**Removal Of Heavy Metals By Using Aquatic Plants – *Limnocharis Flava* In Constructed Wetlands For Wastewater Treatment**

by

**Rosli Bin Mhm Said**

**14680**

Dissertation submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

JANUARY 2014

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# CERTIFICATION OF APPROVAL

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Approved by,

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(AP Ir Abdul Aziz bin Omar)

UNIVERSITITEKNOLOGI PETRONAS

TRONOH, PERAK

Januari 2014

# 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 excepts 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.

ROSLI BIN MHM SAID

# ABSTRACT

Wetlands with aquatic plants have been widely used in industry as the treatment for wastewater because of the availability of the wetland and the costs for maintenance and construction is quite low compared to the others. It is also has been proven to be effective in removing heavy metals from the wastewater compared to other technology that is available. In this project, the effectiveness of aquatic plants - *Limnocharis Flave* is going to study by building a laboratory-model size for the treatment. The experiment will be done with a reactor tank contains aquatic plants and the other one is without aquatic plant as the control. The sample of the aquatic plants (leaf, stem and roots) is going to analyze at the beginning and end of the experiment. In addition, the sample of the wastewater will be taken at the sedimentation tank and will be study and analyze by using atomic absorption spectroscopy. It is observed that greater reduction of heavy metals is achieved with treatment of limnocharis flave with the highest percentage is iron with 61.73%, followed by lead (60.79%) and nickel (35.84%). For without treatment of limnocharis flava, a slightly reduction of heavy metals are observed with the highest percentage is observed by lead (26.07%), followed by iron (20.21%) and nickel (12.37%). Analysis is also been done to the parts of plants – to study the accumulation of heavy metals at different parts of plant and roots have the highest accumulation of heavy metals and followed by stems and leaves. Thus, it can be concluded that limnocharis flava have the potential to reduce heavy metals from wastewater and roots are the most beneficial for photostabilisation of metal contaminants.

# ACKNOWLEDGEMENT

The past 28 weeks of my enrolment in this final year project have been truly a valuable experience to me. Therefore, I would like to take this opportunity to express my gratitude to a number of people that have helped me throughout this project.

First and foremost, all praises to the Almighty, Allah S.W.T. for His mercy and grace, I was able to complete this project in good health and well being. Next, I would like to sincerely thank you my Supervisor, AP. Ir. Abdul Aziz bin Omar who has supervised me throughout my project period. His willingness to guide and advice me had helped me in achieving the goals of my project. Besides, he was constantly supportive of the decisions that I make and always share his knowledge and experiences with me.

Very special thanks to both of my parents and family for their love and supports. Last but not least, thanks to my colleagues, friends and those who involved directly and indirectly towards accomplishing my final year project's objectives. In short, I feel blessed to have successfully completed my final year project and for all help that the aforementioned parties have given me. My final year project only becomes a success with their help.

Thank you.

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# INTRODUCTION

## 1.1 Background

Wastewater from the industry is one of the main concerns that have been highlighted in past over 30 years. This is because wastewater contains organic and inorganic matters as well as heavy metals such as Iron (Fe), Manganese(Mn), Nickel(Ni) and many more. It is vital to treat wastewater before releasing it to environments. According to the American Rivers, the Environmental Protection Agency (EPA) estimates that over 7 million people suffer from the mild to moderate illnesses caused by untreated sewage every year and another half million get seriously ill. This is mainly caused by the pathogens, bacteria and heavy metals that were from the untreated wastewater. So, removal of pathogens and heavy metals can be done by wastewater treatment method like advanced oxidation process, wetlands and many more.

Aquatic plant is one of the methods that are currently developed in developing country as treatment for wastewater in large scale. Natural wetland is a land area that is wet during part or all of the year because of the location in the landscape. Besides, wetlands also contain aquatic plants and microorganisms' species that are involved in the higher rate of biological activity and it has potential to transforms many of the common pollutants that occur in the conventional wastewaters into harmless by-products or essential nutrients that can be used for additional biological productivity.

In 1912, natural wastewater have been used as convenient wastewater discharges sites at old wetland sites in North America include the Great Meadows, natural wetland neat the Concord River in Lexington. However, the study of the natural wetlands as the secondarily treated wastewater was done by Howard T. Odum and A.C. Chestnut (n.d.) from University of North Carolina. They began their five year study of using coastal lagoons with presence of marsh wetland littoral vegetation (Camp et al., 1971; Marshall, 1971; McMahan et al., 1972 Stiven and Hunter, 1976; Kadlec and Wallace, 2009). While in Asia, the function of land treatment systems was affirmed by the Chinese authorities as early in 1986 and after that, China started the joint-pilot-scale studies in Shenyang with a daily treatment capacity of 600 tons of municipal wastewater and as the results, a high removing efficiency for organic compounds and nutrients is observed and starting from 1991, the production-scale application for the wastewater treatment by wetland is introduced to the industry in China (Staudenmann et al., 1995). The root zone method is also one of the treatment that had been done by Hans Brix(1987) and in his studies, the wastewater is cleaned by microbiological degradation and by chemical or physical processes. Nitrogen and phosphorus are two main heavy metals that analyze for the root-zone method. Nitrogen is removed by denitification and phosphorus and heavy metals are bound in the soil.

In developing countries like Malaysia, Vietnam and China, wetland is the most preferred for the wastewater treatment because of its low cost alternative and maintenance. In addition, wastewater treatment by using wetland has been proven to be efficient in reducing different undesired constituents and pollutants such as BOD, COD and heavy metals. The environmental benefit treatment of wastewater in a constructed wetland include; decreased energy consumption by using natural processes rather than conventional, electrically driven wastewater-treatment processes; efficiently removed many pollutants from wastewater and also enhance the environment by providing a habitat for vegetation, fish and other wildlife (Jin et al., 2003). Thus, it is clearly shown that wastewater treatment by using aquatic plants is economic as compared to the current technology that available.

## 1.2 Problem Statement

The level of heavy metals are extremely high in the wastewater and releasing it to the environment without treatment can lead to serious problems such like water pollution and unhygienic water source. However, current technologies that available for wastewater treatment is not able to be remove or reduce certain heavy metals. Thus, aquatic plant is used as the agent for the removal of the heavy metals and pollutants in wastewater treatments.

In a case study done by Ain, Roslaili and M. Faizal (n.d.), the wastewater contained of heavy metals such as managanese, nickel, calcium and magnesium and they were able to reduce the concentration of iron and manganese by using aquatic plants.

Currently, there are several types of physical-chemical wastewater treatment technologies that can be used for wastewater treatment. However, the treatment is not favourable since it is not very economic because the costs operation and maintenance are high. Some of these processes even require extensive pretreatment process (Britz, 1995). Therefore, as the alternative method of physical-chemical wastewater treatment technologies, constructed wetland was developed.

In this project, *Limnocharis Flave* or *keladi itik* is the type of aquatic plant that will be used in this project. It is known for its ability to reduce heavy metals contents in the wastewater. As for this project, the effectiveness of *Limnocharis Flave* in treating wastewater is going to be study and the heavy metals contents is taken at the beginning and at the end of experiment. If the content of the heavy metal is reduced at the end of experiment, *Limnocharis Flave* is said to be effective in wastewater treatment. In numerous studies, wetland systems have shown great potential in removal of heavy metals in landfill leachate (Lu et al., 1984; Qasim and Chiang, 1994; Renee et al, 2001; Nancy, 2004). Due to its high rate of the biological activities, teh wetland can transform common pollutants into harmless byproducts and essential nutrients (Kadlec and Knight, 1996).

### 1.3 Objectives and Scope of Study

In this project, there are some objectives that want to be achieved. They are:

a) To investigate the performance of the sursurface flow constructed wetland system and aquatic plants (*Limnocharis Flave)* in treating wastewater.
b) To examine the amount of heavy metal taken off by roots and leaves of the wetlands plants for constructed wetland system.

This project also involve in set-up a three-stage lab scaled system which consists of three tanks – settling tank, reactor tank and sedimentation tank. Two experiments need to be done where one of the reactors contains aquatic plants and another one without presence of any aquatic plants (control). Also, the aquatic plant that used for this project is *Limnocharis Flave* or in malay's name, *keladi itik*. The efficiency of wastewater treatment is evaluated in terms of heavy metals analysis. The experiment is carried out for duration of 30 days. The experiment is carried out at Chemical Engineering Laboratory, Faculty of Chemical Engineering, Universiti Teknologi Petronas.

# CHAPTER 2

# LITERATURE REVIEW AND THEORY

There are some previous studies that had been done in the past regarding with wastewater treatment by using aquatic plants. In Natural Wastewater Treatment with Duckweed Aquaculture by Poole W.(1996), his study is focused on four parameters; Biochemical Oxygen Demand (BOD) removal, Total Suspended Solid (TSS) reduction, Phosphorus removal and Nitrogen removal. By using duckweed in the wastewater treatment, it will create a surface mat of tiny floating plants over the surface of the lagoon. This will resulted in dark water column and promotes settling and inhibits photosynthesis. In addition, the duckweed mat will acted as thermal barrier to the water below as well as inhibits anaerobic digestion. There are two types of digestion that happens – anaerobic and aerobic digestion. Anaerobic digestion or decomposition of organic materials is occurring in the sediments and in or throughout water column.

While the aerobic digestion is maintain in the water as well as just below the mat to support populations of bacteria which oxidize some of the anerobic by-products. TSS is affected by the reduction of the sunlight and thickness of duckweed mat. Insufficient of sunlight to reach the pond will limit the growth of the algae. In phosphorus removal, there are two mechanisms that is involved in the wastewater treatment – plant uptake and chemical precipitation. Phosphorus removal is utilized by plant tissue in growth process and the amount of duckweed plants can absorb is depending on the concentration of phosphorus and balance of other nutrients. Also, phosphorus removal also can be done by chemical precipitation. Phosphorus will precipitate and settle as calcium phosphate and it is depend on the inorganic chemical makeup of the water. In fact, as a result of plant uptake, removal nitrogen also can be done through that mechanism because nitrogen is one of nutrients that are important to the plants.

In different case studies done by Kamarudzaman, Ismail and Aziz (2011), the efficiencies of wastewater treatment is studies by using different types of aquatic plants – Limnocharis flava and Scirpus atrovirens. The parameters that are used for the research are concentration of ammonia nitrogen ($NH_3$-N) and orthophosphate ($PO_4$-P) before and after treatment. From the results, both planted systems have higher removal efficiency compared to the unplanted control systems. However, Limnocharis flava have shown higher efficiency in removing ammonia nitrogen and orthophosphate with percentage of 91.8% and 95.7%, respectively. In the nutshell, Limnocharis flava and Scirpus atrovirens show suitability to be used in constructed wetlands to treat landfill leachate.

In different studies, Kamarudzaman, Ismail and Aziz also investigate on the removal of heavy metals from landfill leachate using horizontal and vertical subsurface flow constructed wetland. They were using same aquatic plants with their previous study, Limnocharis flava. Their study focussed on comparing efficiency of horizontal and vertical subsurface flow in parameter of heavy metal – iron and manganese. From their result, the horizontal system has higher removal efficiency compared to the vertical system. The analysis done is based on the soil composition as well as plant analysis. For plant analysis, the sample from leaf, root and stem is collected and analyzed. For both systems, the influent concentration of leachate sample is reduced to low amount.From all the previous studies, it is clearly shown wastewater treatments by using aquatic plants have potential in reducing heavy metals contents.

## 2.1 Overview of Wastewater Treatment

Depending on the required process standards, there are many stages that are involved in the wastewater treatments involving physical, biological and chemical process. In the preliminary treatment plant, the first stage involved removals of oils, fats, grease, grit, rags and large solids (EPA, 1995). Activated sludge, biofiltration, rotating biological contractors and constructed wetlands are example of biological treatment of wastewater that are used as the primary treatment. At this stage, the nutrients concentrations of wastewater such as Cadmium, Iron, Nickel and Phosporus are reduced in the outflow in order to vary requirement by the environmental regulations.

Another water quality measurement for the wastewater is total suspended solids (TSS). Basically, chemical treatment is used to improve the settling abilities of suspended solids prior to a solids removal stage or to adjust the properties of components so that less precipitation is formed. Secondary settlement separation is to separate the sludge solids from the outflow of the biological stage while tertiary treatment refers to further reduce parameter below the requirement by national standards.

There are five parameters by which wastewater are measured:

a) Biochemical Oxygen Demand (BOD)

It is the amount of dissolved oxygen needed by aerobic biological organisms in a body of water to break down organic material that is present in a wastewater sample at certain temperature and specific time period. BOD also acted as the indicator of the organic quality of waster.

b) Chemical Oxygen Demand (COD)

The amount of organic compounds in waste is measured indirectly by using COD. Basically, all organic compounds can be fully oxidized to carbon dioxide under the presence of strong oxidizing agent under acidic conditions. The COD test is using oxidising agent potassium dichromate to oxidise organic matter in the sample.

c) Total Suspended Solid (TSS)

TSS gives a measure of the turbidity of the water and it can be observed directly. Due to the light scattering from very small particles in the water, the wastewater tends to be milky or muddy. Basically, a price of filter paper is weighed accurately and one liter of sample of water is filtered through the weighted filter paper. After that process, the filter paper is allowed to dry completely and using a lamp above the filter paper may help to fastening the drying process. Last step is to reqeight the filter paper and the difference weight of filter paper is the weight of the TSS and its commonly expressed in ppm (mg solids per liter of water).

d) pH

Level of acidity or alkalinity of sample is measured by pH value and it indicated the concentration of hydrogen in the sample and the relationship of bacteria growth and pH in wastewater is shown in figure 1:

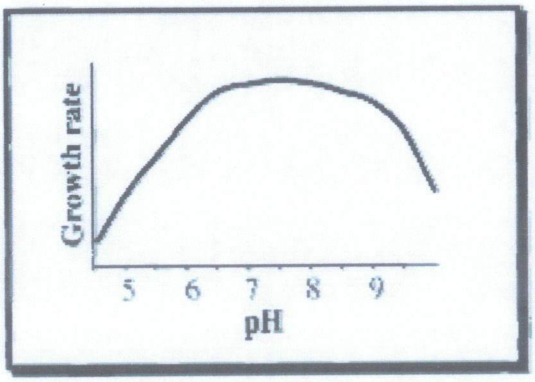



Figure 1 : Relationship of growth rate of bacteria with pH value in wastewater

(From Environmental Protection Agency (EPA) – Wastewater Treatment manuals primary, secondary and tertiary treatment)

e) Total phosphorus

Phosphorus is a naturally limited and not abundantly founded in the wastewater such as Carbon and Nitrogen. The sources of the phosphorus are from soil, rocks, and wastewater treatment plants, runoff from fertilized lawns and crop land and decomposition of organic matters. The acceptable range for total phosphorus as allowed by the regulations is ranged from 10μg/L and 40μg/L. However, higher contents of phosphorus can lead to adverse effect such as algae blooms, accelerated plant growth and low dissolved oxygen.

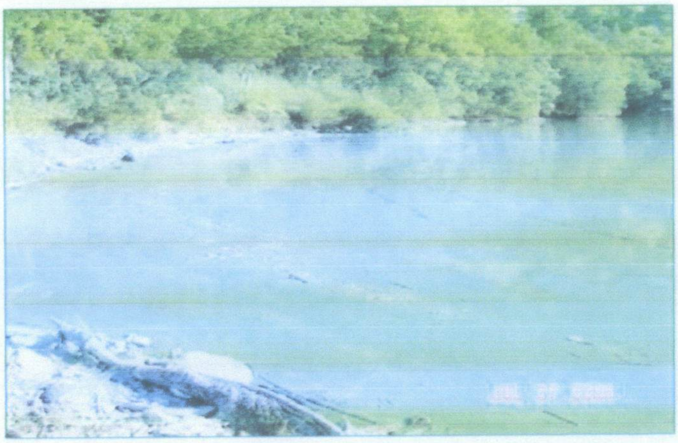


Figure 2: Algae bloom from the Klamath River Basin.

(Retrieved August 12, 2013 from www.epa.gov/region9/water/tribal/training/pdf/TotalPhosphorus.pdf)

f) Total nitrogen

Excess amount of nitrogen in the wastewater will lead to low levels of dissolved oxygen. The total measurement of total nitrogen is a sum up of contents of ammonia, nitrates and nitrites. Basically, the acceptable range for total nitrogen contents in the wastewater is such as 2 mg/L to 6 mg/L. Most of the nitrogen in domestic wastewater is the product of our eating habits and food preparation, body exudates washed off in the bath or shower and products washed from clothes. Cleaning chemicals also contribute organic compounds in varying amounts. These organic compounds require microbial activity to degrade them (Patterson, R.A, 2003).

### 2.2 Constructed Wetlands

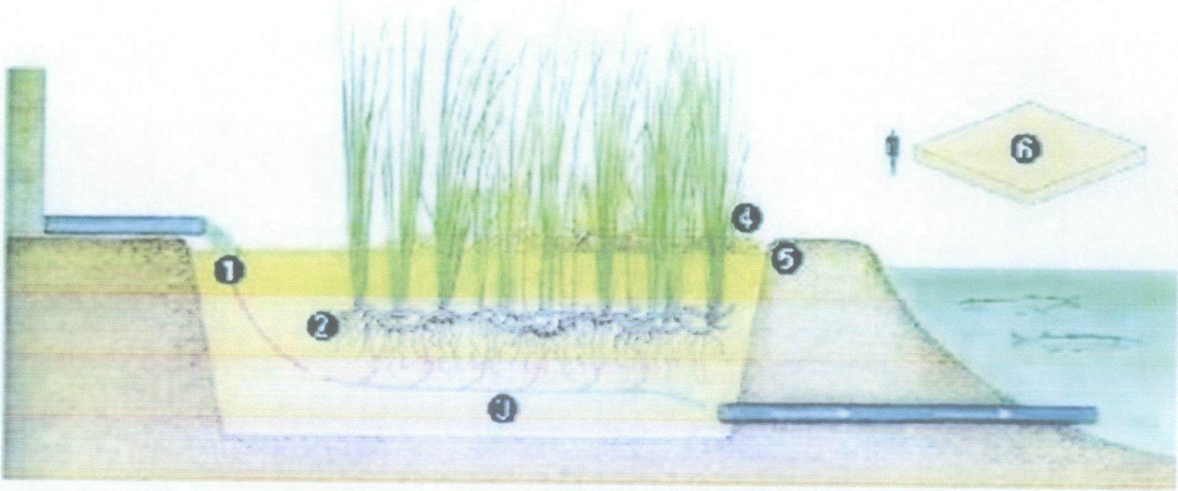


Figure 3: Process of Wetlands from Wastewater from Industry to Enviroment
(Retrieved June 20, 2013., from http://fourthcornernurseries.com/articles/frers.html)

From the industry, the wastewater is released into the wetland. This wastewater contains some of organic and inorganic matters, as well as heavy metals such as cadmium, chromium, iron, manganese and many more depending on the process operations of the plants. The filter consisted of a large vegetative planting which is planted naturally in the wetlands. The nutrients will be absorbed by the aquatic plants and the nutrients absorbed are eliminated with vegetative dieback. The purified water will be rejected to the lagoon.

One of the conventional ways for wastewater treatment is by using wetlands. Wetland is an open space with the animals, plants and microorganism that involve in decomposition of organic and inorganic matters as well as heavy metals. As for today, there are many types of wetland that have been used in industry. The characteristic of the wetland is identified by the flow - surface flow and subsurface flow.

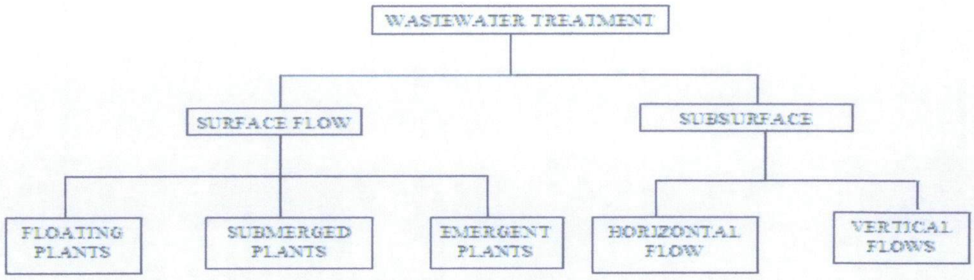



Figure 4: Treatment Wetland Types

(From Robert H.K. & Scott D.W. (2009). Treatment Wetlands, CRC Press Publication. London, United Kingdom.

Figure 4 shows the basic types of constructed wetlands systems that are used. At the current of technology development, there are three types of wetlands that are widely used in industry:

a) Free Water Surface (FWS)

   This type of wetlands contains areas with open water, floating vegetation and emergent plants. The mechanism that used by FWS are sedimentation, filtration, oxidation, reduction, adsorption and precipitation. Usually, FWS is used for secondary or tertiary treatment processes for advanced treatment and it is suitable for all types of climates. Because of its ability to deal with pulse flows and changing water level, FWS is an excellent choice for treatment of urban, agricultural and industrial stormwaters.

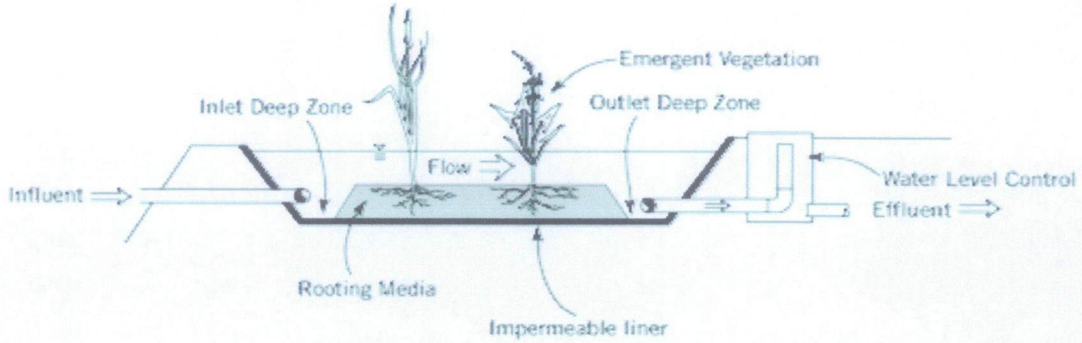



Figure 5: Basic elements of FWS wetland

(From Wallace and Knight.(2006). Basic elements of FWS wetland . Retrieved June 21, 2013, from http://www.naturalsystemsutilities.com/wpcontent/uploads/2012/02/tech_treatment_surface.png)

b) Horizontal Subsurface Flow (HSSF)

HSSF employs with a gravel bed that planted with wetland vegetation while the water is allowed to flow horizontally from the inlet to the outlet but it keeps below the surface of the bed. Basically, HSSF used to treat primary effluent especially soil dispersal or surface water discharge. However, HSSF wetland systems is expensive compared to FWS but the maintenance cost is low.

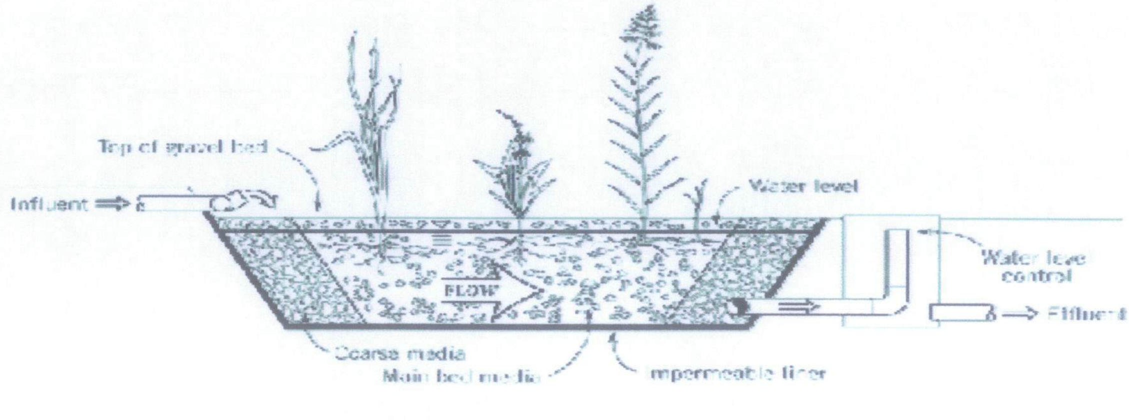



Figure 6: HSSF Wetland Schematic (From Wallace and Knight.(2006).

Retrieved June 21, 2013, from http://www.naturalsystemsutilities.com/wpcontent/uploads/2012/02/tech_treatment_surface.png)

c) Vertical Flow (VF)

VF operated in continuous down flow and usually, VF can be used for very concentrated wastewaters. It is a filter bed that is planted with aquatic plants. The use of overlying water is because to block oxygen transport, so anaerobic conditions can be achieved. VF can be used for treatment of very concentrated wastewaters. Also, VF also have less clogging and require less space than in a horizontal flow constructed wetland. However, pre-treatment is required to prevent clogging and for this design, it will require expert design and supervision.

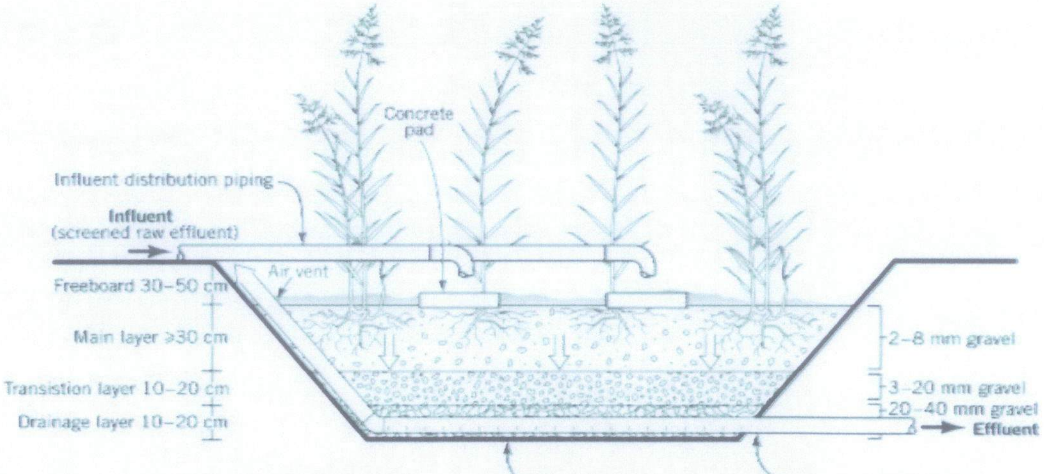



Figure 7: Typical Arrangement of a VF Wetland

(From Paul Knowles, Gabriela Dotro, Jaime Nivala, Joan García. (2011). Retrieved June 22, 2013, from http://www.sciencedirect.com/science/article/pii/S0925857410002600)

## 2.3 Heavy Metals

The presence of metals in the wastewater is one of the main causes of water and soil pollution (Chipasa, 2003; Oliveira et al, 2007). Heavy metals in the water can lead to disease to human society as well as animals such like bird, fish and many more. Initially, most of the wastewater treatment plants are designed to remove organic matter and nutrient load. However, the heavy metals are retained in the wastewater and because of high contents of heavy metals, it cannot be disposed or release to environment. Hence, wetland is one of the possible treatments that can be used to reduce the concentration of the heavy metals.

According to the study conducted by Chanpiwat, Sthuannopkao and Kim, the objective of their research were to determine the metal contents and their variations in influents, effluent and biosludge and to evaluate the potential environmental impact of biosludge generated from Bangkok, Thailand central wastewater treatment plants. Examples of the heavy metals that are detected are such like Cadmium(Cd), Chromium(Cr), Copper(Cu), Iron(Fe), Manganese(Mn) and Nickel(Ni). From the analysis, Iron has the highest concentration in the wastewater released by industry in Thailand. The same findings also founded by Kamarudzaman, Aziz and Jalil and inn their study, iron and manganese are two type heavy metals that have higher concentration as compared to the other. Table 1 shows the result of initial leachate characteristics of their study.

Table 1: Result of Initial Leachate Characteristics

| Parameter | Value(mg/L) |
|---|---|
| Manganese(Mn) | 10.6 |
| Nickel(Ni) | 0.587 |
| Calcium(Ca) | ND |
| Magnesium(Mg) | 0.437 |
| Zinc(Zn) | ND |
| Iron(Fe) | 11.6 |
| Copper(Cu) | ND |
| Chromium(Cr) | ND |
| Cadmium(Cd) | ND |
| Aluminium(Ai) | 0.978 |
| Plumbum(Pb) | 0.653 |

*ND= Not Detected

## 2.4 Aquatic Plants

Aquatic plant is one of the agents that used in the wetland for wastewater treatments. There are many researches were done in identifying the type of aquatic plants that can be used for wastewater treatments. Kanabkaew and Puetpaiboon is using lotus(*Nelumbo Nucifera*) and hydrilla(*Hydrilla Verticillata*) in their study for domestic wastewater treatment for their pilot scale aquatic pond.

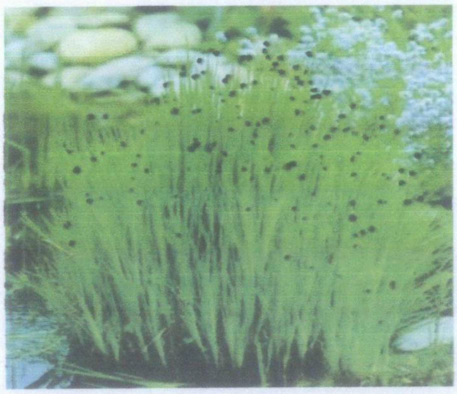


Figure 8: Juncus Ensifolius. Retrieved June 22, 2013

(Retrieved from http://www.wetlandplants.co.uk/acatalog/info_JUNENS.html)

Figure 9: Graves Lovell. 2009. Retrieved June 22, 2013

(Retrieved from http://www.insectimages.org/browse/detail.cfm?imgnum=5400844)

Figure 10: Acorus calamus "Variegatus"

Retrieved June 22, 2013, from
http://www.gardensandplants.com/uk/plant.aspx?plant_id=114; Michael Wolf .
2009.

There are two types of floating plants that used in the wetlands for wastewater treatments. The first one is distinguished by its ability to meet their need for carbon dioxide and oxygen directly from the atmosphere. Another type of treatment is consisted of submerged plants, which is also distinguished by the ability to absorb oxygen, carbon dioxide and minerals directly from the wastewater. Using aquatic plants as the treatment can be low-cost extraction devices to purify polluted water since it is abundant and naturally growth in wetland. The plants also founded to decomposed water faster than microorganism and also, this kind of treatment can be applied to a large area and near the plants if the wetland is available. However, using aquatic plants can be time consuming and it is limited to shallow water or the depth to which roots can penetrate.

There are various processes through with plants can incorporate pollutants and table 2 and figure 11 shows the types of phytoremediation and the location in the plant where the process occurs.

Table 2: Types of Phytoremediation and the Location in the plant where the process takes place.

(Retrieved June 20, 2013., from http://fourthcornernurseries.com/articles/frers.html)

| TYPE | PROCESS INVOLVED | POLLUTANTS TREATED |
|---|---|---|
| Phyto-extraction | The plants are used to concentrate metals in parts harvestable (leaves and roots) | Cadmium, cobalt, chromium, nickel, mercury, lead, selenium, zinc |
| Rhizo-filtration | Plant roots are used to absorb, precipitate and concentrate heavy metals from contaminated liquid effluents and to degrade organic compounds. | Cadmium, cobalt, chromium, nickel, mercury, lead, selenium, zinc radioactive isotopes, phenolic compounds |
| Phyto-stabilization | Metal-tolerant plants are used to reduce the mobility of metals and prevent their passage to groundwater or the air | Lagoons rid of mineral deposits. Proposed for phenolic and chlorinated compounds. |
| Phyto-stimulation | Root exudates promote the development of microorganism (bacteria and fungi) capable of biodegrading compounds. | Petroleum hydrocarbons and polyaromatic, benzene, toluene, atrazine, etc. |
| Phytovolatilization | Plants take up heavy metals and organic compounds, bind or modify them and release the byproducts into the atmosphere via transpiration. | Mercury, selenium and chlorinated solvents (tetraclorometano and trochloromethane) |
| Phyto-decomposition | Both aquatic and terrestrial plants capture organic compounds and store them or decompose them to less toxic or non-toxic byrproducts. | Munitions products (TNT, DNT, RDX, nitrobenzene, nitrotoluene), atrazine, chlorinated solvents, DDT, phosphate pesticides, phenols and nitriles, etc. |

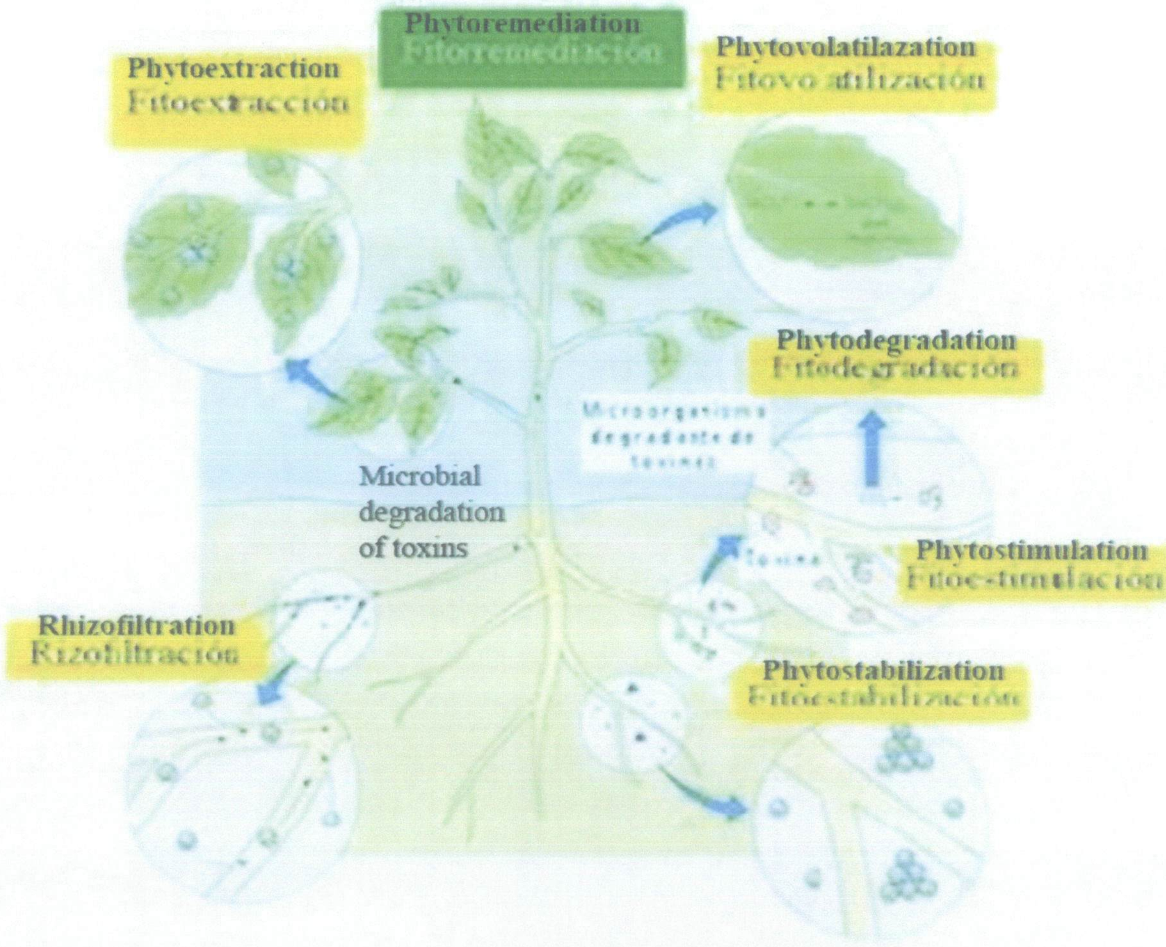



Figure 11: Types of Phytoremediation and the Location in the plant where the process occurs

(Retrieved June 20, 2013., from http://fourthcornernurseries.com/articles/frers.html)

## 2.5 Atomic Absorption Spectroscopy

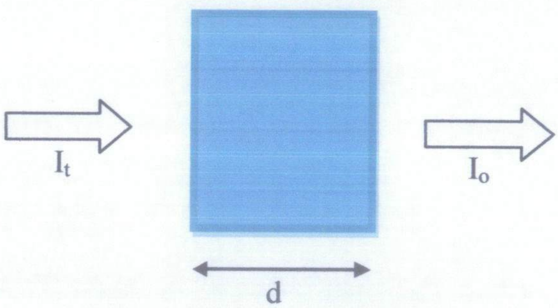
The content of sample analysis from the wastewater is analyzed by using atomic absorption spectroscopy. Basically, atomic absorption spectroscopy uses concept of absorption of light to measure the concentration of analyte in gas phase forms. Thus, for sample that is in solid and liquid forms, it is required to vaporise sample to gas phase forms for analysis using atomic absorption spectroscopy. After vaporisation process, the atoms absorb ultraviolet or visible light and absorb the energy and make transitions to higher electronic energy levels. The absorbance of sample is calculated by:

$$Absorbance = -log\frac{I_t}{I_o}$$

$I_t$ : transmitted radiation

$I_o$: Incident radiation

The concentration of an absorbing species is calculated by using Beer-Lambert concept.




$$Absorbance, A = \varepsilon Cd$$

$\varepsilon$: wavelength-dependent molar absorptivity coefficient

C: concentration

d: path length of the radiation

By applying Beer-Lambert law, it is a linear relationship between absorbance and concentration of an absorbing species. Concentration measurements are usually determined from a calibration curve generated with standards of known concentration.

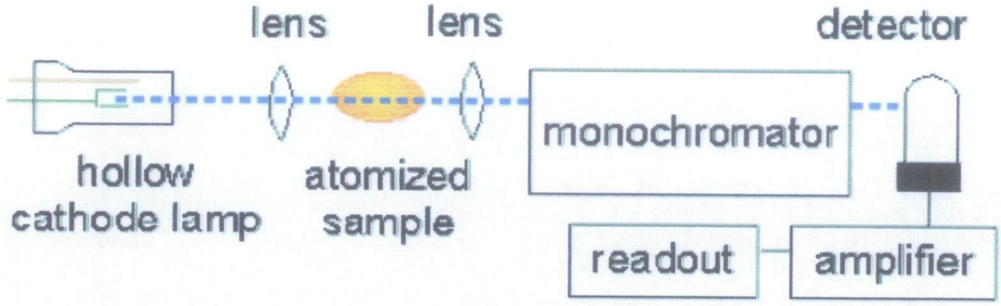



Figure 12: Atomic Absorption Spectroscopy Diagram

Retrieved on August 12, 2013 from
http://faculty.virginia.edu/analyticalchemistry/AAS%20Lead/AAS%20Lead.html

Atomic Absorption Spectroscopy is divided into four main components. They are light source, atomizer, monochromator and detector. Usually, the light source is a hollow-cathode lamp of the element that is being measured.

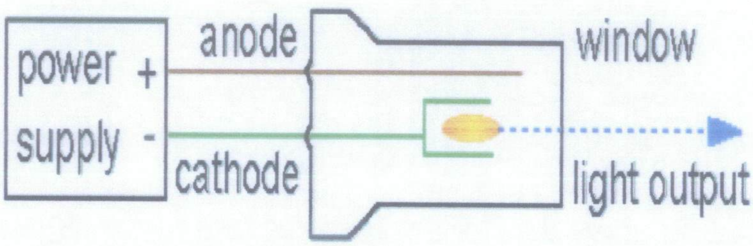



Figure 13: Hollow Cathode Lamp Diagram.

Retrieved on August 12, 2013 from http://www.files.chem.vt.edu/chem-ed/optics/sources/lamps.html

There are two types of light source that can be used in atomic absorption spectroscopy; laser and hollow-cathode lamp. Although laser is more sensitive compared to hollow-cathode lamp, laser has disadvantage that it can only detect one element at a time compared to hollow-cathode lamp. In addition, hollow-cathode lamp also produces narrow emission from atomic species. Ionization of rare gas atoms caused acceleration of gas into cathode and metal atoms of the cathode is sputtered into gas phase. These collisions caused excitation of metal atoms to higher energy levels. The atoms then decayed to lower energy levels by emission of light.

For sample analysis, sample must be in gas phase forms. Thus, atomizer is required to vaporize solid or liquid phase into gas phase. Ions or atoms in a sample must undergo desolvation and vaporization in a high temperature source such as flame atomic absorption. It is a slot type burner that used to increase the path length and according to the Beer-Lambert law, absorptivity is directly proportional to the path length. Thus, increasing the path length travelled by radiation will increased the absorptivity of the sample. Sample solutions are usually aspirated with the gas flow into a nebulising or mizing chamber to form small droplets before entering the flame.Although flame atomic absorption spectroscopy can only analyzed solution and required large amount of sample for analysis, using flame as atomizer is inexpensive and high precision.

Another type of atomizer that can be used also is graphite furnace atomic absorption spectroscopy. Although graphite atomizer is quite expensive and has low precision, it can be used for solutions, slurries and solid samples for analysis. It also has greater sensitivity and typically, 5-50 μL or smaller quantities of sample are required for atomizer process. There are three stages that are involved when using graphite atomizer. Table below shows the three stages and typical conditions of graphite furnace:

Table 3: Stages in Graphite Furnace

| Stage | Temperature and period |
|---|---|
| Drying | 125°C for 20 seconds |
| Ashing | 1200°C for 60 seconds |
| Vaporization | 2700°C for 10 seconds |

The next component is monochromator and it is important to isolated absorption line from the background light as well with from molecular emissions originating in the flame. This is important to isolated lines so that detector will give accurate and precise reading of sample analysis. Last component is detector and a photomultiplier is used to measures the intensity of the incident light and generates an electrical signal proportional to the intensity.

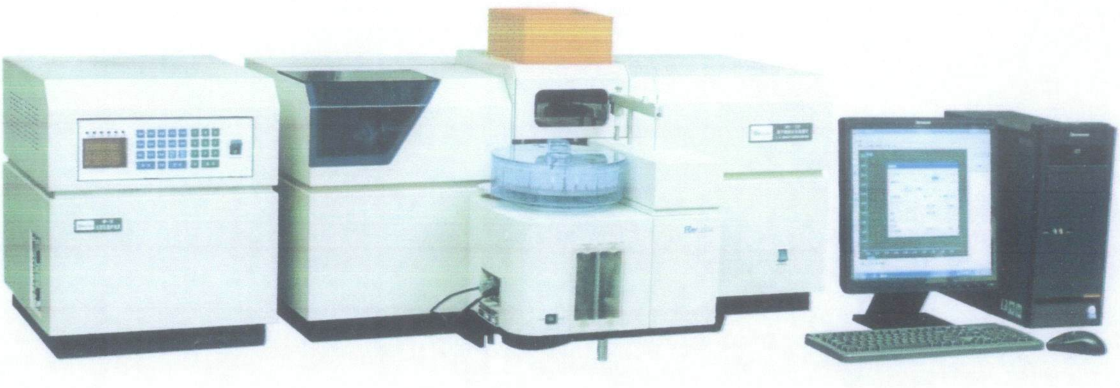


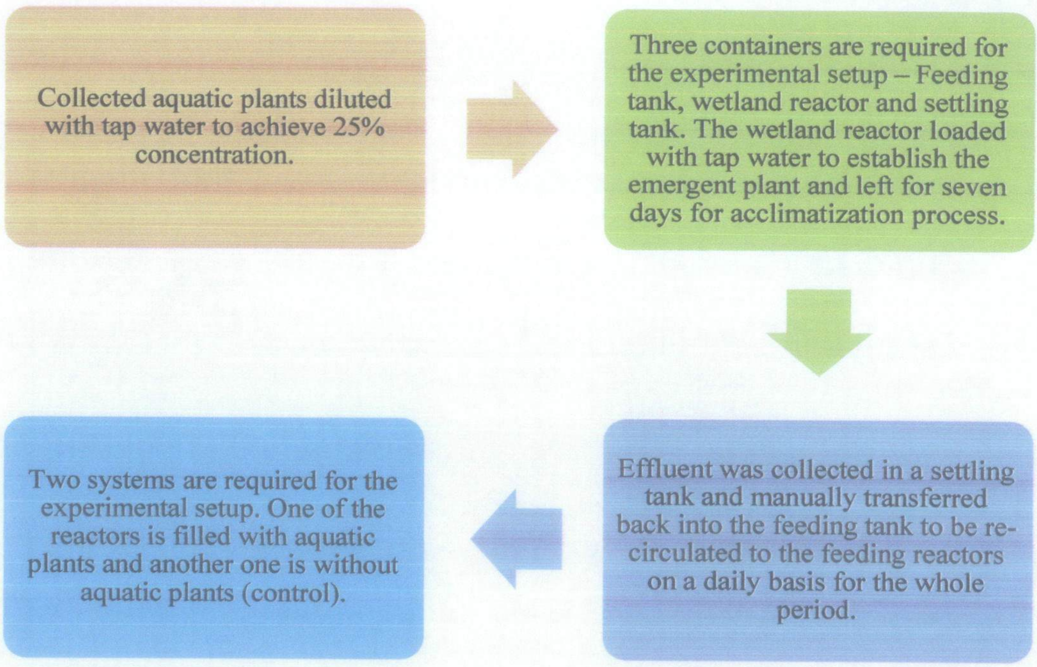
Figure 14: Atomic Absorption Spectroscopy.

# CHAPTER 3

# METHODOLOGY

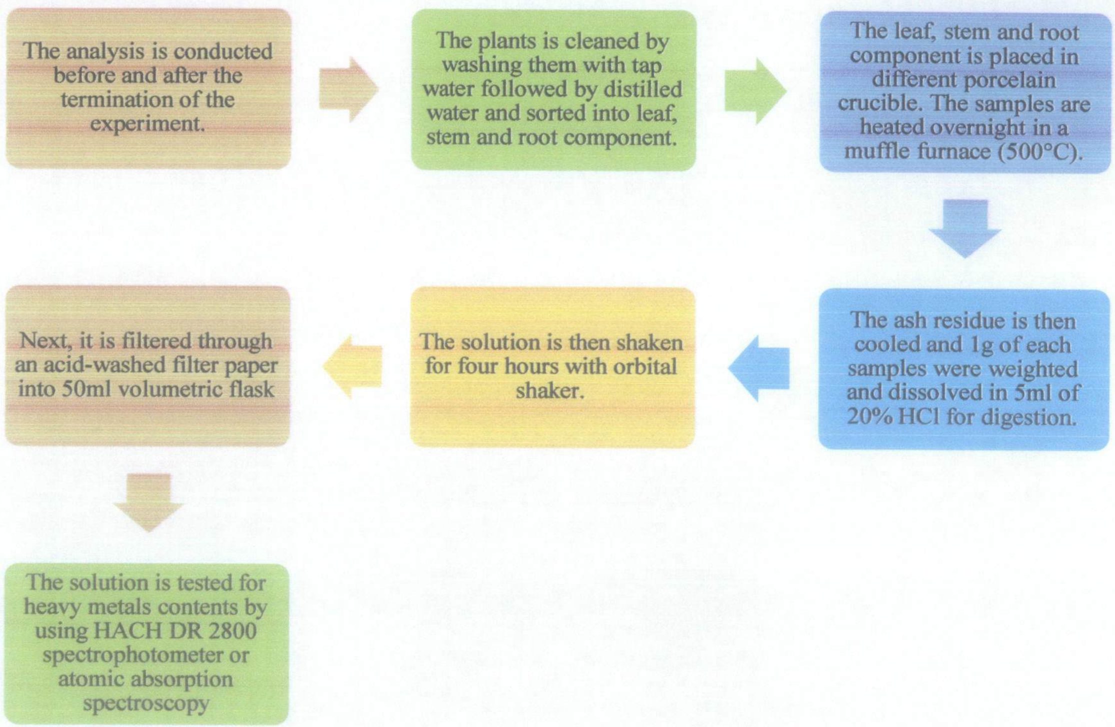
## 3.1 Procedure of Experiment

### 3.1.1 Experimental Setup




For the experimental set up, the experiment is started with acclimatization process, which the collected limnocharis flava is diluted with tap water. This is done to achieve healthy conditions for the roots, stems and leaves to grow before the treatment process is done. Next, it is required to prepare three tanks in sequence – feeding tank, wetland tank and settling tank. Feeding tank is where the wastewater will be feed and after that, treatment process will take place at wetland tank and finally, settling tank function is where all the treated wastewater will be transfer to. After the effluent was collected from the wetland tank, the water will be manually transferred to be circulated to the feeding tank on daily basis for the whole period.

### 3.1.2 Analysis of Plant Tissue




In order to study the accumulation of heavy metals at different parts of plants, it is required to do analysis for roots, stems and leaves. Thus, the analysis will be conducted before and after the termination of the experiment. Samples of plants – limnocharis flava will be collected from the wetland tank and then, cleaned with tap water and sorted into leaves, stems and roots components as shown in figure 15. The samples then are placed at different petri dishes and placed in oven and overheated for one night. After the samples are dried (Figure 16), the leaves, stems and roots were crushed by using agate mortar separately and 1g of each sample is dissolved in 5mL of 20% hydrochloric acid for digestion. Then, the solution is shaken with orbital shaker for four hours and filtered through an acid-washed paper into 50mL volumetric flask. The samples then are transferred into vials and tested for heavy metals contents (Pb, Fe and Ni) by using atomic absorption spectroscopy.

Figure 15 : Leaves, stems and roots of Limocharis Flava

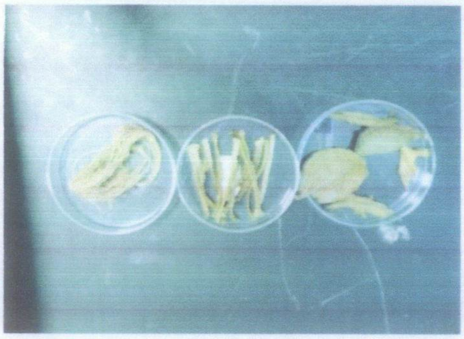
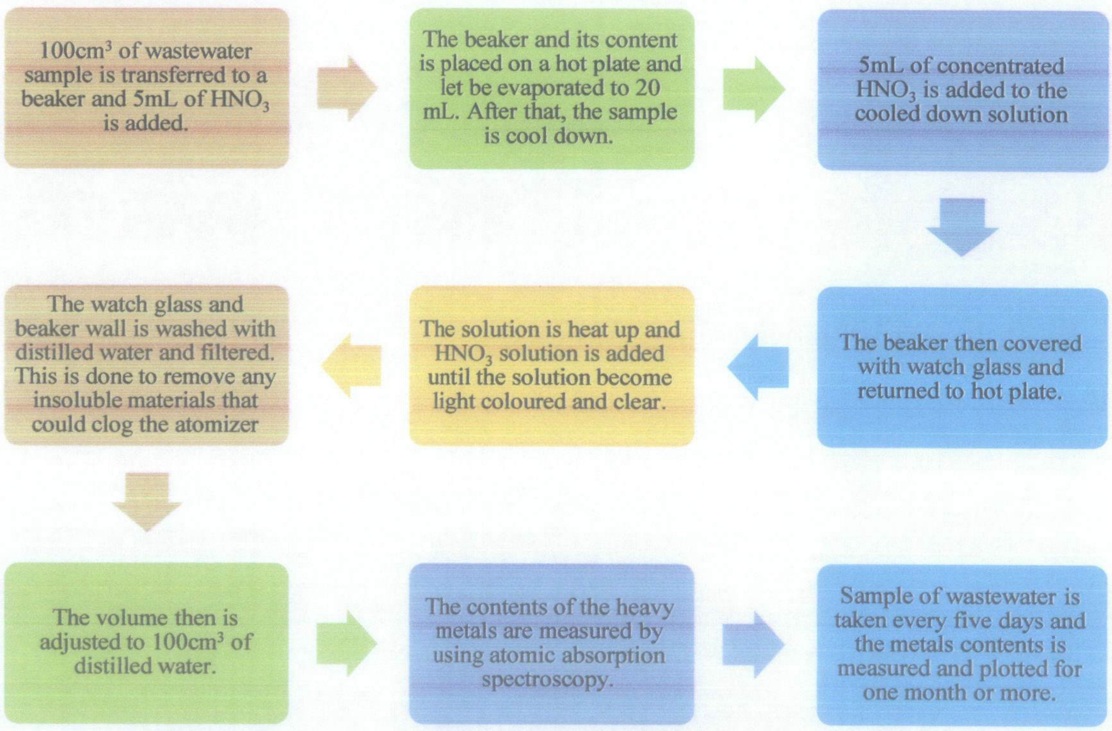


Figure 16 : Leaves, stems and roots after dried in oven for one night

### 3.1.3 Analysis of wastewater




For the analysis of wastewater, about $100cm^3$ of sample is taken from the settling tank and transferred to a beaker which contains of 5mL of acid nitric. Then, the beaker is placed on hot plate and let evaporated until the volume is 20mL and cooled down. After the solution become cool, 5mL of concentrated acid nitric is added and next, the beaker is covered with watch glass and returned to hot plate. This is done to trap the steam for escaped from the beaker and during the heating process, concentrated acid nitric is added to the solution until it become light coloured and clear. After that, the watch glass and beaker wall is washed with distilled water and filtered. It is very important to filter carefully the solution because any presence of insoluble material may damage the atomic absorption spectroscopy. The volume is then adjusted to $100cm^3$ with distilled water and then, the solution is transferred to vial for analysis of heavy metals.

### 3.1.4 Experimental Set-Up

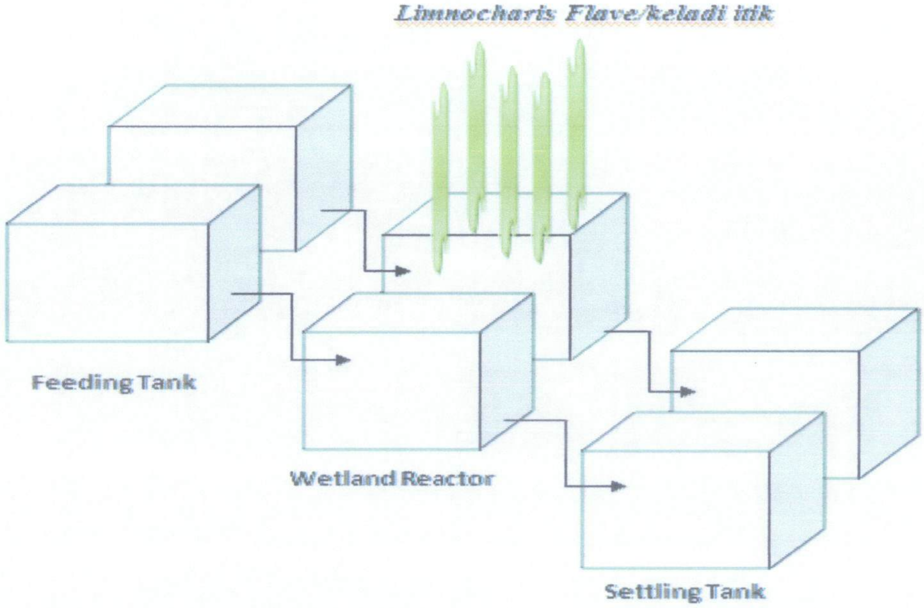



Figure 17: Experimental Set-up

Figure 18: Limnocharis flava.

Retrived June 22, 2013,
https://commons.wikimedia.org/wiki/File:Limnocharis_flava_04.jpg;

### 3.2 Gantt Chart

Table 4: Gantt Chart of FYP1

| No | Details Work | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Selection of Project Topic | | | | | | | | | | | | | | |
| 2 | Preliminary Research Work | | | | | | | | | | | | | | |
| 3 | Submission of Extended Proposal | | | | | | | | | | | | | | |
| 4 | Proposal Defence | | | | | | | | | | | | | | |
| 5 | Project Work Continuous<br>- Listing equipments and chemical reagents<br>- Details experimental set-up<br>- Surveying sources for aquatic plants and wastewater<br>- Cost budgeting<br>- Building Prototype | | | | | | | | | | | | | | |
| 6 | Submission of Interim Draft Report | | | | | | | | | | | | | | |
| 7 | Submission of Interim Report | | | | | | | | | | | | | | |

Table 5: Gantt chart for FYP II

| No | Details/Week | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Collecting materials for prototype | | | | | | | | | | | | | | | |
| | Building Prototype | | | | | | | | | | | | | | | |
| | Preparation of apparatus and chemical reagant | | | | | | | | | | | | | | | |
| | Acclimatization Procedure | | | | | | | | | | | | | | | |
| | Treatment Procedure | | | | | | | | | | | | | | | |
| | Sampling Analysis | | | | | | | | | | | | | | | |
| 2 | Submission of Progress Report | | | | | | | | | | | | | | | |
| 3 | Project Work Continues | | | | | | | | | | | | | | | |
| 4 | Pre-SEDEX | | | | | | | | | | | | | | | |
| 5 | Submission of Draft Report | | | | | | | | | | | | | | | |
| 6 | Submission of Dissertation (soft bound) | | | | | | | | | | | | | | | |
| 7 | Submission of Technical Paper | | | | | | | | | | | | | | | |
| 8 | Oral Presentation | | | | | | | | | | | | | | | |
| 9 | Submission of Project Dissertation (Hard Bound) | | | | | | | | | | | | | | | |

### 3.3 Methodology of Project

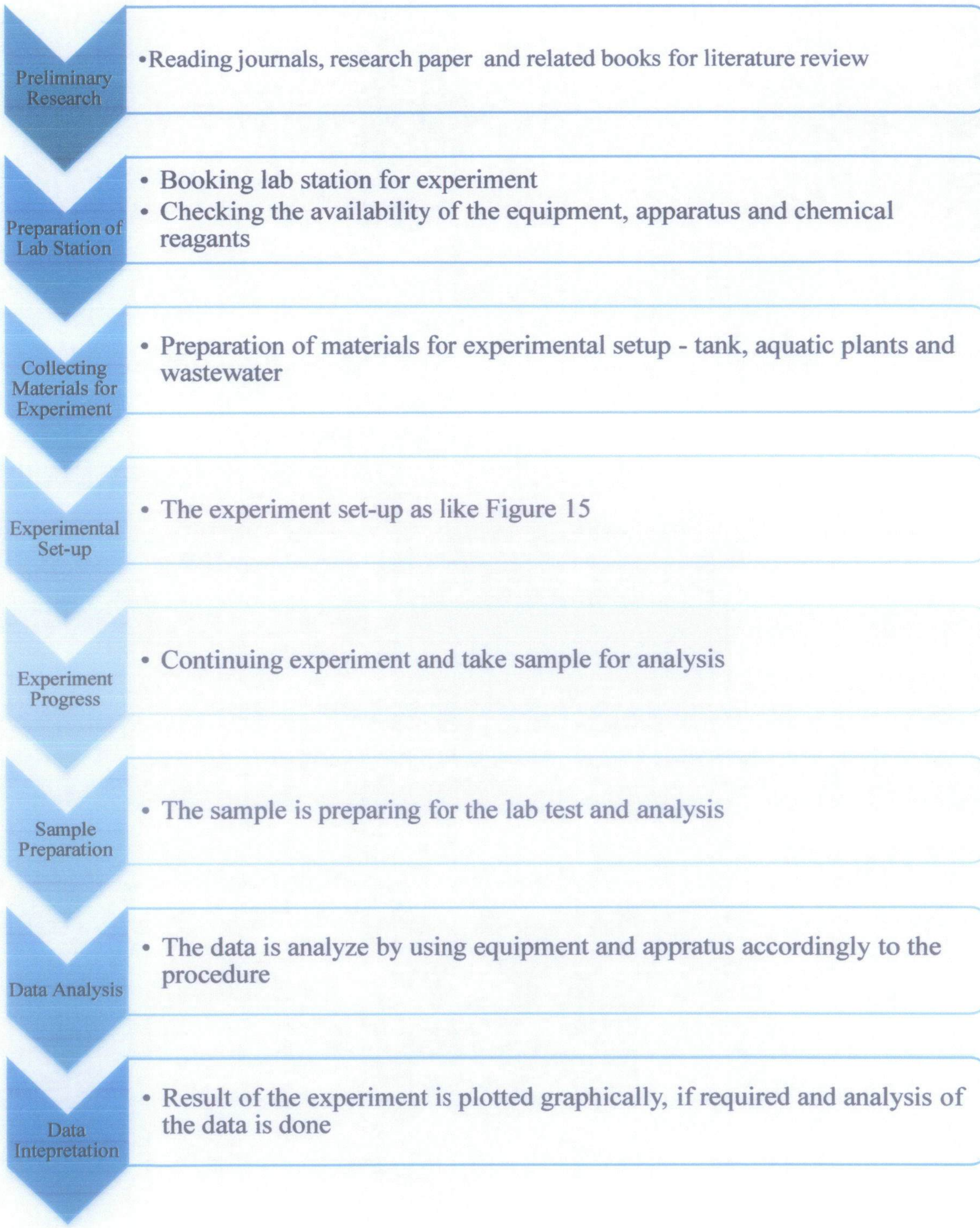



Figure 19: Methodology of the Project

### 3.4 Tools and Equipment

- Atomic Absorption Spectroscopy – to detect heavy metals contents in the wastewater and plant tissue samples.
- Filtering equipment (filter paper, glass funnel, beaker, volumetric flask) – This is done to remove any insoluble materials
- Drying equipment (porcelain cubide, agate mortar, weighing, oven) – a procedure to do analysis of leaf, stem and root which required heating aquatic plants overnight.
- Acclimatization equipment (tank, reactor, tap waters) – to achieve normal and healthy conditions for leaves and stem as well as growth of new leaves. This is done by soaking aquatic plants in tap water for seven days.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Experimental Set-up

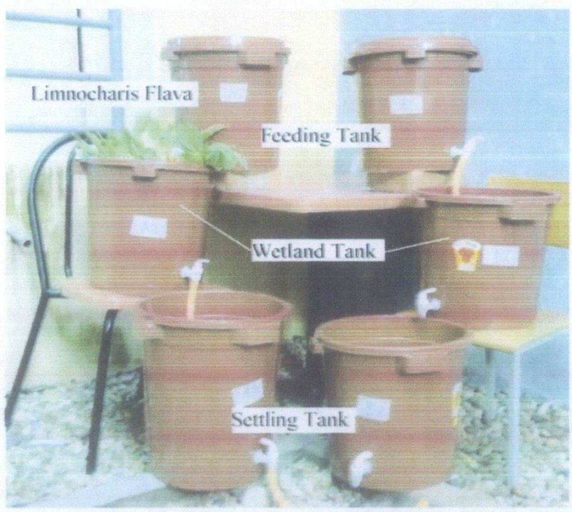



Figure 20: Experimental Set-up for Wastewater Treatment using Limnocharis Flava

Figure 18 shows the arrangement of the wastewater treatment by using aquatic plant – Limnocharis Flave and basically, there are three major tanks that are used for the treatment. They are:

a) Feeding Tank – This is the first part of the treatment where untreated wastewater is keep and all wastewater from settling tank is transferred into the feeding tank on daily basis for circulation purpose.
b) Wetland Tank – This is where the treatment takes place and one of the tanks is planted with aquatic plants – Limnocharis Flava while the other one is planted without aquatic plants and acted as control.
c) Settling Tank – In this tank, all the treated wastewater will be stored in this tank and circulated back to the feeding tank.

In order to investigate the performance of constructed wetland system and Limnocharis Flava in treating wastewater, it is important to use wastewater that is contained higher concentration of heavy metals. For this project, it is decided to treat iron, lead and nickel types of heavy metals. Due to lack of availability of wastewater that contains higher concentration of iron, lead and nickel, synthetic wastewater is used for the treatment.

For Limnocharis Flava, it is obtained from Universiti Teknologi Petronas (UTP) lake (decimal degree coordination: 4.382628,100.975266). According to the procedure, after the emerging plants are collected, they will go through a process which is acclimatization process. It is a process where the limnocharis flave is diluted with tap water and this is done to allowed new leaves to grow before the treatment.

**4.2 Observation during Treatment of Wastewater**

During the treatment of wastewater, it is observed that the number of Limnocharis flava is reduced from day to day. This is happen because of plant uptake on the heavy metals which is higher than required by aquatic plants. When this is happen, the plants are slowly died and finally, settle as accumulated at the bed of soils. This is resulted in increasing amount of heavy metals on the bed of soils and this is only can be proved by doing analysis on the soil itself. For the wastewater treatment analysis, it is required to collect samples every five days. The cloudiness of wastewater is observed and compared. The wastewater becomes cloudier as compared to first and last day of treatment for both tanks (with and without treatment). However, the relationship between the cloudiness of wastewater with the concentration of heavy metals cannot be prove unless the analysis of wastewater is done by using atomic absorption spectroscopy in order to determine concentration of heavy metals.

### 4.3 Results and Analysis

Table 6: Concentration of Heavymetals With and Without Treatment of Aquatic Plants

| | With Treatment (A) | | | Without Treatment (B) | | |
|---|---|---|---|---|---|---|
| | Pb | Ni | Fe | Pb | Ni | Fe |
| 0 | 44.1942 | 78.1208 | 63.3232 | 44.1872 | 77.6975 | 64.3232 |
| 5 | 32.1232 | 75.3232 | 60.3243 | 42.3821 | 77.0212 | 62.3223 |
| 10 | 30.2321 | 69.2323 | 54.4343 | 39.4321 | 74.3223 | 58.3241 |
| 15 | 26.4087 | 65.4953 | 46.3232 | 35.7782 | 70.9664 | 53.4232 |
| 20 | 23.3221 | 62.3212 | 40.3543 | 35.0231 | 69.9323 | 53.0322 |
| 25 | 20.1232 | 54.3233 | 34.5344 | 33.9231 | 69.0032 | 52.4212 |
| 30 | 17.3294 | 50.1234 | 24.2333 | 32.6689 | 68.0895 | 51.3232 |
| Percentage reduction | 60.79% | 35.84% | 61.73% | 26.07% | 12.37% | 20.21% |

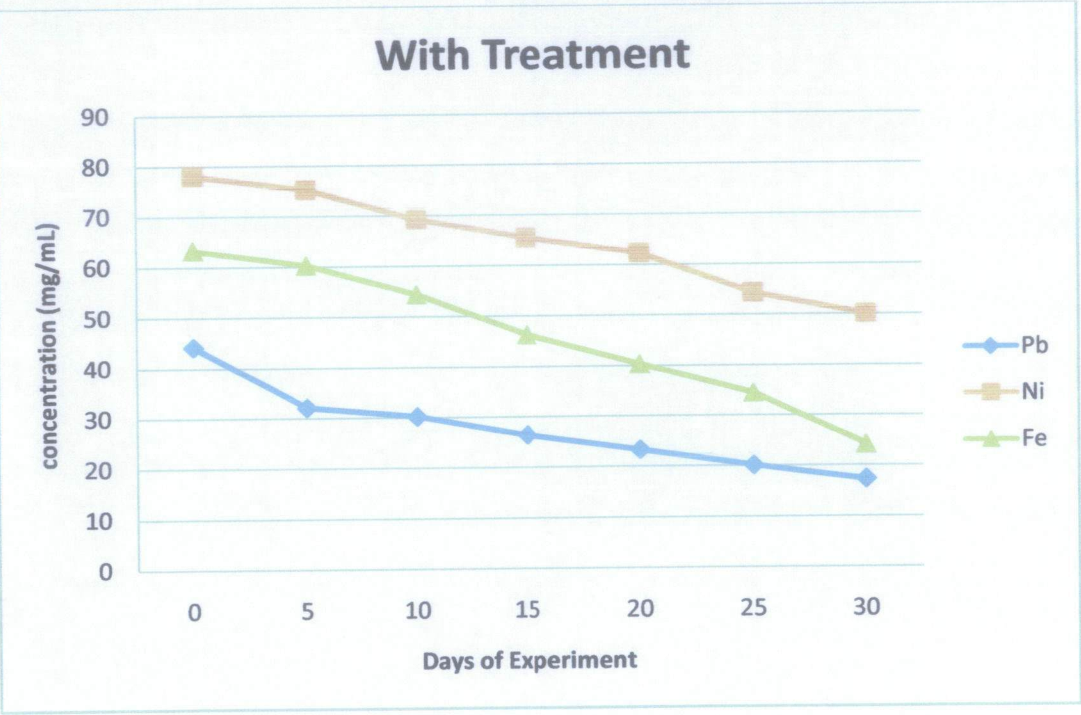



Figure 21: Concentration of Heavy Metals with Treatment of Limnocharis Flava

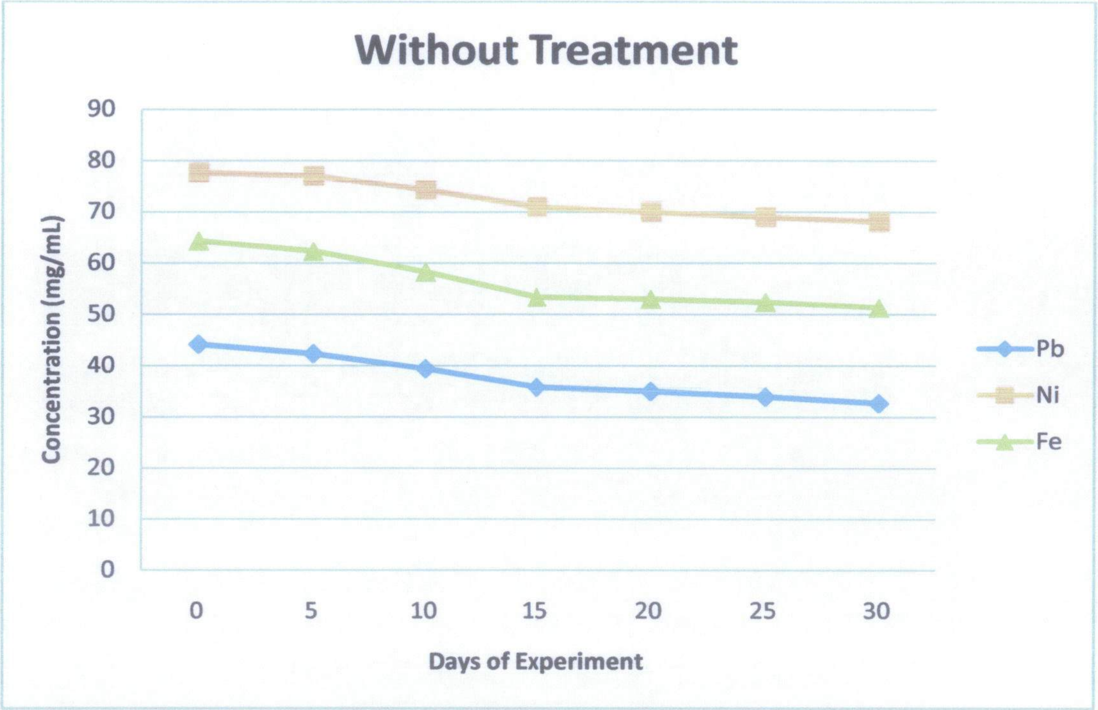



Figure 22: Concentration of Heavy Metals without Treatment of Limnocharis Flava

Table 7: Concentration of Heavy Metals at different parts of Plants (Leaf, Stem and Root)

| | Before Treatment | | | After Treatment | | |
|---|---|---|---|---|---|---|
| | Leaf | Stem | Root | Leaf | Stem | Root |
| Pb | 2.4543 | 1.4323 | 2.3213 | 14.9663 | 19.9724 | 33.4669 |
| Ni | 1.4323 | 1.9283 | 1.4323 | 16.1804 | 21.7359 | 31.7754 |
| Fe | 1.2334 | 2.4323 | 1.3234 | 15.3232 | 20.3242 | 32.4321 |

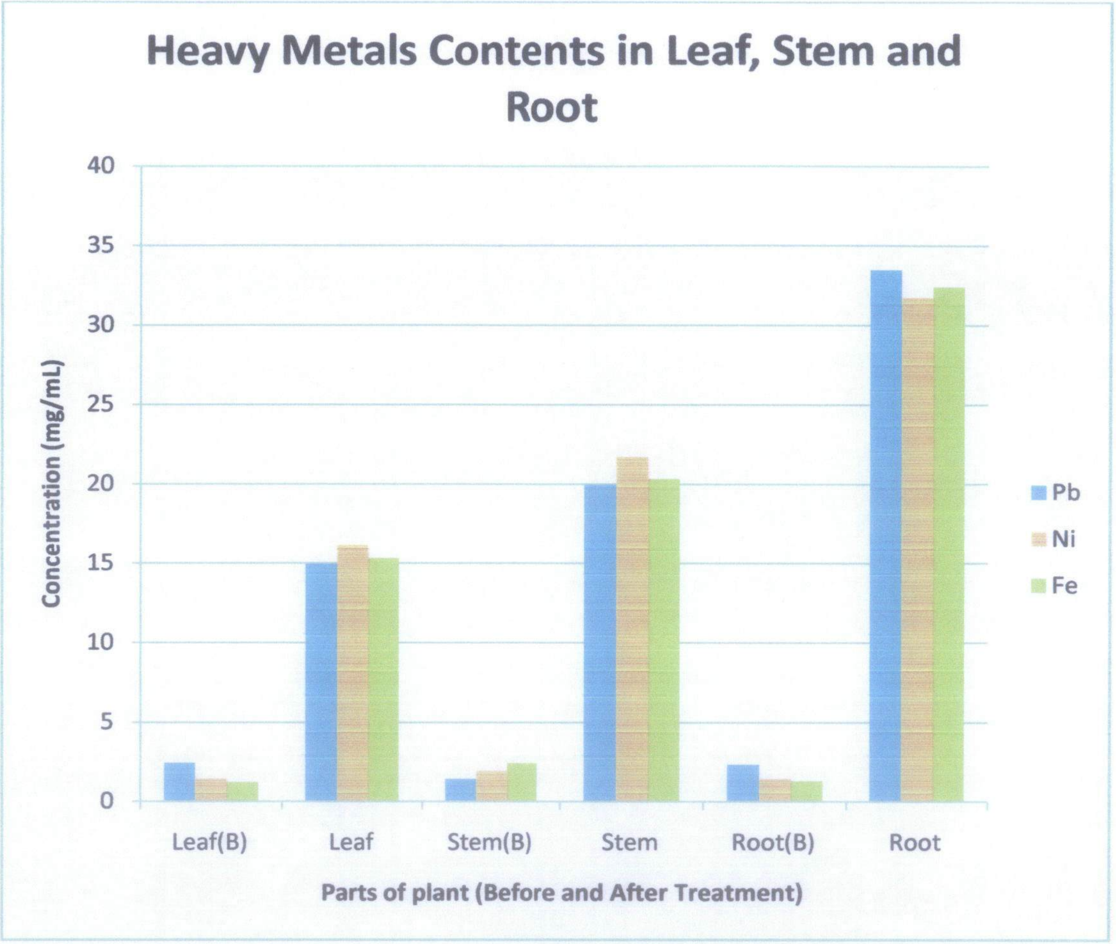



Figure 23 : Accumulation of Heavy Metals at Leaf, Stem and Root.

### 4.3.1 Analysis of Wastewater

At the beginning of treatment, the concentrations of heavy metals are measured for each type of heavy metals – lead, iron and nickel. The concentrations of heavy metals are measured by using atomic absorption spectroscopy. From figure 19 and 20, the initial concentrations of heavy metals for lead, nickel and iron are 44, 78 and 63 mg/mL respectively. After that, the heavy metals contents are measured for every five days for both systems – with and without treatment of limnocharis flava which is done for one month.

After the treatment, the concentrations of heavy metals are observed to decrease for both with and without treatment. But, greater reductions of heavy metals are observed with treatment of limnocharis flava. The highest percentage reduction of heavy metal is recorded by iron with percentage of 62%. It is then followed by lead with 61% of reduction and 36% by nickel. However, for system which is without aquatic plants – a slightly reduction of heavy metals also are observed with lead (26%) as the highest reduction, followed by iron with 20% and nickel with 12% of reduction of heavy metal content from the original value.

From the data analysis, it is obvious that limnocharis flava has potential and significant role in removing heavy metals from the wastewater. Currently, phytoremediation is widely applied in the wastewater treatment industry and it is a bioremediation process to transfer, stabilize, destroy and remove pollutants in the wastewater by using aquatic plants, macrophytes as well as algae and bacteria. There are many mechanisms that are involved in the removal of heavy metals in the wastewater treatment.

First, plant uptake in which the aquatic plants were absorbed heavy metals from the wastewater as nutrient. For example, iron is one of the important nutrients for plants to promote formation of chlorophyll, acts as an oxygen carrier as well as cell division and growth of plants. However, there is a limit in which the plants can absorb heavy metal from the wastewater and it is varies with the nature and characteristics of the plants. Once the plants reached its limit to absorb heavy metals, leaves and stems are slowly becoming yellow and die. After that, it will die and settled at the bottom. This explained the phenomenon during conducted the experiment where the leaves and stems are slowly becoming slowly and at the end of the treatment, the amount of limnocharis flava in the tank is less than at the beginning of the experiment.

In addition, algae and bacteria also plays a significant role in the removal of heavy metals from the wastewater because they are able to consumed and remove heavy metals. Algae are low nutrient requirements and usually do not produce toxic substances which can is beneficially for the industry to remove heavy metals from wastewater by using biosorption process. However, the major focus of this project is to study the removal of heavy metals by using aquatic plants – limnocharis flava and the uptake by algae and bacteria is considered as not significant as the removal by limnocharis flava.

### 4.3.2 Analysis of Heavy Metals Contents in Leaves, Stems and Roots.

After acclimatization process, each part of plants (root, stem and leaf) is taken and prepared for the analysis of heavy metals (Pb, Fe and Ni). This is done to observe which parts of plant is the most significant in removal of wastewater. At the beginning of the experiment, the initial concentrations of heavy metal ranged between 0 to 3 mg/mL. After the treatment process, the contents of heavy metals are measured again to assess the accumulation of heavy metals at three different parts of plants. The lowest accumulation of heavy metals is observed at leaves which the concentration ranged between 15-17 mg/mL and then, it is followed by stems which the concentration of heavy metals is recorded about 20mg/mL for lead, 22 mg/mL for nickel and 20mg/mL for iron. From figure 21, roots have the highest accumulation of heavy metals which is ranged between 30-34 mg/mL for iron, lead and nickel.

As the analysis of accumulation of heavy metals at different part of plants, roots have the highest accumulation of heavy metals as compared to the stems and leaves. Due to the precipitation at the root zone area, the accumulations of heavy metals are concentrated at this part. Since roots have direct contact between wastewater, thus it is easier to absorb and filter heavy metals from the wastewater. In metal contaminants, roots are the most beneficial for photostabilisation. Basically, it is a process in which metal-tolerant plants are used to reduce the mobility of metals and prevent their passage to groundwater or air.

### 4.3.3 Limitations of the Project

There are some limitations that are occur during conducted this project. From the methodology part, it is suggested that the treatment will be done for one month. However, due to the time constraints in submission of the report as well as the documentation, it is preferable to conduct the treatment process for one month. Hence, it is suggested to do the treatment process for more than one month so that the resistant of limnocharis flava towards heavy metals can be studied and proven. Also, it is intended the waste water need to be circulated from the settling tank to feed tank on daily basis. However, for this project – the waste water is transferred manually everyday and this can lead to ineffective and inconsistent of results. Thus, peristaltic pump should be installed to the system so that the wastewater can be transferred daily continuously at the constant volume.

For this project, it is decided to use synthetic wastewater instead of wastewater from the industry. The purpose of this project is to treat wastewater from industry by using aquatic plants in which the concentration of heavy metals should be standardised. Thus, the wastewater sample from industry should to be taken and measured and next, to standardize it with the synthetic wastewater.

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# CHAPTER 5

# CONCLUSION

Wastewaters from the industries need to be treated before releasing it to the environment. However, some of the technology that available is not be able to remove some heavy metals that present in the wastewater. Thus, wetland with aquatic plants as the alternative for the second and tertiary treatment of wastewater is introduced to the industry. In this project, the effectiveness of *Limnocharis Flave* in removing heavy metals such as iron, manganese and chromium is going to study based on the experimental lab project. Application of the wetland as the treatment has been proven to be more effective in removal of heavy metals as well low cost for maintenance and construction but, the process can be time consuming. From this project, it is clearly proven that usage of aquatic plants in removal of heavy metals in wastewater can be applied in the industry. Furthemore, roots have the most significant roles in removal of heavy metals because of greater accumulation of heavy metals is observed in the roots.

As for the recommendations, it is suggested to expand the treatment process to more than one month because this will help to study the behaviour and efficiency in removal of heavy metals from wastewater. Next, peristaltic pump should be used for the project, so that the distribution of wastewater can be efficient. Besides, the study of the project can be further analyse on the effect of soil media used as well as size of gravel in order to optimize removal of heavy metals from the industry.

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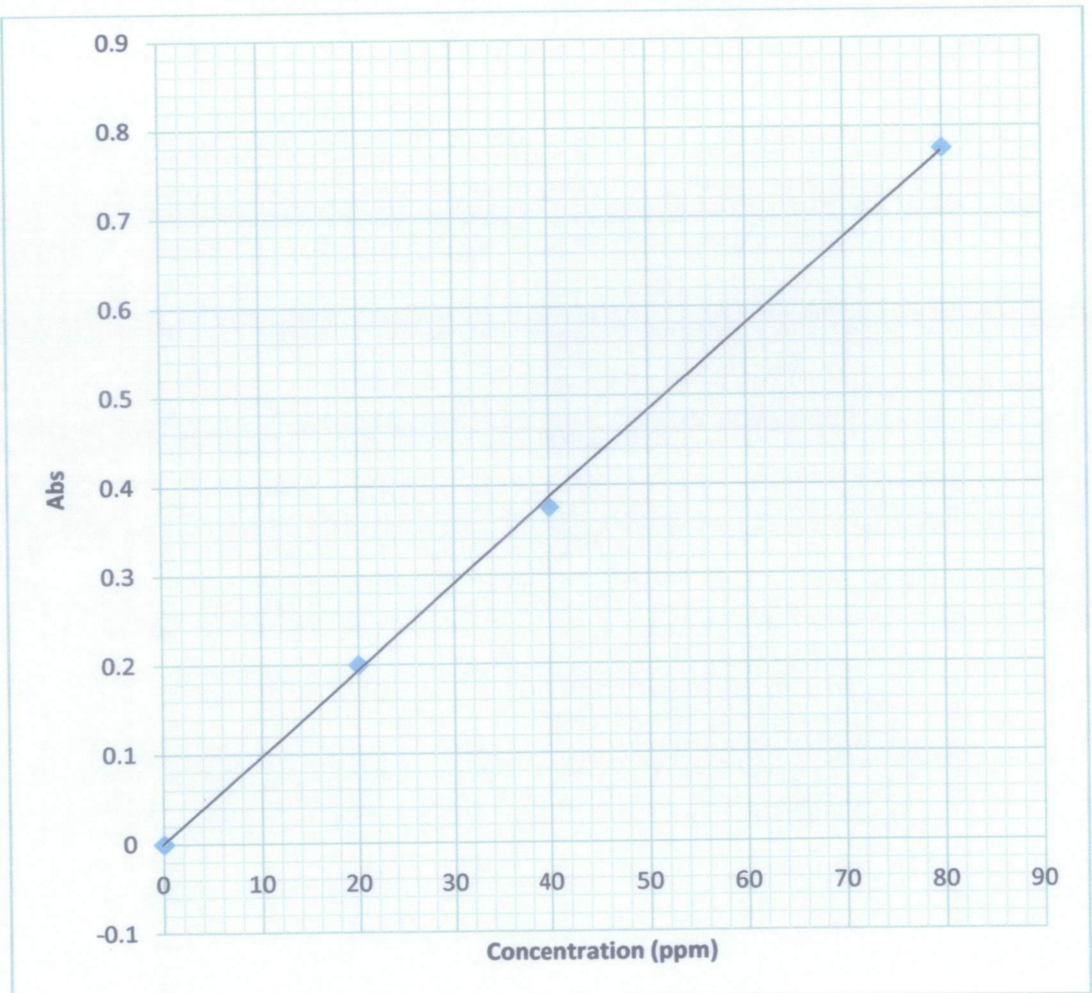
# APPENDICES

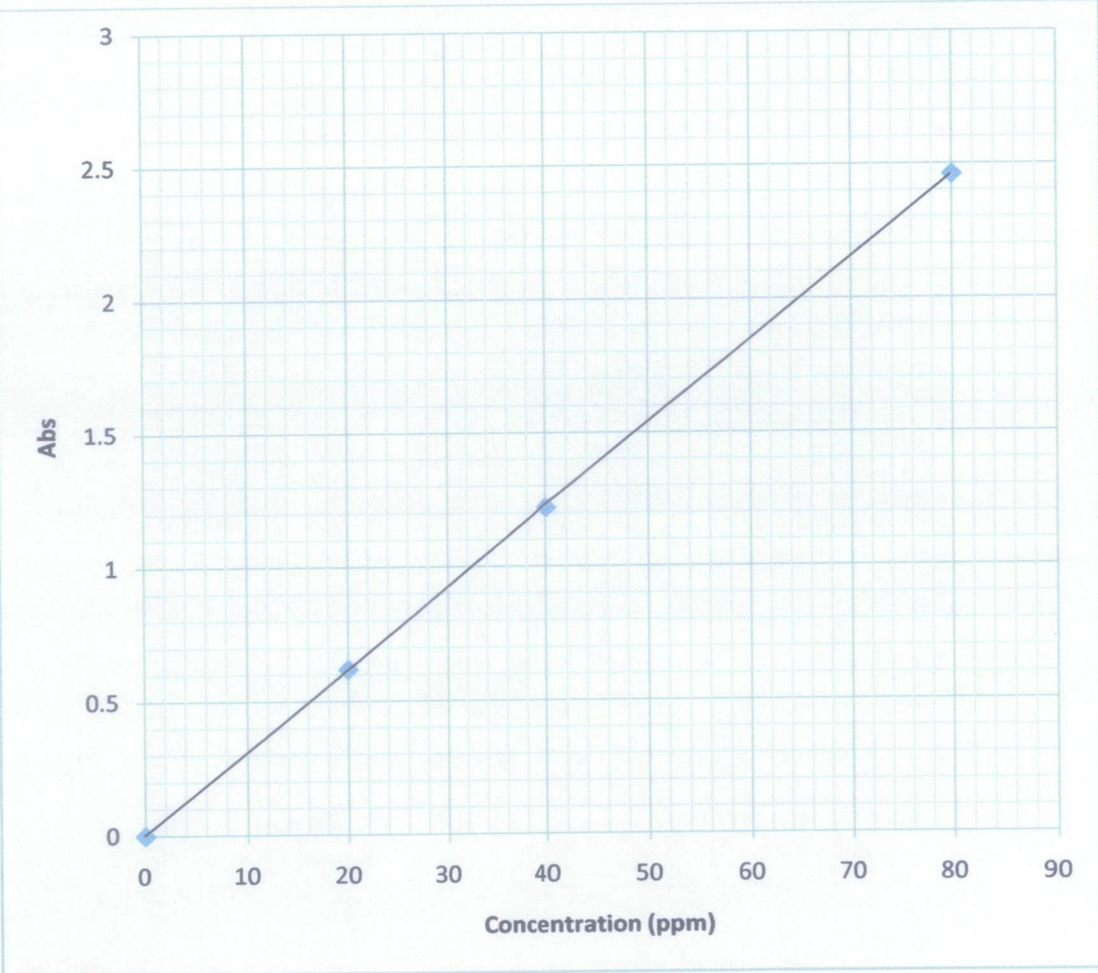
## Appendix 1: Standard Calibration for Lead

| Concentration(ppm) | Absorbance |
|---|---|
| 0 | 0 |
| 20 | 0.2 |
| 40 | 0.375 |
| 80 | 0.775 |



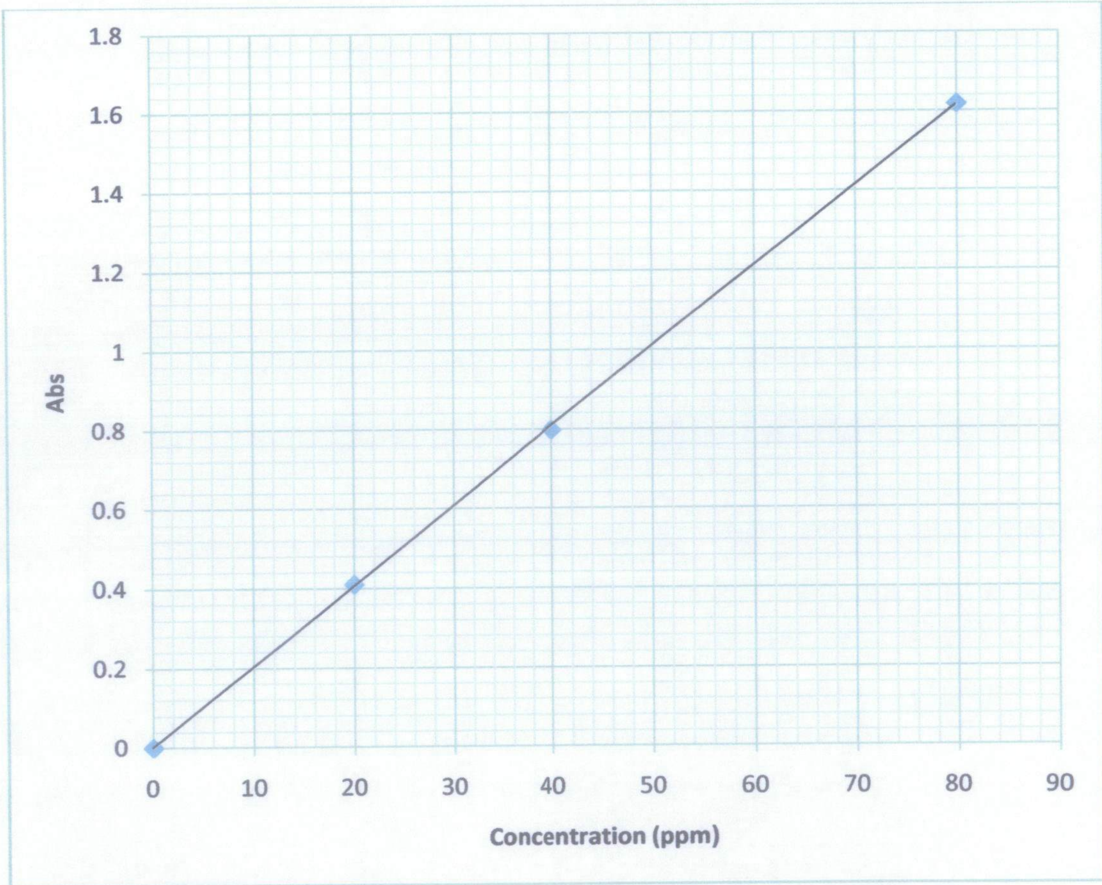
**Appendix 2: Standard Calibration for Nickel**

| Concentration (ppm) | Abs |
|---|---|
| 0 | 0 |
| 20 | 0.62 |
| 40 | 1.22 |
| 80 | 2.46 |



## Appendix 3: Standard Calibration for Iron

| Concentration (ppm) | Abs |
|---|---|
| 0 | 0 |
| 20 | 0.41 |
| 40 | 0.7975 |
| 80 | 1.6175 |



## Appendix 4: Raw Data from Atomic Absorption Spectroscopy

a) Lead

| Samples/Components of Plants | | Absorbance | Concentration (mg/mL) |
|---|---|---|---|
| Leaves | Before Treatment | 0.02209887 | 2.45543 |
| | After Treatment | 0.1347516 | 14.9724 |
| Stems | Before Treatment | 0.0128907 | 1.4323 |
| | After Treatment | 0.1797516 | 19.9724 |
| Roots | Before Treatment | 0.0208917 | 2.3213 |
| | After Treatment | 0.3012021 | 33.4669 |

| Samples/Days | | Absorbance | Concentration (mg/mL) |
|---|---|---|---|
| 0 | With Treatment | 0.3977478 | 44.1942 |
| | Without Treatment | 0.3976848 | 44.1872 |
| 5 | With Treatment | 0.2891088 | 32.1232 |
| | Without Treatment | 0.3809529 | 42.3281 |
| 10 | With Treatment | 0.2720889 | 30.2321 |
| | Without Treatment | 0.3548889 | 39.4321 |
| 15 | With Treatment | 0.2376783 | 26.4087 |
| | Without Treatment | 0.3220038 | 35.7782 |
| 20 | With Treatment | 0.2098989 | 23.3221 |
| | Without Treatment | 0.3152079 | 35.0231 |
| 25 | With Treatment | 0.1811088 | 20.1232 |
| | Without Treatment | 0.3053079 | 33.9231 |
| 30 | With Treatment | 0.1559646 | 17.3294 |
| | Without Treatment | 0.2940201 | 32.6689 |

b) Nickel

| Samples/Components of Plants | | Absorbance | Concentration (mg/mL) |
|---|---|---|---|
| Leaves | Before Treatment | 0.042969 | 1.4323 |
| | After Treatment | 0.485412 | 16.1804 |
| Stems | Before Treatment | 0.057849 | 1.9283 |
| | After Treatment | 0.652077 | 21.7359 |
| Roots | Before Treatment | 0.042969 | 1.4323 |
| | After Treatment | 0.953262 | 31.7754 |

| Samples/Days | | Absorbance | Concentration (mg/mL) |
|---|---|---|---|
| 0 | With Treatment | 2.343624 | 78.1208 |
| | Without Treatment | 2.330925 | 77.6975 |
| 5 | With Treatment | 2.259696 | 75.3232 |
| | Without Treatment | 2.310636 | 77.0212 |
| 10 | With Treatment | 2.076969 | 69.2323 |
| | Without Treatment | 2.229669 | 74.3223 |
| 15 | With Treatment | 1.964859 | 65.4953 |
| | Without Treatment | 2.128992 | 70.9664 |
| 20 | With Treatment | 1.869636 | 62.3212 |
| | Without Treatment | 2.097969 | 69.9323 |
| 25 | With Treatment | 1.629699 | 54.3233 |
| | Without Treatment | 2.070096 | 69.0032 |
| 30 | With Treatment | 1.503702 | 50.1234 |
| | Without Treatment | 2.042685 | 68.0895 |

c) Iron

| Samples/Components of Plants | | Absorbance | Concentration (mg/mL) |
|---|---|---|---|
| Leaves | Before Treatment | 0.024668 | 1.2334 |
| | After Treatment | 0.306464 | 15.3232 |
| Stems | Before Treatment | 0.048646 | 2.4323 |
| | After Treatment | 0.406484 | 20.3242 |
| Roots | Before Treatment | 0.026468 | 1.3234 |
| | After Treatment | 0.648642 | 32.4321 |

| Samples/Days | | Absorbance | Concentration (mg/mL) |
|---|---|---|---|
| 0 | With Treatment | 1.266464 | 63.3232 |
| | Without Treatment | 1.286464 | 64.3232 |
| 5 | With Treatment | 1.206486 | 60.3243 |
| | Without Treatment | 1.246446 | 62.3223 |
| 10 | With Treatment | 1.088686 | 54.4343 |
| | Without Treatment | 1.166482 | 58.3241 |
| 15 | With Treatment | 0.926464 | 46.3232 |
| | Without Treatment | 1.068464 | 53.4232 |
| 20 | With Treatment | 0.807086 | 40.3543 |
| | Without Treatment | 1.060644 | 53.0322 |
| 25 | With Treatment | 0.690688 | 34.5344 |
| | Without Treatment | 1.048424 | 52.4212 |
| 30 | With Treatment | 0.484666 | 24.2333 |
| | Without Treatment | 1.026464 | 51.3232 |