 Age of respondents

The age of the respondents is selected from 20 years old and below, 21 to 30 years old, 31 to 40 years old, 41 to 50 years old and 51 years old and above. Among the respondents shown in figure 4.6, 51.9% are from the age of 21 to 30 years old, 15.9% of the respondents are from 31 to 40 years old, 12.4% are grouped under 20 years old and below, 11.6% of them are 41 to 50 years old and the last 8.2% of the respondents are in the age of 51 years old and above. Majority of the respondents' age are ranging between 21 to 30 years old as most of them are easy to be approached through online medium while respondents in the age of 51 years old and above are the least as they are not familiar with online medium such as WhatsApp and Instagram.

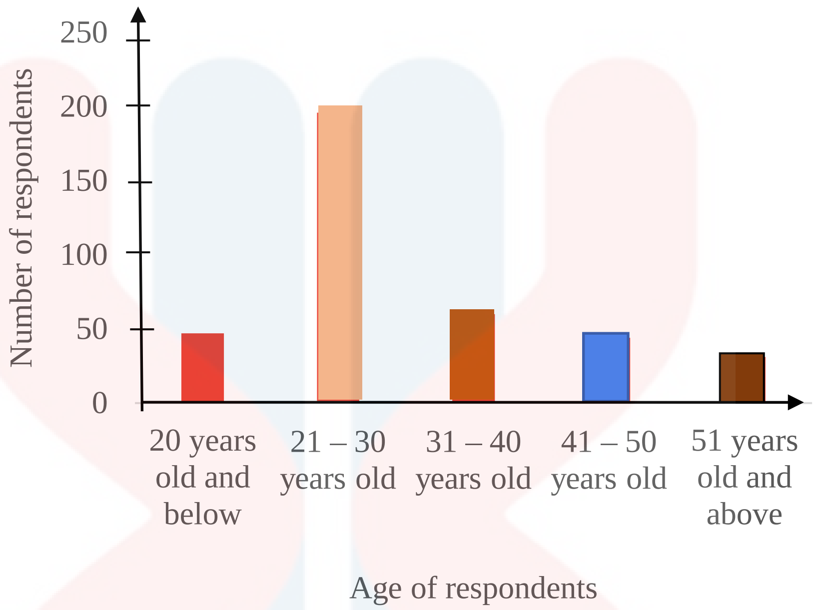


Figure 4.6: Age of respondents

4.8.1.3 Race of respondents

As Malaysia is proudly a multiracial country, the race of the respondents consists of Malay, Chinese, Indian and others. In figure 4.7, majorly 350 people (92.6%) out of 378 respondents who contributed to answer this survey are Malay, followed by Chinese with 4%, Indian with 2.4% and 1.1% (4 people) from other races. Malays turn out to be the majority of race to contributed in this survey as they are easy to be reached out while the other races recorded the least amount of respondents with the total of 4 people because they are low in population and also not that easy to be reached out.

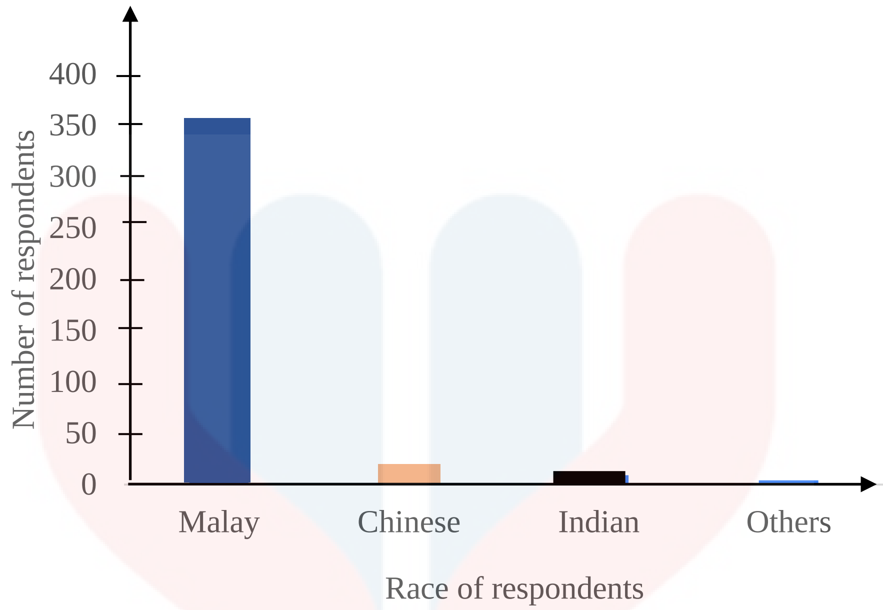


Figure 4.7: Race of respondents

4.8.1.4 Educational Level of respondents

Next, the educational level of respondents which followed Malaysia educational system varies accordingly to SPM (Sijil Pelajaran Malaysia), STPM (Sijil Tinggi Persekolahan Malaysia), Diploma, Degree, Master, PhD as well as none. The results for educational level of respondents varies greatly and are evidently shown in figure 4.8. Among 378 respondents, 44.7% already have Degree or still in the mid of Degree studies. 24.6% have Diploma, 14.8% have SPM, 7.9% owned STPM certificate, 4.5% owned Master certificate, 2.1% have no formal education and 1.3% are honored with PhD. Degree holders are expectedly making up most of the respondents with the total of 169 people because the survey is highly blasted among them and within the institution.

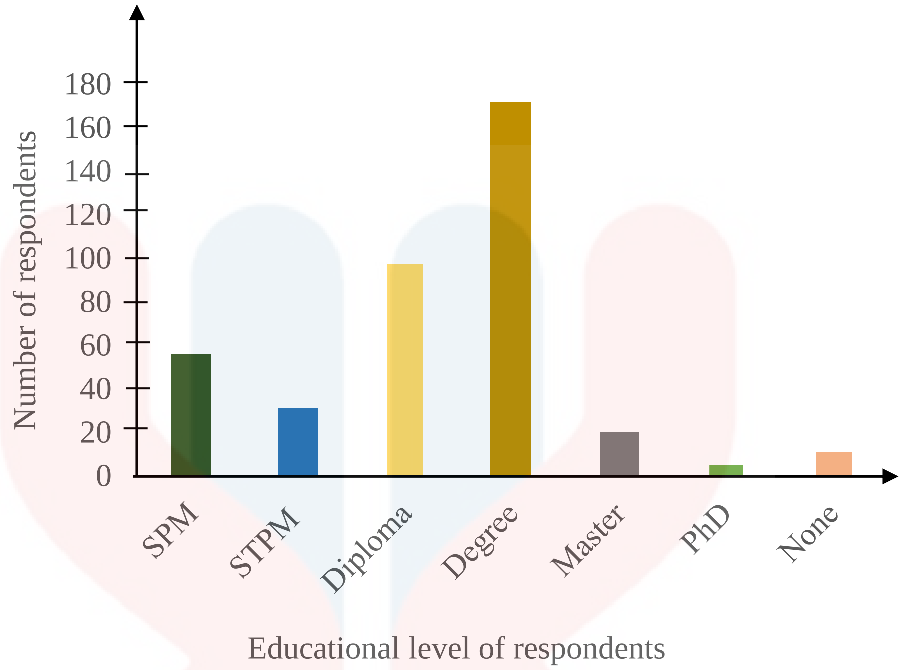


Figure 4.8: Educational level of respondents

4.8.1.5 Income Level of respondents

The income level of respondents is one of the important socio demographics to be observed on as it will indirectly determines the preferences of the respondents on the canned sardine consumption. The income level is grouped into four different group which is from less than RM 1000, RM 1000 to RM 3000, RM 3001 to RM 5000 and last group is more than RM 5000. Based on figure 4.9, respondents with income level less than RM 1000 are the majority with 159 responses which contribute to 42.1%. It is because, most of them are university students and yet to have their own income. Besides, 29.6% have income level between RM 1000 to RM 3000, 18.8% have income level of RM 3001 to RM 5000 and 9.5% of respondents gain more than RM 5000.

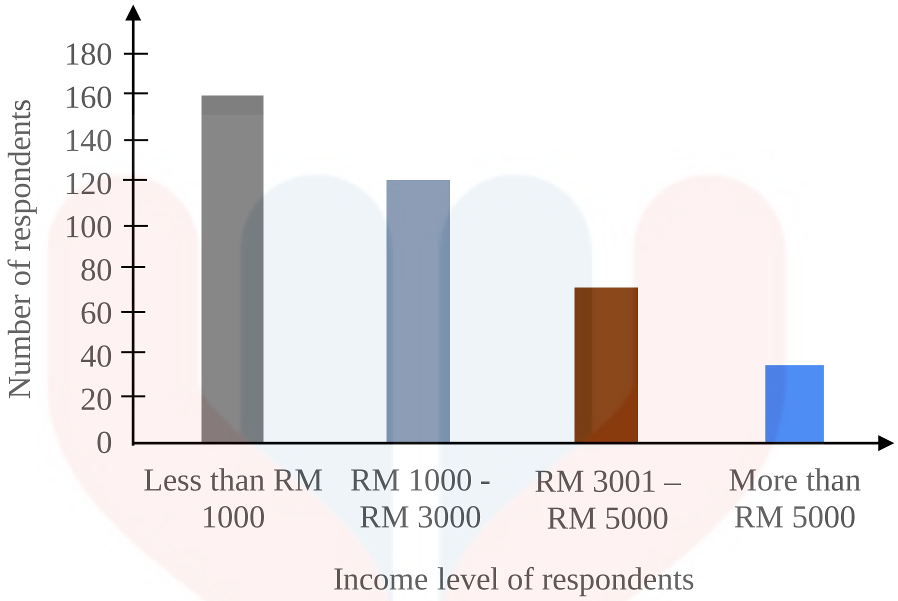


Figure 4.9: Income level of respondents

4.8.1.6 State of respondents

This survey involves respondents who come from 15 states in Malaysia which is Kelantan, Terengganu, Pahang, Perak, Selangor, Wilayah Persekutuan (Kuala Lumpur), Kedah, Perlis, Negeri Sembilan, Melaka, Johor, Pulau Pinang, Sarawak, Sabah as well as Labuan. Respondents who participate in this survey come from different states and are scattered across Malaysia, as shown in the figure 4.10. The total percentage of respondents who live in Kelantan are the highest as compared to the other states with the percentage of 37.8% (143 people). 8.2% are from Pahang, 7.4% come from Selangor, 6.6% come from Wilayah Persekutuan (Kuala Lumpur), 6.3% from Perak, 5.8% live in Johor, 5% are live in Kedah. Negeri Sembilan contribute 4.8%, Melaka contribute 4.6%, Terengganu contribute 4.2%, Pulau Pinang contribute 3.4% while Perlis contribute 2.6% of respondents. Other than that, Sabah and Sarawak each contribute 2.4% and 0.8% of respondents.

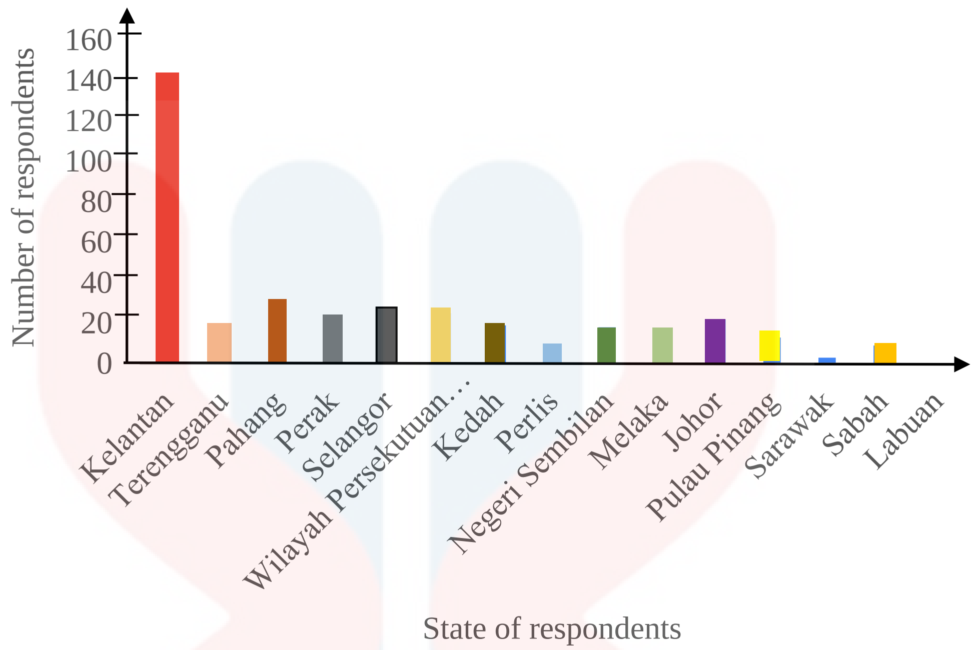


Figure 4.10: State of respondents

4.8.2 Knowledge of respondents about Heavy Metals

4.8.2.1 Familiar with the terms Heavy Metals

The first question in this section asked whether the terms heavy metal is recognizable by the respondents. They are required to answer yes if they are familiar to the terms of heavy metal while no if the term seems unfamiliar to them. Figure 4.11 shows that majority of the respondents are familiar with the heavy metal terms as 285 respondents choose yes as the answer to the question which makes up to 75.4%. Whereas only 93 respondents (24.6%) are unfamiliar with the term. It is because, they are not aware with food safety issues as well as the term are not commonly used in daily life.

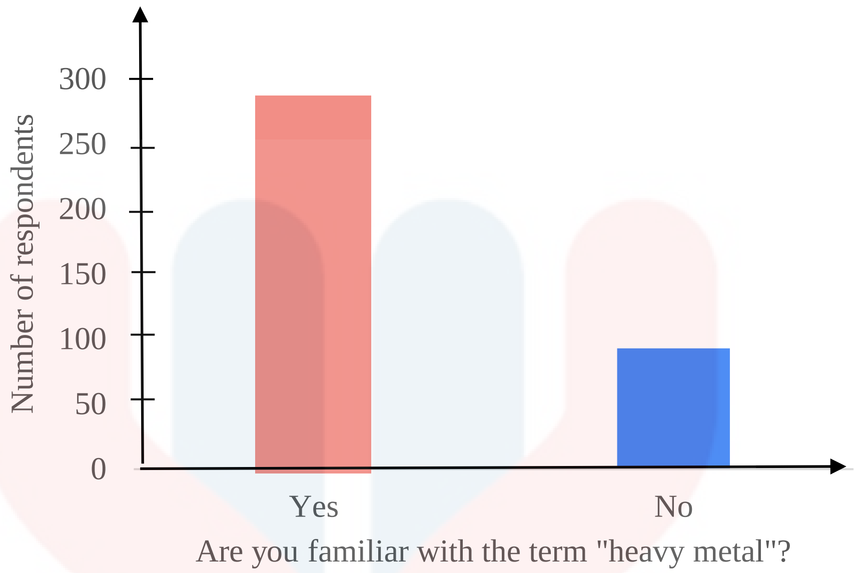


Figure 4.11: Respondents' knowledge about the heavy metal term

4.8.2.2 Opinion on definition of Heavy Metals

The next question is regarding the definition of heavy metals. The definition of the terms is rather important to completely determine the knowledge of the respondents about heavy metal. According to the result obtained in figure 4.12, 55% of the respondents choose to refer heavy metal as an element with high density and toxic at high concentration, 19.3% answered that heavy metal is an element with low density and toxic at high concentration while 5.3% refer heavy metal as an element with low density and toxic at low concentrations. Despite of majority of the respondents are familiar with heavy metals term, merely 20.4% are accurately referring heavy metal as an element with high density and toxic at low concentration. It is believed that respondents do not have information regarding the exact definition of heavy metal. Furthermore, respondents also become confusing with the density or level of toxicity being stated in the definition of heavy metal.

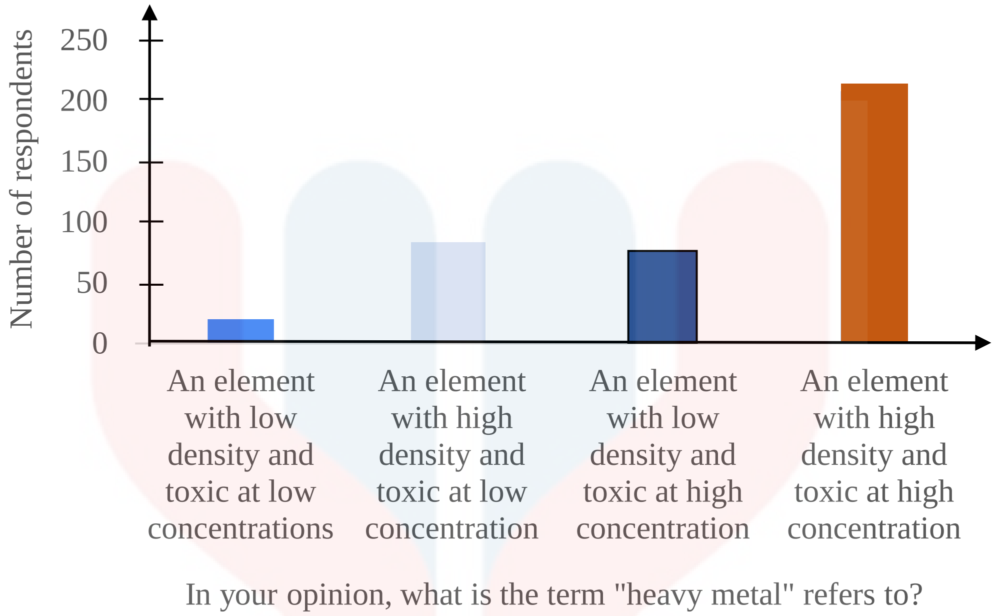


Figure 4.12: Opinion of respondents on the definition of heavy metal

4.8.2.3 Knowledge on the Examples of Heavy Metals

This question calls for the respondents to answer either yes or no in order to confirm their knowledge on the examples of heavy metals being stated which is cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu). As shown in figure 4.13, 356 out of 378 respondents know that Cd, Pb, Zn and Cu are the examples of heavy metal. While, only 22 respondents cannot identify Cd, Pb, Zn and Cu as the examples of heavy metal. 94.2% (356 people) who know the example of heavy metals usually come across it through formal education in educational institute such as schools or universities.

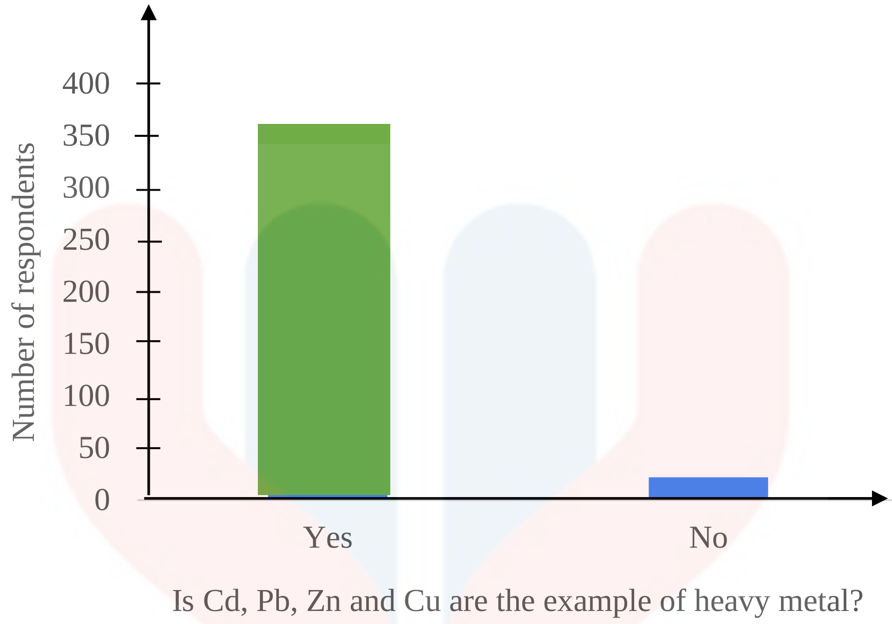


Figure 4.13: Knowledge of the respondents about the example of heavy metal

4.8.2.4 Awareness on the Harms of Heavy Metals Towards the Body

Toxicity of heavy metal can cause various type of health problems such as heart disease, kidney failure and cancer. This question asks whether the respondents are aware that the excessive intake of heavy metal residue in canned sardine can cause sickness and harm their body. The answer provides are multiple choices which either yes or no. Based on figure 4.14, respondents who aware on the harms caused by heavy metal residue in canned sardine to their body are more by 360 respondents than those who are lack the awareness. 97.6% answered yes to portray their awareness and left a total of 2.4% who answered no. It is a great evidence to show that respondents are totally aware on the food safety issue that can harm their body which caused by heavy metal residue.

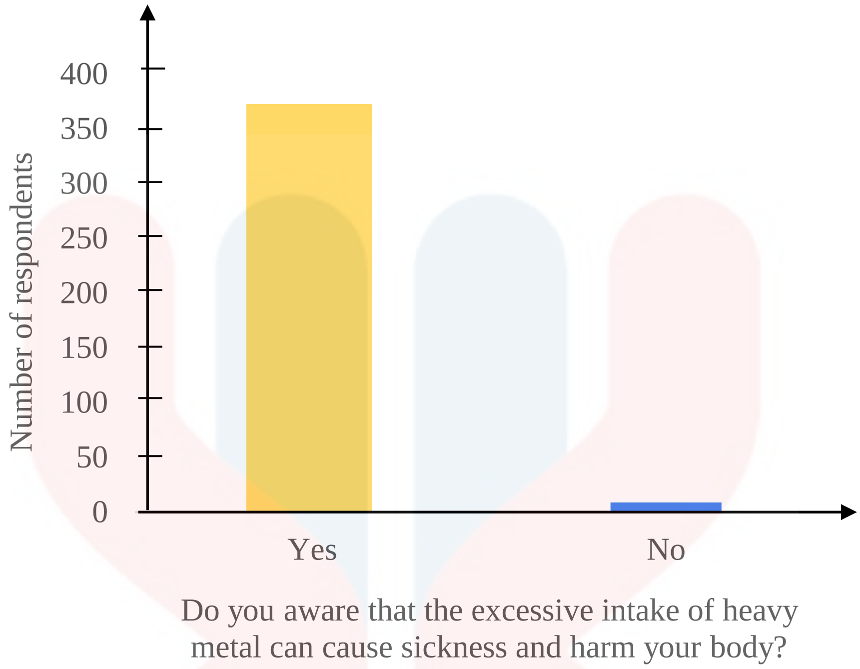


Figure 4.14: Awareness of the respondents towards the harm of heavy metal to the body

4.8.2.5 Opinion on the Safety of Packaging Material Used

Moreover, packaging material of canned sardine is somewhat important to protect the product from the environment as well as to ensure that the products are in good condition. In the last question of this section, the respondents are compulsory to answer either yes or no in order to portray their opinion on the metallic can as packaging material that may also contribute to the contamination of heavy metal residue in canned sardine product. Based on figure 4.15, respondents seem to agree more on the statement where 84.1% (318 people) of the respondents answered yes. Otherwise, 60 respondents who give only 15.9% of the total percentage do not agree that metallic can used for packaging material of canned sardine may contribute to the contamination of heavy metal in the product.

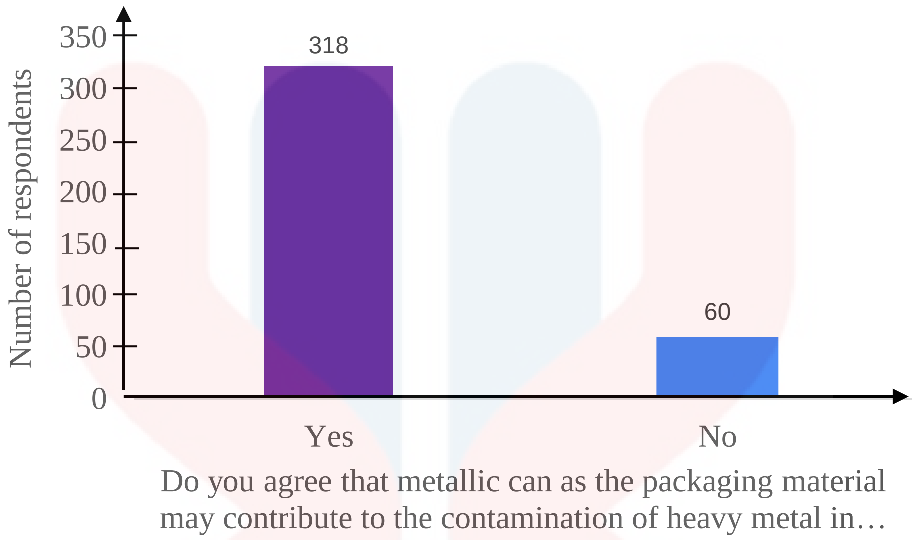


Figure 4.15: Opinion of the respondents on the packaging material used for canned sardine

4.8.3 Frequency of Canned Sardine Consumption by Respondents

4.8.3.1 Frequency of Canned Sardine Consumption in a Week by Respondents

In this section, the question asked intends to observe the frequency of canned sardine consumption by respondents within a week. Respondents are provided with four multiple choices of answer which is none which also means no consumption, 1 to 2 times, 3 to 4 times and more than 5 times consumption in a week. The result for the frequency consumption of canned sardine in a week by the respondents are shown in figure 4.16. It can be analyzed that 56.1% of respondents do not prefer on consuming canned sardine as they might prefer more on the fresh product instead of canned product. While 160 respondents with the percentage of 42.3% consumed 1 to 2 times of canned sardine, few of them which is 5 respondents consumed 3 to 4 times a week and none of the respondents eaten the canned sardine more than 5 times a week.

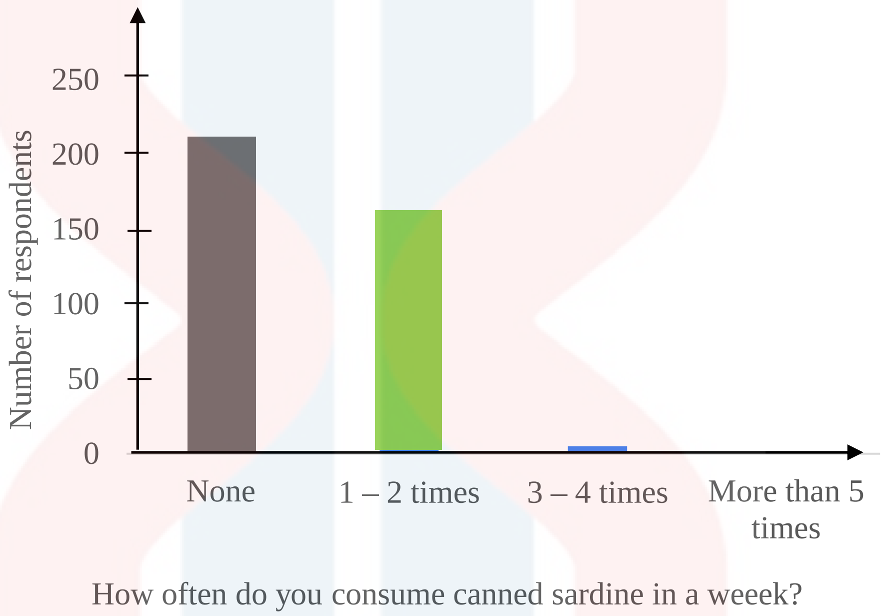


Figure 4.16: Frequency of canned sardine consumption by respondents within a week

4.8.3.2 Frequency of Canned Sardine Consumption in a Month by Respondents

Furthermore, the last question in this section surveyed on the frequency of canned sardine consumption in a month by the respondents. The number of canned sardine consumption within a month are grouped into 5 different groups ranging from none, 1 to 7 times, 8 to 14 times, 15 to 21 times and lastly is more than 22 times. Figure 4.17 below shows more than half of the respondents with 78.6% are consuming canned sardine from 1 to 7 times in a month. Whereas 54 out of 378 respondents answered none and 27 respondents answered 8 to 14 times which contribute only 7.1% of the total percentage. Meanwhile, it is evidenced that respondents do not consume canned sardine regularly as none of the respondents seem to consume canned sardine for 15 to 21 times as well as more than 22 times consumption in a month because it is considered too frequent, and respondents tend to consume other dishes.

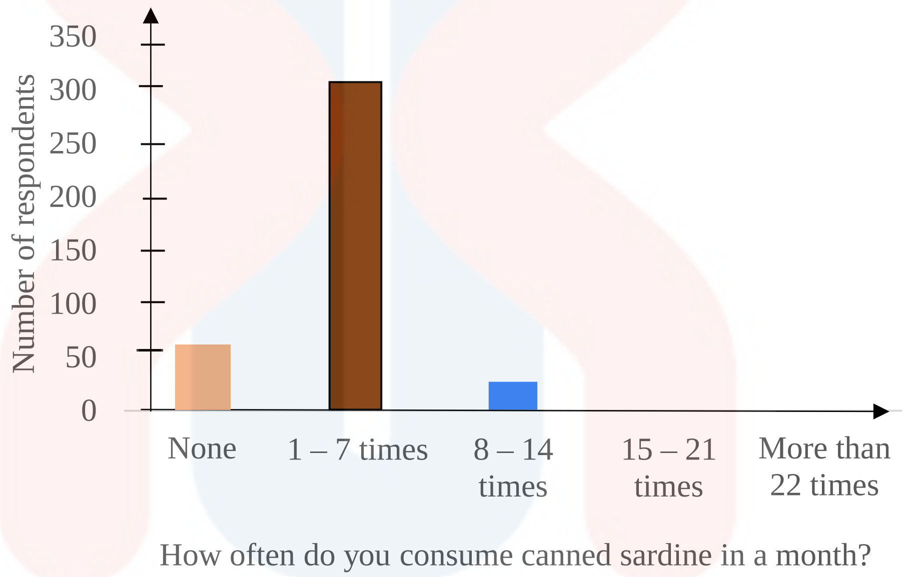


Figure 4.17: Frequency of canned sardine consumption by respondents within a month

4.9 Health Risk Assessment

4.9.1 Estimated Daily Intake (EDI) for Adults in Canned Sardine

Figure 4.18 shows the estimated daily intake (EDI) for heavy metals Cd, Cu, Pb and Zn among the canned sardine samples. The highest EDI for cadmium (Cd) are calculated in both canned sardine B1 and canned sardine B3 with 0.0008 mg/kg/day while the lowest EDI for Cd is in canned sardine B2 with 0.0005 mg/kg/day. Besides, the EDI value for copper (Cu) metal are greatly higher in B1 sample with 0.0082 mg/kg/day but significantly lower in B2 sample with 0.0016 mg/kg/day. Other than that, 0.0061 mg/kg/day is the EDI value for both canned sardine B1 and B3 for heavy metal lead (Pb), much more than EDI value for canned sardine B2 and B5 which is 0.0035 mg/kg/day.

The other metal is zinc (Zn) with the maximum EDI value 0.0963 m/kg/day calculated for canned sardine B1 whereas the minimum EDI value is 0.0154 mg/kg/day that is calculated in canned sardine B2. The EDI of Pb and Cd in canned fish calculated by Gra˙zyna Kowalska (2020) in the previous research is 0.01069 mg/kg/day and 0.00317 mg/kg/day, slightly higher than EDI which determined from this study. Overall, the data imply that canned sardine appears to be a safer seafood product in terms of heavy metal exposure due to the packaging integrity and size of fish selected for processing (Pravina Jeevanaraj et. al, 2020).

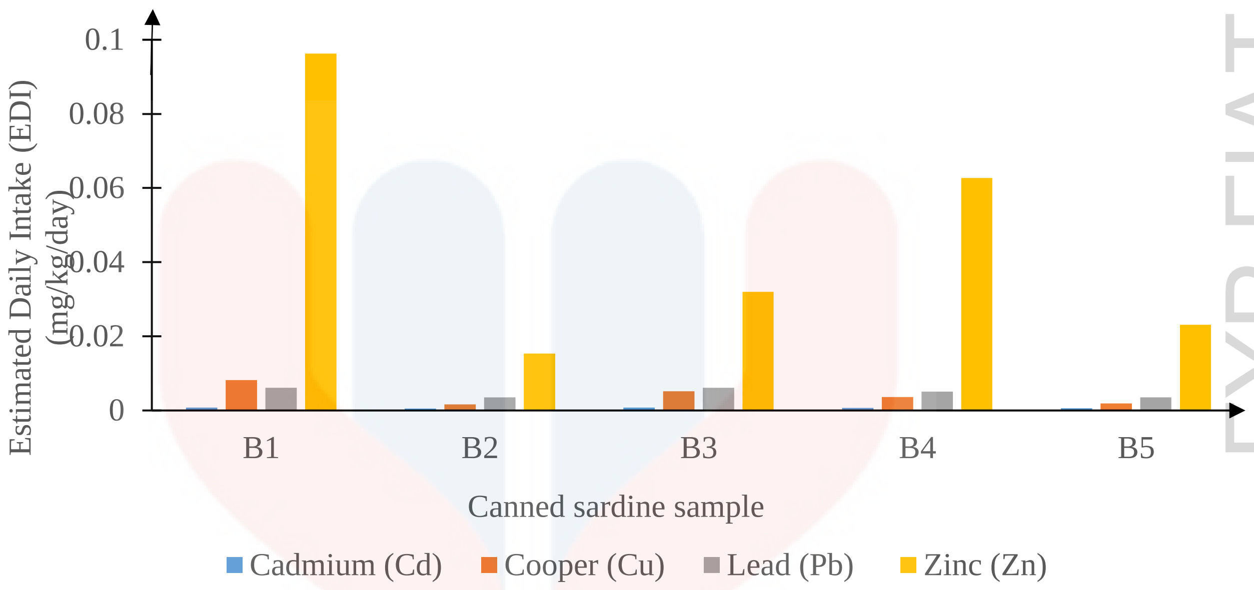


Figure 4.18: Estimated Daily Intake (EDI) (mg/kg/day)

4.9.2 Estimated Weekly Intake (EWI) for Adults in Canned Sardine

The estimated daily intake (EWI) of adults in canned sardine are evidently shown in figure 4.19. From the result gained in this study, zinc (Zn) is considered as the most prevalent metal among cadmium, copper and lead. It is proven by the EWI value of Zn in canned sardine B1 which is 0.1926 mg/kg/week while the B2 sample of canned sardine has the lowest EWI for heavy metal Zn with 0.0308 mg/kg/week. The next EWI value is on the heavy metal cadmium (Cd) where the highest EWI is 0.004 mg/kg/week which calculated for canned sardine B4 and the lowest EWI of Cd is 0.001 mg/kg/week that is determined for canned sardine B2. For heavy metal copper (Cu), the maximum EWI is in B1 sample by 0.0164 mg/kg/week whereas the minimum EWI is evaluated in the sample B2 by 0.0032 mg/kg/week. Then, canned sardine B1 and B3 are both noted a high value of EWI which is 0.0122 mg/kg/week where canned sardine B2 and and B5 noted the EWI 0.007 mg/kg/week, lesser than B1 and B3.

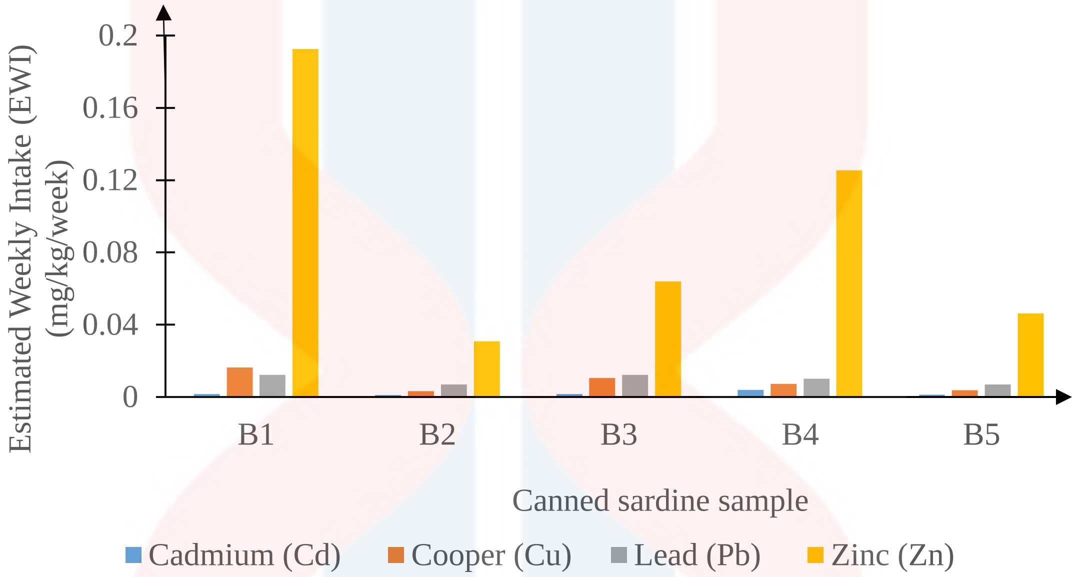


Figure 4.19: Estimated Weekly Intake (EWI) (mg/kg/week)

The Comparison between the Estimated Daily Intake (EDI) and the Estimated Weekly Intake (EWI) for adults are shown in the table 4.6, below. By involving all of the five samples of different canned sardine brands which is B1, B2, B3, B4 and B5, the amount of EWI is greater than the amount of EDI. The association between actual heavy metal intake amounts and the intake safety proposed by The Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) was also studied. The TDI of canned sardine among adults are 1.35 mg/kg/day for Cd, 10 mg/kg/day for Cu, 1.40 mg/kg/day for Pb and 40 mg/kg/day for Zn. Meanwhile, Provisional Tolerable Weekly Intake (PTWI) for adults are 0.42 mg/kg/week for Cd, 210 mg/kg/week for Cu, 1.5 m/kg/week for Pb and 420 mg/kg/week for Zn. Evidently, five different brands of canned sardine studied in this research do not poses health risk and can be consumed by Malaysian people.

Table 4.6: Comparison of Estimated Daily Intake (EDI) and Estimated Weekly Intake (EWI) for adults

Type of canned sardine brands	EDI (mg/kg/day)				EWI (mg/kg/week)			
	Cd	Cu	Pb	Zn	Cd	Cu	Pb	Zn
B1	0.0008	0.0082	0.0061	0.0963	0.0016	0.0164	0.0122	0.1926
B2	0.0005	0.0016	0.0035	0.0154	0.0010	0.0032	0.0070	0.0308
B3	0.0008	0.0052	0.0061	0.0320	0.0016	0.0104	0.0122	0.0640
B4	0.0007	0.0036	0.005	0.0627	0.0040	0.0072	0.0102	0.1254
B5	0.0006	0.0019	0.0035	0.0231	0.0012	0.0038	0.0038	0.0462
TDI (mg/kg/day)	1.35	10	1.40	40	-	-	-	-
PTWI (mg/kg/week)	-	-	-	-	0.42	210	1.5	420

4.9.3 Target Hazard Quotient (THQ) and Hazard Index (HI) for Adults in Canned Sardine

According to table 4.7, Target Hazard Quotient (THQ) and Hazard Index (HI) for adults are determine in canned sardine samples. The highest average value of THQ in canned sardine are in Zn which is 7.65×10^{-4}, followed by THQ of Pb with 6.06×10^{-3}. Moreover, the average value of THQ for Cd is 3.42×10^{-4}, slightly higher than THQ for Cu which is 3.08×10^{-2}. A research formerly conducted by Gra˙zyna Kowalska (2020) showed the rank order of THQ in canned fish for trace element Cd was up to 0.11849 and 0.01292 for trace element Pb. It can be seen that the average THQ value for canned sardine calculated in this study are much lower. Next, the determination is on the value of HI for all five different samples of canned sardine. The result of HI obtained from this study is 0.03, lesser than 1.0. Thus, no cancer risk present for the canned sardine sample. The non-cancer risk proportion of HI is less than 1.0 while HI exceed 1.0 is predicted as cancer risk proportion (Gra˙zyna Kowalska, 2020).

Table 4.7: Target Hazard Quotient (THQ) and Hazard Index (HI) for adults in canned sardine samples

Type of canned sardine brands	**Target Hazard Quotient (THQ)**				**Hazard Index (HI)**
	Cd	Cu	Pb	Zn	
B1	8.77×10^{-4}	2.92×10^{-4}	1.52×10^{-3}	3.21×10^{-4}	3.01×10^{-3}
B2	4.82×10^{-4}	3.95×10^{-3}	8.77×10^{-4}	5.13×10^{-5}	5.36×10^{-3}
B3	7.46×10^{-4}	1.30×10^{-2}	1.51×10^{-3}	1.07×10^{-4}	1.54×10^{-2}
B4	7.02×10^{-4}	8.88×10^{-3}	1.28×10^{-3}	2.09×10^{-4}	0.11×10^{-2}
B5	6.14×10^{-4}	4.71×10^{-3}	8.77×10^{-4}	7.70×10^{-5}	6.28×10^{-3}
Average value	3.42×10^{-4}	3.08×10^{-2}	6.06×10^{-3}	7.65×10^{-4}	0.03

CHAPTER 5

CONCLUSION AND FUTURE RECOMMEDATIONS

5.1 Conclusion

Canned sardines are well-known for being a processed, canned source of protein which is generally available in Malaysia market and is within most people's budgets. The benefits of canned food, particularly sardines, is that it has a longer shelf life, convenient, easy to prepare and thus reducing the rate of food wastage. Still, despite of the advantages, canned food products are concerned of containing chemical contaminants such as heavy metal as it is the major source of pollution in the environment, as well as resulting from technological processing or inappropriate packaging practices. In this study, it can be concluded that the concentration of heavy metals Cd, Cu, Pb and Zn were successfully determined in the five different brands of canned sardine purchased from selected local market in Malaysia.

By the used of Atomic Absorption Spectrometer (AAS), the highest average concentration of heavy metals among canned sardine B1, B2, B3, B4 and B5 samples were detected in metal zinc (Zn) with 1.0464 mg/kg, followed by metal lead (Pb) with

0.1108 mg/kg, copper (Cu) with 0.0932 mg/kg and lastly is heavy metal cadmium (Cd) with 0.0156 mg/kg. Therefore, the ranking of heavy metals was established in decreasing order where Zn > Pb > Cu > Cd. As in comparison to the permissible limit provided by MFA (1985) and WHO/FAO (2011), the concentration of heavy metals contamination in canned sardines are relatively low and below the levels.

Thus, canned sardine sold in Malaysia market can be considered as healthy for consumption. Other than that, the health risk assessment conducted for the canned sardine samples concluded that the value of Estimated Daily Intake (EDI) and Estimated Weekly Intake (EWI) of adults for all the metals involved do not surpass the Tolerable Daily Intake (TDI) and Provisional Tolerable Weekly Intake (PTWI) as referred to Food and Agriculture Organization/World Health Organization (FAO/WHO) and Expert Committee on Food Additives (JECFA). Besides, the Hazard Index (HI) calculated is 0.03 which is not more than 1, no possible health risks are suspected of the consumption of canned sardine.

5.2 Future Recommendations

More comprehensive research on the determination of heavy metal and health risk assessment is suggested to be conducted within different type of canned fish and seafood, as this study is concentrating more on just one type of canned fish which is canned sardine. Several types of canned fish and seafood are such as canned tuna, canned mackerel, canned clam and prawn as well. It is beyond compulsory to test and determine the concentration of heavy metal contamination in variety of canned food products, along with the development and evolution in food technology where the existence of various kind of canned fish and seafood products of numerous brands are showing an increasing pattern in the local market.

Other than that, knowledge on the survey of health risk assessment is comprehensively proposed. Better set of questionnaires which distributed to the respondents involved are most important for further evaluation on the hazard level and safe consumption limit of the canned food. The authorities which responsible for controlling food regulations and health shall spread knowledge and educate the local communities on the risk of frequent consumption of heavy metal contaminated food especially those packaged in can. As much as the higher levels of accumulation, frequent intake of heavy metal accumulated canned food at lower level can possess toxicity properties and also causing harm and contribute all kinds of deleterious health effect to the body such as heart disease, kidney failure and liver damage. In addition to that, knowledge on the safe eating guidelines according to the permissible level as issued by the trusted authorities and organisation should be outlined, as well.

REFERENCES

ABM Helal Uddin, R. S. (2016). Comparative study of three digestion methods for elemental analysis in traditional medicine products using atomic absorption spectrometry. *Journal of Analytical Science and Technology*, 01-07.

Ackova, D. G. (2018). Heavy metals and their general toxicity on plants. *Plant Science Today*, 5(1): 15-19.

Agustina, M., & Tjahjaningsih, W. (2021, February). Assessment of Heavy Metal Lead (Pb) Contents in Canned Crab Products by Atomic Absorption Spectrophotometry (AAS). In IOP Conference Series: Earth and Environmental Science (Vol. 679, No. 1, p. 012012). IOP Publishing.

Ahmed, Q., Levent, B. A. T., Öztekin, A., & Ali, Q. M. (2018). A review on studies of heavy metal determination in mackerel and tuna (Family-Scombridae) fishes. Journal of Anatolian Environmental and Animal Sciences, 3(3), 107-123.

Alaa Eldin M. A. Morshdy, A.-E. S. (2013). Heavy Metal Residues in Canned Fishes in Egypt. *Japanese Journal of Veterinary Research 61*, 54-57.

Chatterjee, S. (2015). Chromium Toxicity and its Health Hazards. *International Journal of Advanced Research Volume 3(Issue 7)*, 167-172.

Christos Noulas, M. T. (2018). Zinc in soils, water and food crops. *Journal of Trace Elements in Medicine and Biology, Volume 49*, 252-260.

Chukwujindu M. A. Iwegbue, G. E. (2009). Characteristic levels of heavy metals in canned sardines consumed in Nigeria. *The Environmentalist*.

D.MacRae, A. T. (2021). A review of chemical and microbial contamination in food: What are the threats to a circular food system? *Environmental Research, Vol. 194*, 1-16.

De Lima, N. V., de Pádua Melo, E. S., Arakaki, D. G., Tschinkel, P. F. S., de Souza, I. D., de Oliveira Ulbrecht, M. O. & do Nascimento, V. A. (2021). Data on metals, nonmetal, and metalloid in the samples of the canned tuna and canned sardines sold in Brazil. Data in Brief, 35, 106865.

El-Dahman, D., Hassan, M., & Eleiwa, N. (2019). Assessment of heavy metal residues in some fishery products. *Benha Veterinary Medical Journal*, *36*(2), 49-56.

Elmer, P. (1996). Analytical Methods for Atomic Absorption Spectroscopy. *Analytical Methods for Atomic Absorption Spectroscopy*, 001-299.

Gjorgieva Ackova, D. (2018). Heavy metals and their general toxicity on plants. Plant Science Today, 5(1), 15-19.

Godwill Azeh Engwa, P. U. (2019, June 19). Mechanism and Health Effects of Heavy Metal Toxicity in Humans. Retrieved from Intechopen:

https://www.intechopen.com/books/poisoning-in-the-modern-world-new-tricks-for-an-old-dog-/mechanism-and-health-effects-of-heavy-metal toxicity-in-humans

Gra˙zyna Kowalska, U. P. (2020). Determination of the Level of Selected Elements in Canned Meat and Fish and Risk Assessment For Consumer Health. *Journal of Analytical Methods in Chemistry*, 01-13.

Guo J, Yue T, Li X, Yuan Y. Heavy metal levels in kiwifruit orchard soils and trees and its potential health risk assessment in Shaanxi, China. Environ Sci Pollut Res Int. 2016 Jul;23(14):14560-6. doi: 10.1007/s11356-016-6620-6. Epub 2016 Apr 12. PMID: 27068913.

Hazrat Ali, E. K. (2019). Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. *Journal of Chemistry*, 01-15.

Hong, Y. J., Liao, W., Yan, Z. F., Bai, Y. C., Feng, C. L., Xu, Z. X., & Xu, D. Y. (2020). Progress in the research of the toxicity effect mechanisms of heavy metals on freshwater organisms and their water quality criteria in China. Journal of Chemistry, 2020.

Jiali Gu, Y. L. (2014). Determination and Safety Evaluation of Heavy Metals in Canned Fish from Liaoning Province. *Asian Journal of Chemistry*, 915-917.

Koleleni, Y. I., & Mosha, P. A. (2018). Evaluation of Essential Elements and Heavy Metals in Sardine Fish from Kivukoni, Kunduchi and Bagamoyo Fish Markets in Tanzania. *Physical Science International Journal*, 1-16.

Mehouel, F., Bouayad, L., Hammoudi, A. H., Ayadi, O., & Regad, F. (2019). Evaluation of the heavy metals (mercury, lead, and cadmium) contamination of sardine (Sardina pilchardus) and swordfish (Xiphias gladius) fished in three Algerian coasts. *Veterinary world*, *12*(1), 7.

Nayara Vieirade Lima, E. S. (2021). Data on metals, nonmetal, and metalloid in the samples of the canned tuna and canned sardines sold in Brazil. *Data In Brief, Volume 35*, 106865.

Noulas, C., Tziouvalekas, M., & Karyotis, T. (2018). Zinc in soils, water and food crops. Journal of Trace Elements in Medicine and Biology, 49, 252-260.

Numbere, C. O. (2020). Heavy metal concentration and public health risk in consuming Sardinella maderensis (Sardine), Sarotherodon melanotheron (Tilapia), and Liza falicipinis (Mullet) harvested from Bonny River, Nigeria. *Journal of Oceanography and Marine Science*, 1-10.

Nurchi, V. M., Buha Djordjevic, A., Crisponi, G., Alexander, J., Bjørklund, G., & Aaseth, J. (2020). Arsenic toxicity: molecular targets and therapeutic agents. *Biomolecules*, *10*(2), 235.

Ojezele, O. J., Okparaocha, F. J., Oyeleke, P. O., & Agboola, H. I. (2021). Quantification of Some Metals in Commonly Consumed Canned Foods in South-west Nigeria: Probable Pointer to Metal Toxicity. Journal of Applied Sciences and Environmental Management, 25(8), 1519-1525.

Othman, L., Nafadi, A., Alkhalid, S. H., & Mazraani, N. (2021). Arsenic poisoning due to high consumption of canned sardines in Jeddah, Saudi Arabia. Cureus, 13(1).

Ouabdesselam, L. (2020). Assessment of Heavy Metal Contamination Levels in Fishes from the Mediterranean Sea. EC Nutrition, 15(12), 30-9.

Petrovic, J., Jovetic, M., Štulić, M., Redžepović-Đorđević, A., Vujadinović, D., Djekic, I. V., & Tomasevic, I. B. (2021). Exposure assessment to essential elements through the consumption of canned fish in Serbia. Theory and practice of meat processing, 6(3), 219-225.

Popović, A. R., Relić, D. J., Vranić, D. V., Babić-Milijašević, J. A., Pezo, L. L., & Đinović-Stojanović, J. M. (2018). Canned sea fish marketed in Serbia: their zinc, copper, and iron levels and contribution to the dietary intake. Archives of Industrial Hygiene and Toxicology, 69(1), 55-60.

Pradip Kumar Mauryaa, D. M. (2019). Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible Human Health Risk Evaluation. *Toxicology Reports*, 472-481. Retrieved from https://www.sciencedirect.com/science/article/pii/S2214750019300733

Pravina Jeevanaraj, A. A. (2020). Heavy metal contamination in processed seafood and the associated health risk for Malaysian women. *British Food Journal*, 3099 3114. Retrieved https://www.emerald.com/insight/content/doi/10.1108/BFJ-03-2020-0280/full/html

Rehman, K., Fatima, F., Waheed, I., & Akash, M. S. H. (2018). Prevalence of exposure of heavy metals and their impact on health consequences. *Journal of cellular biochemistry*, *119*(1), 157-184.

Rewini, W. K., Thomas, N., Silaban, D. P., Iyabu, H., & Taupik, M. (2020, November). Concentration of Pb, Sn and Fe Metals on Milk Products and Canned Fish in Gorontalo City. In IOP Conference Series: Earth and Environmental Science (Vol. 589, No. 1, p. 012033). IOP Publishing.

Shafawati Ismail, S. A. (2018). Determination of selected toxic metal (As, Cd, Pb) and essential (Zn, Cu) elements in local canned seafood products. *AIP Conference Proceedings 1972*, 01-06.

Shathees Baskaran, S. A. (2017). Understanding Purchase Intention of Ready-to-Eat Food among Malaysian Urbanites: A Proposed Framework. *International Journal of Academic Research in Business and Social Sciences*, 566-579.

Shkhaier, K. N. (2016). Determination of some heavy metals in canned Sardines fish from Iraqi markets. *J Fac Med Baghdad*, 387-391.

Sim Siong Fong, D. a. (2006). Evaluation of the Acid Digestion Method with Different Solvent Combination for the Determination of Iron, Zinc and Lead in Canned Sardine. *Malaysia Journal of Chemistry, Vol. 8, No 1*, 010-015.

Sobhanardakani, S., Hosseini, S. V., & Tayebi, L. (2018). Heavy metals contamination of canned fish and related health implications in Iran. Turkish Journal of Fisheries and Aquatic Sciences, 18(8), 951-957.

Tamele, I. J., & Vázquez Loureiro, P. (2020). Lead, mercury and cadmium in fish and shellfish from the Indian Ocean and Red Sea (African Countries): Public health challenges. *Journal of Marine Science and Engineering*, *8*(5), 344.

Thelma Pavesi, J. C. (2020). Mechanisms and individuality in chromium toxicity in humans. *Journal of Applied Toxicology Volume 40, Issue 9*, 1183-1197.

Thomas LiborioDesMarias, M. (2019). Mechanisms of chromium-induced toxicity. *Current Opinion in Toxicology (vol 14)*, 01-07.

Ugonna C. Nkwunonwo, P. O. (2020). A Review of the Health Implications of Heavy Metals in Food Chain in Nigeria. *Scientific World Journal*, 6594109.

Uren, R. C., van der Lingen, C. D., Kylin, H., & Bouwman, H. (2020). Concentrations and relative compositions of metallic elements differ between predatory squid and filter-feeding sardine from the Indian and South Atlantic oceans. *Regional Studies in Marine Science, 35*, 101137.

Yabanli, M. (2013). Assessment of the heavy metal contents of Sardina pilchardus sold in Izmir, Turkey. Ekoloji, 22(87), 10-15.

Yamada, C. P. (2017). Impact of Population Growth on theWater Quality of Natural Water Bodies. *Sustainability*, 01-14.

APPENDIX A

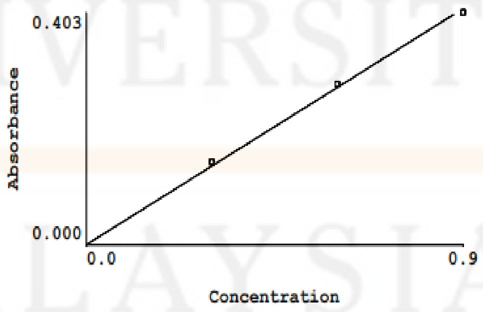
Blank calibration measurement for each heavy metals

Heavy metal	Average concentration (mg/kg)	Standard deviation
Cd	0.00	0.00
Cu	0.00	0.00
Pb	0.00	0.00
Zn	0.00	0.00

APPENDIX B

Standard calibration curve for each heavy metal using AAS.

i. Cadmium (Cd)

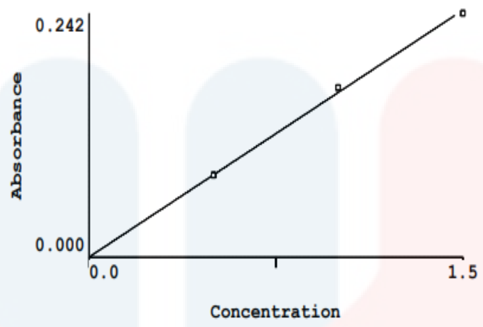


Calibration data for Cd 228.80 Equation: Linear Through Zero

ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	0.000	0.00	>999.9%
Calib Std 1	0.1436	0.3	0.315	0.00	0.56
Calib Std 2	0.2787	0.6	0.612	0.00	0.22
Calib Std 3	0.4034	0.9	0.886	0.00	0.44

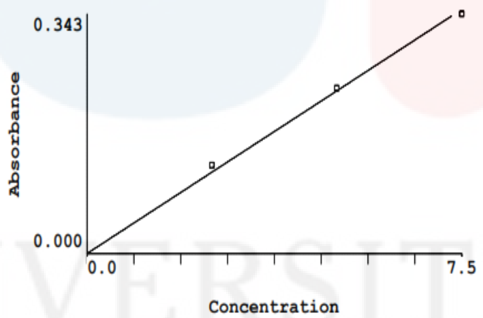
Correlation Coef.: 0.998379 Slope: 0.45523 Intercept: 0.00000

ii. Copper (Cu)



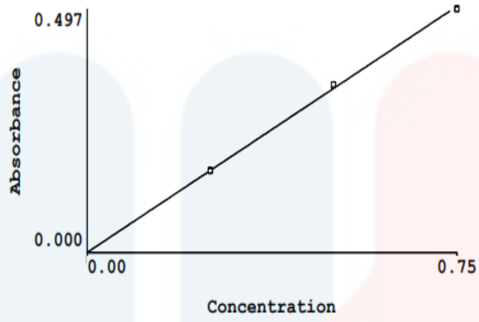
Calibration data for Cu 324.75				Equation: Linear Through Zero	
		Entered	Calculated		
	Mean Signal	Conc.	Conc.	Standard	
ID	(Abs)	mg/L	mg/L	Deviation	%RSD
Calib Blank 1	0.0000	0	0.000	0.00	>999.9%
Calib Std 1	0.0812	0.5	0.497	0.00	1.50
Calib Std 2	0.1680	1.0	1.028	0.00	0.67
Calib Std 3	0.2421	1.5	1.481	0.00	0.30
Correlation Coef.: 0.998851		Slope: 0.16340	Intercept: 0.00000		

iii. Lead (Pb)



Calibration data for Pb 217.00				Equation: Linear Through Zero	
		Entered	Calculated		
	Mean Signal	Conc.	Conc.	Standard	
ID	(Abs)	mg/L	mg/L	Deviation	%RSD
Calib Blank	0.0000	0	0.000	0.00	>999.9%
Calib Std 1	0.1264	2.5	2.711	0.00	0.12
Calib Std 2	0.2370	5.0	5.081	0.00	0.34
Calib Std 3	0.3435	7.5	7.366	0.00	0.37
Correlation Coef.: 0.997233		Slope: 0.04663	Intercept: 0.00000		

iv. Zinc (Zn)



Calibration data for Zn 213.86 — Equation: Linear Through Zero

ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	0.000	0.00	>999.9%
Calib Std 1	0.1671	0.25	0.250	0.00	0.90
Calib Std 2	0.3424	0.50	0.511	0.00	0.34
Calib Std 3	0.4970	0.75	0.742	0.00	0.61

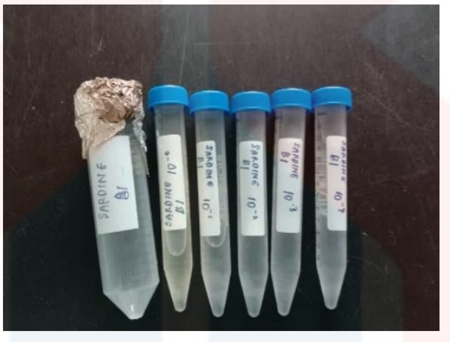
Correlation Coef.: 0.999243 Slope: 0.66954 Intercept: 0.00000

APPENDIX C

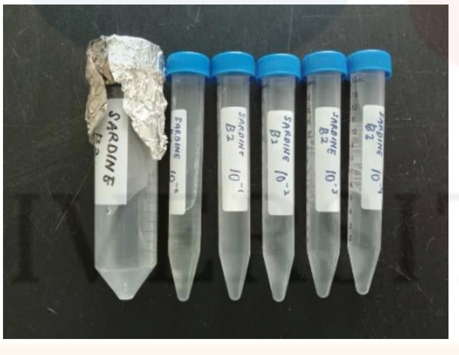
50 mL stock solution after acid digestion, 15 mL sample solution and serial dilution samples of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} from left to right

Colour of sample after acid digestion and serial dilution of canned sardine

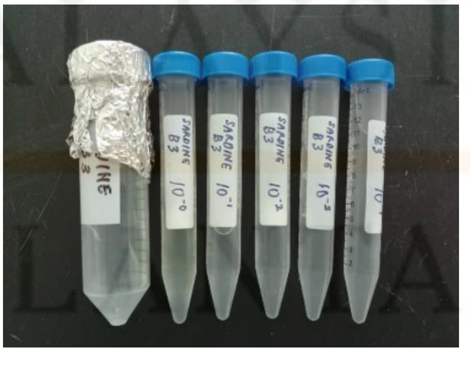
Canned sardine B1:



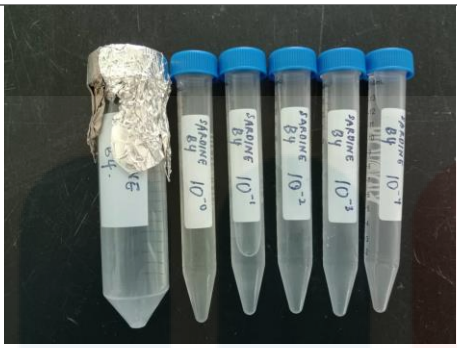
Canned sardine B2:



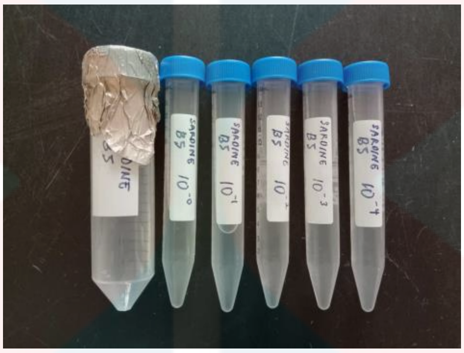
Canned sardine B3:



Canned sardine B4:



Canned sardine B5:



TURNITIN SIMILARITY REPORT

THESIS

ORIGINALITY REPORT

12%	9%	8%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Universiti Malaysia Kelantan Student Paper	3%
2	Shafawati Ismail, Syaza Azhari, Che Wan Zanariah Che Wan Ngah. "Determination of selected toxic metal (As, Cd, Pb) and essential (Zn, Cu) elements in local canned seafood products", AIP Publishing, 2018 Publication	1%
3	www.intechopen.com Internet Source	1%
4	www.science.gov Internet Source	1%
5	Pravina Jeevanaraj, Aliah Ahmad Foat, Halimah Tholib, Nurul Izzah Ahmad. "Heavy metal contamination in processed seafood and the associated health risk for Malaysian women", British Food Journal, 2020 Publication	1%
6	www.ncbi.nlm.nih.gov Internet Source	1%