Heavy Metal Determination and Health Risk Assessment of Edible Mushrooms Collected from Selected Market in Kelantan, Malaysia Using Atomic Absorption Spectrometer (AAS)

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DECLARATION

I declare that thesis entitled "Heavy Metal Determination and Health Risk Assessment of Edible Mushrooms Collected from Selected Market in Kelantan, Malaysia Using Atomic Absorption Spectrometer (AAS)" is the results of my own research except as cited in the references.

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ABSTRACT

Edible mushroom is a fungus that is used as food and medicine with high protein and is widely consumed by humans in daily life, especially in Asia. Despite high protein, edible mushrooms have the potential for accumulating heavy metals. Heavy metal concentration in edible mushrooms can give a negative effect on human health. Furthermore, edible mushrooms can be contaminated by heavy metals from the soil and environment. The aim of this research is to determine the heavy metal extraction (Zn, Pb, Cu, and Cd) in four different samples of edible mushroom species (*Pleurotus ostreatus*, *Lentinus edodes, Agaricus bisporus,* and *Flammulina velutipes*) that were purchased from selected markets in Kelantan. The heavy metal was determined by Atomic Absorption Spectrometer (AAS) and the result was compared to the permissible level by WHO/FAO. Besides, the human health risk was assessed through target hazard quotient (THQ), hazard index (HI), estimated weekly intake (EWI), and provisional tolerable weekly intake (PTWI). The average heavy metals concentrations in four edible mushroom species were found to be in the descending order where Zn > Cu > Pb > Cd. Furthermore, the average THQ for Zn, Pb, Cd, and Cu was 6.413×10^{-5}, 5.877×10^{-4}, 3.05×10^{-4} and 6.55×10^{-5}, respectively, whereas, EWI were found do not exceed the PTWI. There is no significant risk because HI values are less than 1 which is 0.001. Based on the results, we can conclude that the analysed edible mushroom species do not pose a health risk to humans when consumed for a long term due to the relatively low present heavy metals concentration in the investigated mushroom.

Keywords: Edible mushroom, Heavy metal, Atomic Absorption Spectrometry, Health risk, Concentrations

Penentuan Logam Berat dan Penilaian Risiko Kesihatan terhadap Cendawan Boleh Dimakan Dikumpul dari Pasaran Terpilih di Kelantan, Malaysia Menggunakan Spektrometer Penyerapan Atom (AAS)

ABSTRAK

Cendawan yang boleh dimakan adalah sejenis kulat yang digunakan sebagai makanan dan ubat dengan protin tinggi dan banyak dimakan oleh manusia dalam kehidupan seharian terutamanya di Asia. Walaupun protin tinggi, cendawan yang boleh dimakan mempunyai potensi untuk mengumpul logam berat. Kepekatan logam berat dalam cendawan yang boleh dimakan boleh memberi kesan negatif kepada kesihatan manusia. Tambahan pula, cendawan yang boleh dimakan boleh mencemari logam berat dari tanah dan persekitaran. Tujuan penyelidikan ini adalah untuk menentukan logam berat (Zn, Pb, Cu, dan Cd) dalam empat sampel berbeza spesies cendawan yang boleh dimakan (*Pleurotus ostreatus*, *Lentinus edodes*, *Agaricus bisporus*, dan *Flammulina velutipes*) yang dibeli dari pasaran di Kelantan. Logam berat ditentukan oleh Spektrometer Penyerapan Atom (AAS) dan keputusannya dibandingkan dengan tahap yang dibenarkan oleh WHO/FAO. Selain itu, risiko kesihatan manusia dinilai melalui Target Bahaya, Indeks Bahaya, Anggaran Pengambilan Mingguan, dan Pengambilan Mingguan Sementara. Purata kepekatan logam berat dalam empat spesies cendawan yang boleh dimakan didapati dalam susunan menurun di mana Zn > Cu > Pb > Cd. Tambahan pula, purata Target Bahaya untuk Zn, Pb, Cd, dan Cu ialah 6.413×10^{-5}, 5.877×10^{-4}, 3.05×10^{-4} dan 6.55×10^{-5}, masing-masing, manakala, anggaran Pengambilan Mingguan didapati tidak melebihi Pengambilan Mingguan Sementara. Tiada risiko yang ketara kerana nilai Indeks Bahaya kurang daripada 1 iaitu 0.001. Berdasarkan keputusan, kita boleh membuat kesimpulan bahawa spesies cendawan boleh dimakan yang dianalisis tidak menimbulkan risiko kesihatan kepada manusia apabila dimakan untuk jangka masa panjang disebabkan oleh kepekatan logam berat semasa yang agak rendah dalam cendawan yang disiasat.

Kata kunci: Cendawan boleh dimakan, Logam berat, Spektrometer Penyerapan Atom, Risiko kesihatan, Kepekatan

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LIST OF SYMBOL

>	More than
<	Less than
%	Percentage
℃	Degree Celcius
±	Plus minus
µ	Micro
+	Addition
-	Subtraction
×	Multiplication
÷	Division

LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organisation
WHO	World Health Organisation
MFA	Malaysia Food Act
FDA	Food and Drug Administration
AAS	Atomic Absorption Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ANOVA	Analysis of Variance
MRL	Maximum residue limit
PTFE	Polytetrafluoroethylene
HNO_3	Concentrated nitric acid
H_2SO_4	Sulphuric acid
$Cd(NO_3)_2$	Cadmium nitrate
$Zn(NO_3)_2$	Zinc nitrate
$Pb(NO_3)_2$	Lead (II) nitrate
$Cu(NO_3)_2$	Copper nitrate
Zn	Zinc
Cu	Copper
Pb	Lead
Cd	Cadmium

Hg	Mercury
Fe	Iron
Cr	Chromium
Ni	Nickel
As	Arsenic
Sn	Tin
Ag	Silver
Al	Aluminum
Na	Sodium
K	Potassium
Mg	Magnesium
Ca	Calcium
L	Litre
mL	Millilitre
mm	Millimetre
kg	Kilogram
mg/L	Milligram per litre
mg/kg	Milligram per kilogram
µg/L	Microgram per litre
v/v	Volume per volume
mg/kg/day	Miligram per kilogram per day
mg/kg/week	Miligram per kilogram per week
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 Background of Research

Mushrooms are special for their distinct taste, aroma, nutritional value, and medicinal properties. Mushrooms are known for their low energy content and high concentration of important biologically useful elements such as ß-glucans and antioxidants (A. Ihugba et al., 2018). Moreover, mushrooms have been identified as therapeutic foods with antibacterial, anti-mutagenic, and anti-tumoral properties. Most edible mushrooms have a full essential amino acid profile that makes they are a good source of high-quality protein (González et al., 2020). Mushroom is also a component of natural forest ecosystems and plays an important role in element and organic matter cyclic pathways. Macro fungi research in Malaysia focuses on the ecology, cultivation, and health benefits of edible mushrooms (Samsudin & Abdullah, 2019). Mushrooms also contain 5–15% dry matter and have a well-balanced mineral and vitamin composition. Malaysia has a high demand for a mushroom which is expected to increase in line with per capita intake and population growth. In 2018, Malaysia produced a total of 7.772 million tonnes of mushrooms which is up dramatically from 1.995 million tonnes in 2012 and China is the major producer of them.

On the other hand, studies of toxic elements in macro fungi have revealed a connection between mushroom heavy metal concentration and metal contamination sources (Semreen & Aboul-Enein, 2011). In addition, environmental and fungal factors have been shown to influence heavy metal accumulation in macro fungi. It accumulates in varying concentrations of heavy metal in different plants which is some plants will obtain metals more than others. Even though certain heavy metals are needed for plant growth, but with excessive accumulation of these metals by plants grown on polluted land is a sign of increased pollution (Leblebici et al., 2020). Hence, it is crucial to lower the possibility of human toxic metal poisoning and to monitor the heavy metal load in mushroom species regularly.

Cadmium, lead, and mercury is an example of heavy metal that can accumulate at higher levels in mushroom species than in other edible plants that according to numerous studies (Semreen & Aboul-Enein, 2011). All these heavy metals are dangerous to the human body if consumed with a higher amount that can lead to stomach irritation, vomiting, and diarrhea. Since the 1970s, the presence of trace toxic metals in edible mushrooms that could threaten human health has been the subject of much research (Huang et al., 2015). Many factors influence the occurrence of heavy metals in mushrooms including temperature, environmental conditions, and the concentration of macromolecules in each species cell wall. This means that edible mushrooms have a highly efficient mechanism that allows them to easily absorb heavy metals from the environment. Heavy metals commonly accumulate in mushrooms. There are numerous researches about heavy metals accumulation in edible mushrooms including Cd, As, Fe, Pb, Mn, Hg, and Zn have been published (Chen et al., 2009).

Heavy metal accumulation inside the human body is damaging to their health. In addition, toxic metals such as mercury (Hg) and arsenic (As) are not believed to have many positive effects on human health but they can be dangerous if they accumulate in the body over time (Rehman et al., 2018). The appearance of heavy metals in edible mushrooms can cause severe health risks to humans, especially when concentrations exceed the basic requirements of the human body. According to World Health Organization (WHO, 2018), a report addendum in 2016 come out that chemical exposures claimed 1.6 million lives and 45 million confirmed to have a disability. Furthermore, mushrooms can bioaccumulate more heavy metals in their fruit bodies because certain heavy metals are naturally occurring substances in the earth's crust (Sinha et al., 2019). Bioaccumulation shows the heavy metal joins human bodies through a mechanism that is happening at the base of the food chain.

According to James, (2015), maximum amounts of heavy metals in mushrooms are established by Regulation (EC) No. 1881/2006 which is based on wet weight. The maximum levels for common mushrooms such as oyster mushroom (*Pleurotus ostreatus)* and shiitake mushroom (*Lentinula edodes)* are 0.20 mg kg $^{-1}$ cadmium (Cd) and 0.30 mg kg $^{-1}$ for lead (Pb) while the maximum cadmium (Cd) level for all other mushroom is 1 mg kg $^{-1}$ (Cordeiro et al., 2015). The focus of this research was to determine the heavy metal concentration in four species of edible mushrooms and its risk to human health. The amount of heavy metal should not exceed the permitted levels for mushrooms in the human body. When the human body is exposed to heavy metals for a long period, it can cause a health risk and damage many organs in the body.

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1.2 Problem Statement

The majority of people were unconcerned about heavy metals in daily food intake, especially edible mushrooms. They just think the edible mushroom is a valuable health food, high in protein, and easy to consume every day. It is important to raise awareness of heavy metals that give negative impacts and health risks to humans such as interfering with metabolic function. For determining heavy metal health risks in humans, information on heavy metal concentrations in foods and consumer dietary intake is crucial (A. Ihugba et al., 2018). Although eating mushrooms can still be beneficial to human health, but a recent study indicates that taking in moderation may be enough to avoid heavy metal poisoning. Heavy metal toxicity has proved to be a significant hazard with several health risks related to it (Jaishankar et al., 2014).

Heavy metals can contaminate edible mushrooms with metals from the soils that reach the mushroom body and thus can be accumulated at high-level concentrations. Mn> Pb > As > Ni > Cu > Cd were the heavy metal concentrations contained in soil samples taken from agricultural lands (Leblebici et al., 2020). Even the soil contamination is minimal, heavy metal concentration in several edible mushroom species may be higher in natural conditions. As a result, eating mushrooms grown in soils with high metal concentrations could put people at risk for health problems. However, the appearance of heavy metals in the soil will stifle the biodegradation of organic pollutants (Wuana & Okieimen, 2011). It is essential to efficiently track heavy metal concentrations in the soil to minimize the amount and it can reduce the heavy metal that is contaminated in edible mushrooms.

As a final point, the presence of heavy metals in soil, water, and living objects is a major public health issue nowadays as it has been absorbed into plants, animals, and food in the environment. In addition, Zn, Cd, and Pb levels were discovered to be higher than the permissible maximums in some edible mushroom samples (Zhu et al., 2011). Therefore, the mushroom's crop yield is indirectly polluted with heavy metals, causing the quality and protection of edible mushrooms to deteriorate when sold in the market. Furthermore, mushroom demand is increasing as a result of their delicious taste and easy availability. Due to that, human health issues will become more difficult from time to time because the intake of heavy metals is not regulated and consumed in excess. Hence, the appearance of heavy metals should be determined in edible mushrooms to show the local people, especially in Kelantan that it will be a risk when they consumed too much in daily intake.

1.3 Significant of Research

Human always choose healthy foods for their daily intake, but not many people know that some of their foods contain heavy metals that can be harmful and affect on their health. By determining the heavy metal content in edible mushroom species through the current research it could be proved to the local people that some species of mushroom contain high amounts of heavy metal. The heavy metal concentration of Fe was found to be higher than other metals in all mushroom species studied (Sinha et al., 2019). At the same time, this research can help to guide people about the quantity of edible mushroom intake that is suitable for their body with the prescribed amount of heavy metal concentration.

The presence of heavy metals in soil is a major public health issue for environmental health safety due to their negative consequences for human health. The presence of toxic metals in the soil will stifle the biodegradation of organic pollutants (Wuana & Okieimen, 2011). Edible mushrooms will easily be contaminated by heavy metals through the soil that is used. There is need to continuously control heavy metal levels in the soil before planting the edible mushroom species so it can help to reduce the risk to human health when they are consumed. Even the level of contamination in the soil is low, heavy metal concentrations in some edible mushroom species may be higher (Isildak et al., 2007). Furthermore, metal accumulation through mushrooms was discovered to be species-dependent and highly influenced by the chemical composition of the substrate from which the mushrooms obtain their nutrients (Gebrelibanos et al., 2016).

Heavy metals could reach the food chain through edible mushrooms, so it is crucial to examine heavy metal levels and identify any pollution that may be harmful to human health (Huang et al., 2015). However, there is a limitation of the research in Malaysia on edible mushrooms containing high concentrations of heavy metals. This research could assist to prove the presence of toxic metals in edible mushrooms. In general, the deposition of heavy metals by mushrooms is a dynamic mechanism influenced by both environmental factors such as metal content and the amount of organic material present (Kokkoris et al., 2019). Information on heavy metal concentrations in edible mushrooms and the dietary consumption of their consumers is essential in determining the health risks to humans.

1.4 Objective of Study

The particular objective of this study is to

1. Extract heavy metal concentrations from four types of edible mushroom *(Pleurotus ostreatus, Lentinula edodes, Flammulina velutipes, and Agaricus bisporus)* collected from a selected market in Kelantan, Malaysia.
2. Determine heavy metal concentration presence in edible mushrooms using Atomic Absorption Spectrometer (AAS).
3. Evaluate health risk assessment in consuming four different types of edible mushrooms at Kelantan.

1.5 Scope of Study

The four sample edible mushroom species were dry ashing digestion and analyzed by using the Atomic Absorption Spectrometry (AAS) to determine the heavy metal content. At the end of the research, we can gather all the data and statistics about the concentration of heavy metal presence in edible mushrooms collected from selected markets in Kelantan. These four sample of edible mushroom from the selected market is chosen from the demand of consumer in Kelantan. Even though there has been some previous research about mushrooms in Malaysia, but the research on the presence of heavy metals on the edible mushroom is still lacking in Malaysia.

Table 1.1 List of scientific names and common names of edible mushrooms to be chosen as samples.

No	Scientific name	Common name
1.	*Pleurotus ostreatus*	Oyster mushroom
2.	*Lentinus edodes*	Shiitake mushroom
3.	*Flammulina velutipes*	Enoki mushroom
4.	*Agaricus bisporus*	Button mushroom

Determination of heavy metals is important in edible mushrooms to track their high degree of zinc, cadmium, lead, mercury, and other essential metal to give awareness of people in health risk significance (Tchounwou et al., 2012). In this research, data obtained will be compared which the permitted level of heavy metal that is highly suggested by the World Health Organization and Food and Agriculture Organization (WHO/FAO) (2011). To avoid health concerns, the consumer must take the necessary precautions to consume edible mushrooms in accordance with the permissible level.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metal

Heavy metals are major environmental pollutants, and their toxicity is rising due to ecological, evolutionary, biological, and environmental problems (Leblebici et al., 2020). Heavy metals are present in the environment due to natural processes and human activity. Metals include Cr, Cd, Ni, As, Pb, Hg, Ni, and Zn that usually found in food, vegetables, and plant. Al, Cs, Co, Mn, U are some of the other rare metallic pollutants that are present in small quantities but are extremely toxic. In addition, heavy metal is a term that is often used to refer to metals and semimetals that have been linked to pollution, possible toxicity, and ecotoxicity. Heavy metals such as Fe, Cu, Mn, and Zn are essential to humans because they play a significant function in biological processes (Gebrelibanos et al., 2016). Although heavy metals are required in varying quantities by living organisms which extreme levels can be harmful to the organism.

Some heavy metals are bioaccumulative which is they do not break down in the atmosphere and are difficult to metabolize (Ackova, 2018). That is the main reason why heavy metals accumulate in the food chain at primary producers through plant uptake and at consumer levels through ingestion. Heavy metal enters human bodies through a mechanism known as bioaccumulation which occurs at the bottom of the food chain. Toxic heavy metal bioaccumulation in the biodiversity of environmental ecosystems may have negative consequences for animals and humans (Ali et al., 2019). Depending on the dosage and length of exposure, almost any heavy metal may be harmful to ecosystems.

In general, the atomic number, atomic weight, and basic gravity of most heavy metals are all greater than 5.0. Despite the fact that certain metals meet some requirements and others do not, most will agree that mercury and lead are toxic metals with adequate density (Marie, 2018). Some toxic heavy metals like lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) are hazardous to human health even at trace levels, particularly in pregnant women and young children who are more susceptible to toxic metal toxicity. However, when heavy metals such as copper (Cu), zinc (Zn), and nickel (Ni) are present in trace amounts, they serve as micronutrients for human development (Liang et al., 2019).

Moreover, metals have an impact on a variety of physiological and biochemical systems in plants, and their toxicity varies depending on the plant species, metal type, concentration, and chemical state (Ackova, 2018). Heavy metals will reach plants in terrestrial environments mostly through the soil but they may also come from the surrounding atmosphere. However, heavy metals in soils can quickly make their way through the food chain and their concentrations in plants have a negative impact on cellular and physiological processes (Morkunas et al., 2018).

2.1.1 Classification of Heavy Metal

Heavy metal also comes out with any metallic chemical element and there are classified as essential and non-essential in general. Xenobiotics or foreign elements are non-essential metals (Al, Cd, Hg, Sn, Pb, Cr) that have no proven biological role, and their toxicity increases with growing concentrations. Meanwhile, essential metal (Cu, Zn, Ni, Co, Mo, Fe) is considered to play important biological roles and has a toxic at high concentrations (Authman, 2015). In addition, free radicals are known to be generated by some heavy metals that can lead to oxidative stress and types of cell damage.

Heavy metals such as cobalt (Co), nickel (Ni), copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) are essential that needed for growth and stress resistance as well as the biosynthesis and function of a variety of biomolecules (Ali et al., 2019). The different biomolecules consist of carbohydrates, growth chemicals, chlorophyll, nucleic acids, and secondary metabolites. Meanwhile, diverse types of species such as plants, animals, and microbes, may have different lists of essential heavy metals concentrations. Implying that heavy metals may be necessary for one group but not for another. However, when present in low concentrations in the rising medium, most heavy metals are essential to plant but they will only become harmful when a concentration limit is exceeded. On the other hand, a heavy metal such as chromium (Cr) was thought to be essential for humans based on results with experimental animals which are animals lacking in Cr have been shown to have a reduced capacity to use glucose in their diet but recent studies have removed it from the list of essential elements. (Zoroddu et al., 2019).

2.2 Species of Edible Mushroom

There are more than 2,000 species of mushroom in nature, but only about 25 are considered edible and only a small number are used commercially (González et al., 2020). The most consumed edible mushroom in Malaysia is a button species (*Agaricus bisporus),* followed by oyster (*Pleurotus ostreatus),* shiitake (*Lentinus edodes),* and enoki *(Flammulina velutipes)*. In addition, *Lentinula* is the major genus contributing to around 22% of all cultivated mushrooms while *Pleurotus* species which has five or six cultivated species and followed by *Agaricus* species and *Flammulina* species that contribute for 15% and 11% of the overall volume, respectively (Royse et al., 2017). However, several essential amino and mineral supplements such as potassium, phosphorus, zinc, and copper are all abundant in *Agaricus bisporus*. From 2011 to 2020, mushrooms have been designated as one of the high-value commodities under Malaysia's National Agro-Food Policy (Ahmad Zakil et al., 2020).

Besides, mushrooms come in a wide variety of species and differences in shapes and sizes. Interest in edible mushrooms is growing as the need for new forms of food grows and alternative sources of revenue for rural areas are sought. *Pleurotus spp.*, *Auricularia spp.*, *Flammulina spp.*, *Tremella,* and *Grifola* are between edible mushrooms that all have immune system strengthening, lipid-lowering, anti-tumor, microbial and viral properties, heart rate regulating, and other therapeutic effects in varying degrees. All of the mushroom samples that will examine in this research were known as edible mushrooms belonging to the Agaricomycetes class. This research will use four samples of edible mushrooms to determine heavy metals which are *Pleurotus ostreatus*, *Lentinus edodes*, *Flammulina velutipes,* and *Agaricus bisporus*.

2.2.1 *Pleurotus ostreatus*

Pleurotus ostreatus is the second most cultivated mushroom after button mushrooms from all of the mushrooms grown in Malaysia and it is called oyster mushrooms. It is a fungus that can grow on a variety of lignocellulosic substrates according to its lignocellulolytic enzymes, which convert lignocellulosic materials into usable carbohydrates for the mushroom (Koutrotsios et al., 2019). *Pleurotus ostreatus* also provides bioactive compounds including β -glucans that can help with cardiometabolic health. However, in vitro digestion of *Pleurotus ostreatus* generated many bioactive peptides that inhibited the angiotensin-converting enzyme (Dicks & Ellinger, 2020).

Pleurotus ostreatus is a component of natural forest ecosystems and plays a major role in element and organic matter cyclic pathways. *Pleurotus spp.* mushrooms are among the most common edible species, not only because of their properties but also because of their speed of growth and ease of cultivation. Besides, *Pleurotus ostreatus* also can accumulate heavy metals even the soil pollution is minimal but the concentration of heavy metals may be higher in natural conditions (A. Ihugba et al., 2018).

2.2.2 *Lentinus edodes*

Lentinus edodes or also known as shiitake mushrooms that also being famous worldwide. In the most studied species mushrooms, they appear to have antibacterial activities against both positive and negative bacteria. The therapeutic macrofungus of

Lentinus edodes is the first to enter the field of modern biotechnology. However, it is the world's second most common edible mushroom which is according to its nutritious value as well as the possibility of therapeutic applications. *Lentinus edodes* also include other compounds that prevent blood coagulation, lower cholesterol levels, and have antibacterial and antiviral properties (Bisen et al., 2010).

In addition, *Lentinus edodes* protein is made up of 18 different amino acids which is including all of the important ones in proportions that are close to those found in human nutrition. It is may also be used as a protein, either whole or in condensed extract form, or as a dietary supplement. Therefore, the levels of trace metals found in *Lentinus edodes* were high in previous research based on comparisons between organisms and it can absorb significant amounts of heavy metals (Huang et al., 2015).

2.2.3 *Flammulina velutipes*

Flammulina velutipes is a commonly cultivated medicinal mushroom with a pleasant flavour, beneficial nutraceutical capabilities, and a range of important biological functions and also known as enoki mushrooms. *Flammulina velutipes* are a long, thin type of mushroom native to China, Japan, and Korea, with small caps on one end. Furthermore, several studies have shown that *Flammulina velutipes* is a great resource of polysaccharides and fibers that have antioxidant, anti-inflammatory, cholesterol-lowering, and immunomodulatory properties (Dong et al., 2021). *Flammulina velutipes* is high in amino acids, minerals, and vitamins, as well as being low in calories and fat.

Hence, the concentration of heavy metal was found in *Flammulina velutipes* was low in concentrations of Cd in previous research (Huang et al., 2015). According to

Baghel, S. (2019), consumption of *Flammulina velutipes* can cause increased plasma creatine kinase activity, which has negative effects on both skeletal and cardiac muscle. In addition, there has been one confirmed case from previous research of *Flammulina velutipes* poisoning with digestive problems (Mustonen et al., 2018).

2.2.4 *Agaricus bisporus*

Agaricus bisporus or known as button mushrooms is grown in over 70 countries and are one of the most popular and commonly eaten mushrooms worldwide. Furthermore, Agaricus bisporus is the mushroom with the largest global yield. Since there is no protective cuticle layer on the skin of *Agaricus bisporus*, it is vulnerable to physical and metabolic damage (Zhang et al., 2018). Even as *Agaricus bisporus* is low in fat but it does contain essential fatty acids including linoleic acid.

There is more research that has focused on its immune-modulating and therapeutic potential, so it has little information on its health properties is available (Jeong et al., 2010). It is also often used to help with physical and emotional tension, as well as to strengthen the immune system. In addition, the previous research found that *Agaricus bisporus* can be an essential source of nutritional metals elements such as Cu, Fe, and Se which is having an especially high content of these elements (Mleczek et al., 2020)

2.3 Heavy Metals in Edible Mushroom

The heavy metal concentration of edible mushrooms has been found to have both direct and indirect effects on the environment and human health. The concentrations of heavy metals in certain edible mushroom components such as the stipe and cap were also evaluated and the results revealed that Cr, Zn, Fe, Cd, Pb, and Cu easily accumulate in greater amounts in the caps, whereas Ni and Mn appear to accumulate more in the stipes (Mohsen Dowlati et al., 2021). Consumption of these heavy metals is extremely dangerous because their accumulation in the body is linked to a variety of diseases. Many research have looked into the accumulation of heavy metals in the fruiting bodies of edible mushroom species (Nnorom et al., 2020). Therefore, information on the levels of heavy metals in food and the dietary intake of their consumers is essential.

Edible mushrooms have the potential for accumulating heavy metals. The accumulation of heavy metals by edible mushrooms is a complicated process influenced by environmental such as soil, metal content, amount of organic material, and physiological factors. A huge range of environmental pollutants which is including heavy metals that have been found to have a high biosorption ability in mushroom organisms (Kapahi & Sachdeva, 2017). Besides, heavy metals have a negative impact on mushrooms cellular and physiological processes when present in high concentrations. The concentration of heavy metals in the fruiting bodies of certain mushrooms has been focused on much research that heavy metals are absorbed from the substrate by mushrooms through their large mycelium. However, with an increase in metals in the substrate, the concentration of heavy metals in the mushroom bodies continues to increase.

In addition, edible mushroom species that grow near contaminated areas have the potential to accumulate high levels of heavy metals in their bodies. Therefore, an edible mushroom grown in agricultural areas contaminated with heavy metals absorbs these materials over the WHO's allowable range (Leblebici et al., 2020). Edible mushrooms consume and bioaccumulate heavy metals even more quickly than other agricultural products, according to their effective mechanisms. Furthermore, the accumulation of heavy metals by edible mushrooms is mostly genetically determined by the physiology of the organisms, sampling place, mineral composition of substrates, nutrient absorption in mushrooms, and distance from pollution sources (Nnorom et al., 2020). However, there is a growing interest in using mushrooms as biomonitors for trace elements and radionuclides due to their high accumulation potential.

Besides, the amount of heavy metal in the samples varies depending on the mushroom species. Some of the edible mushroom species stored large amounts of heavy metal, but some of them have a low ratio of metals. Heavy metals such as mercury, lead, and cadmium was found as high concentrations in research of mushroom species from an urban area. (Huang et al., 2015). However, there have been a few recent studies on the levels of the heavy metal of edible mushrooms of sampling sites in Malaysia that have been conducted. Thus, it was considered appropriate to be concerned about the heavy metal contents of the most often collected edible mushroom in Kelantan, Malaysia. There was some previous research that has been done to determine the residue of the heavy metal in several species of edible mushroom.

The summary findings of heavy metals in edible mushrooms from previous research and the values of the permissible limit requirements for heavy metals in mushrooms were also compiled in Table 2.1 and Table 2.2. According to previous research by Huang, Jia, Wan, and Jiang (2015), in China, the research indicated that the heavy metals contained in various types of edible mushrooms are high concentrations of some heavy metals such as Pb, Cd, and Hg that exceeded the permissible limits by the Chinese standard (MH 2012) (Huang et al., 2015). Besides, according to research conducted by Cocchi, Luigi, Vescovi, and Luciano (2006), in Italy, the majority of edible mushroom species contain very high amounts of mercury from the maximum acceptable that recommended by WHO which is not more than 0.3 mg. However, edible mushrooms that particularly those found near heavily populated highways, can contain higher levels of heavy metals than plants.

Table 2.1: Summary of previous research on the concentration of heavy metal residues in edible mushrooms.

Edible mushrooms	The concentration of heavy metal residues (mean± standard deviation) (mg/kg)							Location	References
	Zinc (Zn)	Lead (Pb)	Cadmium (Cd)	Copper (Cu)	Mercury (Hg)	Arsenic (As)	Iron (Fe)		
Agaricus bisporus	39.56±3.2	3.8±1.2	2.12±3.3	41.81±1.5	-	-	254.68±3.7	Jordon	Semreen & Aboul-Enein, (2011)
Agaricus bisporus	71.7±16.6	2.63± 3.03	0.386± 0.402	-	0.232± 0.079	0.586± 0.203	175.3± 63.7	Beijing, China.	Huang et al., (2015)
Agaricus bisporus	51.8±4.6	6.9±0.3	0.10±0.01	11.9±1.0	-	-	332±22.6	Kastamonu, Turkey.	Mendil et al., (2004)
Pleurotus ostreatus	86.4±45.8	3.42±3.95	0.753±2.23	-	0.067± 0.028	0.322± 0.164	185.2± 69.1	Beijing, China	Huang et al., (2015)

Pleurotus ostreatus	1.02±0.001	0.36±0.01	-	0.06±0.01	-	-	-	Imo State, Nigeria.	A. Ihugba et al., (2018)
Flammulina Velutipes	62.7± 12.0	2.56±2.29	0.160± 0.173	-	0.049± 0.018	0.132± 0.034	153± 34.4	Beijing, China	Huang et al., (2015)
Lentinus edodes	61.3 ± 4.5	0.92 ± 0.05	0.21 ± 0.01	7.47 ± 0.55	-	-	67.5 ± 5.4	Yunan, China	Zhu et al., (2011)
Lentinus edodes	111±37.0	2.85±4.11	1.67±1.92	-	0.055± 0.049	0.636± 0.320	194±104	Beijing China	Huang et al., (2015)

Table 2.2: Show all the Maximum Residue Limit (MRL) of heavy metal concentrations (mg\kg) in edible mushrooms that had been reported by WHO/FAO (2011).

Types of Heavy Metals	WHO/FAO
Zinc (Zn)	60
Lead (Pb)	0.3
Copper (Cu)	40
Cadmium (Cd)	0.2
Arsenic (As)	0.01
Mercury (Hg)	0.01- 0.02

(Source: ((WHO) & (FAO), 2011))

2.4 Toxicity of Heavy Metals

Toxicity of heavy metals refers to the negative consequences of prolonged exposure or ingestion of concentrations that exceed the normal prescribed limits (Onakpa et al., 2018). Any metallic material with a relatively high density that is harmful even at low doses is defined as heavy metal. The toxicity of heavy metals is also harmful to human health depending on the intake daily consumed that has been absorbed by heavy metals in plants and food. However, individual metals have different toxicity signs, but the general signs of lead, cadmium, mercury, arsenic, aluminium, zinc and copper poisoning are the same (Nkwunonwo et al., 2020). As, Cr, Cd, Pb, and Hg are currently classified as priority metals due to their high toxicity, which is becoming a significant public health issue. In addition, if heavy metals are not digested by the organism after entering the body by food, water, air, or skin absorption, they become toxic and accumulate in soft tissues (Ahmad Bhat et al., 2019).

2.4.1 Zinc (Zn)

Zinc is a crucial element of a wide range of proteins, where it serves as a catalytic, structural, and regulatory component. It is a lustrous bluish-white metal that refers to the periodic table of Group 12. In addition, zinc deficiency may cause loss of appetite, growth retardation, fatigue, low mood, and sexual stagnation, especially in children. It is a vital heavy metal in the human body and its stability refers to the equilibrium between dietary zinc absorption and zinc loss from the body (Gu & Ruei-Lung Lin, 2010).

However, the major portion of zinc is used in industrial activities such as mining, coal, waste combustion, and steel manufacturing. Zinc is a very reactive metal that produces hydrogen and dilutes acids when it combines with oxygen and other non-metals (Parmar & Thakur, 2013). Even though humans can handle a significant amount of zinc but if taking too much of it can cause serious health problems. Overexposure to zinc can result in fevers symptoms, nausea disturbances, or liver disease.

2.4.2 Lead (Pb)

Since lead is a particularly dangerous metal to living organisms, it has been phased out of a variety of products in recent years, including paints and gasoline. Lead is a bright silvery metal that is slightly bluish in a dry environment. However, ionic and oxidative stress pathways in living cells cause lead toxicity. Furthermore, industrial activities, food processing, smoking, drinking water, and household sources are the major sources of lead exposure. Even though lead poisoning is preventable, it is still a serious threat because it can affect almost all of the major systems (Parmar & Thakur, 2013).

Lead is mostly absorbed by the inhalation of lead that is contaminated from dust particles or aerosols, as well as through the consumption of lead that contaminated food, drink, and paints. The major sources of lead ions are environmental and domestic that are the primary cause of disease. Moreover, the kidney absorbs the most amount of lead in the human body, followed by the liver and other soft tissues including the heart and brain. But, with adequate precautionary steps, the risk of lead poisoning can be reduced. Lead also is a highly toxic heavy metal that interferes with a variety of plant developmental processes which is unlike the other metals include zinc, copper, and manganese, it has no biological functions (Jaishankar et al., 2014).

2.4.3 Copper (Cu)

Copper is a micronutrient that serves as a biocatalyst and is necessary for body pigmentation. It also has been discovered to play an important role in physiological processes such as photosynthesis, respiration, carbohydrate degradation, nitrogen use, and cell wall metabolism, as well as controlling DNA and RNA processing and playing a role in disease resistance mechanisms (Aricak et al., 2019). Long-term exposure to high levels of copper through polluted food and water supplies may cause copper toxicity. Even copper is essential but in excessive amounts, it can be toxic because it can displace less competitive metal ions from metallic proteins such as zinc and iron (Zoroddu et al., 2019).

Copper can cause a variety of health impacts in excessive amounts but if the copper deficiency can have serious consequences including neurodegeneration. Although copper is found naturally in many plant and animal foods, the human body only stores about 50–120 mg of it (Kim et al., 2019). However, there is much food that contains copper such as animal meat, grains, vegetables, and mushrooms. This makes it very easy for ha humans to be exposed to copper poisoning because foods that have contained copper are easily consumed by them. In addition, copper particles and gases can be inhaled by workers in the agriculture, water treatment, and mining industries because of their environment.

2.4.4 Cadmium (Cd)

Cadmium is a non-essential substance that has no beneficial role in plants, animals, or humans and has no nutritional value due to its extreme toxicity. Cadmium toxicity occurs when a person inhales high levels of cadmium from the air and consumes food and drinks water is contaminated with cadmium. In addition, it can cause serious damage to the lungs when inhaled at high levels (Ahmad Bhat et al., 2019). Moreover, when consumed cadmium in large quantities, it can cause stomach inflammation, vomiting, and diarrhea. In gastrointestinal assimilation, cadmium and micronutrients work well together (Latif et al., 2018).

Although perhaps a little quantity of cadmium stays in the body after consuming food contaminated with cadmium, if consumed in high amounts over time, it can induce kidney failure and damage bones. Human exposure to cadmium can occur from a variety of causes, including working in primary metal industries, consuming contaminated food, smoking cigarettes, and working in an environment that contaminates with cadmium. However it seen very rare, it can be found in soil, minerals, and water in the forms of sulfide, sulphate, carbonate, chloride, and hydroxide salts (Balali-Mood et al., 2021).

2.4.5 Mercury (Hg)

Mercury is a highly toxic metal which toxicity is a major cause of acute heavy metal poisoning. The most common cause of mercury poisoning is the ingestion of too much methylmercury or organic mercury, which is linked to eating seafood. Depression, memory loss, tremors, nausea, headaches, and baldness are all symptoms of organic mercury poisoning. In addition, vaporized mercury can enter the environment through rain, soil, and water which is posing a major threat to plants, animals, and humans. Thus, toxic mercury exposure has resulted in negative neurological and behavioural changes in animals (Jaishankar et al., 2014).

Mercury is a naturally occurring metal that can be used in small quantities in many consumer items. However, mercury can form organic and inorganic mercury by combining with other elements. Exposure to high levels of metallic, organic, and inorganic, can damage the emerging developing fetus's brain and kidneys (Alina et al., 2012). Mercury can be ingested in a variety of forms, and people can be exposed to it in a variety of ways. It is released into the atmosphere through volcanic activity, rock weathering, and human activity.

2.4.6 Arsenic (As)

Arsenic is a semi-metallic substance that is one of the most dangerous heavy metals, posing a threat to both the environment and human health. Arsenic through its inorganic forms, such as arsenite and arsenate, is more hazardous to human health. They are extremely carcinogenic and can cause lung, liver, bladder, and skin cancers (Jaishankar et al., 2014). Arsenic may be ingested among humans by natural resources, industrial sources, or unintentional sources.

In addition, organic and inorganic arsenic have been discovered in food products such as fruit, vegetables, fish, and meat. It is depending on a variety of factors such as soil type, growing climate, water supply, and food processing system. Low-level arsenic exposure can cause nausea and vomiting, irregular heartbeat, and blood vessel damage. However, long-term arsenic toxicity from drinking water and food has been linked to cancer and skin lesions, as well as cardiovascular disease and diabetes (FAO/WHO, 2011). Furthermore, there is no successful early treatment for chronic arsenicosis, which can induce irreversible changes in the human body's major organs and will be increasing the death rate. The most significant step in affected areas is to provide a clean water source to avoid further arsenic exposure.

2.4.7 Iron (Fe)

Iron is the most abundant transition metal in the crust of the earth. Since it is a trace element for many essential proteins and enzymes, it is the most important nutrient for most living beings. However, children are particularly vulnerable to iron toxicity because they are easily exposed to the greatest amount of iron metals from various products (Jaishankar et al., 2014). After high levels of iron are introduced into the human body, the amount of absorption stage becomes saturated, and free iron is absorbed into the cells of the liver, heart, and brain (Ahmad Bhat et al., 2019).

It is also the exposure that can lead to lung cancer including chronic bronchitis and breathing problems. Another main cause of cancer related to iron overdose is the formation of free radicals. Besides, some crops including rice which are particularly vulnerable to iron shortages. Different strategies are used by plants concerning primary iron uptake and bioavailability (Naeem et al., 2017). However, excess iron can be toxic, so iron supplements can only be used if a deficiency has been diagnosed or if the human is at high risk of developing one. When iron is exposed through cutaneous, oral, or inhalation routes, it shows that iron sulphate, iron sulphate heptahydrate, and iron sulphate monohydrate have low acute toxicity (Engwa et al., 2019).

2.5 Effect of Heavy Metal

Due to various their active inhibitory effects on biodegradation activities, these metals are closely linked to environmental contamination and biological toxicity issues.

Metals with high concentrations accumulate in soil that can cause negative effects on production and fertility. However, toxic metals that accumulate in the human body will be causing serious health effects, including growth and development disorders, carcinogenesis, neuromuscular disorders, psychiatric disabilities, and metabolic failure (Mishra et al., 2019).

2.5.1 Effect of Heavy Metals on Human

Heavy metal accumulation in the human body causes significant damage to various organs, especially the respiratory, mental, and reproductive systems, as well as the digestive systems. When heavy metals are consumed more than their prescribed dietary intake, they have a significant impact on human health. Even though these metals have no biological function, their toxic effects persist in some form that is harmful to human health and its proper functioning. Some metals, including aluminum that can be eliminated by normal body systems while others accumulate in the body and the food chain that can cause chronic effects (Jaishankar et al., 2014).

Even though heavy metals have many negative health effects and last for a long time, but the heavy metal still exposure continues and is growing in many parts of the world in uses such as metal plating, fertilizer, mineral processing, and chemical manufacturing. Furthermore, As, Cd, Cr, Cu, Pb, Zn, and Ni are the most commonly contained heavy metals in polluted water, and they all pose health risks to humans. As an example when humans are exposed to heavy metals such as chromium, it may cause inhibition of the enzyme carrier glutamine synthase which can reduce the ability to convert methemoglobin to hemoglobin in the human body (Morais et al., 2012).

It also exposes the health effect of humans such as irritation of the skin, ulcers, and kidney problems. On the other hand, the use of metal coating and metallurgy sectors are exposed to mercury that gives the negative health effect to humans such as memory issues, an increased heart rate, tremors, kidney damage, and brain damage are all symptoms of Schizophrenia. Some essential trace metals, such as Ni, Cu, Zn, and Mo, are needed for enzymatic and physiological functions, but excessive amounts of these metals can harm human health if consumed in large quantities from the environment (Mishra et al., 2019).

2.5.2 Effect of Heavy Metals on Environment

Various toxic metals have polluted the environment, including soil, water, and air. Heavy metals include Zn, Pb, Fe, and Cu are abundant in the environment and play an important role in ecosystem survival and sustainability. Heavy metal pollution is a major environmental health issue that can be harmful due to bioaccumulation through the food chain, which is caused by rapid industrial development and developments in agricultural chemical usage (Onakpa et al., 2018). In addition, because of the widespread use of Cr salts in the polish tanning process, leather factories are mainly responsible for Cr pollution in the environment. This would have contributed to the dispersion of heavy metals in the environment which is resulting in population health problems. Since the fertilizer can contain high levels of toxic metals, polluted food crops may accumulate in the soil and expose the environment to heavy metals. Moreover, heavy metals are toxic to plant life processes due to interactions with functional groups of molecules in the cell, especially proteins and polynucleotides (Pokorska-Niewiada et al., 2018)

Heavy metals used in drinking and natural water sources contaminate the natural environment in both direct and indirect ways. However, heavy metals are major environmental contaminants, and their toxicity is becoming more of a concern for ecological, developmental, nutritional, and environmental reasons (Jaishankar et al., 2014). Heavy metals which are emitted into the environment as a consequence of volcanic activity and various industrial emissions will because contaminate water and soils (Briffa et al., 2020). In addition, heavy metal emissions in the environment also can be increased by solid and liquid waste products emitted by factory industries.

2.6 Influence Heavy Metal on Food Security

Food security has been designated as one of the most important sustainability goals due to its importance to humanity's long-term viability and well-being. However, heavy metal contamination is high due to a variety of anthropogenic activities, as well as agricultural and environmental activities. According to studies, heavy metal levels in food have exceeded standard thresholds and may represent a threat to food security and living forms. The issue of Food insecurity caused by heavy metals found in food has recently pervaded the Sustainable Development Goals (SDGs) actions on sustainable living for current and future generations (Anthony et al., 2020).

Moreover, these issues will be resulting in ill-health and poor well-being, particularly in developing and third-world countries, where knowledge of the impacts is limited. It is because heavy metals persist in the ecosystem due to their habitation potentials and non-degradable natural condition, and they bioaccumulate in food such as plant and animal tissues, posing potential major harm to humans. To complement and strengthen food security, they advocate using a microbial bio-sorption approach to remove excess heavy metals from soil (Ayangbenro & Babalola, 2017).

2.7 Methods of Study in Heavy Metal Residues

A range of instrumental analytical approaches can be employed to determine the concentration level of heavy metals in various samples. Atomic absorption spectrometry (AAS), atomic emission or fluorescence spectrometry (AES/AFS), inductively coupled plasma mass spectrometry (ICP-MS), and inductively coupled plasma optical emission spectrometry (ICP-OES) is the most commonly used to determine heavy metal (Helaluddin et al., 2016). However, correct sampling is one of the most basic stages required to assure accuracy in analysis. Then, to determine the quantities of heavy metals in foods, the techniques such as dry ashing and wet digestion are used.

This method was allowed for the collection of condensed metal extracts to be analyzed using atomic absorption spectroscopy (AAS). Much previous research has shown that using an AAS is essential to determine heavy metal levels in foods. AAS is one of the methods that are important to many industrial and research laboratory processes. In addition, environmental testing, toxicology, and quality control include spectrometers with low detection limits and fast testing methods are among the reasons that AAS is an advanced model. The health risk of heavy metals can be determined by comparing predicted dietary that consumed by Kelantan residents exposure to recommended safe exposure levels. The allowed levels of heavy metals can be compared to those set by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO).

2.8 Atomic Absorption Spectrometer (AAS)

Atomic absorption spectrometry (AAS) is the most commonly used method for quantifying heavy metals in environmental samples. The total quantities of Cd, Cu, Pb, and Zn must be analyzed using the AAS method, which necessitates effective destruction of the organic matrix, according to EU regulations (Turek et al., 2019). An important analytical instrument for both quantitative and qualitative research is the absorption of light by atoms. Furthermore, atomic absorption occurs when a ground state atom enters the excited state and absorbs light energy from a specific wavelength. AAS also is a metal analysis procedure that can be used to determine about 70 elements (Helaluddin et al., 2016).

Depending on the light wavelength and intensity, relevant elements can be identified and their concentrations determined. However, AAS is still a common option for uncomplicated trace elemental analysis because it has an infinite number of applications. AAS has a wide range of applications in chemistry such as environmental analysis, pharmaceuticals, clinical analysis, mining, and industry. In addition, the technique of atomic absorption is widely used to detect metals and metalloids in environmental samples (Ahmed, 2016). In certain cases, atomic absorption spectrometry is used to investigate unusual issues

2.9 Health Risk Assessment

Health risk assessments gather self-reported data that health and fitness practitioners use to identify risks, segment populations, develop and execute interventions, and track outcomes. A human health risk assessment is a procedure for determining the extent and significance of adverse health effects in humans that might be exposed to chemicals in a polluted environment. It also known as the process of assuming any specific amount of adverse health effects occurring over a given period.

The human health risk assessment is an essential part of the environmental protection strategy for protecting people from contaminants in health risks. It is also a tool for gathering health data, usually in conjunction with a method that involves biometric testing. Besides, it consists of 4 fundamental concepts which are hazard identification, the response of assessment, exposure assessment, and characterization of the risk. In addition, carcinogenic and non-carcinogenic effects may be distinguished in human risk (Yi et al., 2011).

However, a variety of researchers have used the probability risk assessment method. In addition, target hazard quotients (THQ) and health index (HI) will be used as a method to measure the potential of health risk. THQ is the ratio of toxic element exposure to the reference dose, which is the maximum amount at which no negative health effects are predicted (Antoine et al., 2017). The THQ identifies the noncarcinogenic health risk presented by exposure to the toxic metal in consideration. While, hazard index (HI) is the amount of the selected target hazard quotients of the elements evaluated for each food category (Antoine et al., 2017). The HI states that eating a certain form of food exposes you to several potentially toxic metals.

Table 2.3: Previous studies on health risk assessment in humans in edible mushrooms consumption for adults.

	Zn	Pb	Cu	Ni	Cd	Location	References
Target Hazard	0.0033	0.01142	0.00875	0.000005	NA	Okigwe, Nigeria	A. Ihugba et al., (2018)
Quotient (THQ)	0.00276	0.01142	0.02075	0.000005	NA	Anara, Nigeria	A. Ihugba et al., (2018)
	0.13	0.25	NA	0.22	NA	Zambia. Africa	Chungu et al., (2019)
	0.01083	0.100	0.001	0.000005	NA	Ohaji, Nigeria	A. Ihugba et al., (2018)
Estimated Weekly	4.41	0.46	0.574	0.00035	NA	Nigeria	A. Ihugba et al., (2018)
Intake (EWI)	2.9	NA	1.4	NA	NA	Tunceli, Turkey	Alp et al., (2020)
(mg/kg bw/week)	0.01052	0.00004	0.00250	0.00005	0.00007	Bangladesh	Rashid et al., (2018)
Health Risk	0.0050	0.0015	0.0089	0.0004	0.0097	Bangladesh	Rashid et al., (2018)
Index (HRI)	0.005289	0.053319	0.005332	0.006803	0.200404	Bulgaria	Dospatliev et al., (2019)

*NA : not available

CHAPTER 3

METHODOLOGY

3.1 Area of Study

Four different samples of edible mushrooms are collected from the selected market in Kelantan. All the four samples (*Pleurotus ostreatus, Lentinus edodes, Flammulina velutipes,* and *Agaricus bisporus*) that were used in this research are chosen by the most consumed types of mushrooms in Kelantan. Before being digested and analyzed, the samples of edible mushrooms are stored in a clean and dry place.

3.2 Instrumentation

The four samples of edible mushrooms are performed by using an Atomic Absorption Spectrometer (AAS) (Perkin-Elmer(PE) 3300) to determine the concentration level of heavy metal. When using the AAS, a microwave sample preparation device was used and then operated by a computer auto-sampler.

3.3 Chemical and Reagents

The chemicals and reagents that were used in this analysis are mentioned in Table 3.1. The chemicals and reagents used were all of the analytical quality unless specified otherwise (Tüzen et al., 2003).

Table 3.1: List of chemicals and reagents used in this research

No.	Chemical and Reagents
1.	Concentrated nitric acid (HNO_3)
2.	Hydrochloric acid (HCL)
3.	Hydrogen peroxide (H_2O_2)
4.	Sulphuric acid (H_2SO_4)
5.	Deionized water
6.	Cadmium nitrate ($Cd(NO_3)_2$)
7.	Copper nitrate ($Cu(NO_3)_2$)
8.	Zinc nitrate ($Zn(NO_3)_2$)
9.	Lead nitrate ($Pb(NO_3)_2$)

3.4 Apparatus

As shown in Table 3.2, all the apparatus that was used in this research.

Table 3.2: List of laboratory apparatus

No.	Apparatus
1.	Volumetric flask 25 mL and 50 mL
2.	Weighing machine
3.	Beaker
4.	0.45 mm PTFE filter
5.	Stainless steel spatula
6.	Pestle and Mortar
7.	Polyethylene bottles
8.	Porcelain crucible
9.	Electric hot plate
10.	Muffle furnace
11.	Atomic Absorption Spectrometry
12.	Winlab 32^{TM} AAS computer software
13.	Laboratory homogenizer
14.	Laboratory micro oven
15.	Laboratory refrigerator

3.5 Collection of Samples

There is a total of four different species of edible mushrooms that were purchased around selected markets in Kelantan which is the main focus region in this research. The four types of species of edible mushrooms that were purchased were *Pleurotus ostreatus, Lentinus edodes, Flammulina velutipes,* and *Agaricus bisporus* which is the most highly consumed of residents in Kelantan to determine their heavy metal.

3.6 Preparation of Blank Reagent Solution

When using techniques like spectrophotometry, reagent blanks are often used to zero the instrument before calculating test samples and other blanks (Cantwell, 2019). Thus, 5 mL nitric acid (HNO_3), 5 mL sulphuric acid (H_2SO_4), and 1 mL hydrogen peroxide (H_2O_2) were used to make the blank reagent solution. In the 50mL volumetric flask, the solution was diluted with deionized water up to 50 mL.

3.7 Preparation of Standard Calibration Solution

For each element, there are instructions for preparing the stock standards. The shelf life of these stock standards, which come with a certificate of analysis and are traceable to NIST standards, is usually one year (Perkin Elmer, 1996). A concentration of 1000 mg/L stock solution for each of the heavy metals Zn, Pb, Cu, and Cd was prepared. The standard calibration solution for each heavy metal is 1.5980g of lead nitrate

($Pb(NO_3)_2$), 2.1032g of cadmium nitrate ($Cd(NO_3)_2$), 3.798 g of copper nitrate ($Cu(NO_3)_2$) and 1.245 g of zinc nitrate ($Zn(NO_3)_2$) will be solving in deionized water and then diluting to 1000 mg/L in a volumetric flask with deionized water (Ribeiro, 2014). Thus, at concentrations ranging from 5 µg/L to 100 µg/L, the normal calibration solution for each heavy metal was prepared.

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

A standard calibration solution is a dilute solution that is used to generate a calibration curve in research. It is necessary to prepare and calculate a calibration blank as well as the calibration standards when deciding the instrument's working range, which is the range of concentrations in processed samples that can be introduced to the instrument for calculation (Cantwell, 2019). From the previously prepared stock solutions, standard calibration solutions for each type of heavy metal were made.

3.8.1 Standard Calibration Solution for Pb

An amount that is set of stock solution of Pb will be diluted with 500 µL of 0.5% v/v nitric acid in a 50 mL volumetric flask which is at a concentration ranging from 0.50 mg/L to 10.00 mg/L. The mixture was diluted with deionized water until it reaches a volume of 50 mL. Then, the Atomic Absorption Spectrometry system was calibrated with 50 mL of Pb standard calibration solution with concentrations ranging from 0.50 mg/L to 10.00 mg/L (Handayani et al., 2019).

3.8.2 Standard Calibration Solution for Cu

An amount that is set of stock solution of Cu was diluted with 500 µL of 0.5% v/v nitric acid in a 50 mL volumetric flask which is at a concentration ranging from 2.00 mg/L to 20.00 mg/L.The mixture was diluted with deionized water until it reaches a volume of 50 mL. Then, the Atomic Absorption Spectrometry system was calibrated with 50 mL of Cu standard calibration solution with concentrations ranging from 2.00 mg/L to 20.00 mg/L (Hu & Qi, 2013).

3.8.3 Standard Calibration Solution for Zn

An amount that is set of stock solution of Zn was diluted with 500 µL of 0.5% v/v nitric acid in a 50 mL volumetric flask which is at a concentration ranging from 0.50 mg/L to 4.00 mg/L. The mixture was diluted with deionized water until it reaches a volume of 50 mL. Then, the Atomic Absorption Spectrometry system was calibrated with 50 mL of Zn standard calibration solution with concentrations ranging from 0.50 mg/L to 4.00 mg/L (Hu & Qi, 2013).

3.8.4 Standard Calibration Solution for Cd

An amount that is set of stock solution of Cd was diluted with 500 µL of 0.5% v/v nitric acid in a 50 mL volumetric flask which is at a concentration ranging from 0.00

mg/L to 1.00 mg/L. The mixture was diluted with deionized water until it reaches a volume of 50 mL. Then, the Atomic Absorption Spectrometry system was calibrated with 50 mL of Cd standard calibration solution with concentrations ranging from m 0.00 mg/L to 1.00 mg/L (Hu & Qi, 2013).

3.9 Preparation of Sample

The samples of each type of edible mushroom were collected from selected markets in Kelantan and have brought to the laboratory. Each of the four samples was washed with deionized water and the sample was dried at 60˚C for 24 hours in a food dehydrator (Leblebici et al., 2020). Then, each of the samples was crushed and blended in a ceramic mortar into a powder of very fine quality and labeled. Each sample was then homogenized with an agate pestle and held in pre-cleaned polyethylene bottles at -20 °C until the analysis started with a dry ashing digestion method. Atomic Absorption Spectrophotometer (AAS) was used and the concentrations of Pb, Cu, Zn, and Cd in the digests were determined (A. Ihugba et al., 2018).

3.10 Sample Analysis for Determination of Heavy Metal Residue by Atomic Absorption Spectrometry (AAS)

In this research, the digestion method that was used is dry ashing digestion that is particularly suitable for biological tissues, plant, and food samples with high organic matter. Many of the related elements in the samples are usually converted to carbonate or oxide types in the residue using a muffle furnace with temperature control systems and oxygen in the atmosphere as the oxidizing agent (Hu & Qi, 2013). First of all, 1.00 g of dried sample of each mushrooms types was placed into a high porcelain crucible. The porcelain crucible will be heated and placed in a muffle furnace at 600°C for 6 hours and steadily increased to make the sample dry ashing (Tsegay et al., 2019). Then, the samples were ashed until they left a white or grey ash residue. Besides, the porcelain crucible is the best vessel for dry ashing because it is unaffected by all of the common acids except concentrated, high-temperature phosphoric acid and aqua regia (Hu & Qi, 2013).

After that, 10 mL of concentrated mixed acid (HCl: HNO_3=1:1) was used to digest the ashed samples. The digestion solution was heated to 150 °C on an electric hot plate for one hour or until it was nearly dry. Then, the residue was filtered using a PTFE filter and syringe filter. The sample was moved into a volumetric flask with added 3% HNO_3 to make up to 50 mL. Move 15 ml from the stock sample into falcon tube for sample analysis and serial dilution. Before the analytical phase, the digested solution was placed in a polyethylene bottle. However, the blank digestion tests were carried out in the same way. All the four heavy metals (Zn, Pb, Cu, and Cd) were analyzed by using the Atomic Absorption Spectrometer (AAS). Furthermore, the data was displayed in μg/L (w/v) units and then, the conversion formula was used to convert (w/v) to dry weight (mg/kg).

3.11 Calculation and Evaluation of Result

Each heavy metal concentration (C) in the sample was determined by using the equation (3.1) below:

$$C = \frac{(a-b) x V}{m x 1000} \quad (3.1)$$

Where,

C= Concentration in the test samples (µg/g)

a= Concentration in the test solution (µg/L)

b= Average concentration in the blank solution (µg/L)

V= Volume of the test solution (mL)

m= Weight of the test portion

3.12 Statistical Analysis

The experiment was repeated three times, with the average data outcome analyzed. One-way analysis of variance (ANOVA) was used to evaluate the mean concentration and significant variations for heavy metals in four samples of edible mushrooms. However, if the measured p values are less than 0.05, the result is considered significant. The determination of concentration heavy metal present in four sample edible mushrooms was in dry weight, mg/kg.

3.13 Survey conducted

There was a survey conducted using Google Forms and the survey was blasted to the respondent in Kelantan. This survey was provided with three sections which are sections A, B, and C. This section consists of social demographic, intentions of Kelantan residents towards edible mushrooms as a source of food, and frequency of intake edible mushrooms in a day, week, and month among Kelantan residents. This survey was a blast to all Kelantan people and students of the University Malaysia Kelantan.

3.14 Health Risk Assessment

The calculated daily intake was used to determine how much edible mushroom that Kelantan residents consume in a single day. Several methods were used to measure the health risk of edible mushrooms consumed by humans (A. Ihugba et al., 2018).

3.14.1 Estimated Daily Intake (EDI)

The following equation was used to measure the estimated daily consumption (EDI) (mg/kg body weight) of trace metals from eating four types of edible fungi (*Pleurotus ostreatus, Flammulina velutipes, Agaricus bisporus, and Lentinus edodes)* (Chungu et al., 2019).

$$EDI = \frac{(Cmetal\ x\ IR)}{ABW} \quad (3.2)$$

Where,

Cmetal = Averaged of heavy metals content in edible mushroom (mg/kg)

IR = Ingestion rate daily intake of edible mushroom (gram/day person)

ABW= Average body weight.

3.14.2 Estimated Weekly Intake (EWI)

The following equation was used to measure the estimated weekly consumption (EWI, mg/kg body weight/week) of trace metals from eating four types of edible fungi (*Pleurotus ostreatus*, *Flammulina velutipes*, *Agaricus bisporus and Lentinus edodes)* (A. Ihugba et al., 2018):

$$EWI = \frac{(CWmushroomxCmushroom)}{BW} \quad (3.3)$$

Where,

Wmushroom = Weight of mushroom consumed (mg/kg)

Cmushroom = Metal concentration of mushroom (µg/g)

BW= Average body weight

3.14.3 Target Hazard Quotient (THQ)

This Target Hazard Quotient (THQ) is symbol links metal concentrations in food to toxicity, quantity, and quality of food consumed, and consumer body mass. The equation below was used to evaluate THQ using the following method (A. Ihugba et al., 2018):

$$THQ = \frac{EF x\ ED\ x\ FIR\ x\ C}{RfD\ x\ WAB\ x\ TA}\ x\ 10^{-3} \quad (3.4)$$

Where,

EF = Exposure frequency (365 days/year)

ED = Exposure duration (70 years) comparable to the human lifetime

FIR = Edible mushroom ingestion rate (g/person/day)

C = Metal concentration in Edible mushroom (mg/kg dry weight)

RFD = Oral reference dose (mg/kg/day)

Copper (0.04), Zinc (0.3), Cadmium (0.001), and Lead (0.0035)

WAB = Average body weight (kg)

TA = Averaged exposure time for non-carcinogens (365 days/year × Exposure duration)

3.14.4 Hazard Index (HI)

The sum of the hazard quotients is the hazard index (HI) derived from THQ (Dee et al., 2019). The following equation below was used to calculate the hazard index in Pb, Cu, Zn, and Cd:

$$HI = THQ_{Cu} + THQ_{Zn} + THQ_{Cd} + THQ_{Pb} \quad (3.5)$$

There is no apparent risk if HI is less than 1.

Where,

HI = Hazard index

THQ= Target Hazard Quotient

RESEARCH FLOW CHART

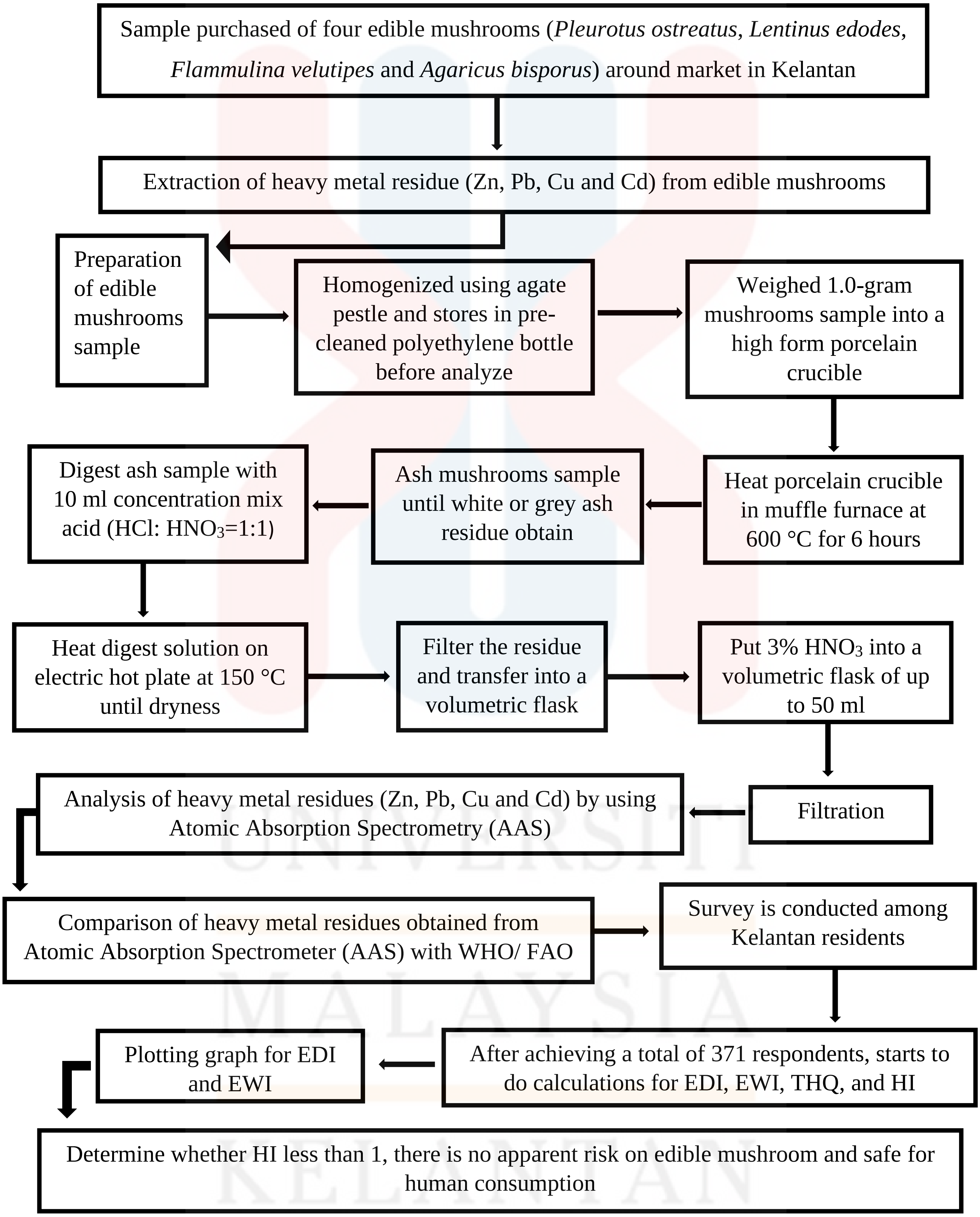

Figure 3.1: Summarize flow chart of research

CHAPTER 4

RESULT AND DISCUSSION

4.1 Samples Collection

A total of four edible mushroom species were purchased from various market places in Kelantan, which is the research's main focus location. The four types of species of edible mushrooms that were purchased were *Pleurotus ostreatus* (Oyster mushroom), *Lentinus edodes* (Shiitake mushroom), *Flammulina velutipes* (Enoki mushroom), and *Agaricus bisporus* which is the most highly consumed by residents in Kelantan.

4.2 Samples Preparation

All of the purchased samples were rinsed with distilled water and chopped into small pieces before being placed in a sample bag and kept in the refrigerator. Several researchers have utilised mushrooms as a bioindicator to evaluate heavy metal contamination levels (Dospatliev & Ivanova, 2017). Each of the four samples was rinsed with deionised water and dried in a food dehydrator at 60°C for 24 hours (Leblebici et al., 2020). The samples were then crushed and mixed into a very fine powder in a blender. Each sample was then homogenised with an agate pestle and stored at -20 °C in pre-cleaned polyethylene bottles until the dry ashing digestion method was used. The amount concentrations of Pb, Cu, Zn, and Cd in the digests were measured using an Atomic Absorption Spectrophotometer (AAS) (A. Ihugba et al., 2018).

4.3 Samples Extraction

The digestion method utilised in this study was dry ashing digestion, which is especially good for biological tissues, plants, and food samples with a lot of organic stuff. This is usually described as the procedure of eliminating all biological matter from a sample, and total combustion of the sample is critical while ashing (Dávila G et al., 2020). A high porcelain crucible was filled with 1.00 g of dried samples of each mushroom species. The porcelain crucible was be heated in a muffle furnace for 6 hours at 600°C, then gradually increased to make the sample dry ashing (Tsegay et al., 2019). The samples were then ashed until a white or grey ash residue remained. After that, the ashed samples were digested with 10 mL of concentrated mixed acid (HCl: HNO_3=1:1). The digestion solution was heated to 150 °C on an electric hot plate for one hour or until it was nearly dry and this method was carried out in a fume hood. Then, the residue was filtered using a PTFE filter and syringe filter. The sample was moved into a volumetric flask with added 3% HNO_3 to make up to 50 mL. Move 15 mL from the stock sample into falcon tube for sample analysis and serial dilution. All the four heavy metals (Zn, Pb, Cu, and Cd) were analysed by using the Atomic Absorption Spectrometer (AAS).

4.4 Metal Concentration in Different Edible Mushrooms Species

The outcomes of this research revealed four different heavy metal concentrations in four different edible mushroom species purchased from a selected market in Kelantan. The amounts of zinc, copper, lead, and cadmium in edible mushroom samples analysed were reported in Table 4.1 in mean values and standard deviations (s.d). After all the test samples were entered, the Atomic Absorption Spectrometer equipment instantaneously calculated the data. The following table 4.1 represents the mean and comparison of heavy metal concentrations for the examined edible mushroom species. All the data measurements were given in microgram per kilogram (mg/kg) and are based on wet weight.

The differences among the concentrations of Cd, Zn, Pb and Cu in four different edible mushrooms were significant in some cases, and this is likely due to its species and cultivated land. All of the metals analysed were found to be not above the detection limit of the FAO/WHO. According to table 4.1, which is the highest concentration is zinc, Zn (1.393 mg/kg), whereas the lowest concentration recorded is cadmium, Cd (0.013 mg/kg). Zinc is encouraged at the highest concentration because it is one of the most important minerals for our bodies and is significantly related to protein and nutrient diets. However, *Pleurotus ostreatus* had higher Zn, Pb, and Cd metal concentrations than *Lentinus edodes, Agaricus bisporus,* and *Flammulina velutipes* while Cu is higher in *Agaricus bisporus.*

The average metal concentrations in four edible mushroom species were used to come up with this result (Table 4.1), the highest level concentration of Zinc, (Zn), (1.393 mg/kg) was found in *Pleurotus ostreatus*; level for copper (Cu), (0.193 mg/kg) was found in *Agaricus bisporus* and level of lead (Pb), and cadmium (Cd), (0.128 mg/kg; 0.02

mg/kg) were found in *Pleurotus ostreatus*. Conversely, the lowest concentration of zinc (Zn), (1.202 mg/kg) was found in *Flammulina velutipes*; level for copper (Cu) and lead (Pb), (0.059 mg/kg; 0.081 mg/kg) was found in *Lentinus edodes* and cadmium (Cd), (0.013 mg/kg) was found in *Lentinus edodes* and *Flammulina velutipes*.

In addition, the results were obtained by calculating the average amounts of means concentrations (Zn, Cu, Pb, and Cd) in the edible mushroom samples in the unit of mg/kg. The average concentrations of heavy metals are calculated as follows; Cu: 0.131 mg/kg; Zn: 1.2953 mg/kg; Cd: 0.01525 mg/kg and Pb: 0.10275 mg/kg respectively. The concentrations of heavy metals in edible mushroom samples were found to be in the following order where Zn > Cu > Pb > Cd. The metal concentrations in four sample edible mushrooms were ranked identically. Moreover, the presence of heavy metals in each of the edible mushroom samples was compared to the authorized levels by World Health Organization/ Food and Agriculture Organization WHO/FAO (2011). According to Table 4.1, all heavy metals (Zn, Cu, Pb, and Cd) in edible mushroom samples did not exceed the WHO/FAO 2011 permitted level respectively.

Table 4.1: Level of heavy metal concentrations (mg/kg) in edible mushroom samples.

Edible mushroom Species	**Heavy metal concentration; Mean ± Standard Deviation (mg/kg)**			
	Vital element		**Toxic element**	
	Zn	**Cu**	**Pb**	**Cd**
Pleurotus ostreatus	1.393 ± 0.0190	0.176 ± 0.0036	0.128 ± 0.014	0.020 ± 0.0011
Lentinus edodes	1.327 ± 0.0061	0.059 ± 0.0032	0.081 ± 0.006	0.013 ± 0.0006
Agaricus bisporus	1.259 ± 0.0084	0.193 ± 0.0013	0.102 ± 0.0135	0.015 ± 0.0008
Flammulina velutipes	1.202 ± 0.0162	0.096 ± 0.0029	0.100 ± 0.0095	0.013 ± 0.0006
Permissible limit (WHO/FAO)	60	40	0.3	0.2

4.5 Result Analysis of Vital Elements

4.5.1 Zinc (Zn)

For living organisms, zinc is important and mushrooms also known to be good zinc accumulators. Zinc is highly connected with protein and carbohydrates that one of the most critical elements required by human bodies. The mean zinc concentration in the edible mushroom analyzed samples in Figure 4.1 did not exceed the WHO/FAO (2011) permissible limit of 60 mg/kg. Pleurotus ostreatus has slightly higher zinc concentrations than the other three edible mushroom species which is 1.393 ± 0.0190 mg/kg.

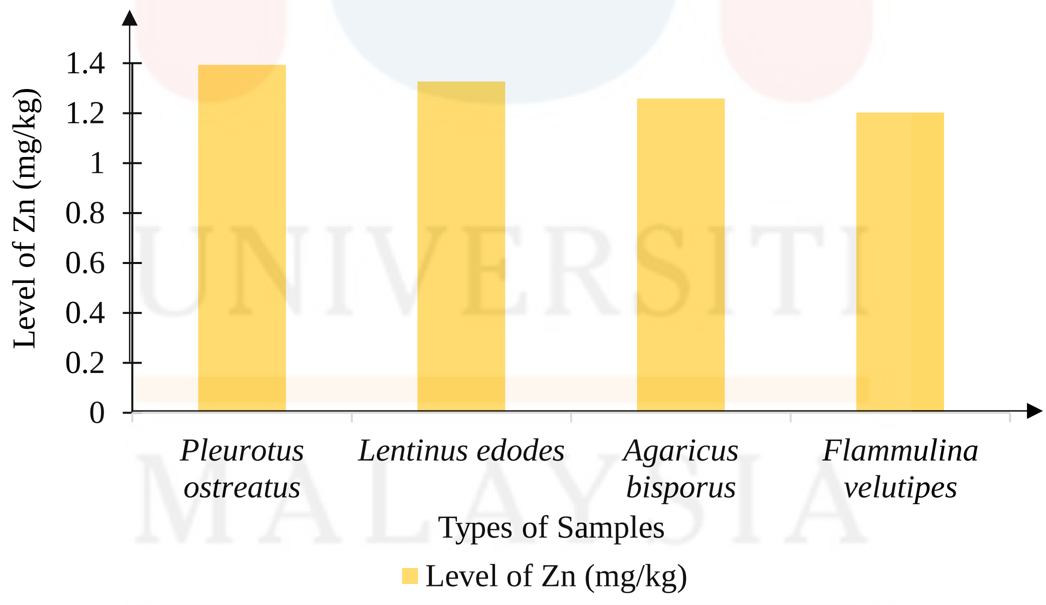

Figure 4.1: Zinc (Zn) concentration in edible mushroom samples

The zinc concentrations in all of the samples analyzed are lower and do not exceed the WHO/FAO (2011) permissible limits for zinc in edible mushrooms, according to the results in Table 4.2. *Pleurotus ostreatus* had the highest mean concentration, with 1.393 ± 0.0190 mg/kg, followed by the mean concentration of *Lentinus edodes* with range 1.327 ± 0.0061 mg/kg. Hence, with a ranging mean concentration of 1.259 ± 0.0084 mg/kg which is determination of mean concentration for *Agaricus bisporus* occurs and the lowest mean concentration of zinc is *Flammulina velutipes* with a ranging concentration of 1.202 ± 0.0162 mg/kg.

Table 4.2: Comparison of the level of Zinc (Zn) detected in edible mushrooms analysed with the maximum permissible level set by regulations.

Edible mushroom Species	**Level of Zn (mg/kg) Mean ± SD**	**Maximum permissible level of Zn in mushroom by WHO/FAO (2011)**
Pleurotus ostreatus	1.393 ± 0.0190	
Lentinus edodes	1.327 ± 0.0061	60
Agaricus bisporus	1.259 ± 0.0084	
Flammulina velutipes	1.202 ± 0.0162	

Zinc is a major aspect of a wide range of enzymes, where it serves as catalysis, architectural, and regulatory element. Furthermore, zinc insufficiency can be caused by a lack of zinc in the diet, poor absorption, excessive output, or hereditary zinc metabolism abnormalities (Zhu et al., 2011). Mushrooms are zinc acquirers, with sporophores and substrate ratios ranging from 1 to 10 mg/kg for Zn (Collection, 2015). Because of the maximum concentration of this element in the cap of the fruiting body for the examined growing edible mushrooms species, zinc has a better characteristics in the analysed species of mushrooms. Zinc was found to be the most abundant heavy metal in all of the edible mushrooms species samples in this research followed by Cu, Pb and Cd. However, zinc amounts reported in this research for edible mushroom species can be regarded as negligible in all samples and are not detrimental to human bodies because it does not exceed the permissible level by WHO/FAO.

This result was matched with a previous study in Nigeria, in which samples of mushrooms were taken from a prominent supermarket in Imo State. According to A. Ihugba et al. (2018), the outcomes are shown that zinc was found to be the most abundant and the highest amounts of Zn ranged from 0.03 mg/kg to 3.25 mg/kg, followed by Cu (0.03 mg/kg to 0.83 mg/kg) and Pb (0.04 mg/kg to 0.36 mg/kg). The average concentration result acquired from this research was approximate to the previous study's observation. Since of crop mineralisation and an environment polluted with zinc based chemical compounds, this observation predicts that zinc will enter the mushrooms from the soil.

4.5.2 Copper (Cu)

Copper is the human bodies natural third most abundant trace element, and it has a nutrient effect on living processes. Copper is essential for a range of metabolic activities, yet it can be harmful to people when concentrations are exceeded (Elements & In, 2019). As can be observed in Figure 4.2, the maximum concentration of cooper was found in *Agaricus bisporus* with the concentration 0.193 ± 0.0013 mg/kg, whereas the lowest concentration was found in *Lentinus edodes* at a concentration of 0.059 ± 0.0032 mg/kg.

The results revealed that the mean concentration of every edible mushroom sample did not exceed the WHO/FAO permissible level of 40 mg/kg.

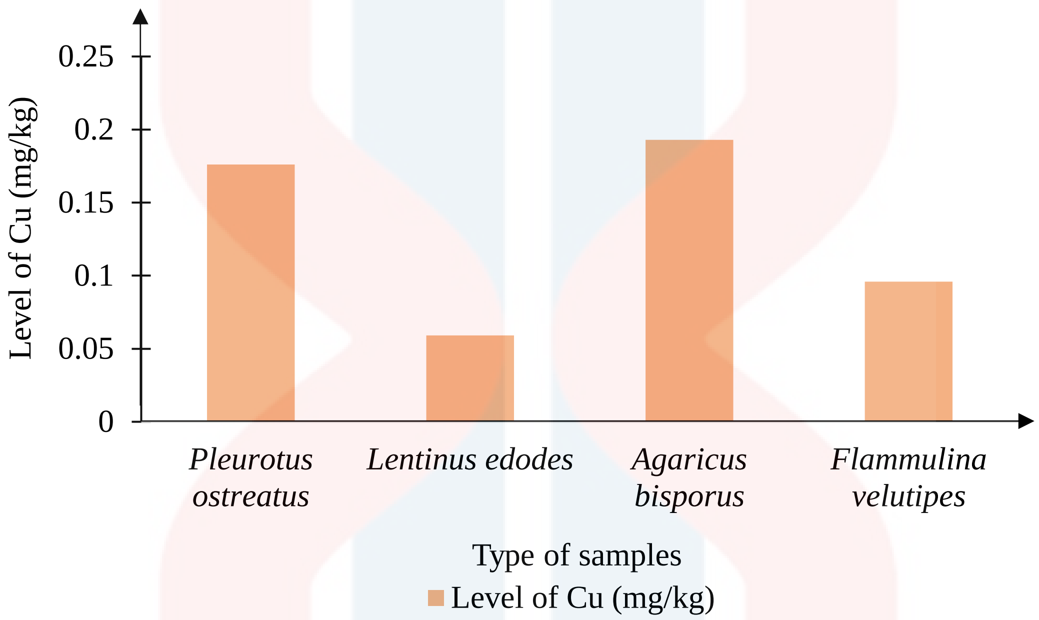

Figure 4.2: Copper, Cu concentrations in edible mushroom samples

Due to the results in Table 4.3, it can be seen that all four test samples of edible mushrooms had a low average concentration of copper, which did not exceed the WHO/FAO permissible limits levels of copper in edible mushrooms. Mean concentrations of copper in four edible mushroom samples were on average 0.176 ± 0.0036 mg/kg for *Pleurotus ostreatus*, 0.059 ± 0.0032 mg/kg for *Lentinus edodes*, 0.193 ± 0.0013 mg/kg for *Agaricus bisporus* which is the higher mean concentration and 0.096 ± 0.0029 mg/kg for *Flammulina velutipes*.

Table 4.3: Comparison of the level of Copper (Cu) detected in edible mushrooms analysed with the maximum permitted level set by regulations.

Edible mushrooms Species	Level of Cu (mg/kg) Mean ± SD	Maximum permissible level of Cu in mushroom by WHO/FAO (2011)
Pleurotus ostreatus	0.176 ± 0.0036	
Lentinus edodes	0.059 ± 0.0032	40
Agaricus bisporus	0.193 ± 0.0013	
Flammulina velutipes	0.096 ± 0.0029	

Copper is found in at least 13 various enzymes, and it is required for each one to work effectively. If copper concentrations exceed the permissible limits, it is known to be hazardous to both living creatures (Kokkoris et al., 2019). In addition, mushrooms have a greater copper concentration than vegetables in general. It is required for a wide variety of biological reactions. Some copper in the environment is less securely bonded to soil particles, and it may be soluble enough in the water for plants such as mushrooms to absorb (Dowlati et al., 2021).

The mean Cu concentration in this study is lower than in a recent study in China, which used mushroom samples from Yunnan Province and found that the mean concentration was greater than in this research. According to the Fangkun Zhu et al. (2011), the mean Cu concentrations in the latest research ranged from 7.44 mg/kg to 31.9 mg/kg. These levels in mushrooms are still below the WHO/FAO acceptable guidelines for both research and safe for consuming by humans.

4.6 Result Analysis of Toxic Elements

4.6.1 Lead (Pb)

Several factors, such as species, ecosystem, anatomical parts, and soil characteristics, such as metal concentrations, pH, and organic matter, may influence lead amounts in edible mushrooms (Elements & In, 2019). Each of the four edible mushrooms samples examined had a significant quantity of lead and the results of the research indicate a low level concentration of lead. Figure 4.3 shows the lead concentrations in four edible mushroom species. *Pleurotus ostreatus* has the greatest concentration (0.128 0.014 mg/kg), whereas *Lentinus edodes* has the lowest concentration (0.128 0.014 mg/kg). All four edible mushroom samples not surpassed the WHO/FAO (2011) permissible limits for lead contents in mushroom, which are 0.3 mg/kg as specified in the rules.

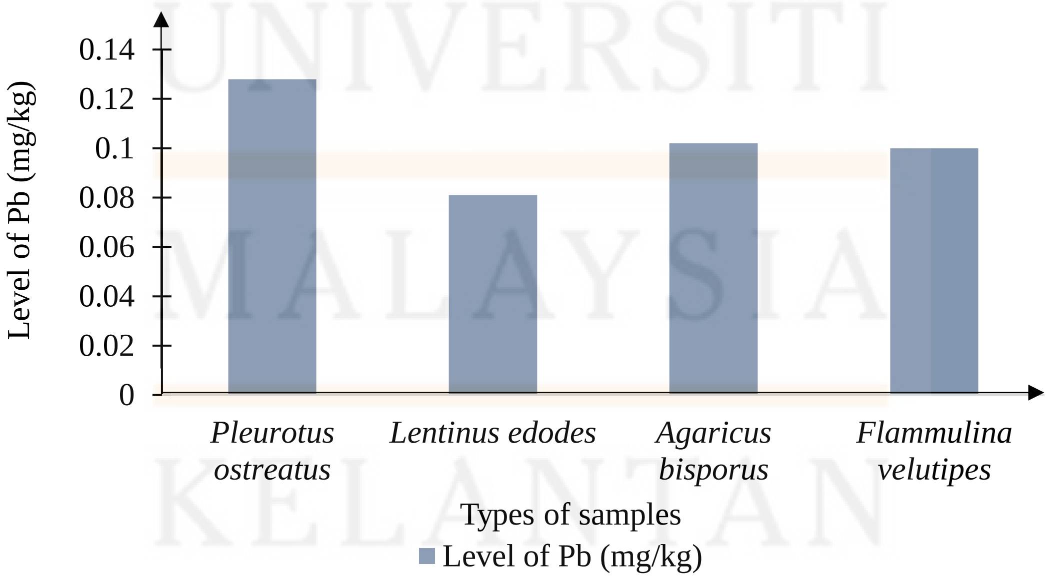

Figure 4.3: Lead, Pb concentrations in edible mushroom samples

Table 4.4 clearly demonstrates that all four edible mushroom samples had a lower mean concentrations of lead. *Pleurotus ostreatus* had the highest lead concentrations, at 0.128 ± 0.014 mg/kg, and was followed by *Agaricus bisporus*, which had 0.102 ± 0.0135 mg/kg. Then, the mean concentrations of lead followed with *Lentinus edodes* and *Flammulina velutipes*, where the concentrations in these samples are 0.100 ± 0.0095 mg/kg and 0.081± 0.006 mg/kg, respectively. Regardless, the lead concentrations in all four edible mushroom samples did not exceed WHO/FAO (2011) permitted levels.

Table 4.4: Comparison of the level of Lead (Pb) detected in edible mushrooms analysed with the maximum permitted level set by regulations.

Edible mushroom Species	**Level of Pb (mg/kg) Mean ± SD**	**Maximum permissible level of Pb in mushroom by WHO/FAO (2011)**
Pleurotus ostreatus	0.128 ± 0.014	
Lentinus edodes	0.081± 0.006	0.3
Agaricus bisporus	0.102 ± 0.0135	
Flammulina velutipes	0.100 ± 0.0095	

After cadmium and mercury, lead is the third most dangerous trace metal found in mushrooms. Lead has no positive function in human metabolism and causes gradual poisoning (Zhu et al., 2011). In addition, lead slowly builds in the bones and can substitute for calcium. It is also can enter the human body through the atmosphere, water, and foods. The digestive tract, consuming contaminated water and contaminated foods, inhalation, and the transdermal pathway are all ways for lead elements to enter the body (Muszyńska et al., 2018). Humans will exposed to insomnia, fatigue, hearing loss, and weight loss which are all symptoms of lead poisoning.

According to the previous study conducted by Semreen & Aboul-Enein (2011) on edible mushrooms that collected from Jordon, where the mean concentrations of Pb range from 2.01 mg/kg to 4.81 mg/kg. In comparison to our study, the results of previous research show significant lead concentrations that exceed the WHO/FAO permissible level which is 0.3 mg/kg. Effective soil quality and assurance control are the improvement efforts that are critical components of growing safe edible mushrooms for consumers. This also can help to reduce lead from accumulating in edible mushrooms.

4.6.2 Cadmium (Cd)

Cadmium is an exposure to the hazardous element that occurs naturally in soil but is also distributed throughout the environment as a result of human activity. It has been found that cadmium accumulates mostly in the kidneys, liver, and organs and blood serum concentration can rise significantly after mushroom consumption (Zhu et al., 2011). All of the edible mushroom samples had Cd concentrations that were consistently below the detection limit. In Figure 4.4, the highest concentrations of Cd are shown in *Pleurotus ostreatus*, with a value of mean concentration is 0.02 mg/kg. However, Cd concentrations were found to be lowest in *Lentinus edodes* and *Flammulina velutipes*, both of each edible mushroom had the same amounts of 0.013 mg/kg. According to WHO/FAO (2011), the maximum allowed level of Cd in edible mushrooms is 0.2 mg/kg.

The levels of Cd found in all edible mushroom samples in this research are still much below the permissible level.

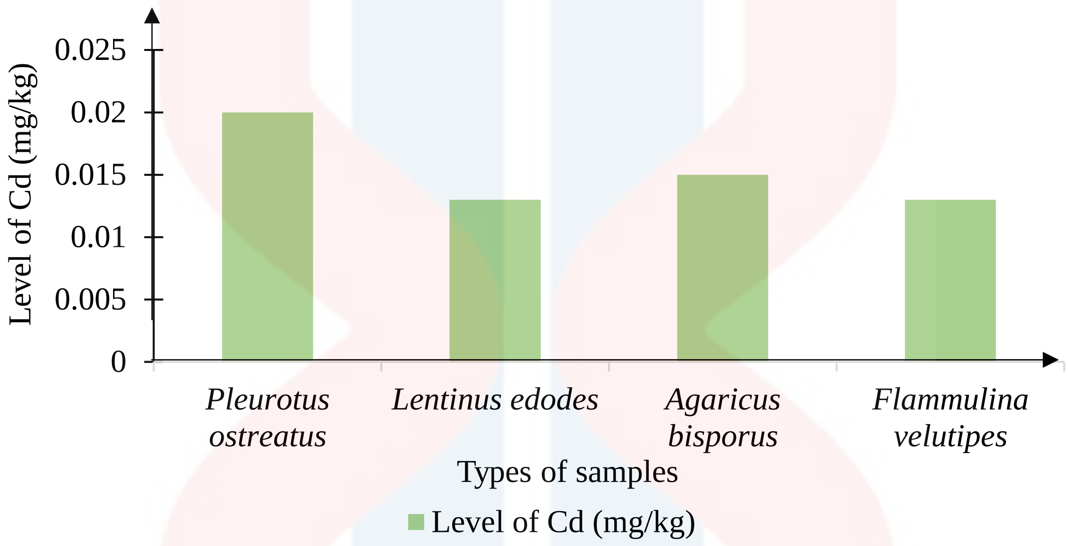

Figure 4.4: Cadmium, Cd concentrations in edible mushroom samples

According to the result findings of this study, which are presented in Table 4.5, it can be concluded that all of the edible mushroom samples contain low levels of Cd and that the levels of Cd do not exceed the limitations set by WHO/FAO regulations. The mean and standard deviation with the highest concentration were found in the *Pleurotus ostreatus*, where the Cd concentration is 0.02 ± 0.0011 mg/kg. Then followed by *Agaricus bisporus* which has the second highest mean concentration of 0.015 ± 0.0008 mg/kg. Lastly, the lowest Cd concentrations were discovered in *Lentinus edodes* and *Flammulina velutipes*, which had the same mean and standard deviation of Cd concentrations of 0.013 ± 0.0006 mg/kg. Most importantly, none of the four edible mushroom samples tested exceeded the permissible limits of Cd concentration which is 0.2 mg/kg that set by WHO/FAO.

Table 4.5: Comparison of the level of Cadmium (Cd) detected in edible mushrooms analysed with the maximum permitted level set by regulations.

Edible mushroom Species	Level of Cd (mg/kg) Mean ± SD	Maximum permissible level of Cd in mushroom by WHO/FAO (2011)
Pleurotus ostreatus	0.02 ± 0.0011	
Lentinus edodes	0.013 ± 0.0006	0.2
Agaricus bisporus	0.015 ± 0.0008	
Flammulina velutipes	0.013 ± 0.0006	

Since cadmium is a hazardous element for humans, acute poisoning is uncommon but chronic poisoning induced by the accumulation of small amounts of cadmium over time is more prevalent such as in food (Muszyńska et al., 2018). Cadmium also a chemical substances have refers to processes and carcinogenic properties, as well as disrupting liver processes, the reproductive organs, and bones. Moreover, Cd is commonly found in soil and is absorbed into the food chain by plants such as mushrooms (Karami et al., 2020). As a conclusion, the rate of absorption, distribution, and bioaccumulation of Cd in mushrooms must be examined.

Aside from that, research carried out by Liu et al. (2021) collected edible mushrooms from Jilin Province, China,also shown that the level of Cd concentrations was lower than the permissible level by regulations. The concentrations of Cd from previous research in mushrooms ranged from 0.007 mg/kg to 0.07 mg/kg (Liu et al., 2021). When the Cd concentrations in edible mushroom samples are compared to the previous studies, it can be concluded that the Cd concentrations in edible mushrooms for this study are significantly the same because it does not exceed the permissible limit by WHO/FAO.

4.7 Accumulation of Heavy Metals by Edible Mushroom Species

The focus of this research was to determine the amounts of heavy metals in four edible mushroom species that coloected from selected market in Kelantan. This research also aimed to determine whether heavy metals cause harm to human health if they are consumed in large quantities in human diets. *Pleurotus ostreatus*, *Agaricus bisporus*, *Flammulina velutipes*, and *Lentinus edodes* were selected for this study because they are popular and commonly consumed among Kelantan residents and are inexpensive. In addition, all these edible mushroom species are consumed by the majority of Malaysians as their regular diet.

Edible mushroom species play an essential role in human consumption, by doing this research study also aids in identifying the quantity of heavy metals that can accumulate in mushrooms. Furthermore, it is vital to assess contamination and human exposure to edible mushrooms since they accumulate heavy metal and cause substantial harm to people. In general, the accumulation of heavy metals by mushrooms is a complex process influenced by both external factors such as soil pH, metal concentration and the amount of organic material present, as well as internal aspects such as taxon and developmental stage (Kokkoris et al., 2019). Besides, the accumulation of toxic metals in the fruiting bodies of various mushrooms has been the subject of numerous research.

Although several mushroom species have been reported to efficiently accumulate metal elements in their fruit bodies, bioexclusion of toxic metals is recognized as a defence mechanism attempting to protect mushrooms against excessive concentrations in soil (Karami et al., 2020). Lastly, metal availability and exposure to mushrooms need to be controlled so that heavy metal levels do not exceed the permissible level by WHO/FAO, where it is influenced by factors such as soil characteristics and environmental sedimentation.

4.8 Survey Conducted

This survey is conducted to acknowledge the perceptions of Kelantan residents towards edible mushrooms as their daily food intake. This survey is specifically for residents of the district in Kelantan only. There are 371 respondents in total that are taking part in this survey, which includes all Kelantan residents from all districts in Kelantan. This survey was created by Google Form and spread via WhatsApp, Instagram, and Telegram for approximately two months. This survey was completed by people of various ages and educational levels to obtain a diverse set of responses. In addition, this survey assists in determining the health risk assessment of Kelantan residents when it comes to the consumption of edible mushrooms. The outcome from this survey is separated into three categories which are socio-demographic of respondents, understanding of respondents toward heavy metal, and the frequency of edible mushroom consumption by respondents. To make it easier for respondents to comprehend and save time answering all of the survey questions, all of the questions are given as multiple choices questions.

4.8.1 Socio-Demographic of Respondents

4.8.1.1 Gender of respondents

The gender of all respondents is the first socio-demographic question shown in Figure 4.5. According to the survey that spread around Kelantan respondents, there are 232 female respondents and 139 male respondents, for a total of 371 people participating in this survey. The percentage of female respondents is higher which is 62.5%, whereas male respondents make up only 37.5% of the total that shows they are difficult to interact with and contribute to answer the survey. This also shows that female is more likely than male to respond to the surveys sent via WhatsApp or Telegram and they are more open to spending more time participating in the survey online.

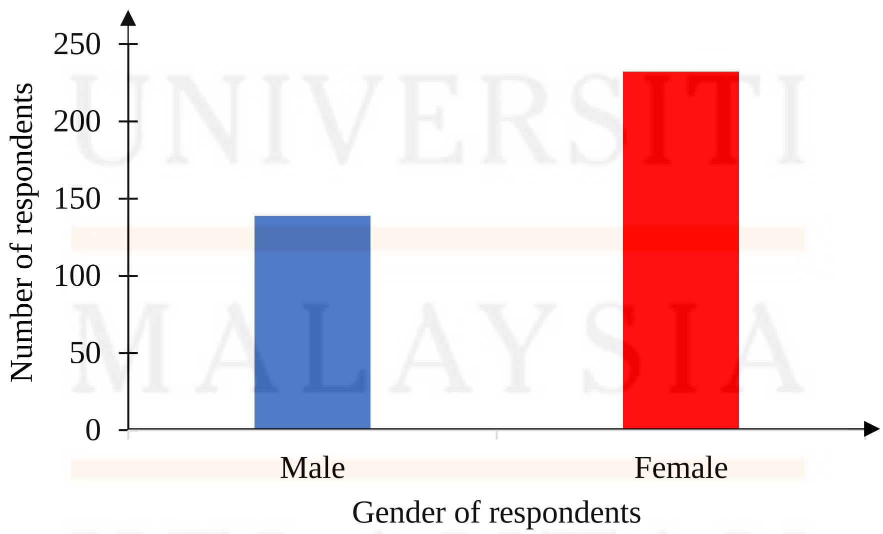

Figure 4.5: Gender of respondents

4.8.1.2 Age of respondents

The age of responders is the next socio-demographic question shown in Figure 4.6. There is a wide range of options in this survey, with the bulk of respondents falling into the age groups of 13-20 years old, 21-30 years old, 31-40 years old, 41-50 years old, and lastly is the age of the respondents from the group more than 50 years old. The highest percentage of respondents is 58.8% which is from the age group of 21-30 years old because the majority of the survey was distributed among university students that live in Kelantan. A total of 21.8% of respondents is the second-highest percentage who is answering the survey are between the ages of 13-20 years old that the respondents mostly from secondary school. 16.7% is the total percentage of respondents that answer this survey ranging from the age of 31- 40 years old. The total lowest percentage is 2.7% respondents consist of age group more than 50 years old which is 6 respondents accounting for 1.6% of the total, while the age between 41-50 years old only consists of 4 respondents accounting for 1.1% of the total that contribute in this survey.

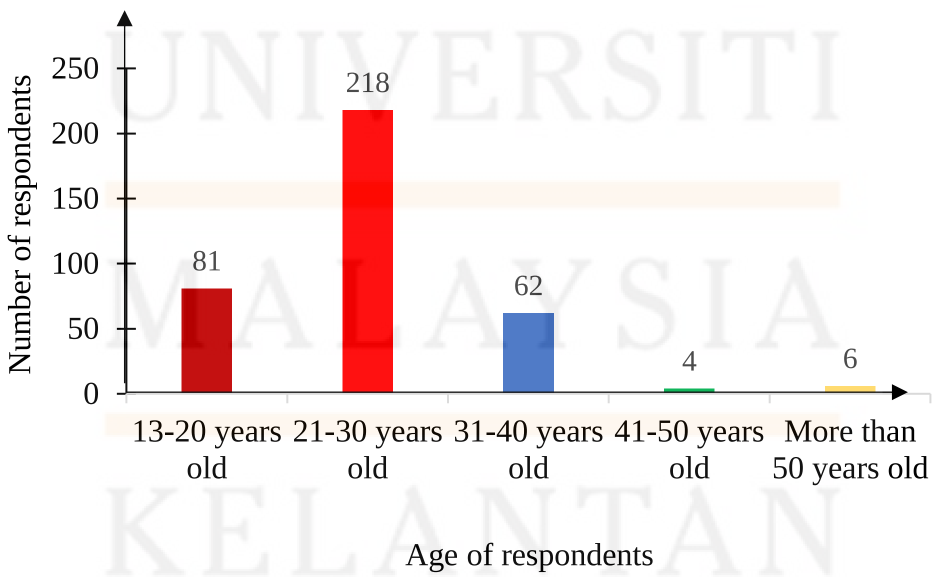

Figure 4.6: Age of respondents

4.8.1.3 Race of respondents

Next, the third socio-demographic question is the race of respondents in Kelantan shown in Figure 4.7. Malays constitute the majority of respondents, followed by Chinese, Others, and Indians. A total of 331 respondents from Malays participate in this survey with the highest of the total percentage of respondents accounting for 89.2%. This indicates that the majority of respondents in this survey are Malays which is showing that Kelantan residents are predominantly Malays. However, with a total of 21 responses, Chinese respondents account for around 5.7% of the total percentage followed by Others race which contributes about 3.2% with a total of 12 respondents that mostly consist of Siamese who stayed in Kelantan. The lowest percentage race of respondents in Kelantan that participate in this survey is Indian, that just 7 respondents accounting for 1.1% of the total percentage.

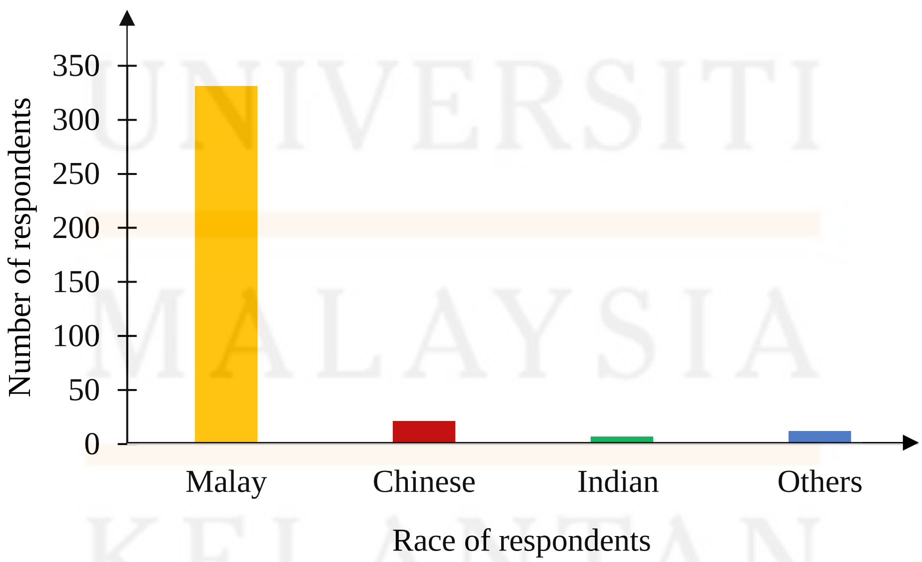

Figure 4.7: Race of respondents

4.8.1.4 Educational Level of respondents

Afterward, there is a question in this survey about the educational levels of the respondents, which is the result shown in Figure 4.8. The purpose of this question is to determine which set of respondents, based on their educational level, are most likely to spend money on mushrooms every weekend and like to consume mushrooms in their weekly and monthly. Based on this survey, the total of 178 respondents is participating in this survey account for around 48% which is the highest total percentage in this survey that shows respondents have a Degree or are currently pursuing one of those institutes at Kelantan or other educational institutions. One of the major reasons that Degree is the most common response would be that this survey is widely distributed to universities and other institutions.

The second highest total of respondents that contribute to this survey is 90 respondents that have a Diploma as their level education, which is accounting for 24.3% and is followed by 14.6% respondents consisting of 54 people that have an SPM certificate. The rest of the respondents that have the lowest percentage is 6.2% that have an STPM certificate followed by 4.6% that consist of the respondent who does not have any certificate and 2.4% that has Master or Ph.D. for their educational level. That shows the respondent with degree level education are most likely to consume and spend money on mushrooms.

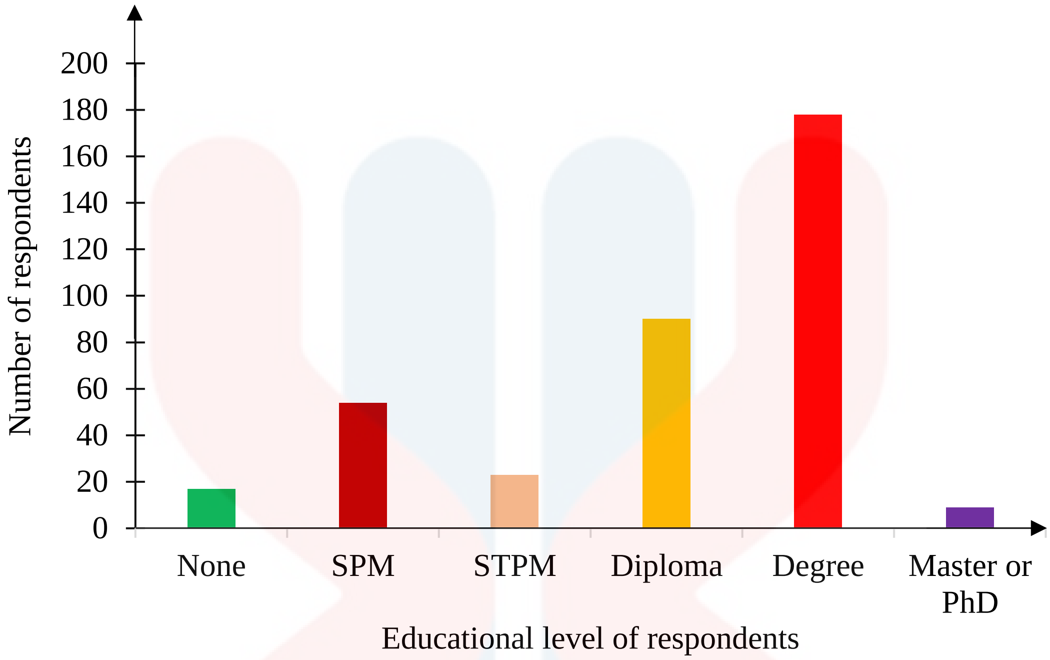

Figure 4.8: Educational Level of respondents

4.8.1.5 Income Level of respondents

Then, the fifth question in this survey is about the income level of respondents with a variety of answer that is shown in Figure 4.9. The answers are sorted into 6 different categories to make it easy to discover which category of income level is more likely to include mushrooms in their daily food. The majority of respondents have an income level of less than RM 500, accounting for 56.9% of the total percentage of respondents who contribute to this survey. This is because the survey is widely distributed among full-time students who do not yet have a stable income. There are 63 respondents who have an income level between RM 500 - RM 2000, accounting for 17% of the total percentage, and 13.2% of percentage contribution in this survey comes from an income level group of RM 2000- RM 3000 that consists of 49 respondents. 7.3% of respondents have an income level of between RM 3000- RM 4000 and 4% of respondents have an income level of more than RM 5000, for a total of 42 respondents in these two categories. The lowest percentage income level of respondents that contributed to this survey is 1.6 percent that consists of 6 respondents having an income level between RM 4000 and RM 5000.

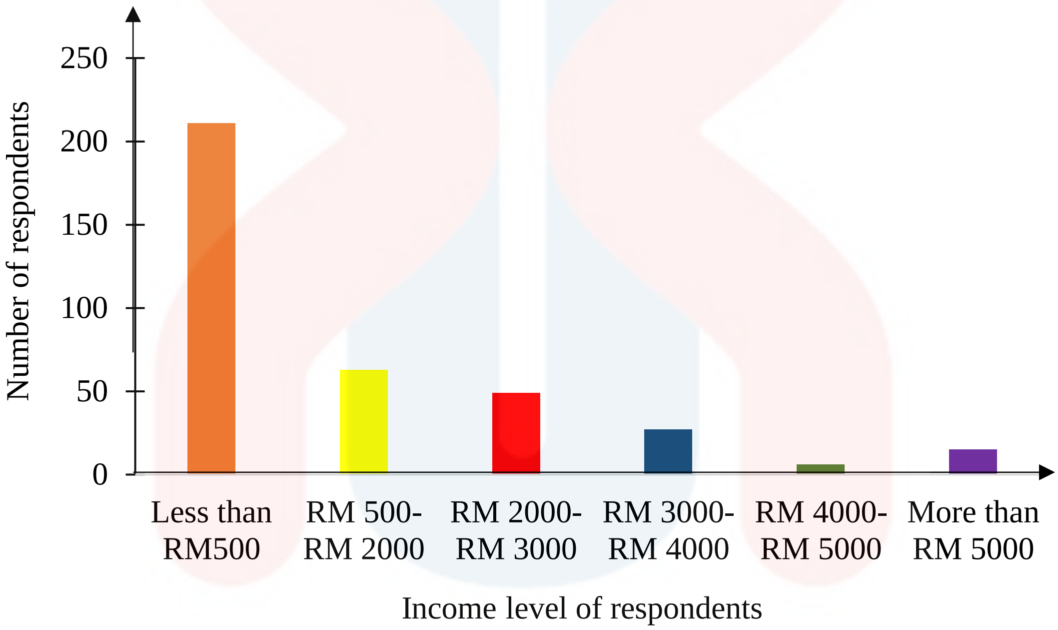

Figure 4.9: Income Level of respondents

4.8.1.6 District of respondents

Next, the question in socio-demographic represent the district of respondents in Kelantan shown in Figure 4.10. For most of the student who takes part in this survey, the district is counted based on where their educational institutes are placed in Kelantan. Kelantan is divided into 10 districts, with Kota Bahru being the highest number of respondents that participate in this survey with 31.3% of the total percentage consist of 116 respondents. 14.8% of respondents are from Tanah Merah and 11.6% are from Jeli that consists of 55 and 43 respondents from Tanah Merah and Jeli who took part in this survey. Both the Pasir Putih and Pasir Mas districts contributed 8.9% equally, given the fact that the total number of respondents was the same in both districts which are 33. In addition, the districts of Tumpat and Bachok also share the same percentage of respondents that contribute to this survey, which is 5.1% with the total number of respondents being 19. There are only have 5.4% of respondents from Gua Musang and 4.9% of respondents from Kuala Krai that contribute to this survey. The district of Machang has the lowest percentage of respondents, with only 4% of the total percentage.

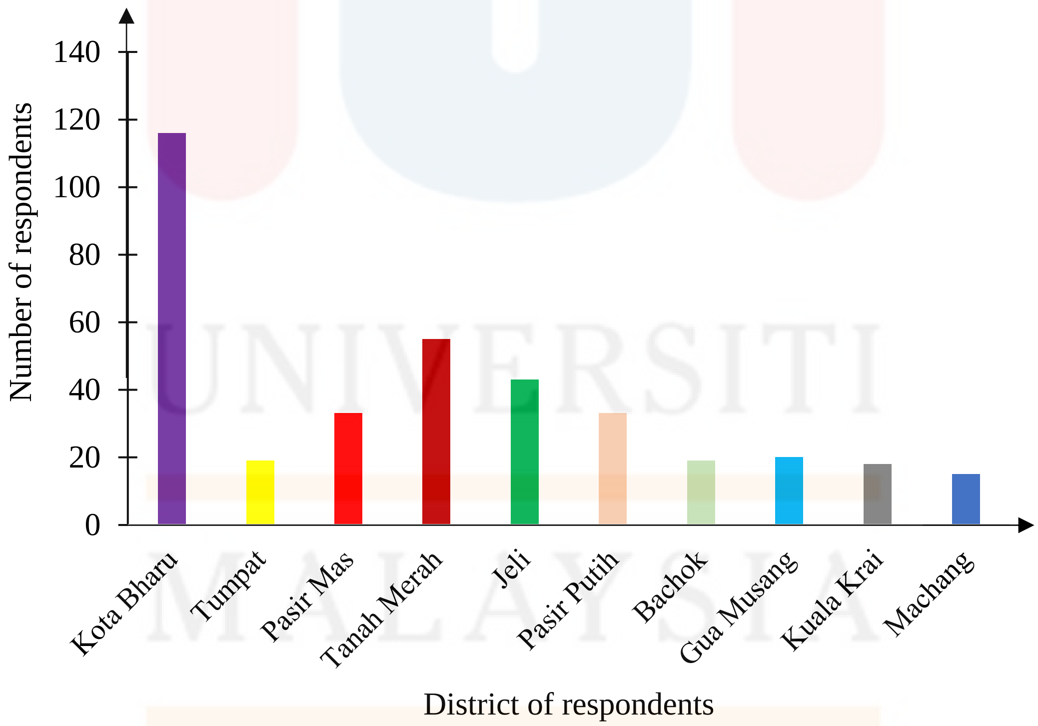

Figure 4.10: District of respondents in Kelantan

4.8.1.7 Average expenditure on mushroom (weekly)

The last socio-demographic question is the average expenditure on mushrooms in weekly that shown in Figure 4.11. The purpose of this question is to acknowledge how much the respondents spend their money to consume mushrooms in weekly. 47.7% of respondents spend less than RM 10 per week on mushroom purchases, which is the highest respondents of those surveyed said to agree. There are 31.5% of respondents spend between RM 10- RM 15 for mushroom in weekly that consist of 117 respondents who are answering this survey. Furthermore, 11.6% of respondents have spent a significant amount of money on mushrooms weekly, which is more than RM 20 and the lowest percentage of average expenditure on mushrooms in weekly is 9.2% of respondents which is between RM15 – RM20.

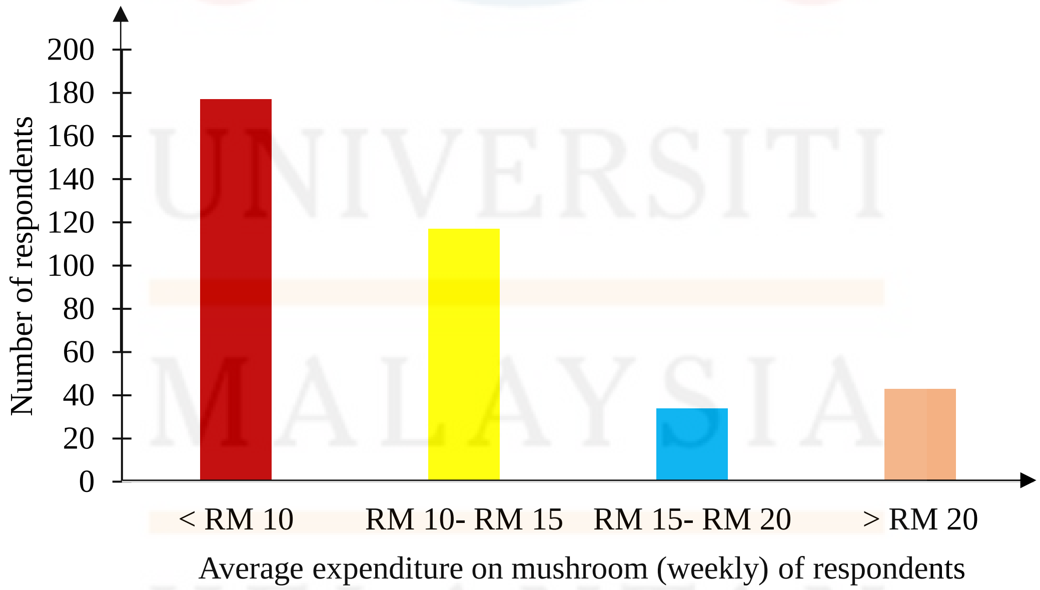

Figure 4.11: Average expenditure on mushroom (weekly) of respondents

4.8.2 Knowledge of respondents towards Heavy Metals

4.8.2.1 Familiar with the term Heavy Metals

This is the first question in Figure 4.12 for the second section of the survey, which is about the knowledge of respondents towards heavy metal. Based on this survey, it was unexpected because the largest number of respondents were knowledgeable of the term heavy metals. There have been more than half of the respondents who said yes, with the highest percentage being 78.7% consist of 292 respondents. This is because a higher percentage of respondents hold a Bachelor of degree and are more likely to be exposed to heavy metal terms. For this part of the survey, the remaining 21.3% of respondents said no and did not comprehend the term of heavy metals which is consists of 79 respondents.

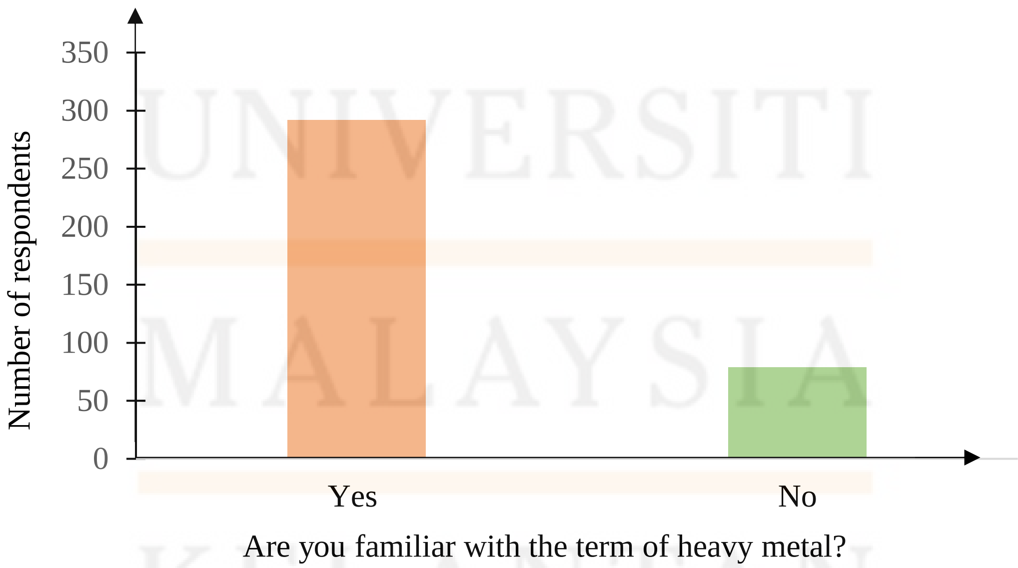

Figure 4.12: Respondents' knowledge towards heavy metal terms

4.8.2.2 Opinion of Meaning of Heavy Metals

Afterward, the second question of this section is about the meaning of heavy metals that are shown in Figure 4.13. Respondents simply need to choose the answer that they believe is correct based on their own opinions because this survey only contains multiple choice questions. Despite this, it is a bit quite surprising because, 43.4% of respondents truly comprehend the concept of heavy metal which is choosing the answer of heavy metal is high density and toxic at a low concentration that consists of 161 respondents. 27.8% of respondents answered that heavy metal is relatively high density and toxic at high concentrations while 15.1% of respondents thought that heavy metal is low density and toxic at high concentration. Heavy metal is low density and toxic at high concentrations receives the lowest percentage of respondents, accounting for only 15.1% of the total percentage of respondents which is consists of 51 respondents.

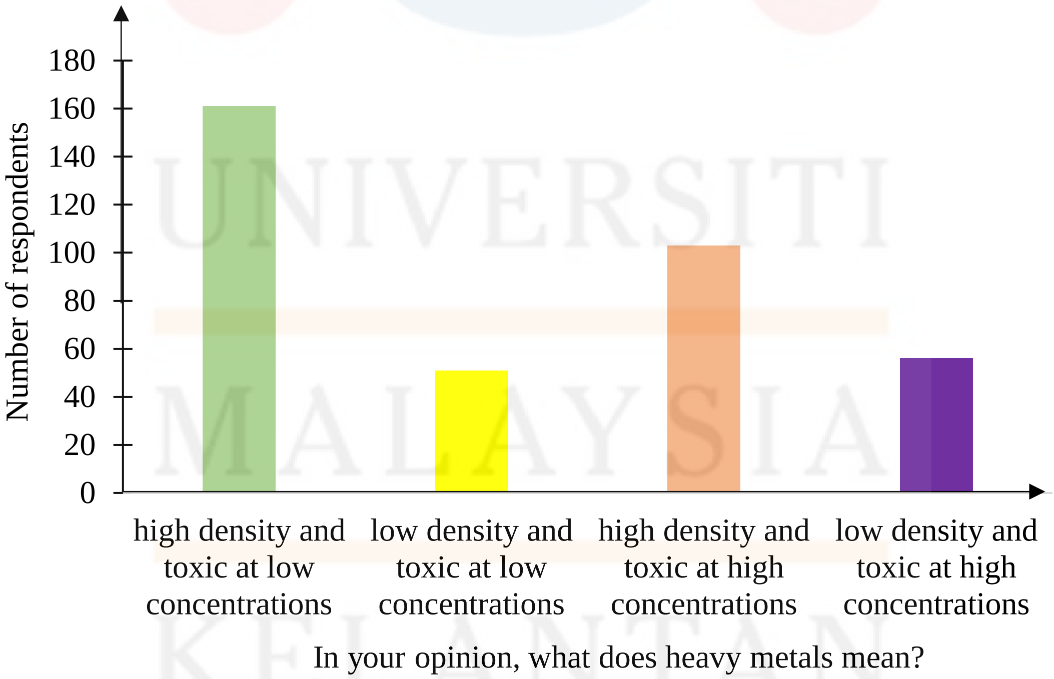

Figure 4.13: Opinion of respondents towards heavy metal meaning

4.8.2.3 Opinion on Examples of Heavy Metals

Next, the third question in this survey is about the opinion of examples of heavy metals that are shown in Figure 4.14. In this question, there are two options for an answer which is yes or no and the question is regarding Lead (Pb), Cadmium (Cd), Copper (Cu), and Zinc (Zn) examples of heavy metal. 89.8% of the respondents from the total percentage are recognizing that heavy metals such as Lead (Pb), Cadmium (Cd), Copper (Cu), and Zinc (Zn) are existing, while the remaining 38 of the respondents is unaware about heavy metals. It is about 10.2% of respondents said no, indicating that they had no awareness of heavy metal and they might not even be aware that heavy metal occurs. In addition, it is a good sign that 333 of the respondents answers yes indicates that they are really familiar with heavy metal.

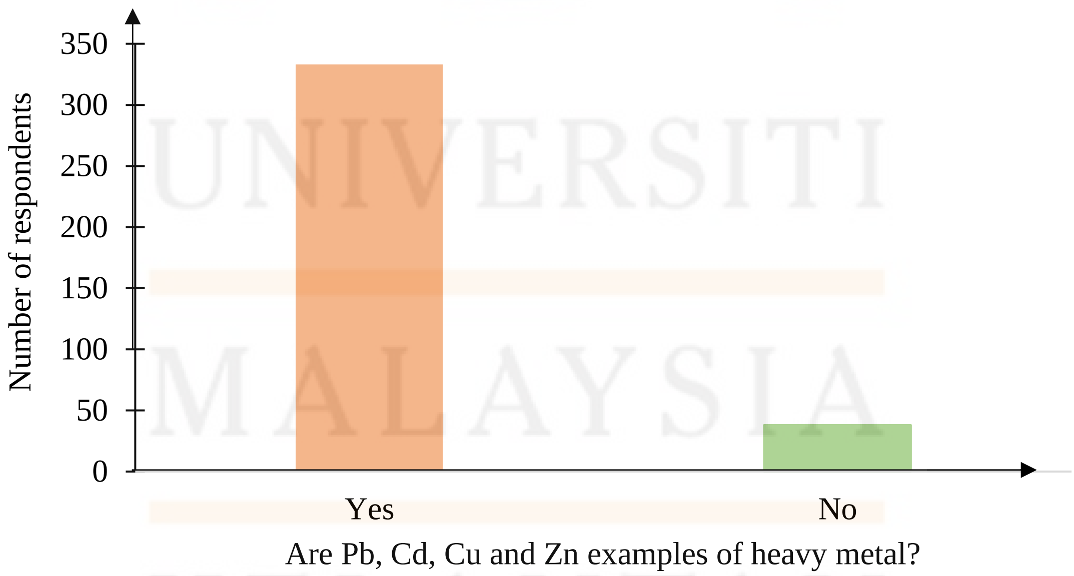

Figure 4.14: Knowledge of respondents towards heavy metals examples

4.8.2.4 Opinion on Heavy Metals Harming Towards Respondents' Body

After that, the fourth question data in this section shown in Figure 4.15 is about respondents' opinions on heavy metal that can harmful to their bodies. In this question is also provide yes or no as answer. This question also wants to know that respondents are aware that high heavy metal in the mushrooms they consume can cause harm and sickness. There are 78.4% of respondents that answer for yes, which they agree that the excessive heavy metal in mushrooms can harm their bodies. It is the highest percentage from the all total percentage that consists of 291 respondents who is really aware of their body and health while only 21.6% of the respondents answered no. It shows that 80 respondents do not know that excessive heavy metal can cause harm and sickness towards their bodies.

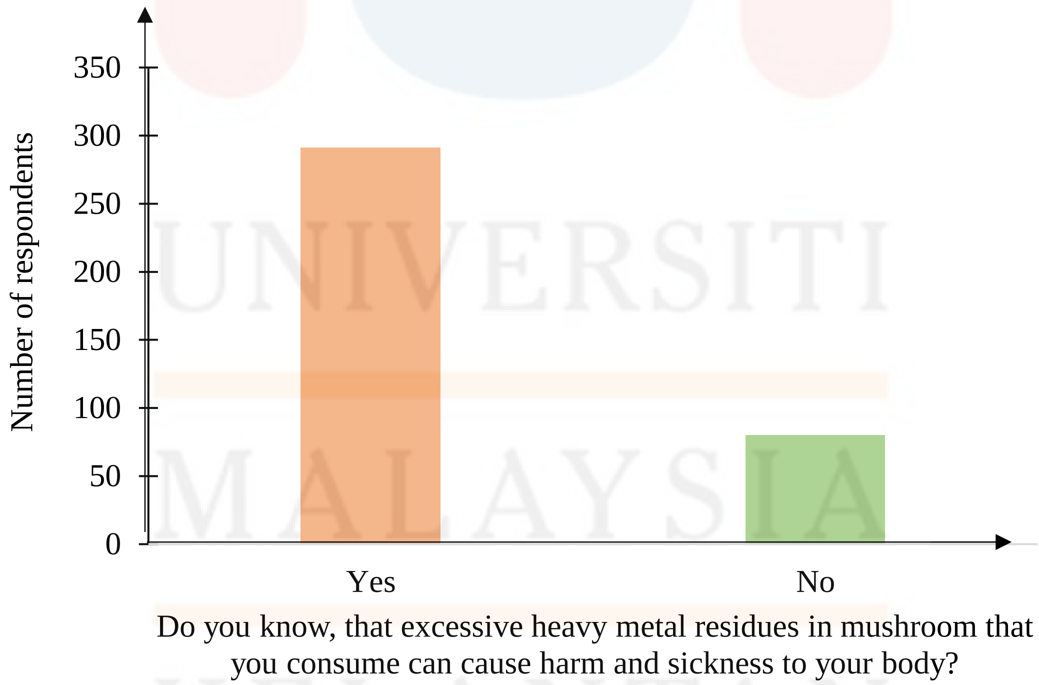

Figure 4.15: Opinion on heavy metals harming towards respondents' body

4.8.2.5 Opinion on Heavy Metal can Accumulate at Higher Levels

The last question in this section shown in Figure 4.16 is about opinion of respondents towards heavy can accumulate at higher levels in mushrooms. In this question is also provide yes or no as answer. This question also wants to know about respondents' knowledge toward heavy metal, which might accumulate at higher levels in mushroom that they consume on a daily basis. Unexpectedly, more than half of the respondents answer yes to this question. There are 65.8% of respondents that answer yes, which they agree that heavy metal can accumulate in mushrooms at a higher level. All the 244 are knowledgeable of heavy metal in mushrooms while 34.2% of respondents are unsure or unaware that heavy metal can accumulate in mushroom. There are 127 respondents who answered no to this question, which is the lowest number of respondents.

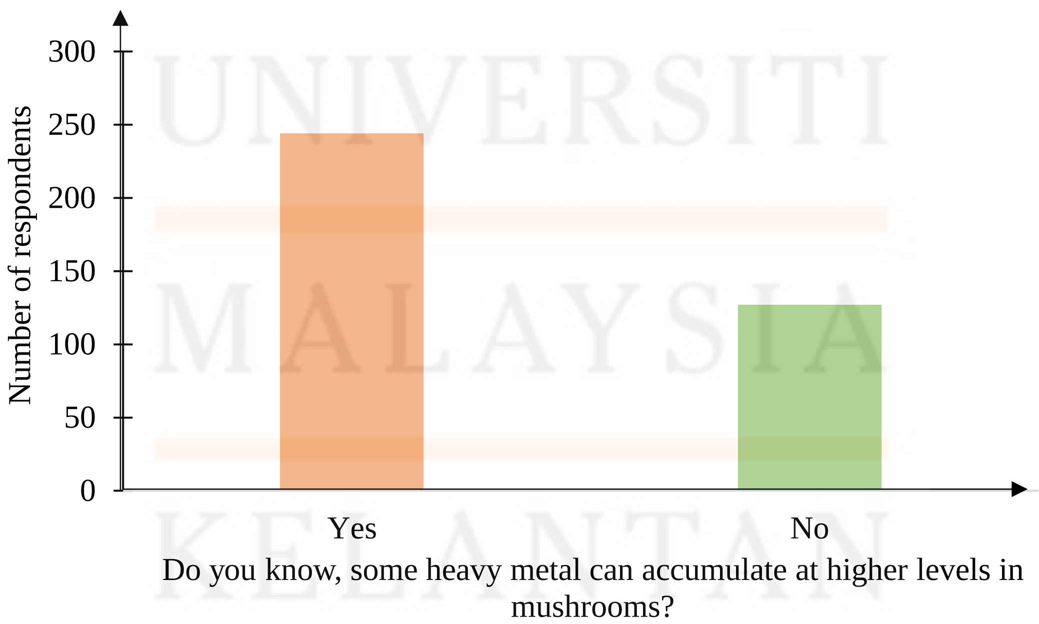

Figure 4.16: Opinion on Heavy Metal can Accumulate at Higher Levels

4.8.3 Frequency of Mushroom Consumption by Respondents

4.8.3.1 Frequency of Mushroom Consumption in a Week by Respondents

The purpose of this section is to find out the frequency of respondents consume mushrooms in a week. The frequency shown in Figure 4.17 is split into four groups which are none, 1-2 times, 3-5 times and 6-7 times. The most average frequency chosen by respondents is 1-2 times a week, which accounts for 53.9% of the total percentage respondents. It consists of 200 respondents that like to consume mushrooms just 1-2 times in a week while 34% respondents do not consume mushrooms at all in a week. All the 126 respondents who is does not consume mushroom might be due of an allergic reaction or do not like to eat mushrooms. However, it is only 10% of respondents choose that they are consumed mushrooms 3-5 times in a week which is consist 37 respondents. The lowest percentage frequency of mushroom consumption is 6-7 times a week, which accounts for only 2.2% that consists of 8 respondents. It indicates that just a small percentage of people enjoy eating a lot of mushrooms on a daily basis

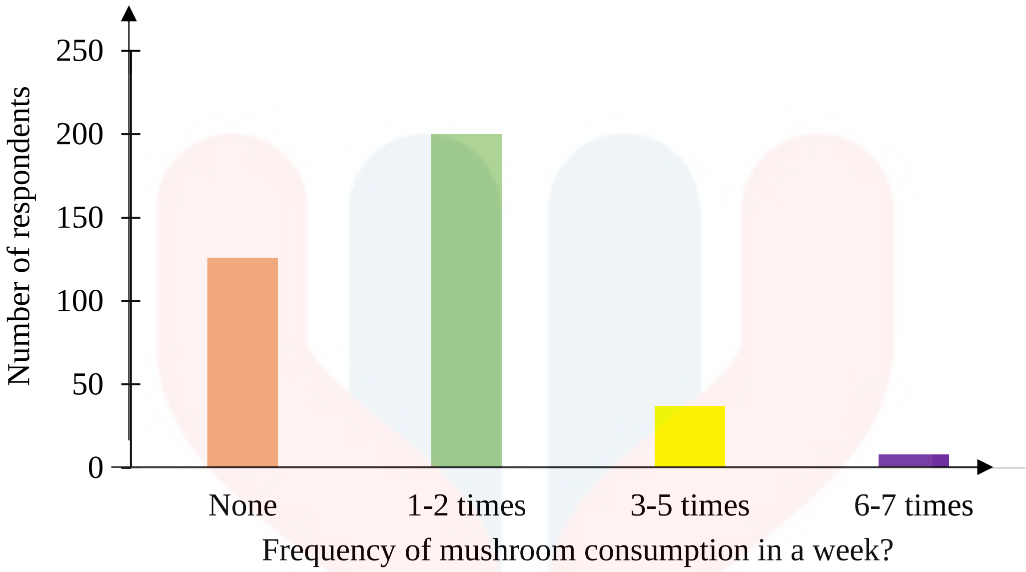

Figure 4.17: Frequency of mushrooms consumption by respondents in a week

4.8.3.2 Frequency of Mushrooms Consumption in a Month by Respondents

The last survey question shown in Figure 4.18 examines the frequency of mushroom consumption in the month by the respondents. The answer of the frequency is splits into seven groups based on their mushrooms consumption in a month which are none, 1-5 times, 6-10 times, 11-15 times, 16-20 times, 21-25 times and more than 25 times. The majority of the respondents consume 1-5 times of mushrooms in a month, accounting for about half of the total percentage which is 41.2% that consists of 153 respondents. However, just 29.1% of the respondents consume mushrooms 6-10 times in a month while just 12.9% of the respondents that not interested at all to consume mushroom in a month. It is consisting of 48 respondents that might be allergic on a mushroom or even does not like to eat. There are also have 44 respondents who consume 11-15 times mushroom in a month, accounting for just 11.9% of the overall percentage and 2.7% who consume 16-20 times in a month, accounting for 10 respondents that has more interested in mushrooms. Only 1.3 % consume mushrooms more than 25 times a month, which accounts for 5 respondents, while 0.8 % prefer to consume mushrooms 21-25 times in a month, which accounts for only 3 respondents.

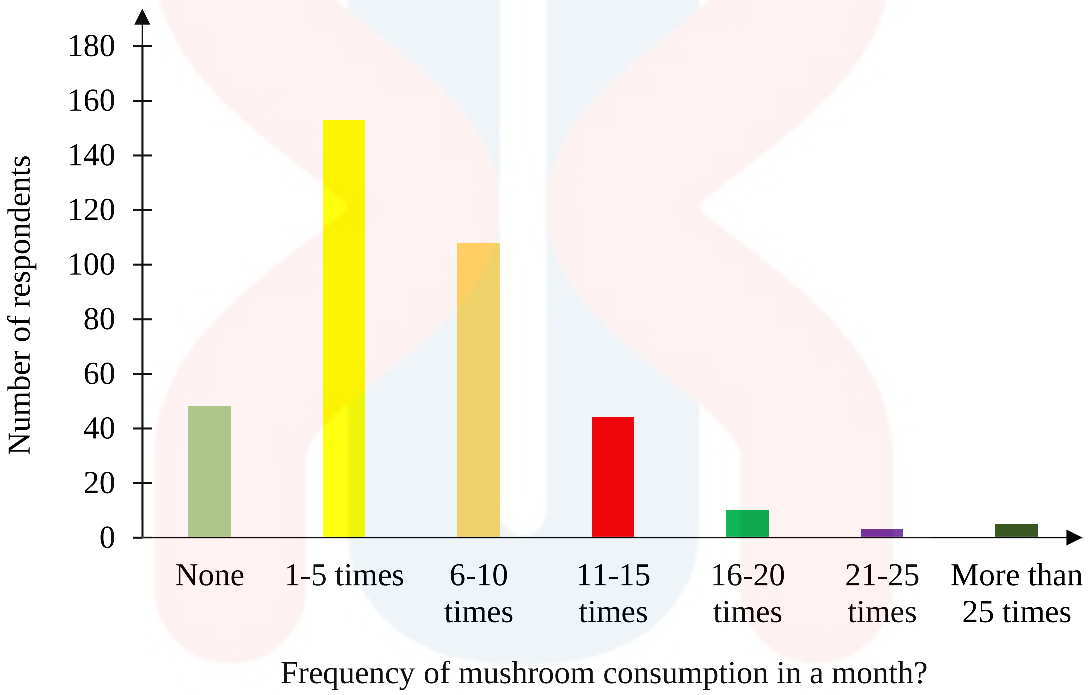

Figure 4.18: Frequency of mushrooms consumption in a month by respondents

4.9 Health Risk Assessment

4.9.1 Estimated Daily Intake (EDI) for Adult in Edible Mushroom Species

According to Figure 4.19, the estimated daily intake (EDI) for adults in edible mushroom species were found that Zinc, Zn is the most average highest EDI that can be claimed as required the most by adults, especially *Pleurotus osteatus* as the EDI needed for adults are 0.007 mg/kg/day, followed by Zn in *Lentinus edodes* with EDI of 0.0066 mg/kg/day estimated daily intake, Zn in *Agaricus bisporus* with 0.0063 mg/kg/day and the last one Zn in *Flammulina velutipes* with EDI of 0.006 mg/kg/day. Copper, Cu is the second metal that has higher of EDI where Cu in *Pleurotus ostreatus* and *Agaricus bisporus* have the same amount of EDI which is 0.0009 mg/kg/day, Cu in *Flammulina velutipes* with EDI of 0.0005 mg/kg/day and followed by *Lentinus edodes* with EDI of 0.0003 mg/kg/day.

Then, the third metal is Lead, Pb where the estimated daily intake (EDI) of Pb for adults in *Pleurotus ostreatus* are 0.0006 mg/kg/day and followed by Pb in *Agaricus bisporus* with EDI of 0.00051 mg/kg/day. Next, 0.0005 mg/kg/day of EDI in *Flammulina velutipes* and finally the lowest EDI in *Lentinus edodes* where the EDI is 0.0004 mg/kg/day. The last metal EDI for adults in edible mushrooms species are Cadmium, Cd. The highest of most estimated daily intake of Cd in adult which are *Pleurotus ostreatus* where the EDI is 0.0001 mg/kg/day, followed by *Agaricus bisporus* with EDI of 0.00008 mg/kg/day. The lowest estimated daily intake of Cd in edible mushrooms was 0.00007 mg/kg/day in *Lentinus edodes* and *Flammulina velutipes.*

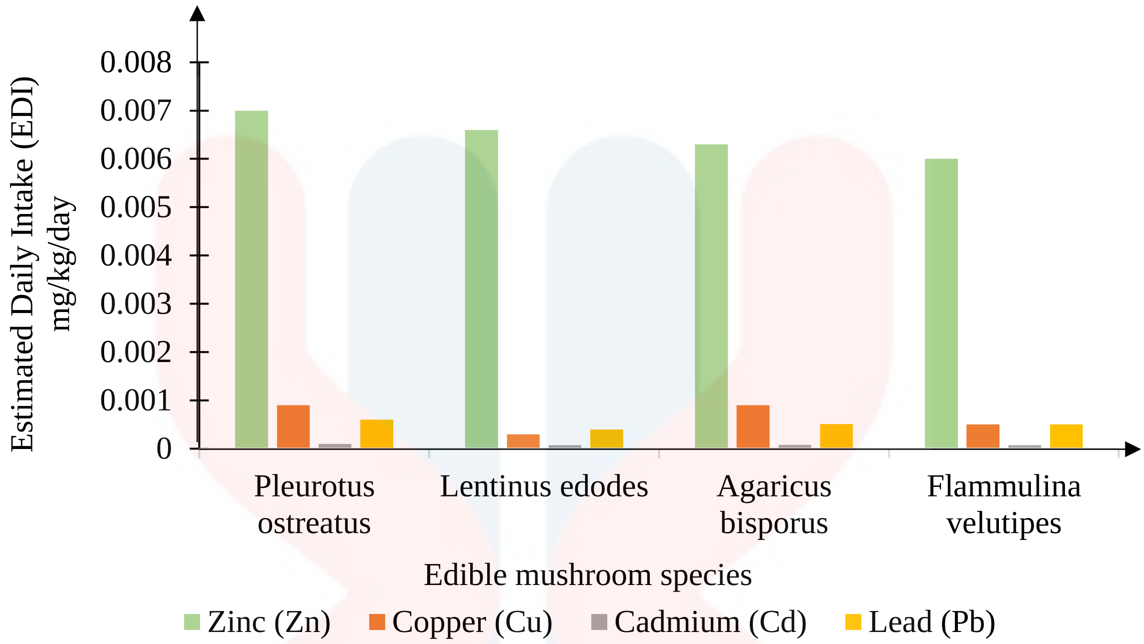

Figure 4.19: Estimated Daily Intake (EDI) (mg/kg/day)

4.9.2 Estimated Weekly Intake (EWI) for Adults in Edible Mushrooms Species

The Estimated Weekly Intake (EWI) for adults in edible mushroom species, as well as four metals found in this study, is shown in Figure 4.20. It may be determined that Zinc is the most abundant metal, followed by Copper, Lead, and Cadmium. The EWI in *Pleurotus ostreatus* were found to be high for the first metal, Zinc which is 0.014 mg/kg/week, followed by Zn in *Lentinus edodes* with 0.0132 mg/kg/week and Zn in *Agaricus bisporus* with 0.0126 mg/kg/week. The last edible mushroom species are *Flammulina velutipes* which is the lowest of EWI for Zn with 0.012 mg/kg/week. The second metal is Copper, Cu, which has the highest EWI in *Pleurotus ostreatus* and *Agaricus bisporus*, both at 0.0018 mg/kg/week, and is followed by Cu in *Flammulina* *velutipes* at 0.001 mg/kg/week. For the last edible mushroom species are Lentinus edodes which is the lowest EWI for Cu with 0.0006 mg/kg/week.

Next, the other metal is Lead, Pb. The Estimated Weekly Intake (EWI) of lead for adults in *Pleurotus ostreatus* has an EWI of 0.0012 mg/kg/week, *Agaricus bisporus* has an EWI of 0.00102 mg/kg/week. Then, followed by *Flammulina velutipes* has an EWI of 0.001 mg/kg/week, and *Lentinus edodes* has an EWI of 0.0008 mg/kg/week. . The last metal of EWI for adults in edible mushroom species is Cadmium. Cd. The most estimated daily intake of Cd n adults are in *Pleurotus ostreatus* with 0.0002 mg/kg/week and followed by *Agaricus bisporus* with 0.00016 mg/kg/week. The lowest estimated weekly intake of Cd in edible mushrooms was 0.00014 mg/kg/week for both in *Lentinus edodes* and *Flammulina velutipes.*

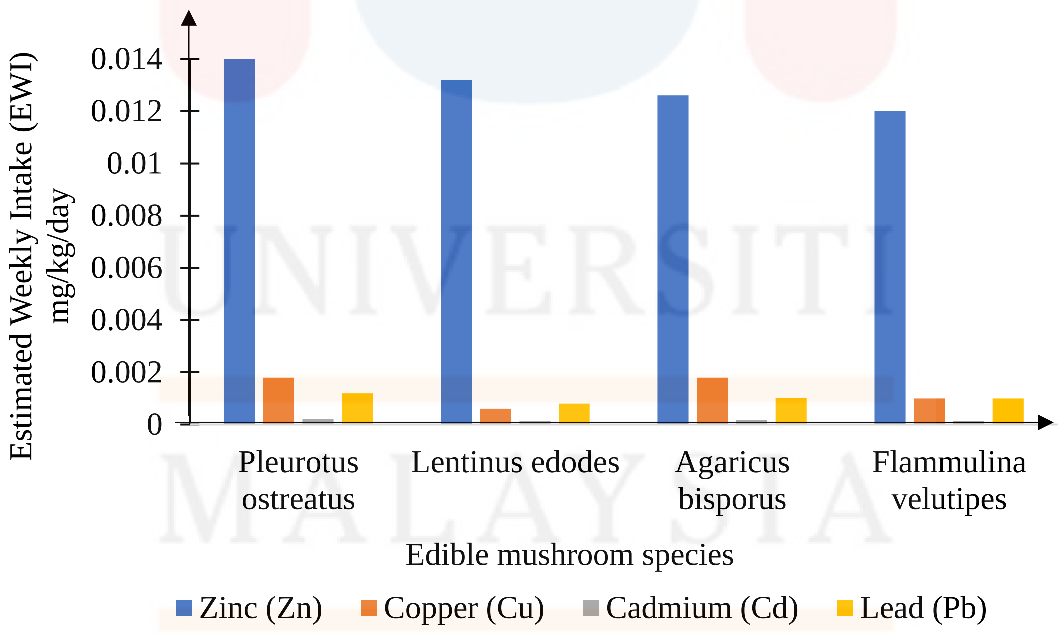

Figure 4.20: Estimated Weekly Intake (EWI) (mg/kg/week)

The comparison of Estimated Daily Intake (EDI) and Estimated Weekly Intake (EWI) for adults in all four edible mushroom samples in this research is shown in Table 4.6. The amount of EDI is lower than the amount of EWI because their time is different. It is because of EDI is used to determine the metal requirements in mushrooms for adults in days, whereas EWI is used to determine the metal requirements in mushrooms for adults in weeks. In addition, EDI of heavy metals was determined using the average mean of each heavy metal in the mushrooms and the consumption rates associated with each heavy metal (Leblebici et al., 2020). Then, according to the body of adult requirements, the Tolerable daily intake (TDI) (mg/kg/day) of edible mushrooms is collected from WHO/FAO (2011) stated that for Zn is 60 mg/kg/day, for Cu are 4.3 mg/kg/day, for Pb are 0.03 mg/kg/day and lastly for Cd in edible muhsrooms are 0.01 mg/kg/day.

In addition, Provisional tolerable weekly intake (PTWI) is the maximum amount of contaminants that a 60 kg consumer can consume in a week which is set by WHO/FAO (A. Ihugba et al., 2018). Whereas, PTWI (mg/kg/week) for adults in edible mushroom species is 360 mg/kg/week for Zn because Zn is essential in human bodies but not excessively, 30 mg/kg/week for Cu, 18 mg/kg/week for Pb, and 7 mg/kg/week for Cd. Table 4.6 shows that the EDI and EWI for the edible mushroom samples in this research do not exceed the TDI and PTWI recommended by WHO/FAO (2011). This indicates that all of the edible mushroom samples were safe for human consumption especially Kelantan residents.

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

Edible mushroom	**EDI (mg/kg/day)**				**EWI (mg/kg/week)**			
Species	**Zn**	**Cu**	**Pb**	**Cd**	**Zn**	**Cu**	**Pb**	**Cd**
Pluerotus ostreatus	0.0070	0.0009	0.0006	0.0001	0.014	0.0018	0.0012	0.0002
Lentinus edodes	0.0066	0.0003	0.0004	0.00007	0.0132	0.0006	0.00014	0.0008
Agaricus bisporus	0.0063	0.0009	0.0005	0.00008	0.0126	0.0018	0.00102	0.00016
Flammulina velutipes	0.0060	0.0005	0.0005	0.00007	0.012	0.001	0.001	0.00014
TDI (mg/kg/day)	60	4.3	0.03	0.01	-	-	-	-
PTWI (mg/kg/week)					360	30	18	7

4.9.3 Target Hazard Quotient (THQ) and Hazard Index (HI) for Adults in Edible Mushroom Species

The results of the Target Hazard Quotient (THQ) and the Hazard Index (HI) for adults in the four edible mushroom species samples are shown in Table 4.7. Target Hazard Quotient (THQ) in all four shrimp species that were evaluated. THQ indicator links metal concentrations in mushroom to their toxicity, as well as the quantity of mushroom consumed and the body weight of consumer. The average value of THQ in Zn is 6.413×10^{-5} , while the average of THQ in Cu are 6.55×10^{-5}. Whereas, the average value of THQ for Pb in the four edible mushroom samples are 5.877×10^{-4} and followed by the last metal which is Cd that show the average value of THQ are 3.05×10^{-4}. The

Hazard Index (HI) for adults in the edible mushroom species that was examined were discussed. However, to determine the HI value, sum all of the THQ in all of the metals together. In this research, the HI result came out to be 0.001. There is no significant risk in four edible mushroom species that purchased from a selected market in Kelantan because the Hazard Index (HI) values are less than 1, and the edible mushrooms are safe to consume.

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

Edible mushroom	Target Hazard Quotient (THQ)				Hazard Index
Species	Zn	Cu	Pb	Cd	(HI)
Pleurotus ostreatus	2.32×10^{-5}	2.2×10^{-5}	1.83×10^{-4}	1×10^{-4}	3.282×10^{-4}
Agaricus bisporus	2.09×10^{-5}	2.41×10^{-5}	1.46×10^{-4}	7.5×10^{-5}	2.66×10^{-4}
Lentinus edodes	2.21×10^{-5}	7.38×10^{-6}	1.16×10^{-4}	6.5×10^{-5}	2.105×10^{-4}
Flammulina velutipes	2.003×10^{-5}	1.2×10^{-5}	1.43×10^{-4}	6.5×10^{-5}	2.4003×10^{-4}
Average value	$\mathbf{6.41 \times 10^{-5}}$	$\mathbf{6.55 \times 10^{-5}}$	$\mathbf{5.88 \times 10^{-4}}$	$\mathbf{3.05 \times 10^{-4}}$	**0.001**

CHAPTER 5

CONCLUSION AND FUTURE RECOMMENDATIONS

5.1 Conclusion

This research provides an overview of heavy metal accumulation in four edible mushroom species which are *Pleurotus ostreatus* (Oyster mushroom), *Lentinus edodes* (Shiitake mushroom), *Agaricus bisporus* (Button mushroom) and *Flammulina velutipes* (Enoki mushroom) that purchased from selected market in Kelantan. In addition, using an Atomic Absorption Spectrometer (AAS) instrument, the heavy metals identified in the four edible mushroom species were Zn, Cu, Pb and Cd. All this edible mushroom species is a common mushroom and the organic matter content of their habitat and soil have the greatest impact on the heavy metal level of mushrooms. According to regulations guidelines, the accumulation of heavy metal in mushrooms is not at a critical level. However, mushroom can accumulate at higher levels of heavy metal if not regulated. Since edible mushrooms are low in calories, they supply a wide range of nutrients in the human diet, therefore it is important to make sure they are safe to consume.

The mean concentrations of heavy metals identified in four edible mushroom species in this research were coming out with different level of concentrations. All the mean concentration were sorted in increasing order, with Cd <Pb < Cu < Zn being the most prevalent. Moreover, the concentrations of zinc accumulating in edible mushrooms were found to be the highest of the four heavy metals observed in all edible mushroom species. While, Copper appeared in the second highest heavy metal, followed by the Lead, and Cadmium had the lowest metals accumulation of mean concentrations. As a result, metal concentration differences are dependent on mushroom species and environments. The average heavy metal concentrations identified in each edible mushrooms species are Zn (1.2953 mg/kg), Cu (0.131 mg/kg), Pb (0.1028 mg/kg) and Cd (0.0153 mg/kg). The levels of heavy metal concentration in all edible mushroom species in this research do not exceed the WHO/FAO (2011) permissible limits.

Furthermore, the Estimated Daily Intake (EDI) for adults observed in all edible mushroom species was determined to be lower than the WHO/FAO recommended Tolerable Daily Intake (TDI) for all heavy metal examined. Besides, the Estimated Weekly Intake (EWI) for an adult consuming 300g of mushroom and weighing 60kg was found to be lower than the Provisional Tolerable Weekly Intake (PTWI) for Zn, Cu, Pb, and Cd levels given by WHO/FAO. As a result, there is no risk to people in Kelantan who consume these four edible mushroom species. The Hazard Index (HI) was 0.001, which indicates that no significant risk exists in all edible mushroom species. Lastly, heavy metal levels in edible mushrooms should be examined more frequently in order to assess the potential risk to human health.

5.2 Future Recommendations

Future extensive studies of heavy metals and other probable contaminants in other types of edible mushroom such as canned mushrooms and mushroom products are recommended. However, make sure that other species are included as samples in future studies, as this will assist to prove the varying levels of metal accumulation in edible mushrooms. After that, compare the different types of mushroom species and explain how mushrooms can accumulate heavy metals in different areas. It is indeed crucial to figure out if the level of heavy metal contamination in edible mushrooms will remain under the limit in the next years. It is to avoid the health risk to the consumers if edible mushrooms are not safe to eat because they exceed permissible levels set by regulations. However, to reduce metal concentrations in the examined mushroom species and investigate their effects on human health, it is crucial to properly monitor heavy metals in the soil and mushroom. This will help to prevent chronic health concerns of heavy metal exposure.

In addition to the questionnaires distributed, it is recommended to conduct a face-to-face interview or distribute a paper form for the survey section. By directly questioning the respondents, it is possible to improve the results and learn why the respondents decided to include mushrooms in their daily diet. Then, we can include more adult respondents by distributing paper forms because not everyone knows how to utilise online question such as google form. Through interviewing, respondents can greatly aid in obtaining the optimum research result. In order to achieve better outcomes in the research, good communication with respondents is needed.

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APPENDIX A

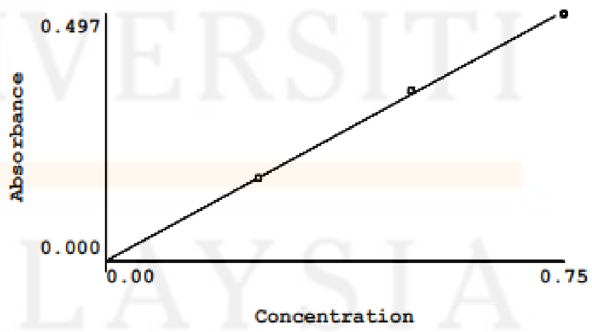
Blank calibration measurement for each heavy metals

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

APPENDIX B

Standard calibration curve for each heavy metal using AAS.

i) Zinc (Zn)

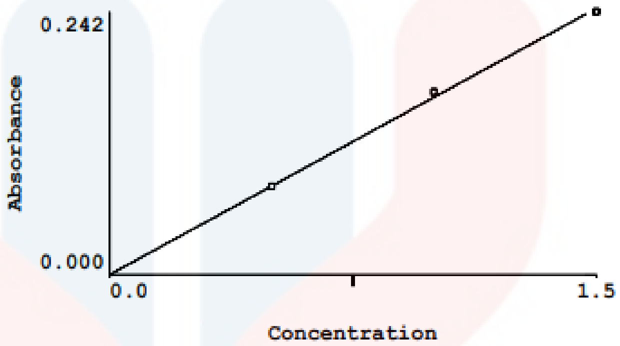


Calibration data for Zn 213.86 Equation: Linear Through Zero

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

ii. Copper (Cu)

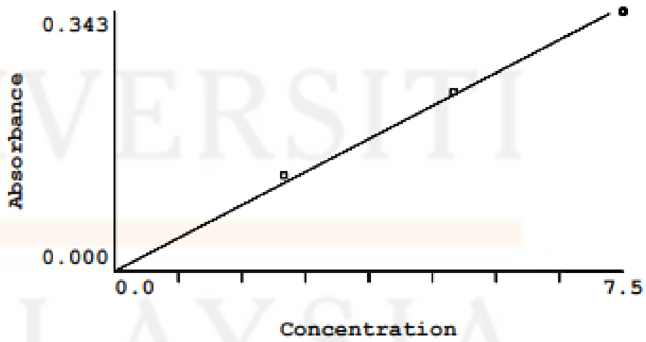


ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	0.000	0.00	20.97
Calib Std 1	0.0812	0.5	0.497	0.00	1.50
Calib Std 2	0.1680	1.0	1.028	0.00	0.67
Calib Std 3	0.2421	1.5	1.481	0.00	0.30

Calibration data for Cu 324.75 Equation: Linear Through Zero

Correlation Coef.: 0.998851 Slope: 0.16340 Intercept: 0.00000

iii. Lead (Pb)

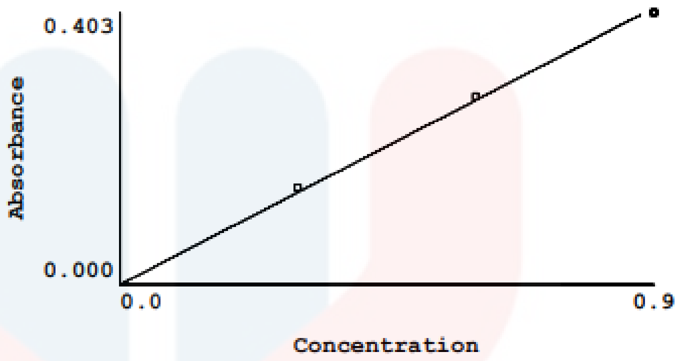


Calibration data for Pb 217.00 Equation: Linear Through Zero

ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard 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. Cadmium (Cd)



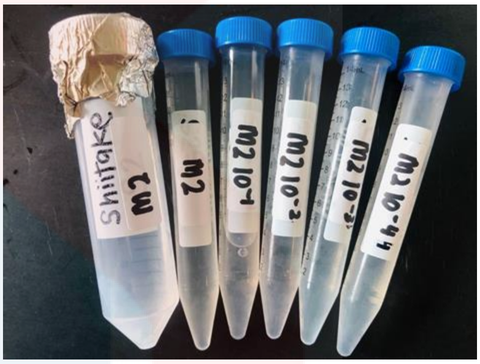
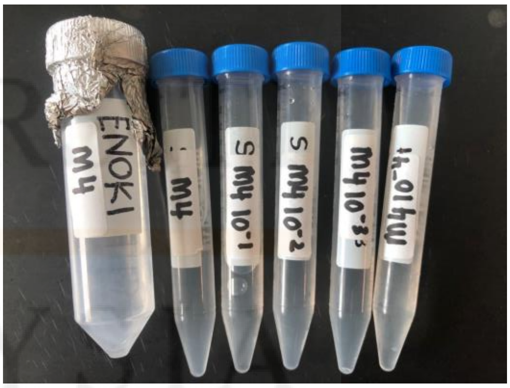
Calibration data for Cd 228.80 Equation: Linear Through Zero

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

APPENDIX C

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

Edible mushroom sample after dry ashing digestion and serial dilution	
i. Pleurotus ostreatus	ii. Lentinus edodes
	
iii. Agaricus bisporus	iv. Flammulina velutipes
	

APPENDIX D

List Question of Edible Mushroom Health Risk Assessment in Kelantan

Section A: Demographic of Respondents

1. Gender
2. Age
3. Race
4. Educational Level
5. Income Level
6. District in Kelantan
7. Average expenditure on mushroom (weekly)

Section B: Knowledge Towards Heavy Metals

1. Are you familiar with the term of heavy metals?
2. In your opinion, what does heavy metals mean?
3. Are Pb, Cd, Cu, and Zn examples of heavy metals?
4. Do you know, that excessive heavy metal residues in mushroom that you consume can cause harm and sickness to your body?
5. Do you know, some of heavy metal can accumulate at higher levels in mushroom?

Section C : Frequency of Mushroom Consumption

1. Frequency of mushroom consumption in a week?
2. Frequency of mushroom consumption in a month?