PHYTOREMEDIATION OF AQUACULTURE WASTEWATER BY *PISTIA STRATIOTES*

by

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DECLARATION

I declare that this thesis entitled Phytoremediation of Aquaculture Wastewater by using *Pistia stratiotes* is the result of my own research except as cited in the references. The thesis has not been accepted by any degree and is not concurrently submitted in any candidature of any degree.

Signature :

Name :

Date :

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LIST OF ABBRERIATIONS

HRT	Hydraulic Retention Time
XRF	X-Ray Fluorescence
BCF	Bioconcentration Factor
DO	Dissolved Oxygen
TDS	Total Dissolved Solid
PPM	Part Per Million
USEPA	United State Environmental Protection Agency
MWW	Municipal Wastewater

Phytoremediation of Aquaculture Wastewater by using *Pistia stratiotes.*

ABSTRACT

Aquatic macrophytes have tremendous potential for remediation of the heavy metal. In this study, *Pistia stratiotes* had been used as phytoremediation agent to remediate the aquaculture wastewater and heavy metals. The objectives of this study were to study the removal efficiency of *Pistia stratiotes* in heavy metal and changing in physico-chemical parameter of aquaculture wastewater, to determine the accumulation of heavy metals in *Pistia stratiotes* and to study the suitability *Pistia stratiotes* for phytoremediation of aquaculture wastewater and heavy metals. Heavy metal pollution is a serious and widespread environmental problem due to their toxicity. They enter the environment through various natural methods and human activities. Thus, *Pistia stratiotes* is chose as a phytoremediation agent to treat aquaculture wastewater and heavy metal. *Pistia stratiotes* is a wide spread emergent aquatic weed. It generally grows on wastewater discharged areas like bogs, lakes, river pools and other places. The aquaculture wastewater was inserted into reactor tank and three reactors (*Pistia stratiotes*) were put into reactor tank. Then, the concentration of pollutants such as heavy metals and aquaculture waste were analyzed based on HRT. The experiment was repeated with 6 cycles. All the samples were analyzed both before treatment and after the treatment by the XRF and YSI 556 multi-parameter to identify the concentration of each pollutant in the water samples and physico-chemical parameters. The data analysis and statistics were calculated by Microsoft Excel 2010. The result of physico- chemical analysis showed that *Pistia stratiotes* has the ability to increase the values of pH, DO and reduce the value of TDS. Furthermore, the experiments demonstrate that *Pistia stratiotes* significantly remove Al, P, Cl, Cd, Fe and Ba respectively in aquaculture wastewater. The average removal efficiencies for these elements were 79.71±8.90, 35.74±12.51, 44.57±26.23, 49.88±37.31, 76.45±28.4, and 7.49±7.76 ppm respectively after three days of HRT. Besides that, *Pistia stratiotes* can accumulate these elements in the plant tissues. The maximum BCF value of Cd was 34.56 mg/L concentrated which points towards the suitability for removing Cd from surface waters by this phytoremediation agent. Thus, *Pistia stratiotes* useful to reduce pollutants water sources and increase water quality.

Air Sisa Akuakultur menggunakan *Pistia stratiotes.*

ABSTRAK

Makrofit akuatik mempunyai potensi yang besar untuk merawat logam berat. Dalam kajian ini, *Pistia stratiotes* telah digunakan sebagai agen fitopemulihan untuk merawat air sisa akuakultur dan logam berat. Objektif kajian ini adalah untuk mengkaji kecekapan penyingkiran oleh *Pistia stratiotes* dalam logam berat dan perubahan parameter fiziko-kimia dalam air sisa akuakultur, untuk menentukan pengumpulan logam berat dalam *Pistia stratiotes* dan mengkaji kesesuaian *Pistia stratiotes* untuk fitopemulihan air sisa akuakultur dan logam berat. Pencemaran logam berat adalah satu masalah alam sekitar yang serius disebabkan oleh kandungan toksik yang tinggi. Mereka mencemari alam sekitar melalui pelbagai kaedah semula jadi dan melalui aktiviti manusia. Oleh itu, *Pistia stratiotes* telah dipilih sebagai ejen fitopemulihan untuk merawat air sisa akuakultur dan logam berat. *Pistia stratiotes* adalah rumpai akuatik yang tersebar luas. Ia biasanya tumbuh di kawasan air sisa yang dilepaskan seperti tanah berlumpur, tasik, kolam sungai dan tempat-tempat lain. Air sisa akuakultur dimasukkan ke dalam tangki reaktor dan tiga reaktor (*Pistia stratiotes*) dimasukkan ke dalam tangki reaktor. Kemudian, kepekatan bahan cemar seperti logam berat dan sisa akuakultur dianalisis berdasarkan HRT. Eksperimen diulang dengan 6 kitaran. Semua sampel dianalisis kedua-duanya sebelum rawatan dan selepas rawatan oleh XRF dan YSI 556 pelbagai parameter untuk mengenalpasti kepekatan setiap pencemar dalam sampel air dan parameter fiziko-kimia. Analisis data dan statistik telah dikira dengan Microsoft Excel 2010. Hasil analisis kimia psiko menunjukkan bahawa *Pistia stratiotes* mempunyai keupayaan untuk meningkatkan nilai pH, DO dan mengurangkan nilai TDS di dalam air sisa akuakultur. Tambahan pula, kajian menunjukkan bahawa *Pistia stratiotes* menghapuskan Al, P, Cl, Cd, Fe dan Ba masing-masing dalam air sisa akuakultur secara ketara. Kecekapan penyingkiran purata bagi elemen-elemen ini adalah 79,71 ± 8.90, 35.74 ± 12.51, 44.57 ± 26.23, 49.88 ± 37.31, 76,45 ± 28.4, dan 7.49 ± 7.76 ppm selepas tiga hari HRT. Selain itu, *Pistia stratiotes* mampu mengumpul elemen-elemen ini dalam tisu tumbuhan. Nilai maksimum BCF untuk Cd adalah 34.56 mg/L menjelaskan kesesuaian bagi menghapuskan Cd dari air permukaan oleh ejen fitopemulihan ini. Oleh itu, *Pistia stratiotes* berguna untuk mengurangkan pencemaran sumber air dan meningkatkan kualiti air.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Recent studies have shown the pollution from pond aquaculture wastewater has become serious problems. Aquaculture continues to grow more rapidly than all other animal food-producing sectors. This sector has grown at an average rate of 8.8% per year since 1970, compared with only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production over the same period worldwide. Moreover, aquaculture industry is expanding in many regions of the world including Malaysia (Mokhtar *et al*., 2009). Aquaculture waste and heavy metal are wastewater related contaminant that is frequently present in aquaculture environment (Cao *et al*., 2007).

Phytoremediation is viewed as the new innovation of remediation technology to remediate sites contaminated with organic and inorganic pollutants. Furthermore, it is one of the biological wastewater treatment methods (Gupta *et al*., 2012). Interestingly, this new technique is an aesthetically pleasing mechanism that can reduce remedial costs and restore habitat (Chavan & Dhulap, 2013).

Phytoremediation used the concept of plants-based systems and microbiological processes to eliminate contaminants in nature a rather than entombing it or transporting the problem to another site (Gupta *et al*., 2012). This technology is rapidly proliferating for growing industries worldwide.

Wetlands are human engineered treatment systems utilizes natural treatment processes with high degree of treatment for pollution control in wastewater (Chavan & Dhulap, 2013). Wetland can often be an environmentally acceptable, cost-effective treatment options, especially for small communities. Wetland system with phytoremediation technique is the most suitable in terms of contaminant removal efficiency, cost reduction and simplicity (Chavan & Dhulap, 2013).

The wetland treatment systems can be established almost anywhere including on lands with limited alternative uses. For example, the increasing growths of wetland systems in Africa and Asia have seen in recent years. Moreover, wetlands are wet long enough to exclude plant species that cannot grow in saturated soils and to alter soil properties because of the chemical, physical, and biological changes that occur during flooding (Kadlec & Wallace, 2009).

Aquatic macrophytes have tremendous potential for remediation of the heavy metal (Das *et al*., 2014). *Pistia stratiotes* is chooses as the plant used for the phytoremediation agent for the wetland system (Prajapati *et al.*, 2012). *Pistia stratiotes* can be easily found in aquatic as well as marshy areas. The plant shows fast growth, which can grow well even without the use of fertilizers and has less harvest time (Das *et al*., 2014).

1.2 Problem Statement

Heavy metal pollution is a serious and widespread environmental problem due to their toxicity. Heavy metals enter the environment through various natural methods and human activities. Besides that, it can accumulate in fish and other organisms (Kalay, 2000). This pollution with toxic metals has become a worldwide crisis by affecting agriculture, contributing to bioaccumulation and biomagnification in the food chain (Sukumaran, 2013). As an alternative to the traditional treatment of pollutants, an ecological approach has been developed using of plants to remediate wastewater contaminated with toxic metals. In this study, *Pistia stratiotes* is chose as a phytoremediation agent to treat aquaculture wastewater and heavy metal in the wetland system since the plant is easily find at lowland wetlands of Malaysia.

1.3 Objective of Study

The objective of the research can be summarized as follows:

i) To study the removal efficiency of heavy metal by *Pistia stratiotes* and changing in physico-chemical parameter of aquaculture wastewater.

ii) To determine the accumulation of heavy metals in *Pistia stratiotes.*

iii) To study the suitability *Pistia stratiotes* for phytoremediation of aquaculture wastewater and heavy metals.

1.4 Expected Outcome

Wetland system through phytoremediation by using the *Pistia Stratiotes* is hypothesized effective for removal the aquaculture wastewater and heavy metal (Ali *et al*., 2013). From this study, *Pistia stratiotes* is expected has high capability in removal of heavy metal in aquaculture wastewater.

1.5 Significant of Study

The purpose of phytoremediation technique using *Pistia stratiotes* in the wetland system is to treat the contamination of aquaculture waste and heavy metal from wastewater efficiently. The increasing levels of heavy metals in the environment, their entry into food chain and the overall health effects is a great concern (Aqeel *et al.*, 2013). Therefore, this research can give benefits particularly to the communities at arid and semi-arid regions to meet their routine water needs which they can experience to treat and reuse the wastewater from various sources by applying this treatment by constructing their own wetland.

Besides that, the research groups have recognized that certain toxic metals may remain in the environment for a long period and eventually bio accumulate to higher levels that could affect human being. Therefore, an ecological approach has been developed using of plants to remediate soils or water contaminated with toxic metals as an alternative. Traditional technologies for removal of pollutants can be successful in specific situations, but they are not cost effective. Phytoremediation is very dynamic effort to develop new, more cost-effective and ecofriendly techniques to remediate contaminants.

1.6 Scope of Study

For this research, it focused on removal of aquaculture waste and heavy metals in aquaculture wastewater collected at Tilapia pond at Agropark UMK Jeli. The phytoremediation agent carried out by to remove the contaminant in wetland system. After the experimental period, the plants were harvested and analyzed for the concentration of aquaculture waste and heavy metals in the wastewater and plant sample. The initial and final concentration of heavy metals and elements in aquaculture wastewater and in whole part of plant samples were analyzed through XRF.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metal

Heavy metals are environmental pollutants thus their toxicity is a problem of increasing significance for ecological, nutritional, evolutionary, and environmental reasons. The concentrations of heavy metals in the environment increase from year to year (Ali *et al.*, 2012). Heavy metal pollution is a serious and widespread environmental problem due to their toxicity. Heavy metals enter the environment through various natural methods and human activities. Besides that, the toxicity can accumulate in fish and other organisms (Kalay, 2000).

Fish are often at the top of aquatic food chain and may concentrate large amounts of some metals from the water (Kalay, 2000). Some metals are essential to human health. Consequentially, heavy metals in aquatic environments are transferred throughout the web chain into humans (Taweel *et al.*, 2011). Furthermore, fish are the final organism in the aquatic food chain and a significant food source for man. Among the animal species, fish are inhabitants that cannot escape the detrimental effects of these pollutants (Basha *et al.*, 2003)

Heavy metals such as cadmium, zinc, mercury, chromium and copper can cause heavy pollution. The pollution is particularly in zones affected by effluents released from industries, sewage and agricultural drains. Metals are naturally occurring elements that become contaminants when human activities increase their concentrations above normal levels in the environment (Taweel *et al.*, 2011).

Unlike organic pollutants, heavy metals are not degraded into harmless forms. Therefore, the heavy metal is persisted in the environment for the long term. The toxicity of heavy metals adversely affects soil quality, agricultural production, human health and the environment (Ahmad & Zia, 2015). Heavy metals tend to accumulate in the environment and subsequently contaminate the food chain (Ali *et al*., 2013b). Table 2.1 summarize the harmful effect of heavy metal toward human health (Ali *et al*., 2013b).

Table 2.1: Harmful effect of heavy metal toward human health (Ali *et al*., 2013b).

Heavy metal	Harmful effect	References
Cd	Carcinogenic, mutagenic, and teratogenic; endocrine disruptor; interferes with calcium regulation in biological systems; causes renal failure and chronic anemia.	(Hanaa *et al.*, 2000)
Cu	Elevated levels have been found to cause brain and kidney damage, liver cirrhosis and chronic anemia, stomach and intestinal irritation.	(Hanaa *et al.*, 2000) and (Wuana *et al.*, 2013)
Pb	Its poisoning causes problems in children such as impaired development, reduced intelligence, loss of short- term memory, learning disabilities and coordination problems; causes renal failure; increased risk for development of cardiovascular disease.	(Hanaa *et al.*, 2000) and (Wuana *et al*., 2013)

2.2 Phytoremediation

Phytoremediation is a recent technology (Ali *et. al.*, 2012) which use of green plants for decontamination of polluted sites (soils and waters). This technology is one of the biological wastewater treatment methods which implemented the concept of using plants-based systems and microbiological processes to eliminate contaminants in nature (Gupta *et al.*, 2012). In addition, phytoremediation basically refers to the use of plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environments (Ali *et al.*, 2013).

The remediation techniques utilize specific planting arrangements, constructed wetlands (CW), floating-plant systems and numerous other configurations (Ahmad & Zia, 2015). The removal of wastewater constituents are achieved by different mechanisms like sedimentation, filtration, chemical precipitation, adsorption, microbial interactions, and uptake of vegetation (Gupta *et al.*, 2012). Among the various mechanisms, the most effective technology is phytoremediation strategy using CW technology. Besides it can be used for heavy metal sand radionuclides as well as for organic pollutants (Ali *et al.*, 2013b).

Plants are used to remove or degrade contaminants with an old process that occurs naturally in ecosystems as both inorganic and organic constituent's cycle through these plants (Chavan & Dhulap, 2013). It can be used for removal of heavy metals and radionuclides as well as for organic pollutants such as polynuclear aromatic hydrocarbons, polychlorinated biphenyls, and pesticides. It is a novel, cost-effective, efficient, environment and eco-friendly, in situ applicable, and solar-driven remediation strategy (Clemens, 2001).

Phytoremediation is one new approach that offers more ecological benefits and a cost efficient alternative. Although it is cheaper method but requires technical strategy, expert project designers with field experience that choose the proper species and cultivars for particular metals and regions. The plant used in the phytoremediation technique must have a considerable capacity of metal absorption, its accumulation and strength to decrease the treatment time (Aqeel *et al*., 2013).

Phytoextraction is the uptake of contaminants by plant roots and movement of the contaminants from the roots to aboveground parts of the plant. Contaminants are generally removed from the site by harvesting the plants. Phytoextraction accumulates the contaminants in a much smaller amount of material to be disposed off than does excavation of soil or sediment (Marques *et al.*, 2009).

2.3 Phytoremediation Agent

Pistia stratiotes is a perennial freshwater weed spread across the world. It is aquatic macrophytes. It is a floating perennial commonly called water lettuce belonging to the family Araceae (Gupta *et al*., 2012). This species carries its entire life cycle as free-floating plant on the surface of the water. Furthermore, only it root system is completely submerged beneath floating leaves. (Prajapati *et al.,* 2012). While it may have originated in South America but the origin of this plant is uncertain (Gupta *et al*., 2012).

The leaves can be up to 14 cm long and have no stem. They are light green, with parallel veins, wavy margins and are covered in short hairs which form basket-like structures and help in trapping air bubbles, increasing the plant's buoyancy (Gupta *et al*., 2012). The flowers are dioeciously and are hidden in the middle of the plant among the leaves. The plant can be reproduced by both vegetative and sexually (Anju *et al.*, 2011).

The ability of Pistia as the phytoremediation is supported based on other study. *Pistia stratiotes* is a suitable candidate for effective removal of heavy metals (Hg, Cd, Mn, Ag, Pb, Zn) (Ugya *et al.*, 2015). This species takes up metals from water, produces an internal concentration several folds greater than their surroundings and shows much higher metal-accumulating capacity than non-hyper accumulating terrestrial plants (Ali *et al.*, 2013b).

Figure 2.1 showed *Pistia stratiotes* in the pond. Especially in tropical or subtropical areas, *Pistia stratiotes* (large-leaved floating plant) is used in phytoremediation water systems (Gupta *et al*., 2012). Compared to native plants, this invasive plant show a much higher nutrient removal efficiency with their high nutrient uptake capacity, fast growth rate, and big biomass production (Reddy & Sutton, 1984).

Pistia stratiotes had chosen as phytoremediation agent for many researches. For example, a study was designed to assess the efficiency of *Pistia stratiotes* in the removal of heavy metals in Romi Stream since Kaduna refinery and petrochemical company discharge it waste water directly into the stream (Ugya *et al*., 2015). In addition, it had been extensively used for phytoremediation. *Pistia stratiotes* was used in laboratory experiments for the removal of several heavy metals (Fe, Cu, Zn, Mn, Cr, and Pb) resulting from anthropogenic activity (Prajapati *et al.*, 2012).

Figure 2.1: *Pistia stratiotes i*n the pond.

2.4 Wetland System

The wetland treatment systems can be established almost anywhere including on lands with limited alternative uses. For example, the increasing growths of wetland systems in Africa and Asia have seen in recent years (Kadlec & Wallace, 2009). Moreover, wetlands are wet long enough to exclude plant species that cannot grow in saturated soils and to alter soil properties because of the chemical, physical, and biological changes that occur during flooding (Kadlec & Wallace, 2009).

Malaysian wetlands are one of the most varied and diverse ecosystems across the country. This is mainly due to heterogeneity of vegetation structure and composition, unpredictable rainfall patterns and occurrence of adjacent different surrounding landscapes. These wetland habitats are dominated by a variety of aquatic vegetation such as emergent, submerged, reedbeds and grasses which are suitable habitat for fish, amphibians, reptiles and invertebrates (Rajpar & Zakaria, 2016). Figure 2.2 showed the wetland ecosystem in Paya Indah wetland.

Figure 2.2: Wetland ecosystem in Paya Indah Wetland (Rajpar & Zakaria, 2016).

The treatment of wastewater using CW has potential to be one of the sustainable solutions in treating and then discharging the huge quantity of wastewater and getting access to safer drinking water (Chavan & Dhulap, 2013). It designed to accomplish natural processes with wetland substrates, vegetation and the associated microbial assemblages to help in treating wastewaters. Furthermore, it take advantages of the processes that occur in natural wetlands within the more controlled environment (Chavan & Dhulap, 2013). Figure 2.3 showed treatment of sewage using plant in the constructed wetland.

Figure 2.3: Treatment of aquaculture wastewater using *Pistia stratiotes* .

CHAPTER 3

METHODOLOGY

3.1 Site Description

The experiment had been conducted at an assign plot at Agropark, Universiti Malaysia Kelantan for 65 days. The aquaculture wastewater from the Tilapia pond was used as wastewater for the phytoremediation treatment. There is one species of Tilapia which is Tilapia Merah (*Oreochromis niloticus*). The coordinate of the Tilapia pond is 05˚44'48.4"N 101˚52'02.3" with elevation at 120m.

3.2 Plant Sampling

The phytoremediation agent, *Pistia stratiotes* was collected around Jeli. The plant samples were collected in the pond. The samples were placed in the sterile plastic bag. In order to avoid excess moisture and vaporization, the plastic bag should not be seal. The plants were acclimatized for one week in the nursery tank before use as phytoremediation agent in whole experiment process. For the whole period of research, the healthier plant samples were selected and used for research purpose.

3.3 Wetland Preparation

The phytoremediation technique using *Pistia stratiotes* was conducted in a wetland system. One set of reactor tank with diameter 15 cm with 45 cm height was used. The reactor tanks were provided with a pipe for outlet flow. Three layers of bed were prepared using pebbles, sand and garden soil. The big size pebbles made bottom layer of 10 cm height followed by sand was added to form a middle layer of 10 cm height and small size and sieved soil forming upper layer of 10 cm height was used for construction of bed. The pebbles and sand materials were neatly wash with tap water and arranged in different layers in each experimental set up (Figure 3.1). A control tank was prepared without *Pistia stratiotes* to correct the experimental result caused by environmental factor.

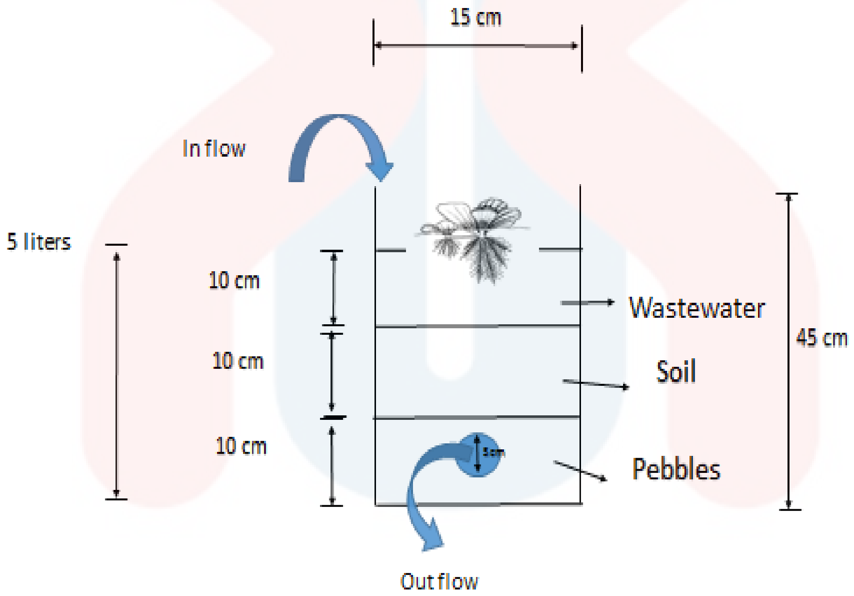


Figure 3.1: Experimental design of wetland.

3.4 Analysis and Characterization

3.4.1 Characterization of Aquaculture Wastewater

3.4.1.1 Physico-Chemical Parameters in Aquaculture Wastewater.

The aquaculture wastewater samples were analyzed based on water quality parameter such as pH, turbidity, DO, salinity and TDS. The 15ml of aquaculture wastewater samples were collected for all parameters. The samples for all parameter were measured using YSI 556 MPS and initial measurement of each parameter was taken.

3.4.1.2 Heavy Metals Characterization and Analysis in Aquaculture Wastewater.

The aquaculture wastewater had been collected for 15ml for heavy metal. The wastewater samples were measured by using XRF. The initial concentration for each heavy metal was taken.

3.4.1.3 Heavy Metals Analysis and Characterization in Plant Sample.

The experiment analyzed the heavy metal that accumulated in the plant sample by using XRF. The plant samples were grinded and put into oven for oven dried with 24 hours at 80ºC (Melorose *et al*, 2015). The plant samples also sieved through a 75-micron sieve with auto-sieve shaker.

3.5 Experimentation

3.5.1 Hydraulic Retention Time (HRT)

The experiment was set at 48 hours of HRT to determine the optimum time need by *Pistia stratiotes* for accumulation of elements. The experiments were conducted for 6 batches using the same plant with two replications. For each time section, a sample of aquaculture wastewater was collected at 15 ml and was analyzed using XRF for initial and final measurement for each set of time.

3.5.2 Parameters Removal

Based on HRT, a reactor tank was set up based on Figure 3.1. The initial and final readings for each batch of wastewater sample analysis were taken. The plant sample analysis of initial reading was taken when the experiment started. Final reading for plant analysis was taken when the whole experiment ended. The experiment was conducted by 6 batches using the same plant samples based on HRT.

3.5.2.1Physio-chemical Analysis in Aquaculture Wastewater

To determine the changes in physico-chemical of aquaculture waste by *Pistia stratiotes*, the reading of physico-chemical parameter of aquaculture waste; pH, Total Dissolved Solid (TDS), and Dissolved Oxygen (DO) for both initial and final concentration were measured based on method mention in section 3.4.1.1.

3.5.2.2 Heavy metals Analysis in Aquaculture Wastewater

To determine the removal efficiency of aquaculture waste by *Pistia stratiotes*, the concentrations of heavy metals and elements (Zn, Cl, Cd, Fe, Ba and Pb) for both initial and final concentration were measured based on method mention in section 3.4.1.2.

3.5.2.3 Heavy metal Analysis Accumulate in Plant Sample

The accumulation of heavy metal was determined based on the whole plant parts of *Pistia stratiotes* by using Bioconcentration Factor (BCF). It is calculated as:

BCF = metal concentration in plant tissue at harvest (ppm)/initial concentration of the metal added in water (ppm) (Das *et al.,* 2014). The accumulation of heavy metal in *Pistia stratiotes* was measured based on this form.

3.6 The Reading Changes of Physico-Chemical, Efficiency of Element Removal and BCF.

The reading changes of physico-chemical in water were calculated.

$$Changes = C_1 - C_{\circ} \qquad (1)$$

Where $C_{\circ}$ and C_1 are initial and final concentration of element in medium (ppm).

The percentage of element removal efficiency in water was calculated according to (Tanhan *et al*, 2007):

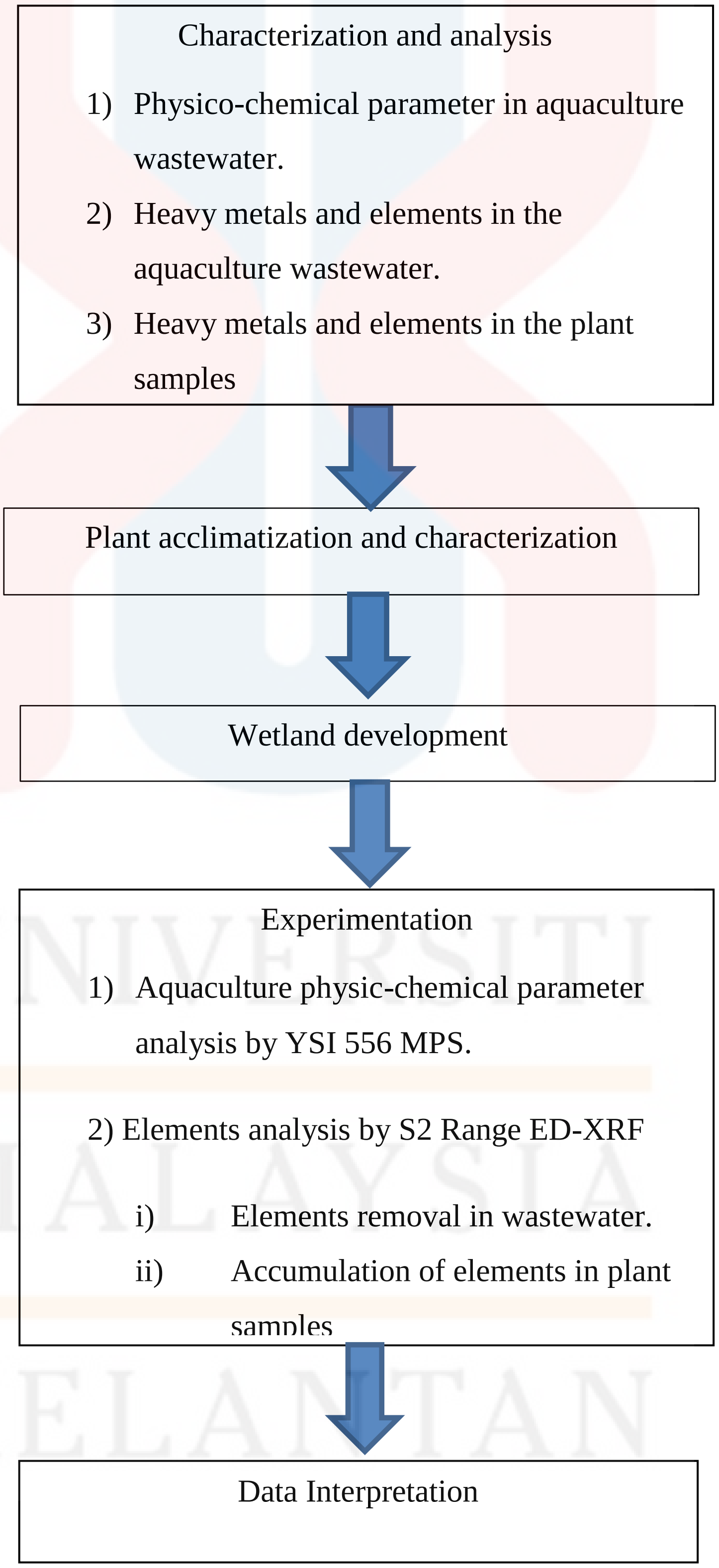
$$\%\ efficiency = \frac{C_{\circ} - C_1}{C_{\circ}}\ x\ 100 \qquad (2)$$

Where $C_{\circ}$ and C_1 are initial and final concentration of element in medium (ppm).

BCF indicates the efficiency of a plant species in accumulating of elements into its tissues from the surrounding environment (Ladislas *et al*, 2012). It was calculated as follows (Rahmani, & Sternberg, 1999):

$$BCF = \frac{Element\ accumulate\ in\ plant\ (ppm)}{Average\ initial\ element\ in\ wastewater\ (ppm)} \qquad (3)$$

3.7 Flowchart of Research Approach



CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 General

The whole data in this chapter are presented as mean and standard deviation were in Part Per Million unit (PPM). The physico-chemical parameters of aquaculture wastewater were calculated by YSI 556 MPS at each of the batches. The data for wastewater and plant samples were calculated by XRF analyzing machines.

The reading of heavy metal was taken for two parts which were the removal of heavy metal in water and the accumulation of heavy metal at the whole part of the plant. The water samples were taken for sixth times for each cycle after 48 hours. The solid plant samples were taken at two times which at the initial and final of the experiment.

4.2 Characterization of Physico - Chemical Aquaculture Wastewater Parameter.

Table 4.1 shows the readings of physico-chemical parameters for aquaculture wastewater parameters characterization. These reading was taken before started the experiment to characterize the physico-chemical parameter for aquaculture wastewater in Tilapia pond.

Table 4.1: Reading for physico - chemical for aquaculture wastewater parameters characterization.

Physico-chemical parameters	**Reading**
DO (mg/L)	2.58
pH	6.76
TDS (g/L)	0.03
Salinity (ppt)	0.02
ORP (mv)	206.60
Conductivity (ms/cm)	0.04
Temperature	30.45

Physico - chemical properties such as pH and conductivity of water in any aquatic system are largely governed by the existing meteorological condition and are essential for determining the structural and functional status of natural water (Aniruddha, 2014). Based on the characterization in Table 4.1, the pH of the pond was in the desirable range (Onada *et al.*, n.d.).

Neutral to slightly alkaline pH ranges for wastewater are considered to be congenial for aquatic production owing to greater availability of most of nutrient elements and also due to increased biological activities under this pH range (Aniruddha, 2014). Based on the reading on Table 4.1, the pond indicated there was less pollution since most of the parameters were within the acceptable limit (Aniruddha, 2014).

4.3 Physico - Chemical Parameter Analysis for Aquaculture Wastewater.

Table 4.2 shows the readings of physico-chemical parameter (pH, DO and TDS) in the aquaculture tilapia pond for sixth batches. Based on the table 4.2, the negative reading indicates that the parameters had undergone reduction while the positive reading shows that the reading of parameters had been increased. After being treated with *Pistia stratiotes* for 48 hours, the changes in TDS had been increased the most at 61.54% showed at Batch 1 while decreased the most with -46.43% at Batch 3. Next, DO increase the most with the 22.87% at Batch 2 and decreased the most at Batch 5 with -47.45. Lastly, pH of aquaculture wastewater increased the most with 13.51% after being treated with *Pistia stratiotes* at Batch 3 while decreased the most at -33.33% at Batch 2.

Table 4.2: Percentage removal of TDS, DO and pH by *Pistia stratiotes* in 6 batches.

Parameter	**Batch 1**				**Batch 2**			
	Initial	Final	changes	% of changes	Initial	Final	Changes	% of changes
TDS (g/L)	2.60	4.20	1.60	61.54	2.70	1.90	-0.8	-29.63
DO (mg/L)	2.58	3.02	0.44	17.05	8.52	12.42	3.9	22.87
pH	6.79	6.78	-0.01	-0.15	7.22	7.27	0.05	-33.33

Parameter	**Batch 3**				**Batch 4**			
	Initial	Final	changes	% of changes	Initial	Final	changes	% of changes
TDS (g/L)	2.80	1.50	-1.30	-46.43	2.70	4.30	1.60	59.26
DO (mg/L)	2.61	2.85	0.24	9.20	6.34	4.99	-1.35	-21.29
pH	7.70	8.74	1.04	13.51	8.05	8.51	0.46	5.71

Parameter	**Batch 5**				**Batch 6**			
	Initial	Final	changes	% of changes	Initial	Final	changes	% of changes
TDS (g/L)	3.50	2.40	1.10	31.43	0.04	0.04	0.00	14.29
DO (mg/L)	1.96	1.03	-0.93	-47.45	4.40	4.67	0.27	6.14
pH	9.05	9.94	0.89	9.83	5.39	6.19	0.80	10.83

4.3.1 DO

The dissolved oxygen (mg/L) obtained from this study was in the range of 1.96– 8.52 mg/L before being treated with *Pistia stratiotes*. These values agreed with those of (Saloom & Duncan, 2005), they also stated that the minimum dissolved oxygen should be 5 mg/L for tropical fish. After being treated by pistia stratiotes for 48 hours, the dissolved oxygen (mg/L) obtained from this study was in the range of 1.03 – 12.42 mg/L. The highest dissolved oxygen recorded was in the Batch 2.

Besides that, Batch 1, 3 and 6 also had been increased in dissolved oxygen after being treated. The higher level of dissolved oxygen recorded in these batches may be as a result of photosynthetic activities by *Pistia stratiotes* during the day when light intensity is high. The increasing of DO give benefit to the pond because it can support aquatic life for respiration. However, the reason for low dissolved oxygen recorded in the Batch 4 and 5 may be due to respiratory activities aerobic organisms (Onada *et al.*, n.d.) in the reactor tank. Furthermore, the increasing DO may prevent the anaerobic conditions and cause bad odour in water (Adekunle *et al.*, 2007) .

4.3.2 pH

The desirable range for pond pH is 6.5 - 9.5 and acceptable range is 5.5 - 10.0 (Bluff & Extension, 1999). The range of the pH obtained from this study was (6.79 – 9.05) (Onada *et al*., n.d.). After being treated by phytoremediation agent, the range of the pH was in 6.78 -9.94 which was slightly alkali. The concentration of carbon dioxide concentrations and pH in the pond are affected by respiration and photosynthesis activities (Onada *et al*., n.d.).

Carbon dioxide is released during respiration and consumed for photosynthesis. The changes in pH caused by plant and phytoplankton absorbed carbon dioxide for photosynthetic production of sugar (Onada *et al.*, n.d.). Moreover, as daylight progressively intensifies, the rate of photosynthesis increases and so does the uptake of carbon dioxide. The removal of carbon dioxide reduces the concentration of carbonic acid, and pond pH rises. This rise of pH can lead to good pond productivity and fish health can be maintained (Onada *et al.*, n.d.).

4.3.3 TDS

Solid contents such TDS estimated before and after the treatment reflected improvement in the quality of wastewater with phytoremediation (Chavan & Dhulap, n.d.). TDS is a measure of all dissolved substances in water, including organic and suspended particles that can pass through a very small filter (CSWRBC, 2016). Based on the figured, the table shows the range of TDS obtained in this experiment is 0.04- 3.5 g/L. After being treated by *Pistia stratiotes* for 48 hours, the range of TDS recorded as 0.04-4.3 g/L.

TDS is frequently used to measure salinity (CSWRBC, 2016). Water uptake by plants can also increase soil salinity. Water percolating through the ground of aquaculture wastewater had salts dissolved in it. Besides that, human activities also affected salinity levels in ground and surface water. For example, the application of synthetic fertilizers and manures in agriculture activities could contribute salt to surface and groundwater (CSWRBC, 2016)

4.4 Characterization of Elements in Aquaculture Wastewater.

In this study, the concentration of 14 different elements including heavy metals were identified in the aquaculture wastewater located at UMK Jeli.

Table 4.3: Reading of elements and heavy metals concentration in aquaculture wastewater characterization.

Element	Concentration (ppm)
P	2.76
Cl	7.16
Fe	2.24
Ba	0.04
Sn	0.01
Na	86.18
S	0.25
Sb	0.01
Mg	17.89
Al	0.67
Si	0.09
Cd	0.02
Ti	0.35
K	11.99

4.5 Removal of Elements in Aquaculture Wastewater.

In this study, the total of 14 different elements including heavy metals were identified in the aquaculture wastewater located at UMK Jeli. However, there were six elements namely aluminum (Al), phosphorus (P), chlorine (Cl), iron (Fe), cadmium (Cd) and barium (Ba) showed the significant removal in the wastewater by using phytoremediation. Apart from that, Ba showed the least removal among the others. These elements were analyzed for 48 hours in 6 consecutive cycles. The mean and standard deviation results of percentage removal of six elements were presented in the Table 4.4.

Table 4.4: Reading of percentage removal of Al, P, Cl, Cd, Ba and Fe in the treated aquaculture wastewater.

Elements	Batch 1 (%)	Batch 2 (%)	Batch3 (%)	Batch 4 (%)	Batch 5 (%)	Batch 6 (%)	Mean ± Sd
Al	67.34	73.08	84.75	86.07	91.03	75.98	79.71± 8.90
P	20.50	21.39	38.74	38.14	43.56	52.13	35.74 ± 12.51
Cl	16.44	18.92	32.28	60.28	56.95	82.53	44.57 ± 26.23
Cd	30.51	37.88	42.80	93.67	94.42	0.00	49.88 ± 37.31
Fe	100.00	37.98	42.64	90.20	88.56	99.30	76.45 ± 28.41
Ba	5.48	13.53	19.63	0.00	0.00	6.31	7.49 ± 7.76

The reading of percentage removal for Al, P, Cl, Cd, Ba and Fe were presented as Mean±S.D. The highest mean for six batches were recorded by Al with 79.71±9.00 ppm. The second highest mean was presented by Fe with 76.45±28.41 ppm followed by Cd with 49.88±37.31 ppm. Next element is Cl with 44.57±26.23 ppm and lastly, P which was 35.74±12.51 ppm. The lowest men for the six batches were recorded by Ba with 7.49±7.76 ppm.

Based on the Table 4.4, Cd recorded the increasing percentage removal pattern range from 30.52% at batch 1 to 91.42% in Batch 5. Al had been presented high percentage removal pattern from the beginning from 67.34% until achieved the maximum percentage removal at 91.03%. However, at Batch 6 the percentage removal was slightly dropped. P and Cl increased in percentage removal but then slightly drop at Batch 4 and Batch 5. Based on the table 4.4, P and Cl had the maximum percentage removal at Batch 6 with 52.13% and 82.53%.

Besides that, Fe had been recorded as the maximum percentage removal at the beginning of the experiment but decreased for the remaining batches. Lastly, Ba had the least percentage removal compared to others. Ba had the maximum removal at Batch 3 with 19.63% and the minimum percentage removal recorded at Batch 4 and 5 with 0.00%.

In this study, heavy metals such as Al, Fe and Cd were detected at low concentration in aquaculture wastewater samples collected from the Tilapia pond located at UMK Jeli. Anthropogenic metal concentrations in aquaculture samples were low indicating that Tilapia pond had not experienced extreme pollution. However, the heavy metals concentration in aquaculture organism can increase several times over the environmental levels which demonstrate their potential as accumulators of heavy metals (Mokhtar *et al*., 2009). Based on the Table 4.4, the order of accumulation of different heavy metals in *Pistia stratiotes* was Al > Fe > Cd > Cl > P > Ba.

It can be observed from the figure above that the percentage removal efficiency of *Pistia stratiotes* for the six different elements for 48 hours in 6 batches. From the Table 4.4, it was cleared that Al had the increasing percentage removal for every batch but dropped in batch six. The main reason for this may be cause by Al is one of the most frequent elements in the biosphere. Al mobilization in its soluble forms from soil to aquatic ecosystems can cause acidification to the water bodies (Rout *et al*., 2001). The decreased in removal cause by the toxicity of Al to *Pistia stratiotes* (Silva, 2012).

In this study, Fe had high percentage removal starting from the first batch. This may be caused by the present the abundant of Fe in organic and inorganic forms in freshwater (Mokhtar *et al*., 2009). Fe acts as micronutrients which are essential to the plant growth. *Pistia stratiotes* removed Fe from the aquaculture wastewater and consume them for it growth. The removal of Fe gives benefit to the pond because when the concentration of Fe increased, it become toxic to the plants. This is supported by the literature, *Pistia stratiotes* commonly used in the experiments for the removal of several heavy metals such as Fe, Cu, Zn, Mn, Cr, and Pb which resulting from anthropogenic activity *(Prajapati et al.*, 2012).

However, on the second and third batch, the result declined rapidly. In the next three batches, the percentage removal by *Pistia stratiotes* started increased consistently for every batch. This was perhaps due to the different concentration of Fe available in the wastewater each of the batches. Moreover, since there were a few of construction site located in UMK, this may contributed to the Fe concentration in this study as supported by (Mokhtar *et al.*, 2009) where Fe was reported to be found naturally in aquatic system, construction and fertilizers usage in agricultural processes could cause the Fe to be leached into aquaculture through runoff.

From this study, it can see clearly from Figure 4.1 that percentage removal of Cd was increasing consistently from the first batch to the last batch. Cd from aquaculture wastewater was efficiently removed by *Pistia stratiotes* in 48 hours. Aquatic macrophytes such as *Pistia stratiotes* have tremendous potential for remediation of the heavy metal cadmium (Das *et al.*, 2014). For example, *Pistia stratiotes* can be considered a hyper accumulator for trace metals such as Cr, Cu, Fe, Mn, Cd , Pb, and Zn (Gupta *et al.*, 2012).

Periodic harvesting of water lettuce was necessary not only for maintaining an optimal growth density, but also for effective removal of nutrients such as N, P and metals from the waters, otherwise the nutrients and metals would be released back into the water system after the plant died and decomposed (Fonkou *et al.*, 2002). Furthermore, it had been successfully used in wastewater treatment systems to improve water quality by reducing the levels of organic and inorganic nutrients (M. I. Lone e*t al*., 2008). The present finding was in consonant with other findings and supported by literature.

Besides accumulated from aquaculture wastewater, plants can also extracted Cd from the soil and transported it via the xylem into shoots and leaves where it accumulated (Lombi *et al.,* 2002). In this study, *Pistia stratiotes* accumulated Cd the most compare with other elements. Based on the literature, his may due to *Pistia stratiotes* was found to accumulate Cd in both its root and shoot in a high degree, although the shoot Cd translocation was not more compared to the root concentration in the present research (Das *et al*., 2016).

The similar results were observed with the removal of Cl by *Pistia stratiotes*. The graph in Figure 4.1 shows that the percentage removal of chlorine was increasing from batch one 1 until batch 6 even though the result was lower than Al, Cd and Fe. Plant uptake of chlorinated solvents influenced by many factors including soil pH, clay content, water content, and organic matter content, as well as the properties of the chlorinated solvent (Pivetz, 2001). The example source of chlorinated solvent can came from chlorinated pesticides. These pesticide were shown to have enhanced degradation in the rhizosphere of the plant (Shann, 1995). Although data were limited, it appeared that both the plants and the associated microbial communities played a significant role in attenuating chlorinated compounds (USEPA, 2005).

Besides that, P had the percentage removal with 35.74% in 48 hours. This is due to because the organic phosphorus compound was decomposed and mineralized by enzymatic complexes such phosphatases produces by microbes in the aquaculture pond such as Tilapia pond (Barik *et al*., 2001). This result shown similarly by other research where *Pistia stratiotes* promoted 50% reductions in nitrogen and phosphorus from freshwater MWW (Municipal Wastewater) (Castine *et al*., 2013).

Lastly, Ba was recorded as the least element removed by *Pistia stratiotes.* The percentage removal of Ba was varied from the Al, Cd, Cl, Fe and P. Since the agriculture pond which located at the remote area, the concentration of Ba in the aquaculture point was low so the removal of Ba was low compared to other elements (Kowalska *et al.*, 2012).

4.6 Accumulation of Heavy Metal in *Pistia stratiotes*.

The accumulation data of Al, Cd, Cl, Ba, Fe and P had been recorded at the initial and final of the experiment. The reading was taken before the aquaculture wastewater being treated and after the aquaculture wastewater was being treated by using *Pistia stratiotes* for 48 hours after 6 batches.

Table 4.5: Initial and final accumulation reading of Al, Cd, Cl, Fe, Ba and P in *Pistia stratiotes.*

Elements	Net accumulation (mg/L)	BCF (mg/L)
Al	1.52	2.03
Cd	0.63	34.56
Cl	0.07	0.00
Fe	1.89	0.01
P	0.42	0.71
Ba	0.00	0.00

This research was performed to determine the accumulation of Al, Cd, Cl, P, Ba and Fe in *Pistia stratiotes.* In the Table 4.5, the reading of BCF showed that Cd accumulated the most by *Pistia stratiotes* at 34.56 mg/L. Besides that, Al accumulated with 2.03 mg/L followed by Fe with 0.70 mg/L, and P with 0.0102 mg/L. Lastly, Cl and Ba recorded 0.00 mg/L accumulation in *Pistia stratiotes.*

4.7 BCF of Elements in *Pistia stratiotes.*

BCF is a useful parameter to evaluate the potential of the plants in accumulating metals. Metal accumulations by macrophytes such as *Pistia stratiotes* could be affected by metal concentrations in water and sediments. The ambient metal concentration in water is the major factor influencing the metal uptake efficiency. In general, when the metal concentration in water increases, the amount of metal accumulation in plants also increases (Sukumaran, 2013).

This research was performed to determine the accumulation of elements in *Pistia stratiotes*. In Table 4.5, the experimental data showed that Cd accumulated the most by *Pistia stratiotes* at 34.56 ppm. The higher BCF of *Pistia stratiotes* makes it more suitable than others for Cd phytoremediation in both aquatic as well as marshy conditions. Some researchers found similar nature of metal uptake in water lettuce for cadmium (Ugya *et al.*, 2015). Metal uptake such as Cd, Al and Fe by the plant involved transport of metals across the plasma membrane of root cells, loading in xylem tissues and translocation and subsequently detoxification and sequestration of metals at the whole plant and at cellular levels (Lombi *et al.*, 2002).

Besides that, Al accumulated with 2.03 ppm. Generally, aluminum is not bioaccumulated to a significant extent due to it toxicity. Similarly, because of its toxicity to many aquatic organisms, including fish, aluminum did not accumulated in aquatic organisms to any significant degree (M. S. Chhatra., 2003). Furthermore, differential tolerances to Al toxicity almost certainly involves differences in the structure and function of roots of the plant (G. R. Rout *et al.*, 2001).

Aluminium interferes with cell division in roots, decreases root respiration and uptake and use of water and nutrients, particularly calcium and phosphorous and metabolic pathway (Silva, 2012). Due to this reason, *Pistia stratiotes* was not able to accumulate the high concentration of Al.

Besides Cd and Al, P recorded 0.71 ppm of BCF by *Pistia stratiotes.* In the aquatic environment P may be encountered in several forms which were dissolved inorganic P (DIP), particulate inorganic P (PIP), dissolved organic P (DOP), and particulate organic P (POP) (Reddy *et al.*, 2016). Accumulation of P in the *Pistia stratiotes* is high emphasizing the critical role played by phosphate in plant metabolism and biomass increase (Reddy *et al.*, 2016). In addition, *Pistia stratiotes* had been widely reported as a P bio remediating agent (Shardendu, Sayantan, Sharma, & Irfan, 2012).

Based on the Table 4.5, Fe accumulated with 0.71 ppm in the plant after being treated by *Pistia stratiotes* for 48 hours. The low concentration of nutrients may reduce the performance of plant in removing nutrients. After being accumulated, the iron acted as essential micronutrient because of it plays critical role in metabolic processes such as DNA synthesis, respiration, and photosynthesis (G. R. Rout & Sahoo, 2015) in order to support *Pistia stratiotes* as a phytoremediation agent. Iron is an essential nutrient for plant growth however when present at high concentrations, they are toxic to plant (He *et al*., 2005).

Cl and Ba had zero accumulation in plant with 0.00 ppm of BCF. The removal through accumulation was directly dependent on amount of nutrient in the water column (Shardendu *et al*., 2012). Furthermore, chlorine is an essential micronutrient for higher plants (Marschner, 1995) so this plant can consumed this element which cause the zero acclamation of Cl. There are only a few reports concerning barium accumulation by plants. Relative to the amount of Ba found in soils, less is accumulate by plants (Kowalska *et al*., 2012). This is may be due to macrophyte like *Pistia stratiotes* not suitable to accumulate Ba.

The final concentration of elements accumulated and removed in wastewater samples and plants were analyzed using XRF. The efficiency of each plant in accumulating heavy metals in their leaf and root were calculated using BCF. This result gave an indication that *Pistia stratiotes* was able to accumulate the element from aquaculture wastewater. Apart from that, *Pistia stratiotes* was suitable as a phytoremediation agent could be used efficiently for heavy metal removal (Sukumaran, 2013).

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

Toxic metal pollution of waters is a major environmental problem since they can affect aquatic lives in enormous ways. However, the most conventional remediation approaches do not provide acceptable solutions. The use of specially selected and engineered metal-accumulating plants for environmental clean-up is an emerging technology of recent years (Prajapati *et al*., 2012). Phytoremediation is an eco-friendly approach for remediation of contaminated water using plants such as *Pistia stratiotes*.

The major conclusions of this study are the removal efficiency by using *Pistia stratiotes* in heavy metal was able to study in this research. Moreover, phytoremediation also changed the physico-chemical parameter of aquaculture wastewater such as TDS, DO and pH. The increased in pH and DO in aquaculture wastewater after being treated give some benefit to the pond by improving the Tilapia pond condition.

Next, the accumulation of elements was determined by using BCF. In this study, *Pistia stratiotes* accumulated Al and Cd the most compared to the others elements. Lastly, based on this study, *Pistia stratiotes* has high suitability as a phytoremediation agent of aquaculture wastewater and heavy metals. The elements including heavy metal were removed prominently by accumulated in this plant from the aquaculture wastewater. So *Pistia sratiotes* can be considered as the good plant for the phytoremediation of heavy metals from aquaculture wastewater in a sustainable way.

As mention earlier, phytoremediation is a relatively recent field of research and application Factors that may affect phytoremediation in the field include variations in temperature, nutrients, precipitation and moisture, plant pathogens and herbivore, uneven distribution of contaminants, soil type, soil pH, and soil structure (Ali *et al.*, 2013b). So as the recommendation, the factors mentioned above must be control by having a control tank. Moreover, if these factors affected the experiment, it will cause inaccuracy.

In addition, the advancement and achievements in such molecular studies will greatly help in understanding the mechanism and enhancing the efficiency of phytoremediation. An improved understanding of heavy metal uptake by plants (Ali *et al*., 2013). Furthermore, phytoremediation is suitable and applicable to sites with low to moderate levels of metal contamination because plant growth is not sustained in heavily polluted soils (Ali *et al*., 2013b). Therefore, phytoremediation is the best way for remediation of contaminated aquaculture water using plants.

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APPENDICES

Appendix-A: Graph of percentage removal

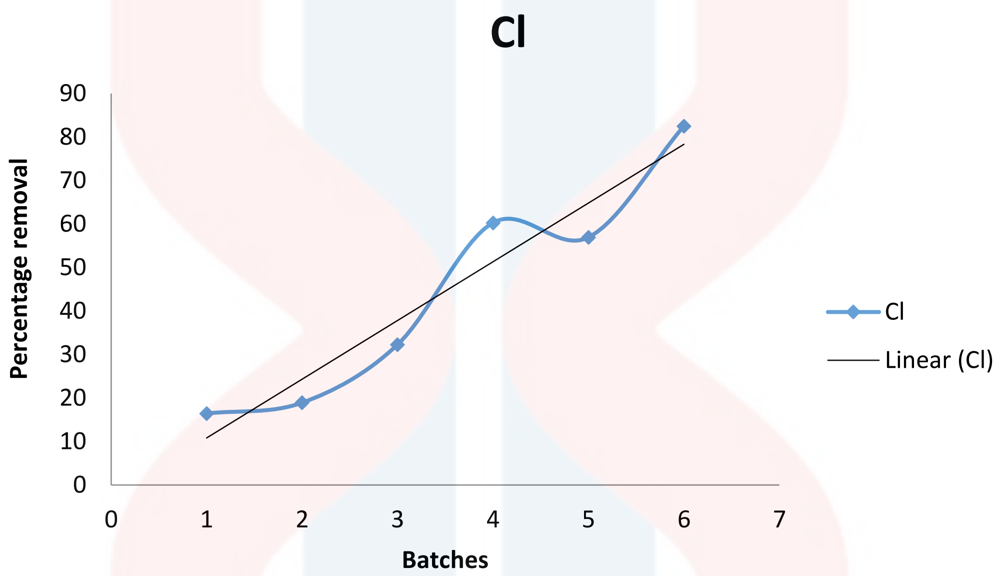

Figure A1:Graph of percentage removal of Cl by *Pistia stratiotes* for sixth batches.

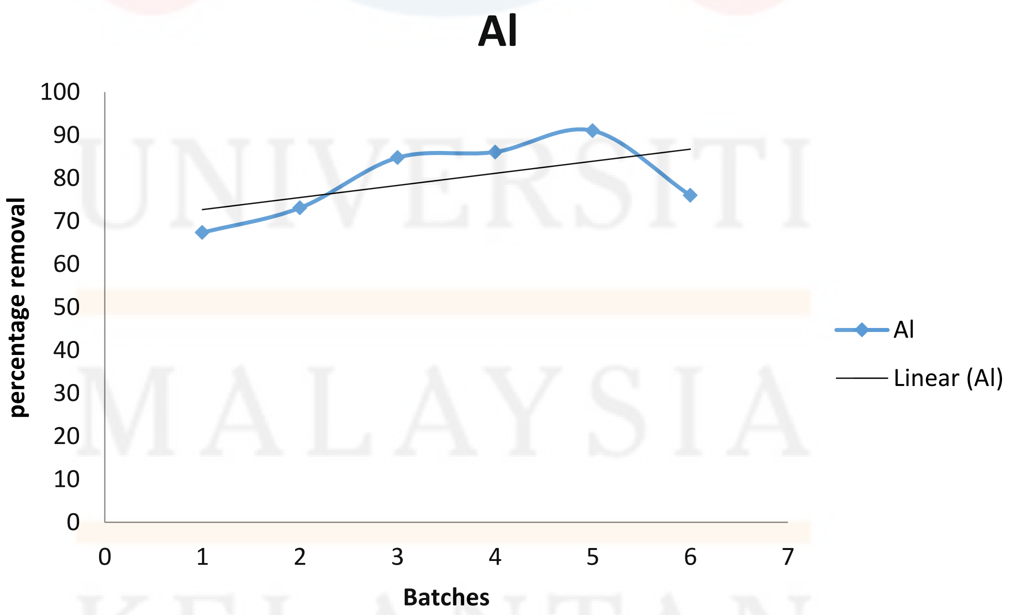

Figure A2: Graph of percentage removal of Al by *Pistia stratiotes* for sixth batches.

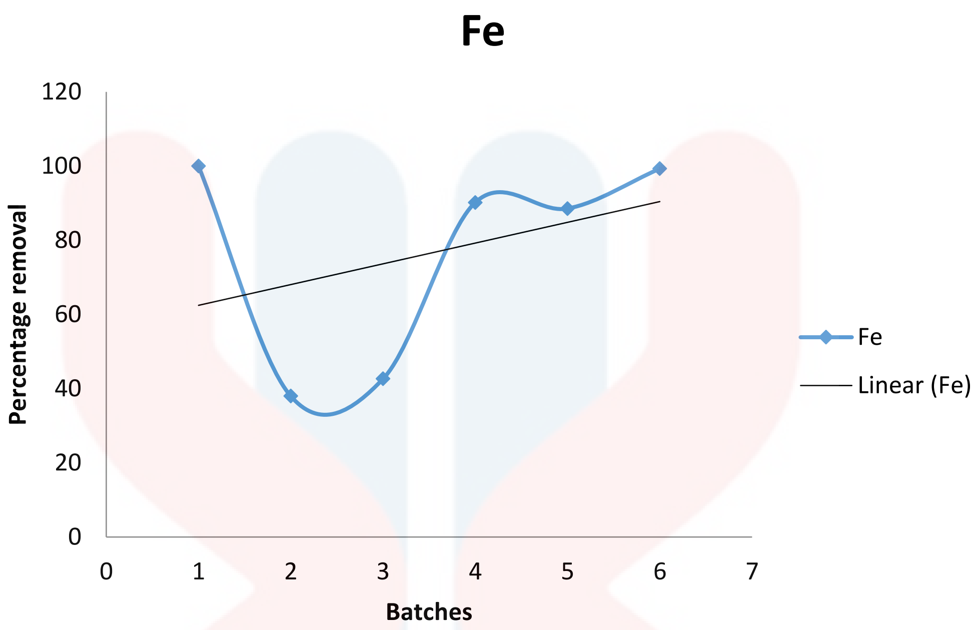

Figure A3: Graph of percentage removal of Fe by *Pistia stratiotes* for sixth batches.

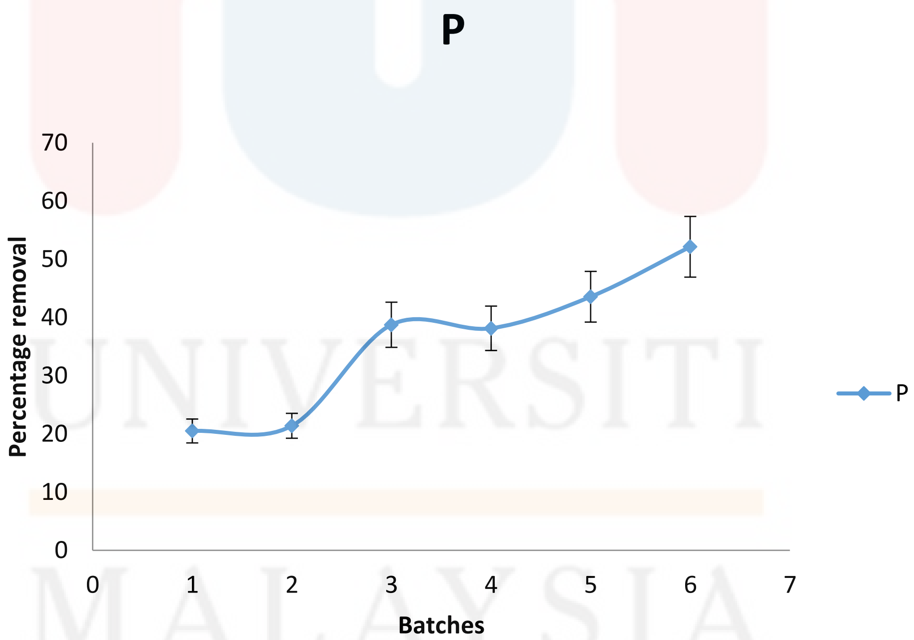

Figure A4: Percentage removal of P by *Pistia stratiotes* for sixth batches.

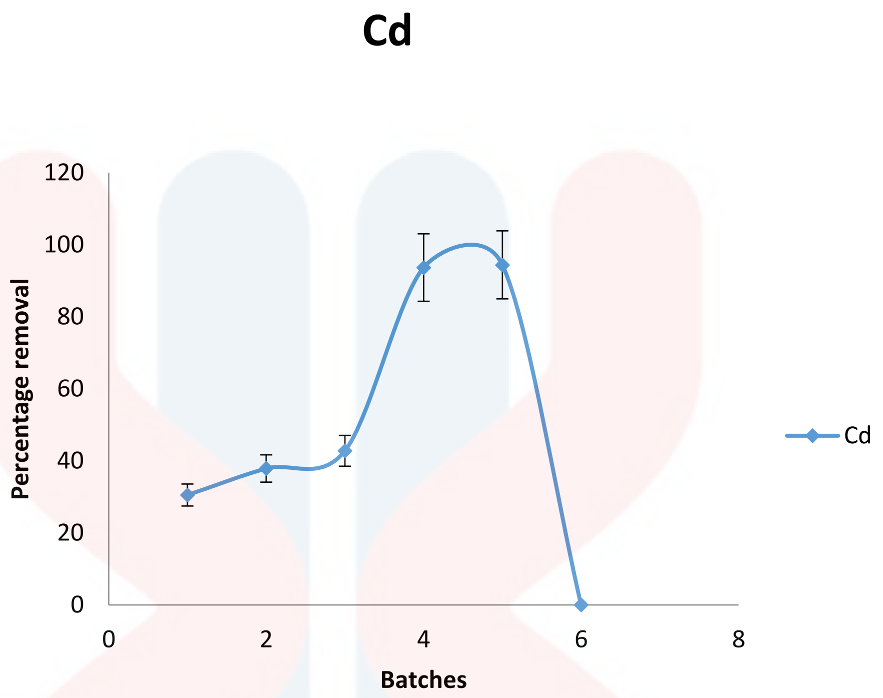

Figure A5: The percentage removal of Cd by *Pistia stratiotes* for sixth batches.

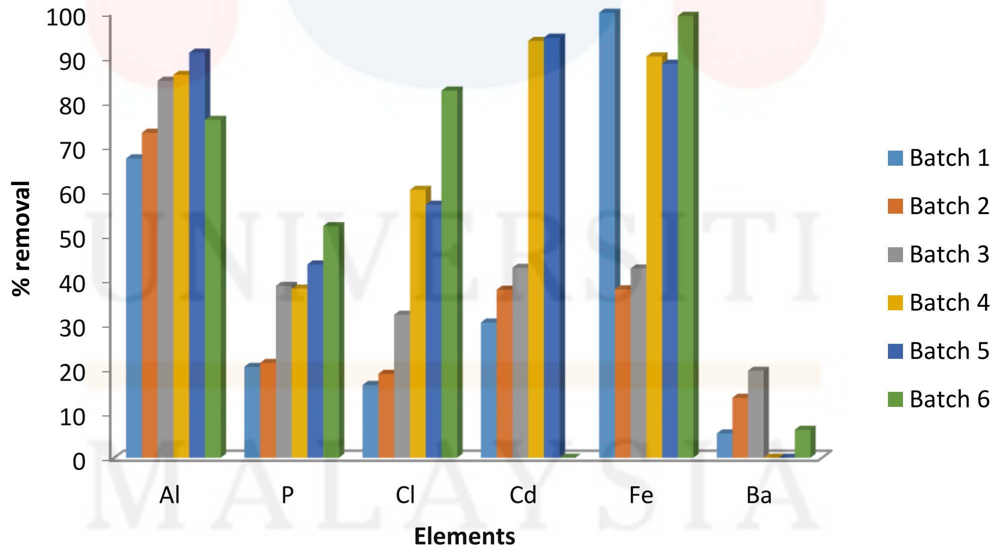

Figure A6: Percentage removal of six elements after being treated with the phytoremediation agent, *Pistia stratiotes.*

Appendix B1: Tables of percentage removal elements and heavy metals by *Pistia stratiotes* for sixth batches.

Elements	Batch 1					Batch 2				
	Initial	Control tank	Final	Changes	% removal	Initial	Control tank	Final	Changes	% removal
Al	0.7738	0.7344	0.2133	-0.5211	67.343	0.7397	0.7293	0.1887	-0.5406	73.0837
P	2.3152	2.4691	1.9945	-0.4746	20.4993	2.2685	2.5263	2.0411	-0.4852	21.3886
Cl	7.0425	44.8885	43.7308	-1.1577	16.4388	4.2336	43.4955	42.6944	-0.8011	18.9224
Cd	0	0.0662	0.046	-0.0202	30.5134	0	0.0623	0.0387	-0.0236	37.8812
Fe	0	4.45	0	4.45	100	3.66	0.0408	0.0269	-0.0139	37.9781
Ba	0.0146	0	0.0008	0.0008	5.4795	0	0.0865	0.0982	0.0117	13.526

Elements	Batch 3					Batch 4				
	Initial	Control tank	Final	Changes	% removal	Initial	Control tank	Final	Changes	% removal
Al	0.7239	0.702	0.0885	-0.6135	84.7493	0.7245	0.7308	0.1072	-0.6236	86.0732
P	2.6812	2.4285	1.4463	-1.0387	38.7401	2.3131	2.3502	1.4681	-0.8821	38.135
Cl	6.6861	8.8245	6.6663	-2.1582	32.2789	4.1862	3.9841	1.4606	-2.5235	60.2814
Cd	0.0243	0.0204	0.01	-0.0104	42.7984	0.0237	0.0233	0.0011	-0.0222	93.6709
Fe	2.2463	1.2162	0.2584	-0.9578	42.639	2.265	0.2077	0.0034	-0.2043	90.1989
Ba	0.1104	0.0217	0	-0.217	19.63	0	0.1183	0	-0.1183	0

Elements	Batch 5					Batch 6				
	Initial	Control tank	Final	Changes	% removal	Initial	Control tank	Final	Changes	% removal
Al	0.7615	0.7432	0.0388	-0.6932	91.0309	0.7623	0.7946	0.2154	-0.5792	75.9806
P	2.6574	2.1628	1.0053	-1.1575	43.5576	2.1303	2.1198	1.0092	-1.1106	52.1335
Cl	44.0952	32.6563	7.5459	-25.110	56.9459	25.0465	23.1641	2.4936	-20.671	82.5285
Cd	0.0609	0.0602	0.0027	-0.0575	94.4171	0.0000	0.0000	0.0000	0.0000	0.0000
Fe	3.4330	3.428	0.0378	-0.305	88.5598	4.425	0.4517	0.0123	-0.4394	99.2994
Ba	7.2540	0.0000	0.0000	-7.254	0.0000	0.226	0.083	0.0688	-0.0142	6.3111

Appendix B2: Table of accumulation of heavy metals and elements in *Pistia stratiotes*

Elements	Initial (mg/L)	Final (mg/L)	Accumulation (mg/L)
Mg	1.265	1.210	0.000
Na	0.654	0.621	0.000
Al	0.012	1.530	1.518
Si	1.040	1.669	0.560
Cd	0.582	1.690	0.629
S	0.470	0.313	0.000
Cl	0.036	0.103	0.067
K	4.120	6.670	2.550
Ca	4.474	2.680	0.000
P	0.356	0.380	0.424
Ti	0.000	0.046	0.046
Mn	0.009	0.000	0.000
Fe	0.480	2.370	1.890
Sn	0.230	0.000	0.000
Sb	0.359	0.000	0.000
Ba	0.090	0.000	0.000

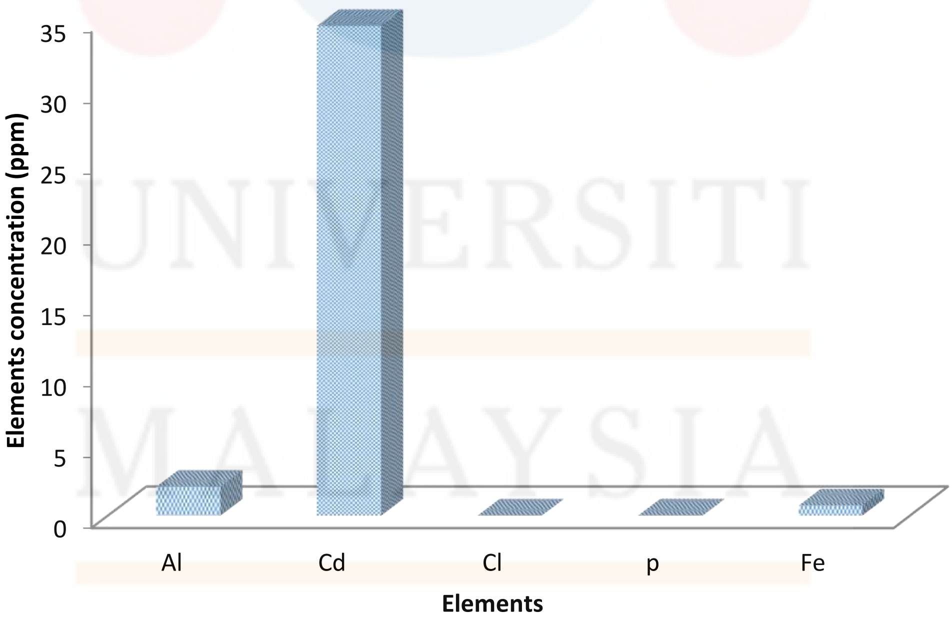

Figure B1: Bioconcentration factor of elements in *Pistia stratiotes* after being treated for 48 hours.

Appendix B3: Table of physico-chemical parameters of aquaculture wastewater.

Batches	TDS (g/L)	DO (mg/L)	pH
Batch 1	61.5385	17.0543	-0.1473
Batch 2	-29.6296	22.8739	-33.3333
Batch 3	-46.4286	9.1954	-13.5065
Batch 4	59.2593	-21.2934	5.7143
Batch 5	31.4286	5.7143	9.8343
Batch 6	14.2857	6.1364	10.8254
Mean	15.07565	6.613483	-3.43552
S.D	45.07852	15.23094	17.14232

Appendix C: Figures

Figure C1: *Pistia stratiotes* in reactor tank

Figure C2: Reactor tank

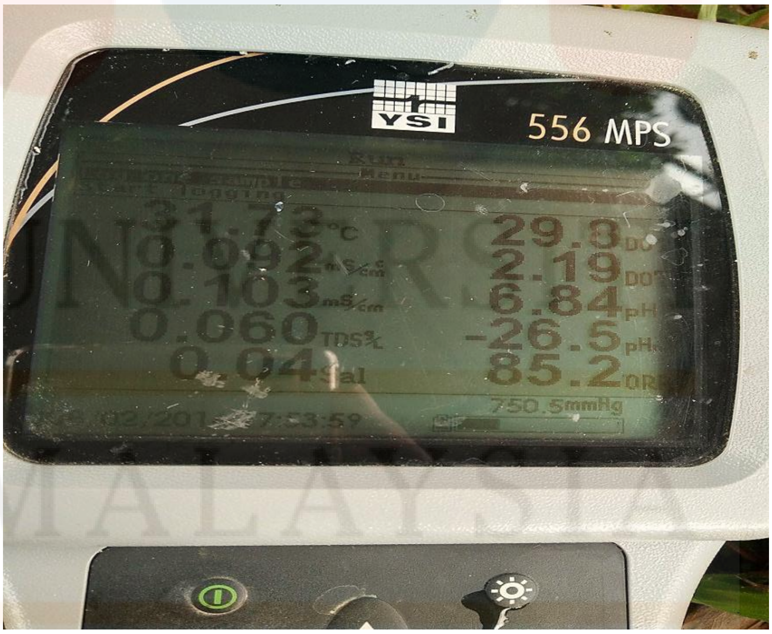

Figure C3: YSI 556 MPS

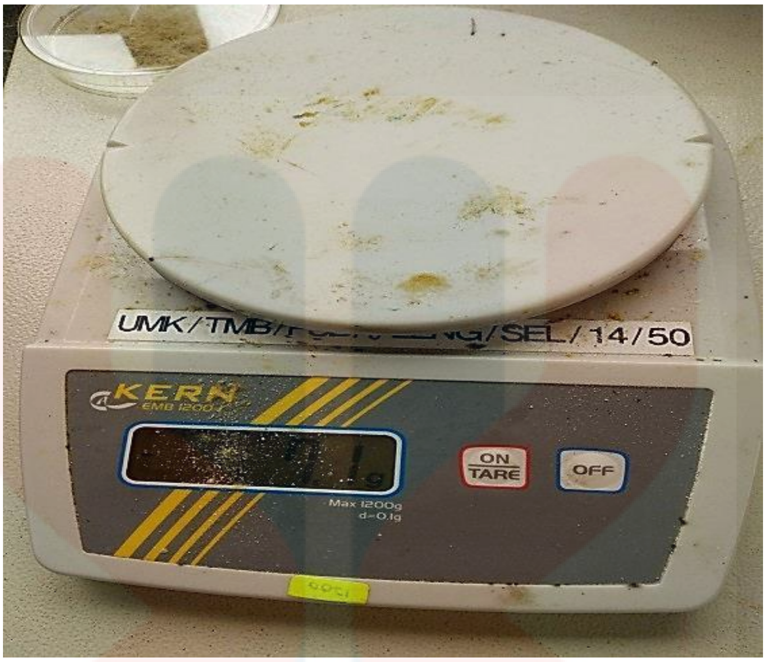

Figure C4: Electronical Balance.

Figure C5: Auto Sieve Shaker

Figure C6: Powdered *Pistia stratiotes* after grinded.

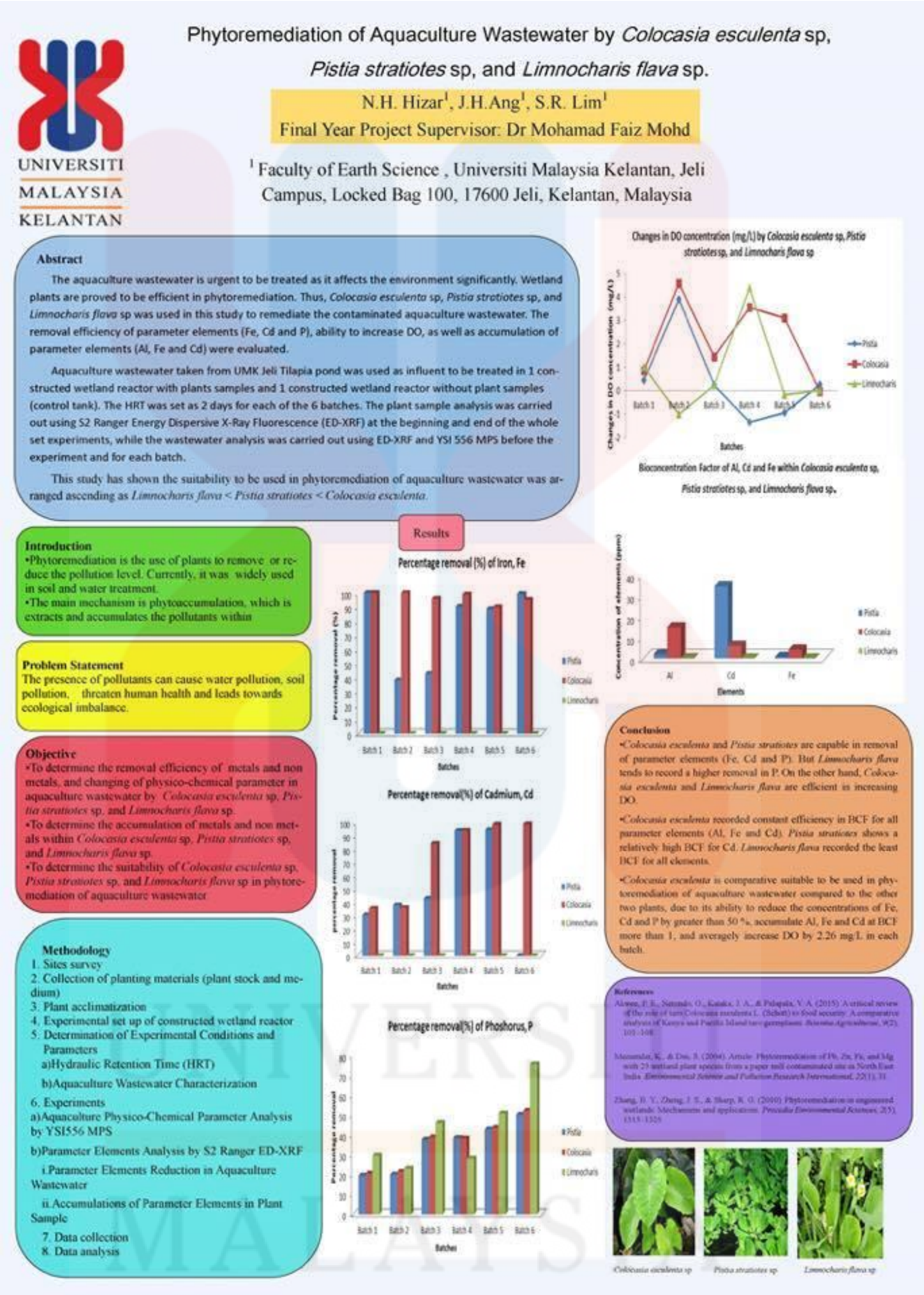

Figure C7: FYP Poster Exhibition for AGX.

Figure C8: Certification for FYP Poster Exhibition for AGX.