Biomarker as Indicator of Aerial Dispersal of Heavy Metal within Remediated and Abandoned Tin Mine Site

By

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

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Chemical Engineering Programme
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Approved by,

(Dr. Nurlidia binti Mansor)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

JUNE 2010

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.

7/6/2010

HAFIZAH BINTI AHMAD AFIF 8142

ABSTRACT

This project aims to investigate the behavior of aerial dispersal of heavy metals in the former tin mine sites in Perak. Research consists of two parts: (a) the study of heavy metal concentrations at remediated sites of former tin mine and (b) the study of heavy metal concentrations at abandoned sites of former tin mine. In this report, the research activity focuses on the application of biomarkers from plants in these sites due to their reliability in environmental monitoring study. Tree barks from several tree species are collected to indicate the concentration level of lead (Pb) and zinc (Zn). Species of plants are chosen based on their availability at the study sites. Accumulation of heavy metals left by mining activities would contribute to the degradation of mineral contents needed by plant for growth in former mining lands. Previous attempts to exploit the idle lands as agro forestry plantation mostly failed to meet the expectation especially in health and safety issues. According to several studies, the crops grown on former mining land are found to contain high level of heavy metals (Reuters, 2002) which could be a threat to human health. To overcome this issue, former mining lands must be remediated to reduce the degree of pollution and establish the accumulation of heavy metals to a tolerable level. Forest Research Institute Malaysia - Bidor Research Station (FRSB) is one of the examples of former tin mine which has had remediation activities such as phytoremediation. Studies are done to determine and investigate the behavior of aerial dispersal of Pb and Zn thus assessing the pollutant effects over the years when remediation started. Tree barks will be collected from the sites and used for laboratory analysis to indicate the Pb and Zn concentration by using Flame Atomic Absorption Spectrometry (FAAS). To study the influence of distance in airborne deposition, samples were collected at three different distances which are 5 m, 50 m, and 100 m from a point source. To study the relation between height and concentration, samples are collected at three different heights which are 0.6 m, 1.2 m and 1.8 m of a single tree. From results analysis, concentration of Pb and Zn at TDH is higher than FRSB mainly at 5 m distance from the point source. Comparison

between concentration of Pb and Zn in 2006 with current concentration referred from this study in year 2010, shows reduction in Pb and Zn concentration. In the context of distance from source aspect, most of the trees located closer to the source point are receiving the mine waste greatly enriched in Pb and Zn. From this study, it shows no relation between height of a single tree with Pb and Zn accumulation.

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List of Abbreviations

As	Arsenic
Cu	Copper
FRIM	Forest Research Institute Malaysia
FRSB	Forest Research Institute Malaysia - Bidor Research Station
ha	hectare
Hg	Mercury
Ni	Nickel
Pb	Lead
TDH	Tanjung Tualang Tin Dredge Heritage
Zn	Zinc
FAAS	Flame Atomic Absorption Spectrometry
PTE	Potential Toxic Elements

INTRODUCTION

Tin is the 49^{th} most abundant element and has, with 10 stable isotopes, the largest number of stable isotopes in the periodic table. Tin is obtained predominantly from the mineral cassiterite, where it occurs as tin dioxide, SnO_2 by reducing the ore with coal in a reverberatory furnace. The main use of tin is to coat other metals to prevent corrosion (Husted, 2003). Tin mining was a big industry once ago and Malaysia had become one of the major contributors of tin in the world economy. However, the number of operating tin mines has gradually decreased and resulting more than 2100 abandoned areas which later then become ponds. Those ponds contain thick layers of slurry slime at the bottom. The slurry contains heavy minerals such as ilmenite, monazite, zircon and xenotime. There are also existence of heavy metals such as arsenic (As), aluminum (Al), chromium (Cr), Zn, cadmium (Cd), Pb, nickel (Ni) and nitrate (NO_3) in former tin mining area (Haidar, 2007). Previous studies such as those of Wang & Mulligan (2005), Navarro et al. (2007), and Schwab et al. (2007) showed that mining is one of the most important sources of heavy metal contamination in the environment. The collapse of mining industries in 1930s resulted in approximately 113 750 ha of degraded land. Part of these former mining lands were transformed into useful land such as housing, recreational parks, industrial park etc. However about 80 000 ha remains unutilized (Ang, 2004). The revitalization and rehabilitation process in those areas should start with land structuralizing and involves remediation activity. For example, FRSB is currently conducting pyhtoremediation techniques with high timber value trees. It has successfully converted the area into a biodiversity park cum small-scale forest plantation with agro forestry on the side (FIF, 2002). Looking at the abundance of trees, it can be said that the land contents have improved hence supports the growth of plantation. This shows that trees are able to take up heavy metals and improve soil quality in former mining land (Ang, 2004). This draws the interest to determine the atmosphere content and conduct a comparative study to see the significant effects of phytoremediation towards heavy metal dispersal in the atmosphere.

1.1 Background

This section describes the selected sites of study, their historical values, and environment surrounding the FRSB and TDH. A general investigation was initially performed in both areas where they fulfill 4 criteria; (1) study site should be a former tin mining land; (2) must has remediation activity on-going (3) must be abandoned, currently none serious remediation is worked on it; (4) site must be far from industrial area to avoid possible effects related to industrial pollution.

1.1.1 FRIM Research Station Bidor

The first selected site is FRSB, geographically located at latitude 4° 7' 12" North of the Equator and longitude 101° 16' 48" East (*see Figure 1.1-1*). It is a mining town about 128 km north of Kuala Lumpur. The active period of mining activities at this site was during the 1950s using gravel pump and dredging methods. Previously the study site was populated by grasses and small pioneer species such as the *Macaranga spp.* and *Vitex spp.* The illegal settlers planted a few of plant species such as the rambutan, coconut, *Melia azadiractha* and *Pterocarpus indicus* for fruits, medicinal products and shade respectively (Ang, 2004). According to Malaysia Meteorological Department (2010), Perak had received the monthly rainfall above average which is 300 mm with mean total annual rainfall of 3218 mm, indicated that the project site was rather wet.

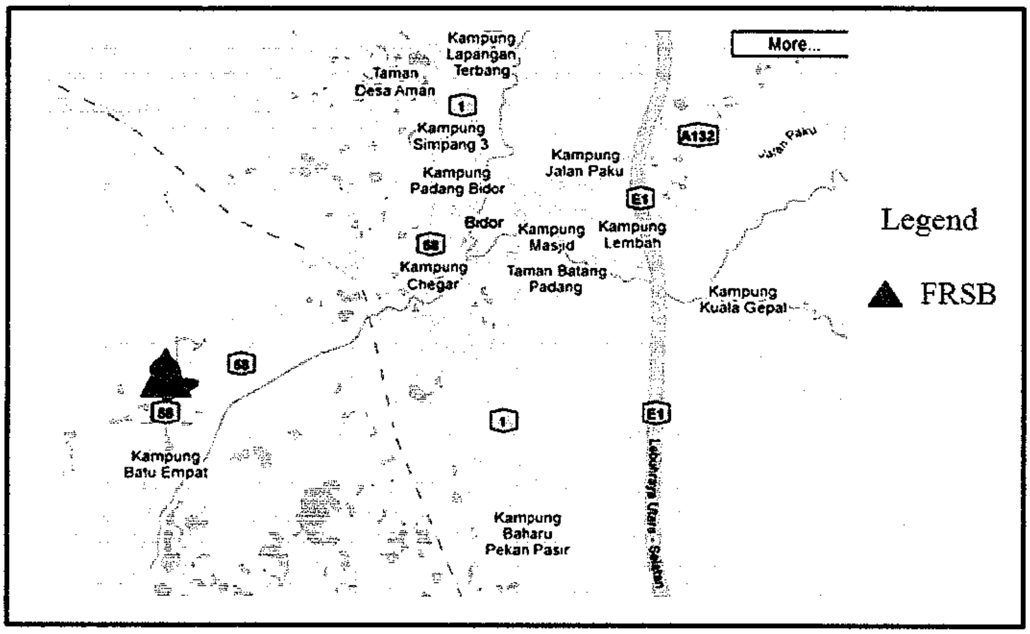

Figure 1.1-1 Location of FRSB with A sign (Google Maps)

Phytoremediation has been one of the accepted approaches of remedial measure. It uses plants to extract the ions from the soils using photosynthetic energy, which accumulate them in the biomass; roots, stems and branches according to nutritional requirements (Prasad, 2007). According to Clemens (2001), the extraction works when the PTE presents at elevated levels, it is able to enter the higher plants but need to refer the chemical similarity to other nutrients ions. For example, ASO_4^{3-} can enter roots through the uptake systems for PO_4^{3-}. Harvesting of the aboveground biomass accumulated with heavy metals will remove the PTE from the contaminated site after successive growth periods. Other advantage of afforestation is that it contributes towards carbon sequestration where trees act as carbon sink thus helping to reduce the global concentration of carbon dioxide.

Green house study has shown that many types of plant may accumulate considerable amount of Pb, Cd, As and Hg from soils. At FRSB, species that are proven to be used as phyto-extractors are *Acacia mangium, Pinus, Flindersia brayleyana,* and *Khaya ivorensis* (Ho & Ang 2003). This approach still has many advantages over the traditional treatment. One of the advantages is this method does not cause further disruption to the soil dynamics because soil is treated in-situ. Moreover, it also sustains the soil nutrients and stabilization by reducing water and wind erosion. Phytoremediation reduces the duration of workers expose to the radionuclides (Negri, 2000) and it can be used as a long-term treatment that is economical.

FRSB with 121.4 ha large is proving that phytoremediation has potential to remove polluted and contaminated elements while slowly producing good timber. A recent survey of *Pinus, Flindersia brayleyana* and *Khaya ivorensis* produced from FRSB estimated yields as high as USD 30 000 per 40 000 ha at 40 years after planting (FIF, 2002). The prospects of converting abandoned former mining land into forest plantations are more significant, compared to agricultural products on tin mine land which have been found to be potentially toxic. This is strongly supported by most studies on former mining lands, confirmed that mining soils are subjected to heavy anthropogenic disturbance which may convert as Potentially Toxic Element (PTE). There is concern that the PTE concentration may exceed human tolerance limit and cause severe health problems.

1.1.2 Tanjung Tualang Tin Dredge Heritage (TDH)

The second study site is a tourist attraction in Tanjung Tualang, about 60 km from Ipoh (*see Figure 1.1-2*) where a heritage tin dredge is preserved for historical value. A dredge (*see Figure 1.1-3*), T.T. No 5 was built in 1938 by W.F. Payne & Sons for Pernas Chartered Management Sdn Bhd. Once belonging to Southern Malayan Tin Dredging (M) Sdn Bhd, the dredge had scoured for tin ore in the Kinta Valley for 44 years until operations stopped in 1983 due to the collapsed tin mining industry. The place started to open for public viewing on Jan 1, 2008. It is undeniably faced with high potential of heavy metals in the atmosphere, resulting from former tin mining activities. However, within the distance of 5 km, the area is dominated by various species of plants such as grass, flamboyant, *delonix regia (see Figure 1.1-4)*, which are generally tolerant of toxic actions of heavy metals. Some part of the area is consumed for agro forestry such as sweetsop (*Annona squamosa*) plantation (The Star, 2008).

Heavy metals dispersion at this site is mainly associated with a static pond of mine waste where the dredge is left idle. The heavy metals are contained in residues coming from mining and metallurgical operation. They are often dispersed by wind and/or water after their disposal.

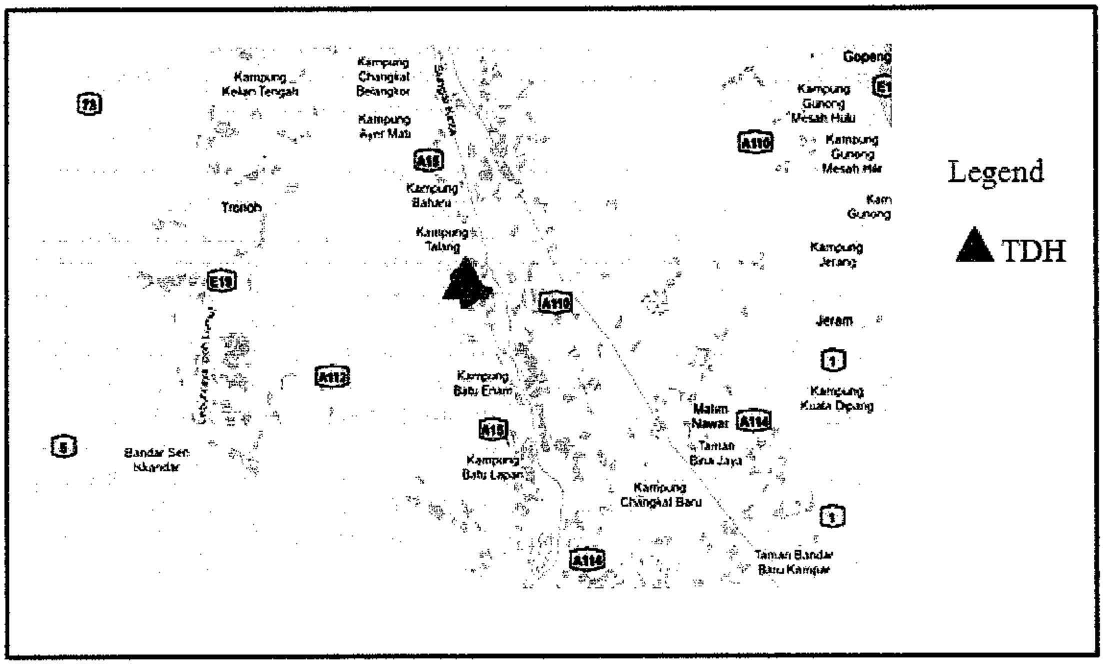

Figure 1.1-2 Location of TDH in Perak (Google Maps)

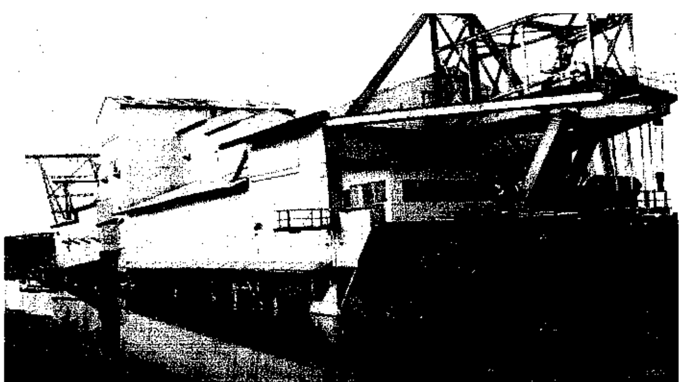

Figure 1.1-3 T.T. No 5 was built in 1938

The environment of TDH is surrounded by several abandoned ponds and idle lands resulting from collapsed tin mine industry. Most of the lands are filled with bushes, small plants and bundles of huge trees. Looking at the dredge, it may assume that the area is affected by corrosion issues.

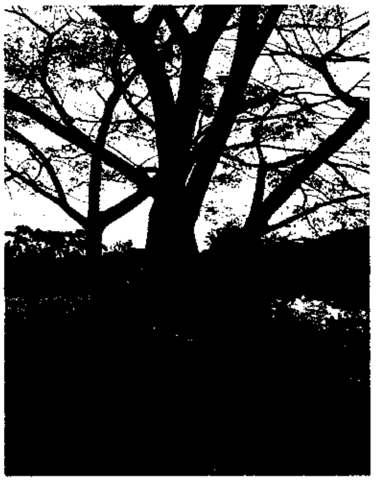

Figure 1.1-4 Delonix Regia

The environment looks healthy and trees grow just like in the non former mine site. Sweetsop plantation is an alternative economic activities managed by Osborne & Chappel Sdn Bhd who responsible for managing the TDH. After successive growth period, sweetsops have been harvested and sell to tourists. The sweetsop plantation is harvested in schedule to hold up the customer demand at peak. A concern also rises on the content or heavy metals accumulation in the fruits. 2 sources of heavy metal emissions were identified from that site which is the tin mine waste ponds; (1) Source Point 1; the pond where the dredge is located, with 5000 m^2 large and (2) Source Point 2; an idle pond located in front of Source Point 1.

1.2 Problem Statement

1.2.1 Problem Identification

The promising result of afforestation at the FRSB signifies improvement quality of soil thus promoting the study of whether dispersal of heavy metal in the atmosphere is also reduced. The site is planted with varies species of timbers which already have matured and may trap the air contaminants within the surface of tree barks. Moreover, one-third of the FRSB area still being abandoned which probably emits heavy metals into the atmosphere. Due to lack researches in Malaysia on the former mining land, those idle lands they have been utilized regardless the adverse effects of heavy metals contamination. Studies have shown that crops grown on former mining sites have high levels of As, mercury (Hg), Cd and Pb (Ho et al., 2000); which are potentially harmful to human health at elevated concentration. It is known that at elevated level of heavy metals could be a threat to human health and cause damage to environment. Human can be exposed to the threat by several means and one of them is through digestive system. This occurs when human consumes fruits or vegetables that are harvested from the plantation on former mining land. Besides that, human can access to the heavy metals source through inhalation system. It is aware that heavy metals are dispersed by air from the sources and less dense heavy metals may stay longer in atmosphere. Those heavy metals will travel by nature transporter such as wind and rain to a certain distance and accumulate in good absorbance material.

1.2.2 Significant of Project

The results from this project will allow a reappraisal of the efficiency of biomarkers as indicator in the context of environment monitoring studies. Biomonitoring is effectively being used in many areas, not only in the environmental industrial study, but globally, as testified by the number papers, books and international conferences on this subject that have taken place over the last few years. Also the issue has a high profile in public awareness because environmental issue has significant effect toward human health and it is aware that health cannot be compromised. When a threat exists, human will strategize measure to control the source of threat and remedial measure will be taken out to absolutely remove the potential threat. This project is intent to get as much as information of aerial dispersal at former tin mine site to reflect the heavy metals concentration in the atmosphere resulting from past mining activities. Therefore it is anticipated that this project would generate a great deal of interest, not only among researchers, but also to the wider public.

1.3 Research Objectives

The main objectives of this research are;

1. to investigate the aerial dispersal of heavy metals at remediated and abandoned former tin mine sites;
2. to conduct comparative study on the level of heavy metals concentration at a remediated site with past studies;
3. to attest the effectiveness of timber barks as a biomarker for air pollution monitoring;

1.4 Scope of Study

This project involves the application of biomarkers in the context of environmental monitoring studies. Biomarker is a measurable physiological change, which can also be seen at a behavioral level that reveals its present or past exposure to pollution (Kaiser, 2001). The origin and the concept of biomarkers in ecotoxicology focus on some points corresponding respectively to terrestrial and aquatic ecosystems and the use of biomarkers for monitoring the health of terrestrial ecosystems relies on animals and on plants. Biomonitoring is the most appropriate one among monitoring methods.

In biomonitoring studies, living species are used to monitor toxic metal pollution in the environment. Most plant species, like moss, lichen, pine barks and pine needles, are a more convenient tool for monitoring studies (Lieth and Weckert, 1996). However, the most suitable species of biomarker for the biomonitoring of toxic metal pollution all over the world has not been found yet. Due to the reason, varieties of biomarkers are used to indicate pollution level in many countries. Tree bark can be a biomarker because of its ability to absorb and accumulate airborne contaminants and they are mostly accumulated in the outer bark of trees (Harju et al., 2002). Therefore tree bark has been widely used for the monitoring of atmospheric pollution (Odukoya et al., 2000).

1.5 Relevancy of Study

This research attempt to investigate the environmental transport pathways, which result in the dispersion of Pb and Zn contaminants within remediated and abandoned tin mine sites. As FRSB is an established research station, it supports numerous research activities related to the reclamation process.

LITERATURE REVIEW

This chapter will discuss on heavy metal availability in plants, its deposition and transport. The study of heavy metals available due to mining activities is performed and presented in this chapter. Information of chemical and environmental effects of those heavy metals is discussed to assist a deeper understanding in heavy metals behavior. The existence of such threaten elements draw out interest to learn the remedial measure to remove threaten level of heavy metals from nature.

2.1 Metals Availability in Plants

One of the more prominent issues for some regions of Central and Eastern Europe is the concentration of heavy metals in the environment, their deposition and long range transport. Biomarker indicates information on the state of ecosystems, so it must be very sensitive to highlight environmental changes as soon as possible. There are two factors that influence metal availability to plants; (1) soil and (2) aquatic systems. In order for a plant to take up metals, the metals has to be in an available form or plants must have mechanism to make metals available (Greger, 2004). Soil consists of inorganic clay minerals and organic substances and it is negatively charged because the clay minerals have structures of hydroxyl groups and electron pairs of oxygen. Positive metal ions are attracted to these charges. Factors that affect the solubility and availability of metals include their chemical characteristics, pH, loading rate, cation exchange capacity (CEC), soil texture, redox potential, clay content, and organic matter content (Lagerwerff, 1972). In aquatic systems which consist of water, metals are also bound to negatively charged small particles and cells, and metals are also found in complexes with anions or humic substances.

2.2 Heavy Metals Emission by Anthropogenic Activities

Anthropogenic activities like mining, combustion of fossil fuels, metalworking industries, phosphate fertilizers, etc. lead to the emission of heavy metals and the accumulation of these compounds in ecosystems (Angelone & Bini, 1992). In this paper, a former mining land which had been remediated is in the concern of study. Historically, Malaysia involved in tin industry and was one of the largest contributors. However, the number of operating tin mines decreased years by years resulting in more than 2000 mined out ponds. The lengths of the ponds are up to 500 and 1000 m for gravel pump and dredge mines, respectively. The mining land cover about 113 750 ha of land surface and about 40 % had been used for cultivation of fruit crop, aquaculture, recreational grounds and housing estates. Recent studies onto former tin mine sites show that the soil of such location are contaminated by heavy metals such as As, Cr, Cu, Ni, Pb, Hg and Zn (Ang, 2007 & Fares, 2009).

Table 2.2-1 Total Contents of Heavy Metals in the Contaminated Soils at Abandoned Tin Mine in Sungai Lembing, Pahang, Malaysia (Fares, 2009)

Location	Heavy Metals Concentration (mg/kg)					
	As	Pb	Ni	Zn	Cu	Cr
SL1	13643.6	250311.3	225351.3	205396	247720.6	101.71
SL2	31680.03	160073.2	144991.7	139839.1	148669.1	142.8
SL3	13817.4	158107.6	145130.2	146285.6	157207.1	104.4
SL4	14302.6	155837.1	145521.7	160672.5	183539.13	104.1
SL5	6096.2	150997.9	142814.9	141578.1	145840.3	108.9
SL6	4906.9	149928.8	141019.9	136688.3	142879.8	102.7
SL7	2009.6	150354.9	140097.3	125886.5	142420	110.5
SL8	941.1	149740.3	140059.9	131537.4	141545.3	205.5
Dutch list	55	530	210	720	190	380
Kelly Indices	50	500	50	500	200	200
SQG	12	70	50	200	63	64

Table 2.2-1 is generated from the risk assessment at abandoned tin mine in Sungai Lembing, Pahang, Malaysia conducted by Fares (2009). Data indicates that the highest concentration of heavy metals exists in that tin mine is Pb with 250311.3 mg/kg. The study reveals that mine waste or tailings at abandoned mine of Sungai Lembing that were left exposed to air

and water has produced acidic water and has increased the level of heavy metals in surface water and soils. Polluted heavy metals dispersion is mainly associated with water transportation of mine waste through the flowing streams. Control measures of pollution routes and remediation measures are urgently required at that site.

In the context of air pollution, if permissible levels of air pollutants for urban, rural environment for the health of man are exceeded, the effects can be demonstrated by health statistics, changes in biodiversity species, declining in forest ecosystems, decreases yield of agricultural crops, corrosion of materials, etc.

2.3 Main Environmental and Health Problem in Former Tin Mine Site

The main environmental problems in most of the former tin mine site arise from atmospheric pollution. It happens due to a combination of unfavorable natural geographic conditions and high concentration of mining emitting air pollutants (gaseous and solid). Temperature inversion causes the creation of a closure above the area followed by rising concentration of air contaminants. However, the mines do not cause the majority of the atmosphere pollution. The main air pollutant originating from the strip mines is dust that is no so harmful to human health and can be controlled to some extent. Generally, the most environment devastating air pollutants are nitrogen and sulphur dioxides, apart from many different organic pollutants (Evdokimova, 1999).

Mining pollution has had a generally low profile within the Environment Agency, but not until specific events have raised interest (Potter, 2004), for example:

i. release of acidic minewater from the abandoned tin/zinc mine at Wheal Jane (Cornwall) in 1992, resulting in a highly visible plume of acidic, metal-laden water in the Carnon River;
ii. significant concerns over risks to human health and livestock, for example at various spoil sites in mid-Wales, and Shropshire; and

iii. minewater rebound following the closure of Whittle Colliery (Northumberland) in 1997 which threatened the River Coquet unless dewatering was undertaken.

The main environmental concerns are metal-contaminated water from the rebound of formerly depressed groundwater and leaching from spoil heaps, and the residual spoil and other solid wastes from the extraction and smelting processes. The areal and temporal extent of mining activities means that in addition to point sources of pollution.

2.3.1 Lead (Pb)

Pb is a highly toxic metal found in small amounts in the earth's crust. It is very resistant to corrosion but tarnishes upon exposure to air. (*See Appendix A for Pb properties*). Native Pb is rare in nature. Currently Pb is usually found in ore with Zn, silver and Cu and it is extracted together with these metals. Extreme Pb exposure can cause a variety of neurological disorders such as lack of muscular coordination, convulsions and coma, much lower Pb levels have been associated with measurable changes in children's mental development and behavior. Chronic Pb exposure in adults can result in increased blood pressure, decreased fertility, cataracts, nerve disorders, muscle and joint pain, and memory or concentration problems (NIEH, 2009).

2.3.2 Zinc (Zn)

Zn is a very common substance that occurs naturally. Zn occurs naturally in air, water and soil, but Zn concentrations are rising unnaturally, due to addition of Zn through human activities. Most Zn is added during industrial activities, such as mining, coal and waste combustion and steel processing. Some soils are heavily contaminated with Zn, and these are to be found in areas where Zn has to be mined or refined, or were sewage sludge from industrial areas has been used as fertilizer. Zn is a trace element that is essential for human health. When people absorb too little Zn they can experience a loss of appetite, decreased sense of taste and smell, slow wound healing and skin sores. Zn-shortages can even cause birth defects.

Although humans can handle proportionally large concentrations of Zn, too much Zn can still cause eminent health problems, such as stomach cramps, skin irritations, vomiting, nausea and anemia. Very high levels of Zn can damage the pancreas and disturb the protein metabolism, and cause arteriosclerosis. Extensive exposure to Zn chloride can cause respiratory disorders.

2.4 Tree Bark as Biomarker for air Pollution Monitoring

It has been observed that aerial heavy metal pollution has biochemical or physiological effects on plants. Local topography and microclimate of the study area also played a role in the dispersion of heavy metals. Air pollution may affect the growth of plants. Toxic heavy metals may affect germination, young or old trees, stem growth, leaf formation, root growth, flowering/fruiting, plant growth rate and biomass, photosynthesis, transpiration, mineral nutrition and secondary metabolism etc. All those responses may be functional for bio-indication of aerial heavy metal dispersal where elemental analysis devices with latest technologies are expensive or time consuming.

Tree bark is selected as the pollution indicator in this study and the basis for selection is the fact that accumulation of heavy metals is different from part to part of a plant. For example, the primary source of Hg and Pb in the higher parts of a plant generally thought to be via aerial deposition and not via translocation and root uptake. Cu accumulation was significantly higher in the roots than in the leaves and Cu concentrations in the leaves elevated significantly when the roots appeared to become saturated. (Zhang et al., 1995). Pilegaard and Johnsen (1984) revealed that Cu and Pb plant concentrations correlated with aerial deposition but not with soil concentrations. In contrast, Ni and Cd content in the plants correlated with deposition and soil content.

The other selection basis for tree bark is its suitability to indicate longer term air pollution. Bark is not for real time monitoring. It is preferable to use bark to investigate the accumulation of heavy metals over certain period of time. Bark is exposed to air pollutants either directly from the atmosphere or from stem flow. Compare to lichens and mosses, barks are more recommended for larger scale analysis because of it greater availability. The collection of a large number of samples is more beneficial for the analysis of data by factor analysis (Kuik & Wolterbeek, 1994). Moreover, the sampling procedures of tree bark are simpler and less time-consuming than those practiced for lichens and mosses.

Concentration of heavy metals in the bark reflects airborne dispersion and generally does not influence by metal uptake from soil. The plant structure xylem and phloem are the parts that directly contact with soil medium. However, xylem rings (Figure 2.4-1) do not involved in the determination of air pollution history because a study of distribution patterns of heavy metals in forest trees on contaminated sites in Germany, Truby (1995) found no relationship between the radial distribution in the tree rings and the historical heavy metal deposition in the area. Moreover, according to Hagemeyer and Schafer (1994) stem wood are not reliable sources of information about former pollution level. In their study, they concluded that radial distribution pattern of element concentration is stem wood can be affected by physiological process.

Another proof of disintegration between bark and xylem-phloem is the mechanism of plant growth. The parts of a tree do not expand from the middle because it grows up from the ends, not the bottom. Most of the parts of tree do not grow at all. Only a thin layer inside the bark, called the *cambium*, actually produces new material. Figure 2.4-2 shows layers of cork tree, which has extra layers of cork in it. This explains the uncorrelated between content in tree bark with xylem and phloem which absorbs heavy metals directly from soils.

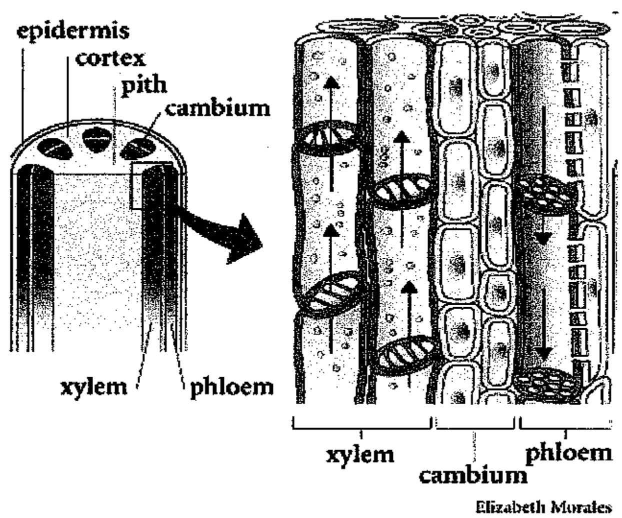

Figure 2.4-1: Location Xylem and Phloem

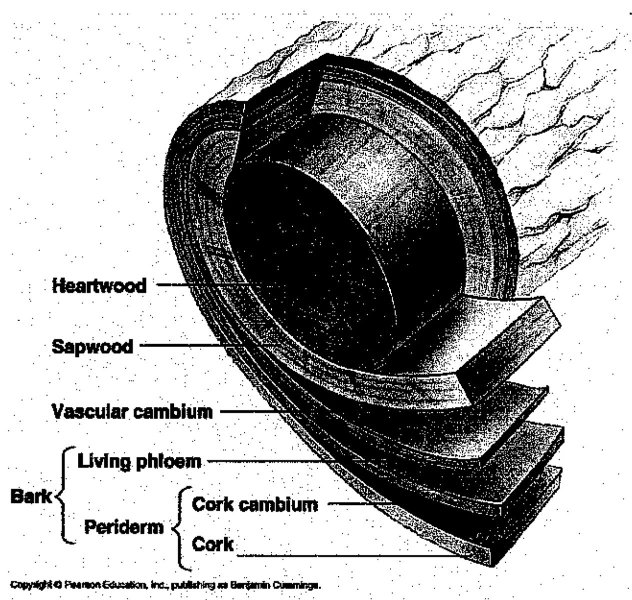

Figure 2.4-2: Layers of Cork Tree

However, there is limitation of using tree barks as biomarker. The deposited particles may be washed by rain into the soil, resuspended or retained on plant foliage. The degree of retention is influenced by weather conditions, nature of pollutant, plant surface characteristics and particle size (Harrison & Chirgawi, 1989).

METHODOLOGY

This chapter describes sampling procedures and the analytical methods applied in this research. First section describes sampling activity conducted in both locations; FRSB (remediated) and DTH (non-remediated). The following section elaborates on the methodology in preparing samples for AAS testing starts from drying, pulverizing, ashing, digestion and lastly testing.

3.1 Sampling Activity

Activities of samplings started on 8 January 2010 at TDH. It took about 40 minutes from UTP campus to arrive at TDH. Before sampling activity started, a briefing was given by a staff about some history views of TDH and also basic safety precautions while working around that area. As being informed, the site has *sweetsop* plantation and it is strongly prohibited to collect any samples from that plants. Refer to Figure 3.1-1 where barks are covered with a spread of lichens, it is clearly signifies less effects of damage by heavy metals pollution to plants. Samples were collected and appropriately sealed before bringing back to UTP.

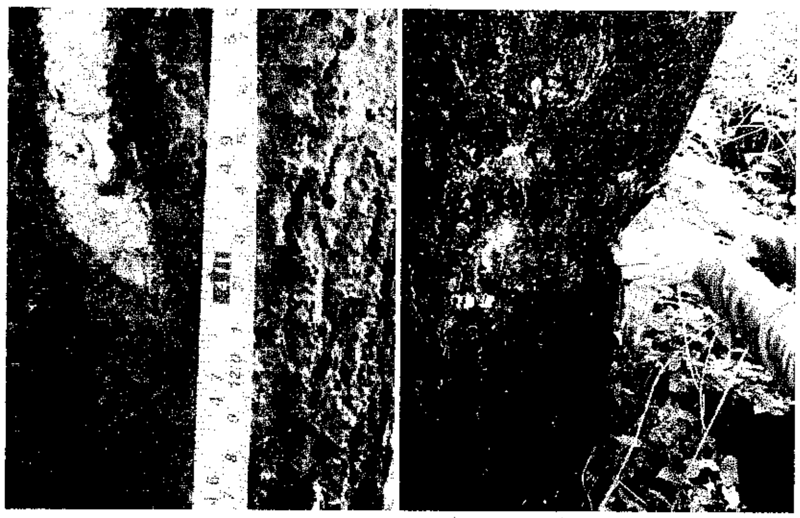

Figure 3.1-1 Collecting barks using hard steel knife at 4 ft of height

Figure 3.1-2 shows the mapping of sampling point locations at TDH. About 4000 gram of tree bark was collected from *Flamboyant* tree (*Delonix regia*) at each three different heights of the same tree which are at 0.6 m, 1.2 m and 1.8 m. The tree is located 5 m close to source point 2. The second location was 100 m close to source point 2. The tree involved was Acasia where 4 gram of tree bark was collected at each three different heights which are at 0.6 m, 1.2 m and 1.8 m.

Total of six samples of barks approximately 3000 g – 5000 g per pack were kept in sampling bags and properly labeled. The exact location of both trees is shown in *Figure 3.1-2*. The *Delonix regia* is located at coordinate of +4° 23' 50.82", +101° 3' 12.64" while the *Acasia* is located at coordinate of +4° 23' 48.90", +101° 3' 9.44.

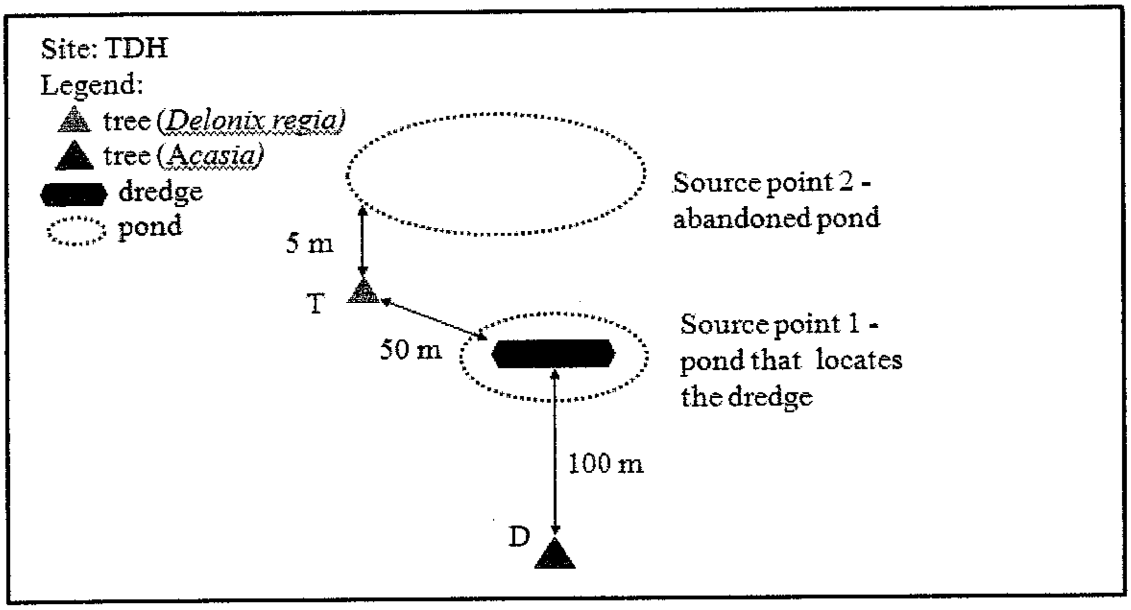

Figure 3.1-2 Map of Sampling Point locations at TDH

The second part of sampling was conducted at FRSB on February 2010. Refer to the *Figure 3.1-3*, two source points of heavy metals could be emitted from, were identified at the site. The identification is made based on finding by Fares (2009) which saying that mine waste at abandoned mine that were left exposed to air and water has produced acidic water and has increased the level of heavy metals in surface water and soils.

Figure 3.1-3 shows the locations of trees labeled as S, R, and F where barks were collected. The S is located at 5 m from source point 1, followed by R and F located at 50 m and 100 m from source point 1. From S, three samples were taken at 0.6 m, 1.2 m and 1.8 m of its height. That step was repeated at R and F. Total of nine samples approximately 3000 g – 5000 g per pack were kept in sampling bags and properly labeled.

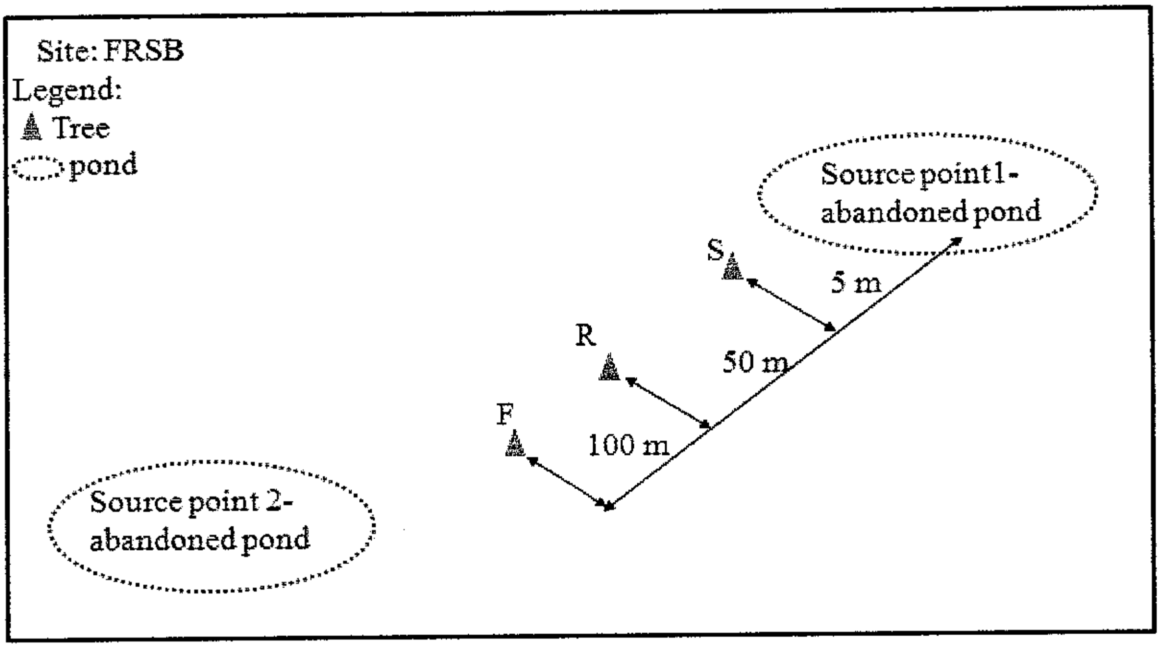

Figure 3.1-3 Map of Sampling Points at FRSB

3.2 Sample Preparation

This section explains the sample preparation methodology for laboratory analysis. All activities involved are summarized in the following figure.

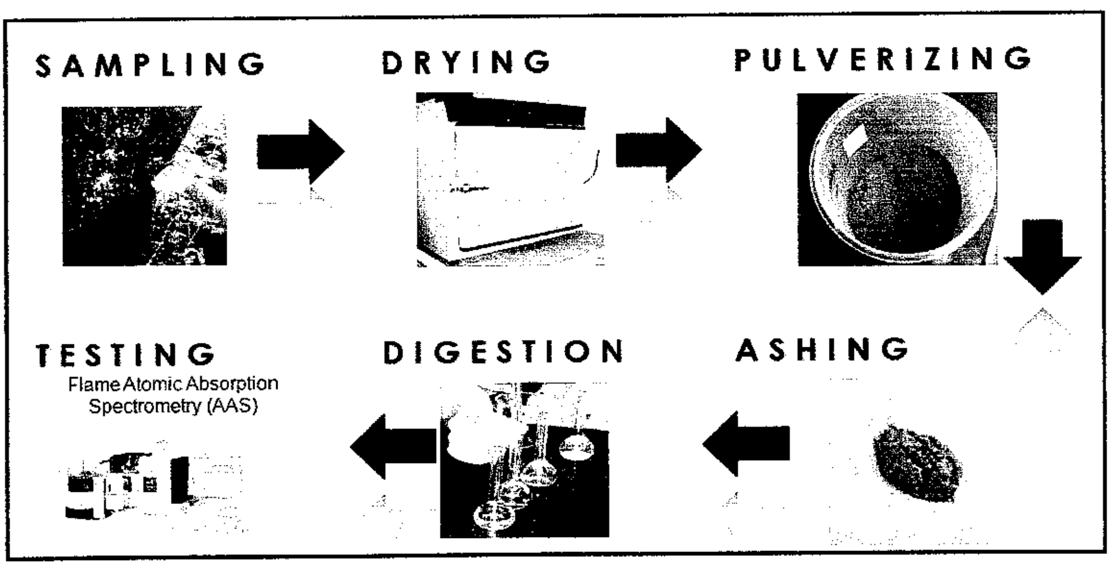

Figure 3.2-1 Flow of sample preparation for laboratory analysis

Drying. Back in laboratory, barks were preliminary dried at room temperature for about 8-9 hours. After that, barks were placed into oven and left for about 8 hours at 110 ^{0}C until they achieved constant weight or they reached high percent of drying. Percent of weight drying signifies amount of moisture being removed from barks. Results were recorded and attached in APPENDIX B.

Pulverizing. For this purpose, barks were crushed into smaller pieces until they can be grinded approximately to 2 mm^2 size using home-grinder (Figure 3.2-2). This would turn the barks into powder.

Ashing. 2 g of bark powder of from a sample was weighed and put into a crucible bowl. The crucible bowl was labeled and ready to be put into a furnace. Procedure was repeated for the remaining 18 samples. All labeled crucible bowls were properly placed into a furnace for about 5 hours at 500 ^{0}C to remove the organic matter (*Figure 3.2-3*). After the powder turned into ash (*Figure 3.2-3*), the ash were kept in a dessicator to isolate them from contacting with water and air.

Figure 3.2-2 samples of dried bark after grinding

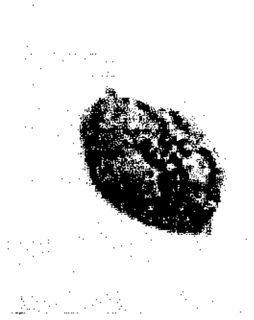

Figure 3.2-3 ash after being heated up at 500 ^{0}C

Digestion. To digest the ash, concentrated nitric acid provided in laboratory that has 65% purity must be diluted. It has to be diluted into 10% volume/volume. To check molarity of acid, following equation can be applied;

$$Molarity = (10 \times \%\ weight / weight \times density) / molar\ mass$$

Equation 3.2-1

When nitric acid 10% was ready, the ash of samples A was poured into 10 ml beaker and 20 ml of acid nitric 10% was added in the beaker. The solution was stirred and later on filtered into 100 ml volumetric flask. The volumetric flask was topped up to the 100 ml level. This step was repeated for the total of 19 samples. Now, the solution was ready to be tested in AAS.

3.3 FAAS Start-up and Preparation of Calibration Curve

1. The flame atomic absorption spectrophotometer will be set up to measure Pb and Zn. Temperature (25 ± 5 ^{0}C)
2. The detection limits for Pb and Zn are illustrated at below;

Table 3.3-1 Equipment Detection Limit of Pb and Zn

Serial No.	Element	Detection Limit
6	Pb	0.5ppm
4	Zn	0.1ppm

3. **Preparation of Zero Calibration Solutions.** Zero calibration solution is prepared by dissolving 8 mL pure conc. HNO_3 in 250 mL double distilled water in a 1000 mL volumetric flask and then diluting it up to the mark by adding double distilled water.
4. **Preparation of working solution.** For 40 ppm working standard solution, 1 mL of standard 1000 ppm stock solution is pipette into clean 25 mL volumetric flask with the help of volumetric pipette.
5. The volume is made up to the mark by adding zero calibration solution to prepare working standard/ stock solution.
6. Standards are ran during the sample analysis.
7. FAAS is switched on and following works are operated for obtaining standard curve.
8. **Standard curve.** The zero calibration solution is aspirated and the instrument is auto zero.
9. Different standard solutions are aspirated in ascending order for preparation of standard calibration curve applying standard conditions for the mineral to be analyzed.
10. **Sample analysis.** The zero Calibration solution or sample blank is aspirated as blank.
11. The sample solution to be analyzed is aspirated into the flame and in case of default of software; data is noted in lab note book.
12. **Quality checks/Verification.** Calibration curve: normally 3 types of working solution (200ppm, 40ppm, and 1000ppb) are made from stock. Diluted standard solutions of 0.5ppm, 1ppm, 2ppm, and 4ppm are made from 200ppm solution while diluted Standard solutions of 0.1ppm, 0.2ppm, 0.4ppm, and 0.8ppm are made from 40ppm solution (Table 3.3-2). A new calibration curve with freshly prepared standard solutions has to be drawn for new estimation.

Table 3.3-2 Diluted Standard Solutions (For Standard Curve)

Working Standard Solution	Diluted Standard Solutions
40 ppm	0.1ppm, 0.2ppm, 0.4ppm, 0.8ppm
200 ppm	0.5ppm, 1ppm, 2ppm, 4ppm
1000 ppb	5ppb, 10ppb, 20ppb, 40ppb

13. **Internal quality control (IQC).** For uniformity and stability of the value among samples and triplicates IQC are run in-between samples and triplicates as well as before and after sample analysis.
14. **Checking Reproducibility/Verification.** Reading of the standard solutions are taken periodically in between the samples to ensure proper functioning and reproducibility of instrument response. Prior to the Pb having low absorption, extra reading of IQC or standards after each sample is taken to verify the instrument performance.
15. **Repeatability of analysis.** Repeatability of analysis is determined through replicate sample; at least three replicates are prepared for each analysis.
16. **Analysis of old samples**: The old sample is analyzed (previously analyzed at the same conditions using the same procedure in order to verify performance qualification of the instrument and to check the test method).
17. **Precautions/ safety requirements.**
    a. All the acids are analytical grade.
    b. Acids and strong/toxic reagents and chemicals are used with care under fume hood with exhaust "TURN ON".

3.4 Flame Atomic Absorption Spectrometry (AAS)

For the elemental analysis laboratory, in order to trace the concentration of Pb and Zn, FAAS is used. AAS is a popular instrumental technique because the instruments are rugged, reliable and capable of quantitative measurements to single digit part per million (mg L^{-1}) concentrations and below for a large number of elements. It is a technique for determining the concentration of a particular metal element in a sample (Sperling et al., 1999). AAS can analyze the concentration of over 70 different metals in a solution. Even though AAS dates to the nineteenth century, the modern form was largely developed during the 1950s by a team of Australian chemists. They were led by Alan Walsh and worked at the CSIRO (Commonwealth Science and Industry Research Organisation) Division of Chemical Physics in Melbourne, Australia (L'vov, B. V. (2005)). In approaching to devise a method for trace element determinations, FAAS should be the first choice of technique considered prior to its advantages such as relatively inexpensive in term of capital, running and maintenance costs. Moreover, they are simple to operate and have numerous safety features. They do not require any special power supplies, cooling water or thermostatically controlled laboratory facilities. The interferences are well time-consuming, calibration procedures such as standard additions or internal standards (Julian et. al, 2004).

3.4.1 Basic Principles

The principle states that "Matter absorbs light at the same wavelength at which it emits light". This means that atoms in the ground state absorb the same radiation as they emit in the excited state. AAS is based on the concept that the extent to which radiation is absorbed by a derivative of the target analyte species might be directly related to the amount of the analyte species in the material under investigation. In ASS, metals will absorb ultraviolet light when they are excited by heat. Each metal has a characteristic wavelength that will be absorbed. The AAS instrument looks for a particular metal by focusing a beam of uv light at a specific wavelength through a flame and into a detector. The sample of interest is aspirated into the flame. If that metal is present in the sample, it will absorb some of the light, thus reducing its intensity. The instrument measures the change in intensity. A computer data system converts the change in intensity into an absorbance (GMU, 1998).

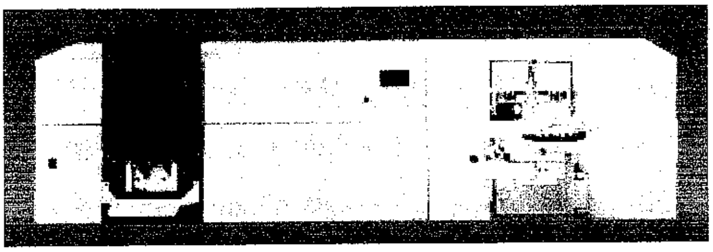

Figure 3.4-1 Flame Atomic Absorption Spectrometry

Generally, AAS applies the principals of quantum chemistry to detect the presence of metals and determines the concentration of those metals in samples. In quantum mechanics, atoms do not increase the energy levels gradually. An atom sets out directly from one state to another without going through intermediates (GMU, 1998).

3.4.2 Instrument Design

There are two ways to add thermal energy into the samples in AAS. For graphite furnace AAS, graphite is used with strong electric current to heat up the samples. Using FAAS, a sample is aspirated into flame by a nebulizer (GMU, 1998). Burners are designed to produce a flame that is long and thin. The supplied thermal energy will make atoms undergo transition from ground state to first excited state. At this moment, atoms absorb some of the light from beam. The more concentrated the solution, the more light energy is absorbed.

The light beams are designed specifically for targeted metals. Greater temperature changes are brought about by changing the oxidant and the fuel. *Table 3.4-1* lists the oxidant, the fuel, the combustion reaction and the theoretical temperature of several flames which can be used for FAAS. The most popular flames are air $-C_2H_2$ and N_2O-C_2H_2. The beam's intensity is reduced when some of the light is absorbed by a metal. That reduction is recorded as an absorption by the detector. That absorption is shown on readout by the data system. From the record, a calibration curve can be constructed by running standards of various concentrations on the AAS and observing the absorbance.

Table 3.4-1 Properties of Common Flames (Alkernade and Herrmann)

Fuel-oxidant	**Combustion reaction***	**Theoretical stoichiometric temperature (K)**
C_3H_8-air	$C_3H_8 + 5O_2 + 20N_2 \longrightarrow 3CO_2 + 4H_2O + 20N_2$	2267
H_2-air	$2H_2 + O_2 + 4N_2 \longrightarrow 2H_2O + 4N_2$	2380
C_2H_2-air	$C_2H_2 + O_2 \longrightarrow 2H_2O$	2540
H_2-O_2	$C_3H_8 + 5O_2 \longrightarrow 3O_2 + 4H_2O$	3080
C_3H_8-O_2	$C_2H_2 + 5N_2O \longrightarrow 2CO_2 + H_2O + 5N_2$	3094
C_2H_2-N_2O	$C_2H_2 + 5N_2O \longrightarrow 2CO_2 + H_2O + 5N_2$	3150
C_2H_2-O_2	$C_2H_2 + 2O_2 \longrightarrow 2CO_2 + H_2$	3342

*N_2 is included in the air mixture reactions so that the stoichiometry can be discerned from the air composition.

RESULT & DISCUSSION

This chapter presents the results for each of the different samples investigated in this study. It is separated into three sections dealing with tree bark at 2 different sites; remediated (FRSB) and non-remediated (TDH). The results are interpreted into the several aspects such as distance from source of emission, relation of distance and comparative study with past data.

4.1 Accumulation of Pb and Zn in Tree Barks of Remediated and Non-Remediated Sites

Table 4.1-1 shows the data generated by AAS. At first, Pb and Zn concentration is in unit mg/L, so to convert from mg/L to mg/kg, below equation is used.

$$concentration\left(\frac{mg}{kg}\right) = AAS\left(\frac{mg}{L}\right) \times Volume\ of\ extraction(L) \times dilution\ factor$$

Table 4.1-1 Data from AAS and pH test

Code	Height (m)	Increase distance from source point (m)	Concentration		pH
			Pb	Zn	
			(mg/kg)		
Site: FRSB					
F4	1.22	5	31.42	27.10	5.11
F6	1.83	5	21.76	11.05	5.20
R4	1.22	50	9.35	12.98	5.12
R6	1.83	50	16.24	16.40	5.53
S4	1.22	100	7.97	11.74	4.56
S6	1.83	100	17.62	9.66	4.60
Site: TDH					
T4	1.22	5	63.14	11.66	6.58
T6	1.83	5	57.62	16.60	6.46
D4	1.22	100	60.38	18.55	6.07
D6	1.83	100	16.24	32.30	5.89
Site: UTP					
U4	1.22	50	71.42	8.72	5.48
U6	1.83	50	72.80	31.56	5.52
Site: Block 4 (UTP)					
B6	1.83	100	23.14	8.47	5.71

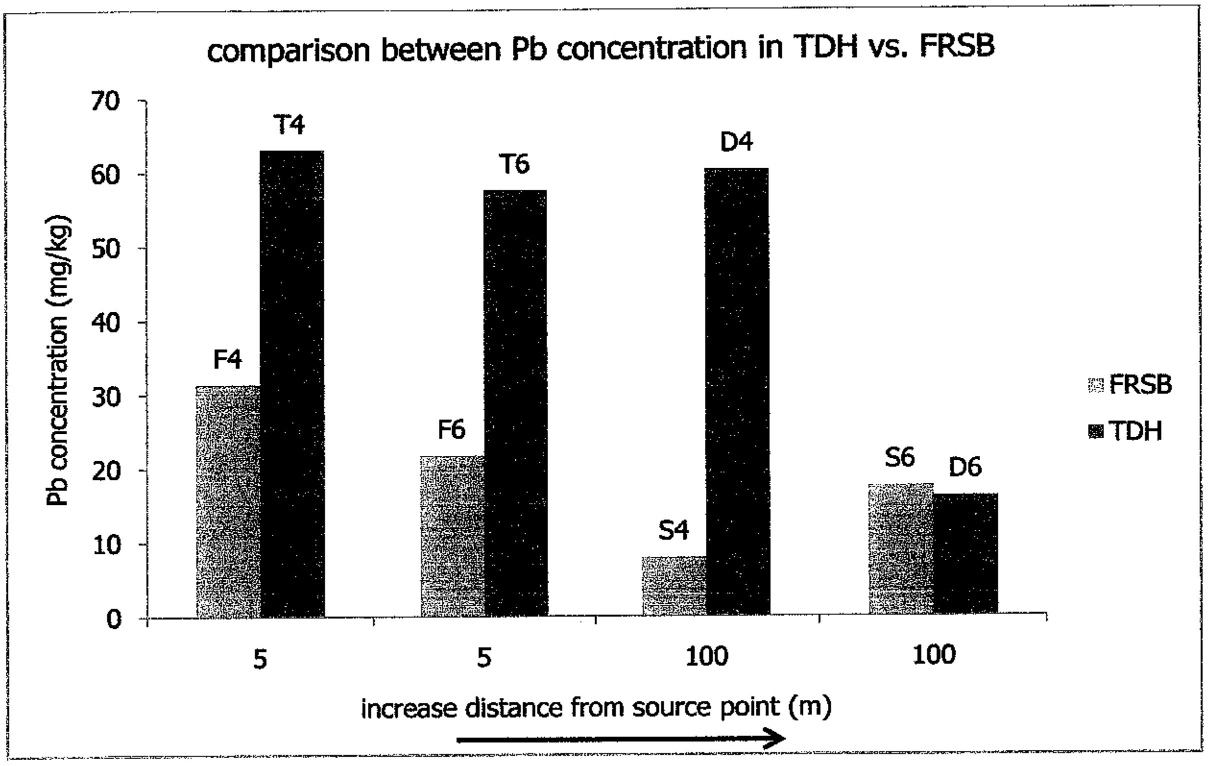

Figure 4.1-1 Comparison of Pb Concentration between TDH vs. FRSB

Figure 4.1-1 shows concentration of Pb at TDH is higher than FRSB mainly at 5 m distance from point of source. It can be assumed that, after more than 10 years of soil remediation conducted in FRSB, the level of Pb concentration in the atmosphere may be reduces due to less emission from the source point. However at 1.8 m of height with 100 m distance of from source point, the concentrations of Pb at TDH and FRSB are nearly close to each other. It can be said, at that height of 1.8 m, Pb is less transported by the natural agent which is wind. It could be the heavy metals that are emitted by source point, deposits most in the closest tree bark while the remaining are transported to the higher point and trapped in the bunch of trees or probably washed by the rain and entering the pond again.

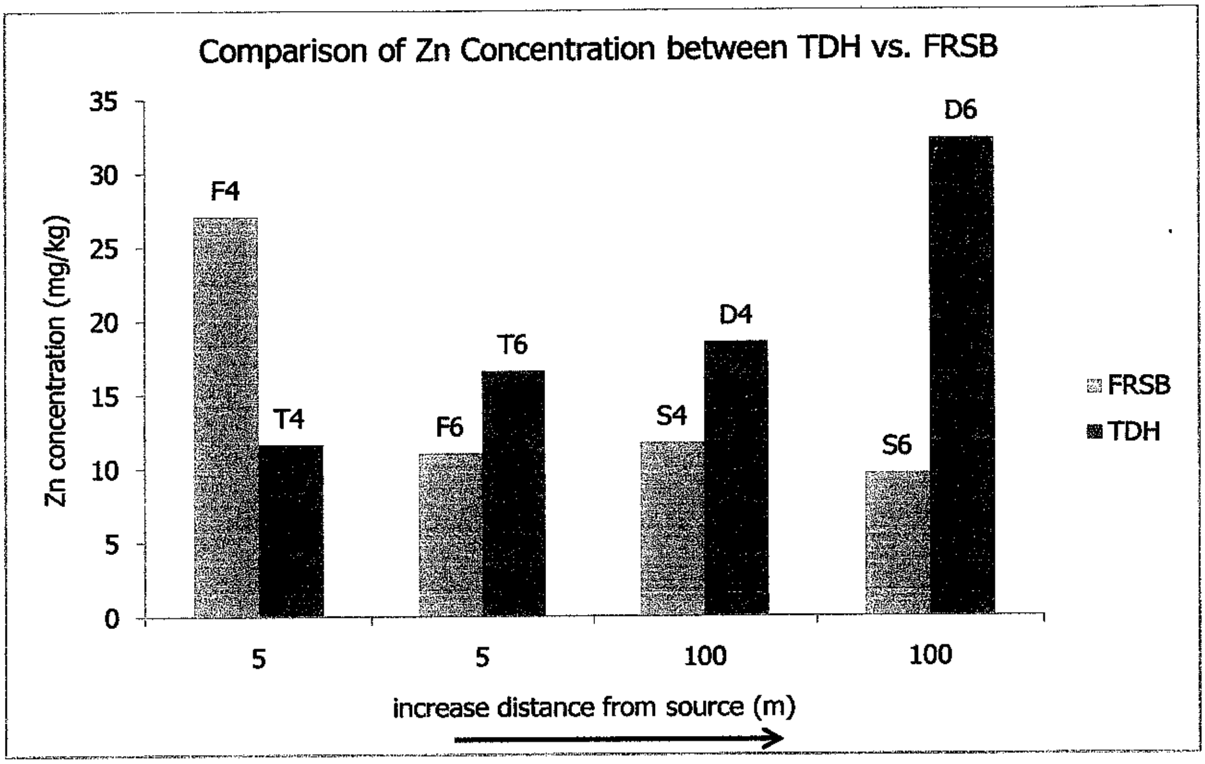

Figure 4.1-2 Comparison of Zn Concentration between TDH vs. FRSB

Comparison of Zn concentration between TDH and FRSB is graphically shown in Figure 4.1-2. From the bar chart it appears that Zn at TDH dominates the highest concentration at the most points except at F4 and T4 (*see table 4.1-1 for further declaration*). This proves that remediation measure conducted in former mining lands could reduce the concentration of Zn which is the waste left from past mining activity. However, for F4 it is assumed that the source point at FRSB still accumulate the high amount of Zn waste. Further investigation relating the source point and soil contents could be carried out to explain the condition.

From both bar charts, it can be concluded that site remediation activity conducted in former tin mines could give advantages to payback the investment made in remediation activity. Even though a process of restoration for chemical and microbiological properties of soil and soil fertility took a long time, remediation could at least eliminate the threat of human health which is heavy metals accumulation in the air. According to Evdikimova (1999) natural process of self-restoration of the soil polluted by heavy metals is an extremely long process and is possible only up to limited concentrations of metals in soil. For the soil to get rid its burden of heavy metals by means of natural processes is 200 years for Cu, 90 years for Ni and 60 years for Cobalt. With phytoremediation, it is expected to increase the rate of removal of heavy metals from the soils through plant uptake.

4.2 Comparative Studies

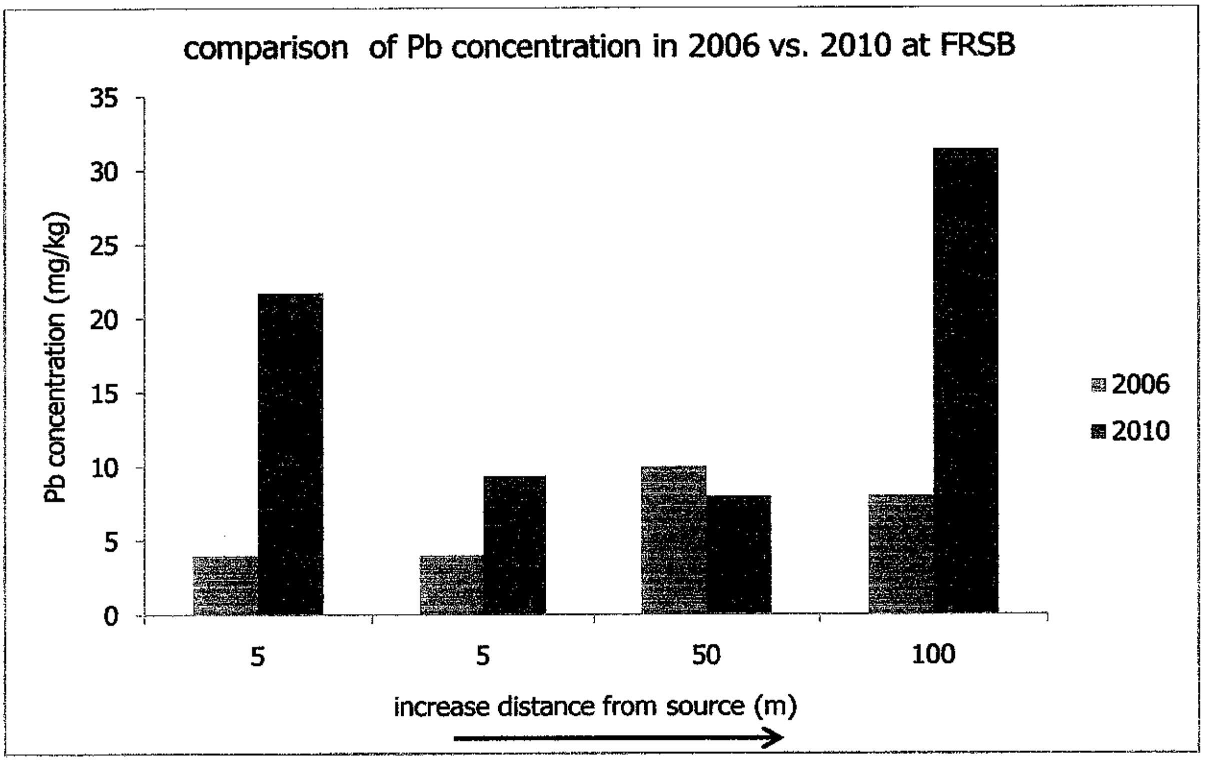

Figure 4.2-1 comparison of Pb concentration in 2006 vs. 2010 at FRSB

Comparative study is done between the results referred by previous study done by Mansor in 2006 with the current result obtained from this study. Figure 4.2-1 shows increment in Pb concentration accumulated in tree bark since the past four years. This is happened maybe due to only part of the site is remediated and remains a part without any activities. It could be the bare soil from nearby abandoned site is more disposed to wind erosion thus spreading the heavy metals by airborne dust to FRSB.

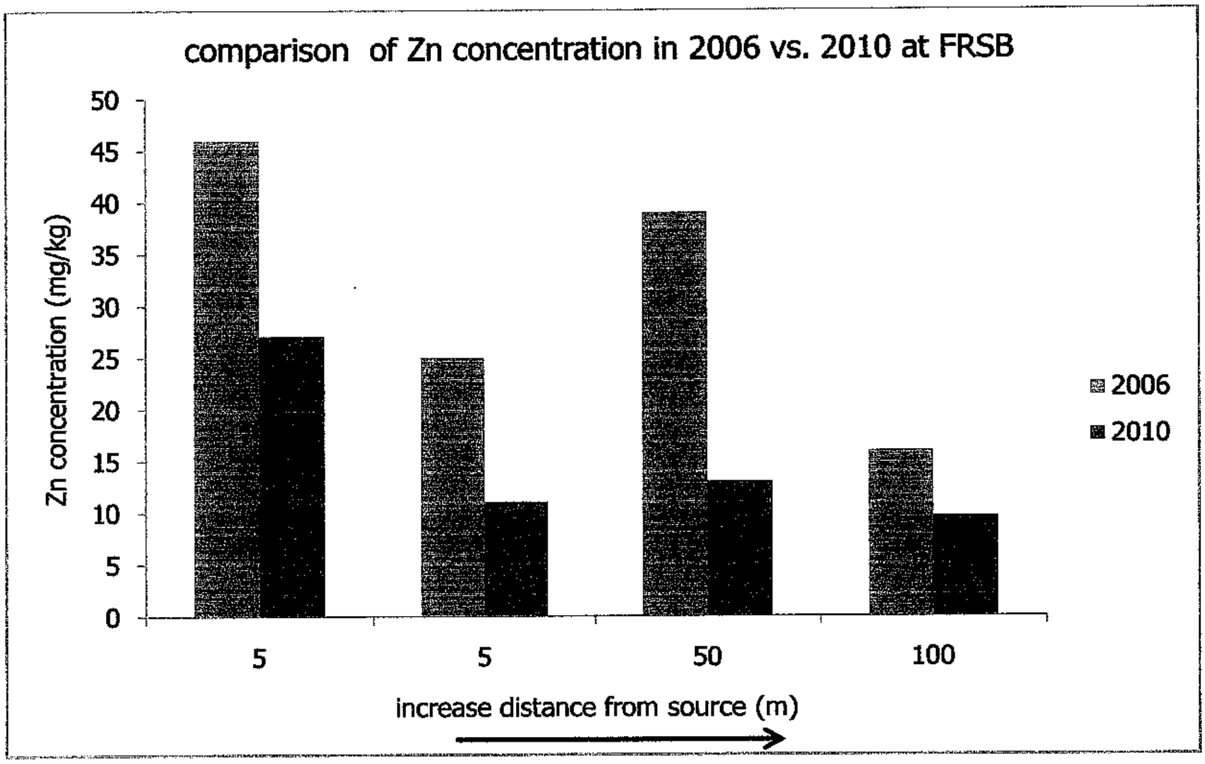

Figure 4.2-2 comparison of Zn concentration in 2006 vs. 2010 at FRSB

From *Figure 4.2-2,* it shows that Zn concentration has reduced from 2006 to 2010. This shows less Zn accumulates in tree bark and reflects the reduction in level of Zn dispersed in the FRSB atmosphere. Zn is light and easy to disperse. Zn has density of 7.1 g cm^{-3} which is slightly lighter that Pb which is 11.4 g cm^{-3} (TED, 2007). Zn is one of the heavy metals which are essential for plants when present in the growing medium in low concentration. Specifically, Zn is required for the activity of various types of enzymes including dehydrogenases, adolase, isomerases, etc (Rengel, 2000). Thus, greater amount of Zn is removed from soil trough the metals uptake by plants. This could indicate that, remediation trough afforestation may minimize soil erosion and pollution spread.

4.3 Distance from Source Aspect

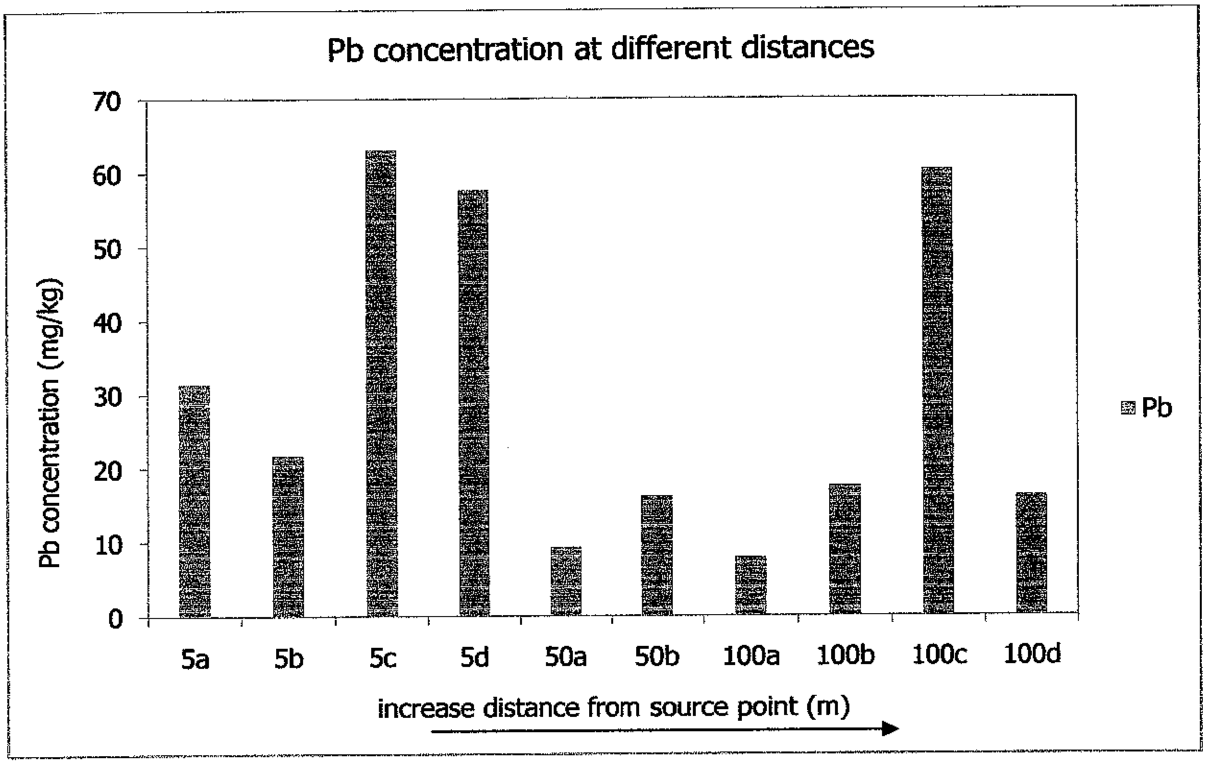

Figure 4.3-1 Pb concentration at different distances

To study the relation between distances and the behavior of heavy metals airborne deposition, samples should be collected at various distances from source point. In this study, barks were collected from 3 different trees which are located at 5 m, 50 m and 100 m from source point. *Figure 4.3-1*, shows the relation between distances from source point with the Pb concentration. Two of the samples at 5 m from source point indicate greater Pb accumulation in tree bark compared to another distances. This may help us to understand the manner of where heavy metals are transported by wind after they were emitted from bare soil and pond. The trend of heavy metals being transported from a distance to another is not solely depending on the wind itself. It could be contributed by other factors such as rains, physical human activities like deforestation and etc. It is assumed that, heavy metals tend to move from the lower part of land structure to the upper part of land which is greater in permeability like pond and river. After a long time of accumulation, heavy metals tend to emit from the pond to the atmosphere and this is where they are being transported by wind to certain distances and heights. Assumption made is illustrated in Figure 4.3-2 for crystal clear understanding.

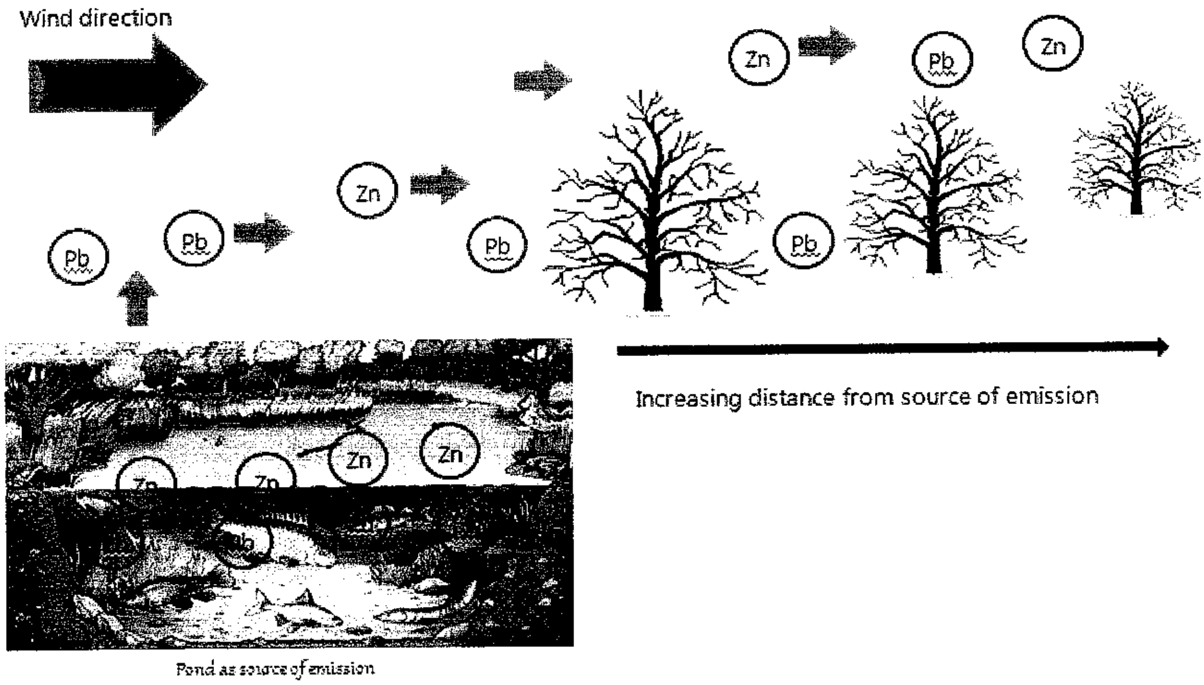

Figure 4.3-2 illustration of heavy metals transportation by wind

Refer to the illustration, Zn and Pb particles have a greater tendency to be trapped in the bark of the closest tree from source of emission.

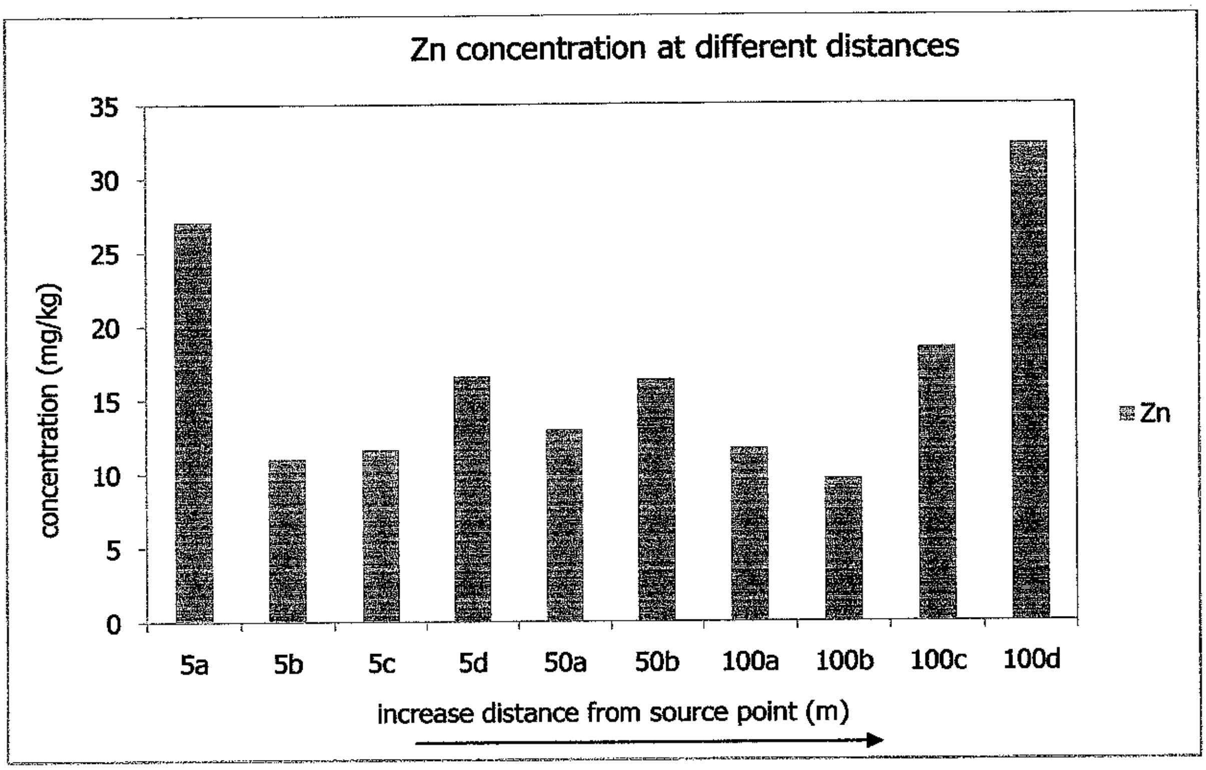

Figure 4.3-3 Zn concentration at different distances

The relation of distance onto the Zn concentrations is presented in Figure 4.3-3 where the small range of Zn concentrations disclose an almost even level with very little variability between each of the tree bark samples. There are several assumptions can be made from the condition. First, the earlier assumptions stated, the source point which is an abandoned pond is the place where heavy metals are suspected to be emitted from is valid. Second, trees located closer to the source point are greatly receives the mine waste enriched in Pb and Zn.

4.3.1 Height Aspects

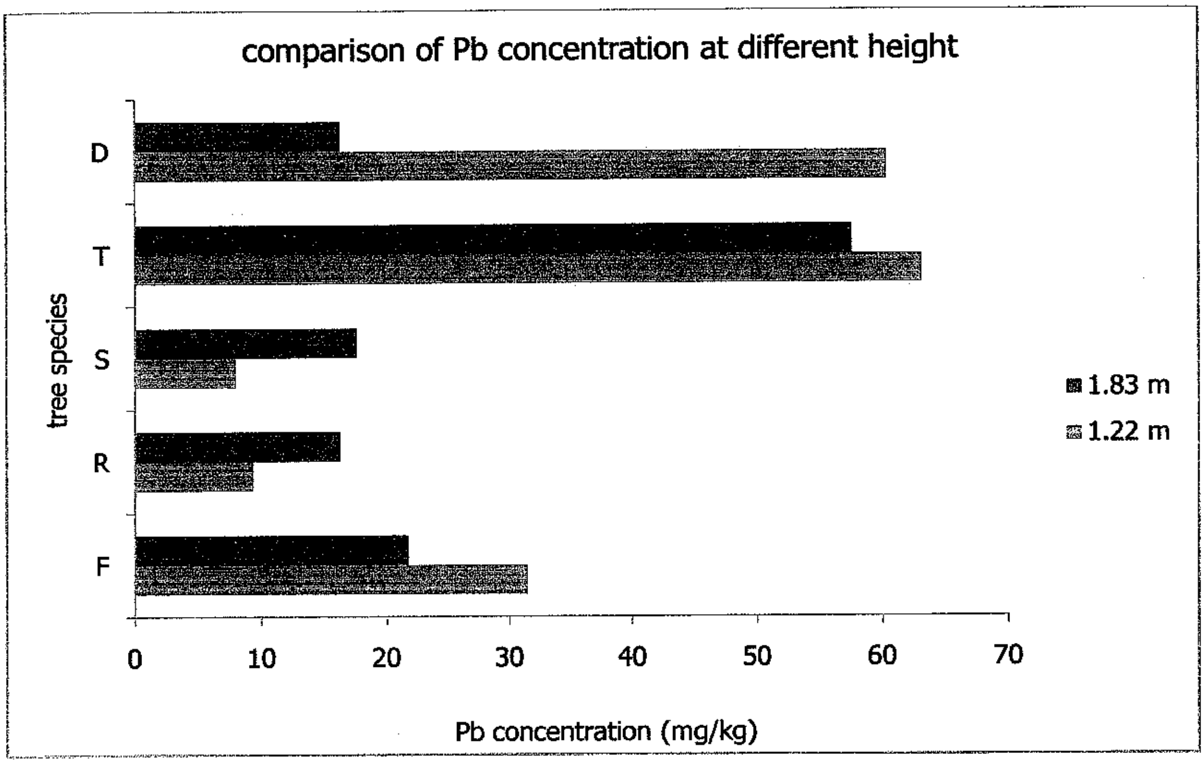

Figure 4.3-4 Comparison of Pb concentration at 1.83 m vs. 1.22 m

Comparison chart of Pb concentration at 1.83 m vs. 1.22 m is illustrated in Figure 4.3-4 which analysis shows three out of five samples at 1.22 m had greater Pb concentration compared to samples at 1.83 m.

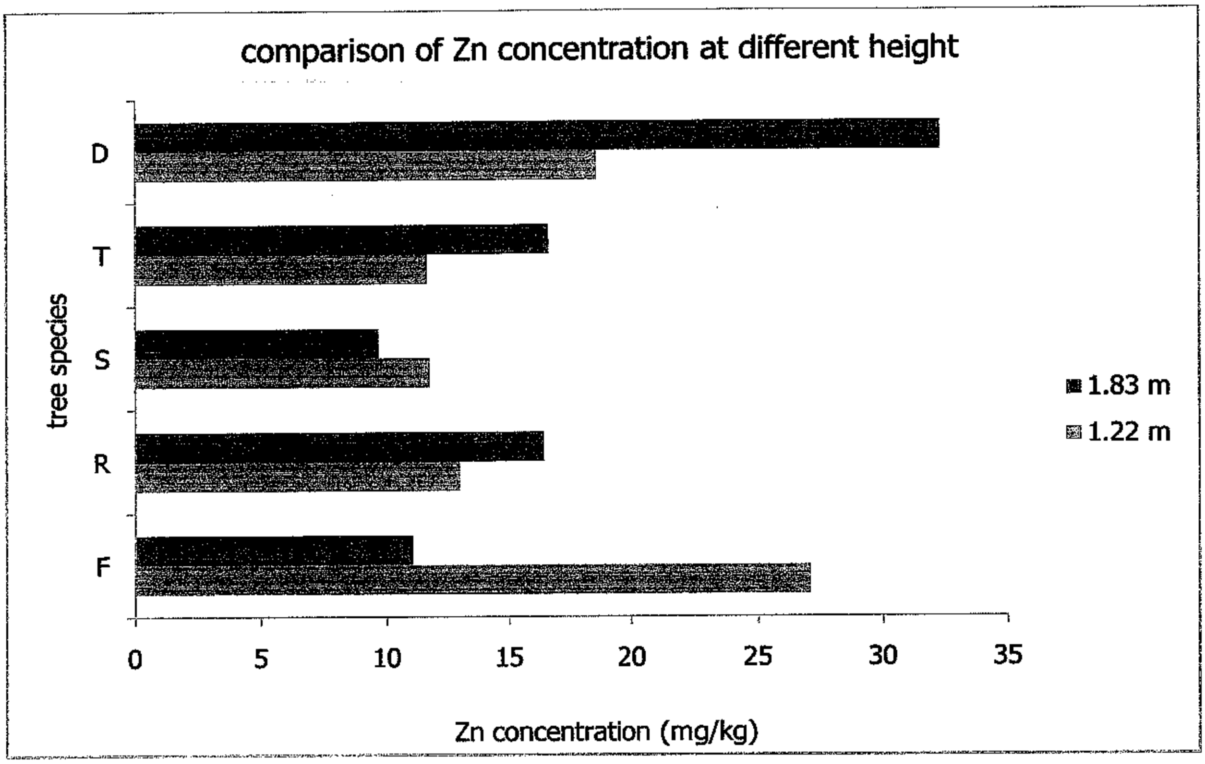

Figure 4.3-5 Comparison of Zn concentration at 1.83 m vs. 1.22 m

Comparison chart of Zn concentration at 1.83 m vs. 1.22 m is illustrated in Figure 4.3-5 which analysis shows three out of five samples at 1.83 m had greater Zn concentration compared to samples at 1.22 m.

According to Mansor (2008), from a tree bark analysis of airborne deposition at different heights, it was found that the concentration of contaminants show gradual reduction at increased heights. It concluded that the airborne contaminants are deposited at increasingly lower concentration as the height increases. However this study shows no relation between concentration and height variance (*see Figure 4.3-4*). This is probably occurred due to experiment was done with limited amount of samples thus could not give the expected trend or at least agree with past studies.

CONCLUSION & RECOMMENDATION

Recommendations

The process of interpreting biomarker data can be difficult; however to facilitate matters, all relevant information for a particular study needs to be identified and incorporated into the process. To ensure that important information is obtained, the implementation of proper approach should be considered. Other sources of ancillary information that should be consulted, as they are often invaluable as well, are past investigations about the study area that contain evidence of historical use, databases from environmental monitoring activities, and previously identified toxic effects observed in bioindicator species. Markers of forest-level responses to air pollutants need to be further developed. One of the examples of promising forest-level marker techniques include stream chemistry analysis and remote sensing from aircraft and satellites. Such techniques will be particularly useful if applied along known gradients of air-pollution intensity to obtain broad information.

Conclusions

The overall results show bark samples from *Hopea ordorata* and *Acasia* is feasible to be used to investigate the occurrence of atmospheric deposition within the former mine site. Reflects to the aim in attesting timber bark as biomarker, generated results are promising like past studies conducted worldwide. Although the study had a limited number of samples collected, it has been shown that tree bark provides valuable information on the source of airborne contamination and the deposition behavior. It is suggested that further studies can be carried out for tree bark at different side aspects for better evaluation of atmospheric dispersal of the site.

Study indicates that the level of Pb and Zn concentration in tree bark is lower in remediated site compared to abandoned site. It proves that, soil remediation would gradually eliminate risk to humans or environment from toxic metals.

After years, remediation activity that has been conducted at FRSB could turn that former mining land into valuable assets with less Pb and Zn in the atmosphere.

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APPENDIX A

Chemical Properties, Lead

- Atomic number 82
- Atomic mass 207.2 $g.mol^{-1}$
- Electronegativity according to Pauling 1.8
- Density 11.34 $g.cm^{-3}$ at 20°C
- Melting point 327 °C
- Boiling point 1755 °C
- Vanderwaals radius 0.154 nm
- Ionic radius 0.132 nm (+2) ; 0.084 nm (+4)
- Isotopes 13
- Electronic shell [Xe] $4f^{14}$ $5d^{10}$ $6s^{2\ 6p2}$
- Energy of first ionization 715.4 $kJ.mol^{-1}$
- Energy of second ionization 1450.0 $kJ.mol^{-1}$
- Energy of third ionization 3080.7 $kJ.mol^{-1}$
- Energy of fourth ionization 4082.3 $kJ.mol^{-1}$
- Energy of fifth ionization 6608 $kJ.mol^{-1}$

Chemical Properties, Zinc

- Atomic number 30
- Atomic mass 65.37 g.mol -1
- Electronegativity according to Pauling 1.6
- Density 7.11 g.cm-3 at 20°C
- Melting point 420 °C
- Boiling point 907 °C
- Vanderwaals radius 0.138 nm
- Ionic radius 0.074 nm (+2)
- Isotopes 10

- Electronic shell [Ar] 3d10 4s2
- Energy of first ionization 904.5 kJ.mol -1
- Energy of second ionization 1723 kJ.mol -1
- Standard potential - 0.763 V
- Discovered Andreas Marggraf in 1746

APPENDIX B

Experimental Data

No	Plant species	Distance from source (m)	Height (m)	Code	Initial weight (g)	After 8 hours in oven at 120 ^{0}C	Percent of drying (%)
1	*Acasia*	50	0.61	U2	67.52	37.00	45.20
2	*Acasia*	50	1.22	U4	71.73	38.20	46.74
3	*Acasia*	50	1.83	U6	65.63	40.77	37.88
4	*Delonix regia*	5	0.61	T2	55.44	23.47	57.67
5	*Delonix regia*	5	1.22	T4	40.47	8.44	79.15
6	*Delonix regia*	5	1.83	T6	50.85	16.25	68.04
7	*Acasia*	100	0.61	D2	47.42	32.30	31.89
8	*Acasia*	100	1.22	D4	39.63	25.88	34.70
9	*Acasia*	100	1.83	D6	43.47	25.52	41.29
10	*Pinus*	100	0.61	F2	51.56	23.94	53.57
11	*Pinus*	100	1.22	F4	37.64	8.61	77.13
12	*Pinus*	100	1.83	F6	47.29	16.58	64.95
13	*Pinus*	50	1.83	R6	44.10	32.95	25.29
14	*Pinus*	50	1.22	R4	51.56	23.94	53.57
15	*Pinus*	50	1.83	R6	37.64	8.61	77.13
16	*Pinus*	5	0.61	S2	47.29	16.58	64.95
17	*Pinus*	5	1.22	S4	44.10	32.95	25.29
18	*Pinus*	5	1.83	S6	44.10	32.95	25.29

APPENDIX C

Satellite view of TDH (a) and FRSB (b), using "Google Maps" to obtain actual coordinate

a)

b)