Investigation of PTE Levels in Plants at Former Mine Site

by

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Dissertation submitted in partial fulfilment of
the requirements for the
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CERTIFICATION OF APPROVAL

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Approved by,

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TRONOH, PERAK
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CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

NAZHATUL SHIMA BINTI ZOLKEFLI

ABSTRACT

Contamination of Potential Toxic Element (PTE) in plants at former mine site has caused an interest in the scientific approach of their remediation since the PTE may cause a lot of health problems to human itself. However, there is lack of complete catalogues of PTE levels at former mine site for classification. PTE levels in plant may differ according to different locations and the concentration will be different depending to the parts of plant. Due to that, this research is to investigate the PTE levels in plant at former site in Perak Malaysia, according to each part plant which are root, stem and leaf. The sampling was taken at two former mine sites with different condition. Location 1 is active with agriculture activity while location2 has been abandoned for many year and currently has been museum galley. In the field of study, common elements in the PTE are heavy metals, and in this scope of fieldwork which involve former mine site, the heavy metals will be referred as PTE. The main PTE commonly found at former mine site are Lead (Pb) and Zinc (Zn). However, Cadmium (Cd), Nickel (Ni), Copper (Cu), Arsenic (As), Chromium (Cr) and others also can be found at former mine site. The types of PTE investigated in this research are Pb, Ni and Cu. The experiment conducted is using hydrochloric acid (HCl) and nitric acid (HNO_3). The samples were analyzed by using Atomic Absorption Spectrometry (AAS). The result has shown that all the plants taken from former mine studied are having higher concentration of PTE. The concentration of Pb, Ni and Cu are higher than Permissible Exposure Limits in plant which are 2mg/kg, 10mg/kg and 10mg/kg respectively.

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CHAPTER 1

INTRODUCTION

1.1 Background Study

Based on research done, tin mining has begun as early on 1616s in Malaysia. However, beginning in the 1820s when the Chinese began to engage in the mining of tin, it is becoming increasingly rapid and progressive. Tin has become the major pillars of the Malaysian economy. However, the tin mining industry has fallen greatly over the last 30 years due to low tin prices and high operating cost. Currently, many industries on tin mining have been shut down and some of it has become abandoned. Though, some of it has been used for farming, agriculture and also be developed as residential area.

PTE is been considered one of the main pollutant in the environment, since it has a significant effect on its ecological quality (Sastre *et al*, 2002). Expanding interest in the field of PTE research is associated with their increasing emissions into the environment. This has resulted in a growing hazard to human health posed by elevated metal concentrations in the air, water and food (Mirjana *et al*, 2009).

The main concern now is the major threat to environment from former mine site is PTE contamination. The effect of PTE on plants result in growth inhibition, structure damage, a decline physiological and biochemical activities as well as of the function of plants. Background concentrations may be used in the determination of contaminants of potential concern for a site. However, in Malaysia, only few numbers of studies has been done on data collection of PTE level in plant at former mine site whereas plant might be one of the source or access to enter the human food chain. From previous research of PTE contamination in Argentina (Raul, 2004), it is known that the major substrates available are Pb, Zn and Cu in the residual tailing of the mine. In addition, it is claimed that As also one of the common heavy metal pollutant from mining activities (Tomoyoki, 2010). The majority of agricultural soils in Asia are contaminated with As. Others PTE might also available depending on the previous mining method and content of the mineral in the soil.

1.2 Problem Statement

Land and water pollution by heavy metals is a worldwide issue. Al countries have been affected, though the area and severity of pollution vary enormously. In western Europe, 1,400,000 sites were affected by heavy metals of which, over 300,000 were contaminated (Lone *et al*, 2008). A variety of organic and inorganic pollutants, including PTE are being mixed with the cultivated soil and water especially at former mine site. Those pollutants are eventually transported to the natural vegetation, cultivated crops and concentrated in food chains, with possible detrimental effects on human health and wild-life (Parisa *et al*, 2010). However, there is lack of complete catalogues of PTE levels at former mine site for classification. Hence, this research is to investigate the level of PTE levels at former mine site which is necessary to assist in future evaluation of former mine site.

1.3 Objective

i. To investigate the PTE levels in plants from various sample areas.

ii. To investigate which part of plants accumulate the highest concentration of PTE.

1.4 Scope of Study

In achieving project objectives, a comprehensive study has to be conducted and it covers particular scopes as per following:

i. Gather information about the former mine site; during tin mining activities and current activities at the former mine site.

ii. Part of the plants which are roots, stems and leaves will be analysed.

CHAPTER 2

LITERATURE REVIEW

2.1 Former Mine Site

Research showed that metal mining is the second largest source of heavy metal contamination in soil and after sewage sludge (Ashraf *et al*, 2011). Mining activities lead to many of hazardous effects on the environment. The negative impact of the mining activities on the surroundings is mainly due to the presence of high volumes of tailings. The tailings usually have unfavourable conditions to natural vegetation growing on it such as low levels of plant nutrients.

This study focuses on former mine site scattered around Perak. After having some observation, two locations have been selected which are the former mine site having agriculture activities and the other one is the former mine site that has been abandoned and now has been museum.

2.2 Potential Toxic Element (PTE)

In this field of study, common elements in the PTEs are heavy metals. Heavy metals are non-degradable and can persist for long period in aquatic and terrestrial environment (Otitoloju *et al*, 2002). These metals will be transported through soils to be taken up by plants.

The availability of metals in soil is a naturally selection phenomenon for plants, that are capable of surviving or reproducing under high metal concentrations. Some plant species can grow in these severe conditions. However, excessive concentration of heavy metals such as Cadmium (Cd) and Nickel (Ni) in plants can cause oxidative stress. Presence of Copper (Cu) may affect photosynthesis process and reproductive processes process. Lead (Pb) may reduce chlorophyll production while Zinc (Zn) stimulates the growth of leaves and shoots. Hence, ultimately plant growth becomes limited or impossible.

In the field of study, common elements in the PTE are heavy metals. Hence, in this scope of fieldwork which involve mining site, heavy metals will be referred to as PTE.

2.2.1 Lead (Pb)

Lead is useful and common metal that has been used by humans for thousands of years. It is also a very dangerous poison when it is accidentally inhaled or ingested. It has been known for a long time that Pb is very toxic to brain, kidneys and reproductive system. Pb can also cause impairment in intellectual functioning, fertility, miscarriage and hypertension. In children, Pb will decrease the muscle & bone growth behaviour issues and reduce the intelligence (IQ). In adults, Pb toxicity usually causes digestive issue, nerve disorders, and memory & concentrations problems. High level of Pb are life threatening and can cause seizures, unconsciousness and death. Contaminated soil is one of the common sources of Pb.

2.2.2 Nickel (Ni)

Ni is a chemical element which belongs to the transition metals. Ni has gained considerable attention in recent years, because of its rapidly increasing concentrations in soil, air, and water in different parts of the world. The main mechanisms by which Ni is taken up by plants are passive diffusion and active transport. Soluble Ni compounds are preferably absorbed by plants passively, through a cation transport system; chelated Ni compounds are taken up through secondary, active-transport-mediated means, using transport proteins such as permeases. Insoluble Ni compounds primarily enter plant root cells through endocytosis. Once absorbed by roots, Ni is easily transported to shoots via the xylem through the transpiration stream and can accumulate in neonatal parts such as buds, fruits, and seeds. Ni deficiency produces an array of effects on growth and metabolism of plants, including reduced growth, and induction of senescence, leaf and meristem chlorosis, alterations in N metabolism, and reduced Fe uptake (Ahmad *et al*,2011). When crops encounter excessive Ni exposures, altered processes produce will reduced yields of agricultural crops.

2.2.3 Copper(Cu)

Copper is a chemical elements where the metal and its alloys have been used for thousands of years. Copper is a very common substance that occurs naturally in the environment and spreads through the environment through natural phenomena. The absorption of copper is necessary, because copper is a trace element that is essential for human health. Although humans can handle proportionally large concentrations of copper, too much copper can still cause eminent health problems. Long-term exposure to copper can cause irritation of the nose, mouth and eyes and it causes headaches, stomachaches, dizziness, vomiting and diarrhea. Intentionally high uptakes of copper may cause liver and kidney damage and even death.
When copper ends up in soil it strongly attaches to organic matter and minerals. As a result it does not travel very far after release and it hardly ever enters groundwater. In surface water copper can travel great distances, either suspended on sludge particles or as free ions. Copper does not break down in the environment and because of that it can accumulate in plants and animals when it is found in soils. On copper-rich soils only a limited number of plants have a chance of survival. That is why there is not much plant diversity near copper-disposing factories. Due to the effects upon plants copper is a serious threat to the productions of farmlands. Copper can seriously influence the proceedings of certain farmlands, depending upon the acidity of the soil and the presence of organic matter. Despite of this, copper-containing manures are still applied. Copper can interrupt the activity in soils, as it negatively influences the activity of microorganisms and earthworms. The decomposition of organic matter may seriously slow down because of this.When the soils of farmland are polluted with copper, animals will absorb concentrations that are damaging to their health. Mainly sheep suffer a great deal from copper poisoning, because the effects of copper are manifesting at fairly low concentrations.

2.3 Permissible Exposure Limits (PEL) of PTE in Plants

Table 1: PEL of PTE in Plants

ELEMENT	PERMISSIBLE LEVEL (mg/kg)
Lead, Pb (WHO,1996)	2
Nickel, Ni (WHO,1996)	10
Copper, Cu (WHO,1996)	10

2.4 Phytoextraction

PTE at former mining area needs bio-monitoring to remediate the soil. Hence, phytoextraction is the best way to use in order to investigate the PTE level. Phytoextraction can be defined as the ability to uptake of contaminant by plant roots and movement of the contaminants from the roots to aboveground parts of the plant. Usually, the plants suitable for phytoextraction are small, have a small and shallow root. Phytoextraction accumulates the contaminant which is PTE in a much smaller amount of material to be disposed of than doing excavation of soil and sediment. This technique is mostly applied to heavy metals in soil, sediment and sludge. Plants have a natural propensity to take up metals. In this approach, plants capable of accumulating high level of heavy metal grown in contaminated soil (Mitch).

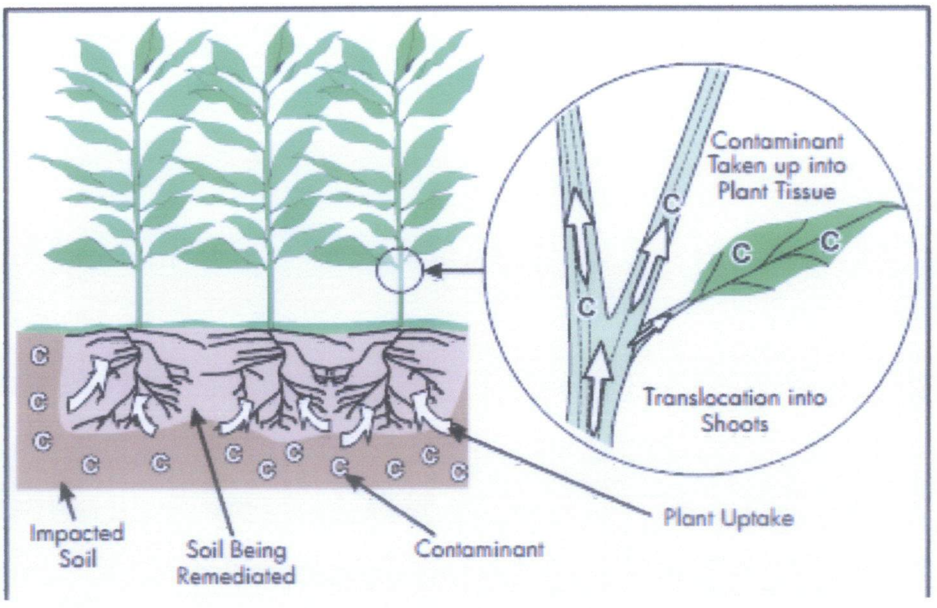


Figure 1: Phytoaccumulation of inorganic contaminants.

By using phytoextraction, it will tell us the PTE levels at three parts of plant which are root, stem and leaf. After knowing the PTE levels, the remediation of soil can initiate by using plant as well through phytoremediation.

2.5 Atomic Absorption Spectrometry

The key component of this research is Atomic Absorption Spectrometry (AAS) in which the level of PTEs' concentration can be tested and determined through this machine. This atomic absorption spectrometry has been used worldwide by scientist 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 element present. AAS will use the concept of atomic absorption and emissions which later produces line of spectrum that can be analysed by collector.

In atomic absorption spectrometry, there are five 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 about absorption of radiation by the sample (Levinson).The light source will emit electromagnetic radiation, depend on the trace metal to be studied, to the excited atoms. Then, 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. By supplying heat to the sample, atoms will start to excite and transit to the higher energy level. At this state, absorption process will occur. After 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 dispersal and monochromator will resolve the lines. In the detection chamber, the photomultiplier tube will amplify the intensity and corrected the ambient wavelength.

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

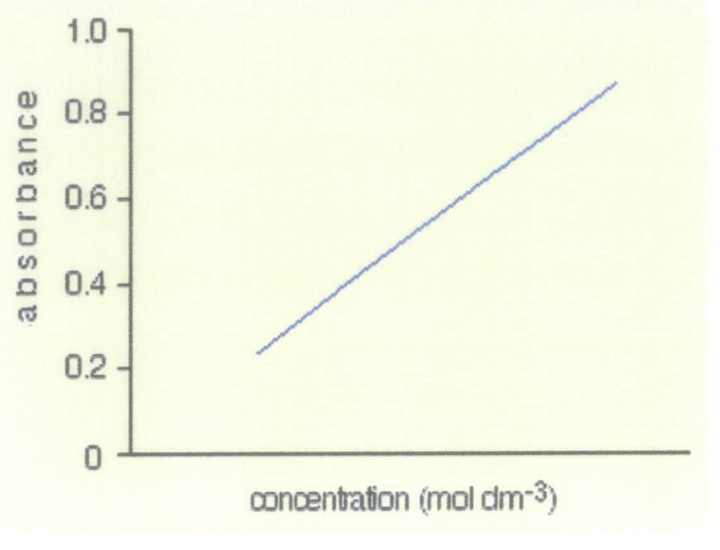


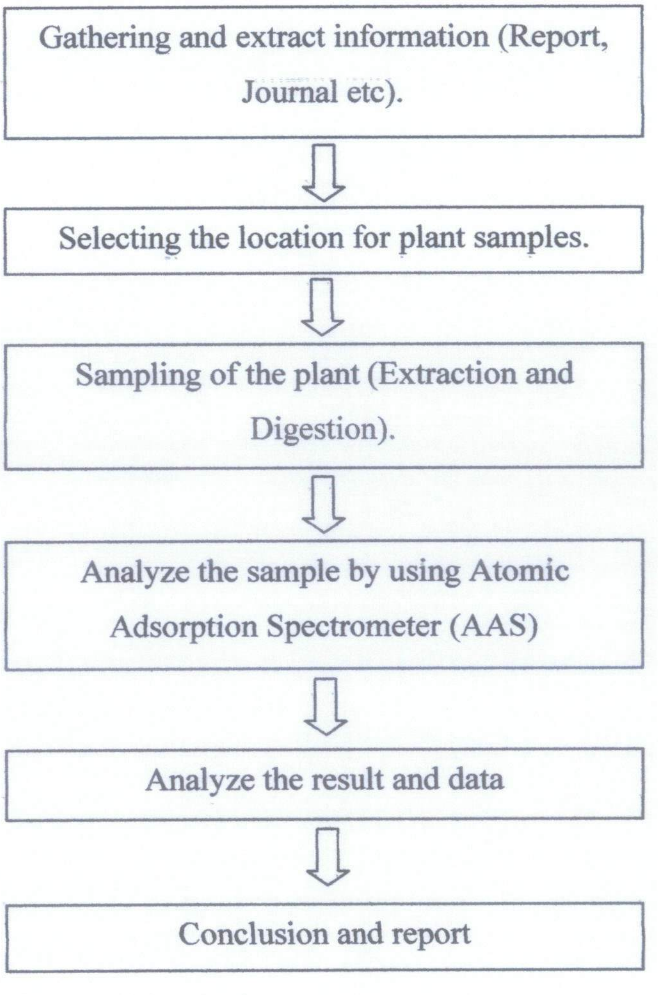
Figure 2: Plot of Absorbance against Concentration

Linear line calibration curve 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 Pb, Pb 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 Pb.

CHAPTER 3

METHODOLOGY

3.1 Project Flow



3.2 Study Site (Sampling Location)

In selecting suitable sample area, various conditions of area will be the priority. This is because of history and current condition of area might be the reason in the trend of result. Example of various condition of areas are the former mine site that has be abandoned or the area having agriculture activities.

The location will be divided into two parts which are active and inactive. The locations are as below:

- Batu 8, Tanjung Tualang (Active): Converted to agricultural land.
- KM9, Tanjung Tualang (Inactive): Abandoned & a museum was built & opened since 2009.

Plants may take up PTE by their roots, stems and leaves (Cheng, 2013). The accumulation and distribution of PTE are depending on the species and element. Due to that, this research will be done to the parts of plant which are root, stem and leaf.

3.3 Plant Sampling

Three species of plants are randomly selected within 1 km diameter of location. (Lai *et al*, 2010)

⇩

The plant samples are harvested by using scythe.

⇩

Then, the plant samples will be kept in a plastic bag and labelled with

Location 1: Sample 1, 2 & 3 ; Location 2: Sample 4, 5 & 6

⇩

The samples will be kept in laboratory for elemental analysis

The types of plants for both locations are listed as below:

Table 2: Type of plants investigated

Location	Sample No.	Name of plants
Location1	1	Achyranthes Aspera
	2	Turnera Ulmifolia
	3	Cuphea Hyssopifolia
Location 2	4	Achyranthes Aspera
	5	Turnera Ulmifolia
	6	Cuphea Hyssopifolia

3.4 Experimental

1. Sample of plant part were weighed to determine the fresh weight and dried in an oven at 80°C for 72 hours to determine their dry weight (Akan *et al*, 2013).

Figure 3: The plant samples were dried in an oven

2. The dry samples were crushed in a mortar and the resulting powder digested by weighing 0.5g of oven-dried ground and sieve (<1mm) into an acid-washed porclain crucible and placed in a muffle furnace for 4 hours at 500°C.

Figure 4: The sample was crushed and become powder form.

3. The crucibles were removed from the furnace and cooled.
4. 10ml of 6M HCL was added, covered and heated on a steam bath for 15 minutes.
5. Another 1ml of HNO_3 was added and evaporated to dryness by continuous heating for 1 hour to dehydrate silica and completely digest organic compounds.
6. 5ml of 6M HCL and 10ml of water were added and the mixture was heated on a steam bath to complete dissolution.
7. The mixture was cooled and filtered through a Whatman no. 541 filter paper into a 50ml volumetric flask and made up to mark with distilled water.
8. Filtered sample is poured into 10ml sample vial.
9. The sample is ready to be analysed by using AAS.

3.5 Materials and Equipment

Table 3: Material and equipment used for experimental purpose

Materials	Equipment
Plant samples	Laboratory apparatus
Nitric Acid (HNO_3)	Oven
Hydrochloric (HCl)	Furnace
Distilled water	Atomic Absorption Spectrometer (AAS)

3.6 Gantt Chart (FYP 1)

No.	Detail/Week	FYP 1													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Selection of project topic														
2	Preliminary Research Work														
3	Submission of Extended Proposal														
4	Project Work -location of sample selection -methodology														
5	Proposal Defense														
6	Taking plant (sample) at former mine site														
7	Lab analysis														
8	Preparation of Interim Report														
9	Submission of Interim Report														

3.7 Gantt Chart (FYP 2)

No.	Detail/Week	FYP 2														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Lab analysis															
2	Preparing analysis and report															
3	Submission of progress report															
4	Pre-EDX															
6	Submission of dissertation (softbound)															
7	Oral presentation															
8	Submission of technical paper															
9	Submission of project dissertation (hardbound)															

CHAPTER 4

RESULTS AND DISCUSSION

The plant samples were taken on August 2013 in two different locations which are:

- Location 1: Batu 8 Tanjung Tualang, Perak
- Location 2: KM9 Jalan Tanjung Tualang, Perak

All of the plant has been labelled as S1, S2 and S3 at location 1, and as S4, S5 and S6 at location 2 as tabulated below.

Table 4: The samples according to their location and description.

Location	Description	Samples Number
Location 1: Batu 8,Tanjung Tualang	**Active**: Converted to agriculture land	1,2 & 3
Location 2: KM9, Tanjung Tualang	**Inactive**: Abandoned for almost 30 years. Then, a museum was built & opened since 2009.	4,5 & 6

In the laboratory, the sampling of plant has been done by divided into three parts which are root, stem and leaf for each plant. Then, the plant was digested by using Nitric Acid (HNO_3) and Hydrochloric Acid (HCl).

The finished sample has been analysed by using Atomic Absorption Spectrometer (AAS) in order to know the level of PTE in parts of plants.

There are three results which are respectively for Pb, Ni and Cu.

The concentration unit from AAS data is mg/L. Hence it will be converted to mg/kg by this equation:

$$Concentration\left(\frac{mg}{kg}\right) = \frac{AAS\left(\frac{mg}{L}\right) \times Volume\ of\ Extraction\ (L)}{Weight\ of\ Sample\ (kg)}$$

Table 5: Pb Average Concentration absorbed by AAS (mg/kg) for leaf, stem and root of each plant

Source	Pb Average Concentration absorbed by AAS (mg/kg)					
	Location 1			Location 2		
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Leaf	25	23	23	24	21	18
Stem	21	24	21	22	18	16
Root	100	10	22	19	5	17

Table 6: Ni Average Concentration absorbed by AAS (mg/kg) for leaf, stem and root of each plant

Source	Ni Average Concentration absorbed by AAS (mg/kg)					
	Location 1			Location 2		
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Leaf	70	171	50	195	502	49
Stem	72	92	36	98	45	47
Root	55	56	45	54	46	48

Table 7: Cu Average Concentration absorbed by AAS (mg/kg) for leaf, stem and root of each plant

Source	Cu Average Concentration absorbed by AAS (mg/kg)					
	Location 1			Location 2		
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Leaf	89	73	100	49	112	87
Stem	74	60	39	24	72	45
Root	31	43	91	91	41	44

The data the will be analysis by referring the graph in figure below:

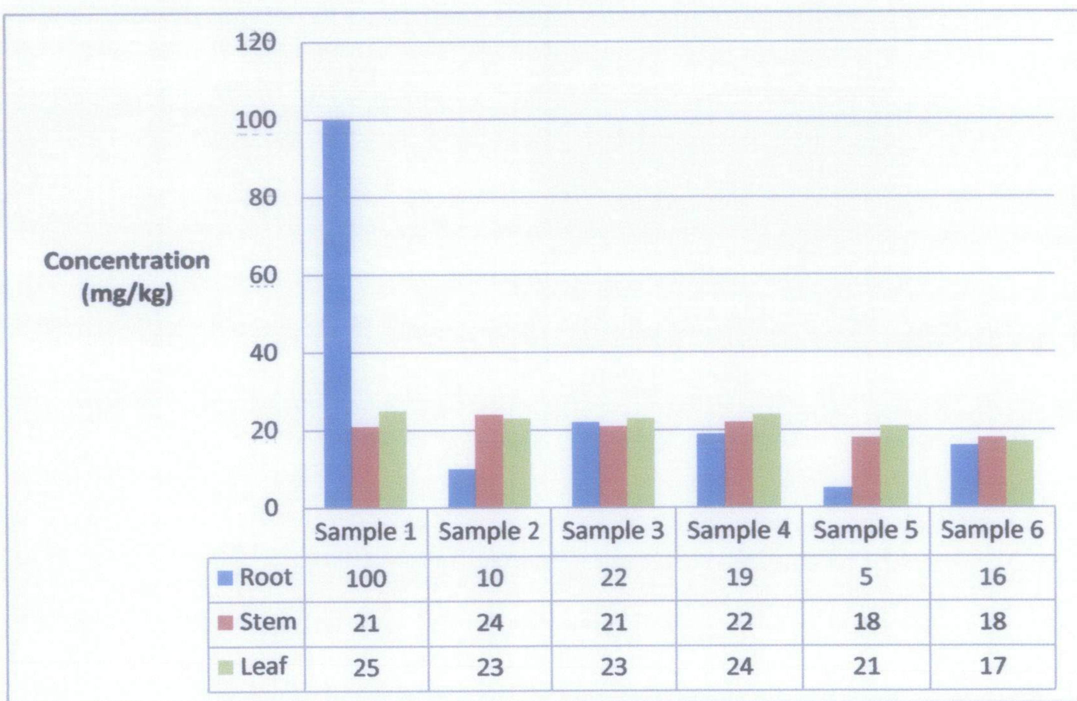
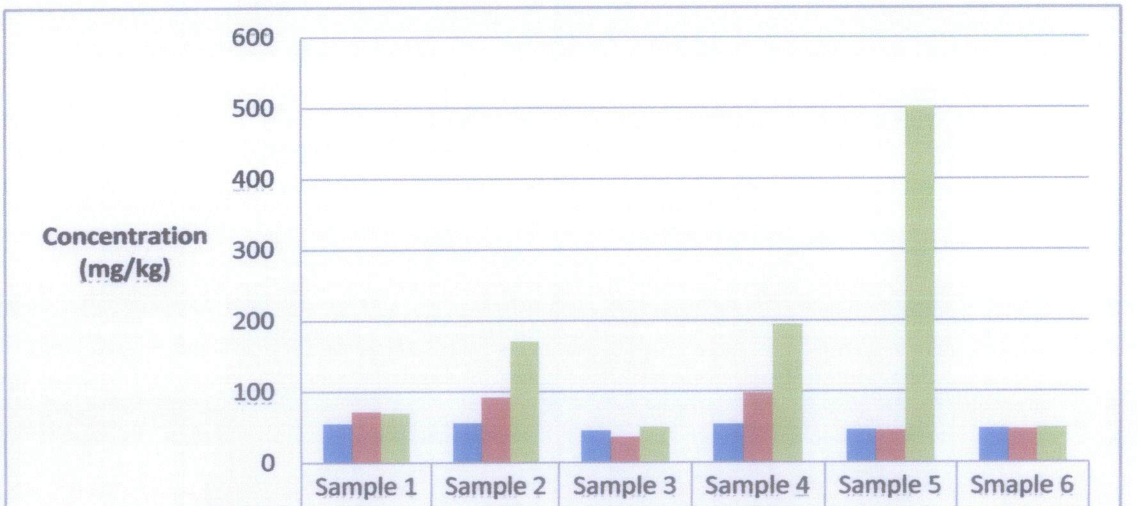


Figure 5: Mean concentrations of PTE (Pb) in different parts of plant samples.

Based on Figure 5, the concentration of heavy metals in sample 1 has highest concentration on root and for the sample 2, the highest concentration is on stem. However, for sample 3, 4, 5 & 6, the highest concentration is on the leaf. The concentration of Pb on the root is lowest for Sample 2, 4, 5 & 6.

As mentioned earlier, Sample 1, 2 & 3 is from location 1, while Sample 4, 5 & 6 is from location 2. It is not too much difference of their concentration of Pb to be compared from location 1 and 2. However, most of the result showed that samples from location 1 have higher concentration of Pb.

The permissible limit of Pb in plants recommended by World Health Organization (WHO) (Iqbal *et al,* 2011) is 2mg/kg. The values of Pb concentrations in plant samples are higher than 2mg/kg, especially for Sample 1, on its root. This means that the plants are unsafe from the hazardous effects of Pb.

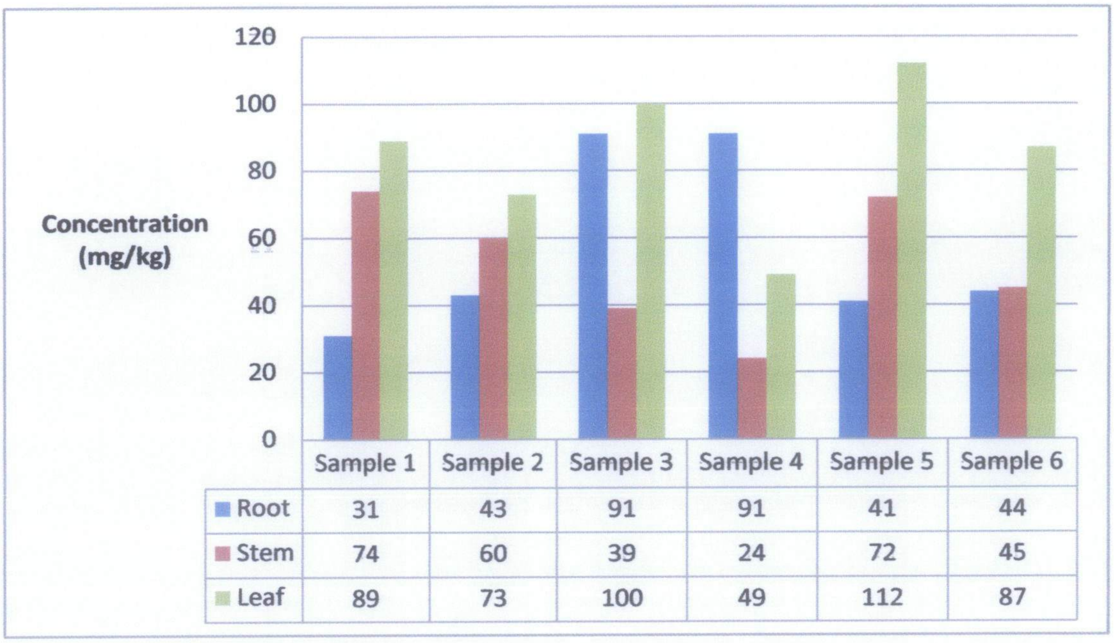


	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Smaple 6
Root	55	56	45	54	46	48
Stem	72	92	36	98	45	47
Leaf	70	171	50	195	502	49

Figure 6: Mean concentrations PTE (Ni) in different parts of plant samples.

Most of the samples have highest concentration on the leaf, except for sample 1. By referring to the Table 6, sample 5 shown the highest concentration of Ni, which is 502mg/kg. This proves that the plants have ability to uptake of contaminant by roots and movement of the contaminants from the roots to aboveground parts of the plant. In the other words, plants have a natural propensity to take up metals.

The values of Ni concentrations in plant samples are higher than 10mg/kg, especially in the leaf for Sample 5. This shows that the former mine site still having high level of PTE after many year has been left over.



	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Root	31	43	91	91	41	44
Stem	74	60	39	24	72	45
Leaf	89	73	100	49	112	87

Figure 7: Mean concentrations PTE (Cu) in different parts of plant samples.

Based on Figure 7 above, the concentration of Cu in leaf is highest for all samples, except for sample 4. Sample 1, 2 5 & 6 have the lowest concentration on the root. This shows that the accumulation of Cu is highest at leaf, followed by stem and root.

The values of Cu concentrations in plant samples are higher than 10mg/kg, especially in the leaf for Sample 1. This means that the plants are unsafe from the hazardous effects of Pb.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

Based on the results obtained, the highest accumulation of PTE is on the leaf followed by stem and root. The results also indicate that all the plant samples analysed in this study have high levels of PTE. The highest PTE contaminant in this study is Ni, followed by Cu and Pb.

In addition, the levels of all the PTE studied were higher than Permissible Exposure Limit for plants which are 2mg/kg, 10mg/kg and 10 mg/kg respectively for Pb, Ni and Cu.

Furthermore, there is not much difference of their concentration of PTE to be compared from location 1 and 2. However, most of the result showed that samples from location 2, which has been abandoned for many years has higher concentration of Pb, Ni and Cu compared to location 1.

Last but not least, all these three plants can be used for remediation of soil as it has been proven that they have ability to take up high concentration of PTE. The plants are Achyranthes Aspera, Turnera Ulmifolia and Cuphea Hyssopifolia.

However, for better improvement in future investigation, it is highly recommended for having a plant as a control to differentiate the contamination in plants taken from mine site and from the site that is not related with mine site.

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APPENDICES

Appendix 1: The calibration curve

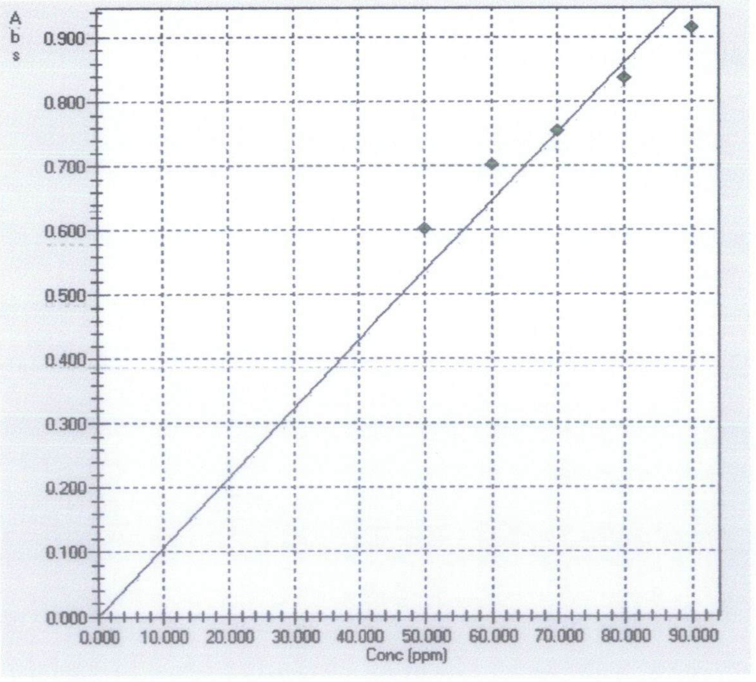

Figure 8 : The calibration curve for Pb with 0.997 slope.

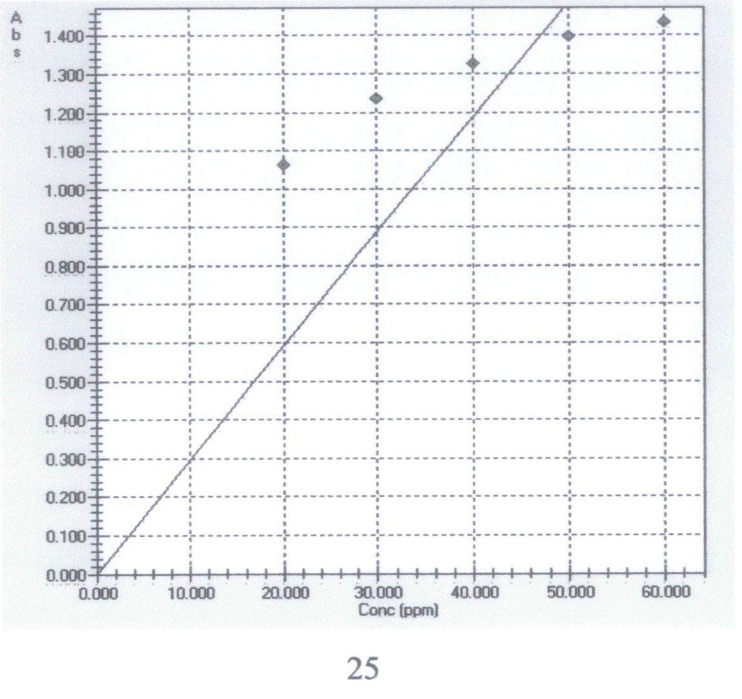

Figure 9: The calibration curve for Ni with 0.964 slope.

Appendix 1: The calibration curve (Continue)

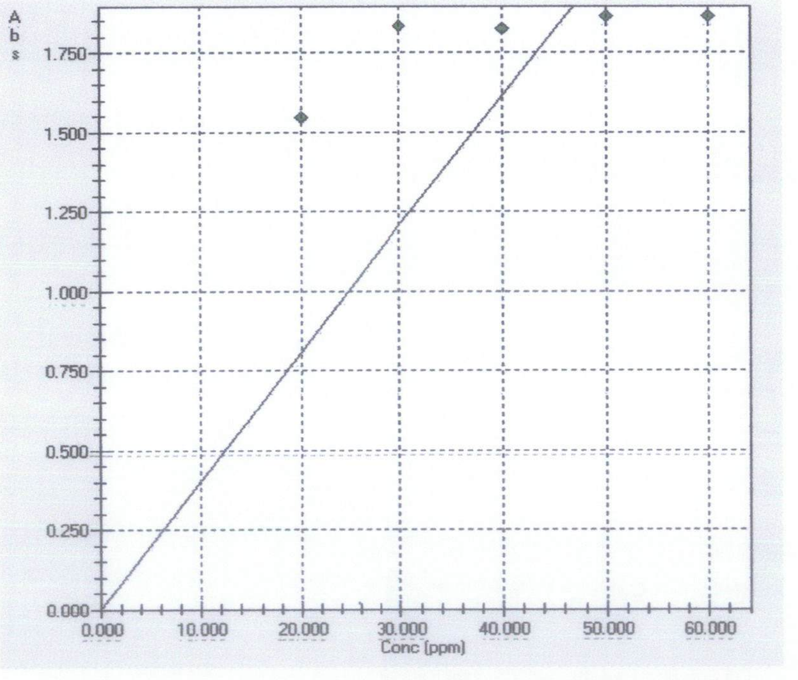

Figure 10: The calibration curve for Cu with 0.778 slope.

Appendix 2: Related Figure

Figure 11: Fish pond at Batu 8 Tanjung Tualang, Perak

Figure 12: Farming site at Batu 8 Tanjung Tualang, Perak

Appendix 2: Related Figure (Continue)

Figure 13: The last dredge at KM9 Tanjung Tualang Perak

Figure 14: Achyranthes Aspera

Appendix 2: Related Figure (Continue)

Figure 15: Turnera Ulmifolia

Figure 16: Cuphea Hyssopifolia

Appendix 2: Related Figure (Continue)

Figure 17: The sampling of plants in the labarotary.

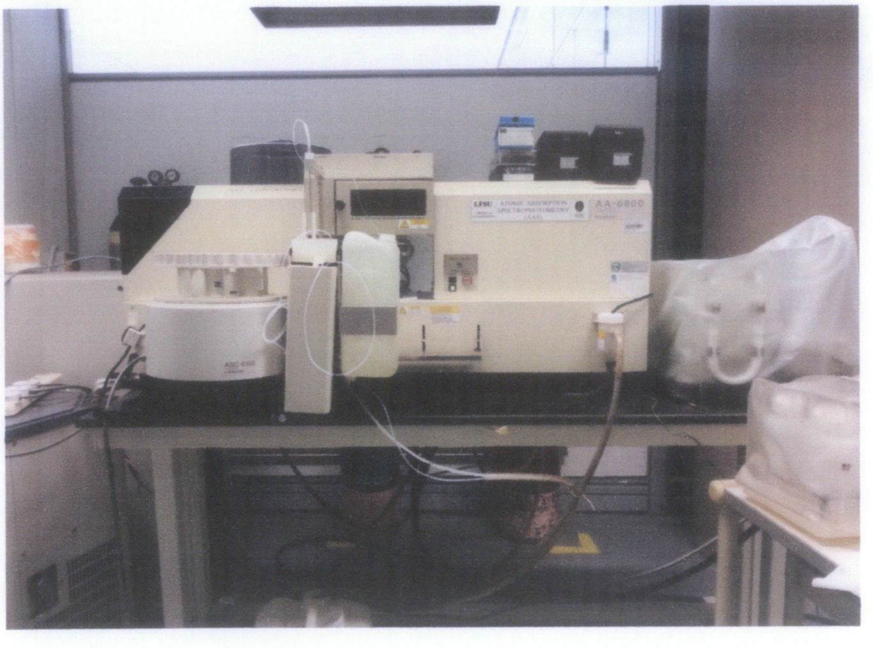

Figure 18: Atomic Absorption Spectrometer (AAS) used to analyse the concentration of heavy metals.

Appendix 2: Related Figure (Continue)

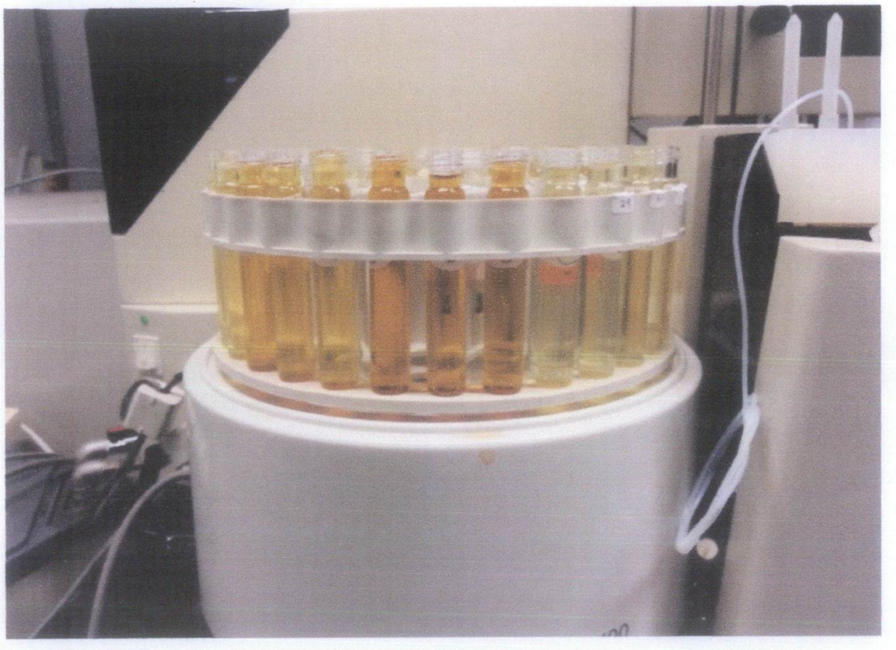

Figure 19: The samples were ready to be analysed by AAS.