PHYTOREMEDIATION OF IRON FROM RED SOIL BY *Ipomoea aquatica*

by

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DECLARATION

I declare that this thesis entitled “title of the thesis”is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : _____

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Date : _____

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PHYTOREMEDIATION OF IRON FROM RED SOIL BY *IPOMOEA AQUATIC*

ABSTRACT

The presence of excessive heavy metal in soil has gained attention from public as it will accelerate human health issues and threaten the environment. Phytoremediation technique is more conducive and preferable technique in terms of monetary and effectivity. Mining activities in Kelantan surge up the iron contamination in soil, water and air. In this study, the potential of *Ipomoea aquatica* as iron hyperaccumulator has been investigated. The phytoremediation mechanism of *Ipomoea aquatica* were analysed by screening the amount of iron (Fe) accumulated in the leaf, shoot and root of *Ipomoea aquatica* plant via X-Ray Fluorescence (XRF) technique. The independent variable in this study was the iron (Fe) concentration levels induced on red soil, ranging from 50 mg/kg, 100 mg/kg, 150 mg/kg to 200 mg/kg. After 40 days of exposure, the plants were harvested and segment accordingly for analysis. Results showed the maximum iron (Fe) accumulation (266.0 ± 4.770 mg/kg) was found on concentration level 150 mg/kg. Where the amount of iron (Fe) found in the root was 158.0 ± 6.083 mg/kg, followed by leaf (71.3 ± 2.443 mg/kg) and shoot (36.7 ± 1.572 mg/kg) [root > leaf > shoot]. There was a strong correlation between soil pH, organic matter, temperature and soil texture to the bioavailability of iron (Fe) in soil for plant uptake. Therefore, the above statements prove that *Ipomoea aquatica* which has phytoremediation characteristics could reduce the iron (Fe) contamination level in soil. Bioconcentration Factor (BCF) and Translocation Factor (TF) values also show vital relationship to the phytoremediation ability of *Ipomoea aquatica*. Based on the BCF and TF values, *Ipomoea aquatica* sh ows a great potential as iron (Fe) hyperaccumulator in applying phytoextraction and phytostabilization mechanisms.

FITOREMEDIASI TANAH MERAH YANG TERCEMAR DENGAN BESI MELALUI *IPOMOEA AQUATICA*

ABSTRAK

Kewujudan pencemaran logam berat yang berlebihan di dalam tanah telah menarik perhatian orang ramai kerana logam bukan sahaja boleh menjejaskan kesihatan manusia, serta boleh menjadi ancaman yang berterusan kepada alam sekitar. Sebagai teknik alternatif yang mempunyai kos rawatan yang munasabah, fitoremediasi telah menjadi pilihan utama. Aktiviti perlombongan di sekitar Kelantan telah mencemarkan tanah, sungai dan udara dengan kandungan besi (Fe) yang tinggi. Dalam kajian ini, potensi *Ipomoea aquatica* sebagai agen fitoremediasi telah dikaji. Mekanisma fitoremediasi *Ipomoea aquatica* telah dianalisis untuk mengkaji jumlah amaun besi (Fe) yang terkumpul di dalam daun, batang dan akar dengan menggunakan teknik XRF. Tahap konsentrasi besi (Fe) di dalam tanah merah, yang terdiri daripada konsentrasi 50 mg / kg, 100 mg / kg, 150 mg / kg dan 200 mg / kg adalah pemboleh ubah bergerak balas dalam kajian. Selepas 40 hari pendedahan, *Ipomoea aquatica* akan dituai dan diasingkan mengikut bahagian tumbuhan bagi tujuan analisis. Hasil kajian menunjukkan pengumpulan amaun besi (Fe) yang maksima (266.0 ± 4.770 mg / kg) boleh didapati pada tahap konsentrasi 150 mg/kg. Manakala akar mempunyai amaun besi 158.0 ± 6.083 mg / kg, diikuti dengan daun (71.3 ± 2.443 mg / kg) dan batang (36.7 ± 1.572 mg / kg) [akar> daun> batang]. Pengujudan korelasi yang kuat antara pH tanah, bahan organik, suhu dan tekstur tanah dengan bioavailabiliti besi (Fe) di dalam tanah bagi penyerapan tumbuhan. Oleh demikian, kenyataan di atas menyokong *Ipomoea aquatica* yang mempunyai ciri-ciri fitoremediasi boleh digunakan dalam proses dekontaminasi dan pengurusan tanah yang tercemar dengan pencemaran besi terutamanya dengan menggunakan *Ipomoea aquatica* yang mempunyai ciri-ciri fitoremediasi. Nilai Biokepekatan Faktor (BCF) dan Translokasi Faktor (TF) juga menunjukkan hubungan yang saling berkait kepada keupayaan fitoremediasi daripada *Ipomoea aquatica*. Berdasarkan nilai-nilai BCF dan TF, *Ipomoea aquatica* sesuai untuk dijadikan sebagai agen fitoremediasi bagi besi dengan menggunakan mekanisma fitostabilisasi dan fitoekstraksi.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BCF	Bioconcentration Factor
CEC	Cation Exchange Capacity
CH_3COOH	Acetic Acid
C_2H_5OH	Ethanol
cm	Centimetre
$cmol_c/kg$	Centimoles per Kilogram
CRD	Completely Randomized Design
dH_2O	Distilled Water
FAO	Food and Agriculture Organization
$FeSO_4$	Iron Sulphate
KCl	Potassium Chloride
kg	Kilogram
mg/kg	Milligram per Kilogram
mg/L	Milligram per Litre
NH_4OH_2	Ammonium Hydroxide
TF	Translocation Factor
WHO	World Health Organization
XRF	X-Ray Fluorescence

LIST OF SYMBOLS

Fe	Iron
%	Percentage
x	Multiply
/	Divide
^{o}C	Temperature (degree Celsius)
=	Equal
>	Greater than
<	Less than
R^2	Linear Regression
r	Pearson Correlation Coefficient

CHAPTER 1

INTRODUCTION

1.1 Research Background

For the last fifty years, the global population is increased by two folded (Ward & Singh, 2004). By 2020, the human population is expected to reach 8 billion. With this increasing human population, the biosphere has received various toxic chemicals and biological substances that exceeded the threshold levels in the form of contaminants (Ali *et al*., 2013). Globalization boost up the contaminants consist of heavy metals, organic compounds and pesticides have been leached to soil in massive quantity, resulted in diverse health difficulties.

Remediation technique has earned the trust from public as a method to expel heavy metals in our biosphere (Salido *et al*., 2003). Phytoremediation as one of the remediation methods to excrete toxic heavy metals from soil. Mohanty *et al*., (2010) justified that this alternative can be carried out by both *in situ* and *ex situ* methods. Typically, green plants with great roots depth, growing rate and wide growing tolerance served as vital criteria in phytoremediation to expel heavy metals from soil by transforming and sequestrating heavy metals into harmless substance (Mudgal *et al*., 2010; Paz-Alberto & Sigua, 2013).

Series of studies carry out by Abioye *et al*., (2013) concluded that, hyperaccumulators families that have been broadly studied includes Fabaceae, Flacourtiaceae, Asteraceae, Brassicaceae and Violaceae. Besides that, *Viola calaminaria* and *Thlaspi caerulescens* plants are species under the families. These families consist of incredibly high number of hyperaccumulators that can compromise

and tolerate with high concentration of toxic metal elements and being used for their environmental friendly and cost effective remediation strategies.

1.2 Problem Statement

The excessive presence of heavy metals, particularly iron (Fe) in red soil has been studied extensively. Their removal from soil is necessary for the sustaining of soil and human health is great effect to the soil ecosystem. Mostly the method of removal of heavy metals is carried out by physical and chemical methods. Whereas the need of time is to adopt a method based on green technology. Thereby, the present study can eventually help in removal of iron from red soil using the concept of phytoremediation.

1.3 Objectives

a) To evaluate the accumulation of iron (Fe) in the roots, stems and leaves of *I. aquatica*.
b) To determine the ability of *I. aquatica* to remove the Fe from red soil.

1.4 Hypothesis

It is hypothesized that *Ipomoea aquatica* will be a good source for removing Fe from contaminated red soil.

1.5 Significance of Study

The findings of this study will contribute to the Fe removal activities in *I. aquatica* by green remediation technology, phytoremediation. Over this research, people will get to know the importance phytoremediation as a natural process in cleaning up and stabilization of contaminated soils.

CHAPTER 2

LITERATUE REVIEW

2.1 Phytoremediation

Phytoremediation itself is a set of organic technologies with low budget in both capital and operation, which predominantly operate with various plant species with an aim to 'clean' up the contaminated site due to uncontrolled leaching of heavy metals. Some plants have natural resistances or characteristics to resist high amount of heavy metal contaminations through tolerance and avoidance (Abioye *et al*., 2013). Nowadays, the soil environment is a huge concern due to excessive chemicals and heavy metals which undoubtedly contaminate the environment complication to fulfil the demand of human in producing up-to-date technologies (Abou-Shanab, 2011). The soil contamination is gradually beyond the environmental threshold level of rejuvenating by natural processes. Hence, phytoremediation techniques able to minimize this dispute effectively.

Besides phytoremediation, micro-organism-based remediation technique like bioremediation is also an attractive and novel technology as there will be an integrative way that mainly adopts biological systems to break down hydrocarbons, expel and degrade pollutants from the soil and water (Ward & Singh, 2004; Jadia & Fulekar, 2008). Consequently, bioremediation utilizing both plants and microorganisms that considered as a safe and can be applied over a large surface area plus it is environmental-friendly and inexpensive if compared to non-biological processes such as physical and chemical remediation (Singh *et al*., 2009). However, bioremediation dealing more with microbiology by using specialized microbial strains that requires

sophisticated technologies and high expertise to restore the healthiness of polluted soil rather than physical plant like phytoremediation. Therefore, phytoremediation is easier to handle and control compared to bioremediation.

Likewise, soil remediation techniques also involved physical and chemical remediation. Physical remediation includes soil vapour extraction and soil washing processes. Soil vapour extraction is a process where a passage from the soil is necessary to extract the contaminants out. These processes will not only alter the harmony ecosystem but also will destroy the habitat of living organism that live on that area, unlike phytoremediation process which has the capability to remain the natural condition of the place. Meantime, injection of active oxidants like potassium permanganate, ozone gas or hydrogen peroxide into contaminated ground just to detoxify pollutants are very risky and demand high level of monitoring procedures. Accurate amount of injection of active oxidants is mandatory to prevent oxidative stress that causing disparity of free radicals and neutralization process by antioxidants for the plants in the soil (Pagliarani *et al*., 2012).

In short, phytoremediation uses the plants to sequester and detoxify the contaminants from the polluted soils (Johnson *et al*., 2011). Plenty of plants carry natural resistances within to endure the high heavy metal contaminations through tolerance and avoidance (Vamerali *et al*., 2013). Abou-Shanab (2011) mentioned that phytoremediation technology is still eagerly being tested and extensively viewed as ecologically responsible substituent to the environmental-friendly remediation methods. Growing of green plants can eventually serve as carbon sink to store surrounding carbon dioxide that primarily causes global warming in the world. The association of plant-microbial is the main reason in the succession of removing toxic metal elements from soil medium. Moreover, combination of air purifier and soil

cleaner is the principal rationale to pinpoint phytoremediation is the most appropriate technique that balancing the economy, environment and social benefits. Figure 2.1 below shows the mechanisms of phytoremediation in sequester the heavy metals from soil to the air.

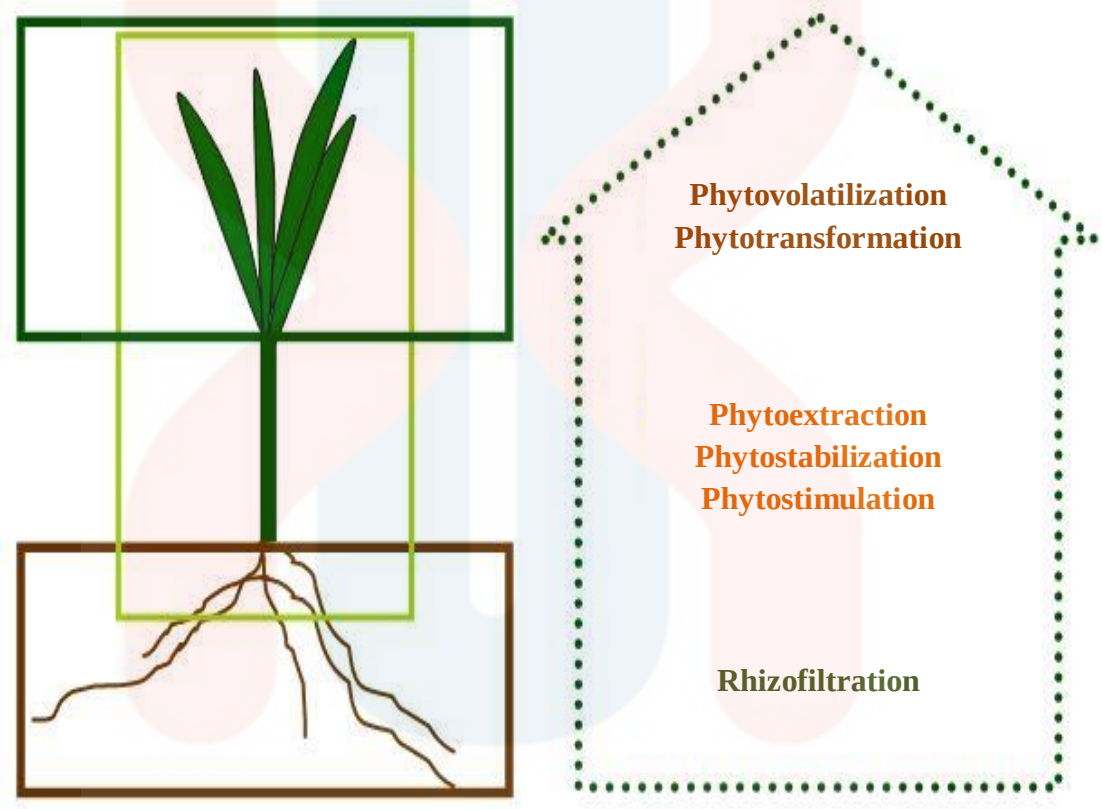

Figure 2.1: The locations of phytoremediation methods in plant. (Source: Yang & Liu, 2011)

2.1.1 Rhizofiltration

This treatment is like the concept of phytoextraction whereas the medium of remediation is more to contaminated groundwater instead of polluted soils (Jadia & Fulekar, 2008; Johnson *et al.*, 2011). This because in this first method, aquatic plants (submerged or floating) is used to absorb, concentrate and remove hazardous substances like heavy metals, trace elements and other radionuclides by their roots. A plant with large root system is more prefer like a plant that capable in producing up to 1.5 kg of dry weight/month per m^2 of water surface will be good enough in this

situation (Vamerali *et al*., 2013). Typically, plants used for rhizofiltration treatment are planted accustom to pollutant in advance but not planted directly to the *in situ* because of the disposal problem.

2.1.2 Phytostimulation

Phytostimulation is based on the secretion of compounds by plants in root exudates which enhance the growth of microbial activities to breakdown the organic contaminants available in the soil by soil dwelling microbes (Vamerali *et al*., 2013). It carries almost the same meaning with bioremediation process because particular soil swelling microbes will digest organic pollutants like fuels and solvents and produce environmental friendly products. Plus, the secreted enzymes can speed up the existence of soil microflora in the rhizosphere by up to 4 orders of magnitude compared to the loose soil and surrounding bulk soil respectively (Johnson *et al*., 2011). Therefore, phytostimulation capable in cultivating new microorganisms in the soil to introduce specific organisms to the growing of rhizosphere.

2.1.3 Phytostabilization

This process involves certain plants to immobilize the contaminants under the soil, sludges and sediment through adsorption and accumulation by the plant roots. These can be done by decreasing the solubility and bioavailability of contaminants to the food chain. Thus, this method prevented erosion, leaching and runoff, thereby preventing them from migrating into food chain and underground. Meanwhile, high concentrations of metal contaminants enable this process to rejuvenate plant community on sites that have been bared earlier. Types of plants that ideal for phytostabilization should be able to develop a comprehensive root system that will

supply a favourable soil colonization, ideal in immobilizing the contaminants in the rhizophere and possess tolerance to various level of contaminant metals (Jadia & Fulekar, 2008; Johnson *et al*., 2011; Etim, 2012).

2.1.4 Phytoextraction

A sub process of phytoremediation in which plant roots take up dangerous heavy metals from soil or water and translocate them to above-ground plant tissues (Etim, 2012). Phytoextraction includes the cultivation of higher plants that capable in concentrating the soil pollutants so that it is safe to be harvested at the end of the growth stage. Hyperaccumulators are the species which enable in accumulating metals at levels 100-fold greater than those typically measured in non-accumulator plants species. The ideal of phytoremediators being used in phytoremediation must have internal capacity to tolerate and possess multiple attributes such as high growing mass, easiness in cultivation, extensively branched plant roots, high tolerance in growing condition and unpleasant to herbivores. Hence, the escape of accumulated metals to ecosystem food chain can be avoided (Vamerali *et al*., 2013).

2.1.5 Phytotransformation

This method is also known as phytodegradation that refers to uplift of contaminants with the consequent breakdown of organic contaminants by both internal and external metabolic processes induced by the plant (Johnson *et al*., 2011; Etim, 2012). Subsequently the direct uptake of substances that will be metabolized into H_2O and CO_2 by enzyme complexes involved in the pant metabolic cycles (Vamerali *et al*., 2013). This is because the small pollutants molecules can be used as metabolites by the plant as it grows and thus associated with the plant tissues. In a field report posted

by Mayer and Staples (2002), laccases are used for the degradation of a variety of persistent environmental contaminants including bisphenol A, synthetic dyes and alkenes.

2.1.6 Phytovolatilization

Phytovolatilization is the process where the plant uptake and transpire contaminants that are water soluble and release them back into atmosphere (Etim, 2012). Phytovolatilization is an alternative for phytoextraction, with just the contaminant is predominantly focuses in above and underground plant. The contaminants may become modified along the way as they will slowly evaporate into atmosphere along the path from roots to leaves. The advantage of this process is that the magnitude of contaminants can be greatly reduce before releasing it to our biosphere. Phytovolatilization is really convincing for mercury, Hg, chlorinated solvents, and selenium, Se in which contaminants are converted into a volatile form for release and dilution into atmosphere (Jadia & Fulekar, 2008; Vamerali *et al*., 2013).

2.2 Heavy Metals

Earth's crust is the main source of many heavy metals. The chemistry and type of parent materials of heavy metals are beneficial to organism in traces. However, anthropogenic activities may most probably increase the heavy metals concentration to exceed the threshold levels (Mirsal, 2008). These activities caused high accumulation of heavy metals in soil that will bring adverse effects to the health ecosystem of living organisms. Besides that, excessive heavy metals exposure also causes oxidative stress to plants (Ruley *et al*., 2006). Laghlimi *et al*., 2015 proclaimed

that most of the anthropogenic activities resulted massive accumulation of heavy metals like Cadmium (Cd), Mercury (Hg), Selenium (Sn) and Lead (Pb) in the soil.

Other than that, we also can find iron (Fe) in the Earth's crust which positioned 2nd after aluminium and it is one of the metals that known in antiquity and great age. The abundance of iron solubility and availability in soil is dependent on several factors like soil pH, soil texture and redox status of Fe in the soil (Radanović & Antić-Mladenović, 2012). Predominantly, physical conditions in soil such as acidic soil will reduce the soil conditions to detain the solution of iron compounds as acidic soil will eventually enhance the solubility of inorganic iron compounds than ordinary neutral soil. Bad soil infiltration rate will reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) due to oxygen deficient condition in waterlogged soil and make the Fe ions to be more soluble in the soil (Bhat, 2016).

In Kelantan, the soil is made up of sandy red clay that contains high content of Fe concentration. Therefore, in Kelantan state there are plenty of mining factories along Ayer Lanas to Tanah Merah to extract the iron from the soil. Despite that, anthropogenic activities like mining activities often result in the formation of acid mine drainage (AMD) that cause chemical reaction with bacteria in both water and air (EPA, 2003). Senese (2015) proves that iron has very high reactivity with the oxygen and water in the air to form hydrated iron (III) oxides which also known as rust that is red colour when dehydrated.

$$Fe(s) \rightleftharpoons Fe^{2+}(aq) + 2\ e^{-}\ \text{(oxidation of Fe by droplet of water)}$$

$$Fe^{2+}(aq) + 4\ H^{+}(aq) + O_2(aq) \rightleftharpoons 4\ Fe^{3+}(aq) + 2\ H_2O(l)$$

$$Fe^{3+}(aq) + 3\ OH^{-}(aq) \rightleftharpoons Fe(OH)_3(s)$$

In conjunction with that, Table 2.1 below shows some hyperaccumulating plants species that corresponding with various heavy metals and amount of accumulation.

Table 2.1: Different types of heavy metals with respective hyperaccumulating plants.

Heavy Metal Pollutants	**Plant Species**	**Level of Accumulation, mg/kg**
Arsenic, Ar	*Pteris vittata*	27, 000 (Wang *et al*., 2002)
Cadmium, Cd	*Thlaspi caerulescens*	1, 800 (Macek *et al*., 2004)
Copper, Cu	*Ipomoea alpina*	12, 300 (Macek *et al*., 2004)
Iron, Fe	*Centella asiatica*	1, 640 (Bhat *et al.*, 2016)
Lead, Pb	*Thlaspi rotundifolium*	8, 200 (Macek *et al*., 2004)
Nickel, Ni	*Psychotria douarrei*	47, 500 (Macek *et al*., 2004)
Selenium, Se	*Stanleya pinnata*	> 1, 000 (Cappa, 2014)
Thallium, Tl	*Iberis intermedia*	13, 430 (Scheckel *et al*., 2004)
Uranium, U	*Helianthus annuus L.*	24.6 % (Lábusová, 2013)
Zinc, Zn	*Thlaspi caerulescens*	51, 600 (Macek *et al*., 2004)

2.2.1 Factors affecting the iron heavy metals phytoavailability in soils

Heavy metals occur originally in soil and they can be split into two major groups based on their physiological activities. They are essential heavy metals and non-essential heavy metals which directly proportional to their concentrations and plants will only react to the fraction of heavy metals that is 'phytoavailable' to them (Rascio & Izzo, 2011). In addition, heavy metals positioned at top list among dominant contaminants of green leafy vegetables (Gupta *et al*., 2013). The bioavailability of heavy metals is a vital dependent variable for the heavy metals uptake by plants root. Yet, there are some direct and indirect factors that will retard the effectiveness of plants to remediate heavy metals besides metal phytoavailability (Chang *et al*., 2014).

a) Soil pH

Soil pH has strong negatively correlated relationship with the metals in plant and plays a role in governing heavy metals uptake by plants (Jung, 2008). Slight alkaline condition (> pH 7) will eventually lowering down the mobility of heavy metals in soil whereas low pH makes metal bioavailability surges to compete with H^+ ions without forming hydroxyl-complexes (Laghlimi *et al*., 2015). Furthermore, when the pH is low H_30^+ will replace the ions as a result of organic acids and H^+ competition, which then mobilize heavy metal ions from soils and increase the probability of getting absorb by the plants (Li *et al*., 2015).

b) Temperature

Temperature has been found to be one of the factors on metal speciation, as chiefly chemical reaction rates are hypersensitive to temperature changes. Every increment of 10 ^oC is sufficient to double up the biochemical process rates and able to act as a kind of driving force in earth surface conditions for a kinetically slow reaction thus boost up the tendency for both efflux and influx rate of metals. In addition, the temperature also will affect the rate of evapotranspiration and absorption rate to reach a balanced output and input state in the plants (Sherene, 2010).

c) Organic matters

Organic matter serves a vital role for the plant in the absorbing phase to control the behaviour of heavy metals in the soil. Formation of metal-organic complexation from organic matter that tend to weaken the mobility of metals and thus decrease the toxicity (Laghlimi *et al*., 2015). Organic ligands in plant roots like phytosiderophores

control the metal solubility in the soil (EPA, 2003). According to Sherene (2010), humic acid by organic matter is responsible in the solubility of heavy metals and directly proportional to the solubility of heavy metals. Soluble heavy metals are hard to remove, thus decrease the adsorption capability of heavy metal by plants.

d) Root zone

Laghlimi and his colleagues (2015), certified that plant root plays a major role in phytoremediation activities as rhizosphere can affect heavy metal phytoavailability by altering the soil properties. Root is the closest part of the plant to direct contact with the heavy metal in soil. Thus, roots will tend to absorb and store most of the heavy metal followed by leaves and stems (Li *et al*., 2015). Furthermore, the root exudates like acetate, succinate, malate, isocitrate, citrate, sugar and amino acids produced by decaying root will associate directly in the metal uptake as positive correlation between the plant root activities and the metal solubility (Macek *et al*., 2004).

e) Plant species

Many plant species which has the ability to grow fast and high biomass are being investigated for their usefulness for phytoextraction purposes (Pinto *et al*., 2015). There are studies reported that submerged plant species can accumulate higher amount of heavy metals like Cd, Cu and Zn compared to emerged plant species (Li *et al*., 2015). This is mainly due to the roots of the emerged plants might be degraded and cannot perform an ideal mechanism in phytoremediation process. Plant species that has good root posture and large root surface for metals uptake will increase the chemistry of rhizosphere to metal uptake (Laghlimi *et al*., 2015).

f) Soil texture

The classification of soil into three partitions, which are sand, slit and clay fractions is the best expression on metal solubility in soils. As the texture reflects the size of the particles distribution of the soil, clay has the smallest particle size among other 2 fractions. In a same volume, soil with fine particles (< 100µm) have more exposed surface areas if compared to bigger and coarser particles like sand (Laghlimi *et al*., 2015). Due to the total exposed surface areas, clay has the highest amount of heavy metals due to the high adsorption rate with the presence of sulphides, organic matter and clay minerals (Rieuwerts *et al*., 1998).

2.3 Hyperaccumulators

Hyperaccumulator describes the internal ability of plants to accumulate and store massive amount of toxic heavy metals from medium like soil and water. Hyperaccumulators have the tendency to resist the phytotoxic effect after exposed to high amount of heavy metals or toxic substances. Phytotoxicity is a type of toxic effect that influences the plant growth. Phytotoxic effects may give stress to the plant by altering the percentage of successful germination, length of shoots and roots of a plant (Rascio & Izzo, 2011). Datta *et al*., 2011 revealed that hyperaccumulator plants will free from such effects due to the extraordinary abilities to sequester toxic heavy metal content in the soil.

Heavy metals are normally stored in soil and absorbed by plant root through numerous physiological processes and defence mechanisms starting from roots before entering the plant and are detoxified or sequestered into vacuoles. Studies carried out by Rascio and Izzo, (2011) stated that hyperaccumulators are primitive to metalliferous

soils but the main procedures in hyperaccumulation is still depending on genes common to both hyperaccumulator and non-hyperaccumulators. The potential of hyperaccumulator capabilities of a plant has drawn attention from public and researches for practical operations to develop green and eco-friendly soil remediation technology, literally phytoremediation. There are three hallmarks that can be significantly differentiate between hyperaccumulator plants and non-hyperaccumulator plant species. The plant transport system basically through heavy metal uptake, heavy metal segregation and root-to-shoot translocation.

Heavy metal uptakes by specific transporters and genes are one of the main hallmarks in differentiation both species. For zinc hyperaccumulator plant *T. caerulescens*, the species contain Zinc-regulated transporter Iron-regulated transporter Proteins (ZIP) to help in transporting the heavy metals from contaminated soil (Manara, 2012). Besides that, the efficiency of translocating heavy metals from soil followed by chelation and storing is a huge concern in hyperaccumulating plants. Metal-binding ligands like free histidine (His) which made up of enormous organic molecules occur in plant roots are crucial in translocation functions. Lastly, segregation or sequestration is the key factor of hyperaccumuators in preventing the phytotoxic effect in plant so that the plant can remediate continuously (Rascio & Izzo, 2011).

2.4 *Ipomoea aquatica* (water spinach)

2.4.1 Description of plant

I. aquatica is one of some members under Convolvulaceae family which has the same genus as *Ipomoea batatas* (sweet potato). Besides Convolvulaceae family, there are a few aquatic plant families such as Pontederiaceae, Araceae, Lemnaceae, Scrophulariaceae, Hydrocharitaceae and Nelumbonaceae also have

hyperaccumulating potential (Das *et al*., 2013). In this Convolvulaceae family of flowering plants, most of them are twining shrubs or herbs comprising about 60 genera along with 1,600 species. Plus, this family is widespread in both temperate and tropical areas like New Zealand and Malaysia respectively.

Figure 2.2: Flower of *I. aquatica.*

I. aquatica is an herbaceous semi-aquatic perennial plant and favourable growing by using both hypotonic and terrestrial methods (Manvar & Desai, 2013). Figure 2.3 shows *I. aquatica* has narrow leaves and white flowers when reaches

maturity stage and prefers damp conditions and hence requires large amount of irrigation. When the plant reaches maturity stage, the hollow stems can reach up to 30.0 cm or more and the length and width of the leaves are around 10.0 cm and 5.0 cm respectively which can be seen in Figure 2.4. The flowers of *I. aquatica* are trumpet-shaped with less than 5.0 cm in diameter and white colour with a purplish colour at the centre as shown in Figure 2.2 below. Meanwhile, the succulent foliage is light green in colour followed by a four-seeded pod which look almost similar as sweet potato plants (Stephens, 2015).

Figure 2.3: Narrow leaves of *I. aquatica*. (Source: Kitsteiner, 2014)

Figure 2.4: Matured hollow stem of *I. aquatica*. (Source: Kitsteiner, 2014)

2.4.2 Common names

I. aquatica is the oldest preferred scientific name among others since 1775. Swamp morning-glory is also another preferred common name. When comes to international common names, *I. aquatica* have names like water spinach (English), *kongxin cai* (Mandarin), *batata acuática* (Spanish), patate aquatique (French), *you-sai* (Japanese) and lastly *batata aquática* (Portuguese). Table 2 below shows other international names for *I. aquatica* plant.

Table 2.2: Various local names in different countries.

Countries	**Local Names**
Germany	*trichterwinde*
India	*vellai kerai, karmi, nali nari*
Malaysia	*kangkong, kangkung*
Peru	*camotillo*
Philippines	*balangog, cancong, tangkong*
Sudan	*argala*
Vietnam	*wau muong*

2.4.3 Traditional uses of plant

The plant has many uses like serves as foods and medicines for living organisms. The young and fresh stem and leaves are cooked with oil and eaten in various dishes as culinary purposes. Young succulent tips are preferred mainly is because older stems will become fibrous and not tender to be eaten (Stephens, 2015). On the other hand, *I. aquatica* buds can used as plant material that can effectively deal with skin diseases such as athletes foot and ringworm. Boiled *I. aquatica* juice can prevent constipation and treat fever due to the fibres within. *I. aquatica* is a very useful traditional medicinal plant that contains wide range of nutrients that able to cure diabetes or cancer.

2.5 Soil

Soil shapes human history. In the early Chinese culture, which began to develop 6,000 to 7,000 years ago on the big flood plains of the Yellow River. Soil can be formed via physical, biological and biological weathering agents (Mirsal, 2008). Those agents are wind, climate, biota, running water, parent material, living organisms and temperature change. The processes of parent materials of soil due to biological weathering agents including rock leaching, modifying and recycling are vital to develop soil to become an organic constituent and non-renewable natural mineral.

The combination of soil from various organic and inorganic matter to serves as a physical support on earth and required much intense land management blueprint to preserve the best quality soil. The creation of soil consists of both organic and inorganic constituents in deviate phases of system. Individual soil particles were known as soil separates. Typically, soil is a natural medium for most of the terrestrial

living organism and providing them a perennial source of organic matter (Dorian, 2012). Therefore, soil is a living object that gives visible support to living organism.

Plant root system needed the physical support of a healthy soil to sustain the anchorage posture of the plant. In turn, a well-functioned soil will make sure the circulation of water and air in a correct manner to prevent any clogging or blockage inside the soil. Recently, the soil has been polluted by leached heavy metals from urbanization and industrial revolution. Awokunmi *et al*., (2010) described that the criticalness of toxic heavy metals pollution caused physical deterioration incidents announced globally including activities like mining, chemical manufacturing, nuclear and other industries.

2.5.1 Red soil

The mining activities in Kelantan area indicates that the state is enriched subsoil dominated by minerals like kaolinite, iron oxides and quartz. These are the parent materials of iron that contribute to acidic red soils which formed from gradual weathering and leaching activities. The natural colour of oxidized iron is red in colour, this can be indicating that the red soil in Kelantan is contaminated with massive amount of iron as iron colours the world red. The colour of subsoil can be examined physically by using Munsell colour system and very vital to understand the soil condition. Drainage condition, degree of oxidation and organic matter content are three main partitions that used to analyse the colour of subsoil (Dorian, 2012).

Ultisol is one of the twelve soil orders and usually red or yellow in colour disclose with highly weathered soil and oxidation of iron and aluminium. Utisol is a typical acidic soil. Well, the relationship between subsoil and aeration of soil is directly

proportional. Red or brown colour of subsoil indicates good drainage yet yellow and grey colour of subsoil represent moderate and poor drainage respectively (Dorian, 2012). Result shown by Prasetyo and the team, (2001) apprised that Ultisol contains low organic matter content, low base saturation and perform acidic reaction. Therefore, red soils serve as a root on soil fertility and stability due to the existence of iron oxides in the soil medium (Trakoonyingcharoen *et al*., 2006).

CHAPTER 3

MATERIAL AND METHODS

3.1 Material

Seeds of *I. aquatica* were bought from Jeli Town, Jeli, Kelantan, Malaysia and used in this research experiment.

3.2 Chemicals

Iron sulphate ($FeSO_4 \cdot 7H_2O$), glacial acetic acid (CH_3COOH), ammonium hydroxide (NH_4OH_2), ethanol (C_2H_5OH) and potassium chloride (KCl) obtain from HmbG Chemicals.

3.3 Instrumentation and apparatus

Black polybags, mask, glove, knife, tissue paper, aluminium foil, oven (Binder), Bunsen burner, Whatman No. 4 filter paper, crucible with cap, Buchner funnel, Volumetric flask, Erlenmeyer flask, mortar & pestle, test tube with screwed cap, retort stand clamp screw, test tube rack, beakers, plant plug trays, electronic balance, 1 Stage vacuum pump Model VE135N, Direct Soil pH meter (Hanna HI 99121 Romania), vortex mixture (VELP Scientifica) and Energy Dispersion X-Ray Fluorescence (XRF) S2 Ranger were used.

3.4 Plant sample preparation

The seeds of *I. aquatica* were washed under running distilled water to clean up the physical impurities stick on the seeds. Soon after, seeds were sown on cotton wool

in plant plug trays and irrigated with distilled water at 25-35 ^{o}C for 14 days (Sewalem *et al*., 2014). Two-week-old seedlings of similar growth (10.0 cm shoot and 5.0 cm root) will then transferred to black polybags filled with red soil containing five different concentration levels of $FeSO_4$ solution, 0 mg/kg (control), 50 mg/kg, 100 mg/kg, 150 mg/kg and 200 mg/kg and irrigate with tap water daily (Ruley *et al*., 2006).

After 40 days, the plants were gently removed from the black polybags and washed thoroughly with distilled water. After drying the plants with filter papers, plant samples were sorted into roots, stems and leaves respectively. Then all three parts were put into 70 ^{o}C oven for two days. The dried parts of plant were then evaluated on Energy Dispersion X-Ray Fluorescence (EDXRF).

3.5 Water sample preparation

In this experiment, tap water from Universiti Malaysia Kelantan (UMK) campus Jeli lab BAP 1.1 were used to irrigate the plants for 40 days continuously. Therefore, tap water from BAP was collected and undergone chemical analysis by XRF to determine the concentration of Fe existing in the tap water. Meanwhile, the water was tested on YSI Multiparameter to determine the temperature, pH, Dissolved Oxygen (DO), Electrical Conductivity (EC) and Total Dissolved Solids (TDS) of the water. Besides that, the $FeSO_4$ solution was prepared by mixing with distilled water according to dilution equation, $C_1V_1 = C_2V_2$ where the

C_1 – concentration of the stock solution in mg/kg

V_1 – volume of the stock solution in litre, l

C_2 – concentration of the prepared solution in mg/kg

V_2 – volume of the prepared solution (3.1)

The concentration of the stock solution was still be 200 mg/kg as only 1 kg of red soil was used in the experiment.

3.6 Red soil preparation

In this experiment, the soil sample obtained from AgroPark Universiti Malaysia Kelantan (UMK) campus Jeli. The red soil was screened through a 2.0 mm stainless steel sieve and keep in a zip-lock plastic bag for further use (Jadia & Fulekar, 2008; Rezvani & Zaefarian, 2011). Then the soil was undergone both chemical and physical analysis for evaluation purposes. Approximately 20.0 g of dry soil was screened through 75 µm stainless steel sieve and send to X-ray Fluorescence (XRF) to determine the percentage of Fe in the soil before treatment (Mukhtar et al., 2010). The Loss on Ignition (LOI) method was a wide and commonly used procedure to estimate the organic matter of dry soil by using crucible and Bunsen burner (Heiri *et al*., 2001). The formula used was shown below:

$$\text{LOI, \%} = [(DW_{0hour} - DW_{4hours}) / DW_{0hour}] \; x \; 100 \; \% \qquad (3.2)$$

Then, the soil was subjected to Ammonium Acetate at pH 7 to analyse the Cations Exchange Capacity (CEC) within (Ross & Ketterings, 2011).

Physical analysis on soil also very crucial as one of the factors affecting the phytoavailability of Fe in soil is the soil texture. Therefore, Bottle Test and Feel methods were used to determine the soil texture. Next, Gravimetric method was used to determine the percentage of water content and the formula used was shown below:

$$\text{Moisture Content, \%} = [(mass\ of\ water, g) x\ (mass\ of\ dry\ soil, g)] \; x \; 100 \; \% \qquad (3.3)$$

The mass of water was discovered by finding the mass difference between saturated soil and oven dry soil after putting in the oven at 110 ^{o}C for 24 hours. On the other hand, the pH of the soil was determined by using pH meter.

This experiment setup was in randomized block design, using a 3 x 1 factorial scheme with triplet method (Romeiro *et al*., 2007). Then 1 kg of sieved red soil was placed in each of the 15 in x 10 in black polybags. After that the polybags were arranged into 5 rows with 3 columns formation with various $FeSO_4$ concentrations as shown in Figure 3.1 below.

Concentration of (FeSO4) solution, ml/kg	1st Trial	2nd Trial	3rd Trial
0 (Control)			
50			
100			
150			
200			

Figure 3.1: The 2-D of 5 x 3 formation of experimental setups.

After 40 days of treatment, approximately 50 g of hydrated red soil from each treatment was collected in a small zip-lock bag and analysed by XRF Analyzer afresh

to investigate the existing Fe content in the soil after the experiment. This can ensure the capability and effectivity of *Ipomoea aquatica* plants in the extraction of Fe from the soil.

3.7 Translocation Factor (TF) and Bioconcentration Factor (BCF)

TF is the translocation of Fe from the root to shoot of the *Ipomoea aquatica* plant and was calculated by TF formula given below:

$$\text{TF} = \frac{total\ concentration\ of\ Fe\ in\ shoot,\ \text{mg/kg}}{total\ concentration\ of\ Fe\ in\ root,\ \text{mg/kg}} \quad (3.4)$$

When the TF value is less than 1, it carries the meaning that the tendency of translocation of heavy metals from root to shoot is effective (Rezvani & Zaefarian, 2011; Majid *et al*., 2012). On the other hand, BCF is the tendency of the plant root to uptake heavy metals from soil medium. The efficiency of Fe removal from the contaminated soil was calculated as follows:

$$\text{BCF} = \frac{total\ concentration\ of\ Fe\ in\ root,\ \text{mg/kg}}{total\ concentration\ of\ Fe\ in\ soil,\ \text{mg/kg}} \quad (3.5)$$

When the ratio of BCF is greater than 1, it indicates that the plant has the potential to remediate heavy metals from the soil.

3.8 Daily Intake of Metal (DIM)

In terms of toxicological study, the value of Daily Intake of Metal, DIM is a parameter to measure the safety of heavy metal intake to avoid hazard of iron (Fe) overload (Bonglaisin *et al*., 2015). The formula of DIM was calculated as follows:

$$\text{DIM} = \frac{Conc.Fe\ x\ Cf\ x\ Wplant\ intake}{Avg.Weight} \quad (3.6)$$

Where conc. of Fe is the concentration of Fe in *Ipomoea aquatica* consumable plant parts (mg kg^{-1}), C_f is the Conversion factor is the average fraction of dry matter Fe consisted in *Ipomoea aquatica* which is 0.085, $W_{plant\ intake}$ is the weight of *Ipomoea aquatica* intake daily (g) and lastly is the average of body weight (kg).

3.9 Data analysis

Data analysis is an effective tool to analyse and evaluate the results from this experiment. All comparisons between data were subjected to One-Way analysis of variance (ANOVA) analysis with confident level of 95% by using the SPSS Statistics version 20. Tukey's test at $p < 0.05$ was used on the comparison between the variance and means of data collected (Romeiro *et al*., 2007). This analysis can accurately determine the efficiency of iron removal at different parts of *Ipomoea aquatica* plants from treated soil sample. The relationship between various parts of *I. aquatica* was interpreted by Pearson Correlation coefficient on the iron accumulations at the significant level of $p < 0.01$ (Poniedzialek *et al*., 2010). All the comparison data was transferred into graph format to ease the analysis of data in next chapters.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Assessment on plant growth

The plants were observed at different iron (Fe) concentrations for 40 days continuously. The height and number of shoot and leaves were not significantly increased. Yet, the plants seem healthy and germinate new shoots. This results proves that *Ipomoea aquatica* plants were able to survive and withstand in the treated red soil with different Fe concentrations starting from 50 mg/kg to 200 mg/kg. Besides, there was no chlorosis symptom found in the plant shown in Figure 4.1 as the colour of leaf margin was evenly fresh green but not any darkening of leaf margins was found in the plant leaves (Bhat *et al*., 2016).

Figure 4.1: Physical condition of *Ipomoea aquatica* after 40 days of Fe treatment.

Fe chlorosis is a popular Fe deficiency syndrome that caused by the failure of chloroplast development, especially in young leaves. This condition often occurs in plants with calcareous soil, which has a high pH than neutral soil (pH 7.8 – 8.2). This may predominantly due to an excess of bicarbonate ions, HCO^{3-} that immobilizes and inhibits Fe in the plants. Similarly, the high hydroxyl and bicarbonate ion concentration associated with the high pH soil solution responsible to keep available ferrous iron, Fe^{2+} concentration low for insufficient normal plant uptake as Fe^{2+} is more bioavailable and soluble than ferric iron, Fe^{3+}. The presence of manganese and copper which are oxidizing agents might oxidize Fe^{2+} to Fe^{3+} too (EPA, 2003). Plus, the solubility of Fe in soil are controlled by the pH of the growth medium, which alternate the availability of Fe to the plants (Alam *et al*., 1999). In fact, there were some leaf holes left by insects like grasshopper, caterpillars or leaf chewers even on leaves treated under high Fe concentrations. Therefore, the treated *Ipomoea aquatica* plants were capable to accept certain amount of metals (Mokhtar *et al*., 2011).

4.2 Chemical and physical analysis of mediums used

Water and soil were among the two mediums that have directly and close contact with the plant. Therefore, both mediums show a very crucial role to identify the Fe accumulation in the plant parts.

4.2.1 Soil medium

Ordinarily, iron concentration in soils range up to 550, 000 mg/kg and most of the iron occurs mainly as ferric iron, Fe^{3+} which is insoluble to plant uptake (EPA, 2003). Red soil from AgroPark UMK Jeli was selected as the only medium for plant growth. The sieved red soil sample was used to ease *Ipomoea aquatica* plant roots to expose more Fe ions in the soil as smaller soil particles have larger total surface area

for roots adsorption (Rieuwerts *et al*., 1998). The soil pH measured in Table 4.1 shows neutral (pH = 6.86). Soil pH with greater acidic properties is more suitable to enhance and trigger vegetable growth as this condition will favour the bioavailability of most crucial nutrients in the soil for plants uptake (Chuan *et al*., 1996; Bhat *et al*., 2016). The pH of soil brings massive impact as alternating factor on metal bioavailability in soils as both solubility and pH influencing each other negatively (Rieuwerts *et al*., 1998). On top of that, the red soil was obtained from about 5.0 cm depth so there was a great possibility the status of the soil was frequently eluted by rain water thus free from any corrosive contaminants that will alter the pH of the soil.

Figure 4.2: Physical appearance of freshly obtained red soil sample from AgroPark.

The colour of red soil was observed from Munsell Soil Colour Chart concurrently in the field side by comparing the fresh red soil sample shown in Figure 4.2 with the colour in the book according code to observe the most accurate soil colour at that time. Subsequently, the moisture content of the soil sample was 19.49%. Under

field conditions, soil moisture alters with precipitation and temperature. Thus, moisture fluctuations capable in regulating the availability of nutrients for plant species. The reason behind was that an increase in soil moisture will cause a surge in HCO^{3-} concentration that collaborate strongly with several ions, especially Fe^{3+}. This phenomenon often distributes to be the predominant factor responsible for plant chlorosis (Mirsa & Tyler, 2011). Overly wet soil can eventually reduce the Fe^{3+} in the soil to Fe^{2+} in the soil which will more readily soluble for the adsorption of plant roots.

Table 4.1: Chemical and physical properties of soil medium.

Parameters	Results
Cation Exchange Capacity (CEC), $cmol_c/kg$	16.36
Organic Matter, %	9.4
Total Nitrogen, mg/kg	4332.46
Total Phosphorus, mg/kg	2490.28
Total Potassium, mg/kg	765.8
N : P : K Ratio	5 : 3 : 1
Iron, mg/kg	9018.09
pH	6.76
Munsell Colour Test	10 YR, 5/6 (Yellowish Brown)
Moisture Content, %	19.49
Soil Textural (Sand : Silt : Clay)	50 : 10 : 40 (Sandy Clay)

The soil texture of red soil was sandy clay with the composition of 50% sand, 10% silt and lastly 40% of clay. Soil organic matter tends to upsurge along with clay content in the soil (EPA, 2003). As shown in Table 4.1, the organic matter of red soil calculated was about 9.4%. In the view of the fact that, the potential for aggregate formation in the soil increases followed by the retention of decomposition process due to the strong bonding between clay particles and organic matter (Bot & Benites, 2005). Meantime, the soil has a Cation Exchange Capacity (CEC) due to the presence of organic matter and clay particles as these make the soil has a great tendency to become negatively charged. Even though clay particles and organic matter have strong

correlation, whereas organic matter might consist 4 to 50 times higher CEC per given weight if compared to clay. Furthermore, since soil pH has direct influence to the organic acid dissociation, CEC also known as pH-dependent CEC (Cornell University Cooperative Extension [CUCE], 2007).

The CEC value obtained from the soil was 16.36 $cmol_c/kg$ and the Nitrogen-Phosphorus-Potassium (N-P-K) ratio of the soil was 5-3-1. Due to the presence of clay and organic matter in soil, the soil shifts to negatively charged and this results the soil to have CEC values which positively depending on the number of clay particles and organic matters within the soil. The soil not only serves as a support medium to plants and living organism, soil also play a role as storehouses for plant nutrients. The most common nutrients exist as soil cations are ammonium (NH_4^+), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and hydrogen (H^+). Normally, soil has high concentration of silica oxide (SiO_2) and aluminium oxide (Al_2O_3) because of the substitution of aluminium (Al^{3+}) and silica (Si^{4+}) in the clay structure (Mengel, n.d.). Therefore, CEC value of the soil will eventually determine the capability of soil to hold the positively-charged ions.

Besides, N-P-K ratio is another important chapter in growing plants. Nitrogen able to assist the plants to generate new tissues through the making of specific proteins. Next, phosphorus prompts the plant root growth and the pH of the soil must at the range of 6.5 – 6.8 in order to absorb phosphorus from soil. Lastly, plant also relies on the potassium content as potassium will ensure plant has enough carbohydrates plus increase the disease resistance by regulating the metabolic activities within the plant. *Ipomoea aquatica* able to absorb average amount of phosphorus (772.7 mg/kg) constantly in all 3 parts of the organs. This may chiefly due to the soil pH which was pH 6.76 that lies between the range where plant able to absorb phosphorus at the peak

amount. Meanwhile, macronutrients such as calcium and magnesium also play a significant role in plant growing. This is because calcium serves as an intermediate medium to bind inorganic and organic particles together plus neutralizing toxic materials in the plant cell membrane. In addition, every green plant produce photosynthesis with the help of chlorophyll, yet chlorophyll cannot process the sunlight without the metallic component, magnesium that built up in chlorophyll. Sufficient and adequate amount of N-P-K in the soil is imperative for sustainable growth of plants or crops (Liberte, n. d.).

4.2.2 Water sample

The physical and chemical parameters of tap water obtained from the Lab BAP 1.1 were analysed by using YSI Multiparameter Model 556 MPS along 40 days of experiment and the mean results were shown in Table 4.2.

Table 4.2: Chemical and physical properties of water sample.

Parameters	Results
pH	6.50
Temperature, ^{o}C	23.68
Electrical Conductivity (EC), mS/cm	0.046
Total Dissolved Solids (TDS), g/L	0.030
Salinity, Sal	0.02
Dissolved Oxygen (DO), mg/L	1.53
Iron (Fe), mg/kg	0.00

From the Table 4.2, the water sample pH used for irrigation was slightly skewed to acidic (pH 6.50) and zero Fe content. Therefore, this can be suggested that the plants uptake iron ions solely from the soil medium. In conjunction, the pH value for rainwater in UMK Jeli shows great acidic properties because heavy construction works are carrying out day and night and produced plenty of sulphur and nitrogen emissions to bind with rain water to form acid rain (Singh & Agrawal, 2008). Meanwhile the

mean Electrical Conductivity (EC), Total Dissolved Solids / Salts (TDS) and Salinity of water sample were 0.046 mS cm^{-1}, 0.030 g L^{-1} and 0.02 Sal respectively. According to Iyasele and Idiata (2015), both EC and salinity were interrelated as salinity can be well stipulated by EC. Salinity can be defined as the total concentration of all dissolved salts in the solution, particularly water. Thus, salinity also can be narrated with TDS. On the other hand, TDS also can be a strong contributor to electrical conductivity. Table 4.3 shows the pH, EC, TDS and Salinity of water samples from another 7 sites followed by correlation graph between EC and TDS in Figure 4.3.

Table 4.3: Physical and chemical properties of water at different sites.

Sites	Parameters			
	pH	EC, mS cm^{-1}	TDS, g L^{-1}	Salinity, Sal
Clogged Rainwater in Trolley, A	6.41	0.023	0.012	0.01
Wood Lab Water Tank, B	6.69	0.007	0.004	0.00
AgroPark Dam, C	6.61	0.042	0.024	0.02
Ex-Butterfly Farm Stream, D	6.90	0.072	0.046	0.03
Tap Water Lab BAP 1.1 (A), E	6.50	0.046	0.030	0.02
Tap Water Lab BAP 1.1 (B), F	6.61	0.047	0.031	0.02
Rainwater at IBS, G	5.41	0.027	0.017	0.01
Rainwater at Block B, H	7.17	0.007	0.004	0.00

Based on the Figure 4.3, the EC was surged simultaneously with TDS content (R^2 = 0.9913). The EC and TDS for water sample site D was the highest among the 8 sites, 0.072 mS cm^{-1} and 0.046g L^{-1} respectively. Basically, the water source at site D was accumulate from leaching process of red soil sampling area due to heavy rain. Hence, the soil sample used in the experiment was an ideal medium for plant growth as the water sample nearby has a high EC and TDS values.

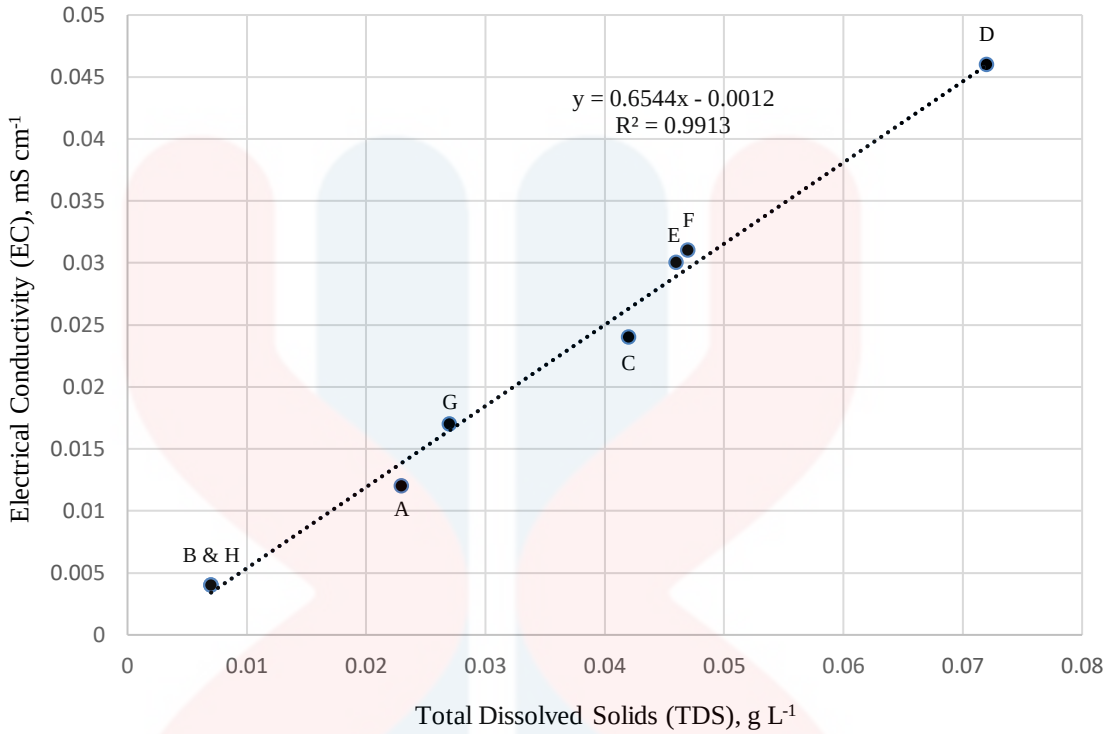

Figure 4.3: Correlation between Electrical Conductivity, EC and Total Dissolved Solids, TDS of various water sources.

4.3 Removal of iron from soil by *Ipomoea aquatica*

The plants were collected after 40 days of growing within treatment soil with Fe concentrations 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg and control (0 mg/kg). Deionised water was used in the washing process to ensure the outer part of the plants are free from red soil particles before blotted with filter paper. Consequently, the plant parts were segmented carefully as shown in Appendix C, Figure C.2 in order to make sure the leaves, shoots and roots of *Ipomoea aquatica* were differentiated distinctly for most accurate results (Appendix C, Figure C.3). The fresh weight for all parts of plant was weighed in advance before XRF analysis. Since there were triplicate for 5 different treatments, Completely Randomized Design (CRD) was carried as CRD experimental design which is meant to be applied for total Fe accumulation in plant parts. Standard statistical methods like Tukey Test and One-Way Analysis of Variance (ANOVA)

were used to vindicate the result by giving a more reliable and convincing results with statistical support (Anamika *et al*., 2009; Bhat *et al*., 2016).

Table 4.4: Mean Concentration of Fe in the different parts of *Ipomoea aquatica* (mg/kg).

Soil Treatment, mg/kg	Fe Concentration, mg/kg			Total Fe Accumulation, mg/kg
	Leaves	Shoots	Roots	
0 (Control)	35.7 ± 1.664^{c}	0.0 ± 0.000^{d}	84.7 ± 1.752^{c}	120.4 ± 2.646^{d}
50	28.0 ± 2.646^{d}	27.0 ± 2.000^{bc}	128.0 ± 11.533^{b}	183.0 ± 10.583^{c}
100	51.7 ± 1.300^{b}	23.0 ± 3.606^{c}	159.5 ± 5.122^{a}	234.2 ± 9.182^{b}
150	71.3 ± 2.443^{a}	36.7 ± 1.572^{a}	158.0 ± 6.083^{a}	266.0 ± 4.770^{a}
200	21.0 ± 2.646^{e}	32.0 ± 3.605^{ab}	140.0 ± 14.422^{ab}	193.0 ± 12.530^{c}

Means with different superscript letters are significantly different at $P < 0.05$ (Tukey Test).

Meantime, the dependent and independent variables were the mean accumulation in plant parts (leaves, shoots and roots) and Fe concentration of soil treatments (0, 50, 100, 150 and 200 mg/kg). Table 4.4 shows the accumulation of Fe in three different parts, which are leaves, shoots and roots in plant according to varying iron concentrations levels of soil treatments followed by the total accumulation of Fe by the plant (Appendix A, Table A.1). Figure 4.5, the mean total accumulation of Fe from 0 to 200 mg/kg were 120.4 ± 2.646 mg/kg, 183.0 ± 10.583 mg/kg, 234.2 ± 9.182 mg/kg, 266.0 ± 4.770 mg/kg and 193 ± 12.530 mg/kg respectively. Surprisingly the total Fe accumulation for soil treatment 200 mg/kg dropped to 193 ± 12.530 mg/kg which decreased by about 27.4% from previous amount in Fe concentration level, 150 mg/kg. This can be explained that *Ipomoea aquatica* shows a certain characteristic on the limit of accumulation capacity within the plant itself. As for the plants with another level of artificial Fe treatment, the amount of Fe accumulation surged steadily in each treatment ranging from 50 mg/kg until 150 mg/kg. Reeves (2006) reported that hyperaccumulator plants must capable to accumulate more than 1000 mg/kg of heavy metal concentration in the dry mass. Whereas the full remediation potential of *Ipomoea aquatica* has been limited due to the limited time given to the experiment.

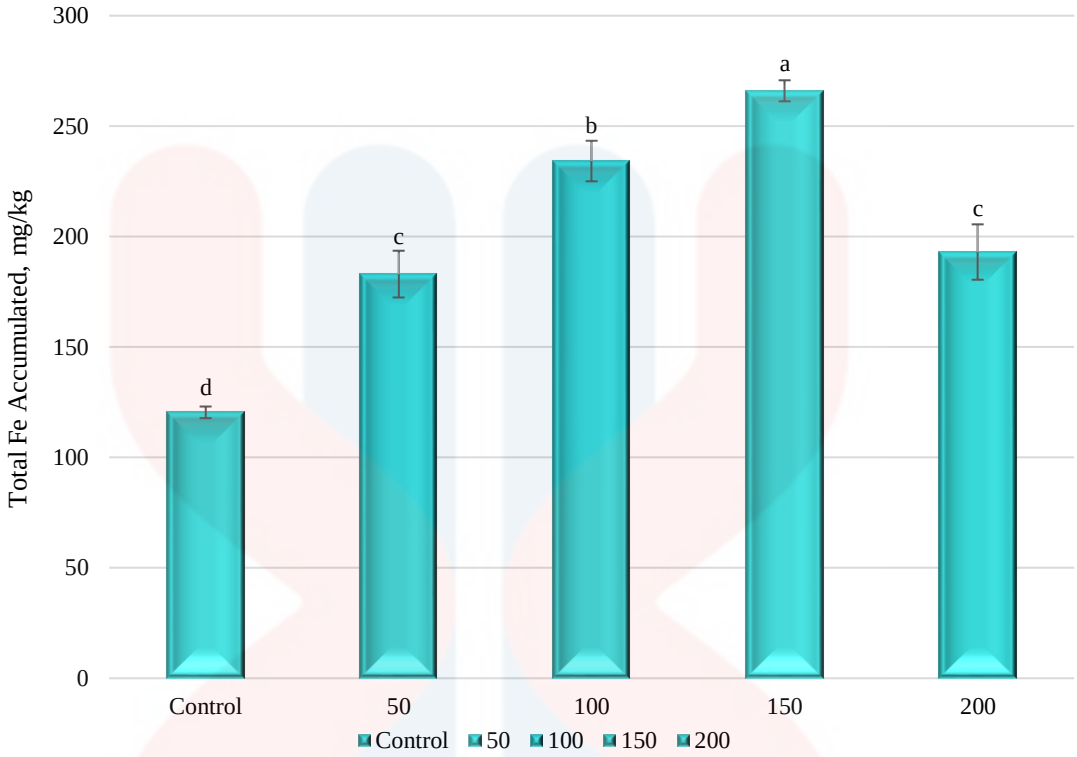

Figure 4.4: Total Fe accumulation of *Ipomoea aquatica* plant. Means with different superscript letters are significantly different at P < 0.05 (Tukey Test).

After 40 days of uptake, heavy metal was drained from the red soil which indicating effective absorption of Fe by *Ipomoea aquatica.* The scaling down in terms of concentration levels of Fe in red soil was attributed to the uptake capability by the plants. Indeed, generally there was a statistically significant difference between the leaf and root and between root and shoot at all soil treatments ($p < 0.05$). Also, none of the data in Table 4.4 showed any statistically significant difference in regard to the Fe distribution between the leaves and shoot except for control and 150 mg/kg soil treatments ($p > 0.05$). Meanwhile, based on Figure 4.6 the roots contain highest Fe accumulation by accumulating more than half of the total accumulation in each treatment. Mokhtar and his colleagues (2011) reported the reason behind storing of heavy metals occur predominantly in the roots of plant because of the mobility of metal transport was very slow pace. The Fe accumulation in the plant roots shows a parabolic

curve with a decreasing gradient as the peak value of accumulation lies at Fe concentration level 150 mg/kg.

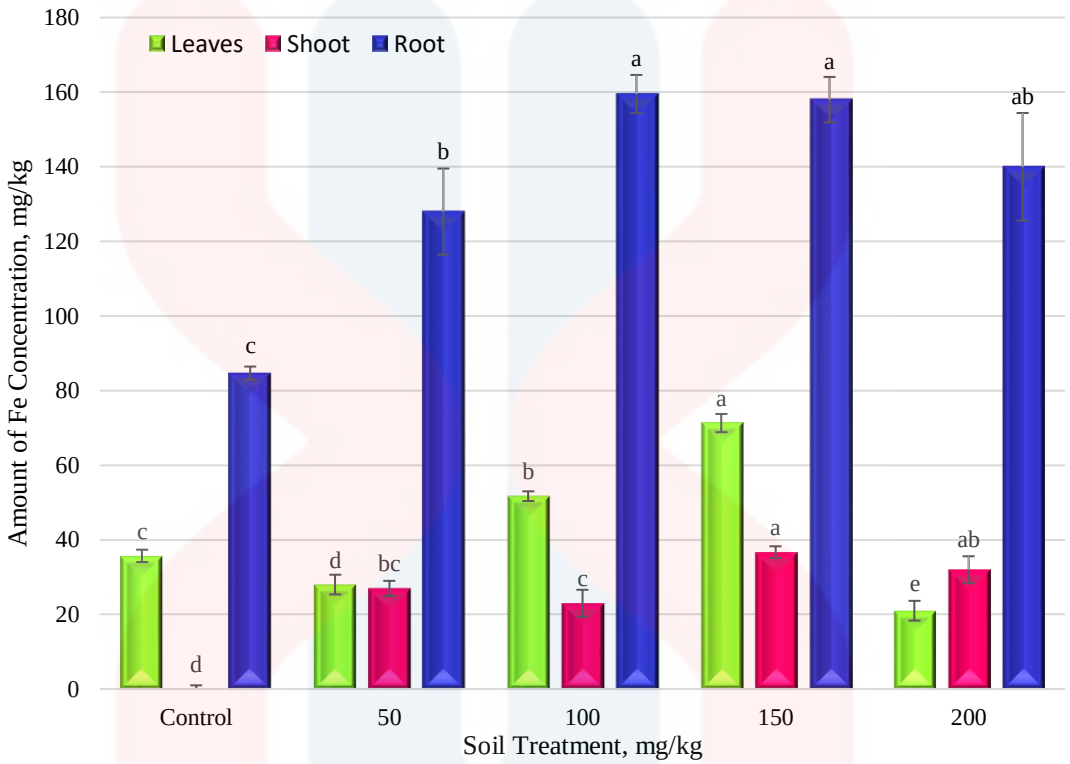

Figure 4.5: Iron adsorption in different parts of *Ipomoea aquatica* plant. Means with different superscript letters are significantly different at $P < 0.05$ (Tukey Test).

From Figure 4.6, *Ipomoea aquatica* can survive through various Fe concentrations by carry out phytoremediation mechanism in plant roots as roots are the only medium for heavy metals translocation solely from soil via numbers of potential tolerance mechanism (Anamika *et al*., 2009). At the same time, plants were well-grown and not diagnosed with any plant stress or diseases like yellowing leaves and plant chlorosis. From the XRF analysis, there was no cadmium, Cd present in the original soil sample before the treatment. Cd is a heavy metal that capable to retard the plant root metal uptake by constraining metal translocation from root to shoot. Consequently, Fe will fail to loading to plant xylem as blocked by Cd with which Cd can parcel out a common translocation system as Fe or Ca-like metals (Solti *et al*., 2011). At the same time, there was no detection of Cr as Cr is a strong oxidant that

will inhibit root cell division and extension of cell cycle through the oxidation of root cells (Ton *et al*., 2015). Therefore, no plant stress issues prevailed in the experiment.

Table 4.5: Mean moisture content in the different parts of *Ipomoea aquatica* (mg/kg).

Soil Treatment	Plants Parts	Mean Moisture Content, %
Control	Leaves	78.55 ± 6.954
	Shoots	71.88 ± 7.364
	Roots	52.61 ± 24.402
50mg/kg	Leaves	63.77 ± 13.761
	Shoots	69.77 ± 16.325
	Roots	54.43 ± 15.132
100mg/kg	Leaves	67.72 ± 7.101
	Shoots	70.53 ± 8.039
	Roots	59.22 ± 13.807
150mg/kg	Leaves	56.70 ± 13.190
	Shoots	66.12 ± 1.499
	Roots	61.12 ± 15.299
200mg/kg	Leaves	70.39 ± 3.420
	Shoots	73.90 ± 3.034
	Roots	60.90 ± 7.133

Besides that, *Ipomoea aquatica* has high capability to become Fe metal accumulator as the plant is able to survive for 40 days continuously and hyperaccumulate high concentration level of Fe from treatment soil. As Table 4.2 shows zero Fe concentration in the water sample used for irrigation, thus the total Fe concentration in red soil can be estimated by adding up the total Fe accumulated in plant parts and the remaining Fe in the control soil treatment. Therefore, there was a negative correlation between the total Fe accumulation in plant parts and the Fe remaining in red soil because the more Fe successfully accumulated in plant, the lesser the Fe will be left in the soil. In addition, reduction in moisture content of plant roots also designates for plant stress responses like the physiological reactions due to high heavy metals accumulation in that particular area (Bhaduri & Fulekar, 2012). In conjunction, the moisture content of the root plant shown in Table 4.5 has the lowest value due to the high accumulation of iron within.

Undeniably there are plenty of factors such as oxygen content, moisture content, bacteria, organic substance and pH that can affect the phytoavailability of heavy metals in soils, yet the unique characteristics of root zone also capable to fix the plant and adsorb dissolved minerals from soil because root exudates are the first line of defence opposed heavy metals (EPA, 2003; Manara, 2012; Radulescu *et al*., 2013) The term 'hyperaccumulator' relate to the plant abilities to survive on metalliferous soil and store abnormal quantity of heavy metals in their plant biomass without showing significant phytotoxic effects like plant chlorosis (Zitka *et al*., 2004). Therefore, a preliminary step to classify whether the plants are suitable to be an hyperaccumulator or not is through their survivorship on metalliferous soil. *Ipomoea aquatica* plants have a strong tolerance to iron contaminant soil along these 40 days of treatment. Indeed, the expression and regulation of genes found in the plant parts via physiological and molecular analyses are still the most primary step a hyperaccumulation processes rely on. These genes encoding transmembrane transporters like members of ZIP, Multi-antimicrobial Extrusion Protein (MATE), Arabidopsis Yellow Stripe-Like (YSL) and Membrane Transport Protein (MTP) families play crucial role in hyperaccumulators through constitutive overexpression of genes (Rascio & Izzo, 2011). In particular, this type of plant will make these extraordinary abilities as a defence mechanism inimical to natural foes like herbivores because typically hyperaccumulator plants will have high heavy metals concentration in leaves which make the leaves poisonous to herbivores.

Table 4.6: Types of Fe transporters in plant along with their functions in phytoremediation process.

Iron Transporters	Significant Function	References
AtATM3	Responsible for the transportation of Fe-S from Mitochondria cell.	Conte & Walker, 2011

AtFPN1	Loading of Fe ions into xylem via Fe efflux across plasma membrane.	Conte & Walker, 2011
AtNAP14	Chloroplasts influx of Fe.	Conte & Walker, 2011
VIT1	Fe influx to plant vacuole.	Conte & Walker, 2011
ZmYS1, OsYSL15 & HvYS1	Responsible for the main Fe uptake from soil medium.	Conte & Walker, 2011
OsYSL2	Work on the TF of Fe from roots to shoots.	Conte & Walker, 2011
IRT1	Reduction of insoluble Fe to make it soluble to plant uptake.	Manara, 2012
FRO2	Encode the root ferric chelate reductase.	Connolly *et al*., 2003

Hyperaccumulator plants can effectively and efficiently compartmentalize heavy metals into 3 main partitions of plant, leaves and shoots and roots respectively (Zitka *et al*., 2004; Manara, 2012). Plants have 2 special strategies to achieve Fe uptake and curb lack bioavailability of Fe. Both natural strategies required the assistance of Iron Regulated Transporter1 (IRT1) and Ferric Reductase Oxidase (FRO2) transporters in order to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) and move the Fe^{2+} across plant root epidermal plasma membrane (Connolly *et al*., 2003; Conte & Walker, 2011). Although pH of soil has strong correlation to the heavy metal uptake as the H^{+} availability in soil will lower the soil pH and triggers heavy metals absorptions from soil medium, yet the transporters in plant parts especially the roots provide a more promising proves that responsible for high affinity metal uptake even under iron deficiency phenomenon. (Vert *et al*., 2002). Next, in consideration of treating shoot as transporting heavy metals to the above-ground plant parts via the xylem, level of citrate in plant is crucial as there is positive correlation between citrate amount and levels of Fe available in xylem. Rellan-Alvarez and his colleagues (2008) prove that citrate is the essential complexor of Fe in xylem when take metal chelator and soil pH into theoretical calculations.

Apart from that, massive efficiency in heavy metals sequestration is the primary characteristic of plants. Typically, heavy metals accumulate differently between leafy vegetables and non-leafy vegetables (Song *et al*., 2015). Hence, sequestration process is carry out in leaves especially in epidermis, cuticle or even trichomes where photosynthesis is carried out. In leaf, the heavy metals have been moved by heavy metal complexation with ligands from metabolically active cytoplasm into vacuoles and cell wall which known as inactive compartment in plant (Zitka *et al*., 2004; Rascio & Izzo, 2010). The reason ligands or organic acids play a vital role as detoxifying factors because ligands enable heavy metals to entrap in vacuoles where chelates situated. For instance, hyperaccumulators form a strategy to enhance cell antioxidant system or regulation of hormone synthesis to adapt heavy metal stress (Manara, 2012). For example, Se hyperaccumulators will get rid of selenoamino acids (selenocysteine, Se-Cys) in leaf chloroplasts as primary detoxification strategy. Figure 4.6 illustrated that amount of Fe accumulated in leaves positioned second after roots. This is predominantly due to the evapotranspiration process carried out by leaves and eventually create an adhesive and cohesive process to pump the Fe ions from stems to leaves. Plus, adequate amount of Fe proficient to promote the development of chloroplast in plant leaves for photosynthesis process (Tangahu *et al*., 2011; Bhat *et al*., 2016).

Table 4.7: Pearson correlation coefficients between different plant parts and varies Fe concentration levels.

	Leaf	Shoot	Root
Leaf	1	0.261	0.462
Shoot	0.261	1	0.820**
Root	0.462	0.820**	1

** Correlation is significant at the 0.01 level (2-tailed).

Table 4.7 above shows the correlation between plant parts of *Ipomoea aquatica* in different Fe concentration levels soil treatment after 40 days of exposure. The correlation between root and shoot was the highest, r = 0.820 followed by correlation between root and leaf which was r = 0.462. Meanwhile, the correlation between shoot and leaf was the lowest, r = 0.261 only. Therefore, the correlation was significant for root and shoot and this can draw a conclusion that high accumulation in roots will afterward source high accumulation in stems in terms of relationship but not in terms of quantity absorbed.

Nevertheless, human requires a certain amount of iron to sustain daily chores in life. Iron is so important as one of the most crucial component in haemoglobin formation and haemoglobin represent about two/thirds of human body's iron. This will ensure body to form sufficient healthy oxygen-carrying red blood cells. A lack of red blood cells may cause a phenomenon called iron deficiency anaemia. Thus, immune system's ability and brain function might also have degraded as the iron content in human body decreases. According to WHO/FAO (2011), the average daily iron intake was approximately 17 mg/day for men and 12 mg/kg for women where adults shouldn't take more than 45 mg of iron daily. In Table 4.4, the maximum amount of Fe accumulated in consumable plant parts (leaves and shoots) was 108 mg/kg in 150 mg/kg soil treatment. By using the equation 3.4, the calculated $DIM_{150mg/kg}$ was just 14.123 mg Fe kg^{-1} $person^{-1}$ d^{-1}, assuming 100 g of vegetable consumption in a day by a person weight 65 kg and conversion factor of 0.085 (Khan *et al*., 2009). Therefore, the *Ipomoea aquatica* plant is safe to be consumed even with high iron treatment. For instance, food chain is the key passage of heavy metal exposure to humans (Jolly *et al*., 2013; Song *et al*., 2015; Balkhair & Ashraf, 2015). According to Stephanie (2011),

ordinary people only able to absorb about 10% of the iron consume except for people with hemochromatosis condition, which can absorb up to 30% of iron consume.

4.4 Effectiveness of iron accumulation in *Ipomoea aquatica* plant

Bioconcentration Factor (BCF) and Translocation Factor (TF) are collaborating with each other to evaluate the possibility and suitability of the plant to absorb and transfer Fe ions from roots in soil to upper part of plant parts, shoot and leaves. Hyperaccumulator plants are those who able to accumulate massive amount of heavy metals in plant, this will only be done when the roots have the capability to diffuse the cations from the soil to xylem (Syam *et al*., 2016). Normally TF value is less than 1 and BCF value is more than 1 as hyperaccumulator plant will have more Fe deposited on roots and has greater amount of Fe accumulated in plant rather than soil respectively (Manan *et al*., 2015). The correlation coefficient between TF and BCF was 0.647 with a significant difference of $p < 0.01$.

Table 4.8: Translocation Factor (TF) of *Ipomoea aquatica*.

Soil Treatment, mg/kg	Fe Concentration in, mg/kg		Translocation Factor, TF
	Shoots	Roots	
50	27.0 ± 2.000	128.0 ± 11.533	0.211
100	23.0 ± 3.606	159.5 ± 5.122	0.144
150	36.7 ± 1.572	158.0 ± 6.083	0.232
200	32.0 ± 3.605	140.0 ± 14.422	0.229

From Table 4.8, the highest translocation factor occurs in soil treatment with concentration level 150 mg/kg, which was 0.232. The TF value is directly proportional to the total iron accumulated in shoot as the total Fe accumulated in 150mg/kg soil treatment was the highest among the 5 soil treatments (36.7 mg/kg). Furthermore, the correlation between Fe concentration levels with TF was 0.756 under a significant

difference of $p < 0.01$. Meantime, the Pearson Correlation between BCF and TF was 0.565 under a significant difference of $p < 0.05$. Thus, in this case the p-values have enough evidence to suggest that the Fe concentration levels has significant positive correlation to BCF and TF. In other words, increase in Fe concentration levels do significantly relate and give positive increase in BCF and TF and vice versa.

Table 4.9: Bioconcentration Factor (BCF) of *Ipomoea aquatica.*

Soil Treatment, mg/kg	Total Fe Accumulation in Plant Tissue, mg/kg	Remaining Fe Concentration in Soil, mg/kg	Bioconcentration Factor, BCF
50	183.0 ± 10.583	8885.09 ± 128.553	3.660
100	234.2 ± 9.182	8883.89 ± 140.698	2.342
150	266.0 ± 4.770	8902.09 ± 205.790	1.773
200	193.0 ± 12.530	9025.09 ± 228.090	0.965

The highest BCF was recorded in soil treatment of 50 mg/kg with 3.66 and 0.965 was noted as the lowest BCF value in soil polluted with 200 mg/kg. The BCF were decreasing steadily with the increasing of iron concentration levels of soil treatments ($R^2 = 0.9702$) as shown in Figure 4.7. The BCF value between 0.1 to 1 indicated that the plant is a moderate accumulator species (Manan *et al*., 2015). Thus, *Ipomoea aquatica* is a good accumulator species as the BCF value shows mostly more than 1 except for soil treatment with iron concentration level 200 mg/kg. Meanwhile, the iron concentrations levels of soil treatment which exceed 200 mg/kg caused the calculated BCF values to be less than one ($BCF_{200mg/kg} = 0.965$). This may have suggested that the tolerance level of *Ipomoea aquatica* plant in remediating iron from red soil has reaches the certain limitation. Therefore, macronutrients like phosphorus, nitrogen and potassium may affected and lead to stunning of plant growth to accumulate higher level of iron from contaminated soil (Bhat *et al*., 2016).

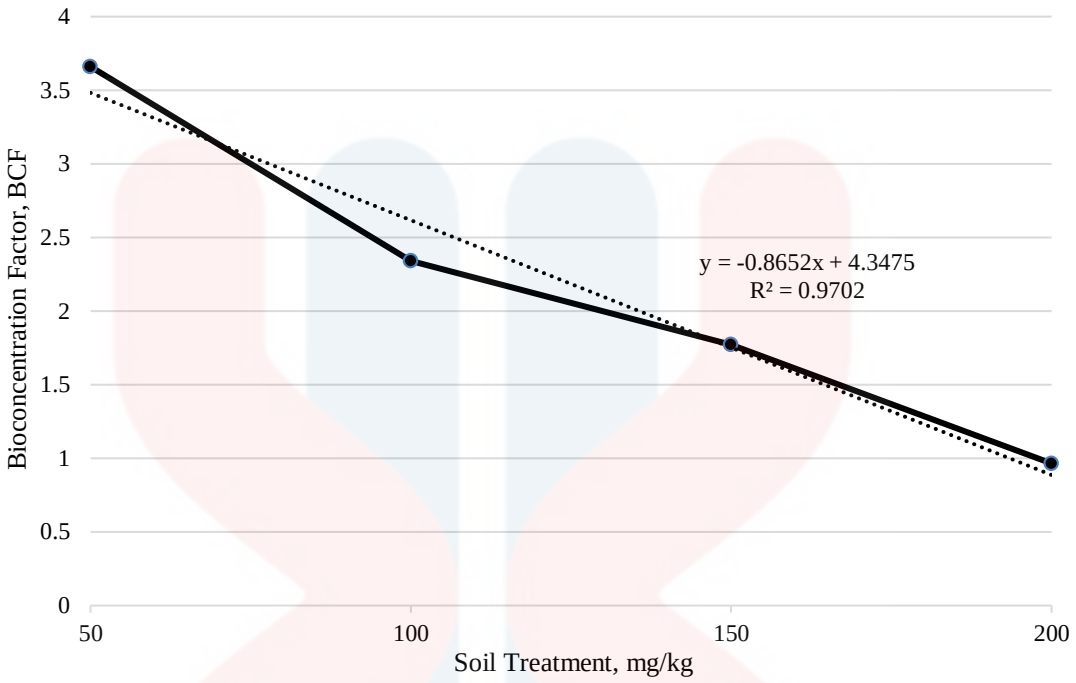

Figure 4.6: The Bioconcentration Factor (BCF) of *Ipomoea aquatica*.

One-way Analysis of Variance (ANOVA) was used to determine whether the level of iron concentration differed among soil treatments. Test of homogeneity of variances was carried out to ensure there is homogeneity variance. Result shown there was no significant difference ($p > 0.05$), hence the homogeneity variance is not violated and the assumption was failed to reject. In Appendix B, Table B.1, the ANOVA analysis showed significant differences among the groups ($F(4,10) = 119.408$, $p < .001$). Control soil treatment shows the greatest accumulation ($M_{control} = 120.1$, S.D. = 2.646), followed by soil treatment 150 mg/kg, 100 mg/kg, 50 mg/kg and 200 mg/kg, with ($M_{150mg/kg} = 266.0$, S. D. = 4.770), ($M_{100mg/kg} = 234.2$, S.D. = 9.182), ($M_{50mg/kg} = 183.0$, S.D. = 10.583) and ($M_{200mg/kg} = 193.0$, S.D. = 12.530) respectively. Since there was statistically significant differences between groups, Post-hoc test can be interpreted. Post-hoc Tukey tests was performed as there is an equal sample size (triplicate was performed). Tukey tests showed that the iron accumulation for all soil treatments differed statistically from every soil treatments, but the difference between soil treatment 50 mg/kg with 200 mg/kg was not statistically significant, where the p >

0.05. Furthermore, the effect size was determined, the effect size tells how significant a significant result is. The size of effect of this experiment is very large as there are 98% ($\eta^2 = 97.949$).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study was carried out to screen *Ipomoea aquatica* plant growing on a contaminated site to determine its potential for iron accumulation. The iron was deposited in different parts of plant in different amounts through chemical mechanisms and transport strategies in their roots. The highest iron accumulation was found in the root and lowest accumulation was in the shoot (Root > Leaf > Shoot). Based on the results, *Ipomoea aquatica* has statistically proven to remediate iron contaminated soil more significantly. Literally, both phytostabilization and phytoextraction mechanisms are suitable to describe the phytoremediation technique shown by *Ipomoea aquatica* aquatic plant. This is because *Ipomoea aquatica* mainly stored iron in its roots and this could be further use as a bioindicator in monitoring the water quality with heavy metals issues.

The most vital factor is using a suitable plant to remediate the right heavy metals in the soil medium. Overall, *Ipomoea aquatica* can withstand different iron concentration levels of soil treatments with zero mortality rate. Hence, phytoremediation of iron by using *Ipomoea aquatica* seems to be a lucrative way to remediate contaminated soil in environment. Protract study can be carry out by diminishing the limitation of phytoremediation so that phytoremediation can be carry out efficiently and effectively.

5.2 Recommendations

The aim of the study was focus solely on the phytoremediation of iron with different concentration levels by using *Ipomoea aquatica* as remediation agent. Therefore, the future study can be carry out by using *Ipomoea aquatica* to remediate different types of heavy metals, such as manganese, aluminium, selenium, zinc and also lead. The experiment also can be conducted in the way by comparing the amount of heavy metal uptake by inducing another heavy metal (cadmium - Cd) with different doses, this can be tested whether the deposition of chromium at the shoot can bring any impact to the translocation factor (TF) of *Ipomoea aquatica* (Bah *et al*., 2011).

Meanwhile, there are thousands of plant hyperaccumulator with some have heterogeneous hyperaccumulating abilities, such as Cu/Co hyperaccumulator and Zn/Cd hyperaccumulator. A study revealed that, Zn/Cd hyperaccumulator like *Thlaspi caerulescens* or *Sedum alfredii* got Cd amount decreases in plant root when the concentration of Zn increases (Rascio & Izzo, 2011). Therefore, *Ipomoea aquatica* can use another heavy metal to test on the effectivity of iron remediation followed by the growth condition by comparing the plant parts. This is valid when heavy metals involved do not support the growth and development of plants (Chibuike & Obiora, 2014). This can only apply phytoremediation technique in a wider field with lesser limitation.

Furthermore, more research should be stimulated on phytoextraction-inducing substances. Chelating agent is a type of catalyst to enhance the ability and speed to remediation in plant. These chelating agents vary with different affinities for different metals because chelation involves bonding of molecules or ions to metal cations. Examples of chelators can be like EDTA, DTPA, HEDTA, NTA and also citric acid.

Consequently, adding of chelating agent into *Ipomoea aquatica* can be further increase the efficiency of the phytoremediation technique (Ruley *et al*., 2005). This can be performed by comparing the plants with chelating agent and without chelating agents in remediating heavy metals.

Environment or physical growing conditions is crucial for plant growth and subsequently remediation capability as well. Thus, to bring the precision of the experiment to a higher level, growing of *Ipomoea aquatica* in a greenhouse with strict surveillance of dependent variables like water source, soil type and air quality are mandatory. Besides that, growing of *Ipomoea aquatica* in water rather than soil can be put into comparison to the one growing in soil. As typically *Ipomoea aquatica* is an aquatic plant that able to adapt hypotonic growing method. This is because by doing so, the efficiency of phytoremediation mechanisms like phytoextraction and phytostabilization with phytodesalination and phytofiltration of *Ipomoea aquatica* can be investigated (Bhat *et al*., 2016).

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APPENDIX – A

Table A.1: XRF results include the Mean (M) and Standard Deviation (S.D.) of Iron Accumulation (mg/kg) in Leaf, Shoot and Root of *Ipomoea aquatica* under varying Iron Concentration Levels of Soil Treatments.

Soil Treatments (mg Fe/kg)	Plant Parts			Total Iron Accumulation (mg/kg)
	Leaf	Shoot	Root	
0 (Control)	35.0	0.0	86.4	121.40
	34.5	0.0	82.9	117.40
	37.6	0.0	84.8	122.40
M	35.7	0.0	84.7	120.4
S.D.	1.66433	0.00000	1.75214	2.64575
50.0	31.0	29.0	119.0	179.00
	26.0	25.0	124.0	175.00
	27.0	27.0	141.0	195.00
M	28.0	27.0	128.0	183.0
S.D.	2.64575	2.00000	11.53256	10.58301
100.0	51.0	19.0	153.6	223.60
	50.9	26.0	162.8	239.70
	53.2	24.0	162.1	239.30
M	51.7	23.0	159.5	234.0
S.D.	1.30000	3.60555	5.12152	9.18205
150.0	68.5	35.0	162.0	265.50
	73.0	37.0	161.0	271.00
	72.4	38.1	151.0	261.50
M	71.3	36.7	158.0	266.0
S.D.	2.44336	1.57162	6.08276	4.76970
200.0	19.0	31.0	156.0	206.00
	24.0	29.0	128.0	181.00
	20.0	36.0	136.0	192.00
M	21.0	32.0	140.0	193.0
S.D.	2.64575	3.60555	14.42221	12.52996

Table A.2: The Test of Homogeneity of Variances between Total Iron Accumulation and Iron Concentration Levels in Soil Treatment

Levene Statistic	df1	df2	Sig.
1.718	4	10	.222

Table A.3: Homogeneous Subsets of Tukey HSD for Total Iron Accumulation

Concentration	N	Subset for alpha = 0.05			
		d	c	b	a
0 mg/kg	3	120.4000			
50 mg/kg	3		183.0000		
200 mg/kg	3		193.0000		
100 mg/kg	3			234.2000	
150 mg/kg	3				266.0000
Sig.		1.000	.642	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

APPENDIX – B

Table B.1: Table of One-Way ANOVA of Mean Total Accumulation of Iron in *Ipomoea aquatica* from Soil Treatment of Different Concentration Levels.

	Sum of Squares	df	Mean Square	F	Significance
Between Groups	36592.464	4	9148.116	119.408	0.000
Within Groups	766.120	10	76.612		
Total	37358.584	14			

* The mean difference is significant at the $p < 0.05$ level.

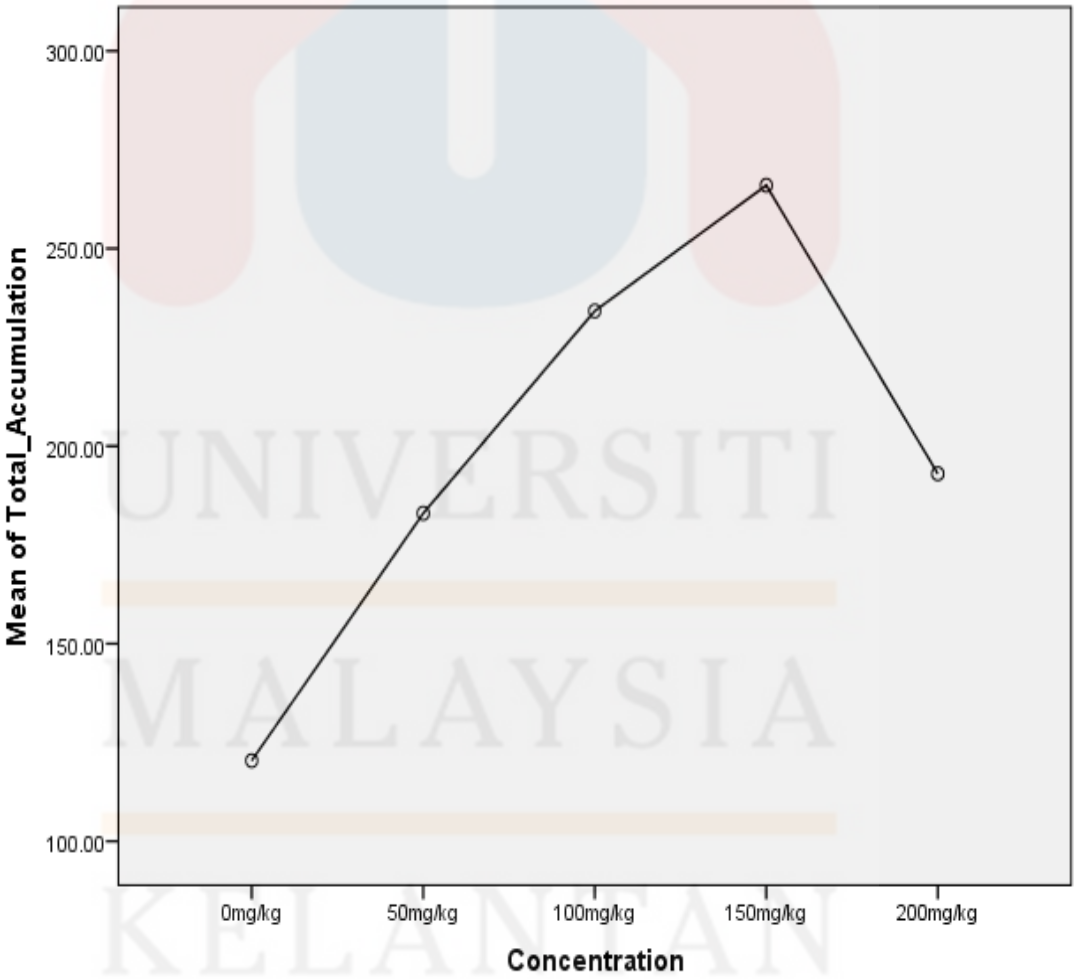


Figure B.1: The Mean of Total Iron Accumulation in *Ipomoea aquatica* (mg/kg) Dry Mass.

Table B.2: Multiple Comparison (Tukey HSD) for Iron Accumulation in Leaf.

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 mg/kg	50mg/kg	7.70000*	1.80481	.011	1.7602	13.6398
	100mg/kg	-16.00000*	1.80481	.000	-21.9398	-10.0602
	150mg/kg	-35.60000*	1.80481	.000	-41.5398	-29.6602
	200mg/kg	14.70000*	1.80481	.000	8.7602	20.6398
50 mg/kg	0mg/kg	-7.70000*	1.80481	.011	-13.6398	-1.7602
	100mg/kg	-23.70000*	1.80481	.000	-29.6398	-17.7602
	150mg/kg	-43.30000*	1.80481	.000	-49.2398	-37.3602
	200mg/kg	7.00000*	1.80481	.020	1.0602	12.9398
100 mg/kg	0mg/kg	16.00000*	1.80481	.000	10.0602	21.9398
	50mg/kg	23.70000*	1.80481	.000	17.7602	29.6398
	150mg/kg	-19.60000*	1.80481	.000	-25.5398	-13.6602
	200mg/kg	30.70000*	1.80481	.000	24.7602	36.6398
150 mg/kg	0mg/kg	35.60000*	1.80481	.000	29.6602	41.5398
	50mg/kg	43.30000*	1.80481	.000	37.3602	49.2398
	100mg/kg	19.60000*	1.80481	.000	13.6602	25.5398
	200mg/kg	50.30000*	1.80481	.000	44.3602	56.2398
200 mg/kg	0mg/kg	-14.70000*	1.80481	.000	-20.6398	-8.7602
	50mg/kg	-7.00000*	1.80481	.020	-12.9398	-1.0602
	100mg/kg	-30.70000*	1.80481	.000	-36.6398	-24.7602
	150mg/kg	-50.30000*	1.80481	.000	-56.2398	-44.3602

* The mean difference is significant at the 0.05 level.

Table B.3: Homogeneous Subsets of Tukey HSD for Leaf

Concentration	N	Subset for alpha = 0.05				
		e	d	c	b	a
200 mg/kg	3	21.0000				
50 mg/kg	3		28.0000			
0 mg/kg	3			35.7000		
100 mg/kg	3				51.7000	
150 mg/kg	3					71.3000
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table B.4: Multiple Comparison (Tukey HSD) for Iron Accumulation in Shoot.

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 mg/kg	50mg/kg	-27.00000*	2.08071	.000	-33.8478	-20.1522
	100mg/kg	-23.00000*	2.08071	.000	-29.8478	-16.1522
	150mg/kg	-36.70000*	2.08071	.000	-43.5478	-29.8522
	200mg/kg	-32.00000*	2.08071	.000	-38.8478	-25.1522
50 mg/kg	0mg/kg	27.00000*	2.08071	.000	20.1522	33.8478
	100mg/kg	4.00000	2.08071	.366	-2.8478	10.8478
	150mg/kg	-9.70000*	2.08071	.006	-16.5478	-2.8522
	200mg/kg	-5.00000	2.08071	.191	-11.8478	1.8478
100 mg/kg	0mg/kg	23.00000*	2.08071	.000	16.1522	29.8478
	50mg/kg	-4.00000	2.08071	.366	-10.8478	2.8478
	150mg/kg	-13.70000*	2.08071	.000	-20.5478	-6.8522
	200mg/kg	-9.00000*	2.08071	.010	-15.8478	-2.1522
150 mg/kg	0mg/kg	36.70000*	2.08071	.000	29.8522	43.5478
	50mg/kg	9.70000*	2.08071	.006	2.8522	16.5478
	100mg/kg	13.70000*	2.08071	.000	6.8522	20.5478
	200mg/kg	4.70000	2.08071	.235	-2.1478	11.5478
200 mg/kg	0mg/kg	32.00000*	2.08071	.000	25.1522	38.8478
	50mg/kg	5.00000	2.08071	.191	-1.8478	11.8478
	100mg/kg	9.00000*	2.08071	.010	2.1522	15.8478
	150mg/kg	-4.70000	2.08071	.235	-11.5478	2.1478

* The mean difference is significant at the 0.05 level.

Table B.5: Homogeneous Subsets of Tukey HSD for Shoot

Concentration	N	Subset for alpha = 0.05			
		d	c	b	a
0 mg/kg	3	.0000			
100 mg/kg	3		23.0000		
50 mg/kg	3		27.0000	27.0000	
200 mg/kg	3			32.0000	32.0000
150 mg/kg	3				36.7000
Sig.		1.000	.366	.191	.235

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table B.6: Multiple Comparison (Tukey HSD) for Iron Accumulation in Root.

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0 mg/kg	50mg/kg	-43.30000*	7.36931	.001	-67.5530	-19.0470
	100mg/kg	-74.80000*	7.36931	.000	-99.0530	-50.5470
	150mg/kg	-73.30000*	7.36931	.000	-97.5530	-49.0470
	200mg/kg	-55.30000*	7.36931	.000	-79.5530	-31.0470
50 mg/kg	0mg/kg	43.30000*	7.36931	.001	19.0470	67.5530
	100mg/kg	-31.50000*	7.36931	.011	-55.7530	-7.2470
	150mg/kg	-30.00000*	7.36931	.015	-54.2530	-5.7470
	200mg/kg	-12.00000	7.36931	.513	-36.2530	12.2530
100 mg/kg	0mg/kg	74.80000*	7.36931	.000	50.5470	99.0530
	50mg/kg	31.50000*	7.36931	.011	7.2470	55.7530
	150mg/kg	1.50000	7.36931	1.000	-22.7530	25.7530
	200mg/kg	19.50000	7.36931	.134	-4.7530	43.7530
150 mg/kg	0mg/kg	73.30000*	7.36931	.000	49.0470	97.5530
	50mg/kg	30.00000*	7.36931	.015	5.7470	54.2530
	100mg/kg	-1.50000	7.36931	1.000	-25.7530	22.7530
	200mg/kg	18.00000	7.36931	.181	-6.2530	42.2530
200 mg/kg	0mg/kg	55.30000*	7.36931	.000	31.0470	79.5530
	50mg/kg	12.00000	7.36931	.513	-12.2530	36.2530
	100mg/kg	-19.50000	7.36931	.134	-43.7530	4.7530
	150mg/kg	-18.00000	7.36931	.181	-42.2530	6.2530

* The mean difference is significant at the 0.05 level.

Table B.7: Homogeneous Subsets of Tukey HSD for Root

Concentration	N	Subset for alpha = 0.05		
		c	b	a
0 mg/kg	3	84.7000		
50 mg/kg	3		128.0000	
200 mg/kg	3		140.0000	140.0000
150 mg/kg	3			158.0000
100 mg/kg	3			159.5000
Sig.		1.000	.513	.134

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

APPENDIX – C

1st Day of Treatment

40th Day of Treatment

Figure C.1: The Growth Process of *Ipomoea aquatica* in treatment black polybag.

Figure C.2: Segmentation of Plant to Distinguish Leaves, Shoots and Roots respectively.

Figure C.3: The Arrangement of Segmented Plant Parts for Oven.

APPENDIX – D

Figure D.1: The Feel Method to determine the Soil Texture of Red Soil Sample.

Figure D.2: The Grounding of Soil Sample by Pestle and Mortar into Fine Powder for XRF Analysis.

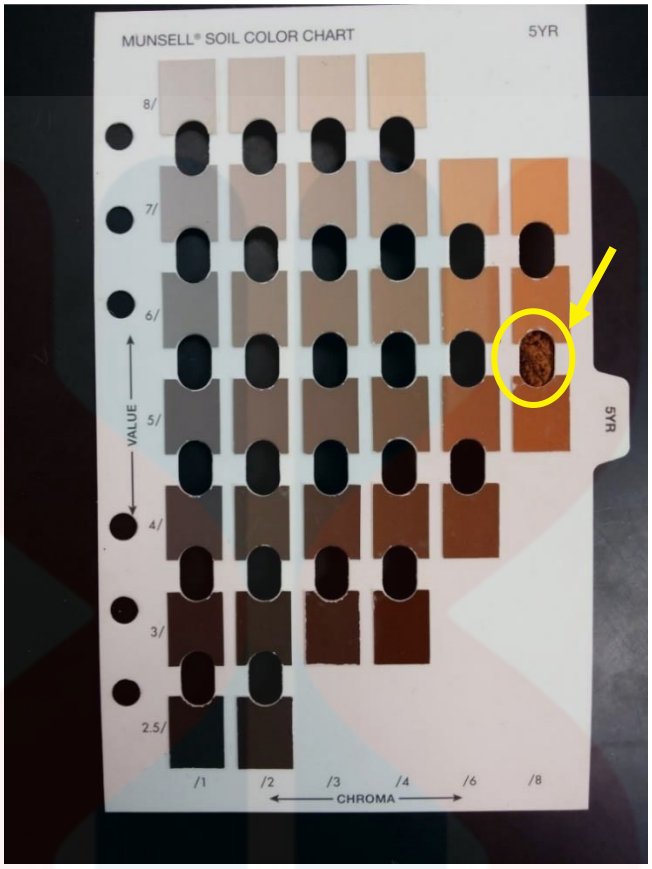
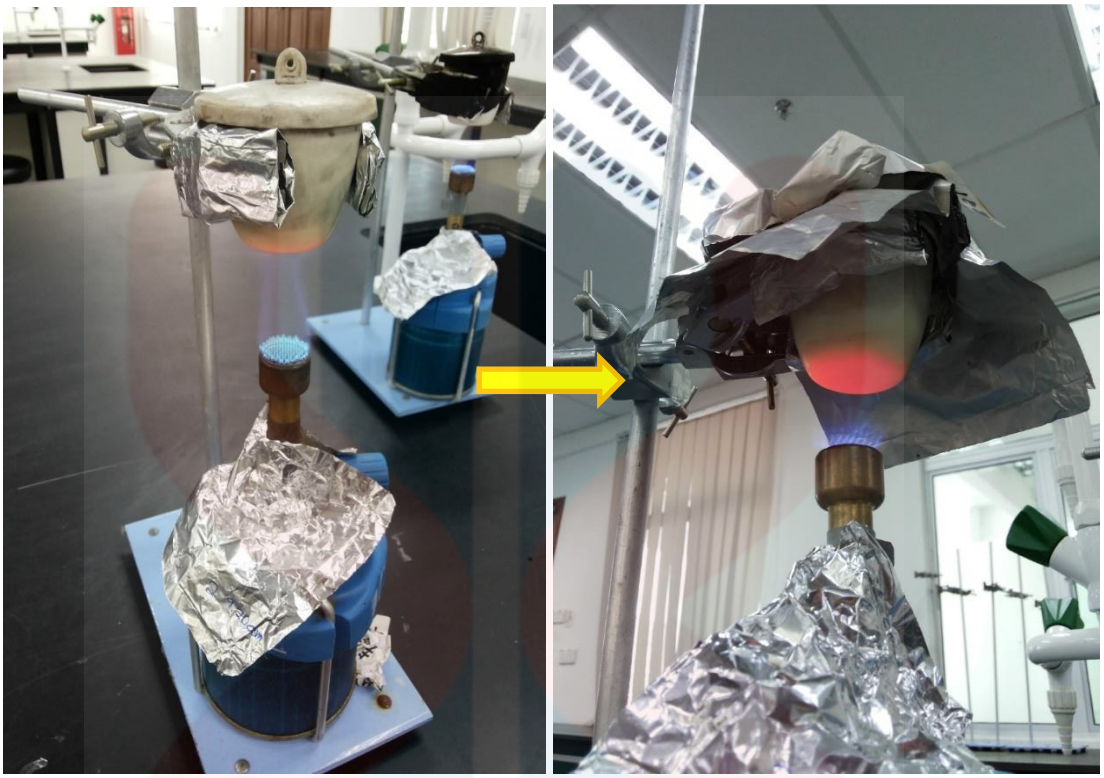


Figure D.3: The Comparison between the Colour of Fresh Red Soil with Munsell Colour Book.

Figure D.4: Soil pH Meter to determine the pH of Red Soil at AgroPark UMK Jeli.



1st Hour of Burning 4th Hour of Burning

Figure D.5: Bunsen Burner and Retort Stand Clamp Screw used to Determine the Organic Matter in the Red Soil.

Figure D.6: The 1-Stage Vacuum Pump used to Filter the Soil Sample in Determination of CEC by using Ammonium Acetate Method.

APPENDIX – E

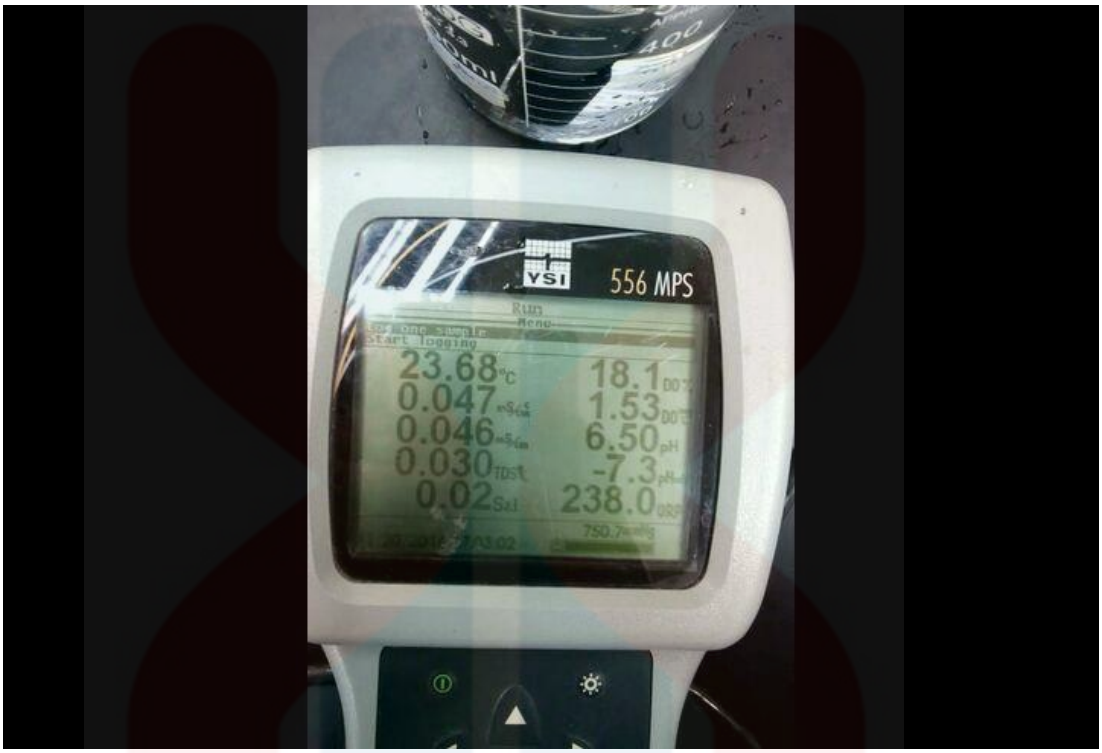


Figure E.1: YSI Multiparameter to Determine the Physical and Chemical Properties of Water Sample.