**SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING**

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# THE REMOVAL OF HEAVY METALS BY PHYTOREMEDIATION USING CYPERUS ALTERNIFOLIUS

By

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# DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled **"The Removal of Heavy Metals by Phytoremediation using Cyperus Alternifolius"**. I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or university.

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Date: 21 June 2017

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# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# PENYINGKIRAN LOGAM BERAT MELALUI PHITOREMEDIASI MENGGUNAKAN CYPERUS ALTERNIFOLIUS

## ABSTRAK

Keupayaan Cyperus alternifolius bagi merawat logam berat telah dikaji. Tiga set uji kaji (1,2 dan 3) setiap set uji kaji mengandungi berlainan jenis sampel air iaitu air paip, larutan logam- logam berat dan larutan ferum sulfat. Dari kajian, set uji kaji 1, 2 dan 3 telah menunjukkan penurunan kadar pH sepanjang proses rawatan. Analisis unsur bagi logam Cu, Mn, Ni dan Fe telah ditentukan dengan menggunakan AAS. Di pengakhiran phitoremediasi kandungan logam semakin sikit sekiranya dibandingkan dengan kandungan di permulaan rawatan justeru menunjukkan keberkesanan Cyperus a. dalam menyingkirkan logam-logam berat. Dalam uji kaji 1, Mn > Cu = Fe > Ni dengan peratusan 89 > 84 > 80. Uji kaji 2, dalam antara Cu > Fe > Mn > Ni dengan peratusan 90 > 83 > 81 > 72. Logam yang terkumpul di dalam akar, dahan dan daun dianalisis untuk mendapatkan faktor pengumpulan (A) dan faktor pemindahan (TF). Ia telah didapati faktor pengumpulan kuprum adalah tinggi pada bahan uji kaji 2 pada dahan dengan kadar 66 %. Pada ferum, mangan dan nikel pula faktor pengumpulan (A) adalah paling tinggi di akar pada set uji kaji 1 dengan bacaan masing-masing 43 %, 34 % dan 50 %. Kuprum dan nikel kedua-duanya menunjukkan kadar faktor pemindahan yang paling tinggi di dahan dengan bacaan masing-masing 5.66 di set uji kaji 1 dan 0.36 di set uji kaji 2. Faktor pemindahan kuprum dan mangan kebanyakkannya di dedaun pada set uji kaji 2 dengan kadar 0.62 dan 0.91. Secara keseluruhannya, faktor pemindahan pada set uji kaji 1, 2 dan 3 boleh diringkaskan secara berikut : Cu > Mn > Fe > Ni (1.34 > 0.77 > 0.45 > 0.2) , Mn > Fe > Ni > Cu ( 0.91 > 0.62 > 0.22 > 0.01 ) dan bacaan 0.17. Oleh itu, daripada keputusan yang diperoleh dapat disimpulkan Cyperus a. adalah sejenis pokok pengumpulan yang dapat menyingkirkan logam-logam berat daripada persekitarannya.

# THE REMOVAL OF HEAVY METALS BY PHYTOREMEDIATION USING CYPERUS ALTERNIFOLIUS

## ABSTRACT

The ability of Cyperus alternifolius was studied in the removal of heavy metals. Three sets of heavy metals removal experiment were set up (1, 2 and 3), where each set contain containing tap water, heavy metals solution and iron sulfate solution. This research, experimental set-up 1, 2 and 3 showed a drop in pH throughout the treatment process. Elemental analysis of Cu, Mn, Ni and Fe ions were determined by AAS. At the end of the phytoremediation, concentration of metals in set-up 1, 2 and 3 were lesser compared to their initial metal concentration indicating the efficiency of Cyperus alternifolius in the removal of heavy metal. In set-up 1, Mn > Cu = Fe > Ni by percent of 89 > 84 > 80. Set-up 2, within the range of Cu > Fe > Mn > Ni within the percent of 90 > 83 > 81 > 72. Element accumulated at the roots, stems and leaves were analyzed to obtained accumulation factor (A) and translocation factor (TF). It was found that the accumulation factor of copper was highest in the experimental set up 2 in the stems by 66%. For iron, manganese and nickel the accumulation factor was highest in roots in the set-up 1 by 43%, 34% and 50% respectively. Both copper and nickel showed the highest translocation factor value of 5.66 at the stems for set-up 1 and 0.36 respectively for set up 2. Translocation factor of iron and manganese in the leaves gave values of 0.62 and 0.91 respectively. Overall, the translocation factor in set-up 1, 2 and 3 can be summarized as follow: Cu > Mn > Fe > Ni (1.34 > 0.77 > 0.45 > 0.2), Mn > Fe > Ni > Cu (0.91 > 0.62 > 0.22 > 0.01) and value of 0.17 respectively. Therefore, from the results obtained it can be concluded that Cyperus alternifolius is a hyperaccumulator that able to remove metals ions from its environments.

# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

Mining practices contribute to a lot of environmental issues in all over the countries. Local environment surrounding is at a high risk of potential hazard and acid mine drainage is one of the major problem related to it. Outflow of acidic water into the source of water surrounding is accompanied by harmful heavy metal that eventually endangered nearby provincial. Water pollution is the most widespread and easily affected problem commonly associated with mining activity. Significantly, this paper will be focusing on treating wastewater by phytoremediation.

Acid mine drainage (AMD) occurred when acidic solution is formed characterized by its low pH as the water flow over or through sulfur-bearing materials and rich in heavy metals. Heavy elements such as Copper, Manganese, Nickel, and Iron are non-biodegradable and accumulate in the environment. Therefore, various treatments have been developed to actually save the environment but so far it has yet to be effective. AMD mitigation technology can be divided into two categories, active and passive treatment. Active treatment makes use of chemical reagents such as lime, caustic soda, soda ash briquettes and ammonia. This type of treatment needs constant monitoring and maintenance. Meanwhile, passive treatment relies on biological, geochemical and gravitational process. Passive treatment is considered more cost effective, eco-friendly and simple as it utilized the naturally occurring matter to modify the influence characteristic of acid mine drainage.

Phytoremediation indeed is a passive treatment. The medium used to treat mine effluent is by taking the benefit of specific plant species. The selected plant has the ability to decontaminate heavy metal accumulated within the soil and water as well as increase the pH level of mine waste water. Selection of species must be certain such characteristic as a hyper accumulator. Significantly, it be able to translocation pollutant element through roots, stems and leaves as well as modify them into less harmful substance. Therefore, phytoremediation is termed more environmental-friendly, hassle free, less expensive and does not required maintenance. Yet, proper disposal of the plants must be carefully taken care of and must make sure throughout the treatment process other living beings such as animals or human does not consume them as it compromises with an aerial concentration of harmful elements.

This research paper focused on the ability of Cyperus alternifolius a plant species to treat heavy metal from synthetic waste water and improve the solution pH value. Tolerance of the plant towards heavy metal will be determined by how much the concentration of those elements can be removed from the synthetic mine waste water. The synthetic waste water prepared consisted of copper, iron, nickel, and manganese. Cyperus alternifolius is commonly known as umbrella grass or umbrella papyrus. The plants have numerous numbers of roots, long leaves and a hollow like stem structure which is best suit the characteristics of plant species in phytoremediation.

## 1.2 Problem Statement

Outflow of acidic water from mine contains aerial concentration of heavy metals and metalloids (White., n.d.). These elements are harmful to living organisms if the amount at a certain area exceeds the allowable limit. Hence, to control the widespread of AMD where it is the major contributing of heavy metal into the environment, various technology treatments must be implemented. Addition of reagent into waste water, formed secondary wastes and highly cost. The approach is deeming ineffective and therefore mining industry is in need of more reliable AMD mitigation.

Phytoremediation is an emerging technology that have been discovered which is cost effective, environmental friendly and safe by making use of plants to remove contaminant. The technique is also referred as botanical bioremediation because this method utilized green plant to decontaminate pollutant in the soils and waters where it can be applied to both organic and inorganic at a large scale (Priscila., 2005). On that account, only selected plant species is effective in phytoremediation.

Mamut Copper Mine which is located in Sabah, Malaysia is the most prominent example of an abandoned mine that still suffers from AMD. Even hundreds of millions ringgit has been invested to reclaim the location, yet no improvement can be observed. This is because of the large amount of polluted mine pit water, very high rainfall and large volumes of sulphidic mineral face at the site contribute to difficulties in restoring the mine (Ent and Edraki., 2016). Apart from that, the abandoned mine are also experiencing water quality degradation and siltation issues (Rahman et al., 1984: Jopony and Murtedza., 1994).

In relation to the issues in Mamut Copper Mine, numerous AMD technological approaches must be taken into consideration. Chances to reduce pollution related to mining industry become wider. Umbrella grass a common name for Cyperus alternifolius is chosen in this research. This is because by utilizing a local plant species the method is considered more affordable and hassle free. All in all, as phytoremediation is one of the passive treatments that are environmental friendly, simple and effective then it is the best remediation technique to be applied.

## 1.3 Objectives

The objectives of this research are:

1. To monitored and study the change in pH within 1 weeks of treatment.
2. To study the effectiveness of removal of heavy metals by using Cyperus alternifolius on metal ions solution which contain copper, nickel, manganese and iron ions.
3. To study the accumulation factor and ratio of translocation of metal from roots to shoot to determine the effectiveness of the plants as an hyperaccumulator.

## 1.4 Significance of research

Heavy metal and acidic water destroy aquatic organism, soil and local surrounding. The presence of heavy metals and metalloid in the environment affect crop yields, soil biomass, aquatic life, fertility and human where contributing to bioaccumulation in the food chain (Priscila., 2005). Therefore, various physiochemical and biological techniques are implemented to neutralize and remove metals and sulfate from mine waste water. The most widely used treatment technology is based on addition of chemical and hydroxide precipitation of metals. The disadvantages of the method include high cost and produce secondary wastes.

On the contrary, phytoremediation is considered more reliable, cost effective, eco-friendly and safe to decontaminate soil and water from heavy metals and metalloids. This emerging technology is either absorb or breaks downs those elements in the soil, store them in the stems or leaves of the plants (Rungwa et al.,2013). Significantly to achieve the goal, selective plants species must be carefully chosen as phytoremediation candidate.

### 1.5 Scope of work

In order to achieve the objectives, pH reading of each set will be taken at day 0, day 1, day 5 and day 7 during 1 weeks of treatment. Changes of pH level will be observed and compared from a plot of pH against time graph. Meanwhile, effectiveness of heavy metal removal by Cyperus alternifolius will be determined by taking water sample from each set at initial and after 7 days of treatment to be analyzed by AAS. The concentration of heavy metals from AAS will show changes of each metal concentration in the water throughout the 7 days of treatment.

Three sets of different types of water sample will be treated for 1 week by Cyperus alternifolius. At the end of each treatment process, plant in each set will be cut into three parts roots, stem and leaves. The weight of each part of the plant will be taken and digested separately. Concentration of heavy metals and arsenic stored in each part will be analyzed by Atomic Absorption Spectrometry (AAS) test. Percent removal of copper, nickel, manganese and iron by Cyperus alternifolius from synthetic mine water sample will be determined based on its initial concentration. Overall, objectives of this research will be achieved by proper treatment preparation and the end result obtained.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

Environmental pollution due to heavy metal is a worldwide issue especially in countries associated with mining activities. These issues become a threat to land, aquatic environment and living organism when the level of heavy metals released to the local surrounding are above the certain allowable limit. The elements such as copper, manganese, nickel and iron are discharged into the environment due to the mobilization of heavy metals through extraction from ores and subsequent processing for different application (Ali et al., 2013). Consequently, these elements accumulated in the soil and water due to its property which is nonbiodegradable. Therefore, reliable and efficient approaches are discovered through time to solve the issues in maintaining good quality of soils and water as well as keeping them free from contamination (Lone et al., 2008).

## 2.2 Land and water pollution by heavy metal is a worldwide issue

As time passed, the concentration of heavy metal in the environment has increased tremendously (Govindasamy et al., 2011). Heavy metal present in the environment naturally or exists due to manmade processes which is considered as by products (Harvey and Julie K., 2011). Due to the characteristic of heavy metals that are not degradable through microbiological and chemical process (Mahmood et al., 2014), consequently they are eventually stored in the soil and then diffused into groundwater table (Luo et al., 2016). It was recognized by researchers that certain chemicals such as toxic metals, could harm humans as they remain in the environment for a long period of time as from accumulation to a dangerous level (Priscila et al., 2005).

The issues spread widely due to the industrial activity worldwide as consequences from mining wastes, agricultural, paper mills and toxic elements from atmospheric emission (Priscila et al., 2005). It was discovered that the campine region in Belgium and Netherland was contaminated by atmospheric deposition of Cd, Zn, and Pb within the area of 700 $km^2$ (Meers et al., 2010). Meanwhile, in China as consequences from mining activities a total area of 2.88 x $10^6$ ha of destroyed land is produced and mean area of 46 700 ha is established annually. In the other part of the world, there are 600 000 brown field in USA which are in needed of reclamation due to heavy metal contamination (Mckeehan and Brownfields., 2000). It was discovered that more than 19 000 km of US streams and rivers are contaminated by heavy metals, acid mine drainage and polluted sediment which according to the government statistic the source is coming from coal mine (Iqbal et al., 2008).

As, Cu, Cd, Pb, Cr, Ni, Hg and Zn are the few heavy metals that have been discovered in polluted environment. However, different site may present different heavy metals depending upon the source of individual pollutant.

### 2.3 Conventional treatment to treat heavy metals and acidic water.

The conventional process to treat heavy metals from wastewater or mine effluent can be achieved through many processes. The most commonly practice approach is physico-chemical removal method, chemical precipitation, coagulation and flocculation, electrochemical deposition and ion exchange (Barakat., 2010).

Physico-chemical methods are primarily applicable to particulate forms of metals, discrete or metal bearing particle (Dermont et al., 2008). The method is achieved through the separation of colloidal particles and addition of chemical known as coagulants and flocculants.The efficiency of the treatment is based on the soil characteristics such as particle size, moisture and clay content and metal contamination (Smith., 1995; Williford et al., 2000).

Chemical precipitation is the most favorable method used for heavy metal removal from inorganic effluence due to its operation simplicity (Williford et al., 2000). The conceptual mechanism can be presented based on the equation 1.1 below: (Wang et al., 2004).

$$M^{2+} + 2(OH)^{-} \longleftrightarrow M(OH)_2 \downarrow \quad (1.1)$$

The reactants represent dissolved metal ions and the precipitants, respectively meanwhile the product indicate insoluble metal hydroxide. The major parameter that significantly amend heavy metal removal through chemical precipitation is the alteration of pH to the basic condition (9-11) resulting from the addition of precipitant agent such as lime. Even though the method is considered cost effective but due to a large amount of chemical required, other various problems arise. A few of them are excessive sludge released, slow metal precipitation, the aggregation of metal precipitates and the sludge disposal causes long term environmental impacts (Aziz et al., 2008).

Removal of heavy metal through electrolysis is the process where it uses electricity to pass a current by a cathode plate and an insoluble anode through an aqueous metal bearing solution (Gunatilake., 2015). Heavy metals are treated by precipitating the elements in a weak acidic or neutralizing the catholyte as hydroxide. Positively charged metallic ions attract to the cathode and leaving behind a strippable and recoverable metal deposit (Kurniawan et al., 2006). Wastewater treatment using electrochemical process involved electro-deposition, electro-coagulation, electro-flotation and electro-oxidation (Shim et al., 2014).The obvious shortcomings of employing this method is that corrosion can become a significant limiting factor, as it requires frequent replacement of electrodes.

Ion exchange is another commonly remediation in removing heavy metals from effluent. Ion exchange is capable to attract soluble ions from liquid phase to solid phase (Gunatilake., 2015). Removal of metal ion from the solution is done by a special ion exchanger contain in anions or cations. Synthetic organic ions resins are the most typically used matrices for ion exchange (Barakat., 2010). Effectiveness of the technology is more prominent in treating water with low concentration of heavy metals and comprises low cost materials as well as convenient operations (Dizge et al., 2009; Hamdaoui., 2009).Other solids and organics contain in the wastewater may spoiled the matrix therefore this method is unreliable in handling concentrated metal solution.

## 2.4 Passive treatment is considered more reliable than active treatment

Mine effluent can be either treated with active or passive treatment. This is commonly associated with the term AMD which is known as acid mine drainage. Acid mine drainage (AMD) are defined based on high level of heavy metal, sulfate concentrations and low acidity level (Gate., 2008). AMD is known as an outflow of acidic water that either comes from currently operating or abandoned mine. It occurred due to the exposure of sulphide mineral in the presence of water and air which then oxidize to form sulphuric acid that resulting in releasing heavy metals and other pollutants to the drainage (Sheoran and Sheoran., 2006). This is however, an unfortunate event as aquatic systems and biological community structure are negatively affected by bioaccumulation of species in the food chain (da Cruz et al., 2010).

AMD can be treated by using chemicals like lime, calcium carbonate, hydrated lime, caustic soda and soda ash. This is most commonly known as active treatment where the technology employed a large amount of chemical in treating mine effluent. However, the treatment is unreliable as it produces a high amount of sludge and represents an addition to environmental issues (Fiset et al., 2003). Apart from producing secondary pollution problems, the conventional remediation method also suffers from certain limitations such as high cost, intensive labor, irreversible changes in soil properties and disturbance of native soil micro flora (Ali et al., 2013).

The passive treatment is a treatment which employed the nature surrounding such as gravity and biological processes to treat polluted water which are acidic and contaminated with heavy elements thus the use of chemical is almost to none. Passive treatment is considered more economic than active treatment in the long run (Trumm.,2002). As the technique depend on natural physical, biological and geochemical process therefore the technique is hassle free and reliable. The technology does not need constant maintenance and operation just only needed a onetime installation .

The best well known method of passive treatment method in the mine industry worldwide are constructed wetland (Cws), anoxic limestones drains (ALD), vertical flows systems and open limestone channels (OLC).

## 2.5 Phytoremediation as a green technology to treat heavy metal

The world is in need for an alternative method to treat AMD which are cost effective, eco-friendly and safe to operate. Therefore, a more modern technological approach has been discovered which are cost effective, eco-friendly and safe. This method is known as phytoremediation which utilize plant in the removal of heavy metals and neutralize acidic water.

Plant decontaminates heavy metal without affecting topsoil and conserve its utility and fertility (Mench et al.,2009). The concept is employed by introducing plant into wastewater and contaminant assimilation occurred into roots and leaves (Priscila et al.,2005). Heavy metal is either break in the soil by plant roots or they are absorbed, stored in the stem and leaves of plant (Rungwa et al.,2013). Generally speaking, phytoremediation is defined as “the use of green plants and their associated microbiota, soil amendments and agronomic technique to remove, contain or render harmless environmental contaminants” (Costello et al.,2003).

Phytoremediation utilized plant to treat waste water and heavy metals through absorption. Therefore, suitable plant must be chosen for the remediation to be successful. Solubility and complexity of heavy metal affect the process of absorption and transformation of these elements into plant system (Stanley Rungwa et al.,2013). Heavy metals from soil solution will be extracted and accumulated by plant where it passes through the surface of the root in which special plant membrane protein recognized the chemical structure of essential metals where these proteins bind the metal for uptake and transport (Axelsen and Palmgren.,2001). Vascular system is the medium to transport these absorbed elements from roots to above soil biomass (shoots) where the shoot is harvested, incinerated to reduce volume, disposed of as hazardous waste or precious metals can be recycled. On the other hand, by using selective plant roots might either break the contaminant down in the soil or absorb it in the stems and leaves (Adams., 2000).

Uptake of heavy metal normally occur in the wetland as emergent plant and surface floating plants have the best capacity to absorb these elements that are present in the water from AMD. Based on the growth cycle of the plant and the concentrations of the metal, the rate uptake of heavy metal are varied.

## 2.6 Techniques of phytoremediation

Phytoremediation are categorized on various strategies such as phytoaccumulator, phytofiltration, phytovolatilization, rhizodegradation, phytodesalination, phytostabilization and phytodegradation (Alkorta et al., 2004).

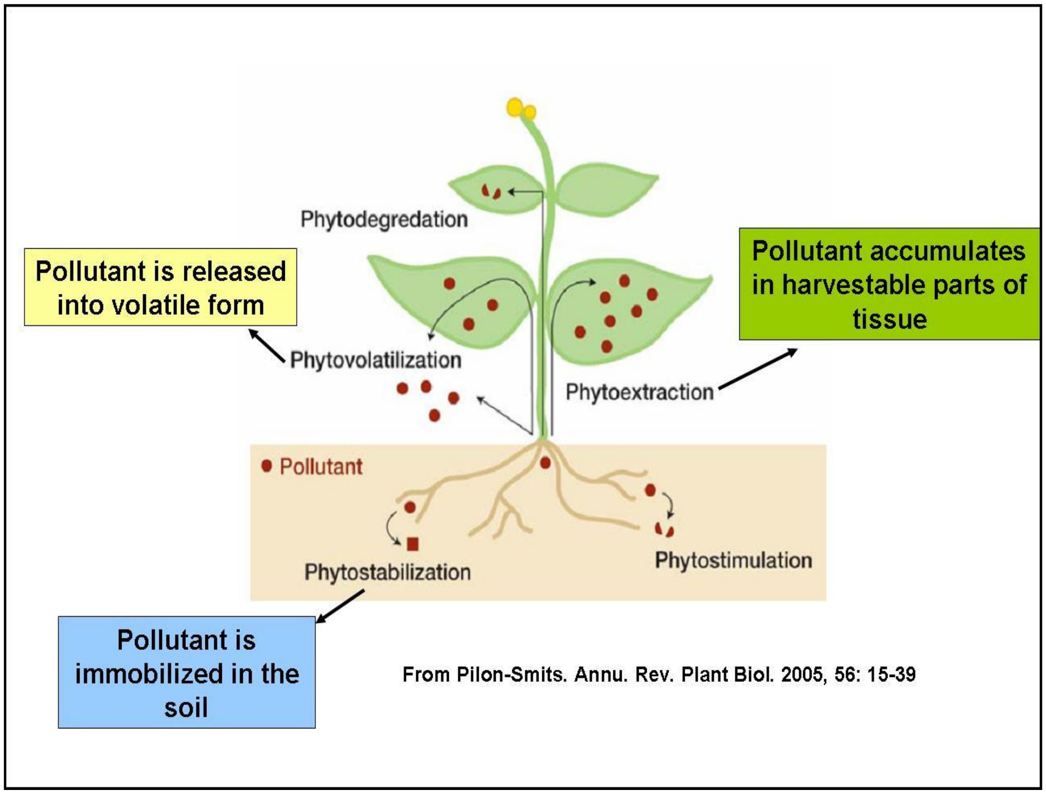


**Figure 2.1** Illustration of various phytoremediation techniques (Pereyra., 2006).

Phytoaccumulation or best known as phytoextraction is based on the ability of the plants roots to uptake contaminants from the soil or water and able to translocation the accumulation to other parts (Sekara et al., 2005; Yoon et al., 2006; Rafati et al., 2011). Significant amounts of metal from polluted soils or water can be taken up by the hyperaccumlators plants where it is able to clean up the contaminated sites (Zhuang et al., 2007).

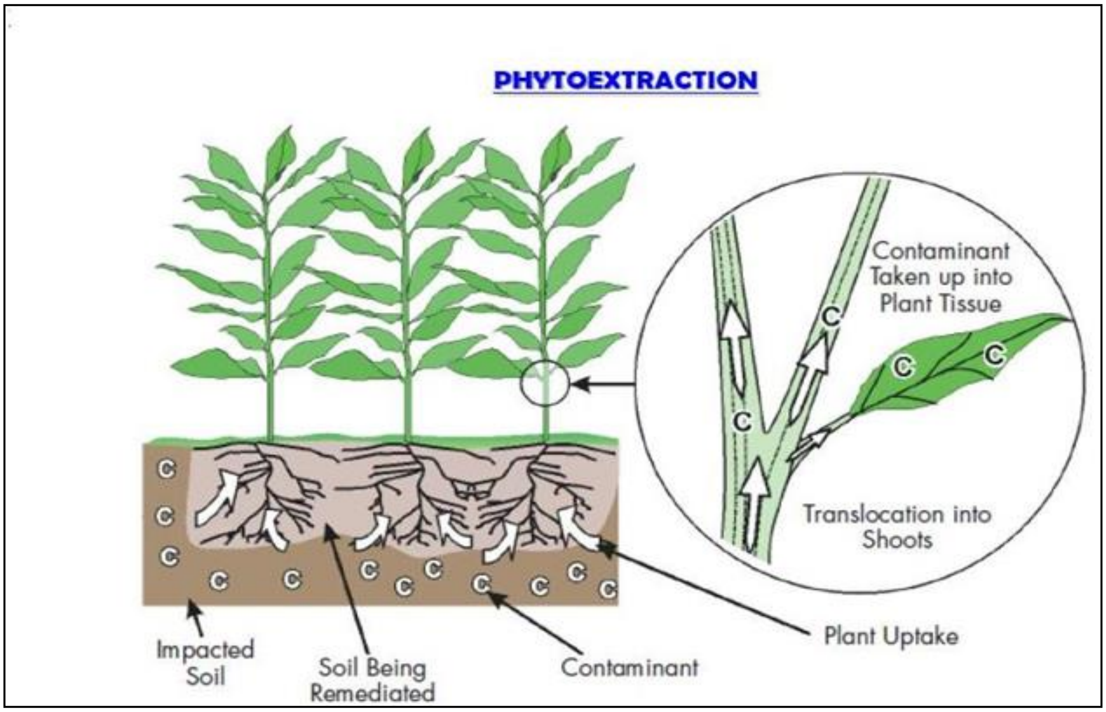


**Figure** 2.2 Illustration of phytoextraction technique (Chyn., 2011).

The efficiency of phytoextraction is measured by the capacity of metal hyperaccumulator and the production of biomass (McGrath and Zhao., 2003). If either one of the factor can be optimized, undoubtedly phytoremediation effectiveness can be accelerated. As to enhance phytoextraction process, addition of chemical reagents to the soil can increase the ability of the plants to uptake heavy metal. Hence, based on several researches, these are a few of chemical mobilizing agent that can increase capability of phytoextraction include ethylene diamine triacetic acid (EDTA), *N*-(2-hydroxyethyl)-ethylene-diaminetriacetic acid (HEDTA) and diethylene-tetramine-pentaacetate acid (DTPA) (Nowack et al., 2006 ; Turgut et al., 2004; Chen and Cutright., 2001; Huang et al., 1997).

On the other hand, phytofiltration or rhizofiltration is the capability of plants to remove pollutants from contaminated surface waters or waste waters (Mukhopadhyay and Maiti., 2010). The technique employed a combination between aquatic plants and biogeochemical processes where complex ecosystem is formed. One of the most practice is constructed wetland (Cws) in which the method stimulates natural system to counter-act against environmental degradation (Fonder and Headley., 2013; Nyquist and Greger., 2009; Sobolewski., 1999). In a simple word, the contaminants are either absorbed or adsorbed and its distribution in the underground water is lessened (Ali et al., 2013).

Phytovolotalization is the uptake of toxins from soil and the conversion into volatile form then subsequently release into the atmosphere. The method is also suited for organic pollutant and some toxic element such as Hg and Se. Unfortunately, the techniques are considerably unfavorable as the fact it can only transferred pollutant to one segment (soil) into another (atmosphere) without actually removing the contaminant. Thus, the ability of the method is the most controversial (Padmavathiamma and Li., 2007).

Rhizdegradation is the breakdown of contaminant by the aid of microorganisms in the rhizosphere (Mukhopadhyay and Maiti., 2010). Stimulation of microbial activity is stimulated by the plant up to 100 times higher in the rhizosphere. This is possible through the secretion of exudates containing carbohydrates, amino acids and flavonoids. The nutrients release from the plants provide carbon and nitrogen sources to the soil microbes and creates a nutrient rich environment in which microbial activity is stimulated (Kuiper et al., 2004; Yadav et al., 2010).

Phytodesalination is the ability to remove salts from salt-affected soils by using halophytic plants as to enable them to support normal plant growth (Manousaki and Kalogerakis., 2011; Sakai et al., 2012). Glycophytic plants is naturally less capable to cope with heavy metal compared to halophytic plants (Manousaki and Kalogerakis., 2011). According to a research, two of halophytic plants Suaeda maritima and Sesuvium portulacastrum in a period of 4 months are able to remove 504 and 474 kg of sodium chloride respectively from 1 ha of saline soil (Ravindran et al., 2007).

Phytostabilization or also known as phytoimmobilization is refer to plants that able to even out contaminants in pollutant soils (Singh., 2012). The technique reduces contaminants either through accumulation by roots or immobilization within the rhizophere (Bolan et al., 2011). Therefore, the migration of contaminant to groundwater or into the food chain can be prevented (Erakhrumen., 2007). Toxins is immobilized through the process of transpiration and roots growth that reduce leaching, creating an aerobic environment in the root zone, controlling erosion and adding an organic matter to the substrate that bind the contaminant. In other words, the accumulation of heavy metals in biota is limited and their leaching in the underground waters is minimized through the process of phytostabilization. However, the technique is only limiting contaminants movement but the elements remains in the soil (Vangronsveld et al., 2009).

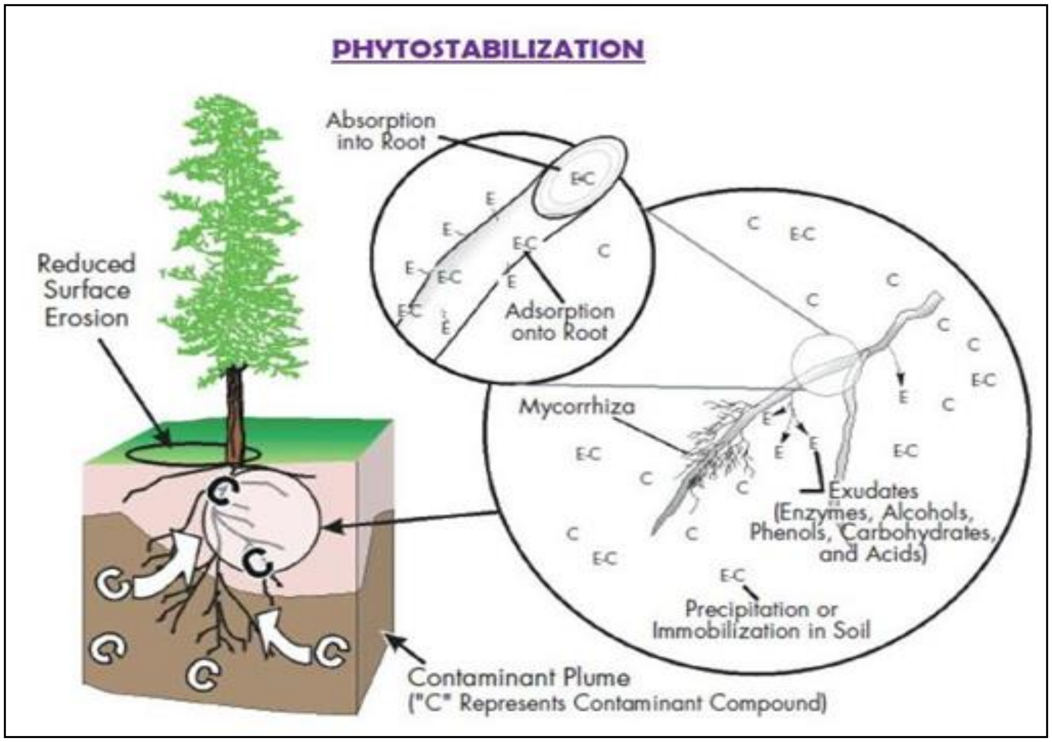


Figure 2.3 Illustration of phytostabilization technique (Chyn., 2011).

The other technique is through the aid of enzymes to degrade the organic pollutants. Phytodegradation is not dependent on rhizopheric microorganisms but through the help of enzymes such as dehalogenase and oxygenase (Vishnoi and Srivastava., 2008). The plants itself is able to produce the enzymes that catalyze and accelerate degradation thus organic pollutants are simplify into more simpler form and implemented into plants tissues to help plant growth (Chyn., 2011). Organic xenobiotic found in the contaminants environments are accumulated and is detoxified through their metabolic activities (Ali et al., 2013). However, phytodegradation abilities is limited to organic pollutants only as heavy elements are nonbiodegradable.

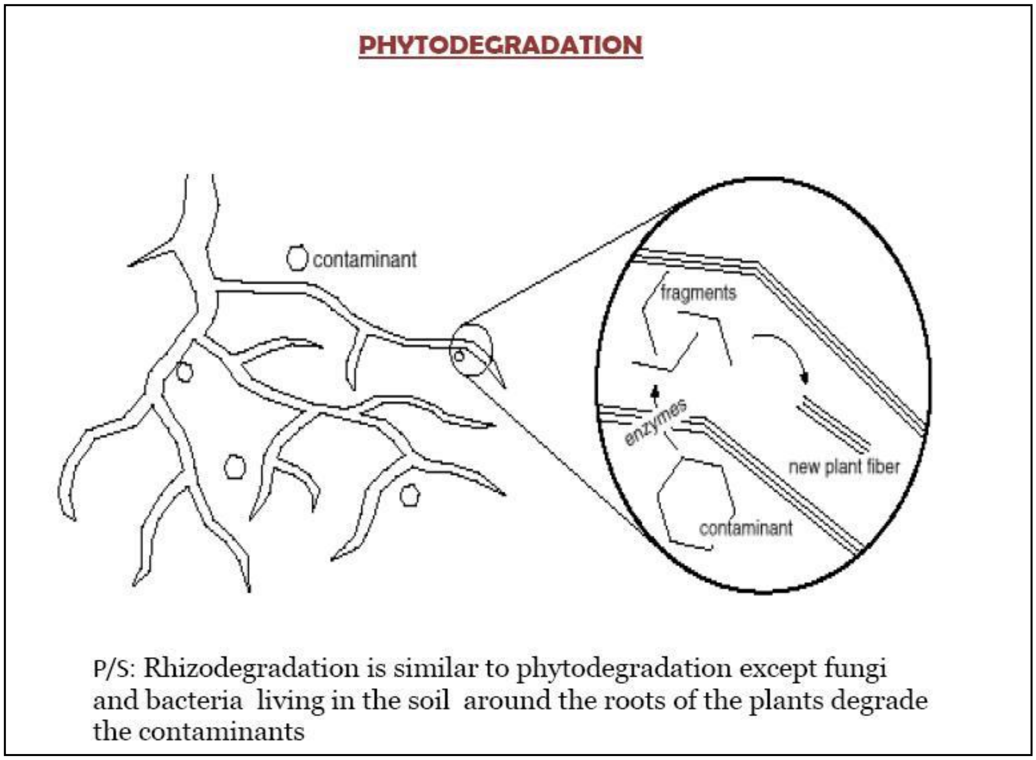


Figure 2.4 Illustration of phytodegradation technique (Chyn., 2011).

## 2.7 Phytoremediation by using Cyperus alternifolius.

Cyperus alternifolius which is also known as umbrella papyrus is a type of perennial herb plant which has strong underground root and erect aerial system, with hollow core construction and without branching (Xindi Liao et al., ,n.d.).It is a type of Cyperaceae plant species where aquatic environment such as freshwater pond, lake and river are the native habitat (Flora Fauna Web., 2017). The plant has been widely used for landscaping, fencing, paper hat, basket making and as a cover plant for protecting slope from soil erosion.

The used of umbrella grass in treating heavy metal is hardly known. However, based on a few research that has been done it shows that umbrella grass could remove about 36% COD (Chemical Oxygen Demand) from pig farm wastewater (Neural et al., 1986). Other example in treating acid mine drainage for the uptake of cadmium, copper, manganese, zinc and lead, umbrella grass and erect marsh-flower are the best-chosen plant (Tara Sadak., 2008).

## 2.8 Techniques for plants digestion

Plants should be digested before it was taken for elemental analysis such as Atomic Absorption Spectrometry (AAS) or Inductively Coupled Plasma (ICP). Based on Campbell and Plank, 1992 there are several options for plant digestion; dry ashing, wet ashing and accelerated wet digestion.

Dry ashing make used of muffle furnace by maintaining temperature between 500˚C to 550˚C in about 4 to 8 hours. According to Horwitz (1980) cited by Campbell and Plank (1992) in order to achieve complete digestion specifically for plant tissue high in carbohydrates and oil, it may require dry ashing aid. Addition of either nitric acid or hydrochloric acid is needed to preserve the sample after ashing is done. Final dilution is made to a volume as to meet range requirement of the analytical instrument.

Wet ashing used the combination of acid such as sulphuric acid ($H_2SO_4$), nitric acid ($HNO_3$), hydrogen peroxide ($H_2O_2$) and perchloric acid ($HClO_4$) (Lambert., 1976). Addition of nitric acid into dry and ground sample as a predigested step is necessary to minimize frothing and amount of organic materials. Hence, risk of explosion can be reduced if perchloric acid is employed in the digestion procedure (Ali et al., 1987). In relation to that, due to the risk of explosion the used of perchloric acid in most laboratories is rejected. Hydrogen peroxide is a strong oxidizing agent thus it is employed to boost reaction speed and complete digestion (Lambert., 1976; Campbell and Plank., 1992). Temperature is maintained between range 80˚C to 125˚ by using either hot plates or digestion blocks (Campbell and Plank., 1992).

Another organic matter destruction method is accelerated wet digestion where time is shortened as pressure and high temperature are implemented. According to White and Douthit (1985) as cited by Campbell and Plank (1992), closed or open vessel are utilized whether by using microwaves ovens or conventional hot plate. Closed vessel is better compared to the used of open vessel. This is because, loss of element is controlled in the closed vessel due to reaction rate is increased and digestion time is decreased. On the contrary, pressure container is not utilized and careful monitored is necessary to evade excess frothing and sample loss if open vessel is practiced.

In comparison, dry ashing is not suggested if the plant material have high Silica (Si) due to the end result obtained of low micronutrient values especially zinc (zn) and the method also give lower value of iron (Fe) and aluminium (Al) compared to wet digestion (Jones and Case., 1990). However, dry oxidation is commonly practiced because of the simplicity, safer and more economic procedure. This is recommended basely due to the risk of explosion and carcinogenic effect that may occurred consequences from the used of one of the digestion materials such as perchloric acid (Andrejko et al., 1983).

## 2.9 Elemental analysis to determine heavy metal concentration

In the determination of heavy elements concentration, atomic absorption spectrometry (AAS) is an analytical technique that is been adopted. Each chemical element that has been tested by AAS released amount of absorbed radiation that can be measured. Photon of light is a form of energy that is been absorbed by samples is measured. Through radiation the samples are at an excited state and produced spectra which is then read by AAS. Ultraviolet or visible light is absorbed by the atoms and transition to higher energy levels is made (Garcia and Baéz., 2012).

When light energy of a specific wavelength enters the excited state, it is then absorbed by the ground state atom, refer to the Figure 2.5 as shown below. The amount of light absorbed increases as the number of atoms in the light path increases. A quantitative determination of the amount of the analyte can be made by measuring the amount of light absorbed. The specific determination of certain elements can be measured with special light sources and careful selection of wavelength (The Perkin-Elmer Corporation., 1996).

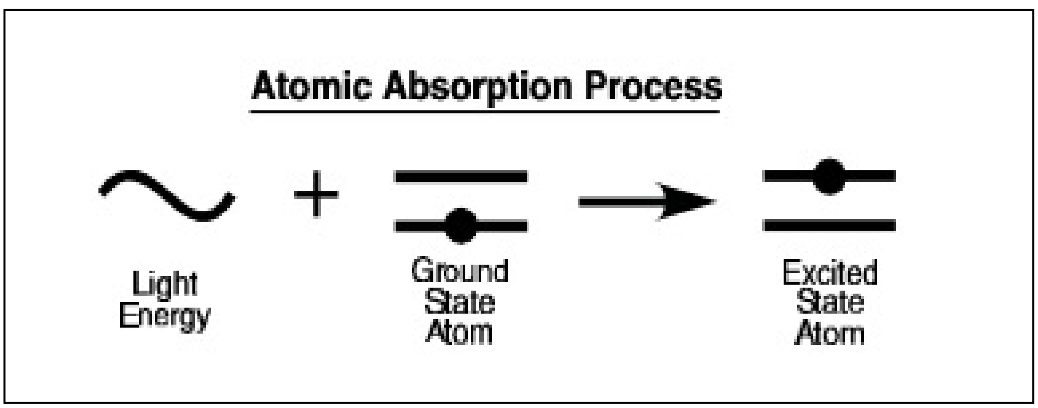


**Figure 2.5** Atomic absorption process (The Perkin-Elmer Corporation., 1996)

According to Menzies (1960), the flame is treated by which spray is injected as it were a trough of absorbing solution in the spectrophotometry and absorbance of the flame is measured. The concentration of absorbing atoms in the flame, and concentration of atoms in the dissolved materials were measured through the absorbance of the flame for light of resonance wavelength directly.

# CHAPTER 3

# METHODOLOGY

## 3.1 Introduction

The research work involved several steps. First and foremost were the preparation of type of waste water solution followed by the treatment process by cyperus alternifolius. This is the heavy metal removal process through the absorption of waste water into the cyperus alternifolius for one whole weeks. Mass before and after treatment process of each plant were taken. At day 0, day 1.day 5 and day 7, pH of different set of waste water were measured to monitor changes in the acidity level of the solution. After the 7 days' treatment, the current concentration of heavy metal was preserved by adding 1% of nitric acid until the solution lowered to below pH 2 and stored them in the refrigerators.

Plants from blank set, heavy metal set and iron sulfate after the absorption process were going through the process of organic matter destruction before they were taken for elemental analysis. Decontamination, drying and particle destruction were necessary to ensure homogenization in plant digestion. In this research, wet ashing was employed and the elemental technique used is atomic absorption spectrometry (AAS) by Perkin Elmer.

## 3.2 Work procedure in the heavy metal removal process by Cyperus alternifolius

The overall work procedure in the heavy metal removal experiment by Cyperus alternifolius is shown in the Figure 3.1 below:

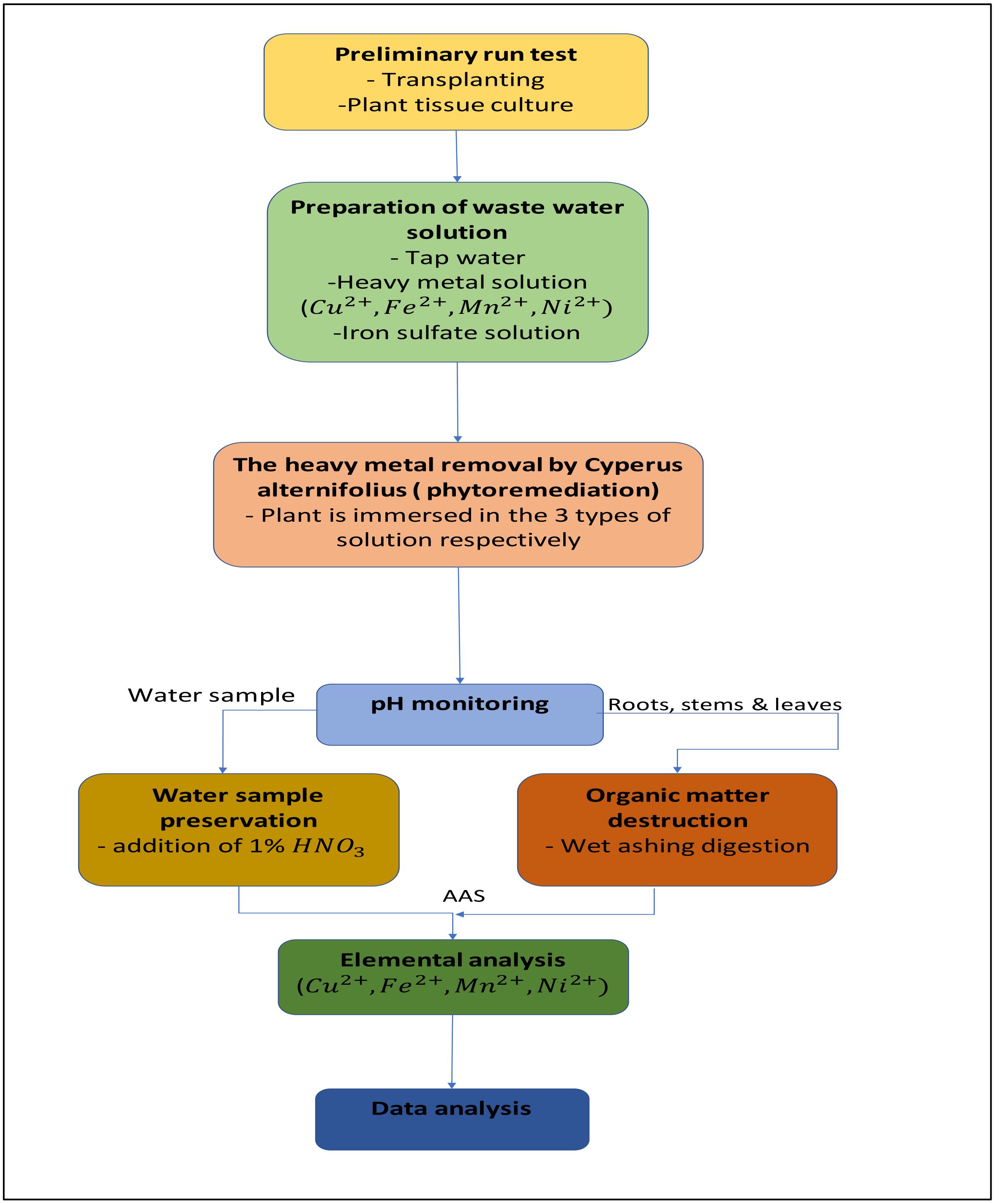


**Figure 3.1** Overall flow chart in the heavy metals removal experiment by phytoremediation using Cyperus alternifolius