

CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

1.1 Background Study

Tin mining is one of the oldest industries in Malaysia. As what had been researched by James R.Lee (2000), the tin mining started since 1820s in Malaysia after the arrival of Chinese immigrants. The Chinese immigrants settled in Perak and started tin mines. Their leader was the famous Chung Ah Qwee. Their arrival contributed to the needed labour and hence the growth of the tin mining industry.

Tin was the major pillars of the Malaysian economy. Tin occurs chiefly as alluvial deposits in the foothills of the Peninsular on the western side. The most important area is the Kinta Valley, which includes the towns of Ipoh, Gopeng, Kampar and Batu Gajah in the state of Perak. But that was long ago before price of tin had fallen greatly and high cost of mining had shutdown the industry. What is left now is the abandoned mine site scattered all over Perak. Some mines are far from populated area and some are in the population itself such as Universiti Teknologi PETRONAS (UTP). The campus is located on land that was once an active tin mining site. The residual of past activities can be seen where number of mining ponds can be found scattered in the vicinity of UTP. The main concern from geologist and nature agencies are the identification of Potentially Toxic Elements (PTEs) and the level of concentration for each element found in the area. Until now, there are only a few numbers of studies in Malaysia that has been done on data collection of PTEs and elevated content of PTEs in former mine. From previous research of PTEs contamination in Argentina (Raúl 2004) and Malaysia (Ang et al. 1998) it is known that the major substrate available is lead (Pb), copper (Cu), zinc (Zn) in the residual tailing of the mine. Other PTEs such as mercury (Hg), selenium (Se) and arsenic (As) might also available depending on the previous mining method and content of the mineral in the soil.

1.2 Problem Statement

Currently there are no complete catalogues of heavy metals (PTEs) identification at former tin mining site for classification. Research studies from previous researchers provide inadequate data for determining the level of contamination of PTE in the former tin mine. The problem arises when agricultural activities such as farming, fish pond breeding and small scale orchard had emerged for the past 10 years near the abandon site. Previous research had shown significant amount of PTEs available in any vectors coming from tin mining site. However, the research has not thoroughly been done since there are places and vectors which in this case aquatic life and plant that were still unknown of its actual concentration data. The concentration data of PTEs from the remaining unknown site may provide characterization of place for future evaluation. The biggest concern of all is that contamination in both aquatic life and crops at former tin mine sites actually can affect the health of human. Tin mines are now actively used for agricultural functions which include growing crops and breeding aquatic life. Product from those agricultural activities will eventually end up in human food chain since the purposes of those activities are to supply commercial food to consumer (human). Without adequate research, the hazard of this PTEs contamination from the former tin mining and its effect towards the human body will never be explained transparently and systematically due to the fact that the concentration of PTEs in food product from said area is unknown. Thus, thorough research on determining and identifying PTEs in the scope of area (former tin mine) is necessary and procedure developed from this research will help future evaluation on the other abandon mining site.

1.2.1 Significance of Study

Contamination from the past activities of mining had been a major concern for long time since the earlier age of commercial mining were introduced to the industry. However, in

certain places where the mining activities are known to be at mild stage and had been stopped for a long time, the hazard that may remain at the abandoned mining site may not be known to the public. However, in recent years, studies and researches on the effects of PTEs resulted from the mining activity started to emerge due to the discovery that some of the food sources (aquatic life and crops) grown and bred from this former tin mines. In Perak, after the tin mining industry having break down and most of the mining sites had to be shut down, local settlers started to grow vegetables, breed fish and open an orchard/farm at the former mining site due to the lot of space available. However, it is known that common PTEs will not be diminished over time. PTEs from the point source (mining location) will contaminate the living things living near or/and at the point source. These living things include fish, vegetables that were grown near the point source where PTE transported to the carrier through soil and water. Fish and vegetables distributed to the consumer will continue to carry the chain of transportation for PTEs and started to accumulate in the human body through ingesting the products. Implication from the PTEs accumulation in the body will cause severe damage and various health problems depending on the type and concentration accumulated. These health problems include cancer, mental loss, organ deterioration and other implications as what had been explained by Schmitt et al. (2006). In Perak, there are many former tin mining site scattered all over and almost 80% of this site has been developed as farming site. And from the farming site, it is estimated 75% of the total farm production (including fish) will go to the consumer as food material. In other words, the final destination in the food cycle is for human consumption. These figure shows that monitoring on unreported contaminated former mining site should be done now as to obtain data on how much of concentration of PTEs had been elevated over time and to study its significance on human health. Through this study, concentration of PTEs can be determined through bioindicator and provide data essential to the objectives of this research. Since this type of research is very limited in Malaysia, it is hope that this research will also provide a model as a research tool for future research. Only then, further steps can be carried out on identified location to monitor and control the condition.

1.3 Research Objectives

The main objectives for this study are:

- 1) To investigate the effects of former tin mine at Bidor, Bemban, Malim Nawar and Tronoh, Perak.
- 2) To determine the concentration of PTEs in aquatic life and crops at the former tin mine sites.
- 3) To provide data of elements found in *Tilapia* sp. (*Talapia*), *Cichla* (*Peacock Bass*), *Melastoma* (*Senduduk*) and *Benincasa* (*Winter Melon*).

1.3.1 Scope of Study

The scope of this study will involve investigating plant's and fish's organs that consumed and stored the most of the nutrients from the food eaten. These organ/parts will act as marker for monitoring level of concentration in the fish and crops. For fish, the parts that will be used are gills, muscle tissue near the *viscera* (head) and liver (Barry 2002). For plant, the main focus will be on stem (*xylem* line) and root (Olle et al. 2005). Through this bio markers, concentration of concerned elements can be monitored more frequent as to observe the changes of the concentration over time. In this study, the concerned elements will involve elements that have toxin effect to the human body. There are numerous trace elements that are present naturally that posed potential health hazard to humans such as Cd, Pb, Hg, As, Se, Zn, Cu and Ni (Chaney 1983). From the natural occurrences of PTEs in nature such as zinc (Zn), lead (Pb) and copper (Cu), only Pb is chosen for the study accompanied other material, zinc (Zn). Both are chosen because with only a small amount, they posed a significant amount of toxicity and can be very harmful. In addition, both element (Pb and Zn) is the highest heavy metal pollutant in the environment. By monitoring level of concentration of this element from this markers, health of the ecosystem can be determined thus provide recommendations on how to reduce the concentration of PTEs on the specified territory.

1.3.2 Scope of Subjects

One of the objectives of this research is to study availability of PTEs in two subjects which are aquatic life and crops. Both are subjected for analysis for concentration determining which later provide data for PTEs content in the subjects. In this research, species for aquatic life will be **Tilapia hybrid** (*Tilapia sp.*) from *Chicliidae* family and **Peacock bass** (*Cichla ocellaris*) from *Cichlidae* family. Species for crops will be **senduduk** (*Melastoma malabathricum*), **winter melon/kundur** (*Benincasa hispida*). All of these subjects were chosen based on their availability at the former tin mine, the adaptations to surrounding ecosystem and focal location from the source of PTEs.

1.3.3 Research Location

In Perak, there are many locations can be used as a location for this research. Booming tin industries during the earlier times had produced vast amount of land that are capable to be candidate for this research. However, several factors such as period of idle time, distance from main road, current operation and also products yield from that site are need to be considered as to choose the best spot for this study. Hence, four different locations have been identified to be studied in this research.

Bidor

Located 72.3km SE (Latitude 4.1167, Longitude 101.2833) from Universiti Teknologi Petronas, Bidor has its own field research station owned by Forest Research Institute Malaysia (FRIM). A number of studies regarding tin tailings had been done near/at this place since Bidor had a history of one of the major tin mining sites during the booming tin industry era around 1950s (Telecentre 2009). Most of the abandon tin mine sites has been converted to agricultural land due the closing of tin industry in Perak around 1980s. This area had been chosen as controlled condition for crops. Controlled condition means that the site has significant number of PTEs concentration but remediation process has been done by previous researchers.

Tronoh

Exact location of the field site is at the ex-mining pond scattered around Universiti Teknologi Petronas. Last reported activities of mining is during the same time as the collapsing of tin-industries which is around 1980s. No agricultural sites were observed at these ex-mining ponds which makes it suitable location to study the concentration of PTEs in crops and aquatic life without disturbance from agricultural factor.

Bemban

Located approximately 9.3km NE (Latitude 4.667, Longitude 101.05) from Universiti Teknologi Petronas, Bemban offers agricultural site for breeding fish. No major mining activities were reported during 1950s but residual activities due to the location from Kinta to the mining town in Tronoh, had made Bemban to be included in the mining activities. Newly formed fish breeding site at 2007 makes it eligible for studying the presence of PTEs in that area.

Malim Nawar

Located 25.5km East (Latitude 4.35, Longitude 101.167) from Universiti Teknologi Petronas, offers a variety of research mode on that area. Malim Nawar consists of vast land that is currently operational for various activities such as palm plantation, duck breeding, sand mining, and agricultural. There are also large spots of abandoned land at this site. According to worker at one of farming site in the area, Malim Nawar no longer been used for mining activities for almost 20 to 25 years which is suitable for this study.

CHAPTER 2

LITERATURE REVIEW/THEORIES

2.1 LITERATURE REVIEW

2.1.1 Potential Toxic Elements (PTEs)

Heavy metals had been known to exist naturally in the environment, whether in soil, water, plants and even air. Some researchers refer the term PTEs to be associated with heavy metals due to the wide range of heavy metals are classified as PTEs (Helle 2007). The concentration of heavy metals at natural level are still considered safe since the toxicity of the elements does not elevated for a long period of time. The external activity to the environment (mining) had caused significant changes in heavy metals concentration which had caused the level of heavy metals become toxic.

Potential toxic elements generally are term used to refer to the elements that are known to be potentially toxic at an elevated concentration (Marta and Raúl 2008). These elements can be nickel, arsenic, lead, zinc and other element exists (Ang et al. 1998). In the field of study, common elements in the PTEs are heavy metals, and in this scope of fieldwork which involve mining site, the heavy metals will be referred to as PTEs.

In this study, zinc (Zn) and lead (Pb) are chosen as focal PTEs to be studied in the test subjects. Availability of said elements in the environment near former tin mine sites (Ang et al. 2000) enable the identification of Zn and Pb can be done accurately. Both heavy metal potentially able to inflict serious health hazard if consume by human body more than standard regulation limit. In addition, with only small amounts of concentration of Pb and Zn on human body is enough to deteriorate key organs and body's functionality (WHO 2001).

2.1.2 Health hazard caused by Zn and Pb

2.1.2.1 Lead

Environmental Defences Canada (2003) reported that lead has no known biological function in humans. Due to poor absorptivity in human body after being consume, lead will later build up in the bones and red blood cell, regardless of the absorb rate of children and adults. There are various adverse effects on health caused by lead. Presence of lead in human body will interfere with creation of oxygen-carrying hemoglobin, leading to anemia. Affected hemoglobin later will induce neurobehavioral problems to developing fetus and children which leads to lack of attentiveness, low IQ development and also the behavior of the human. Affected neuro (brain) system will then affect the functionality of main organs such as kidney, heart (cardiovascular) and also liver.

2.1.2.2 Zinc

Zinc however exhibits different behavior from lead, which it has known function to human body if consume within regulation limit, but will expose human body serious health hazard if consume in excess (Simon-Hettich et al. 2001). Concern in this study is when the PTEs concentration exceed the safe limit, thus the focus will be on the health hazard caused by zinc if consume excessively. On the same report submitted by Simon et al. under World Health Organization (WHO), supported by Lenntech BV (1998), minor effect on health hazard will be muscle cramps, skin irritation, vomiting, nausea and anemia. High level of contamination of zinc will lead to serious health hazard such as arteriosclerosis (stiffening of arteries, affect blood flow) and potentially of damaging pancreas. Medium level of contamination in human body also is associated with respiratory problems which can lead to damaging functionality of lung.

2.1.3 Types of former tin mine

This study focuses on former or abandoned tin mine scattered all around Perak. However, there is difficulty in selecting suitable area since there are many former tin mine with various conditions. Some places are near to residential area with waste exposure, some are busy with sand digging activity, and some are too far from main roads which are difficult to access but mostly are congested with farming activities. Suitable with the objective of the study, location with minimal activity of agriculture had been selected due to the factors such as accessible, availability of test subjects, reduced obstructions from surrounding, and ease of sampling.

Location with minimal activity in this context can be narrowed down as place where farming activities/ fish breeding are at lower level where native/conventional method are used for farming/breeding, no toxic pesticides had been used, no further excavation of the former mining pond, far from residential area buffer zone (approximately 500 meters radial from the point source; e.g. mining pond).

2.1.4 Quantitative mode of analysis

There is no basis on measuring the level of toxicity for certain elements which make the data compilation become difficult. Strategy on providing range for the results will be by choosing a controlled condition as the limit. Sample will be collected from site with activity and compared to the site with no current activity (exploration, digging, exposure etc.). This will provide data to support for both conditions which later on provide trend on concentration of heavy metals at that specific site.

2.1.5 Biological indicator and biomonitoring

Definition from Mikhail (2007) outlined the terminology of biomarker in various fields. The biological marker in general means a tool use for detection involving living things and consists of various chemical/substance to monitor the progression/advancement of certain target disease (medical terminology) or elements of target through living things. In term of this study, it is best to define that biological marker as tool or 'carrier' to gives information on the content of toxic elements contamination in the test subjects (aquatic life and crops) where the test subjects itself is the biological marker. This also can be explained further by definition by Theresa, where biomarker/bioindicator are traditionally defined as organisms used to monitor the health of, or changes in, their surroundings or ecosystem. These can be plants, animals or bacteria that regularly produce certain molecular signals in response to changes in their environmental conditions. It is useful if the response is proportional to changes in environmental conditions (i.e. chemicals, heat, fatigue or injury), but they must always be reliably induced and indicative of the particular state of the cells or organism.

Heavy metal uptake for fish and plants are suitable bioindicators due to the high metabolism and flexibility of each organism in surviving through contamination Through the presence of bioindicator in the research, where in this case is the aquatic life and crops, it will help to improve the biomonitoring at the study location. Biomonitoring provide the overview of the condition for the said ecosystem by studying the subjects that live within. Since this research is not a constant monitoring on the subjects, and the test subjects will be studied spontaneously without creating controlled condition of the ecosystem, it had been defined by Arndt et al.(1987) as passive mode of biomonitoring.

2.1.6 Heavy metal uptake in plants and fish

PTEs in soil were absorbed to the plant via roots which then transported to the whole part of plants via xylem (Ang et al. 1999). Different parts of plants, associated with several factors such as root pattern, branching habits, leaf size, shape, orientation, the biomass, etc as explained by Aidid (1988) affect the level of concentration at each individual part of the plant. Thus, in this study, the sample will be taken from the whole plants/crops and will then separate into three parts (roots, stem and leaves/shoots) to study the pathway of heavy metals in plants. Most concentration of PTEs is expected to be at the root system where, absorption and accumulation happens in this part of plants.

In aquatic life, namely fish. The pathway of PTEs is mainly through ingestion and filtration (gills). PTEs absorbed through ingestion which enter the blood stream and accumulate in most body part of fish such as muscle, intestines and liver. PTEs absorbed through filtration however will accumulate at the head part of fish called viscera (Annune 1992). Gbem et al. (2001) proved that most heavy metal uptake will accumulate at the viscera while kidney and liver are known to accumulate high amounts of metals. In this study, the sample of fish will dissect into two parts (viscera and muscle) to study the level of concentration at both parts,

2.1.7 Atomic Absorption Spectrometry (AAS)

The key component for this research is the atomic absorption spectrometry analysis, where level of concentration of PTEs contained in the test subjects can be determined through this machine. Atomic spectrometry analysis had been used worldwide by scientists to analyze material same as the usage of Real Time X-ray (RTX), Scanning Electron Microscopy (SEM) and Field Emissions Scanning Electron Microscopy (FE-SEM) analysis in the general research to determined type of element present at the targeted area. Atomic spectrometry used the concept of atomic absorption and emissions which later produces line of spectrum that can be analyzed by collector (Hardy 2000).

AAS uses atomization as part of its analyzing process. There are two approaches of atomization as explained by Hardy (2000) which are flame atomization and cold vapor atomization. For this research, flame atomization will be used due to the reliability and suitability of liquid sample (after wet digestion) and gas sample (after atomization). Cold vapor requires a higher temperature range from 2000°C-3000°C which is not suitable for this method where the sample will be destroyed. The selection of using AAS as the analyzer is because of the range of sensitivity which allows the detection limit to be controlled sensibly according to the type of heavy metals studied. AAS also has been used worldwide to study the concentration of trace elements in samples.

2.1.8 Wet Digestion

Compliance with AOAC Methods (1980) and recommended by Zeng-Yei (2003), the best possible digestion mode for biological structures is by using nitric-perchloric acid digestion. The purpose of digestion is to destroy/decompose all biological matrices in the sample and finally reduce the sample to heavy metals only since heavy metals will not decompose by digestion. However, 72% perchloric acid as proposed by AOAC is difficult to handle and poses a working hazard to the lab operator. Knowledge on handling this reagent is required to ensure the safety of the handler and laboratory (DiBerardinis 2001). 65% nitric acid is known as a strong oxidant. It has very good oxidizing power (dissolution) in combination with other acids such as HCl & HF where volatile/unstable species are formed in solution (white fumes). Hydrochloric acid is a non-oxidizing acid which reduces all inorganic elements after being oxidized by nitric acid. Hence, perchloric acid has been substituted with hydrochloric acid (37%) for safer handling.

2.1.9 Research on tin-tailings effect in Malaysia

Tin tailings effect had been discussed long ago by many researchers and journalists. It is also the major factor that brings down the tin mining industry in Malaysia (Wong 1964). In Malaysia alone, since 1980s numerous research had been done, and only few were reported. Most research had been done to investigate content of the tailings and concentration of PTEs in the soil. And mostly involved biological subject such as aquatic life, crops/plants, tree bark, and soil contamination (Ang 1998,1999,2000). Fares et al. (2009) for instance had used the procedure and methodology and implement that for the research at Sg Lembing, Pahang. During 26 February 2002, Ang Lai Hoe, researcher from FRIM finally made it public when the news titled 'Food from Malaysia's ex-mining land toxic' was reported (Reuters 2002). Aware from this breaking news, a lot of researcher surfaced afterwards to gain knowledge on how to cope with such surroundings in Malaysia, in Perak specifically since there are lots of abandoned tin mine in the state. Majid et al. (1994) had proposed on rehabilitate this land into something beneficial by studying problems identified in afforestation in sand tailings produced by Ang (1994).

CHAPTER 3

METHODOLOGY

3.1 SAMPLING SITES

Sampling sites for this study will consist of two parts – active and inactive. Criteria of differentiation will include activity period, current condition, and location of the site. Activity period explains the period of current activity being active before it changes to other activity. For example, Tronoh has been active for mining site for 20 years before some of the land being abandoned 30 years. Current condition explains the current activity of the site at the moment when samplings are being done. Location of the site indicates whether the site is nearing disturbance factors such as housing estate, access road, distance from the main road, area of the site and also external tailing source (rivers, reservoir, monsoon drains and etc.). The tabulate criteria for each site and selection for the sites are as shown below:

Criteria Site	Activity period	Current Condition	Location of the site	Type of site
Tronoh	<ul style="list-style-type: none"> • Abandoned for 30 years 	<ul style="list-style-type: none"> • Abandoned mining pond. • Some lands were converted to housing estate. 	<ul style="list-style-type: none"> • Easy access by main road. • Irrigation system from UTP and housing estate did not affect mining pond. • Size and number of ex-mining site covers large portion of Tronoh. 	Inactive
Bemban	<ul style="list-style-type: none"> • Converted to agricultural land for 6 years. Some parts were converted into housing estate for 3 years. 	<ul style="list-style-type: none"> • Agricultural land used to grow vegetables and breeding fish by local farmers. 	<ul style="list-style-type: none"> • Easy access by main road. • Irrigation and drain system from nearby housing estate did not affect sampling site. 	Active

Criteria Site	Activity period	Current Condition	Location of the site	Type of site
Bidor	<ul style="list-style-type: none"> Converted to agricultural land for 30 years. 	<ul style="list-style-type: none"> Major agricultural land for commercial and research purpose. 	<ul style="list-style-type: none"> Small road to access sampling site. Remote location, no housing area nearby and no water tailings at the site. 	Active
Malim Nawar	<ul style="list-style-type: none"> Part abandoned for 27 years. Some part converted to agricultural land for 20 years. 	<ul style="list-style-type: none"> Small part of the land has been used for agricultural purpose. Other part is still abandoned. 	<ul style="list-style-type: none"> Vast area of ex-mining site. Majority size of the ex-mining site has been abandoned. Kinta River did not affect the mining pond. 	Inactive

Table 3.1: Criteria for sampling sites

3.2 EXPERIMENT ACTIVITIES

Overall processes for experimental works cover 6 stages of activities.

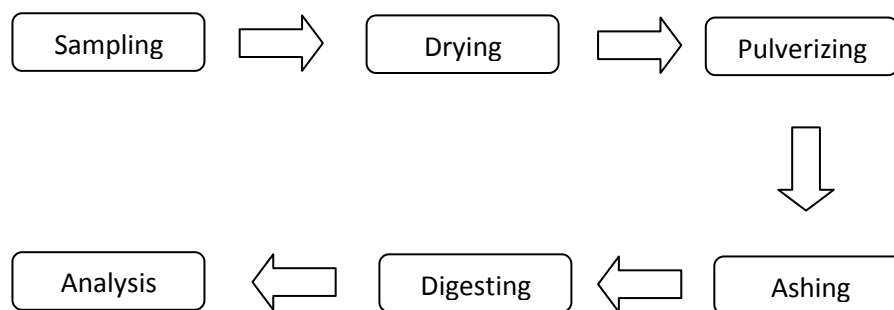


Figure 3.1: Flow activity for experimental works.

Samples were gathered and insert into the oven to get rid of the surface humidity. Samples were then pulverized to reduce the size and increase the surface area. Ashing process eliminate all humidity completely by inserting the samples into the furnace for higher temperature. Via digestion, biological matrices were destroyed leaving heavy

metals behind. Samples were then analyzed for PTEs concentration through absorption spectrometer.

3.2.1 Experimental Procedures for Crops

3.2.1.1 Sampling Procedures (Crops)

Procedure will be carried out according to Zeng-Yei (2003), Devagi et al. (2007), and Subramanian (2005). Aerial part of the plant should be cut using sterilized secateurs at least 3cm above the ground with composite weight of 0.5kg for lab treatment. Root part should be handled carefully where the soil where the roots are will be dug first and carefully remove the soil attached to the root. Aerial part of the plant (leaves, stems, shoots) should be stored separately from roots during transportation, and sample should be immediately transported to the lab after extraction.

3.2.1.2 Sample Preparation and Experiment (Crops)

1. Wash sample with tap water followed by distilled water to eliminate attached soil particulates.
2. Parts of plants such as leaves, stems & roots were separated and sliced into smaller pieces by using stainless steel laboratory scalpel/blade.
3. The samples are then freeze-dried and ground into powder by using pestle and mortar.
4. Place 2 gram of sample in crucible for ashing process in furnace with temperature of 500°C for a minimum period of 5 hours.
5. Weigh the sample produced from ashing process and place it in a small beaker.
6. Add 10ml of 65% HNO₃ boil the mixture gently for about 10 – 15 minutes at temperature not exceeding 160°C. Cover the beaker with watch glass and allow the reaction to subside.

7. Allow the sample to cool down for a while. Add another 5ml of 65% HNO_3 to the beaker and heat the mixture back for another 20-30 minutes at the same temperature as previous.
8. Allow the sample to cool down after the heating process. Add 5ml of 37% HCl into the mixture. Shake it gently and heat the mixture back for another 15 minutes at the same temperature as previous.
9. Filter the solution after cooling by using Whatman No.2 filter paper.
10. Transfer the filtered solution quantitatively to a 100ml volumetric flask and add deionized water until volume limit.
11. Determine the concentration of PTEs in the solution by using Flame Atomic Absorption Spectrometry.

3.2.2 Experimental Procedures for Aquatic Life

3.2.2.1 Sampling Procedures (Aquatic life)

Fish taken from source location (either caught or taken from the net) should be stored on ice in insulated box and immediately transferred to the laboratory. 3 – 5 samples of fish of different species are required for composite sample regardless of the raw weight. Procedure are carried out according to Nnaji et al. 2007 and Olaifa et al. 2004.

3.2.2.2 Sample Preparation and Experiment (Aquatic life)

1. Measure the length of the fish samples (standard and total lengths) with centimeter rule. Weigh the whole fish and fish head/*viscera* with digital analytical balance.
2. Dissect the fish samples with plastic knife on a polyethylene board to separate fish head/*viscera* from other parts.
3. Wash the fish head/*viscera* thoroughly with distilled water before digestion.
4. The samples are then freeze-dried and ground into powder by using pestle and mortar.
5. Place 2 gram of sample in crucible for ashing process in furnace with temperature of 500°C for a minimum period of 5 hours.
6. Weigh the sample produced from ashing process and place it in a small beaker.
7. Add 10ml of 65% HNO₃ boil the mixture gently for about 10 – 15 minutes at temperature not exceeding 160°C. Cover the beaker with watch glass and allow the reaction to subside.
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10. Filter the solution after cooling by using Whatman No.2 filter paper.
11. Transfer the filtered solution quantitatively to a 100ml volumetric flask and add deionized water until volume limit.
12. Determine the concentration of PTEs in the solution by using Flame Atomic Absorption Spectrometry.

3.2.3 Preparation for AAS working standard solution

AAS equipment that will be used in the experiment is type FAAS Shimadzu AA-6800. Before analysis using AAS can be done on the sample collected from the field, standard solution for calibration references is needed for that certain element that are being studied.

1. Standard solutions in UTP's lab generally are in 1000 ppm concentration. From this concentration in the sample standard solution, it needs to be diluted to about 40 – 200 ppm for working standard solution 1.
2. From the working standard solution, the concentration will be diluted further to the range of 0.1 ppm to 4 ppm depending on the type of trace metal to study.
3. In this experiment of tracing lead and mercury, it is advisable to set the working standard solution 2 at 0.1 ppm, 0.2 ppm, 0.4 ppm and 0.8 ppm.
4. The concentration are reduce by using molar concentration formula:

$$C_1 V_1 = C_2 V_2$$

5. Where c_1 is the initial concentration of standard solution, v_1 is the initial volume of standard solution and c_2 is the concentration to prepare, and v_2 is the volume for the working standard solution for 1 and 2.
6. From 1000 ppm to working standard solution 1, the 25ml volumetric flask will be used for the preparation.
7. From working standard solution 1 to working standard solution 2, the 10ml test tube will be used for the preparation.
8. For zero calibration, 1% conc. of HNO_3 will be used.

3.3 ATOMIC ABSORPTION SPECTROMETRY OPERATION PRINCIPLE

In atomic absorption spectrometry, there are 5 main parts in the machine that are necessary for the analysis to be done. This part start with the source which contains the bulb for emissions, the chopper to produce single wave, the platform where the sample will be placed and atomization process will take place, the monochromator which will be used to resolve and modulate line and lastly the detector where resulted ray from atomization will be detected and recorded here.

The concept of operation is mainly about absorption of radiation by the sample ([18] Levinson). The light source will emit electromagnetic radiation (depend on the trace metal to be studied) to the excited atoms. Excited atoms will absorb the emitted ray and the collector will detect level of intensity of the electromagnetic after the absorption. The more radiation absorb by the sample, the more element of element of the cathode are present in the sample. Excited atoms are vaporized sample form after the atomization took place. Normally, atoms in the sample are in the ground state and emission from the light source will not provoke the absorption of electromagnetic ray. By supplying heat to the sample, atoms will start to excite and transit to the higher energy level (free atomic state). At this state, absorption process will occur.

After the absorption process, the remaining radiation will be modulate by a high resolution, holographic grating before entering the detection chamber. This is the monochromator. Light wave from the atomization are in random dispersial and monochromator will resolve the lines. In the detection chamber, the photomultiplier tube will amplify the intensity and corrected the ambient wavelength.

The analysis of AAS relies on the Beer-Lambert law. Beer-Lambert is a linear relationship between absorbance and concentration of the absorbing material.

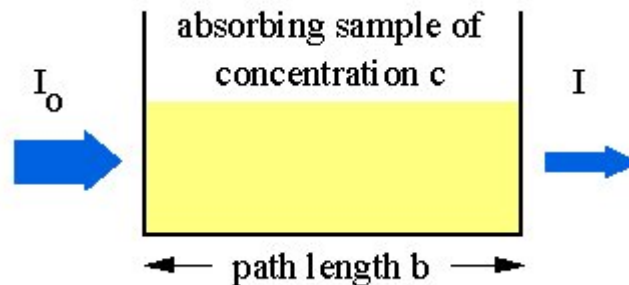


Figure 3.2: Representation of Beer-Lambert Law

The general Beer-Lambert law is usually written as:

$$A = a(\lambda) * b * c$$

where A = the measured absorbance,

$a(\lambda)$ = wavelength-dependent absorptivity coefficient,

b = the path length,

c = the analyte concentration.

When working in concentration units of molarity, the Beer-Lambert law is written as:

$$A = \epsilon * b * c$$

where ϵ is the wavelength-dependent molar absorptivity coefficient with units of $M^{-1} \text{cm}^{-1}$

Result from the above procedure will be in term of absorbance correlated with concentration.

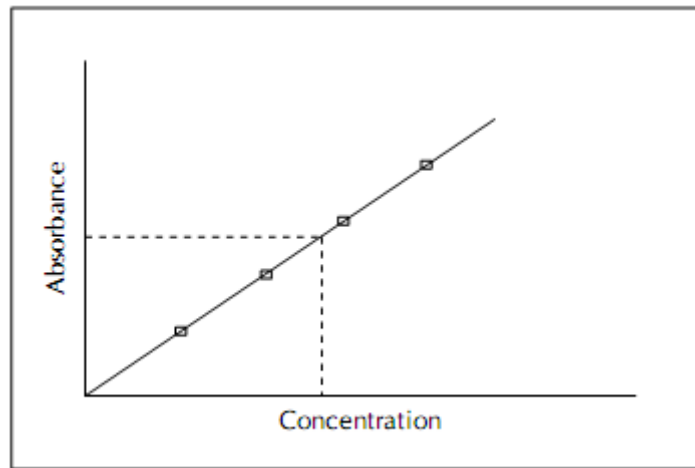


Figure 3.3: Plot of Absorbance against Concentration

Linear line calibration produced from the AAS analysis provides agreeable and reliable data of the sample contain element similar as the light source metal. For example, if the element to be tested is lead, lead cathode will be used as the light source and high absorbance of light during analysis confirm the analysis that the sample contains high concentration of lead.

CHAPTER 4

RESULTS & DISCUSSION

4.1 SAMPLES

Shown below in Table 4.1 is the list of all samples taken for this study according to its location and the part of the sample. The samples then were tagged with code name according to its type and location and this tag name will be used in the result in Table 4.2 and 4.3. Previously in chapter Methodology, the identification of inactive site and active site has been explained. The classification of the site also is mentioned in the 'Remark' tab in Table 4.1.

Table 4.1: List of Samples

Tag	Sample - Part	Location	Remark
SMN1	Senduduk - Leaves	Malim Nawar	Inactive Site
SMN2	Senduduk – Stem	Malim Nawar	
SMN3	Senduduk – Roots	Malim Nawar	
KMN1	Kundur – Fruits	Malim Nawar	Active Site
KMN2	Kundur – Leaves	Malim Nawar	
KMN3	Kundur – Stem	Malim Nawar	
KMN4	Kundur –Roots	Malim Nawar	
PTM	Peacock bass – Muscle	Tronoh	Inactive Site
PTV	Peacock bass – Viscera	Tronoh	
PBM	Peacock bass – Muscle	Bemban	Active Site
PBV	Peacock bass - Viscera	Bemban	
TTM	Tilapia – Muscle	Tronoh	Inactive Site
TTV	Tilapia – Viscera	Tronoh	
TBM	Tilapia – Muscle	Bemban	Active Site

TBV	Tilapia – Viscera	Bemban	
SB1	Senduduk – Leaves	FRIM Bidor	Inactive Site
SB2	Senduduk – Stem	FRIM Bidor	
SB3	Senduduk - Roots	FRIM Bidor	

4.2 RESULTS

Results shown below in Table 4.2 and Table 4.3 is the concentration of Pb and Zn obtained after the analysis using FAAS. The results from FAAS are in mg/L which we need to convert it to mg/kg to compare it with current standards for each element. The conversion of the concentration is being done by using the formula:

$$\text{Concentration} \left(\frac{\text{mg}}{\text{kg}} \right) = \frac{\text{AAS (mg/L)} \times \text{Extraction Volume (0.1L)} \times \text{Dilution Factor}}{\text{Weight (kg)}}$$

Dilution factor is the amount of dilution needed by sample to be within the range of detection. The range of detection for Pb of using Shimadzu AA-6800 FAAS is in between 0.1 mg/L and 0.4 mg/L. While for Zn is 0.2 mg/L and 1.0mg/L. Beyond this range, it is called beyond detection limit (BDL). BDL is the term for when the specified concentration of element cannot be clearly defined as it is out of range. If the reading exceeded the maximum range, then the sample can be diluted to ensure the concentration of the sample is within range. If it is lower than the minimum range, then it is specified as BDL. Weight of the sample is taken before the digestion process which is the dry weight of the sample after ashing process. And the final concentration in mg/kg is obtained by using above formula.

4.2.1 Pb Concentration

Table 4.2: Result for Pb

Sample Tag	Weight (kg)	Dilution Factor	AAS (mg/L)	Concentration (mg/kg)
SMN1	0.002	Nil	0.9317	46.59
SMN2	0.002	Nil	0.9317	46.59
SMN3	0.002	Nil	1.1524	57.62
KMN1	0.002	Nil	0.7938	39.69
KMN2	0.002	Nil	0.7386	36.93
KMN3	0.002	Nil	0.4903	24.52
KMN4	0.002	Nil	0.6007	30.04
PTM	0.002	Nil	0.5455	27.28
PTV	0.002	Nil	0.3428	17.14
PBM	0.002	Nil	0.4903	24.52
PBV	0.002	Nil	0.5455	27.28
TTM	0.002	Nil	0.3248	16.24
TTV	0.002	Nil	0.3248	16.24
TBM	0.002	Nil	BDL	BDL
TBV	0.002	Nil	0.3248	16.24
SB1	0.002	Nil	0.4076	20.38
SB2	0.002	Nil	BDL	BDL
SB3	0.002	Nil	0.1593	7.97

4.2.2 Zn Concentration

Table 4.3: Result for Zn

Sample Tag	Weight (kg)	Dilution Factor	AAS (mg/L)	Concentration (mg/kg)
SMN1	0.002	10	0.2256	112.80
SMN2	0.002	10	0.2313	115.65
SMN3	0.002	10	0.2192	109.60
KMN1	0.002	10	0.1975	98.75
KMN2	0.002	5	0.1820	45.50
KMN3	0.002	5	0.1890	47.25
KMN4	0.002	10	0.2111	105.55
PTM	0.002	10	0.3129	156.45
PTV	0.002	10	0.4073	203.65
PBM	0.002	10	0.2874	143.70
PBV	0.002	10	0.4151	207.55
TTM	0.002	5	0.1844	46.10
TTV	0.002	10	0.3390	169.50
TBM	0.002	10	0.2755	137.75
TBV	0.002	10	0.3003	150.15
SB1	0.002	10	0.2254	112.70
SB2	0.002	10	0.1838	91.90
SB3	0.002	10	0.1846	92.30

4.2.1 Concentration of Pb in plant sample

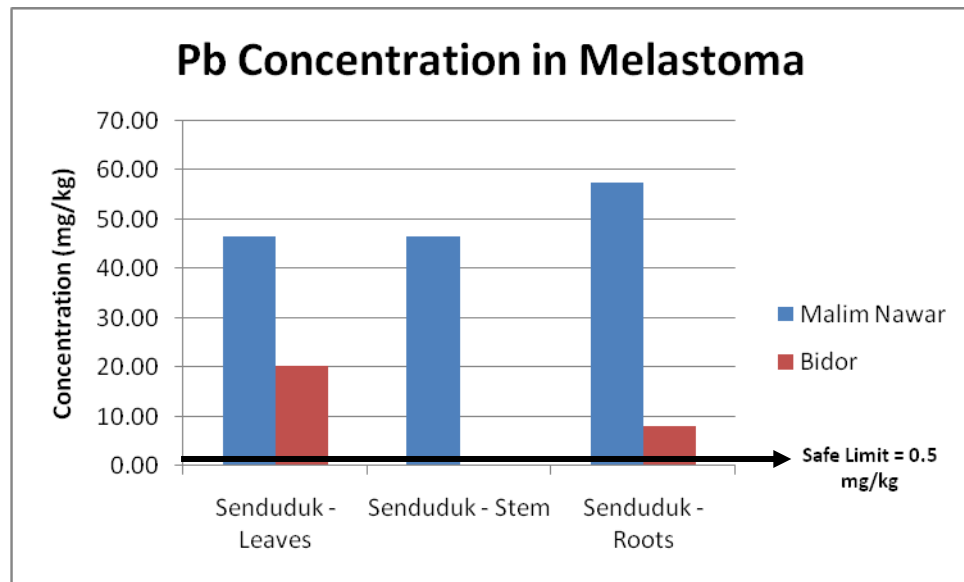


Figure 4.1: Pb concentration in Melastoma in Malim Nawar and Bidor

From Figure 4.1, Range of Pb concentration are between 45 ~ 60 mg/kg in the sample from Malim Nawar. Sample from Bidor had a concentration of Pb ranging from 8 ~ 20 mg/kg. However, no Pb could be detected at the stem part for the sample from Bidor. Results obtained from both location shows different trend where concentration of Pb is quite high in melastoma obtained from Malim Nawar compared to the sample obtained from Bidor. Heavy metal uptake the plant shows the average level at all three parts of the plant (roots, stem and leaves). The part with the highest Pb concentration is at the root which is 57.62 mg/kg. This is approximately 115 times above the safe limit issued by the FAO (1983). From literature review, it is expected that active site shown higher PTEs concentration than inactive site. However, from Figure 4.1, the result shown different wise. This is probably due to remediation process currently being done in the area.

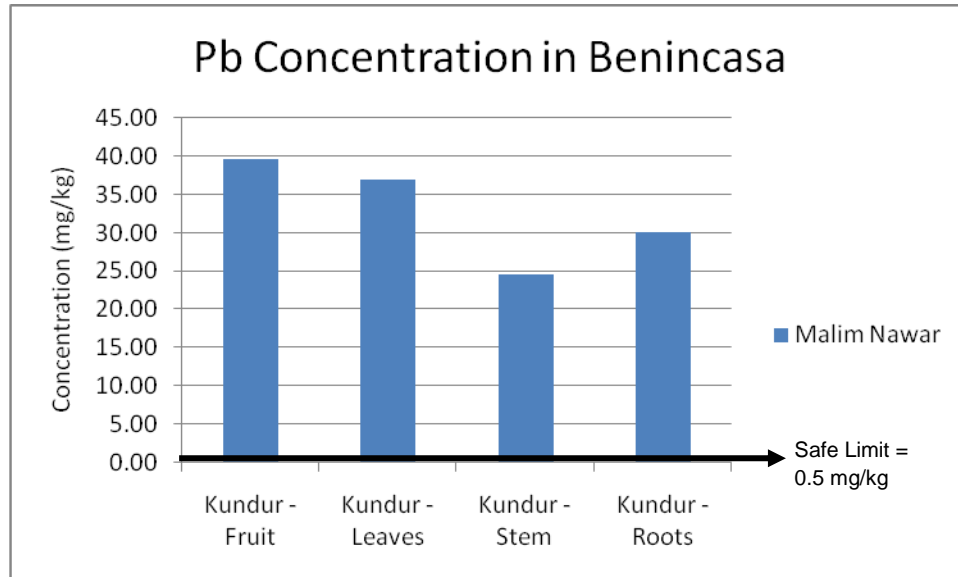


Figure 4.2: Pb Concentration in benincasa in Malim Nawar

From Figure 4.2, the concentration of Pb is observed to be ranging from 25 ~ 40 mg/kg. From the graph also, it is signify that accumulation of Pb is high at fruit part compared to others. Eventhough it has smaller stem diameter compared Melastoma, it posed averagely same value for Pb accumulation in stem. Different species, but still comparable to Melastoma obtained from the same place, Malim Nawar. The highest Pb concentration at fruit part which is 39.69 mg/kg is approximately 80 times higher than the safe limit of 0.5 mg/kg issued by FAO (1983). This finding gives a great impacts fruits are directly consumed and allows heavy metal to enter the food cycle.

4.2.2 Concentration of Pb in fish sample

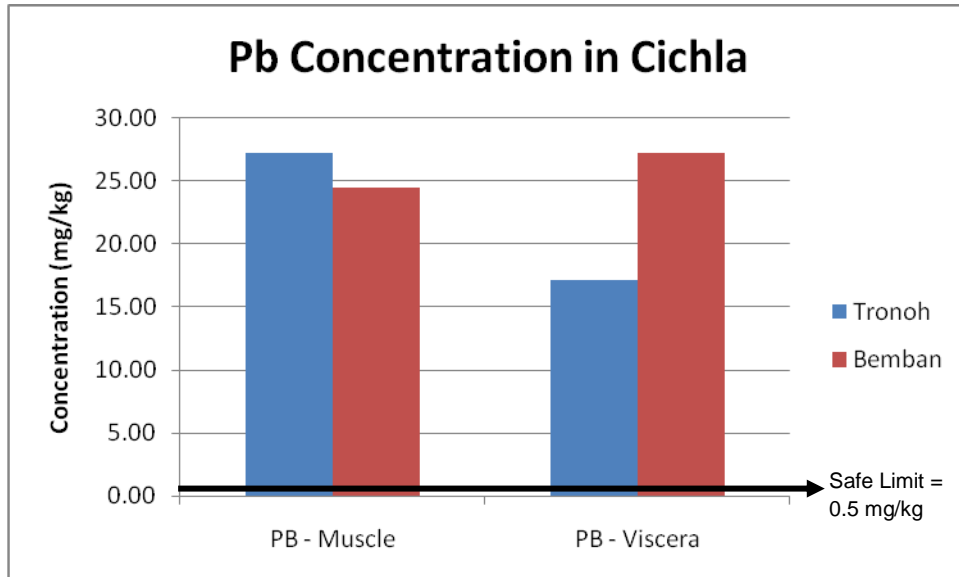


Figure 4.3: Concentration of Pb in Chichla from Tronoh and Bemban.

Figure 4.3 shows averagely same range of values for both sites. Highest concentration of Pb in sample from Tronoh is observed to be more than 25 mg/kg at muscle part and its lowest concentration is about 17 mg/kg in the viscera. For sample from Bemban, the highest concentration is in the viscera with concentration more than 25 mg /kg but has almost same value of concentration in muscle with concentration nearing 25 mg/kg. Pb accumulation in muscle tissue is quite high for specimen from Tronoh compared to its viscera. However, specimen from Bemban exhibit value with small range or margin for its muscle tissue and viscera. Still, the part being consumed is the muscle part which is averagely 52 times higher than the safe limit of 0.5mg/kg issued by FAO (1983). Again, no clear correlation can be observed between active site and inactive site.

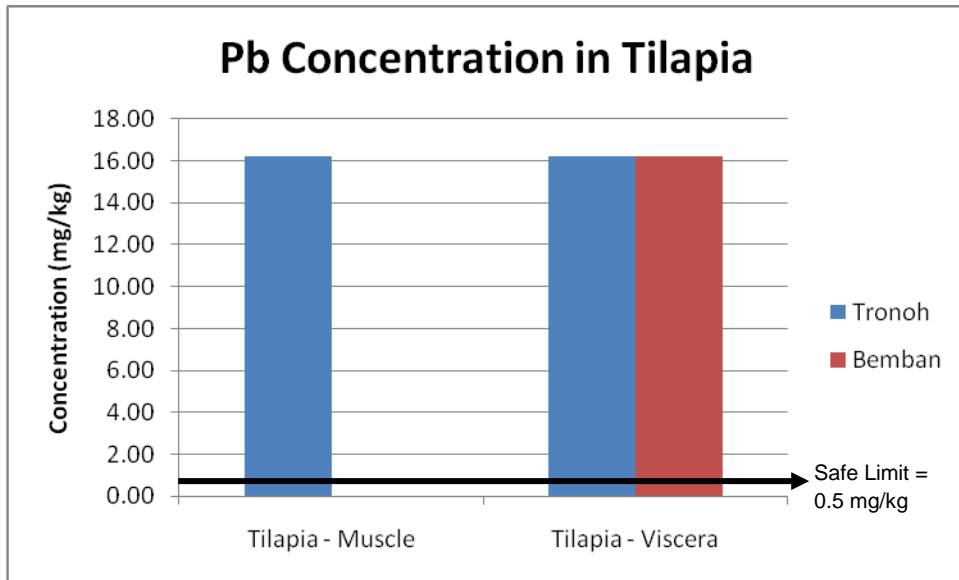


Figure 4.4: Concentration of Pb in Tilapia from Tronoh and Bemban

Concentration of Pb in Tilapia from Tronoh and Bemban however, posed a similar result (~16 mg/kg) for muscle tissue and viscera. However, concentration of Pb for muscle tissue from Bemban's specimen is beyond detection limit which is why no value is shown here. No error has been detected during experiment and analysis which confirmed that the samples have exactly the same amount of Pb concentration. The highest concentration from this result is 16.24 which is approximately 32 times higher than the safe limit of 0.5 mg/kg issued by FAO (1983).

4.2.3 Concentration of Zn in plants sample

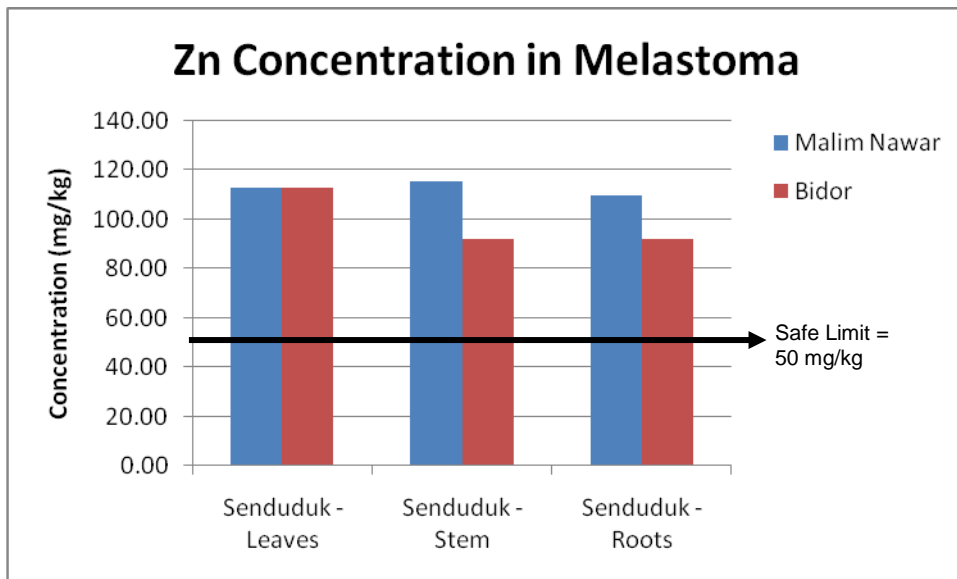


Figure 4.5: Concentration of Zn in Melastoma from Malim Nawar and Bidor

Figure 4.5 above shows high reading of Zn accumulated (~110mg/kg) in Melastoma from both area compared to Pb accumulation in the same species from both sites. However, concentration for Zn at stem part and roots from sample obtained from Bidor is quite less compared to the one obtained from Malim Nawar. The highest concentration of Zn is at the stem of the sample obtained from Malim Nawar which is 115.65 mg/kg. This has exceeded the safe limit of 50 mg/kg issued by WHO (2001) by two times. From this result, it can be observed that concentration of Zn from Malim Nawar is higher than Bidor with averagely small margin.

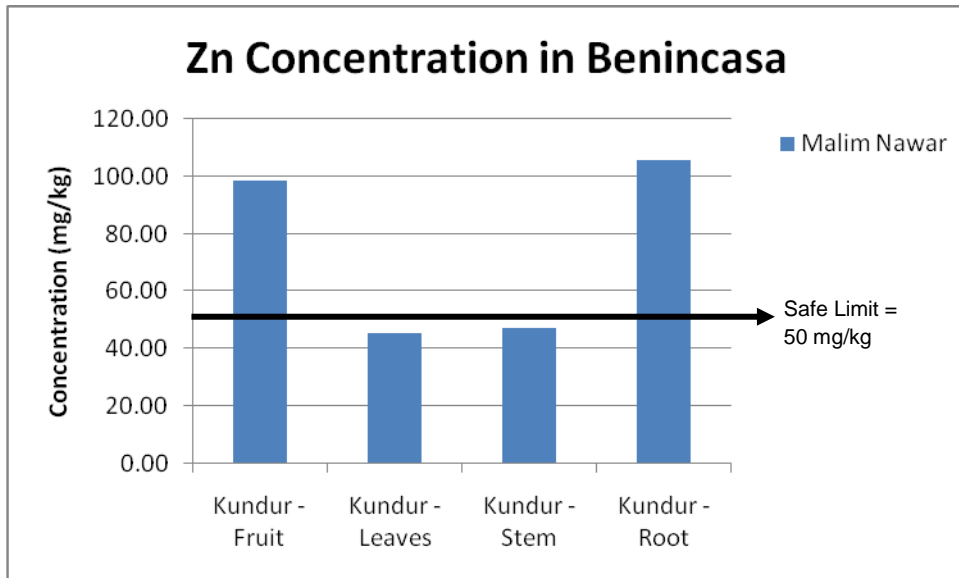


Figure 4.6: Concentration of Zn in Benincasa from Malim Nawar

Zn accumulation in benincasa shows almost the same trend as Pb accumulation in the same species except that the concentration of Zn in the leaves is less compared to concentration of Pb. High accumulation point can be seen at fruit with Zn concentration of second highest of ~100mg/kg and roots with highest concentration compared to other parts with concentration exceeding 100mg/kg. Different from concentration of lead, Zn shows low value of concentration in stem probably due to different ionization process occur in *xylem*. The part being consumed by human is the fruit which have concentration of 98.75 mg/kg. This value is approximately two times higher than the safe limit issued by WHO (2001) of 50 mg/kg.

4.2.4 Concentration of Zn in fish sample

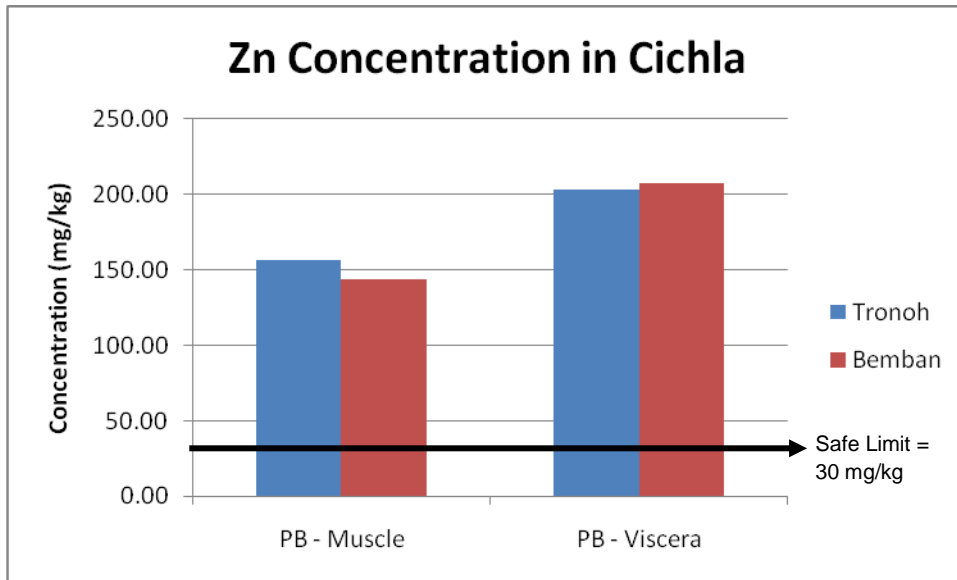


Figure 4.7: Concentration of Zn in Cichla from Tronoh and Bemban

Concentration of Zn on both specimen (from Tronoh and Bemban) shows almost exactly the same trend where Zn accumulated more in viscera compared to muscle tissue. However, concentration of Zn in viscera for both specimens exceeded 200mg/kg which shows quite high value for commercial fish. Still, the concentration of Zn in muscle (~150mg/kg) also is considerably high even it is lower than viscera. Different from concentration of Pb, the accumulation pattern is being observed high at the viscera part compared to muscle for both locations. The highest concentration of Zn is from the sample obtained from Bemban at viscera part with 207.55 mg/kg which is 7 times higher than the safe limit issued by FAO (1983) of 30 mg/kg. Clear correlation in term of location is not signified. However, the correlation of the heavy metal uptake pathway can be observed where concentration of Zn from both samples is higher in viscera rather than muscle.

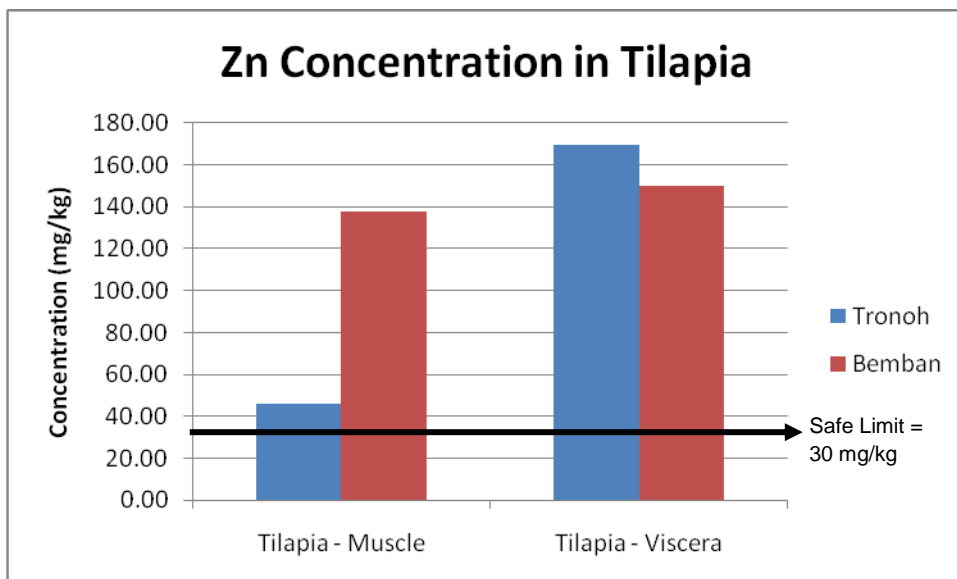


Figure 4.8: Concentration of Zn in Tilapia from Tronoh and Bemban

Figure 4.8 above shows a concentration of Zn in specimen obtained from Tronoh and Bemban which shows that concentration Zn in viscera still higher than Zn accumulated in muscle tissue. However, compared to the specimen obtained from Bemban, the one from Tronoh shows large margin between concentration of Zn (highest is >160 mg/kg and lowest is less than 50 mg/kg) in viscera and muscle tissue probably due to habitual behavior. The highest concentration of Zn was found in viscera of Tilapia obtained from Tronoh with 169.50 mg/kg which is approximately 6 times higher than the safe limit of 30 mg/kg issued by the FAO (1983). No correlation between active site and inactive site can be found in the finding.

4.3 DISCUSSION

Safe limit for each PTEs involved in this study according to its subject are as follows:

- Safe Limit for Pb (Fish) (FAO 1983): 0.5 mg/kg
- Safe Limit for Pb (Crops) (FAO 1983, FAR 1985) : 0.5 mg/kg
- Safe Limit for Zn (Fish) (FAO 1983) : 30 mg/kg
- Safe Limit for Zn (Crops) (WHO 2001) : 50 mg/kg

Based on the results obtained from all of the specimens, it is clearly shown that almost all subjects have PTEs contamination unacceptably over the safe limit (FAO 1983, WHO 2001). Heavy metal uptake pathway is observed to behave almost the same as in research in literature review. However, the results obtained are very high compared to any previous research documentation in ex-mining land.

To clearly visualize the content of PTEs consumed by the general public, it is estimated that an adult human will eat averagely 60 – 70 g of fish per day. Since Tilapia is a popular species to be sold locally, it is estimated by using the formula below, what a normal adult human consume:

$$PTEs (mg/day) = Concentration (mg/kg) \times 0.07kg/day$$

to equal for about 1.1368 mg/day for Pb and 11.865 mg/day for Zn which has exceeded minimum risk limit produced by Agency of Toxic Substances and Disease Registry (ATSDR) of US Department of Health and Human Services:

- 1) **Lead: 0.0000785 mg/day**
- 2) **Zinc: 0.2 mg/day**

Realizing such finding, prompt and strict actions are needed to give people awareness of what they are eating and consuming because such PTEs (Zn and Pb) had been known to contribute to health hazard as mentioned previously. However, real connection between health hazard and PTEs that they may consume in daily food are not visible since other factors such as eating behavior, life style may also contribute to the illness. Nevertheless, part of the contributor for health hazard may come from PTEs consumed in food products cultivated from agricultural site at ex-mining land.

CHAPTER 5

CONCLUSION

5.0 CONCLUSION

The study indicates that there are PTEs, specifically Pb and Zn, in plants and aquatic life grown and bred in ex-mining land regardless of whether that site has activity or not. In all species involved regardless of parts, concentration of Pb and Zn are unacceptably high compared to the safe limit issued by WHO and FAO for food contamination. The concentration of PTEs is at a level that could cause health hazard to humans. Further studies with more systematic and precise approach needs to be done on those areas to ascertain the PTE levels.

Recommendations on this matter will includes giving awareness to people of the danger of consuming food products from former tin mine sites. Consumer has the right to know what they eat thus, time by time, exposure are crucial to avoid future generations still repeating the contaminated food chain. From this study, the level of concentration of PTEs were obtained from large areas former tin mine sites, which may also indicates the same level of PTE's concentration for other ex-mining sites that are not involved in this study. This leads to pre-discussion that former tin mine sites are not safe for any agricultural and aquaculture activities. Thus, the responsible parties should take initiative to find alternate solution of replacing the agricultural activities with other economical activities such as recreational park, tourism sites, which also would be beneficial for the current workers working there (ex-mining site cum agricultural land). For instance, Taiping Lake Garden (Malaysia Vacation Guide 2006) was once a tin mining site, which was converted into tourism spot. And Mines Wonderland in Klang Valley is one of the famous recreational parks in Malaysia which was once a former tin mining site (Wijnen 2001)

As for more technical solution, the former tin mine sites still can be used for agricultural purposes through remediation of the land (US-EPA 2000) but it will take long time to be completed and it may incur high costs. However, for large scale agricultural project and long term vision, this solution is technically feasible to be implemented.

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