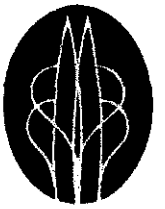

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The potential of *Pandanus Amaryllifolius* as a heavy metal accumulator

by

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Bachelor of Engineering (Hons)

(Chemical Engineering)

DECEMBER 2011

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CERTIFICATION OF APPROVAL

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A project dissertation submitted to the
Chemical Engineering Programme
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Approved by,

(Dr Nurlidia Mansor)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
DECEMBER 2011

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.

MUHAMMAD FAIDHAH BIN HASSAN

ABSTRACT

Over the years, former tin mine site are actively used as agriculture and aquaculture activities. Contamination from past mining activities had been a major concern since the early times when commercial mining were introduced. Studies have indicated that crops cultivated on tin tailings have been found to contain alarming levels of Potentially Toxic Elements (PTEs).

Phytoextraction is the name given to the process where plant roots uptake metal contaminants from the soil and translocate them to their above soil tissues. As different plant have different abilities to uptake and withstand high levels of pollutants many different plants may be used. This is of particular importance on sites that have been polluted with more than one type of metal contaminant. Phytoremediation is an energy efficient, aesthically pleasing method of remediating sites with low to moderate levels of contamination and it can be used in conjuction with other more traditional remedial methods as a finishing step to the remedial process. In many cases phytoremediation has been found to be less than half the price of alternative methods.

Pandanus Amaryllifolius or locally known as 'pandan' can be found rare in the wild and is used widely for cooking and traditional medical treatment. The innovation of technologies has proved that pandan can also be used for insect repellent. This research will look into the potential of using pandan as a heavy metal accumulator in the remediation of contaminated former mining land. The heavy metal in focus is Lead (Pb).

The experiment will involve different concentrations of Pb in the soil and analyse the uptake from different part of the plant The concentration for Pb are 2500 ppm, 3750 ppm and 5000 ppm and the parts investigated will be the leaves and root of the plant.

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CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

1.1 Background of Study

There are approximately 114 000 ha of former mining area left derelict after the tin mining industry collapsed in Malaysia [1] These lands are currently turned into agriculture and aquaculture farms. Unfortunately, studies have indicated that crops cultivated on tin tailings have been found to contain alarming levels of Potentially Toxic Elements (PTEs). Soil at former mining pond has high concentration of heavy metal which will affect planted crop properties and effect consumer's health by circulation in food cycle.

Tin occurs chiefly as alluvial deposits in the foothills of the Peninsular on the western site. The most important area is the Kinta Valley, which includes the towns of Ipoh, Gopeng, Kampar and Batu Gajah in the state of Perak. Over the years, these former tin mine site are actively used as agriculture and aquaculture activities. Contamination from past mining activities had been a major concern for a long time since the early times when commercial mining were introduced. The process of mining exploitation and ore concentrating, mine tailing and wastewaters are created, and dust is emitted.

In recent years, studies and research on the presence of PTEs due to mining activity gained interest due to discoveries that aquatic life and crops bred and grown from former mines contain elevated levels of PTEs. Research of PTEs mining contamination in Malaysia shows that the major elements available are lead (Pb), zinc (Zn) and copper (Cu), in the residual tailing of the mine. After the tin mining industry ceased in the

state of Perak, most of the mining sites had to be shut down. Local settlers grew crops, breed fish and open orchards at the former mining sites due to the available spaces. However, most PTEs exposed during mining activities still exist and may not diminish over time. PTEs may be transported through soil and water and are accumulated within the living organisms. Products of vegetables and crops from the mine are eventually distributed to consumers, thus allowing PTEs to enter the food chain. Implication from PTE accumulation in the body will cause various health problems depending on the type and concentration accumulated. [1]

Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and far away from their optimum performance. The chemical technologies generate large volumetric sludge and increase the costs [2]; chemical and thermal methods are both technically difficult and expensive that all of these methods can also degrade the valuable componentsoils [3]. Conventionally, remediation of heavy-metalcontaminated soils involves either onsite management or excavation and subsequent disposal to a landfill site. This method of disposal solely shifts the contamination problem elsewhere along with the hazards associated with transportation of contaminated soil and migration of contaminants from landfill into an adjacent environment. Soil washing for removing contaminated soil is an alternative way to excavation and disposal to landfill. This method is very costly and produces a residue rich in heavy metals, which will require further treatment. Moreover, these physio-chemical technologies used for soil remediation render the land usage as a medium for plant growth, as they remove all biological activities [4].

Recent concerns regarding the environmental contamination have initiated the development of appropriate technologies to assess the presence and mobility of metals in soil [5], water, and wastewater. Presently, phytoremediation has become an effective and affordable technological solution used to extract or remove inactive metals and metal

pollutants from contaminated soil. Phytoremediation is the use of plants to clean up a contamination from soils, sediments, and water. This technology is environmental friendly and potentially costeffective. Plants with exceptional metal-accumulating capacity are known as hyperaccumulator plants [5]. Phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body [3].

Southeast Asia is very rich with herbs, spices and other beneficial exotic plants.. These plants to grow fast and in large quantity within the rainforest. Malaysia as part of outheastasia have dozens of wild plants available. One of the well grown plant in theMalaysian forest is pandan or its scientific name *Pandanus Amaryllifolius.* It is an upright green plant with fan-shaped sprays of long, narrow, bladelike leaves and woody aerial roots. The plant is sterile, flowers only very rarely, and is propagated by cuttings.

Usually pandan is use in cooking as food flavouring. Due to its fragrance it can increase the taste of the food. The other usage of pandan which is traditionally applied is it can help hair and dandruff problem by making simple process on them. Besides, it also can be natural insect repellent.
Since pandan can grow almost on all types of land in Malaysia, it is an experiment to investigate if pandan can be a good accumulator of heavy metal when it is planted at former mining pond.

1.2 Problem Statements

i. Most of soil remediation techniques are costly and less efficient.
ii. Plants used for phytoremediation has low commercial value.

1.3 Objectives

i. To investigate the potential of pandan as a heavy metal accumulator.
ii. To investigate the part of the plant accumulates the highest concentration of lead (Pb).
iii. To investigate the resistivity of Pandan towards high Pb concentration.

1.4 Scope of Study

In order to complete this research, several scope of study has to be achieved. The major scopes are as follows:

i. To get information about pandan properties and characterization
   - There will be a research towards the elements that exist in pandan leaves
   - The characterization of pandan component such as leaves and root
   - To gather information on technics of plantation, duration of planting and how long it can sustain in the heavy metal soil.

ii. To investigate the contents of former mining pond soil
   - This research will focus more in Tronoh soil since it is one of former mining pond area.
   - The contain of the soil will be more focusing on the heavy metal exist in it

minerals, is another major route of exposure. Despite some noted improvements in worker safety and cleaner production, mining remains one of the most hazardous and environmentally damaging industries. In Bolivia, toxic sludge from a zinc mine in the Andes had killed aquatic life along a 300-kilometer stretch of river systems as of 1996. It also threatened the livelihood and health of 50,000 of the region's subsistence farmers. Uncontrolled smelters have produced some of the world's only environmental "dead zones," where little or no vegetation survives. For instance, toxic emissions from the Sudbury, Ontario, nickel smelter have devastated 10,400 hectares of forests downwind of the smelter.

Heavy metals are conventionally defined as elements with metallic properties and an atomic number >20. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Zn. Metals are natural components in soil [6]. Some of these metals are micronutrients necessary for plant growth, such as Zn, Cu, Mn, Ni, and Co, while others have unknown biological function, such as Cd, Pb, and Hg. Metal pollution has harmful effect on biological systems and does not undergo biodegradation. Toxic heavy metals such as Pb, Co, Cd can be differentiated from other pollutants, since they cannot be biodegraded but can be accumulated in living organisms, thus causing various diseases and disorders even in relatively lower concentrations[4]. Heavy metals, with soil residence times of thousands of years, pose numerous health dangers to higher organisms. They are also known to have effect on plant growth, ground cover and have a negative impact on soil microflora. It is well known that heavy metals cannot be chemically degraded and need to be physically removed or be transformed into nontoxic compounds [4].

Lead (Pb), with atomic number 82, atomic weight 207.19, and a specific gravity of 11.34, is a bluish or silvery-grey metal with a melting point of 327.5°C and a boiling point at atmospheric pressure of 1740°C. It has four naturally occurring isotopes with atomic weights 208, 206, 207 and 204 (in decreasing order of abundance). Despite the fact that lead has four electrons on its valence shell, its typical oxidation state is +2 rather than +4, since only two of the four electrons ionize easily. Apart from nitrate, chlorate, and chloride, most of the inorganic salts of lead2+ have poor

solubility in water [7]. Lead (Pb) exists in many forms in the natural sources throughout the world and is now one of the most widely and evenly distributed trace metals. Soil and plants can be contaminated by lead from car exhaust, dust, and gases from various industrial sources.

Pb2+ was found to be acute toxic to human beings when present in high amounts. Since Pb2+ is not biodegradable, once soil has become contaminated, it remains a long-term source of Pb2+ exposure.Metal pollution has a harmful effect on biological systems and does not undergo biodegradation [7]. Soil can be contaminated with Pb from several other sources such as industrial sites, from leaded fuels, old lead plumbing pipes, or even old orchard sites in production where lead arsenate is used. Lead accumulates in the upper 8 inches of the soil and is highly immobile. Contamination is long-term.Without remedial action, high soil lead levels will never return to normal. In the environment, lead is known to be toxic to plants, animals, andmicroorganisms. Effects are generally limited to especially contaminated areas. Pb contamination in the environment exists as an insoluble form, and the toxicmetals pose serious human health problem, namely, brain damage and retardation [8].

Lead is still widely used as an additive in gasoline. Increased use of coal in the future will increase metal exposures because coal ash contains many toxic metals and can be breathed deeply into the lungs. For countries such as China and India, which continue to rely on high-ash coal as a primary energy source, the health implications are ominous. Lead (Pb) can affect every organ and system in the body. Long-term exposure of adults can result in decreased performance in some tests that measure functions of the nervous system; weakness in fingers, wrists, or ankles; small increases in blood pressure; and anemia. Exposure to high lead levels can severely damage the brain and kidneys and ultimately cause death. In pregnant women, high levels of exposure to lead may cause miscarriage. High level exposure in men can damage the organs responsible for sperm production.[9]

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, stomach-aches, dizziness, vomiting and diarrhoea. Intentionally high uptakes of copper may cause liver and kidney damage and even death. Whether copper is carcinogenic has not been determined yet.

Phytoremediation techniques have been briefly depicted in many literatures or articles. The generic term "phytoremediation" consists of the Greek prefix *phyto* (plant), attached to the Latin root *remedium* (to correct or remove an evil). Generally, phytoremediation is defined as an emerging technology using selected plants to clean up the contaminated environment from hazardous contaminant to improve the environment quality. Figure 2.1 depicts the uptake mechanisms of both organics and inorganics contaminants through phytoremediation technology.

For organics, it involves phytostabilization, rhizodegradation, rhizofiltration, phytodegradation, and phytovolatilization. These mechanisms related to organic contaminant property are not able to be absorbed into the plant tissue.

For inorganics, mechanisms which can be involved are phytostabilization, rhizofiltration, phytoaccumulation and phytovolatilization.

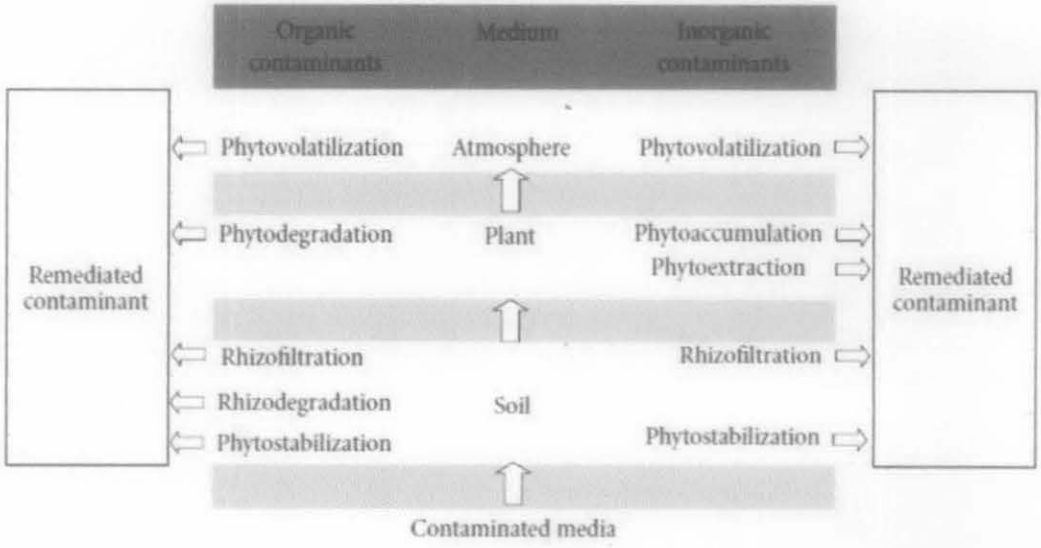

Figure 2.1: Uptake mechanisms on phytoremediation technology. Source: [10].

The root plants exudates to stabilize, demobilize and bind the contaminants in the soil matrix, thereby reducing their bioavailability. These all are called as phytostabilization process. Certain plant species have used to immobilize contaminants in the soil and ground water through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone. This process is for organics and metals contaminants in soils, sediments, and sludges medium [11, 12]. Specific plant species can absorb and hyperaccumulate metal contaminants and/or excess nutrients in harvestable root and shoot tissue, from the growth substrate through phytoextraction process. This is for metals, metalloids, radionuclides, nonmetals, and organics contaminants in soils, sediments, and sludges medium [11, 12].

Phytovolatilization process is the plants ability to absorb and subsequently volatilize the contaminant into the atmosphere. This process is for metal contaminants in groundwater, soils, sediments, and sludges medium. Since phytotransformation/phytodegradation process is the breakdown of contaminants taken up by plants through metabolic processes within the plant or the breakdown of contaminants externally to the plant through the effect of compounds produced by the plants. This process is for complex organic molecules that are degraded into simpler molecule contaminants in soils, sediments, sludges, and groundwater medium [11, 12].

Plant roots take up metal contaminants and/or excess nutrients from growth substrates through rhizofiltration process, the adsorption, or, precipitation onto plant roots or absorption into the roots of contaminants that are in solution surrounding the root zone. This process is for metals, excess nutrients, and radionuclide contaminants in groundwater, surface water, and wastewater medium [11, 12].

The breakdown of contaminants in the soil through microbial activity that is enhanced by the presence of the root zone is called rhizodegradation. This process uses microorganisms to consume and digest organic substances for nutrition and

roots, translocation into shoots is desirable because the harvest of root biomass is generally not feasible. Little is known regarding the forms in which metal ions are transported from the roots to the shoots [14].

Plant uptake-translocation mechanisms are likely to be closely regulated. Plants generally do not accumulate trace elements beyond near-term metabolic needs. And these requirements are small ranging from 10 to 15 ppm of most trace elements suffice for most needs [14]. The exceptions are "hyperaccumulator" plants, which can take up toxic metal ions at levels in the thousands of ppm. Another issue is the form in which toxic metal ions are stored in plants, particularly in hyperaccumulating plants, and how these plants avoid metal toxicity. Multiple mechanisms are involved. Storage in the vacuole appears to be a major one [14].

Water, evaporating from plant leaves, serves as a pump to absorb nutrients and other soil substances into plant roots. This process, termed evapotranspiration, is responsible for moving contamination into the plant shoots as well. Since contamination is translocated from roots to the shoots, which are harvested, contamination is removed while leaving the original soil undisturbed. Some plants that are used in phytoextraction strategies are termed "hyperaccumulators." They are plants that achieve a shoot-to-root metalconcentration ratio greater than one. Nonaccumulating plants typically have a shoot-to-root ratio considerably less than one. Ideally, hyperaccumulators should thrive in toxic environments, require little maintenance and produce high biomass, although few plants perfectly fulfill these requirements [15].

Metal accumulating plant species can concentrate heavy metals like Cd, Zn, Co, Mn, Ni, and Pb up to 100 or 1000 times those taken up by nonaccumulator (excluder) plants. In most cases, microorganisms bacteria and fungi, living in the rhizosphere closely associated with plants, may contribute to mobilize metal ions, increasing the bioavailable fraction. Their role in eliminating organic contaminants is even more significant than that in case of inorganic compounds [16].

Heavy metal uptake by plant through phytoremediation technologies is using these mechanisms of phytoextraction, phytostabilisation, rhizofiltration, and phytovolatilization as shown in Figure 2.2.

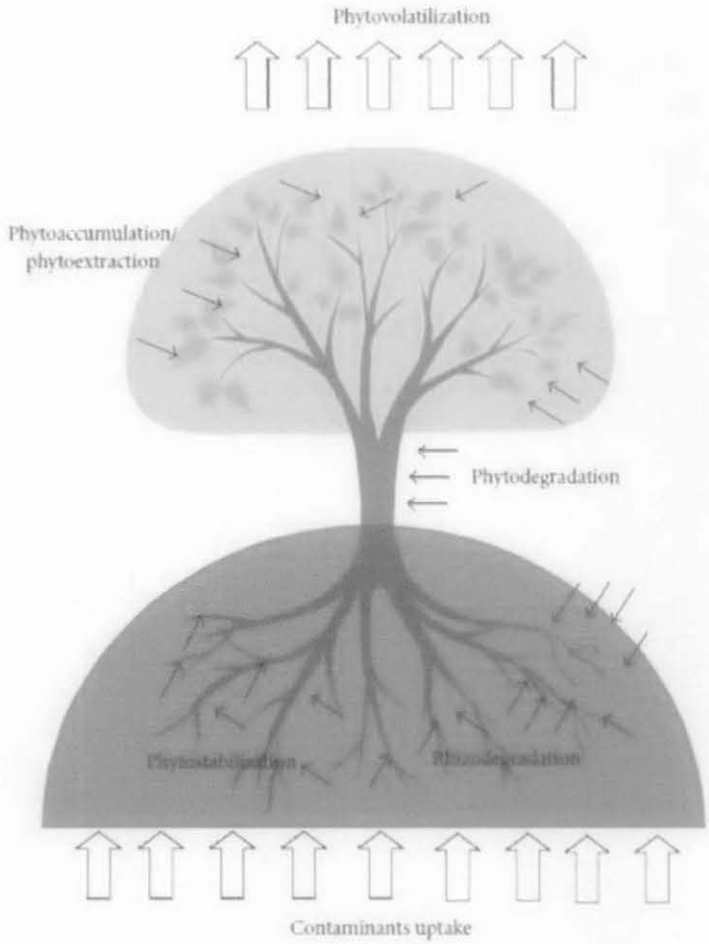

Figure 2.2: The mechanisms of heavy metals uptake by plant through phytoremediation technology.

Phytoextraction. Phytoextraction is the uptake/absorption and translocation of contaminants by plant roots into the above ground portions of the plants (shoots) that can be harvested and burned gaining energy and recycling the metal from the ash [17, 39–42].

Phytostabilisation. Phytostabilisation is the use of certain plant species to immobilize the contaminants in the soil and groundwater through absorption and accumulation in plant tissues, adsorption onto roots, or precipitation within the root zone preventing

their migration in soil, as well as their movement by erosion and deflation [17, 39–42].

Rhizofiltration. Rhizofiltration is the adsorption or precipitation onto plant roots or absorption into and sequesterization in the roots of contaminants that are in solution surrounding the root zone by constructed wetland for cleaning up communal wastewater [17, 39–42].

Phytovolatilization. Phytovolatilization is the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to the atmosphere from the plant. Phytovolatilization occurs as growing trees and other plants take up water along with the contaminants. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations [17, 39–42].

Plants also performan important secondary role in physically stabilizing the soil with their root system, preventing erosion, protecting the soil surface, and reducing the impact of rain. At the same time, plant roots release nutrients that sustain a rich microbial community in the rhizosphere.

Bacterial community composition in the rhizosphere is affected by complex interactions between soil type, plant species, and root zone location. Microbial populations are generally higher in the rhizosphere than in the root-free soil. This is due to a symbiotic relationship between soil microorganisms and plants. This symbiotic relationship can enhance some bioremediation processes. Plant roots also may provide surfaces for sorption or precipitation of metal contaminants.

In phytoremediation, the root zone is of special interest. The contaminants can be absorbed by the root to be subsequently stored or metabolised by the plant. Degradation of contaminants in the soil by plant enzymes exuded from the roots is another phytoremediation mechanism.

CHAPTER 3

METHODOLOGY

3.1 METHODOLOGY

This experiment is considering 3 main parameters which are soil, root and leaves with different concentration of lead (50ppm, 75ppm, 100ppm). The procedures and material used for soil sample pre-treatment are as follow:

Pre-treatment for soil sample.

i. The samples are put into petri dish and dry it by using oven at 50°C for one night.
ii. 1.0g of soil sample is weighed and put into 100ml digestion tube.
iii. 10.0ml of nitric acid is added into the sample.
iv. The sample is stand overnight.
v. The sample is evaporated in hot block digestion at 95°C for 1.5 hours. Cover the digestion tube with watch glass cover.
vi. 5.0ml of hydrogen peroxide is added into the sample.
vii. Continue evaporating the sample in hot block digestion at 95°C for 2.0 hours. Cover the digestion tube with watch glass cover.
viii. 2.5 ml aqua regia is added into the sample.
ix. Watch glass cover is washed with de-ionized water and let the water flow into the digestion tube.
x. The sample is filtered into 100ml conical flask with filter paper.
xi. The volume of the solution is brought up to 100ml with de-ionized water.
xii. The filtered sample is poured into 100ml sample bottle.
xiii. Sample ready to be analysed in Atomic Adsorption Spectrometer (AAS).

There are some different procedure and material used for root and leave digestion sample have explained as follow:

Digestion for plant (roots and leaves) sample.

i. The sample is washed, dried and cut into small chips.
ii. 5.0g of sample is weighed and put into 100ml digestion tube.
iii. 10.0ml of nitric acid is added into the sample.
iv. Stand the sample overnight.
v. The sample is evaporated in hot block digestion at 95°C for 1.5 hours. The digestion tube is covered with watch glass cover.
vi. 5.0ml of hydrogen peroxide is added into the sample.
Note: Hydrogen peroxide actively reacts with organic. For roots sample, add twice (2.5ml per addition).
vii. Evaporating the sample is continued in hot block digestion at 95°C for 2.0 hours. Cover the digestion tube with watch glass cover.
viii. 2.5 ml aqua regia is added into the sample.
ix. Watch glass cover is washed with water and let the water flow into the digestion tube.
x. The sample is filtered into 100ml conical flask with filter paper.
xi. The volume of the solution is brought up to 100ml with de-ionized water.
xii. The filtered sample is poured into 100ml sample bottle.
xiii. Sample ready to be analysed.

Plant Propagation and Spiking Process

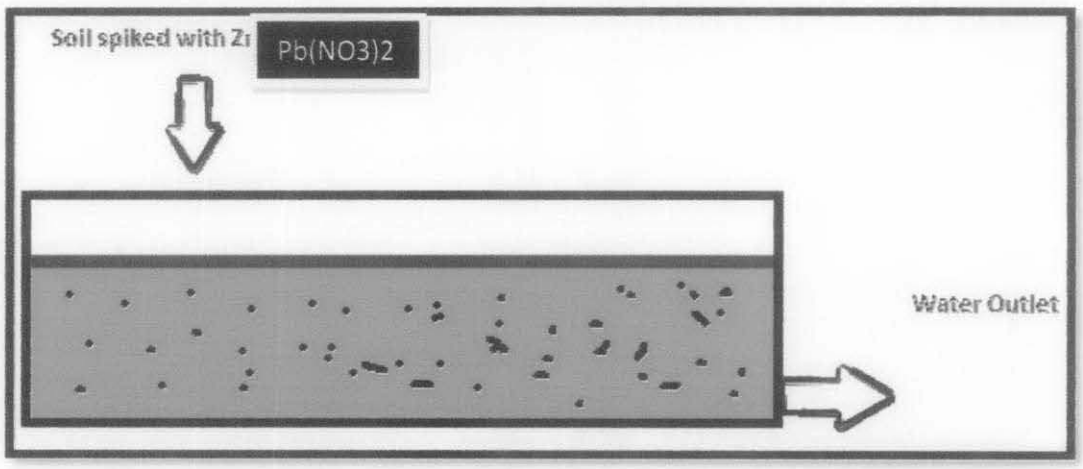

Figure 3.1: Soil Sample Preparation

Materials

i. Soil (top soil, sand and decompose)
ii. Zinc sulphate

Equipment

i. Bucket
ii. Plastic crate
iii. Measuring cylinder

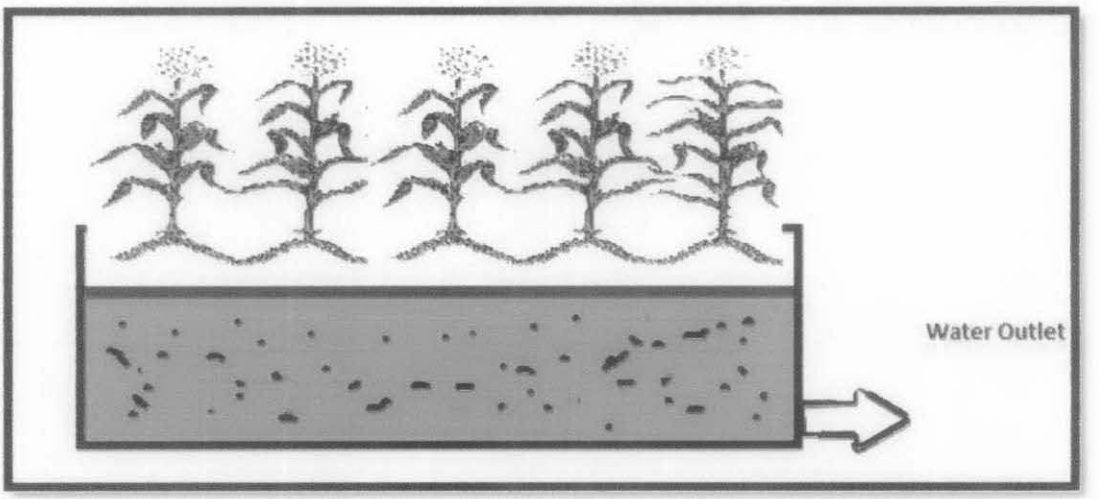

Figure 3.2: Plant Sample Preparation

Materials

i. Soil (top soil, sand and decompose)
ii. Pandan plants

Equipment

i. *Bucket*
ii. Plastic crate
iii. Measuring cylinder

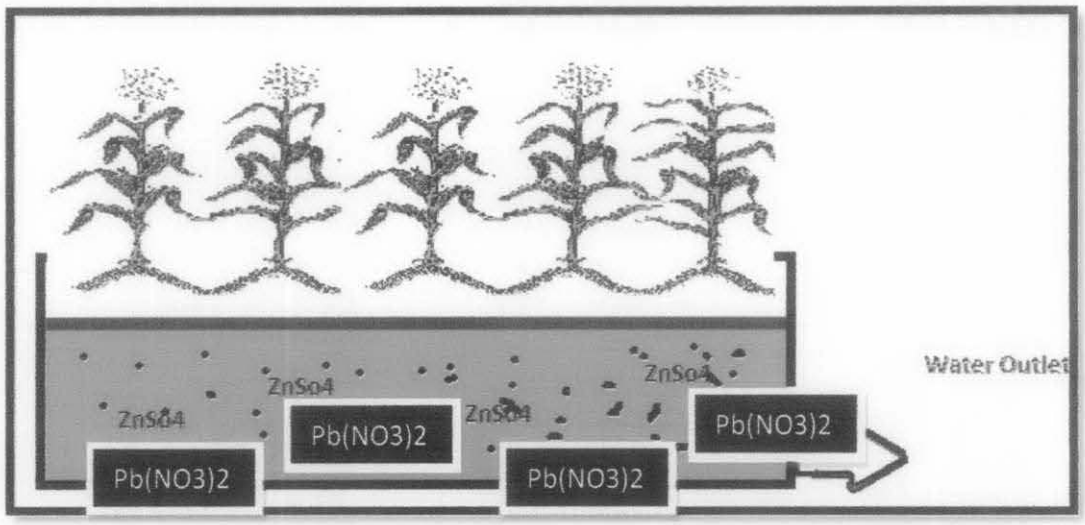

Figure 3.3: Plant Sample After Spiking

Materials

i. Soil (top soil, sand and decompose)
ii. Kenaf plants
iii. Zinc sulphate

Equipment

i. Bucket
ii. Plastic crate
iii. Measuring cylinder

After all samples are prepared, they are analyzed using Atomic Adsorption Spectrometer(AAS). There are several type of AAS such as Flame Atomic Adsorption Spectrometer (FAAS) and Granite Atomic Adsorption Spectrometer (GAAS). For this experiment FAAS is chosen because of it can detect higher concentration of heavy metal (ppm) compared to GAAS which only detect lower concentration (ppb).

Sample Preparation and Plant Analysis

The vegetables were washed with tap water followed by distilled water to eliminate attached soil particulates.

1. Samples were divided for application of two types of analysis. Some samples were used for metal analysis of all the edible parts.
2. For metal analysis in different parts of the vegetables, leaves, stems and roots were separated and sliced into smaller pieces. Subsequent to this, the samples were freeze-dried to reduce the risk of losing volatile elements and ground into powder using pestle and mortar.
3. Two types of dissolution methods were applied to assess metals recovery in the samples, namely wet digestion and dry ashing.
4. In the wet digestion method, 1 g of sample was digested with a mixture of HClO4 and HNO3, while for dry ashing 1 g of sample was placed in a crucible and heated at 450oC in a muffle furnace for 2 hours.
5. The ash was dissolved in 5 mL of concentrated HCl solution. Recovery analysis was done based on amount recovered from the spiked concentrations. It can be seen that the wet digestion method produced better recovery results for the metals.
6. Hence, this method was employed to test the samples. Metals, Fe, Co, Zn, Mn, Cu and Pb
7. Pb were analysed using Flame Atomic Absorption Spectrometry (FAAS).

Figure 3.4: Atomic Absorption Spectrometry (FAAS)

3.2 Gantt Chart and Key Milestones

		May Semester																
lo	Detail	1	2	3	4	5	6	7	8	9	10	11	12	13	14	SW	EW1	E
1	Selection of Project Topic																	
2	Preliminary Research Work																	
3	Submission of Extended Proposal Defence																	
4	Proposal Defence																	
5	Project work continues																	
6	Listing and Finalize Equipment and Methodology																	
7	Purchasing Equipment																	
8	Sample Set Up																	
9	Submission of Interim Draft Report																	
10	Submission of Interim Report																	
11	First Spike																	
12	Taking Sample (every Wednesday)																	
13	Lab Analysis																	
14	Project Work Continues																	
15	Submission of Progress Report																	
16	Preparing Analysis and Report																	
17	Pre-EDX																	
18	Submission of Draft Report																	
19	Submission of Dissertation (soft bound)																	
20	Submission of Technical Paper																	
21	Oral Presentation																	
22	Submission of Project Dissertation (Hard Bound)																	

Table 3.1: Gantt chart and Key Milestone for FYP 1

		September Semester												
No	Detail	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Selection of Project Topic													
2	Preliminary Research Work													
3	Submission of Extended Proposal Defence													
4	Proposal Defence													
5	Project work continues													
6	Listing and Finalize Equipment and Methodology													
7	Purchasing Equipment													
8	Sample Set Up													
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17	Pre-EDX													
18	Submission of Draft Report													
19	Submission of Dissertation (soft bound)													
20	Submission of Technical Paper													
21	Oral Presentation													
22	Submission of Project Dissertation (Hard Bound)													

Specific Work
Routine Work (suggested in manual)
Submission n milestone

Table 3.2: Gantt chart and Key Milestone for FYP 2

CHAPTER 4

RESULT AND DISCUSSION

4.1 Preparation of Sample Result and Discussion

This experiment is started in week 2 (week od study) where pandan are planted using recommended ratio of mixed soil which are top soil, sand and decompose (3:2:1)[19][20]. However, this mixture is not suitable to the plant which they cannot survive. This is because the amount of decompose is too much and make the mixed too hot where all six first sample are all died. Some changes had been made with the mixture where the amount of decompose is removed as much as can.

Figure 4.1: Plant dying in 3:2:1 ratio

Figure 4.2: Removed Decompose

With new ratio of soil mixture, one crate of pandan is planted once again in week 3. They are grown until week 4 with a good care and other three crate of pandan is planted after observing that new ratio of mixed soil is suitable with the plant. Lead Nitrate (Pb(NO3)2) is used as the source of Lead (Pb) because it has characteristic of solubility in water. The first spike of Lead (Pb) with different concentration (2500 ppm, 3750 ppm and 5000ppm) is done in week 5. The experiment is done in 35 days period.

Figure 4.3: Plant Sample in New Soil Mixture

Figure 4.4: Plant Sample after Spiking

4.2 Result and Discussion for Soil Sample

1. The sample is taken once a week starting from week 6. Five reading manage to be taken in 35 days period of experiment. Flame Atomic Adsorption Spectrometer (FAAS) is used to analyze the result. The concentration unit is mg/L from AAS data, converted to the mg/Kg by this equation

   AAS (mg/L) x Volume of extraction (L) x Dilution factor /weight of sample(Kg)

   Example of calculation:

   Control Soil Sample:

   AAS: 0.35 mg/L

   Volume of extraction: 0.1 L

   Weight of Sample: 1.0043 E-3 kg

   $$= \frac{0.35 \frac{m}{L} x\ 0.1\ L}{1.0043\ e-3\ kg}$$

   $$= 34.85 \frac{mg}{kg}$$

2. Results for Soil Sample are as follow:

No	Source	Parameter	Week 6	Week 7	Week 8	Week 9	Week 10
1	Soil	Control	34.85014	0	0	-5.93247	5.55908
2		2500 ppm	350.7654	322.6128	209.134	140.0359	112.604
3		3750 ppm	159.7125	155.9376	204.7007	68.57724	14.1103
4		5000 ppm	320.7755	249.6574	175.1826	218.6797	32.666

Table 4.1: Result for Soil Sample

3. Negative sign in the result shows that no Lead (Pb) is detected. It can be assumed that the contain of Pb in that particular soil sample is zero. To make

the result easier to be understand and analyzed, it is presented in graph form as follow:

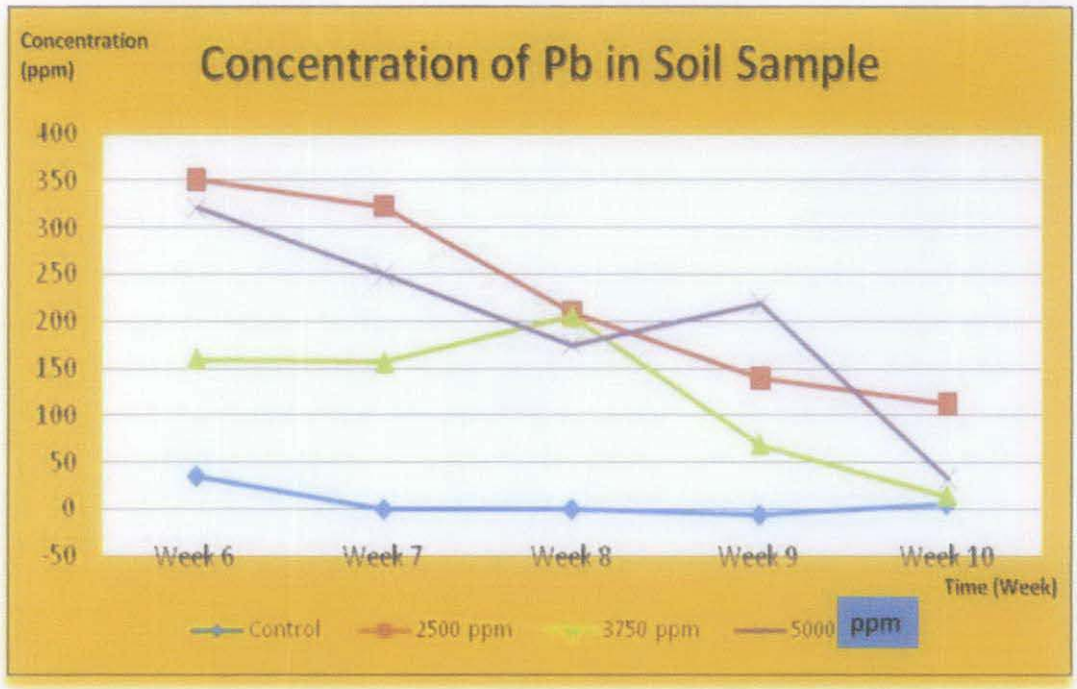

Figure 4.5: Graph of Soil Sample Result

4. Control line maintains the lowest concentration of Pb. The value also almost zero in every week. Control soil sample is the sample which no Pb is spiked on it. It indicate that the original mixed soil (top soil, sand, decompose) has very minimum amount of Pb which will not affect the result of other concentration sample.

5. Concentrations of other soil sample are observed decreasing in every week readings. Concentration of 100 ppm had the largest margin different which is 288.11 ppm. This observation approved that Pb in the soil is accumulate by the plant.

6. According to research, Lead accumulates in the upper 8 inches of the soil and is highly immobile [6]. The concentration result of soil sample for this experiment is taken randomly from different level of the crate. It is better if the sample is taken from upper level of the crate where the higher Pb accumulates. On the other hand, samples need to be taken from all level of the crate and average reading is made to have more precise result.

results from the table are presented in graph to make it easier to understand and analyze.

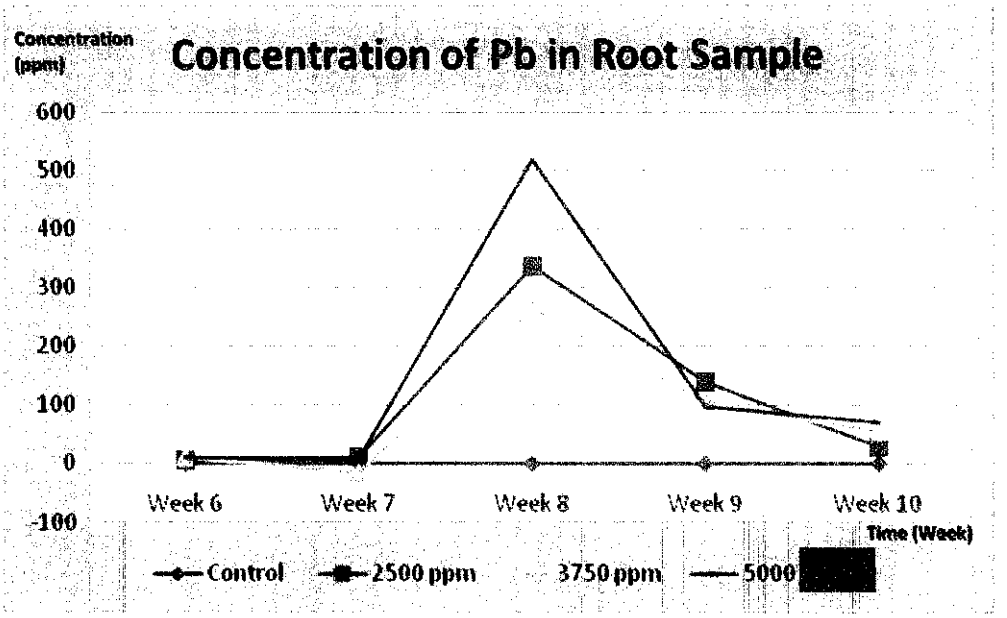

Figure 4.6: Graph of Root Sample Result

4. According to the procedure, weight of the sample need to be around 0.5 to 1.0 g. The main problem for this part is weight of the samples usually below 1.0 g and sometime below 0.5 g. It is because the size of the plant is different with each other while period of planting also taken into consideration.
5. Control Root sample reading is zero in every reading indicates that no lead in the sample since there is no contaminant in the soil sample as discussed in part 4.2.
6. From the graph, we can see the pattern on how root accumulate the Pb in the soil. It is increasing from first until third reading then decreasing to fifth reading. It shows that optimum accumulation occurs after 3 week of the experiment.
7. From the result and graph above, it can be conclude that role of root is as transporter of the accumulation where it transfer the contaminant from soil to other part of the plant which in this experiment is leave. It will be explain in detail in part 4.4.

8. On the other hand, the root plant exudates to stabilize demobilized and bind the contaminants in the soil matrix, thereby reducing their bioavailability [6]. It is one of phytoremediation process known as phytoextraction. Phytoextraction is the uptake or absorption and translocation of contaminants by plant roots into the above ground portion of the plant that can be harvested [7].

4.4 Result and Discussion for Leave Sample

1. The concentration unit is mg/L from AAS data, converted to the mg/Kg by this equation

   AAS (mg/L) x Volume of extraction (L) x Dilution factor /weight of sample(Kg)

   Example of calculation:

   Week 8 75 ppm Leave Sample:

   AAS: 1.1715 mg/L

   Volume of extraction: 0.1 L

   Weight of Sample: 1.01 E-3 kg

$$= \frac{1.1715 \frac{m}{L} x\ 0.1\ L}{1.01\ e-3\ kg}$$

$$= 115.91 \frac{mg}{kg}$$

2. Results for Leave Sample are as follow:

No	Source	Parameter	Week 6	Week 7	Week 8	Week 9	Week 10
1	Leave	Control	0	0	0	0	0
2		2500 ppm	22.764942	-5.3772	110.8696	136.1508	152.3616
3		3750 ppm	16.985732	5.382882	115.9098	139.4969	160.4673
4		5000 pmm	18.52947	21.66094	294.7085	320.1256	400.3745

Table 4.3: Result for Leave Sample

3. Negative sign in the result shows that no Lead (Pb) is detected. It can be assumed that the contain of Pb in that particular root sample is zero. The results from the table are presented in graph to make it easier to understand and analyze.

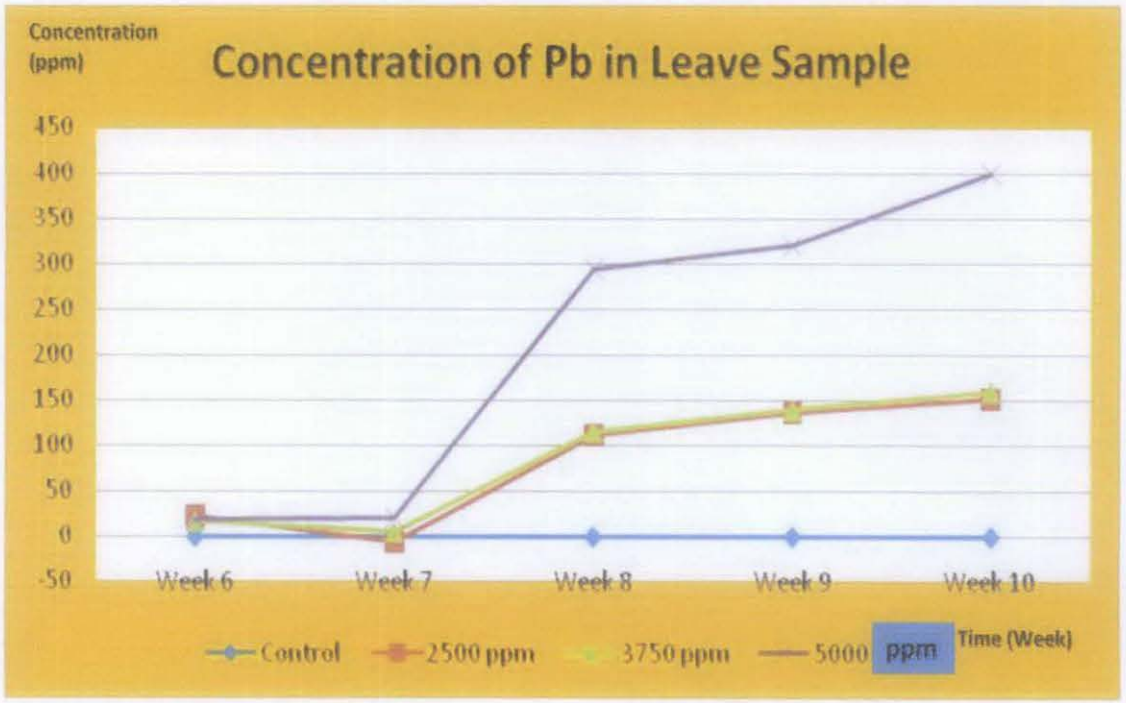

Figure 4.7: Graph of Leave Sample Result

4. Control Root sample reading is zero in every reading indicates that no lead in the sample since there is no contaminant in the soil sample as discussed in part 4.2.

5. The result and graph analysis show that the concentration of Pb is increasing every week. It indicate that leaves is the best part of Pandanus Amaryllifolius that can accumulate heavy metal. As discussed in part 4.3, root role as the transporter for phytoextraction process while leave can be considered as the storage of accumulated contaminant.
6. The smell of pandan from its leave which contain high amount of 2-acetyl-1-pyrroline (2AP) remain after the accumulation. It is approved that it remain its potential to be insect repellent. Previous studies have established significant repellent activity of *P. amaryllifolius* against American cockroaches [4].

4.5 Percentage Pb Accumulated by the Plant

To investigate the efficiency of Pandanus Amaryllifolius as heavy metal accumulator for phytoremediation, some calculation is done by finding the percentage of the accumulation. Total (root and leave) accumulation in week 10 is taken for this method since it is the final reading for this experiment. The data of total accumulation are as follow:

No	Parameter	Root	Leave	Total
1	Control	0	0	0
2	2500 ppm	29.36876	152.3616	181.7304
3	3750 ppm	56.22836	160.4673	216.6957
4	5000 ppm	70.0936	400.3745	470.4681

Table 4.4: Total of Accumulation (Root and Leave)

Concentration of lead that spiked at the early stage of the experiment is considered as the initial concentration. For control, there is no accumulation since the reading is zero.

Percentage of accumulation for 2500 ppm:

$$\frac{Total\ of\ Accumulation\ (Week\ 10\ Reading)}{Initial\ Concentration}\ x\ 100\% = \%\ of\ Accumulation$$

$$\frac{181.73\ ppm}{2{,}500\ ppm}\ x\ 100\% = 7.27\ \%$$

Percentage of accumulation for 3750 ppm:

$$\frac{Total\ of\ Accumulation\ (Week\ 10\ Reading)}{Initial\ Concentration}\ x\ 100\% = \%\ of\ Accumulation$$

$$\frac{216.70\ ppm}{3{,}750\ ppm}\ x\ 100\% = 5.78\ \%$$

Percentage of accumulation for 5000 ppm:

$$\frac{Total\ of\ Accumulation\ (Week\ 10\ Reading)}{Initial\ Concentration}\ x\ 100\% = \%\ of\ Accumulation$$

$$\frac{470.47\ ppm}{5{,}000\ ppm}\ x\ 100\% = 9.41\ \%$$

Summary of accumulation percentage explained in table below:

No	Parameter	Root	Leave	Total	Percentage (%)
1	Control	0	0	0	0
2	2500 ppm	29.36876	152.3616	181.7304	7.269215367
3	3750 ppm	56.22836	160.4673	216.6957	5.778550842
4	5000 ppm	70.0936	400.3745	470.4681	9.409362

Table 4.5: Total of Accumulation (Root and Leave) with percentage

It is proved that within 35 days of experiment, Pnadanus Amryllifolius manage to accumulate about 5.78% to 9.41% from the initial concentration. The plant can

accumulate higher percentage when the concentration of contaminant in the soil is higher.

CHAPTER 5

CONCLUSION & RECOMMENDATION

4.1 CONCLUSION

1. Based on the results, *Pandanus Amaryllifolius* is able lives and survive in high heavy metal concentration condition. It is approved where the experiment is done in 35 days period of time and the plants can grow normally with high concentration of Pb contaminant soil.

2. Potential of *Pandanus Amaryllifolius* to be insect replelent remain after the accumulation. It is approved from the observation where the aroma of 2-acetyl-1-pyrroline (2AP) still exist after the accumulation.

3. *Pandanus Amaryllifolius* has the potential as an alternative to accumulator heavy metal where leaves accumulate higher concentration of Pb compared to root which only act as transporter to the process.

4. Phytoremediation is an energy efficient, aesthically pleasing method of remediating sites with low to moderate levels of contamination.

4.2 RECOMMENDATION

1. 2-acetyl-1-pyrroline (2AP) is the main compound in Pandanus Amryllifolius that make it a potential insect repellent. Due to time constrain, no data is available to measure the quantity of 2-acetyl-1-pyrroline (2AP) remain in the leave after the accumulation process although it is observed that the aroma of pandan still exist and remain after the accumulation. 2-acetyl-1-pyrroline (2AP) compound can be identified using Gas Chromatography. It is used to separate volatile components of a mixture. A small amount of the sample to

be analyzed is drawn up into a syringe. The syringe needle is placed into a hot injector port of the gas chromatograph, and the sample is injected. The injector is set to a temperature higher than the components' boiling points. So, components of the mixture evaporate into the gas phase inside the injector. A carrier gas, such as helium, flows through the injector and pushes the gaseous components of the sample onto the GC column. It is within the column that separation of the components takes place. Molecules partition between the carrier gas (the mobile phase) and the high boiling liquid (the stationary phase) within the GC column. [8]

2. This experiment have approved that *Pandanus Amryllifolius* can be agood heavy metal accumulator. This experiment is focusing on Lead (Pb) which one of main contaminant in former mining pond soil. The other heavy metal consist in the contaminant are Cadmium (Cd), Zinc (Zn) and Copper (Cu) [9]. It is recommended that the experiment can be proceed to investigate the potential of *Pandanus Amryllifolius* to accumulate other element of heavy metal.
3. 3) Local plant has potential to be a good heavy metal accumulator. Further investigation can be done with other plant such as Pteris vittata, clover, radish, carrots, and spinach [9].
4. 4) Effective way to extract contaminant from the plant that had accumulate the contaminant from the soil.

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