# PHYTOREMEDIATION POTENTIAL OF WATER SPINACH, *IPOMOEA AQUATIC* AND AQUAPONIC FARMING SYSTEM IN IMPROVING WATER QUALITY OF HEAVY METALS CONTAMINATED WATER RESOURCES

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# PHYTOREMEDIATION POTENTIAL OF WATER SPINACH, IPOMOEA AQUATIC AND AQUAPONIC FARMING SYSTEM IN RESTORING WATER QUALITY OF HEAVY METALS CONTAMINATED WATER RESOURCES

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Thesis submitted in fulfillment of the requirements

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Special dedication to my beloved father and mother,

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#### ABSTRACT

Water spinach, ipomoea aquatic which is grows largely in Southeast Asia, Southern China and India may contain heavy metals that come from their surroundings habitats. This study was conducted to determine the concentration of the heavy metals contain in water sample and water spinach sample after growing it in the aquaponic system for 50 days. The study also about the phytoremediation potential of water spinach, ipomoea aquatic in restoring water quality of heavy metals contaminated water resources. Two system of aquaponic system were built and using two different type of water which was one using normal water and one from the lake of Universiti Malaysia Pahang (UMP), Pekan Campus which contained metals such as Barium (Ba), Antimony (Sb) and Lead (Pb). To study the capability the water spinach, the plant been collected, dried, grinded and undergo wet digestion process before did the sample analysis. The presence of Lead (Pb) in water spinach sample showed that the plant is actually can absorb the metals contaminated in water sources instead of soil and actually the aquaponic system help in findings the relation in close scale farming system. The results also shows that, for industrial site and manufacturing site mostly is dangerous place to grows plants for consuming by people but suitable as a tools to clean up the nature that environmental friendly.

Key: heavy metals, aquaponic system, phytoremediation

#### ABSTRAK

Kangkung, Ipomoea akuatik yang tumbuh sebahagian besarnya di Asia Tenggara, Selatan China dan India mungkin mengandungi logam berat yang datang dari persekitaran habitatnya. Kajian ini dijalankan untuk menentukan kepekatan logam berat dalam sampel mengandungi air dan sampel kangkung selepas berkembang dalam sistem aquaponic selama 50 hari. Kajian ini juga mengenai potensi Fitopemulihan daripada kangkung, Ipomoea akuatik dalam memulihkan kualiti air dari sumber air yg tercemar dengan logam berat. Dua sistem sistem aquaponic dibina dan menggunakan dua jenis yang berbeza air yang merupakan satu daripadanya menggunakan air biasa dan satu lagi menggunakan air dari dalam tasik Universiti Malaysia Pahang (UMP), Kampus Pekan vang mengandungi logam seperti Barium (Ba), Antimoni (Sb) dan Lead (Pb). Mengkaji keupayaan kangkung, tumbuhan itu telah dikumpulkan, kering, dikisar dan menjalani proses penghadaman basah sebelum melakukan analisis sampel. Kehadiran Lead (Pb) dalam sampel kangkung menunjukkan bahawa tumbuhan sebenarnya boleh menyerap logam tercemar dalam sumber air selain daripada tanah dan sebenarnya sistem bantuan aquaponic dalam dapatan hubungan di dekat sistem pertanian tertutup. Keputusan juga menunjukkan bahawa, untuk tapak perindustrian dan tapak pengeluaran kebanyakannya adalah tempat berbahaya kepada tumbuh tumbuhan untuk dimakan oleh manusia tetapi sesuai sebagai alat untuk membersihkan kerana ia bersifat mesra alam sekitar.

Kata kunci: logam berat, sistem aquaponic, fitopemulihan

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# LIST OF SYMBOLS

g	-	grams
ppm	-	part per million
ppb	-	part per billion

# LIST OF ABBREVIATIONS

Ba	-	Barium
Sr	-	Antimony
Pb	-	Lead
DO	-	Dissolved Oxygen
E.C	-	Electrical Conductivity
TDS	-	Total issolved Oxygen

#### **CHAPTER 1**

#### **INTRODUCTION**

#### **1.1 BACKGROUND RESEARCH**

In this world, the nature has been polluted everyday and the solutions to overcome it are too slow than the pollution itself. The seriousness of pollutions becomes the major issues in all the countries around the globe with debating cases between them. The pollutions are continuing and started new problems like the global warming, flora and fauna extinctions. In the developing world, the industry become bigger each day and mostly this is become the major contributor in polluting the nature. This contributor is polluting mainly in the water resources and this is become serious issues since water is the source of life that needed by all organism and to balance the ecosystem.

Currently, the uses of biotechnology are widely developed in order to overcome many issues that rise. Many tools and application had been used to restore water quality of polluted water resources. In research history, there were many researches had been done to provide the information on how to cleanup or restoring the quality of polluted water which include the heavy metals for the better environment. The use of plant in phytoremediation is one of the best ways to be applied in this condition. The reason is mostly because of the ability of the plant in absorbing and restoring the pollutants in their biomass.

Plants have several technologies that include the process in handling the pollutant which are Phytoextraction, Rhizofiltration, Phytostabilization, Rhizodegradation, Phytodegradation and Phytovolatilization (Kadukova, Manousaki, Kokkali and Nikolopoupou, 2000). This is obvious to use plants as one of the nature

component to deal with the pollutions as easily grows and have great deal in balancing the environment by absorbing the pollutants (heavy metals) to be stored or transforming it to be release into the harmless form back to the nature.

Plants are been used widely in handling contaminant in land (soil) compared to water. Since the plants are the safest ways in handling contaminant, it becomes the alternatives to be used rather than others technology (Ullah, A., et al, 2015). Heavy metals as one of the elements that can be found in water and they will become treating to the nature if the content are too high. It is also have the effect on the water user or the drinker, by relating it with the research, there are the connection of the chronic disease with the contaminated water supply that contain the heavy metals (Hanaa, M., et al 2000).

Aluminium (Alzaimer's and Parkinson's disease), Arsenic (cancer), Cadmium (kidney damage), Lead (poison to human body) (as cited in Bakare-Odunola, M.T., 2005) and Mercury (effects the hearing, vision, speech and movements)(as cited in Hammer, M.J., and M.J. Hammer, Jr., (2004)), are several common heavy metals that have their own toxicity that will gives the serious problems if they been absorbs by water user (Momodu, MA., and Anyakora, CA. (2010)). Based on the many problems faces by all the country, researchers develop methods where can the heavy metals be removed from the water. Here, phytoremediation plays the important role in balancing the nature by applying their effecting mechanism in dealing with the heavy metals.

Hydroponic has been used widely and also help in restoring water quality become the reason of the research of phytoremediation in hydroponic system at first. The combination of these two are used because phytoremediation is widely used in soil or land, bring the method of using plant as the agent in handling heavy metals in contaminated water will become huge changes towards the nature. Generally, the hydroponic system work by pumping the water (containing nutrients) up from the fish tank at the bottom to the grow bed container to be use by the plants as the nutritional supply and making the suitable condition for the plants growth, then the water will flow back to fish tank through siphon at certain water level.

#### **1.2 PROBLEM STATEMENT**

In industrial area, lots of industrial in the world produce waste and pollutants towards the nature. The bigger problem is the industries pollute the water resources including the lake, river and even the ocean by releasing the waste. However there is no effective method that can be used in order to overcome and directly helping in restoring the water quality. The major problems in waste containing in water resources is the heavy metals that are hardly compound to be remove from water. In this case, the methods that had been accepted by humans are importance to be used such as aquaponics system. Aquaponicsystems that have directly contact with the water are believed to be able to restore the water quality by absorbing the heavy metals in its plants biomass. The ability of the plants in absorbing and restoring the water quality in aquaponic system will be observed. The detection of heavy metals in plants sample and water sample using inductively coupled plasma mass spectrometry (ICP-MS) for heavy metals contain in water sample will become major issue here.

### **1.3 RESEARCH OBJECTIVES**

- 1.3.1 To examine the water spinach ability in absorbing heavy metals of polluted water resources by analysing the heavy metals content in water and water spinach, *Ipomoea aquatic*
- 1.3.2 To assess the capability of water spinach, *Ipomoea aquatic* and aquaponic system in restoring natural water resources

## **1.4 SCOPE OF STUDY**

The main focus of this research is to study the phytoremediation potential of aquaponics farming system by using water spinach, *Ipomoea aquatic* for restoring water quality of polluted water resources. The methods used to study the phytoremediation potential are by the analysis of heavy metals content in the water and water spinach, *Ipomoea aquatic* in 50 days. The analysis of heavy metals content can be done by using inductively coupled plasma mass spectrometry (ICP-MS). The results in the heavy metals content analysis will be as an indicator of phytoremediation potential that been applied in hydroponic system.

#### **CHAPTER 2**

#### LITERATURE REVIEW

### 2.1 WATER SPINACH, IPOMOEA AQUATIC

Ullah, A., *et al* (2015), around 45 plant families of plants can tolerate with heavy metals including the families of Brassicaceae, Euphorbiaceae, Asteraceae, Fabaceae, Lamiaceae and Scrophulariaceae (as cited by Ghosh, M., Singh, S., 2005). From the research also, that 500 plant species can deal with the high amounts of heavy metals (as cited by Jaffré, T., Pillon, Y., Thomine, S., Merlot, S., 2013) and the most effective one is Thlaspicaerulescens (Alpine pennycress) and Alyssum bertolonii (as cited by Glick, B.R., 2010).

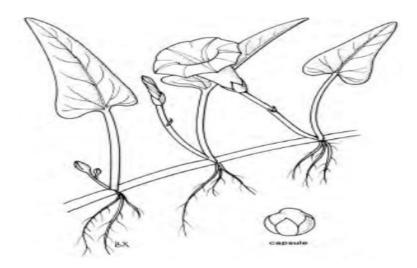


Figure 2.1: Water Spinach, *ipomoea aquatic* 

Source: Risk Assessment for Chinese Water Spinach (Ipomoea aquatica) in Oregon (Harwood & Sytsma, 2003) Water spinach, *ipomoea aquatic* is used traditionally in Cambodia as a vegetable for consumption by people and animals which it has short growth period and is resistant to common insect pests, (Samkol, 1997). It is a semi-aquatic vascular plant native that grow wild and some become food source in India, Southern China and Southeast Asia, (K.J. Umar, L.G. Hassan, 2007) (as cited by Gothberg, A., Greger, M., & Bengtsson, B. E. (2002)). Water spinach is the herbaceous plants that belong to Convolvulaceae family which has along and hollow stem, spread roots and this plant come from the nodes. For water spinach, it can grow in the waters that flows from domestic and other types of waste water which these waters may contain not only nutrients, but often also a wide variety of pollutants, such as heavy metals from various human activities, many people risk poisoning after consuming it, .

### **2.2. PHYTOREMEDIATION**

According to Etin, EE. (2012), phytoremediatioin is the best ways in order to prevent, control, and reduce the effects of the contamination of heavy metals in the nature. Phytoremediation is the use of green plants to remove, stored, degrade or detoxifying the harmful environmental pollutants before it been released back to the nature. This includes the ability of phytoremediation as one of the safest methods in include handling water contaminant which Phytoextraction, Rhizofiltration, Phytostabilization, Rhizodegradation, Phytodegradation Phytovolatilization and (Kadukova, Manousaki, Kokkali and Nikolopoupou, 2000) as shown in Figure 2.2.

# Phytoremediation processes

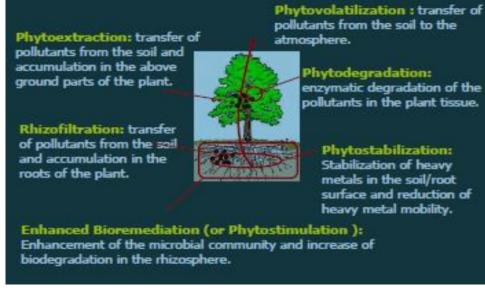


Figure 2.2: Phytoremediation process

Source :Kadukova, Manousaki, Kokkali and Nikolopoupou, 2000

According to Ullah, A., *et al.*, 2015, the most effective method by using the plants is using the plants growth promoting bacteria that will help in degrading the heavy metals. The help by the bacteria will help in transforming the heavy metals to the less harmful form and will be use by the plants to growth. From various researches, the phytoremediation is safe method to be used and well accepted by the human as it does not harmful to the nature and see as the best ways in handling the contaminant including the water resources.

#### **2.3 AQUAPONIC SYSTEM**

Aquaponics is a method of growing green plants in a water solution without soil that is also known as hydro culture, water culture or soil-less culture (Berndsen, C., Gardener, M. (2014)). The system does not using the soil as a medium for growth. For many years, the hydroponic system used the hydroton (clay) to replace soil as it have it owns benefit which it can contain water for longer time and be able to contain bacteria inside of it.

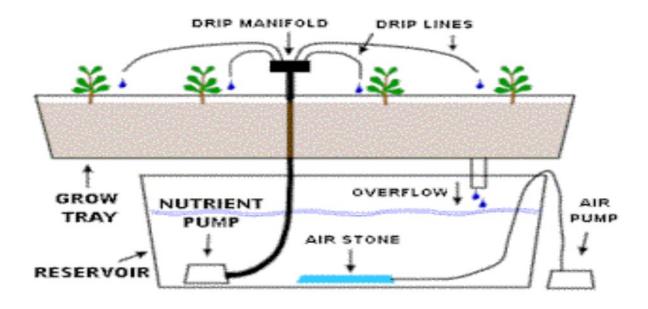


Figure 2.3: Drip aquaponics system which will drip water after pump it from fish

Source: Basic Hydroponics System http://www.simplyhydro.com/hydrou.htm

From Figure 2.3, hydroponic is system using the water to be pump flow into growth medium by water pump. The medium containing the hydroton will filter the water and water will flow back to the container. The medium containing the hydroton is actually medium that been able to filter the water and trap water to keep the humidity of medium and also high in containing oxygen. The hydroton also act as the medium to hold the plant as it can grow upright. By doing this o replace the soil, the water will

flow back to the container with clear after been filtrate by the hydroton. The waste produce and heavy metals will be filter by the plants as it been absorb and used for growth or be release back to the nature in other form. The aquaponics system is chosen because it is promises in flow the water to the plants and medium by cycling the water system during running.

#### 2.4 COMBINATION OF PHYTOREMEDIATION AND AQUAPONIC SYSTEM

Phytoremediation is been study in handling heavy metals mostly in soil. Heavy metal poisoning due to contamination of groundwater, surface water, and soil has become a serious global issues (Wang, Forzani, & Tao, 2007). The research of the phytoremediation in dealing with heavy metals been done by using various type of plant. According to Ullah, A., *et al* (2015), the phytoremediation potential can be increase by using plant growth promoting (PGP) bacteria in the system which is been able to transform metals into bio available and soluble forms through several action. PGP also improve plant growth and increase plant biomass by using the product from the process to be used as the source of its own nutrients.

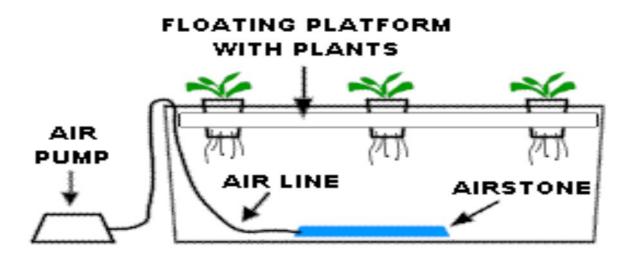


Figure 2.4: Basic Floating farming system

Source: Basic Hydroponics System http://www.simplyhydro.com/hydrou.htm The research is to combine the two technologies that are phytoremediation and the aquaponics system. The example of the floating farming system is just like the Figure 5, combination between two technologies that will help in restoring water quality. The working is of aquaponics are still the same but the use plants here are as the main elements that will store the quality of water resources. According to Putra, Cahyana, &Novarita (2014), the using of hydroponic EAPR system which is the combination between electro-assisted system and hydroponic phytoremediation are used to remove lead and copper by water lettuce (*Pistiastratiotes*Linn.). In this research, the results been compared and from the results, they get the high accumulation of the lead and copper is higher in the roots.

In conclusion, the combination of two different systems, phytoremediation and hydroponic basically can give a good results in restoring water quality even though it is related to the heavy metals problems.

#### **2.5 HEAVY METALS**

According to Duruibe*et al.*, 2007, the poison, high density and toxicity of the heavy metals is the reason why even the small amount of them in the nature is still be danger to the water consumer (as cited by Lenntech,2004). They also stated that the emission of the heavy metals majorly are causes by mining activities (as cited by Hutton M, Symon C (1986)) and the contribution of mercury in the nature is the cosmetics products. The exposures of humans to the heavy metals are mostly from the food, water, air, industrial products and occupation exposure.

In this nature, according to Momodu and Anyakora, 2010, water is the element that is impure due to the contamination by its surrounding environment including the heavy metals and others waste causes by the users (as cited by Mendie, U., 2005). Here also they stated that even in low concentration in the water, they still have the high toxicity (as cited by Marcovecchio, J.E., S.E. Botte and R.H. Freije, 2007) and become the problems to the nature (ground water contamination issues) (as cited by Vodela et al., 1997).

Yap *et al* (2009), the reason of pollutions in the environment by the heavy metals are causing by the human activities including the agriculture, mining, construction and through the industries In this research, they had been using the paddy plants in study the uptake of the heavy metals which include cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), lead (Pb) and zinc (Zn).

From the several researches, there are few methods in identifying and detecting the heavy metals in plants. According to Wang, S. Forzani and Tao (2007), the detection is by using the high-resolution surface Plasmon resonance spectroscopy with the combination of Anodic Stripping Voltammetry. Different with that method, by using flame atomic adsorption spectrophotometer, Syah, Cahyana and Novarita (2014) and atomic absorption spectrophotometer (AAS), Yap *et al* (2009) been able to detect the heavy metals concentration. These techniques require the cut of different part of the plants for example shoots and roots before the detection of heavy metals been read. The heavy metals in water generally been study by taking the water sample trough out the lake or the river generally. By the combination of the phytoremediation and the use of plants, the data will be obtain by analysis the concentration of the heavy metal containing in the biomass of the plants, roots, shoots and stem inductively coupled plasma mass spectrometry (ICP-MS) because of its capability to detects several metals in the sample and can detect the quantity from lower range, ppb to high range, ppm, (Bazilio & Weinrich, 2012).

## CHAPTER 3

## METHODOLOGY

## **3.1 DESCRIPTION OF METHADOLOGY**

## 3.1.1 Aquaponics System Set up

Aquaponic farming system had been set up to include fish tank, water pump, grow bed, electronic circuit and hydroton (clay) to replace the soil. The grown water spinach, *ipomoea aquatic* were grown for one week on the grow bed before starting the day 0 (1<sup>st</sup> harvest), 7<sup>th</sup> September 2016 and system ran for 50 days before harvesting for second time, 26<sup>th</sup> October 2016 and did the analysis on heavy metal contain.





Figure 3.1.1: Two aquaponic farming system in contact with water from the tanks by using pump and ran for 50 days

The aquaponic system was setup and placed at the greenhouse of University Malaysia Pahang (UMP), Gambang Campus. There were two aquaponics system build which was one for control that used normal water and one for sample where the water was taken from one of the lake water located at UMP Pekan Campus. The two tanks were filled up with these types of water and 15 fish were placed at both of the system.

## **3.1.2 Water Quality Analysis**

The water quality analysis for the two aquaponic system were done for five (5) weeks starting from Day 0 where first, 1<sup>st</sup> harvest been done until Day 50 for second, 2<sup>nd</sup> harvest. There were five (5) data analysis were measured for systems which were pH, dissolve oxygen (DO), total dissolved solids (TDS), electrical conductivity (E.C.) and salinity.

### **3.1.3 Water Sample Collection**

At Day 0 for first, 1<sup>st</sup> harvest, the water samples also were taken for both aquaponic systems. The samples were taken and were sent to central laboratory of UMP Gambang Campus for heavy metals detection by using inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS can detects multiple type of heavy metals contain in water samples. The step was repeated ad Day 50, during the second, 2<sup>nd</sup> harvest of water spinach, *ipomoea aquatic*.



Figure 3.1.3: Water sample collection from two aquapponic systems for heavy metals detection using ICP-MS

#### **3.1.4 Sample Collection and Preparation**

The samples collection and preparation were made as preparation before it can be used using atomic inductively coupled plasma mass spectrometry (ICP-MS) to detect the heavy metals contain in the plant. The samples were collected was the roots, shoots and the stem from the water spinach, *ipomoea aquatic* during first harvest (Day 0) and second harvest (Day 50) according to The samples will be dried for one week after harvest in the oven and were grind using mortar and pastel.



Figure 3.1.4: Samples of water spinach, *ipomoea aquatic* from harvest, dried and after grind

The samples were digested using wet/acid digestion method, (Sukender, Jaspreet, Sneha, & Munish, 2012). 10 ml of nitric acid, HNO3 was added to 2g of weight sample in 100 ml beaker. The mixture was heated on hot plate at 95°C for 15 minutes. The mixture next will be cooled down and was added with 5 ml of concentrated nitric acid, HNO3 and heated for 30 minutes at 95°C. The last step was repeated until the solution was reduced to 5 ml without boiling. The sample then will be cooled down and 2 ml deionized water and 3

ml of 30% hydrogen peroxide, H2O2 were added. The beaker were covered with aluminium foil and mixed gently to start peroxide reaction. The samples then were removed from the hot plate and if the sample shows the effervescence and add again 30% hydrogen peroxide, H2O2 during heating until it disappear. 5 ml of hydrochloric acid, HCl and 10 ml deionized water were added and heated for another 10 minutes without boiling. The samples were cooled and filtered using filter paper and did dilution up to 50 ml.

## 3.1.5 Sample Analysis

After the sample and standard preparation, the samples undergo the sample analysis process for heavy metals detection. This step was using the inductively coupled plasma mass spectrometry (ICP-MS) to detect the heavy metals which was been selected (Lead (Pb)).

## **3.2 FLOW CHART AND RESEARCH ACTIVITIES**

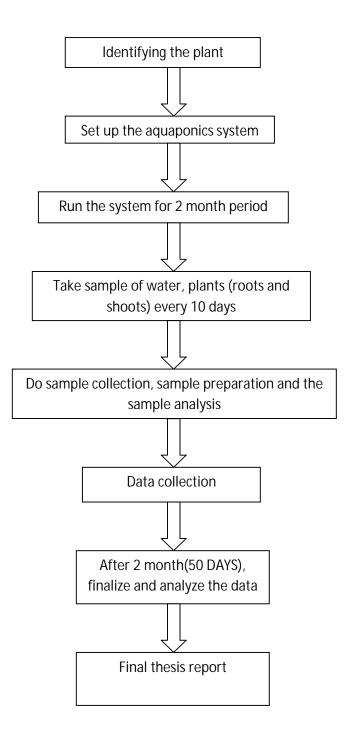


Figure 3.2: Flowchart of research activity

## **3.3 GANTT CHART OF RESEARCH ACTIVITIES**

PROJECT	M1	M2	M3	M4	M5	M6	M7	<b>M8</b>	M9	M10	M11	M12
ACTIVITIES												
1) Literature												
survey												
2) Set up the												
Aquaponic system												
3) Run the system												
4) Do sample												
collection, sample												
preparation and the												
sample analysis												
sumpre unuryons												
5) Data collection												
and analysis data												
6) Final thesis												
report												

Table 3.3: Gantt chart of Research Activities

#### **CHAPTER 4**

#### **RESULTS AND DISCUSSION**

#### 4.1 WATER ANALYSIS USING ICP-MS

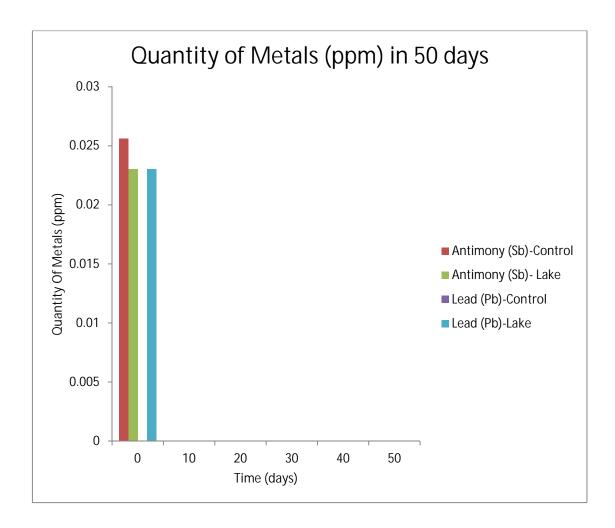
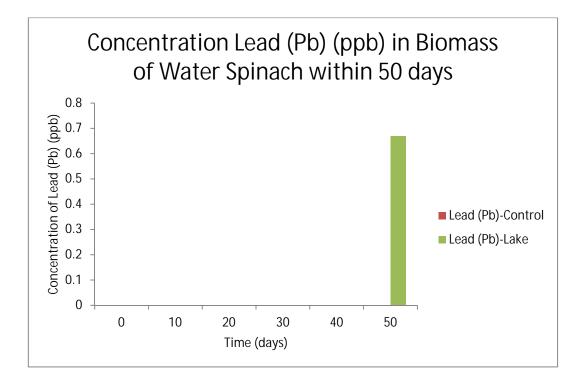


Figure 4.1: Quantity of Metals in Water using ICP-MS

Based on the water analysis at Day 0 and Day 50 by using inductively coupled plasma mass spectrometry (ICP-MS) for the both water sample which is for control and lake water, there are list of few metals that found during Day 0 and after Day 50. At Day 0, Antimony (Sb) and Barium (Ba) were presence in the both type water sample but for lake water, it containing the Lead (Pb), 21.9 ppb. At Day 50 of water sample, there are changes happen in the quantity of metals which is for Antimony (Sb), it decrease from 25.6 ppb to 0.00 ppb for control water and for the lake water, the quantity is also decrease from 23.0 ppb to 0.00 ppb. Next, for Lead (Pb), the quantity also decreased for the lake water quantity from 21.9 ppb to 0.00 ppb. Unfortunately, the quantity of Barium (Ba) increase for both water samples which for control from 327.0 ppb to 9.8 ppm and for lake water from 398.5 ppb to 12.6 ppm might cause by the external factors.

The decrease of metals in the water is because of the phytoremediation process done by the plant, water spinach, *ipomoea aquatic* which be able to absorb heavy metals in water culture and it remediate the heavy metals and stored in it biomass (Sun & Wu, 1998). Water spinach had been able to absorb the water that contain the heavy metals into its system or change the heavy metals to more harmless form back to the nature because according to AbidUllah et al., 2015, the most effective method by using the plants is using the plants growth promoting bacteria that will help in degrading the heavy metals. For the Lead (Pb) quantity which is among the harmful heavy metals is decrease, it shows by the results, water spinach can be use to as the tools to clean the water source but it is harmful for human to consume it (Gothberg, Greger, & Bengtsson, 2002).

#### 4.2 WATER SPINACH ANALYSIS BY USING ICP-MS



# Figure 4.2: Concentration of Lead (Pb), ppb in Biomass of Water Spinach within 50 days

Based on the results, we can see that the connection of the element of Lead (Pb) that contain in water spinach and the water analysis from Table 4.0. the Lead is been chosen because of the presence in lake water from one of the lake in UMP Pekan Campus, and based on its characteristics that characterize as one on the harmful heavy metals and can course cancer, (Salem *et al.*, 2000). In Figure 4.2, we can see only the lake water on Day 50 contained Lead (Pb). The relation between the results between Figure 4.0 is the decreasing amount of the Lead (Pb) in the water but increase in the plant biomass of water spinach.

From the observation, within 50 days, the water spinachable to absorb metals contained in the lake water and the results shows by analysis. The ability of the water spinach to absorb heavy metals is proven when it is contain high amount of element such as the Lead and Cadmium in Laguna de Bay, (Baysa, Anuncio, Chiombon, Dela Cruz, & Ramelb, 2006).

#### **4.3 WATER QUALITY ANALYSIS**

#### 4.3.1 Dissolve Oxygen (DO)

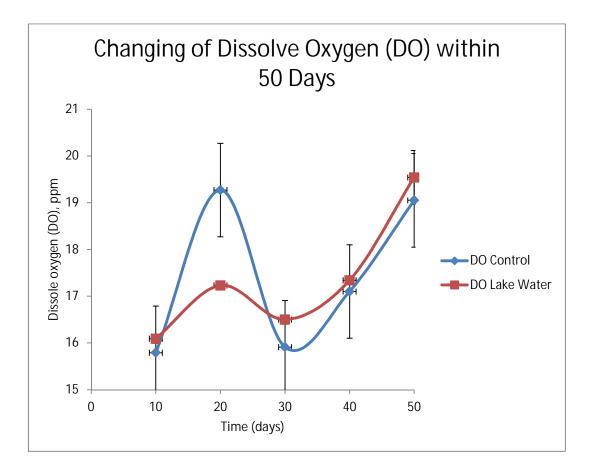


Figure 4.3.1: Dissolve Oxygen (DO) Values within 50 days

Based on the Figure 4.3.1, there is change in the Dissolve Oxygen values within 50 days and it because of the environmental factors such as the photosynthesis of the algae, use of oxygen by fish, addition of water cause by rain and temperature of the water, (Lewis, 2006). Based on the observation, from the algae that cause the water turn green and the addition of water level cause by rain are the contributor for the DO changes in the both system. The water become clearer from day 0 in day 50 also shows the decreasing amount of algae in both systems.



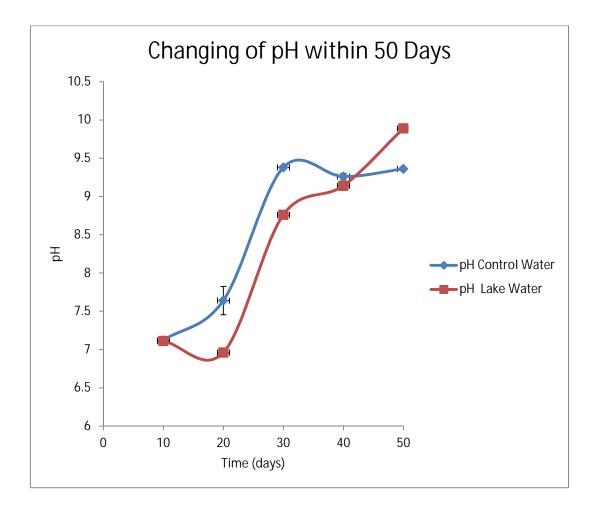


Figure 4.3.2: pH Values within 50 days

From the Figure 4.3.2, most of the pH values during the 50 days of the observations are within the range of normal pH value which is pH 6.5 to pH 9.0, and the exceeding pH above the pH 9.0 is because of the lower presence carbon dioxide, CO2 in the water causing by lower photosynthesis, (Wurts & Durborow, 1992), causing the increasing in pH value.

#### 4.3.3 Electrical Conductivity (E.C)

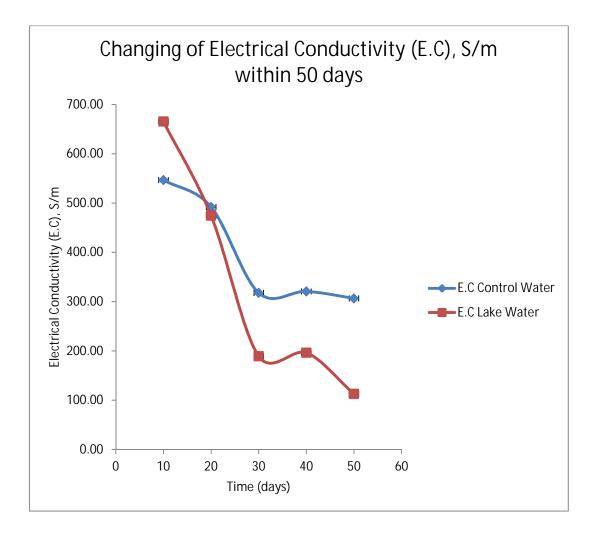


Figure 4.3.3 Electrical Conductivity (E.C) Values in Five Weeks

Based on the Figure 4.3.3, the decreasing of electrical conductivity within 50 days is because of the increasing of water level in the fish tank due to rain ad rain water containing the low electrical conductivity, (US Environmental Protection Agency, 2013). The water in the tanks getting raised by days because of the tanks is open.

#### 4.3.4 Total Dissolve Solids (TDS)

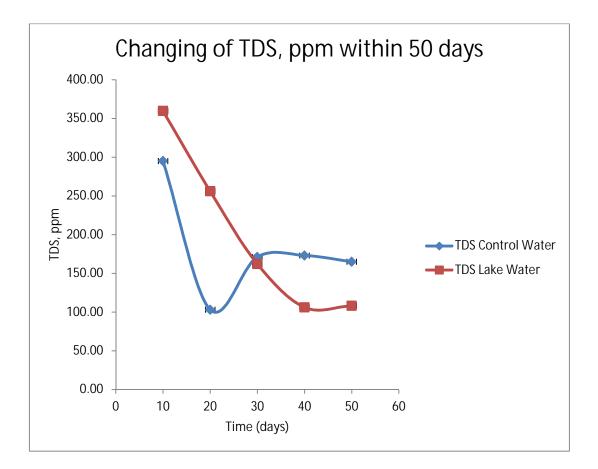
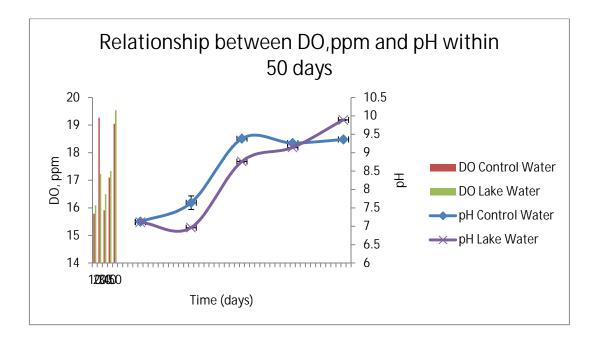


Figure 4.3.4: Total Dissolved Solids (TDS) Values in Five Weeks

From Figure 4.3.4, generally the total dissolved solids (TDS) is decreasing due to same factor as the electrical conductivity (E.C) which is the increase of the water level that lowering the values, (WHO, 1996). The increase of water level lowering its concentration in both aquaponic systems thus affects the TDS values.

#### 4.3.5 Relation between DO and pH Values



#### Figure 4.3.5: Relationship DO and pH Values

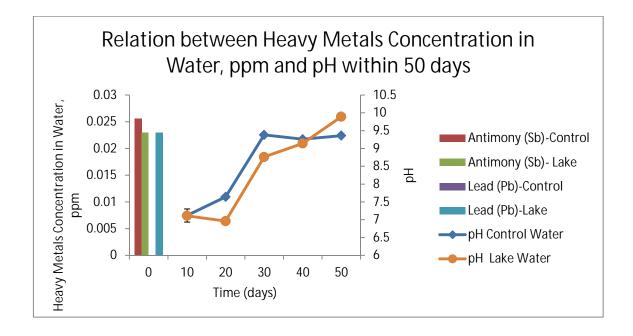
Table 1. Relative concentration changes for dissolved oxygen, carbon dioxide and pH in ponds over 24 hours.						
		Change				
Time	Dissolved Oxygen	Carbon Dioxide	pН			
Daylight	Increases	Decreases	Increases			
Nightime	Decreases	Increases	Decreases			

Table 4.3.5: Relation	betweeen	the DO	and pH values	5
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# Source: Interactions of pH, Carbon Dioxide, Alkalinity and Hardness in Fish Ponds by Wurts&Durborow, 1992

Based on the Figure 4.3.5 and Table 4.3.5, according to Wurts &Durborow, 1992, the increase of the DO values is directly proportional to the increasing of the pH value and the results also shows the relation for both data.

# 4.4 RELATION BETWEEN HEAVY METALS CONCENTRATION, PPM IN WATER, Pb IN BIOMASS OF WATER SPINACH AND pH



# Figure 4.4 (a): Relation between Heavy Metals Concentration in Water, ppm and pH within 50 days

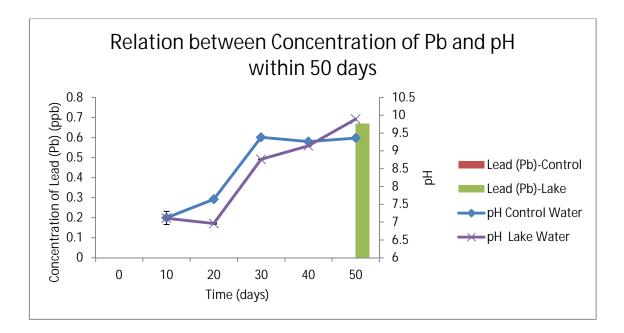


Figure 4.4 (b): Relation between Concentration of lead (Pb) in biomass of water spinach, ppb and pH within 50 days

Based on the Figure 4.4 (a), in water for both water samples, the concentration of metals was decreasing within time and reverse from the pH value which is increased within 50 days. According to Wurts and Durborow, 1992, the pH is affecting the removal of metals contained in water where at certain pH, the metals contained been remediated by plants. In this case, the metals contain in water was decreased but increased in the biomass of the water spinach especially for lead Pb which 0.67 ppb contain in the biomass.

According to Figure 4.4 (b), the Tangahuet al., 2011, there is certain pH for plants to uptake the metals into its biomass by phytoremediation. In this case, the changing in pH is because of the phytoremediation which is lowering the metals contained in water but increased in biomass of water spinach especially lead, Pb. In conclusion, the higher pH is suitable for the uptake for the heavy metals by water spinach from water.

4.5 RELATION BETWEEN HEAVY METALS CONCENTRATION, PPM IN WATER, Pb IN BIOMASS OF WATER SPINACH AND E.C, S/m

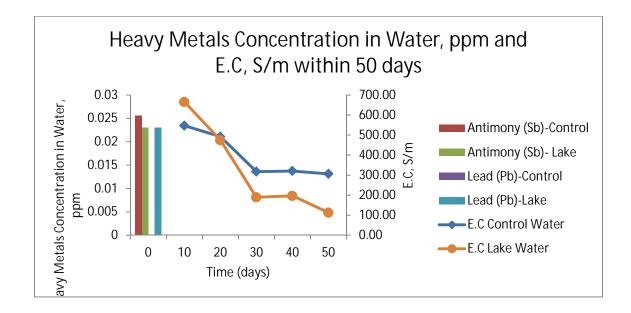


Figure 4.5 (a): Relation between Heavy Metals Concentration in Water, ppm and E.C, S/m within 50 days

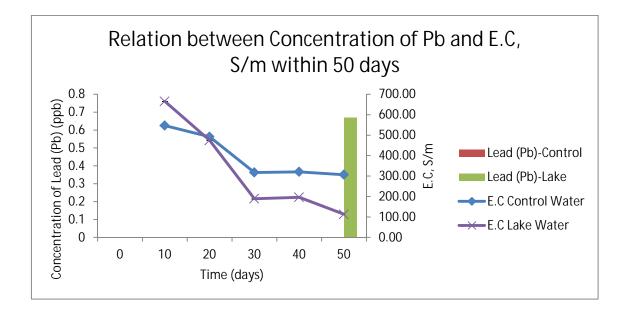


Figure 4.5 (b): Relation between Concentration of lead (Pb) in biomass of water spinach, ppb and E.C, S/m within 50 days

Based on the Figure 4.5 (a), the electrical conductivity of the water was decreased in the control water, 43.9% and for the lake water was 83% as the metals contained in the water also decreased because of the ion particles interaction between the metals ion in the water become lower, Etin, EE. (2012). For the Antimony (Sb) and Lead (Pb), the concentration of these metals getting decreased and become 0 ppm within 50 days which is related to the phytoremediation by the plant, water spinach that use its potential in absorbing metals into its biomass.

According to Harwood and Systama, 2003, the researcher study the risk of the Chinese Water Spinach (Ipomoea aquantica) in Oregon which results was the presence of metals contaminant in the water as well as the in the plants. They also study the effects on the electrical conductivity of the water which containing high metals at the beginning and after the phytoremediation which was deceased by time as the metals decreased in the water. In this case, the water spinach absorb the Lead (Pb) into its biomass might contribute to the decreasing of the electrical conductivity of the water in the both aquaponic tanks.

# 4.6 RELATION BETWEEN HEAVY METALS CONCENTRATION, PPM IN WATER, Pb IN BIOMASS OF WATER SPINACH AND TDS, PPM

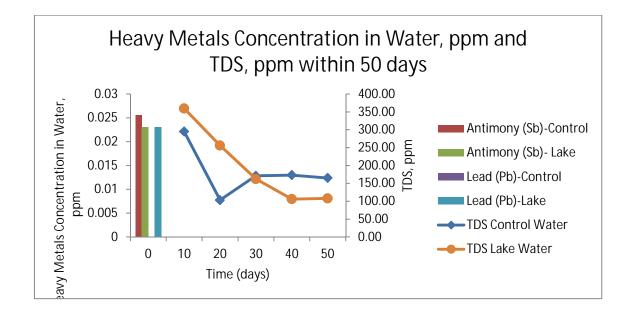


Figure 4.6(a): Relation between Heavy Metals Concentration in Water, ppm and TDS, ppm within 50 days

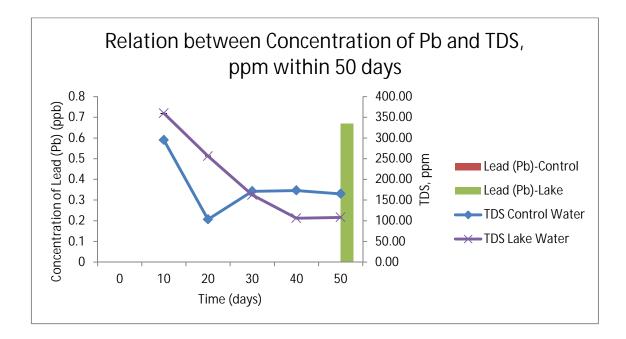


Figure 4.6 (b): Relation between Concentration of lead (Pb) in biomass of water spinach, ppb and TDS, ppm within 50 days

Based on the Figure 4.6 (a) and (b), the concentration of the total dissolve solids, TDS, ppm decreased for both of the water which is control water decreased almost 44% and for lake water was decreased about 70% of its total dissolved solids. The reason is also because of the effects of the heavy metals removals from the water in both aquaponic farming systems. Ions of the metals affecting the presence of the total dissolved solids at the beginning but become decreased by days as it had been adsorb into water spinach biomass by the phytoremediation process, Kodukova, Manousaki, Kokkali and Nikolopoupou, 2000.

#### **CHAPTER 5**

#### **CONCLUSION AND RECOMMENDATION**

#### **5.1 CONCLUSION**

This thesis help me in research and analysis the process from built the aquaponic system in contact with water resources until the analysis the data related based on the results obtain. The development of aquaponics system required the hard work outdoor activities consisting the combination of two system which is aquaponics system and phytoremediation system. Two complete cycle of aquaponics system were built to study the relation of the phytoremediation in the aquaponics systems.

By using water spinach, *ipomoea aquatic* on the aquapnic system to study the phytoremediation process occurred in the two system which using the two different water source, one using the normal water and one using the lake water taken from one of the lake from UMP Pekan Campus. The relationship of phytoremediation and the aquaponic system studied is achieved when there is different in the elements contain at Day 0 and Day 50. Some of metals including the Barium (Ba) and Lead (Pb) are decreased in water and for lead presence in plant sample for lake water approved the capability of the aquaponic system in restoring the natural water resources.

#### 5.2 **RECOMMENDATION**

For future studies, the manipulated variables should be considered including the environmental factors. The addition of water control level variables on the aquaponic farming system should give attention to it because it gives many different and might be the contributing for the valid data. For water quality analysis, the temperature should be includes in the data as it is the major contributor of the adsorption of heavy metals in the plants and removals of heavy metals in the water.

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#### APPENDICES

# APPENDIX A

# Table A: The Metals Elements Presence in Water Samples at Day 0 and Day 50

ELEMENT	Quantity of Metal (ppm)					
	Day 0		Da	y 50		
	Control water	Lake water	Control water	Lake water		
Antimony	0.0256	0.023	0.00	0.00		
(Sb)						
Lead (Pb)	0.00	0.0219	0.00	0.00		

#### **APPENDIX B**

# Water Spinach weight

# Table B: Water Spinach Weight (g)

Weight	Water Spinach Weight (g)					
	Day 0		Day	y 50		
	Control water	Lake water	Control water	Lake water		
Wet	13.9	12.6	15.7	13.9		
Dry	3.9	2.9	5.2	4.15		

#### **APPENDIX C**

ELEMENT	Quantity of Metal					
	Day 0		Day 50			
	Control water	Lake water	Control water	Lake water		
Lead (Pb)	0.00 ppb	0.00 ppb	0.00 ppb	0.67 ppb		

# TABLE C: Metal Contain in Water Spinach Samples from Both System atDay 0 and Day 50

# APPENDIX D

# Water Quality Analysis

Dissolve oxygen (DO)

# 1) Control water

Data	Data 1	Data 2	Data 3	Average	StdDev
Weeks					
1	16.02	15.62	15.72	15.79	0.21
2	19.23	19.29	19.28	19.27	0.03
3	15.92	15.89	15.91	15.91	0.02
4	17.12	17.10	17.08	17.10	0.02
5	19.19	19.01	18.95	19.05	0.02

Data	Data 1	Data 2	Data 3	Average	StdDev
Weeks					
1	16.16	16.12	15.98	16.09	0.1
2	17.24	17.23	17.22	17.23	0.0
3	16.53	16.58	16.39	16.50	0.0
4	17.32	17.31	17.38	17.34	0.0
5	20.20	19.15	19.26	19.54	0.6

# 1) Control water

Data	Data 1	Data 2	Data 3	Average	StdDev
Weeks					
1	7.12	7.12	7.12	7.1	0.00
2	7.48	7.59	7.84	7.6	0.18
3	9.35	9.40	9.39	9.4	0.03
4	9.28	9.21	9.28	9.3	0.04
5	9.36	9.38	9.35	9.4	0.02

Data	Data 1	Data 2	Data 3	Average	StdDev
Weeks					
1	7.09	7.12	7.12	7.11	0.02
2	6.97	6.96	6.96	6.96	0.01
3	8.76	8.77	8.74	8.76	0.02
4	9.14	9.14	9.15	9.14	0.01
5	9.90	9.88	9.89	9.89	0.01

# Electrical Conductivity (E.C)

#### 1) Control water

Data	Data 1	Data 2	Data 3	Average	StdDev
Weeks					
1	546.5	546.7	546.8	546.67	0.15
2	491.9	491.9	491.9	491.90	0.00
3	317.6	317.6	317.6	317.60	0.00
4	320.7	320.7	320.7	320.70	0.00
5	306.5	306.5	306.5	306.50	0.00

Data	Data 1	Data 2	Data 3	Average	StdDev
Weeks					
1	663.3	666.5	666.7	665.50	1.91
2	474.5	474.5	474.5	474.50	0.00
3	189.2	189.1	188.9	189.07	0.15
4	196.3	196.3	196.3	196.30	0.00
5	112.6	112.7	112.8	112.70	0.10

# Total Dissolve Solids (TDS)

#### 1) Control water

Data 1	Data 2	Data 3	Average	StdDev
295	295	295	295	0
103	103	103	103	0
171	171	171	171	0
173	173	173	173	0
165	165	165	165	0
	295 103 171 173	295       295         103       103         171       171         173       173	295295295103103103171171171173173173	295         295         295         295           103         103         103         103           171         171         171         171           173         173         173         173

Data	Data 1	Data 2	Data 3	Average	StdDev
Weeks					
1	360	359	360	359.67	0.57735
2	256	256	256	256.00	0
3	162	162	162	162.00	0
4	106	106	106	106.00	0
5	108	108	108	108.00	0

# Salinity

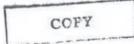
# 1) Control water

Data	Data 1	Data 2	Data 3	Average	StdDev
Weeks					
1	0.26	0.26	0.26	0.26	0
2	0.22	0.22	0.22	0.22	0
3	0.15	0.15	0.15	0.15	0
4	0.15	0.15	0.15	0.15	0
5	0.14	0.14	0.14	0.14	0

Data	Data 1	Data 2	Data 3	Average	StdDev
Weeks					
1	0.26	0.26	0.26	0.26	0
2	0.22	0.22	0.22	0.22	0
3	0.00	0.00	0.00	0	0
4	0.00	0.00	0.00	0	0
5	0.15	0.15	0.15	0.15	0

CENLAB / F / 007





CENTRAL LABORATORY Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Kuantan, Pahang Darul Makmur, Tel : 09-5493351 Fax : 09-5493353 E-mail : ucl@ump.edu.my

# **CERTIFICATE OF ANALYSIS (COA)**

To / Attn	FIST/Azam			
Address	FIST, Universiti Mala	iysia Pahang		
Tel No	-	Fax No		
Sample Lab No	2016/335	No of sample	2	

Sample marking Sample description Date of sample received Date reported

2016/335(15) \$1 Control 25-08-2016 09-09-2016 ----

#### RESULTS:

No	Parameter	Results	Unit	Test Method
1,	Beryflium (Be)	Not Detected (Less than 0.5)	dqq	CENLAB/WI/CHEM-TM/008
2.	Sodium (Na)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
3.	Magnesium (Mg)	(Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
4.	Aluminium (Al)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
5.	Pottassium (K)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
6.	Calsium (Ca)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
7.	Vanadium (V)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
8.	Chromium (Cr)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
9,	Manganese (Mn)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
10.	Iron (Fe)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
11.	Cobalt (Co)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
12.	Nickel (Ni)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
13.	Copper (Cu)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
14.	Zinc (Zn)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

2016/355 Page 1 of 3

15.	Arsenic (As)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
16.	Selenium (Se)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
17.	Molybdenum (Mo)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
18.	Silver (Ag)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
19,	Cadmium (Cd)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
20.	Anfimony (Sb)	25.6	ppb	CENLAB/WI/CHEM-TM/008
21.	Barium (Ba)	327.0	ppb	CENLAB/WI/CHEM-TM/008
22.	Lead (Pb)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

Sample marking Sample description Date of sample received Date reported 2016/335(16) S2 Lake 25-08-2016 09-09-2016

#### RESULTS:

.

No	Parameter	Results	Unit	Test Method
1.	Beryllium (Be)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
2.	Sodium (Na)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
3.	Magnesium (Mg)	(Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
4.	Aluminium (Al)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
5.	Pottassium (K)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
6.	Calsium (Ca)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
7.	Vanadium (V)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
8.	Chromium (Cr)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
9.	Manganese (Mn)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
10.	Iron (Fe)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
11.	Cobalt (Co)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
12.	Nickel (Ni)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
13.	Copper (Cu)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

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22.	Lead (Pb)	21.9	ppb	CENLAB/WI/CHEM-TM/008
21.	Barium (Ba)	398.5	dad	CENLAB/WI/CHEM-TM/008
20.	Antimony (Sb)	23.0	ppb	CENLAB/WI/CHEM-TM/008
19.	Cadmium (Cd)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
18.	Silver (Ag)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
17.	Molybdenum (Mo)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
16.	Selenium (Se)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/0081
15.	Arsenic (As)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
14.	Zinc (Zn)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

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2016/355 Page 3 of 3 CENLAB / F / 007



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# **CERTIFICATE OF ANALYSIS (COA)**

To / Attn	FIST		
Address	FIST, Universiti Malaysia	Pohona	
Tel No	-	Fax No	
Sample Lab No	2016/335 (32-33)	No of sample	-
	1	No or sumple	2

Sample marking
Sample description
Date of sample received
Date reported
Date reported

2016/335(32) Control 04-11-2016 09-11-2016

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RESULTS:

No	Parameter	Results	Unit	Test Method
1.	Beryllium (Be)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
2.	Sodium (Na)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
3.	Magnesium (Mg)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
4.	Aluminium (AI)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
5.	Pottassium (K)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
6.	Calsium (Ca)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
7.	Vanadium (V)	89.4	ppb	CENLAB/WI/CHEM-TM/008
8.	Chromium (Cr)	Not Detected (Less than 0.5)	dqq	CENLAB/WI/CHEM-TM/008
9.	Manganese (Mn)	Not Detected (Less than 0.5)	dqq	CENLAB/WI/CHEM-TM/008
10.	Iron (Fe)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
11.	Cobalt (Co)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
12.	Nickel (Ni)	Not Detected (Less than 0.5)	dqq	CENLAB/WI/CHEM-TM/008
13.	Copper (Cu)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
4.	Zinc (Zn)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

2016/355

15.	Arsenic (As)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
16.	Selenium (Se)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
17.	Molybdenum (Mo)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
18.	Silver (Ag)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
19.	Cadmium (Cd)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
20.	Antimony (Sb)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
21.	Barium (Ba)	9.8	ppm	CENLAB/WI/CHEM-TM/008
22.	Leod (Pb)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

Sample marking Sample description Date of sample received Date reported 2016/355 (33) Lake Water 04-11-2016 09-11-2016

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RESULTS:

No	Parameter	Results	Unit	Test Method
1.	Beryllium (Be)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
2.	Sodium (Na)	Not Detected (Less than 0.1)	mqq	CENLAB/WI/CHEM-TM/008
3.	Magnesium (Mg)	Not Detected (Less than 0.1)	mqq	CENLAB/WI/CHEM-TM/008
4.	Aluminium (Al)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
5.	Pottossium (K)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
6.	Calsium (Ca)	5.1	ppm	CENLAB/WI/CHEM-TM/008
7.	Vanadium (V)	89.9	ppb	CENLAB/WI/CHEM-TM/008
8.	Chromium (Cr)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
9.	Manganese (Mn)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
10.	Iron (Fe)	Not Detected (Less than 0.1)	ppm	CENLAB/WI/CHEM-TM/008
11.	Cobalt (Co)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
12.	Nickel (Ni)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
13.	Copper (Cu)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

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21.	Barium (Ba)	12.6	ppm	CENLAB/WI/CHEM-TM/008
20.	Antimony (Sb)	Not Detected (Less than 0.5)	dad	CENLAB/WI/CHEM-TM/008
19.	Cadmium (Cd)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
18.	Silver (Ag)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
17.	Molybdenum (Mo)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
16.	Selenium (Se)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/0081
15.	Arsenic (As)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
14.	Zinc (Zn)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

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2016/355

CENLAB/F/007



#### CENTRAL LABORATORY

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# **CERTIFICATE OF ANALYSIS (COA)**

To / Attn	FIST/Azman FIST, Universiti Malaysia Pahang			
Address				
Tel No	-	Fax No		
Sample Lab No	2016/504	The second se		
the case its	2010/004	No of sample	4	

Sample marking Sample description Date of sample received Date reported	 2016/504(71) Pb1 C1 29-11-2016 01-12-2016
Dole lebolied	 01-12-2016

RESULTS:

No	Parameter	Results	Unit	Test Method
t,	Lead (Pb)	Not Defected (Less than 0.5)	ddd	CENLAB/WI/CHEM-TM/008

Sample marking Date of sample received Date reported 2016/504(2 29-11-2016 01-12-2016

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11.11

RESULTS:

No	Parameter	Results	Unit	Test Method
1.	Lead (Pb)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

Sample marking
Sample description
Date of sample received
Date reported

2016/504(73) Pb3 L1 29-11-2016 01-12-2016

RESULTS:

No	Parameter	Results	Unit	Test Method
1.	Lead (Pb)	0.67	dqq	CENLAB/WI/CHEM-TM/008

2016/504

Sample marking Sample description Date of sample received		2016/504(74) Pb4 L2 29-11-2016 01-12-2016	
Date of sample received Date reported	:		

#### RESULTS:

No	Parameter	Results	Unit	Test Method
1.	Lead (Pb)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

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C . MOHD RAFIE ROSLY SCIENCE OFFICER

2016/504 Page 2 of 2