PHYTOREMEDIATION OF HEAVY METALS AND DESALINATION OF BRACKISH WATER WITH *IPOMOEA AQUATICA* USING AQUAPONICS SYSTEM

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ABSTRACT

This study presents the phytoremediation and desalination of heavy metals in brackish water with *Ipomoea aquatica* in an aquaponics systems. The study of phytoremediation by *Ipomoea aquatica* in saline condition had not been study extensively. *Ipomoea aquatica* was separated into roots and shoots and the scales of tilapia fish was harvested at Day-0 and Day-30 from two aquaponics tanks. The purpose for this research was to investigate the phytoremediation and desalination capability of *Ipomoea aquatic*. Synthetic saline water and field water collected from Kuantan Estuary were sent for ICP-MS analysis at Day-0 and Day-30 to determine the concentration of 22 types of metal elements contained. The field water sampled from Kuantan Estuary showed a decrease in Na, Mg, K and Ca from Day-0 to Day-30. This indicated that *Ipomoea aquatica* was able to uptake major constituents that contributes to the salinity and has potential in desalination. Na was decreased to 4692.0 ppm from 14070.0 ppm, showing a percentage of 66.65%. Mg had reduced as much of 77.47%, from 1630.0 ppm to 367.2 ppm. K had a reduction rate of 64%, depleting from 446.0 ppm to 159.0 ppm while Ca had a reduction rate of 68.54% when the initial concentration of 486.7 ppm had plunged to 153.1 ppm at Day-30. Cr decreased 33.33%, from 84.0 ppb to 56.0 ppb. There was a total of 12 samples and the samples were subjected to wet digestion. The samples were then analyzed for its Mg content. The samples generally showed an increase in Mg concentration from Day-0 to Day-30. The parameters of water from both tanks including pH, DO, EC, TDS, salinity and temperature were taken as background data throughout the commence of experiment. Conductivity, total dissolved solids and salinity reduced from Day-0 till Day-35 in both tanks as the metal ions were being absorbed by plant biomass and fish scales. In conclusion, the built aquaponics systems were able to phytoremediate heavy metal-contaminated field water by translocating the pollutants within the tissues of *Ipomoea aquatica* and the scales of tilapia.

ABSTRAK

Kajian ini membentangkan *phytoremediation* dan penyahgaraman logam berat dalam air payau dengan *Ipomoea aquatica* menggunakan sistem aquaponics. Kajian *phytoremediation* menggunakan *Ipomoea aquatica* dalam keadaan limpahan belum dikaji secara mendalam. Tujuan kajian ini adalah untuk menyiasat keupayaan *phytoremediation* dan penyahgaraman tumbuhan tesebut. Air limpahan sintetik dan sampel air dari muara Kuantan telah dihantar untuk analisis ICP-MS pada hari-0 dan hari-30 untuk menentukan konsentrasi 22 jenis unsur-unsur logam yang terkandung. Sampel air dari muara Kuantan menunjukkan penurunan dalam Na, K, Mg dan Ca daripada hari-0 hingga 30 hari. Ini menunjukkan bahawa *Ipomoea aquatica* adalah mampu untuk mwnyerap juzuk utama yang menyumbang kepada kemasinan dan mempunyai potensi penyahgaraman. Na telah menurun daripada 4692.0 ppm dari 14070.0 ppm, menunjukkan peratusan sebanyak 66.65%. Mg telah berkurangan sebanyak mungkin ini merupakan 77.47%, daripada 1630.0 ppm kepada 367.2 ppm. K memiliki kadar pengurangan sebanyak 64%, berkurangan dari 446.0 ppm kepada ppm 159.0 manakala Ca memiliki kadar pengurangan sebanyak 68.54% apabila konsentrasi awal 486.7 ppm telah menjunam ke 153.1 ppm pada hari-30. CR menurun 33.33%, daripada 84.0 ppb untuk 56.0 ppb. *Ipomoea aquatica* telah dipisahkan kepada akar dan pucuk dan sisik ikan tilapia adalah dikumpulkan pada hari-0 dan hari-30 dari dua tangka aquaponics. Terdapat sejumlah 12 sampel dan sampel tersebut diprocess melalui cara *wet digestion*. Sampel kemudiannya dianalisis untuk kandungannya Mg. Sampel secara amnya menunjukkan peningkatan pada konsentrasi Mg dari hari-0 hingga 30 hari. Ia didapati bahawa antara sisik ikan, akar dan pucuk, sisik ikan menunjukkan bacaan konsentrasi tertinggi Mg dalam kedua-dua sistem pada hari-0 dan hari-30. Pucuk mengandungi konsentrasi yang lebih tinggi Mg manakala akar mempaparkan bacaan terendah. Parameter air dari kedua-dua sistem termasuk pH, kelarutan oksigen, kekonduksian elektrik, jumlah pepejal terlarut, kadar kemasinan dan suhu diambil sebagai data latar belakang sepanjang eksperimen. Kekonduksian, jumlah terlarut pepejal dan kemasinan berkurangan dari hari-0 hingga hari-35 dalam kedua-dua sistem kerana logam telah diserap oleh *Ipomoea aquatica* dan sisik ikan tilapia.

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LIST OF SYMBOLS

m   milli

g   gram

L   litre

μ   micro

S   siemens

N   number of samples

LIST OF ABBREVIATIONS

Be	Berylium
Na	Sodium
Mg	Magnesium
Al	Aluminum
K	Potassium
Ca	Calcium
V	Vanadium
Cr	Chromium
Mn	Manganese
Fe	Iron
Co	Cobalt
Ni	Nickel
Cu	Copper
Zn	Zinc
As	Arsenic
Se	Selynium
Mo	Molybdenum
Ag	Silver
Cd	Cadmium
Sb	Antimony
Ba	Barium
Pb	Lead
Cs	Cesium

U	Uranium
Sr	Strontium
Hg	Mercury
TPC	Total petroleum hydrocarbon
PCB	Polychlorinated biphenyls
PCP	pentachlorophenols
Fl	Floride
Cl	Chloride
NO_3	Nitrate
SO_4	Sulphate
AAS	Atomic Absorption Spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
SE	Standard error
SD	Standard deviation
ppm	parts per million
ppb	parts per billion
DO	Dissolved oxygen
EC	Electrical conductivity
TDS	Total dissolved solids

CHAPTER 1

INTRODUCTION

This research was conducted to study the use of *Ipomoea aquatica*, an aquatic plant, to remove magnesium, a type of heavy metals in brackish water via phytoremediation means using an aquaponics system. Fish scales of the tilapia in the aquaculture tank would be the secondary subject studied for its properties to accumulate the stated metal element. This chapter briefly explained the background of this research, problem statements, research objectives and the scope of study.

1.1   RESEARCH BACKGROUND

Industrialization has modernized and improved the living style of mankind by exploiting the natural resources that are available on earth's crust. However, this rapid development process resulted in massive waste production which contained organic and inorganic pollutants that were hard to remove or degrade. Soil and water, two of the fundamental resources required to sustain aquatic and terrestrial life, had been contaminated by these waste. Researchers had been conducting extensive studies to remove these toxic pollutants via one of the bioremediation means, which was phytoremediation. This recent technology was non-intrusive. The eco-friendly properties had contributed to the reason phytoremediation had been gaining public acceptance.

Phytoremediation technique using aquaponics system to remove heavy metals had not been studied extensively. It could be scaled up accordingly. The principle of phytoremediation involved the cleaning up contaminated site, could be soil or water, using plants to remove, immobilize, sequester, accumulate or biodegrade the pollutants

(Kiraly, 2003). Aquaponics system was the combination of hydroponics, the farming of plants in water without the need of soil, and aquaculture which reared aquatic animals in tanks. The combination of these two systems created a symbiotic environment to produce fish and plants in an enclosed system.

During phytoremediation application, the contaminated site was first located and identified as the area of study. Sample collection, or biomass harvesting, was done on site by analyzing the potential heavy metal bioaccumulators. These bioaccumulators possessed the traits to grow under harsh conditions, especially when the surrounding environment was polluted by toxic waste, and these plants were able to uptake certain amount of heavy metals. The harvested biomass was sent for lab analysis to determine the concentration of heavy metals accumulated within the plants.

There had been a research that studies the phytoremediation of freshwater crayfish culture wastewater using spinach in aquaponics system (Hefni Effendi, 2015). The waste water contained in the aquaculture tank contained high amount of nitrogen and phosphorus due to the excretion from crayfish. The integrated aquaponics system with phytoremediation technology was capable in removing excess nitrogen and phosphorus by utilizing it as a source of nutrient for the growth of spinach, *Ipomoea aquatic*. However, the availability of studies regarding heavy metal removal in saline condition, or brackish water using phytoremediation technology integrated with aquaponics system had yet to be studied extensively.

The effects of anthropogenic pollution had taken a toll to the aquatic ecosystem as well. Public concerns had been growing as aquatic life was one of the most important source of protein, minerals, trace elements and vitamin contents. As heavy metals were constantly released from man-made activities into the aquatic environment, our food source had been contaminated with these toxic as research had shown that heavy metals accumulate in the tissues of edible fish (Alturiqi and Albedair, 2012). The high level of toxic heavy metals found in the tissues of fish endangers human health as human is the last consumer in the food chain.

1.2   PROBLEM STATEMENT

Phytoremediation in brackish water was lacking in research. Hence for this research, *Ipomoea aquatica*, or better known as water spinach or *kangkung* by Malaysians was used to study the efficiency of heavy metals removal in brackish water collected from Kuantan Estuary. The roots and shoots of *Ipomoea aquatica* and the scales of tilapia were determined for its concentration of magnesium.

The aquaponics system with *Ipomoea aquatica* had the potential to desalinate the brackish water. The outcome of this experiment provided insights for the future as solutions to reduce salinity of contaminated water supply in terrestrial area. This desalination technology integrated with aquaponics system was cost-effective and eco-friendly.

The outcome of the research served as an evidence to prove practicality of a floatable farming system in field application for phytoremediation purpose.

1.3   RESEARCH OBJECTIVES

I.   To evaluate quantitatively the phytoremediation capability of the system in reducing pollutants from the collected water sample.

II.   To investigate the uptake of magnesium by *Ipomoea aquatic* and fish scales of tilapia growing in a recirculating system with brackish water.

III.   To evaluate the desalination property by observing the changes of Na^+, Mg^{2+}, K^+ and Ca^{2+}, which are the components that contribute to salinity.

1.4   SCOPE OF STUDY

This research project mainly studied the uptake of magnesium in contaminated brackish water by *Ipomoea aquatica*. The phytoremediation strategy was integrated with aquaponics system, which involved the combination of a hydroponic technology and a traditional aquaculture. The effluent from the fish tank containing fish excretion served as a nutrient source to sustain vegetative growth in the hydroponic grow bed located above. The brackish water sampled from Kuantan Estuary which believed to contained

heavy metals pollutants. The aquaculture used field water sample to rear marine life, and the brackish water was circulated to the above grow bed. *Ipomoea aquatica* was studied for its property as an agent to remove heavy metals from the field water collected via possible different phytoremediation strategies such as phytoextraction and rhizoflitration.

Atomic absorption spectrophotometry had been used for the approach to determine the trace elements in the tissues of *Ipomoea aquatica* due to its high level of sensitivity, precision, specificity and simplicity (Enders and Lehmann, 2012). The plant tissues and fish scales were subjected to decomposition prior conducting the analysis using atomic absorption spectrophotometry.

Inductively coupled plasma mass spectrometry or ICP-MS had been used as another alternative to determine the trace of heavy metals in the water samples of both tanks. It became one of the most renowned technique for trace elements detection in liquefied samples, for its high selectivity, sensitivity and lower detection limits (Voica, Dehelean, Iordache, Geana, 2012) as compared to other existing techniques.

CHAPTER 2

LITERATURE REVIEW

2.1   BRACKISH WATER

When the salinity of a water source was higher than freshwater, but lower than that of seawater, it was known as brackish water. Commonly, brackish water contained between 0.5 grams to 30.0 grams of salt per litre or could be expressed as 0.5 to 30 parts per thousands. Brackish water was found at area where a river meets the sea, such as estuaries. The uptake of heavy metals by a plant for phytoremediation purpose was influenced by the surrounding temperature and the salinity of the area to be treated. There was a finding stated that the uptake of metal increased with decreasing salinity and increasing temperature in two tested submerged, aquatic plants (Fritioff, Kautsky and Greger, 2005). It was believed that there might be a possibility that a certain degree of salinity might affect the uptake of metals due to the presence of abundant Na^+ and CL^-.

2.2   PHYTOREMEDIATION

2.2.1   Overview

For the past few decades, rapid industrialization had caused the rising of environmental degradation issues. Two of the most important natural resources on Earth to sustain all terrestrial life – soil and water – might had adverse consequences for the ecosystem once contaminated with potential pollutants. This would further lead to the deterioration of human food security and health (Willey, 2007). Key activities from the

industrialization process had left our nature in a state of devastation, with the excessive emission of organic and inorganic contaminants such as pesticides and herbicides, salts, hydrocarbon compounds including petroleum, acids, heavy metals and so on (Ashraf, Ozturk, Ahmad, 2010).

As a result of the rapid accumulation of toxins, our environment ended up in soil degradation, deforestation, and loss of species diversity, pollution, acid rain, greenhouse effects and other environmental issues. In the recent years, researchers had been looking for a more eco-friendly, biological means to remediate this situation to sustain terrestrial ecosystem and to avoid further damages to the environment.

The term "phytoremediation" rised from the combination of the word "phyto-" which means plant in ancient Greek and "-remediation" which meant restoring balance (Etim, 2012). This term was coined in 1980s, and was referred to a set of technologies that remediate a contaminated area by utilizing plants instead of using the conventional physico-chemical method, which cost at a higher rate and its usage might result in an adverse effect to the nature. Fields to had known to apply the technique of phytoremediation including agriculture and horticulture to tackle environmental pollution issues. The information, knowledge and principles of this particular technique were gathered and established through a variety of thorough research areas which comprised of constructed wetlands, oil spills, and accumulation of heavy metals using plants.

Phytoremediation worked on the idea of using a biological approach to remove contaminants at polluted sites by applying plant-based environmental technologies. Generally, the various processes of phytoremediation could be classified into a few categories according to the contaminant fate: degradation, extraction, containment, or a combination of these. The strategies of phytoremediation were classified into a few classes, which would be discussed further in the next subtopic.

2.2.2 Phytoremediation Strategies

This section discussed about the various types of phytoremediation mechanism briefly (United States Protection Agency, 2000). As the contaminants' properties varied

from site to site, there were different types of approach in phytoremediation as options for site managers to choose the most suitable strategy for sites clean-up.

2.2.2.1 Phytoextraction

The uptake of contaminants through plant root system and translocation within the plant was known as phytoextraction (USPA, 2000). When the plants mature, they were then harvested and thus, removing the contaminants as these harmful toxins were contained within the plant (Khan, 2005). The applicable contaminants that were usually removed via this mechanism were heavy metals such as silver (Ag), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb) and zinc (Zn). Contaminants were transferred from a concentrated, large areas into smaller mass, which allowed easier disposal rather than excavating soil with heavy metals. It could also be used to treat sediments, sludge and contaminated water streams at a lesser extent.

2.2.2.2 Rhizofiltration

Rhizofiltration, or also be as phytoflitration, described the action of contaminants found in solution adsorping or precipitating onto the plant roots, or being absorbed completely into the root system of that particular plant (USPA, 2000). The contaminants in solution from the surrounding root system were contained as these harmful particles were immobilized in or within the plant itself. Phytofiltration involved aquatic plants as their roots were longer compared to terrestrial plants, thus a larger surface area for metal sorption (Rezania, Mat Taib, Md Din, Dahalan and Kamyab, 2016). The constituents that were amenable to this phytoremediation method were heavy metals in water bodies as well as radionuclides. The heavy metal ions included Pb, Cd, Cu, Ni, Zn and Cr; while examples of radionuclides that can be remediated are cesium (Cs), uranium (U) and strontium (Sr). This technology was generally used to treat extracted groundwater, waste water, surface water or any water bodies with low concentration of contaminants yet high water content conditions. Such treatment did not perform well with polluted soil, sludge, or sediments as the contaminants must be in the form of ions (or in solution) to be sorbed on or into the plant system.

2.2.2.3 Phytostabilization

Phytostabilization was another option to remove contaminants such as heavy metals including As, Cd, Cu, Cr, Ni, mercury (Hg), Pb or Zn when found in soil, sediments or sludge – via phytostabilization (USPA, 2000). In this process, the harmful toxins was localized within the plant through the action of adsorption and accumulation by roots. To prevent the contaminants from migrating to other parts of the plant, these particles could also be adsorbed onto roots or precipitated within the root zone of the plants. Basically, this mechanism involved the using of plants and plant roots to immobilize the contaminants. Another form of phytostabilization, known as phytolignification, was a term used to define the incorporation of organic pollutants into plant lignin. The removal of organic compound is possible by incorporating these particles into humic material in soils. Humic materials made up the major part of the natural organic matter in soil and water (geological organic deposits too) and they were responsible in contributing the darkening of surface soil, either browning or blacking.

2.2.2.4 Rhizodegradation

When the breakdown of an organic pollutant in soil through microbial activity, usually involve enzymatic reaction, was assisted and enhanced by the presence of the root zone, the mechanism was known as rhizodegradation (USPA, 2000). The interaction between roots and microorganism such as fungi, bacteria, nematodes and so on were triggered by the root-producing compounds, root exudates. Examples of root exudates including sugars, amino acids, organic acids, sterols, growth factors, enzymes and so on. Root exudates served to maintain, support a highly specific yet diversified population of microorganisms in the rhizosphere of a plant species. The biodegradation process occurring in the rhizosphere was mediated in the presence of the secreted root exudates by the plant, whereby the organic contaminants were dissimilated biologically by the high microbial populations. Furthermore, microbial degradation could be stimulated at a higher rate with the increase surface area of rhizosphere. Even in the absence of root exudates, indigenous microbes could carry out biodegradation of organic contaminants in soil by providing a suitable, cohesive environment. The contaminated site surrounding the root zone was made favourable for the occurrence by aerating the soil and moderating the soil moisture content. This method of phytoremediation was not limited only to toxic

organic compounds in contaminated soil, but applicable sediments, groundwater and surface water treatment. The treatment made use of plants transpiration bringing the pollutants from the water body to the root zone for degradation to occur. Successful removal of organic pollutants included total petroleum hydrocarbons (TPC), polychlorinated biphenyls (PCBs), pentachlorophenols (PCPs), pesticides and herbicides, chlorinated solvents and surfactants.

2.2.2.5 Phytodegradation

Unlike rhizodegradation, where the biodegradation process was an interaction between the indigenous microorganisms in soil and the root system, phytodegradation involved the uptake of the organic and inorganic contaminants by the plant (USPA, 2000). The breakdown of the organic and inorganic pollutants occurred within the plant through metabolic processes along with associated microbes (Khan, 2005). It was vital to consider the properties of contaminants (its solubility, polarity and hydrophobicity) as well as the characteristics of the particular plant used for phytoremediation as the uptake of contaminants relied mainly on these two aspects. Other factors including the properties of contaminated site and the age of contaminants did play a role in affecting the rate of uptake. The breaking down of contaminants occurred externally - outside of the plants - through the secretion of substances by that plant such as enzyme for degradation although it was less likely to happen. Commonly, phytodegradation was involved in the treatment of soil, sludge, sediments, groundwater and surface water. The constituents that were removed via this mechanism consisted of two classes: organic and inorganic matter. The examples stated were chlorinated solvent, insecticides, herbicides, phenolic compounds, munitions for organic pollutants while inorganic compounds comprise of various nutrients.

2.2.2.6 Phytovolatilization

Volatilization described the process of a dissolved sample vaporizing. Phytovolatilization involved the bioremediation process with the assistance from a particular plant for its uptake of contaminants (USPA, 2000). The contaminants underwent plant metabolism after the uptake, and was subsequently released back into the atmosphere (Khan, 2005) in either its original form or as a modified by-product which

was less harmful to the environment through transpiration. Common contaminants that could be removed through phytovolatilization include organic compounds such as chlorinanted solvents; and inorganic matters such as Se, Hg and As.

2.2.2.7 Hydraulic Control

Another unique strategy of phytoremediation by controlling the migration of pollutants by the means of restraining water movement using plants uptake and consumption (USPA, 2000). This method was known as hydraulic control, or phytohydraulics or hydraulic plumes control. Since this technology was used mainly for the treatment of groundwater and soil water, the major requirement of the involving plants responsible for remediation must possess the trait of deep root system. Such treatment was used to remove water soluble, leachable organic and inorganic pollutants at a concentration that would not harm the plants.

2.2.2.8 Phytocover

Phytocover, which was a vegetative cover system, manipulated the self-sustaining system of plants growing in and/or over material that posed potential threats to the environment (USPA, 2000). The environmental risk was lowered to an acceptance level, requiring only minimal effort for maintenance through vegetative cover system. Basically, there were two types of phytocover, namely evapotranspiration cover and a phytoremediation cover. In evapotranspiration, factors such as the storage capacity availability of the soil, rate of evaporation, transpiration processes of plants had been maximized by using a combination of soil and modified plants. This served to keep water infiltration at minimal, its mechanism is as similar to that of hydraulic control by plants. On the other hand, phytoremediation cover also minimized water infiltration just like evapotranspiration cover. The main difference between these two phyytocovers was phytoremediation cover assisted the degradation of buried, underlying contaminants by biological means. This method was used to uptake infiltrating surface water, removing organic and inorganic contaminants from the surface water or in soil, sediments or sludge (for phytoremediation cover).

2.3   HEAVY METALS

Within the past two centuries, the sudden spike in industrial processes for development had been contributing a considerable amount of potential environment pollutant – heavy metals. Industries that focused on the manufacturing of batteries, alloys, electroplated metal parts, herbicides and pesticides, textile dyes and steel and etcetera had caused metal pollution to the environment through their many key activities. Large scale agricultural practices should be also held responsible for the widespread of the contaminants into soil, sediments and water streams.

The improper and poor management for disposal of industrial wastes had impaired the nature by causing the accumulation of pollutants in two of the most important natural source to sustain all living organisms on this planet, which were soil and water. A variety of organic and inorganic compounds had caused the pollution of the natural source, resulting in the scarcity in clean, usable resources. A variety of organic and inorganic compounds were the main culprits behind the occurring of soil and water pollution. However inorganic contaminates which were heavy metals, posed a greater threat as compared to organic contaminants. Unlike organic contaminates that could be removed via biodegradation by soil microorganism, heavy metals required to be immobilized or removed physically.

Proper methods of disposing these heavy metals and other inorganic pollutants were available, by using physical or chemical approach. However due to its high cost and its applications would have negative side effects towards the environment, researchers were looking into methods that were milder, usually involves biological metabolisms.

Metals, chemical elements with metallic properties, were classified into two categories based on their density. Those with a density less than 3.5 g cm^{-3} fell in the category of light metal, while those with relatively high density, more than 5-6 g cm^{-3}, were known as heavy metals. Never the less, these metal elements were considered as macroelements, micronutrients or trace elements based on the needs and necessity for living organisms.

Occupational Safety and Health Administration or OSHA had labeled 35 metal elements as heavy metals. Among the listed heavy metals, the common ones include are As, Pb, Hg, Cd, Ni, Fe and Al which would result of toxicity in plants and animals. Though heavy metals once contained at a saturated concentration might cause detrimental effects towards environment, some metal elements acted as micro-elements which were essential nutrients for plants and living organism in a lower concentration. Fe, Cu, Zn and Mn were vital elements for plant metabolism only at low concentrations; Co, Ni, Sn and Cr were necessary in human dietary; As and V were required to maintain the good health of animals.

The discharge of heavy metals into the environment, especially into aquatic system had significantly affected the balance of marine living organism, aquatic plants and marine microorganisms, thus jeopardizing the balance of aquatic ecosystem. Aquatic ecosystem once contaminated by various heavy metals it could lead to detrimental consequences towards human health as aquatic ecosystem played the role as the main source of food and water.

The common detoxification procedure of heavy metals from water bodies included chemical precipitation, solvent extraction and reverse osmosis (Chandra Sekhar, Kamala, Chary and Anjaneyulu, 2003). The efficiency of heavy metals removal via these techniques did not fulfill the expectation as these techniques did not remove the metal completely; the processes required a variety of reagents and energy; and might have resulted in the production of toxic sludge or other waste products which required proper disposal.

2.3.1 Magnesium

OSHA had enlisted magnesium along with other 34 elements as heavy metals (Podsiki, 2008). The permissible exposure limit regulated by OSHA was $15mg/m^3$. In Malaysia, the recommended acceptance value for raw water quality and the drinking water quality standard should not exceed 150 mg per litre or 150 ppm (Engineering Services Division, 2010).

The presence of abundant magnesium in water source contributed to its hardness. Water suppliers were recommended to soften and to condition the hard water as to consider the nutrition value, health-related and other pertinent aspects (World Health Organization, 2009). The water quality might have been negatively impacted and the integrity of piping system might be jeopardized due to corrosion and scaling.

2.4 AQUAPONICS

Aquaponics system was the combination of hydroponics agriculture and traditional aquaculture (Rakocy, Masser, and Losordo, 2006). This integrated system allowed recycling of nutrient waste from the tank and conserves water. The effluent waste was recirculated to the hydroponic grow bed as it was used as an input to stimulate and enhance vegetative growth.

Aquaponics was environmental compatible as it did not discharge the waste into the environment unlike traditional aquaculture, and energy-conserving as this was an enclosed system. The crops grown in this system were free from synthetic fertilizer as the nutrient would be supplied from the fish excretion contained in the water, while the waste water could be reused through biofiltration (Radosavljević *et al.,* 2014*)* . The waste products were converted into organic fertilizer by a biofilter, which could be used readily by plants through absorption. Now that the waste products had been removed by passing through the biofilter, the "clean" water was returned back to the tank.
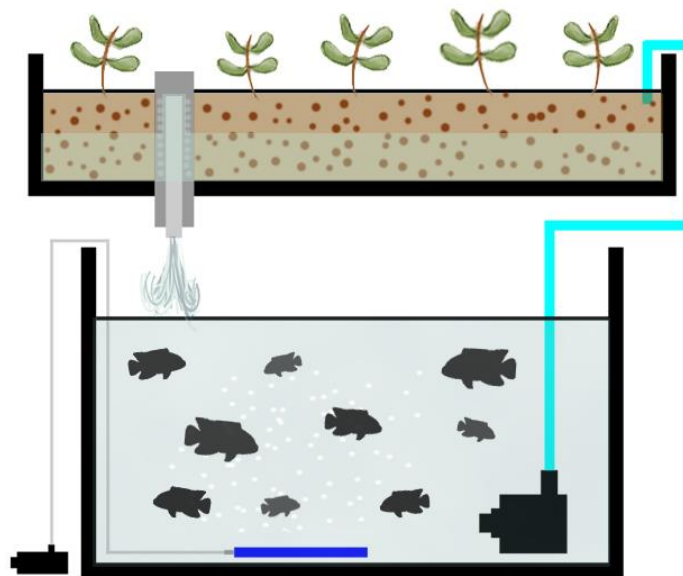

Figure 2.1. A basic aquaponics system diagram.

Source: www.hydroponicsworld.co.za

A review had stated that there was a bright prospect of combining wastewater treatment in recirculating aquaculture system with the production of vegetative crops

(Graber and Junge, 2009). The plants grown in the systems were tomato, aubergine, cucumber and these plants played the role as biofilter, removing a high rate of nutrient waste such as nitrogen, phosphorus and potassium.

2.5   WATER SPINACH, *Ipomoea aquatica*

2.5.1   Background

Water spinach was a semi-aquatic and tropical plant which went by the scientific name of *Ipomoea aquatica*. It was commonly grown in the regions of tropical and subtropical and was known by different callings by the locals based on the country. In Malaysia, locals regarded water spinach as *kangkong*, the cheapest vegetable (The Star, 2014).

Although water spinach was known to be a water-loving plant, it grew well while being cultivated in soil as long the plant was watered heavily on daily basis. The length of its stems in average is 2-3 metres long, and the inner of it is hollow and contributed to its floating characteristics when it grew along swamp or riverside areas. The leaves of water spinach took various forms, from arrow-headed shape to lanceolate, with the general length ranged from 5-15 centimetres long and the breadth from 2-8 centimetres. When the plant matured, it bloomed white trumpet-shaped flowers, with a pale purple center. There were a few methods to propagate water spinach, either by direct planting of stem cuttings or planting from seeds.

2.5.2   Water Spinach for Heavy Metals Removal

It had been a growing concern for environmental authorities as freshwater sources as well as seawater had been polluted heavily by heavy metals since the past few decades. This was due to the rapid industrialization and their improper, poor management of waste products. Researches and studies had been conducted to remediate the contaminated aquatic environment as to prevent further detrimental effects to terrestrial beings and marine life. Aquatic macrophytes had been identified as an agent to restore contaminated waterbodies via phytoremediation as these plants contained the necessary mechanism to

absorb and bioaccumulate soluble or any bioavailable contaminants from the water source (Rahman and Hasegawa, 2011; Kamal, Ghaly, Mahmoud and Cote, 2004).

Though the capacity of accumulating heavy metals within the plant system of an aquatic plant was lower than terrestrial plant, researchers believed that this category of plants had the potential to be an excellent hyperaccumulator. A plant had been identified as an ideal plant used for phytoremediation or hyperaccumulator, with its bioconcentration factor exceeding the value of 1.0 (Duman, Leblebici and Aksoy, 2009).

Several studies had been conducted to study the uptake of different contaminants by *Ipomoea aquatic*. *Ipomoea aquatica* had been proven to be a potential aquatic plant in remediating wastewater containing Cd as implied by its high bioconcenration factor for Cd (Wang, Huang, Lee, Chen and Chang, 2008). In another study conducted in Sri Lanka, *Ipomoea aquatica* was tested for its capacity for Cr (VI) removal (Weerasinghe, Ariyawnasa and Weerasooriya, 2008). This study had shown an outcome of 75% of chromium (VI) was absorbed in a simulated contaminated condition. A study reviewed the possibility of utilizing *Ipomoea aquatica* for phyto-remediate lead contaminated wastewater (Loitonjam and Gupta, 2016). This research found that water spinach was capable in accumulating excess lead at its roots and lower stem parts. Several scientists had carried out an experiment and found that water spinach was also capable in the uptake of di-*n*-butyl phthalate in contaminated soil (Cai *et al*, 2007).

2.6   TILAPIA

The fish chosen to rear in the aquaponics systems was tilapia. Despite tilapia was a type of freshwater fish, it was believed to evolved from marine ancestors (el Sayed, 2006). A study was conducted to test the salt tolerance of tilapia fish, and from the research outcome, there were three species of tilapia fish that could adapt and survive under high salinity condition. Hence in this experiment, tilapia fish was chosen as a suitable choice to be reared in synthetic saline water and field water collected from Kuantan Estuary for study purpose.

In this experiment, fish scales from tilapia were harvested and analyzed for the trace of heavy metal elements. The tissues of marine life had been able to absorb various

heavy metals and other contaminants (Yang, Yao, Xu, Jiang and Xin, 2007). Organic pesticides and heavy metal elements such as Cu and Zn had been found on the gills, liver, brain and muscle of fish with various levels.

CHAPTER 3

METHODOLOGY

3.1   OVERVIEW

This chapter described the methods applied to study the phytoremediation process in removing the targeted heavy metal element, magnesium using *Ipomoea aquatica*. The aquaculture tank was filled with brackish water, sourced from Kuantan estuary. The water was recirculated to reach the hydroponic grow bed to supply nutrients that was obtained from marine fish excretion for plant growth.

The plant in the grow bed and the marine fish grown in the aquaculture tank were harvested to determine the content of the magnesium. *Ipomoea aquatica* was separated into two parts, which were the roots and shoots respectively to study the accumulated amount of heavy metals at different parts of the plants tissue. This plant had the capability to perform phytoextraction, by immobilizing or adsorbing inorganic pollutants from a contaminated water source. The scales of marine fish harvested from the aquaculture tank were studied for its bioaccumulation properties as a study suggested that aquatic animals could take up metals at a high concentration (Al-Weher, 2008).

The essential elements for this study were the field water collected from Kuantan estuary and the biomass gained from the aquaponics, which included *Ipomoea aquatica* and tilapia. We set up an integrated aquaponics system of an ideal size and dimension; a suitable laboratory equipment, apparatus and chemical reagents to determine the different types of heavy metals contained in the collected field water sample and for biomass metal analysis.

3.2   PRE-PREPARATION

This section described the procedures in setting up the aquaponics system to study the phytoremediation process in removing heavy metals using *Ipomoea aquatica*. The scales of the tilpaia reared in the aquaculture tank were collected to study its ability to bioabsorb trace elements contained in the water.

In this study, two aquaponics tanks were set up with one served the purpose as the subject of study while the other tank was the control experiment. The plants and fish in the control tank served as the reference material in a pollutant free condition.

3.2.1   Water Source Collection

Commonly, there were three typical water sources available for study and analyze the efficiency of phytoremediation using living aquatic plants. The three options were lab water, synthetic waste water and field water. In this experiment, we used field water as the water source for this study was taken directly from Kuantan Estuary, a site to be treated. It was a suitable choice as to gain site-specific data.

The water sample collected from Kuantan Estuary had a salinity higher than that of fresh water, yet lower than sea water. Such water sample is known as brackish water. The collected brackish water was analyzed for the trace of heavy metals using inductively coupled plasma mass spectrometry or atomic adsorption spectrophotometer (Mishra and Tripathi, 2008).

Meanwhile, a control was established as a baseline. The establishment of this experiment control was by inducing a known amount of tap water to have the same salinity as the field water collected from Kuantan estuary. The ideal control of this experiment was expected to be magnesium-free but it was impossible as tap water contained slight amount of different heavy metals. However, as the traces of magnesium in tap water was too little that it is not detectable, we assumed that the tap water is free from magnesium. This non-contaminated saline conditions allowed us to monitor and compare the results of the main experiment.

Figure 3.1. Collecting water from Kuantan Estuary.

3.2.2   Purchasing *Ipomoea aquatica*

Ipomoea aquatica was purchased from a local wet market. The upper shoots were removed, leaving only the lower stem with attached roots to be integrated into the grow bed for vegetative growth.

3.2.3   Setting up Aquaponics System

Aquaponics was a combination of hydroponic agriculture and traditional aquaculture, with the tank installed below the hydroponic grow bed. The aquaculture tank was filled with the brackish water from Kuantan Estuary and tilapia fish was reared. In order to sustain the growth and survival of the aquatic beings, an air pump was installed for aeration purpose as to provide sufficient oxygen for respiration. Since there might be a possibility that "algae-bloom" might occur in the tank, a board was placed over the fish tank to prevent sunlight exposure (Hu *et al*., 2015).

The hydroponic grow bed consisted of hydrotons, which acted as a media for nitrogenous bacteria to adsorb onto; and as a solid support for plant growth. The *Ipomoea*

aquatic with its roots attached to the lower part of the stem, was transferred to the grow bed. Water containing the nitrogenous waste due to fish excretion was pumped up to the grow bed to provide nutrients for plant growth. The plants obtained light source directly from the sun for photosynthesis since this aquaponics system was built in a greenhouse, located behind Kolej Kediaman 3 in Universiti Malaysa Pahang, Gambang campus.

Since aquaponics was an enclosed system, water was recirculated from the aquaculture tank to the grow bed. Subsequently the water was drained back to the tank after treated by the aquatic plants in the hydroponic bed. There was no plan of changing water throughout the commencing of this experiment. However, water was replenished for the loss due to evapotranspiration.

3.3   SAMPLE PREPARATION

The fully integrated set up aquaponics system was allowed to run for 14 days to allow the system to stabilized. *Ipomoea aquatica* and the tilapia were transferred into the aquaponics system. The plants and marine fish were harvested after 30 days for heavy metal analysis. Samples were harvested twice which are at Day-0, the day when the plants and fish was introduced into the system and Day-30, one month after the integration. The harvested samples were subjected to wet digestion method using acid prior analyzing with atomic adsorption spectrophotometer.

Samples were subjected to wet digestion procedure using acid as this procedure allows the determination of many elements, including the potential toxic inorganic metals. Compared to other procedures such as dry ashing and microwave digestion, this method prevented the loss of elements at high temperature due to volatilization (Perkin Elmer Coorporation, 1996). Wet digestion did not require high temperature for sample decomposition. The weighed samples were digested by adding acid and heating it for several hours. Upon completion, the mixture containing acid and the digested plant tissues were diluted to a certain volume before proceeding with atomic absorption spectrophotometer analysis. In certain cases, if the concentration of element of interest is low for detection, one may extract it into an organic solvent. This method however, required operator attention as the procedural steps were tedious and complicated and the mixture was subjected to reagent contamination.

3.3.1   Plant Sample Preparation

Ipomoea aquatica was separated into roots and leaves as to study the uptake of heavy metal in different parts of the plant tissue. The plant samples were subjected to wet digestion approach by using nitic acid that has been modified slightly (Hseu, 2004).

The plant biomass was rinsed under running tap water and subsequently with distilled water to remove any remnants. The plants were dried in an oven at 70°C until a constant mass was obtained. The dried plant samples were grounded with a pestle and mortar, and stored in a suitable vessel for further attention (Tüzen, 2003).

Plant samples was weighed for 1.0 g and was added with 10.0 mL of concentrated HNO_3. The mixture was left to stand overnight at room temperature to allow pre-digestion. The ideal weight of samples weight between 0.5 g to 2.0 g to prevent a vigorous reaction from occurring while adding concentrated acid for treatment.

Using a hot plate, the mixture was heated up to 90°C in a water bath. The heating process continued until the emission of red fumes halted, and a clear transparent solution was obtained. The heating process might take up to 8 hours. During the heating process, 5.0 mL of concentrated HNO_3 was added at intervals for at least three times. This step was to ensure the plant tissues had been completely digested, allowing a higher recovery rate of heavy metals accumulated in that part of the plant. The sample was then filtered to remove the remains of the biomass. The filtrate was stored in a chiller for further usage.
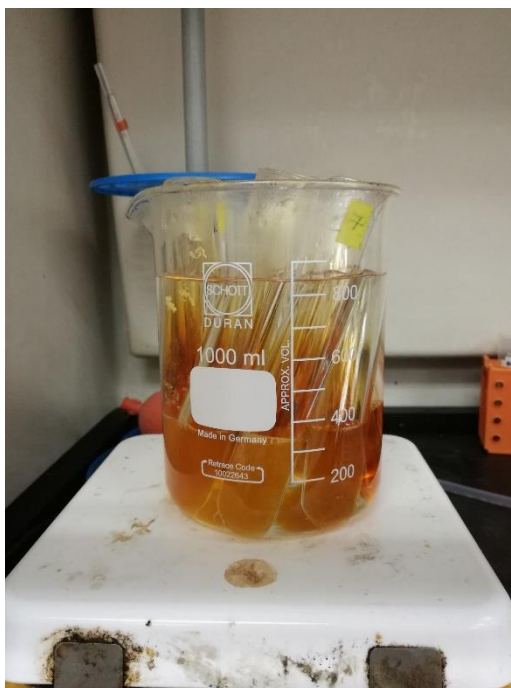
Figure 3.2. Heating plant samples in a water bath at 90°C.

3.3.2   Fish Sample Preparation

The scales of tilapia was used to study for the trace of heavy metals as it has the capability to accumulate trace elements from its surrounding environment. Similar to the approach used to digest plant tissue samples, the scales of marine fish were subjected to wet digestion using acid (Khalifa *et al*., 2010; Akan and Mohmoud, 2012).

The fish scales were collected, washed under running water and lastly rinsed with distilled water. The scales were dried by placing in an oven at 70°C until a constant weight was obtained. The dried tissues were homogenized and grinded using a pestle and mortar, to obtain a powder form which was then digested using nitric acid.

Ten mL of 1 M HNO_3 was added to 1.0 g of dried sample and the mixture was placed in a water bath set at a temperature of 100°C. The heating process continued until dissolution completed and a clear transparent solution was obtained. The solution was filtered once it had been cooled to room temperature. 1 M of HNO_3 was used to wash the obtained precipitate and the walls of the tube to prevent loss of heavy metals. The filtrate was stored for further usage.

3.4   SAMPLE ANALYSIS

Determination of heavy metals in the digested plant biomass and fish scale was done using atomic adsorption spectrophotometer. Standard solutions of magnesium were prepared to determine the concentration of heavy metals deposited in the plant tissues and fish scales. The concentration of standard solutions prepared was 1ppm, 2ppm, 3ppm, 4ppm and 5ppm of a volume 50 mL each.

The water samples from both tanks were subjected to ICP-MS to determine the concentration of contained trace elements at Day-0 and Day-30.

Figure 3.3. Atomic Absorption Spectrophotometer in ANNEX lab at FIST.

3.5   WATER QUALITY PARAMETERS

Water parameters such as DO, pH, EC, TDS, salinity and temperature were monitored using suitable probes throughout this experiment (Lawson and Lawson, 2011). This was to ensure a constant, optimum environment in the aquaculture tank. Six water quality parameters were taken for 5 consecutive weeks as background data using Atlas Scientific Probes. The readings were taken in triplicates. The mean, standard deviation and standard error were calculated accordingly.

Figure 3.4. A laboratory-grade pH sensor kit from Atlas Scientific.

Source: http://www.atlas-scientific.com

Figure 3.5. Recording water quality parameters readings using Atlas Scientific probes.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter explained the outcome of the experiment in terms of ICPMS analysis, AAS analysis and the measured water quality parameters in the aquaponics system.

Two aquaponics systems were set up as shown in **Figure 4.1**. In the grow bed, *Ipomoea aquatica* with it lower part of stems along with its roots was planted in the grow bed and tilapia fish was reared in the aquaculture tank. The control tank was filled with synthetic saline water while the study tank contained filed water collected from Kuantan Estuary.

Figure 4.1. The aquaponics systems set up in this experiment.

4.1   ICPMS ANALYSIS

In this experiment, two types of water were used: one was the synthetic saline water and the other was field water. The synthetic saline water was prepared by inducing a salinity of 2.62% in a tank containing 60 L of tap water with NaCl. This salinity level was the same as the field water collected Kuantan Estuary. As mentioned in the previous chapter, water samples were collected from both tanks, the control tank and the one containing the field water, which would be known as the studied tank. The collected water samples were subjected to inductively coupled plasma mass spectrometry or ICP-MS test for elemental analysis. Water samples were collected at Day-0 and Day-30 from both aquaponics tanks, hence there would be a total of four samples.

A total of 22 heavy metals were determined for their concentration in the sample from the ICP-MS test, which included Be, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Sb, Ba and Pb. At Day-0, the synthetic saline water in the control tank which was made up of tap water and NaCl was tested. Only 6 types of elements were detected as the concentration exceeded the lowest limit of detection for ICP-MS test, which were Na, V, Cr, Mn, Cu and Zn. The water from studied tank collected at Day-0 was tested showed 9 types of elements were present, including Na, Mg, K, Ca, V, Cr, Mn, Cu and Pb. Other elements were deemed not detected as the concentration did not exceed 0.5 ppb. The certificate of analysis for both water samples collected on Day-0 with the complete list of metal elements along with their concentrations was attached at the **Appendix A**.

Water sample was collected from both aquaponics system and sent for ICP-MS analysis after 30 days. Surprisingly, the result outcome for water sample collected at Day-30 showed unexpected differences as compared to the results from Day-0. The water from control tank had more than 5 metal elements: Na, Mg, Al, K, Ca, V, Cr, Fe, Ni, Co, Zn, As, Se, Ag, Ba and Pb. The same observation was seen from the analysis of water sample from the studied tank, more elements were detected in the sample in a trace amount. There were Na, Mg, Al, K, Ca, V, Cr, Mn. Ni, Zn, As and Ba. The certificate of analysis for both water samples on Day-30 with the complete list of metal elements along with their concentrations was attached at the **Appendix B** as well. **Appendix C** showed the

comparison of metal element concentrations of the water sample from both tank taken at Day-0 and Day-30.

Na, Mg, K and Ca were more significant as their respective concentrations were much higher than the other metal elements as stated in the grey shading of Appendix C. Hence, **Figure 4.2**, **Figure 4.3** and **Figure 4.4** only illustrated the difference of Na, Mg, K and Ca concentration in the two tanks at Day-0 and Day-30.
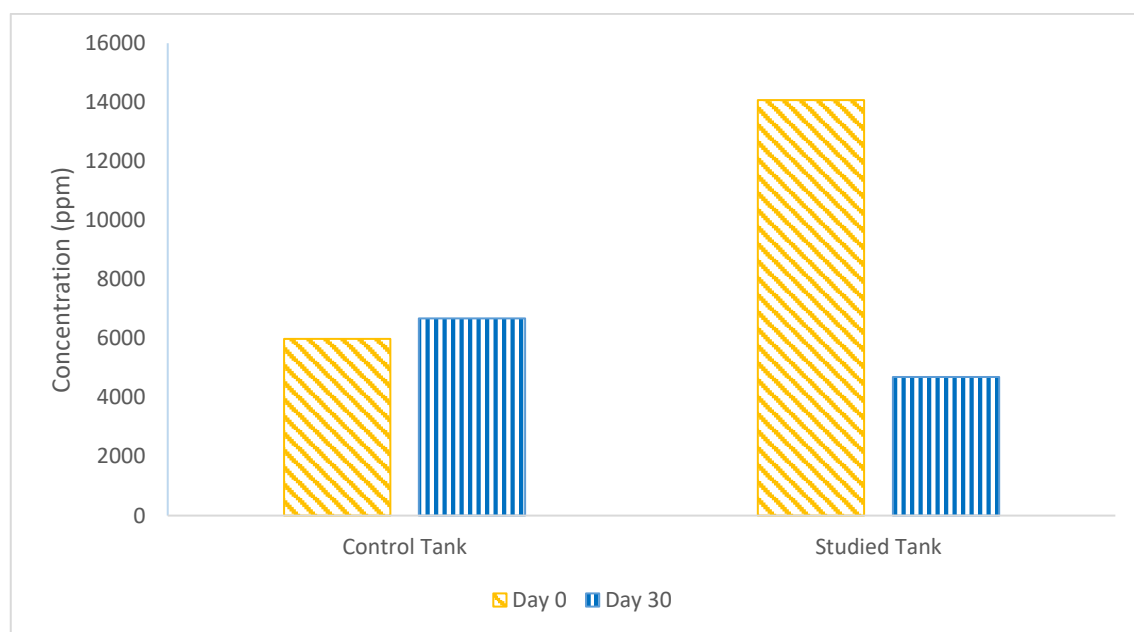

Figure 4.2. The comparison of Na concentration in both aquaponics tanks at Day-0 and Day-30.
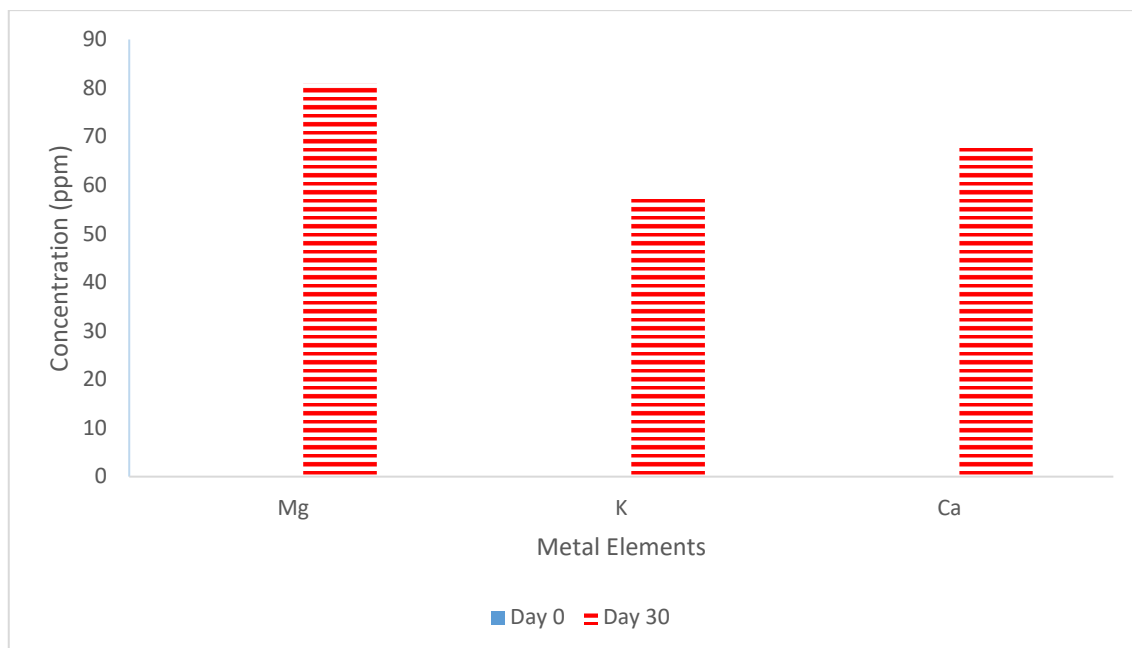
Figure 4.3. The concentration of Mg, K and Ca in control tank at Day-0 and Day-30.
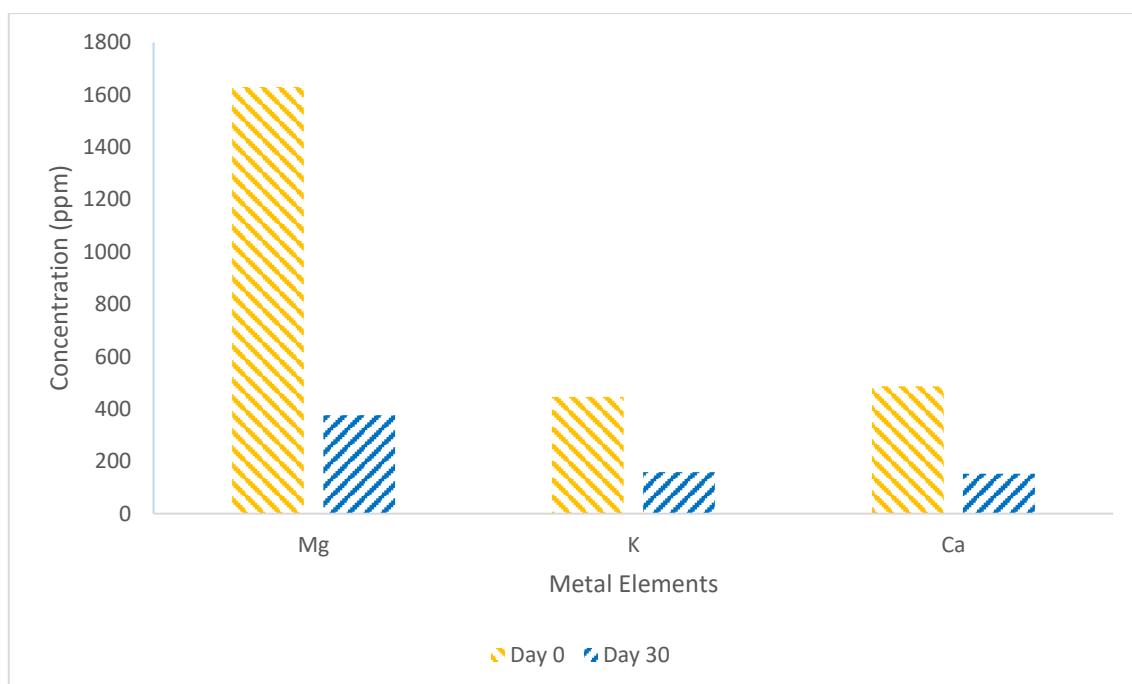

Figure 4.4. The concentration of Mg, K, and Ca in studied tank at Day-0 and Day-30.

Salinity was an environmental stress that could cause structural and ultrastructural changes in species that were sensitive to salt (Pouladi, Anderson, Wootton and Rozema, 2016). The presence of high concentration of ions including Na^+, Mg^+, Ca^+, Cl^- and SO_4^{2-} in water source used for farming might result in stunted plant growth and development. The decrease of Na concentration in both tanks and the plummeting concentration of Mg, K and Ca in the studied tank from Day-0 to Day-30 might be related to the potential of the plant, *Ipomoea aquatica,* in phytodesalination. In the studied tank from Day-0 to Day-30, Na reduced to 4692.0 ppm from 14070.0 ppm, Mg was reduced from 1630.0 ppm to 367.2 ppm, K from 446.0 ppm to 159.0 ppm and Ca was reduced to 153.1 ppm from 486.7 ppm.

The decrease for the 4 types of metal elements were calculated in percentage. Na was decreased to 4692.0 ppm from 14070.0 ppm, showing a percentage of 66.65%. Mg had reduced as much of 77.47%, from 1630.0 ppm to 367.2 ppm. K had a reduction rate of 64%, depleting from 446.0 ppm to 159.0 ppm while Ca had a reduction rate of 68.54% when the initial concentration of 486.7 ppm had plunged to 153.1 ppm at Day-30. Cr decreased 33.33%, from 84.0 ppb to 56.0 ppb.

There was a research that studied about the potential of halophytes in soil phytodesalination and the plants were determined for it concentration of Mg^{2+}, Cl^-, Ca^{2+}, Na^+ and K^+ in its underground and above ground biomass (Alizadeh, Motamedi and Erfanzadeh, 2014). Among the four species of halophytes tested, two were found to have the highest magnesium and chlorine per gram of biomass. In Australia, the saline soil and grasses grown in that area were harvested and determined for the concentration of Na, Mg, Ca and K (Bhuiyan, Raman, Hodgkins, Mitchell and Nicol, 2015). The roots and shoots of the grass contained high levels of Na, Mg, K and Ca, which was absorbed from the saline surroundings. These studies suggested that all plants have the potential to absorb Na, Mg, Ca and K, thus explained the decrease of Na in both aquaponics systems and the decrease of Mg, Ca and K in the studied tank after 30 days.

The sudden appearance of certain metal elements in water samples from Day-30 that had never been detected in Day-0 might be due to certain reasons. **Appendix C** showed water sample collected in water tank had no traces of Mg, Al, K, Ca, Fe, Ni, Se, Ag and Ba at Day-0 but present at Day-30. The water sample collected from studied tank

also shown similar pattern, where Al, Ni, Zn, As and Ba were found at Day-30 but none of them were present at Day-0.

Tap water was replenished in both tanks from time to time to make up the loss of water in the tank due to evaporation. This might due to the content of heavy metals in the tap water source. A study had found that major inorganic elements contained in the tap water samples in Pennisular Malaysia included Na, Mg, K, Ca, Fe, Zn, Cu, Cr, Mn, As, Cd, Ni, Pb, Fl, Cl, NO_3 and SO_4 (Azrina, Khoo, Idris, Amin and Razman, 2011). In Malaysia, water supply from surface water and groundwater was treated prior distributing to consumers as tap water for domestic use or as drinking water, either bottled drinking water or bottled mineral water. A review had showed that the supplied drinking water in Malaysia contained Al, Fe, Cu, Zn, Cr, Mn, Ni, As, Cd and Pb (Ab Razak, Praveena, Ariz and Hashim, 2015).

Moreover, the concentration of dissolved heavy metals in water source fluctuated due to climate changes. A research showed that samples collected from recreational park tributaries in Pahang, Malaysia and in Tasik Chini, Malaysia at different sampling period had difference in the concentration of dissolved heavy metals. Water sampling was conducted before monsoon and after monsoon season, and the outcome indicated that Ni, Cr, Fe, Cu, Pb and Zn increased after the monsoon season and vice versa for Ag and Cd (Rajan, Mohd Firdaus, Appukatty and Ramasamy, 2012). Season influenced not only the heavy metals concentration, but also the water quality (Othman, Ahmad, Mushrifah and Lim, 2008). The emergence of metal elements for both water samples at Day-30 including Al, Fe, Se, As and Pb could have resulted due to the tap water used to replenish the evaporated water containing slight amount of the above elements. These findings could have explained the presence of metal elements at Day-30 that were never found at Day-0 for both water sample.

Other possible sources of the sudden increase of metal elements might be due to the leaching of hydrotons and pipe. Hydrotons were made up of compressed clay and it was used as a substitute for soil in the aquaponics system. Clay was rich in minerals, and composed of SiO_2, Al_2O_3, Fe_2O_3, MgO, Na_2O, K_2O, CaO, TiO_2, Cu, Mn and Zn (Sturz, Itoh and Smith, 1998). There might be a possibility that the minerals from the hydrotons had leached into the aquaculture tank as aquaponics is a recirculating system. The pipe

used for the construction of the system was made of metals, which implied that the metal ions from the pipes might have leached into the water as well since the system ran 24 hours and the pipes were constantly intact with the recirculating water.

In the studied tank at Day-30, there were reduction in certain metal elements. Cr had shown a significant decrease too, from 0.084 ppm to 0.056 ppm. Cu was present at Day-0 with a reading of 0.156 ppm ppb but the concentration was not detected as it was less than 0.5 ppb or 0.005 ppm at Day-30. This might be due to the uptake mechanism by *Ipomoea aquatica* through phytoremediation and the absorption of metal elements into the tissues of fish. *Ipomoea aquatica* had been showing its capability in absorbing Pb, Cu, Mn, Cr, Zn, and Fe (Geeganage, Mohotti, Ariyarathne, Mohotti and Chandrajith, 2011).

4.2   AAS ANALYSIS

Ipomoea aquatica and tilapia fish were harvested at Day-0 and Day-30 for the determination of magnesium uptake by atomic absorption spectrometry. *Ipomoea aquatica* was separated into two parts; roots and shoots; while only the scales of tilapia fish were used for analysis purpose. **Figure 4.5** showed the powdered plant biomass and fish scales while system while **Table 4.1** tabulated the dry biomass of the samples.

(a)

(b)

(c)
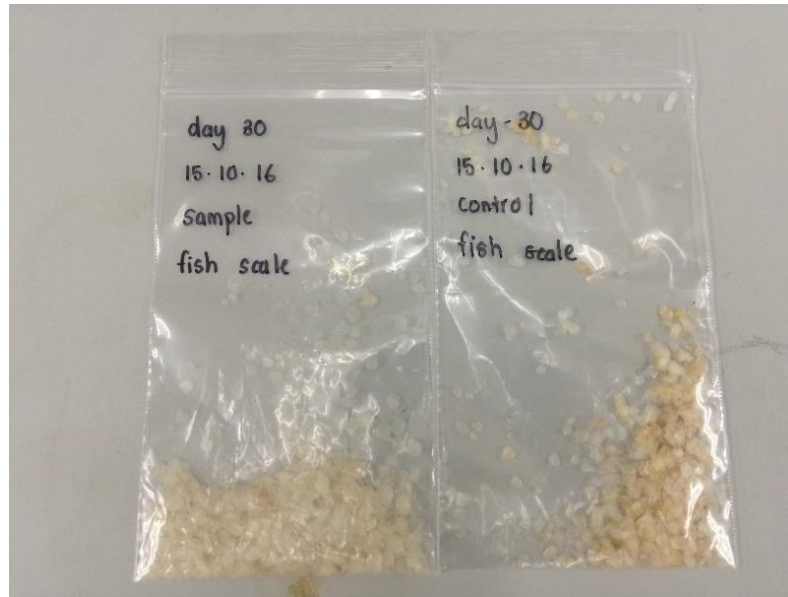
(d)

Figure 4.5. The harvested samples. (a) showed the plant biomass and fish scales at Day-0 for both tanks, (b) and (c) showed the plants roots and shoots while (d) showed the fish scales respectively samples at Day-30.

Table 4.1
The dry mass of samples collected from both systems at Day-0 and Day-30

Sample	Dry mass (g)			
	Control tank		Studied Tank	
	Day-0	Day-30	Day-0	Day-30
Roots	0.4676	0.8715	0.1437	0.5614
Shoots	0.8130	1.1607	0.4519	0.8715
Fish scales	0.1548	0.4824	0.1410	0.7773

As being compared to water spinach that is being grown in fresh, less polluted water source, this plant sample had proven itself to be able to tolerate a moderate amount of salinity but with slower growth rate. A study had shown that *Ipomoea aquatica* when grown in soil irrigated with salt solution of increasing concentration, the growth rate decreased markedly as compared to another halophilic plants under the same growth conditions (Yousif, Nguyen, Fukuda, Hakata, Okamoto, Masaoka, Saneoka, 2010). *Ipomoea aquatica* in the control tank had a higher increase in biomass from Day-0 to

Day-30 as compared to the harvest from studied tank. This might due to the presence of metal elements contained in the field water used in the studied tank that hindered or slowed down the growth of plants and fish in that tank.

The samples were subjected to wet digestion as shown in **Figure 4.6** prior analyzing for the magnesium content by using atomic absorption spectrometry or known also AAS as its abbreviation. AAS had been widely used to determine metal concentrations, especially at trace amount and its success rate is high. The working principle of an AAS was by passing an appropriate wavelength of light through vaporized atoms of interest (Moffett, 2010). The common source of light was the hollow cathode lamp. Emitted light was absorbed by unexcited atoms, hence the more the number of unexcited atoms, the more light was absorbed. Prior measuring the magnesium content in the samples, AAS had to be calibrated by preparing a series of calibration standard solutions. Standard solutions of magnesium were prepared from stock solution of a concentration of 1000 mg/ml or 1000 ppm and the range of concentration for standard solutions prepared was listed in **Table 4.2** and a calibration curve with the absorbance against the different concentration of magnesium standard solutions in **Figure 4.7**.
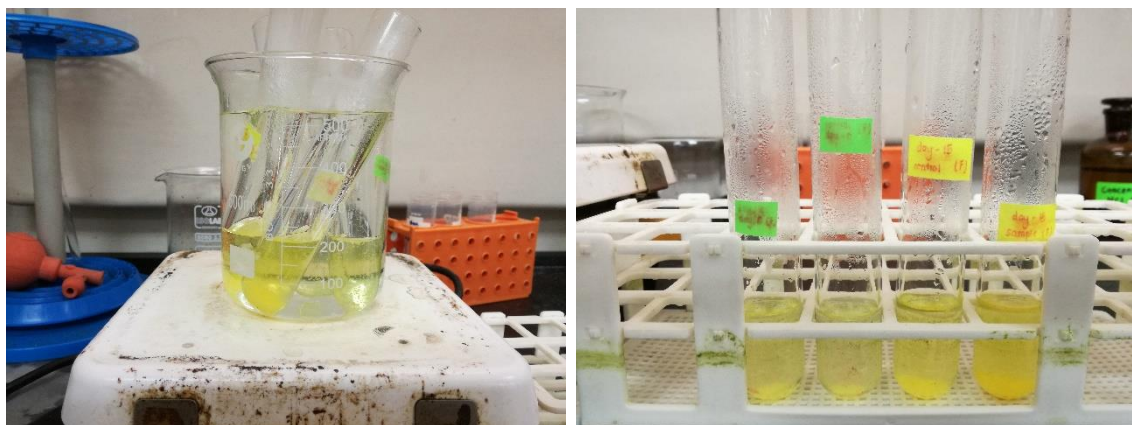
(a)
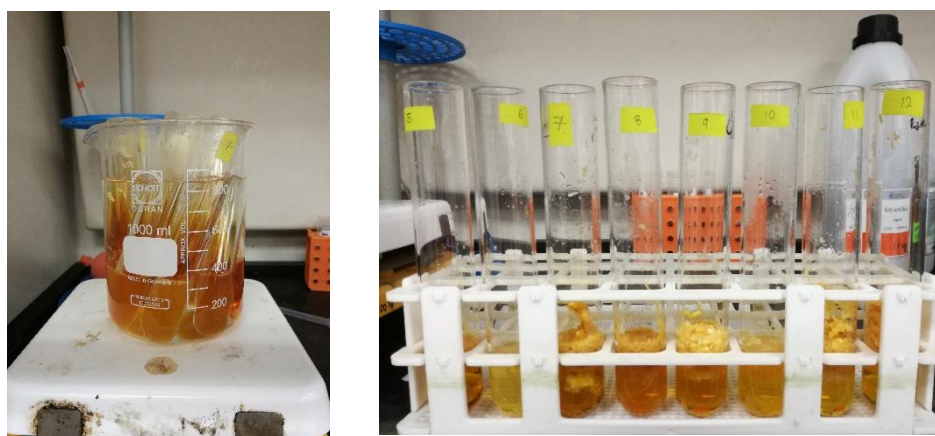
(b)

Figure 4.6. The wet digestion process. (a) showed the samples of fish scales being digested with HNO_3 while (b) showed the plant samples being digested with the same acid.

Table 4.2
The standard solutions of magnesium prepared.

No.	Vol of magnesium standard (ml)	Vol of deionized water (ml)	Final conc of magnesium solution (ppm)	Final vol of solution (ml)	Absorbance value at 285.6 nm
1	0	50	0	50	0.000
2	0.05	49.95	1	50	0.325
3	0.1	49.90	2	50	0.511
4	0.15	49.85	3	50	0.689
5	0.20	49.80	4	50	0.857
6	0.25	49.75	5	50	1.124
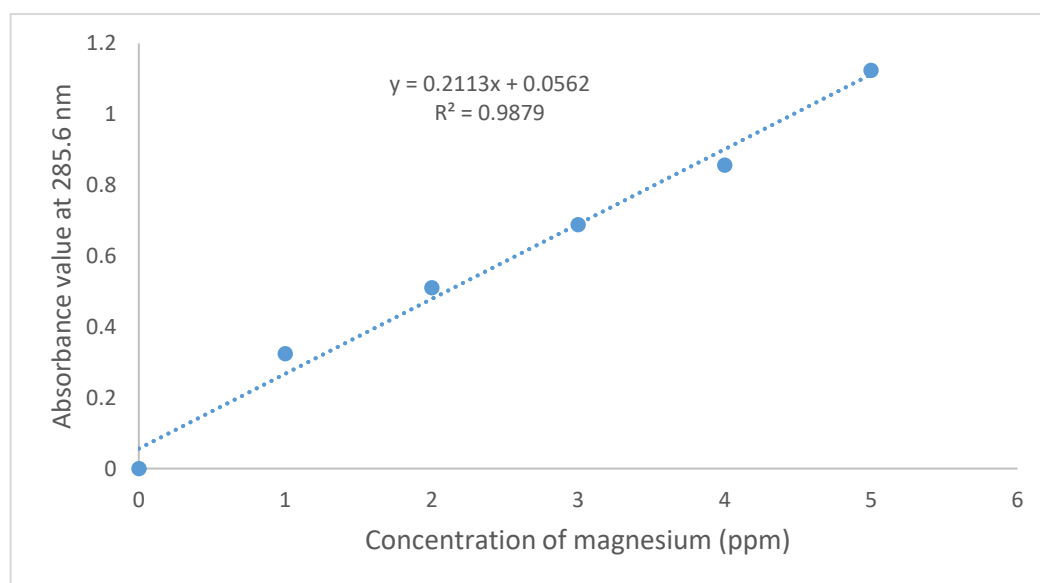
Figure 4.7. Calibration curve of the different concentration of magnesium standard solutions.

After obtaining the absorbance value for each standard solution and the correlation coefficient was more than 0.9600 which was 0.9879, the 12 samples were determined for the concentration of magnesium. Prior running the sample with AAS, 1 mL from each sample was diluted with 14 mL of deionized water to a volume of 15 mL. The correlation coefficient had to be more than 0.96 as to ensure the validity of the sample

readings in a linear curve. The absorbance value of the 12 samples and the measurement of sample concentration was listed in **Table 4.3.** The original concentration in 1 mL of sample was calculated and listed in **Table 4.3**.

Generally, the concentration of magnesium in most samples harvested at Day-30 was higher than the samples harvested at Day-0. However, the sample concentration reading for roots harvested from the studied tank at Day-30 might not be accurate due to certain error. All samples were dissolved by using concentrated HNO_3 to form a liquid solution before introducing to the flame using AAS for elemental detection. The dissolution process was performed using mineral acid digestion. This process however, was time-consuming and might cause result error by introducing impurities or losing of analytes (Robinson, Frame, Frame II, 2005). The dissolution might have caused error of reading for magnesium concentration in the roots sample collected from the studied tank at Day-30.

Table 4.3
The absorbance value and concentration of magnesium in the samples.

Day	Aquaponics Systems	Samples	Absorbance value at 285.6 nm	Diluted sample concentration (ppm)	Sample concentration in 1 mL of sample (ppm)
0	Control Tank	Fish scales	1.602	7.316	109.740
		Roots	1.322	5.991	59.865
		Shoots	1.493	6.800	102.000
	Studied Tank	Fish scales	1.571	7.169	107.535
		Roots	1.163	5.238	78.570
		Shoots	1.480	6.738	101.07

Table 4.3 Continued
The absorbance value and concentration of magnesium in the samples.

Day	Aquaponics Systems	Samples	Absorbance value at 285.6 nm	Diluted sample concentration (ppm)	Sample concentration in 1 mL of sample (ppm)
30	Control Tank	Fish scales	1.718	7.865	117.975
		Roots	1.530	6.975	104.625
		Shoots	1.654	7.562	113.430
	Studied Tank	Fish scales	1.742	7.978	119.670
		Roots	0.154	0.463	6.945
		Shoots	1.607	7.339	110.085

Ipomoea aquatica and tilapia absorbed magnesium into their tissues as the concentration of magnesium content increased from Day-0 to Day-30 in general. The magnesium concentration in fish scales from both tanks harvested at Day-0 and Day-30 showed the highest concentration as compared to roots and shoots samples collected from both tanks at the 2 stated days. The concentration of magnesium in fish scales, roots and shoots harvested in both tanks was presented in **Figure 4.8** and **Figure 4.9** for better understanding.
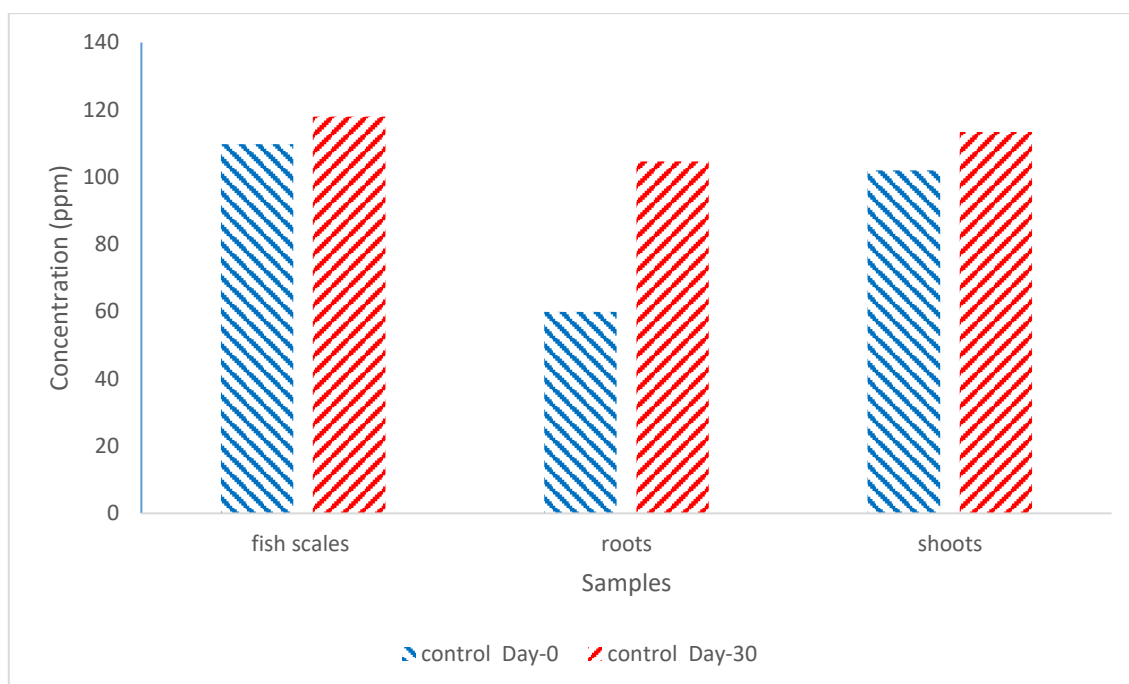
Figure 4.8. The concentration of Mg in scales, roots and shoots harvested from control tank at Day-0 and Day-30.
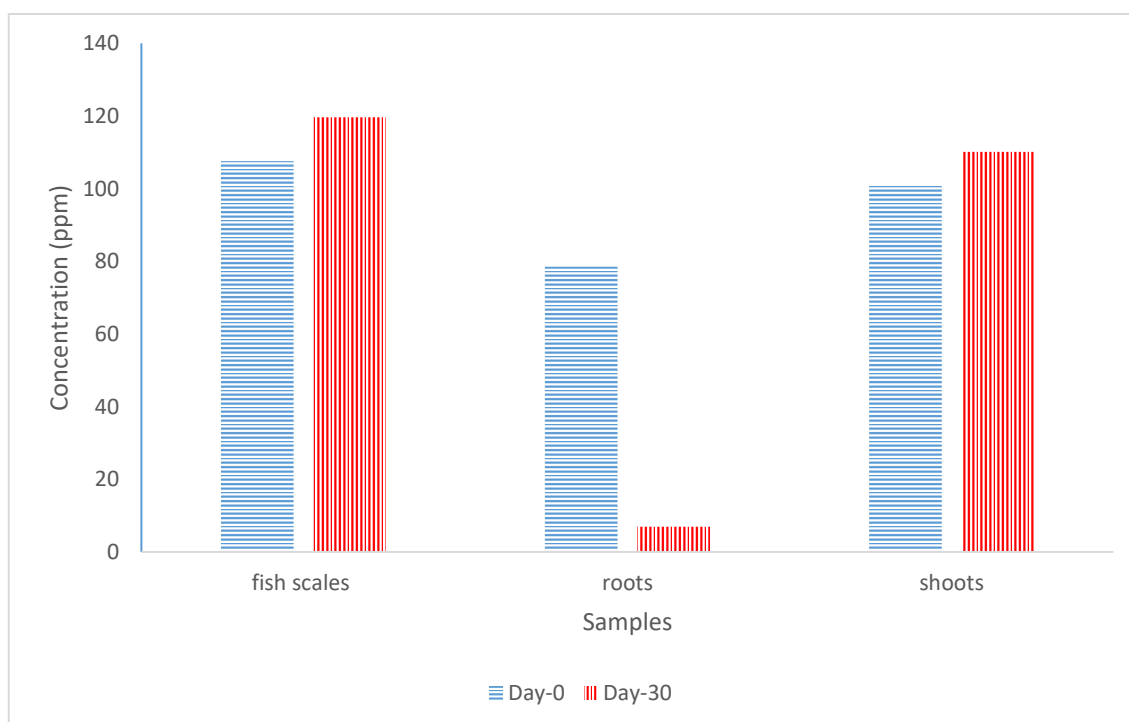

Figure 4.9. The concentration of Mg in scales, roots and shoots harvested from studied tank at Day-0 and Day-30.

A finding had stated that magnesium was distributed in various tissues of tilapia fish, including muscle, liver, kidney, liver, brain, bones and scales (van der Velden, Kolar, Flik, de Goeji and Wendelaar Bonga, 1989). This distribution of magnesium could have explained the high initial magnesium concentration of fish scales taken from control tank and studied tank at Day-0. Comparing the fish scales at Day-0 and Day-30, there was an increase in magnesium concentration. The scales from the control tank increased 7.5% from Day-0 to Day-30 while the scales in the studied tank increased 11.28%. Magnesium was able to deposit onto the scales, bones and muscles of tilapia since the parts contained ambient amount of magnesium (Bijvelds, Flik, Kolar and Wendelaar Bonga, 1996). The concentration of magnesium of fish scales at Day-30 in both control and studied tank was higher due to the presence of magnesium in the tap water used to replenish the evaporated water in control tank and existing magnesium contained in the field water.

Roots and shoots of *Ipomoea aquatica* sampled at Day-30 from both aquaponics system generally showed a higher reading of magnesium concentration as compared to those harvested at Day-0. Results indicated that more absorbed magnesium was localized at the shoots than the roots of the water spinach. In this study, the magnesium content was much higher in shoots than in roots. In the control tank, the sample harvested contained 59.865 ppm for shoots and only 102.000 ppm at Day-0. Though at Day-30 there was an increment in the concentration, still the shoots sample displayed a higher concentration of 114.430 ppm while the roots only contained 104.625 ppm.

The similar trend was observed for the water spinach sampled from the studied tank too. At Day-0, the shoots were determined to have 101.07 ppm magnesium while roots had 78.570 ppm. The concentration of magnesium increased for both roots and shoots after 30 days. Shoots still had a higher concentration, which was 110.085 ppm. The roots sampled at Day-30 from studied tank had an unexpected reading, which might be due to error arose from the dissolution process. Overall, the plant samples at Day-30 had a higher concentration of magnesium for both roots and shoots when compared to those collected at Day-0 because *Ipomoea aquatica* had taken up the magnesium from water in the aquaponics system. At Day-30, the samples in the studied tank had a higher amount of magnesium when compared to the plant samples collected on the control tank. This was because the water used in the studied tank was collected from Kuantan estuary,

which originally contained 14070.0 ppm. Hence, there were more magnesium available for water spinach to absorb.

Aquatic plants performed phytoremediation at its best by means of rhizofiltration mechanism as the roots were constantly intact with waste water, which was high in pollutants concentration. Roots were the site for primary uptake of pollutants (Vymazal, 2016). However, the trend displayed otherwise, where the shoots of *Ipomoea aquatica* accumulated more magnesium than roots. There was a study that supported the phytoremediation trend displayed in this study, where more heavy metals were accumulated at the shoots. *Ipomoea aquatic* was able to absorb Pb, Zn, Fe and Mg at a paper mill contaminated site in North East India. Water spinach localized more of the elements at the upper parts as compared to the aerial roots (Mazumdar and Das, 2015). In this study, *Ipomoea aquatica* was analyzed for it leaves, roots and stems for magnesium content and roots had a highest concentration of magnesium at 550.85 ppm, followed by leaves at 421.15 ppm and lastly stems at 213.2 ppm.

4.3   WATER QUALITY PARAMETER ANALYSIS

Water samples from both tanks were measured for its DO, pH value, EC, TDS, salinity and temperature for five weeks. Readings were taken in triplicates, and the mean and standard variation were measured. Constant qualitative and quantitative measurements were necessary to monitor the water quality from various source of water supply (Food and Agriculture Organization of The United Nations, 1999).

Electrical conductivity indicated the ability of the water sample to conduct an electric current as this parameter was related to its ionic content. It was also known as specific conductance. Conductivity could gauge the hardness, alkalinity and the total dissolved solids of the water sample (Environmental Protection Agency, 2001). An increase of conductivity reading might indicate the presence of polluting discharges in the water sample. The range of conductivity might estimate the salinity of the water sample. The pH value measured the acidity of the water in control tank and studied tank, while dissolved oxygen indicated the amount of oxygen available in both systems.

The measurements of parameters for water from control and studied tank were taken in triplicates, with their respective means and standard errors were calculated and tabulated as shown in **Appendix D**. The data were presented in graphs for better understanding as shown in **Figure 4.10**, **Figure 4.11**, **Figure 4.12**, **Figure 4.13**, **Figure 4.14** and **Figure 4.15**. From the data obtained from the measurements, the water quality was determined by referring to the standards set by Water Environment Partnership in Asia or WEPA in short.
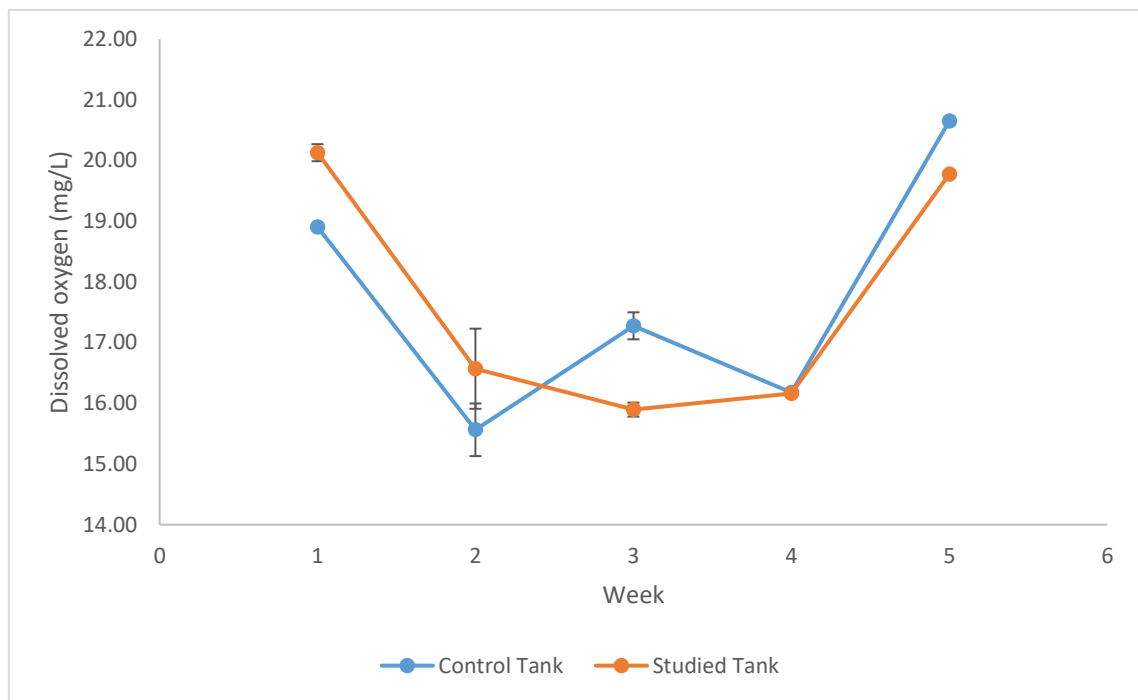
Figure 4.10. The average value of dissolved oxygen for both aquaponics systems for 5 weeks.

Figure 4.11. The average value of pH for both aquaponics systems for 5 weeks.
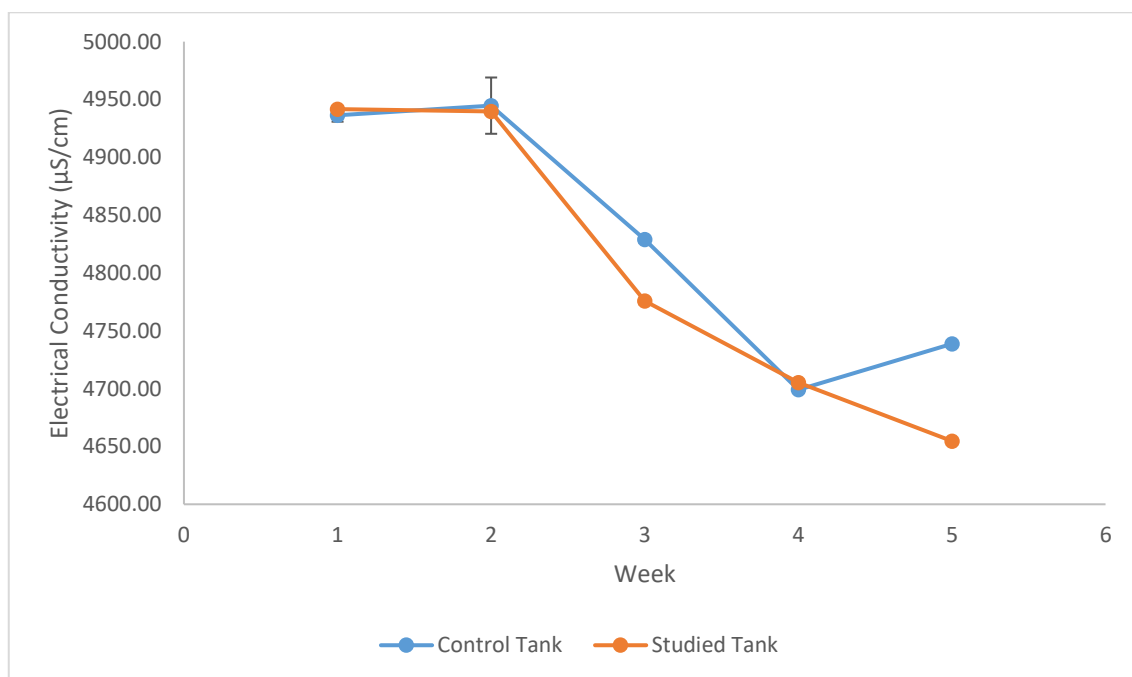
Figure 4.12. The average value of electrical conductivity for both aquaponics systems for 5 weeks.

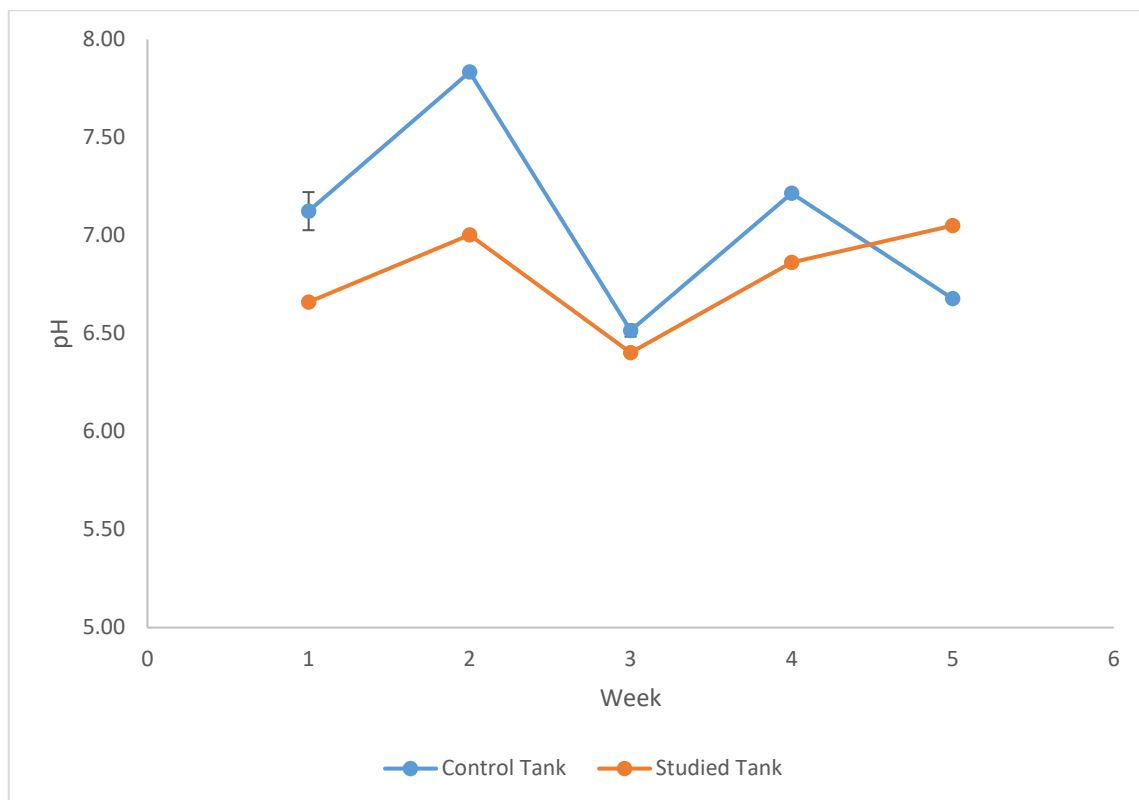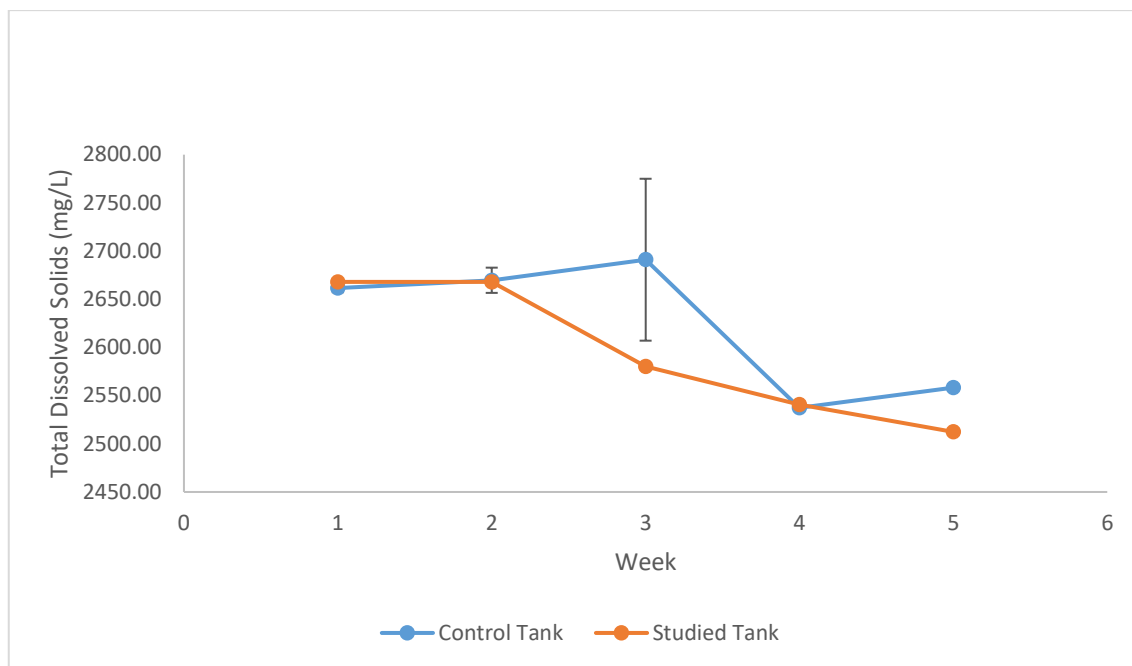**Figure 4.13.** The average value of total dissolved solids for both aquaponics systems for 5 weeks.
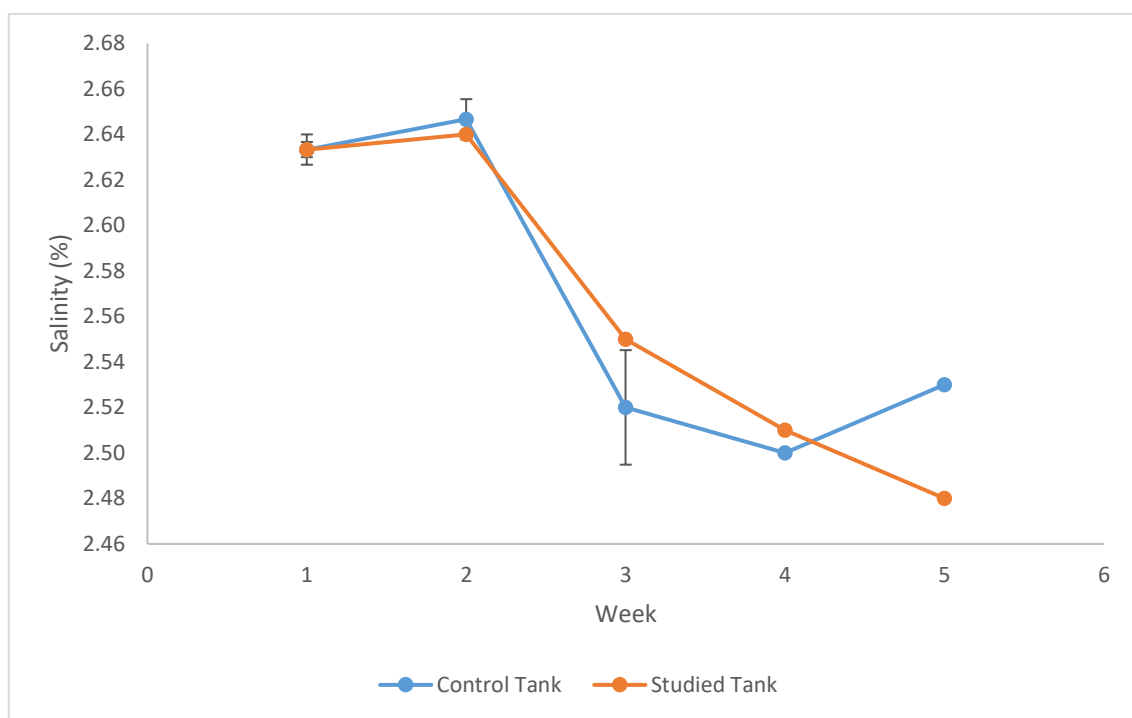
Figure 4.14. The average value of salinity for both aquaponics systems for 5 weeks.

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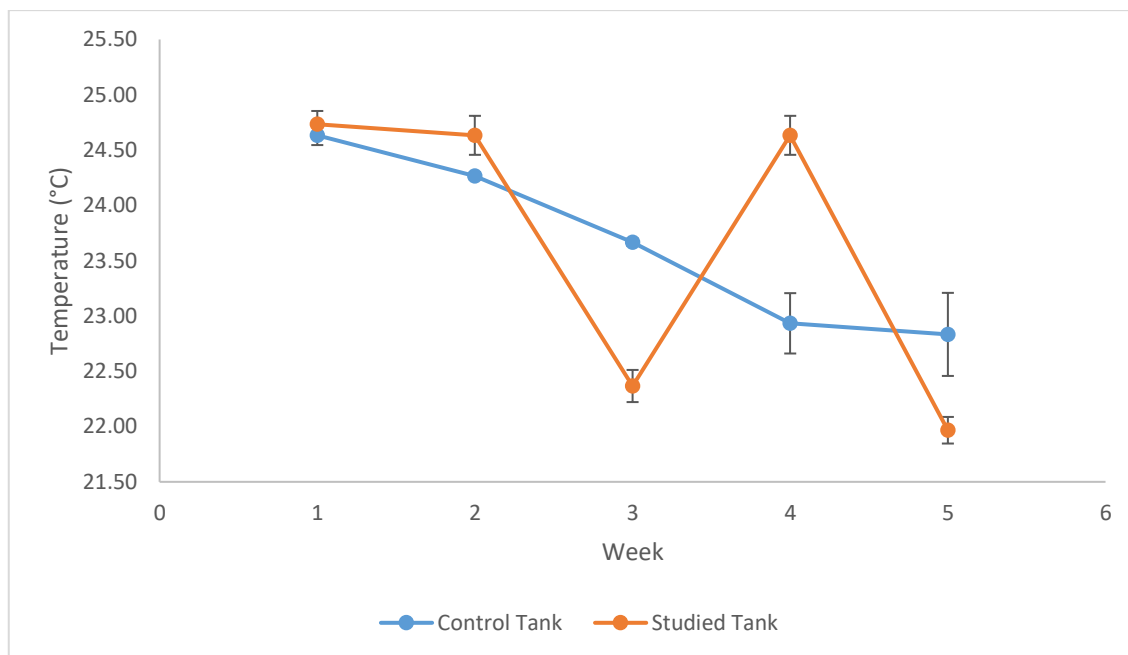
Figure 4.15. The average temperature for both aquaponics systems for 5 weeks.

The average value of dissolved oxygen and pH value for water sample in both systems was between the range of 15.56 to 20.65 µS/cm and 6.401 to 7.834 for the five consecutive weeks. The range for both parameters is within the range of acceptable value for recommended raw water quality as stated by the Malaysia Health Ministry.

Yet, the synthetic saline water in the control tank and the collected field water from Kuantan Estuary was not classified as Class I water but Class IV water (Water Environment Partnership in Asia, 2006) as other considered parameters had exceeded the acceptance value. The conductivity for water samples collected from both systems ranged from 4657.67 µS/cm to 4944.67 µS/cm; total dissolved solids ranged from 2512.33 mg/L to 2669.67 mg/L while the salinity ranged from 2.48% to 2.65%. **Figure 4.16** stated the classification of water based on the National Water Quality Standards for Malaysia by WEPA.

National Water Quality Standards For Malaysia

PARAMETER	UNIT	CLASS					
		I	IIA	IIB	III	IV	V
Ammoniacal Nitrogen	mg/l	0.1	0.3	0.3	0.9	2.7	> 2.7
Biochemical Oxygen Demand	mg/l	1	3	3	6	12	> 12
Chemical Oxygen Demand	mg/l	10	25	25	50	100	> 100
Dissolved Oxygen	mg/l	7	5 - 7	5 - 7	3 - 5	< 3	< 1
pH	-	6.5 - 8.5	6 - 9	6 - 9	5 - 9	5 - 9	-
Colour	TCU	15	150	150	-	-	-
Electrical Conductivity*	µS/cm	1000	1000	-	-	6000	-
Floatables	-	N	N	N	-	-	-
Odour	-	N	N	N	-	-	-
Salinity	%	0.5	1	-	-	2	-
Taste	-	N	N	N	-	-	-
Total Dissolved Solid	mg/l	500	1000	-	-	4000	-
Total Suspended Solid	mg/l	25	50	50	150	300	300
Temperature	°C	-	Normal + 2 °C	-	Normal + 2 °C	-	-
Turbidity	NTU	5	50	50	-	-	-
Faecal Coliform**	count/100 ml	10	100	400	5000 (20000)ᵃ	5000 (20000)ᵃ	-
Total Coliform	count/100 ml	100	5000	5000	50000	50000	> 50000

Notes :
N : No visible floatable materials or debris, no objectional odour or no objectional taste
* : Related parameters, only one recommended for use
** : Geometric mean
a : Maximum not to be exceeded

Figure 4.16. National Water Quality Standards for Malaysia

Source: Water Environment Partnership in Asia (WEPA)

Based on the standards shown in **Figure 4.16**, the water quality of synthetic saline water and field water collected from Kuantan Estuary could be deduced. The electrical conductivity for both water samples was more than 1000 µS/cm but less than 6000 µS/cm, total dissolved solids was more than 4000 mg/L and the salinity was more than 2%. Water from Kuantan Estuary had been classified as Class IV.

The fluctuation of dissolved oxygen in both tanks was due to the aeration pump installed in the aquaponics tanks. The pump was set to run at all time to provide oxygen to the fish in the tank. It was vital to provide high oxygen level in the aquaculture water to ensure optimal vegetative growth as hydroponics plants underwent intense root respiration. This metabolism process required high amount of oxygen from the surrounding water.

The electrical conductivity, total dissolved solids and salinity in the synthetic saline water and field water decreased slightly throughout the 5 weeks. The heavy metals were being translocated along the roots and shoot of *Ipomoea aquatica* and on the scales of tilapia. This resulted in the reduction of dissolved ions, which played the role of a conductors. The uptake of dissolved ions by the plants and fish might explained the slight

decrease of conductivity, total dissolved solids and salinity as these three parameters were closely related (Clean Water Team, 2004).

The average pH value was plotted in **Figure 4.17** along with the difference in concentration of Na, Mg, K, and Ca at Day-0 and Day-30. This was to observe the fluctuation of pH when various metal elements were being absorbed by the *Ipomoea aquatica* and tilapia fish. The surrounding pH affected the uptake of metals by the plants and there were a few studies that had suggested that the most suitable range for higher uptake of metal elements within plant tissues is within pH 6 to 8. Increasing alkalinity or environment that had pH below 3.5 resulted in a decreased amount of Zn taken up by *Valeriana officinalis* (Adamczyk-Szabela, Markiewicz and Wolf, 2015) when compared to those who grew in a much more neutral environment. Water flea was able to absorb similar concentrations of of Cd, Cu, Ni, Pb and Zn at pH 6 and 7 but at pH 8 the concentration of the ions decreased (Komjarova and Blust, 2009).

Figure 4.17 (a). The concentration of Na and pH of the water from both aquaponics systems.

Figure 4.17 (b). The concentration of Mg and pH of the water from both aquaponics systems.
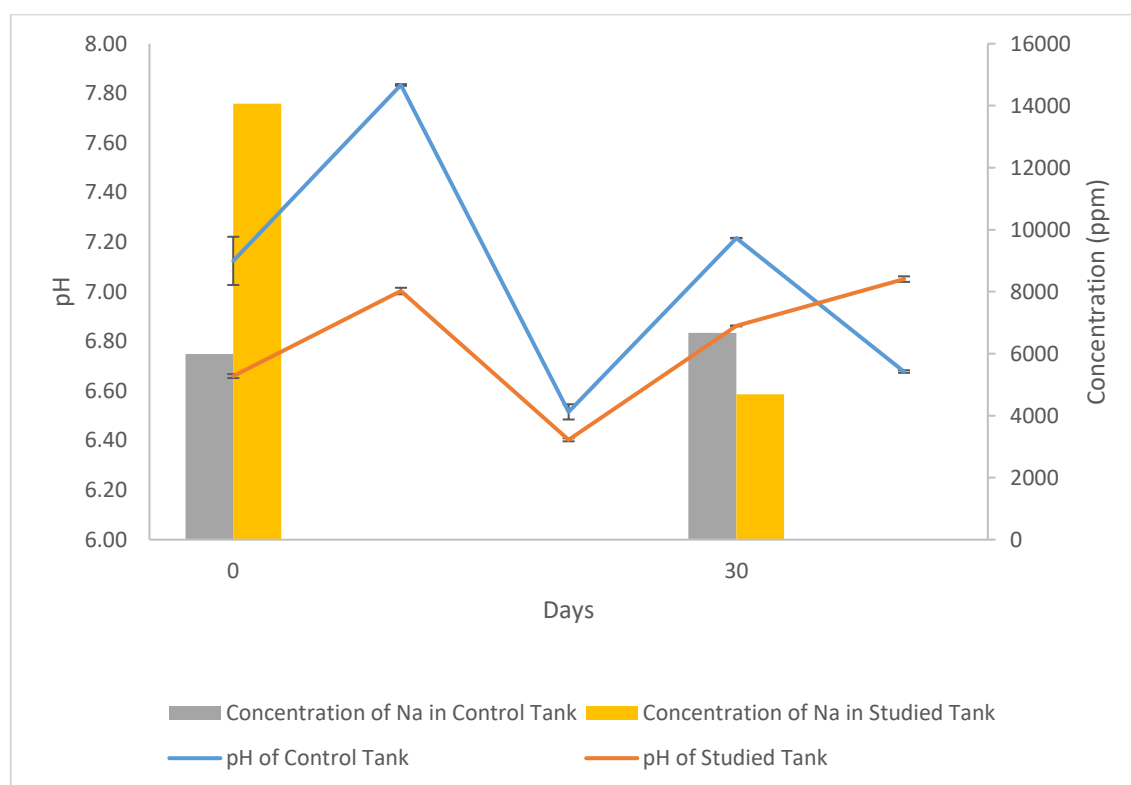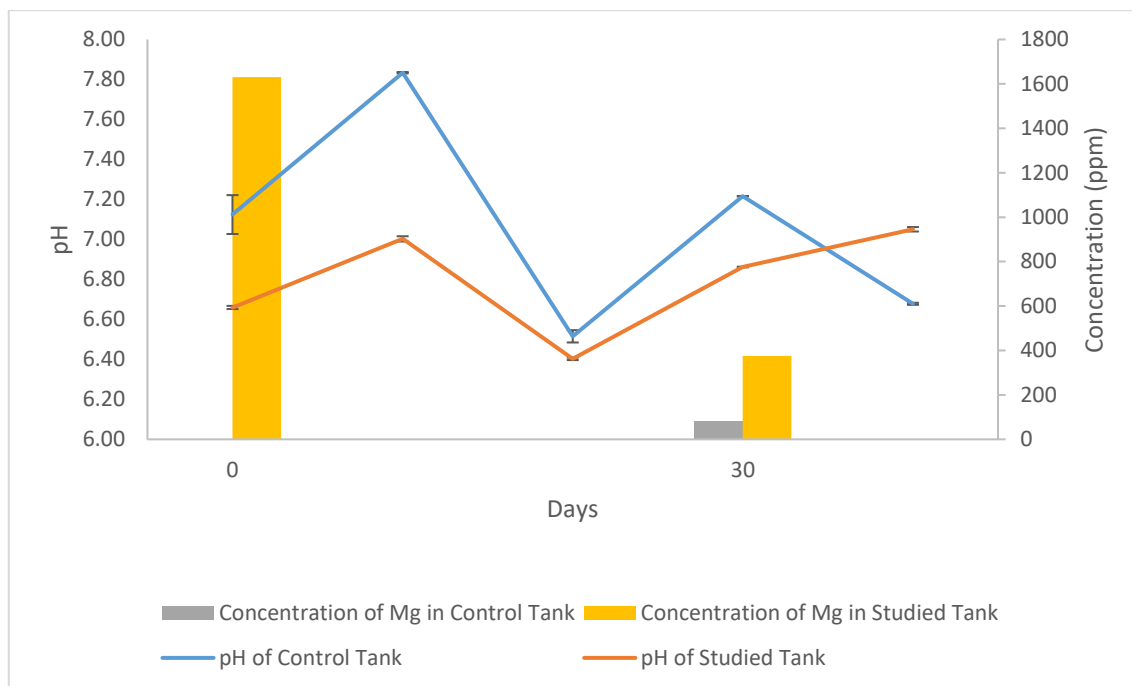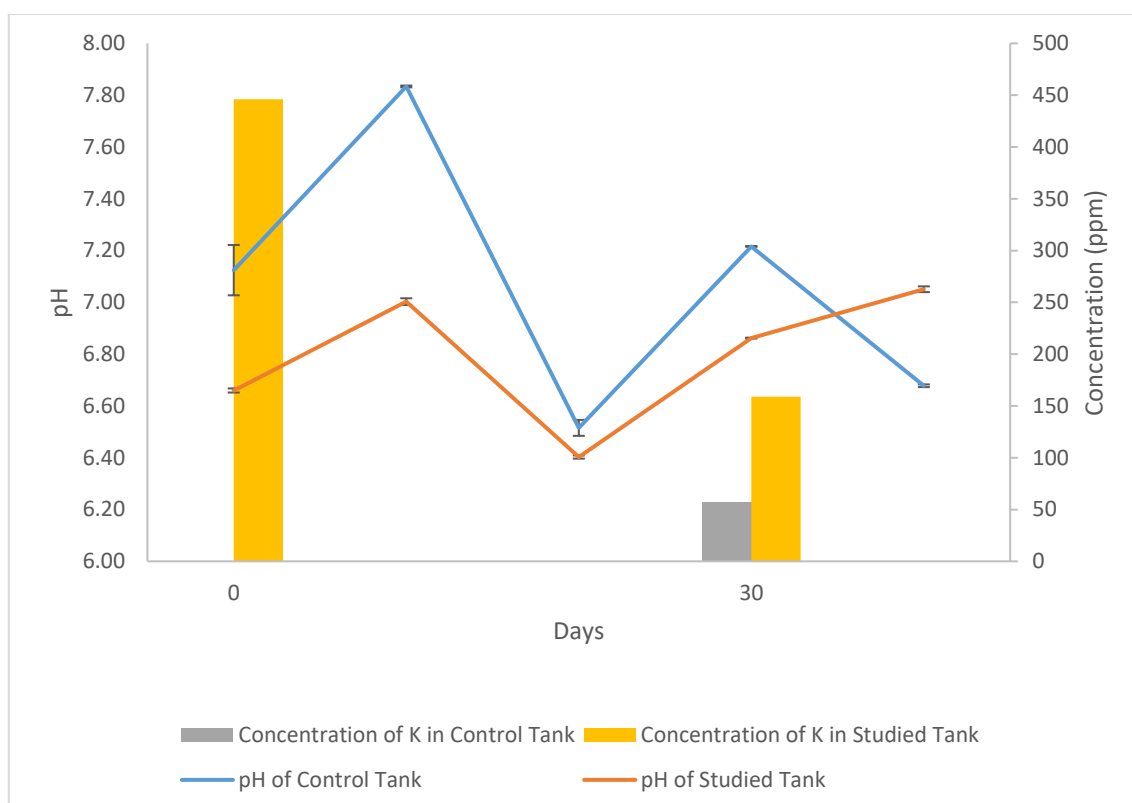

Figure 4.17 (c). The concentration of K and pH of the water from both aquaponics systems.
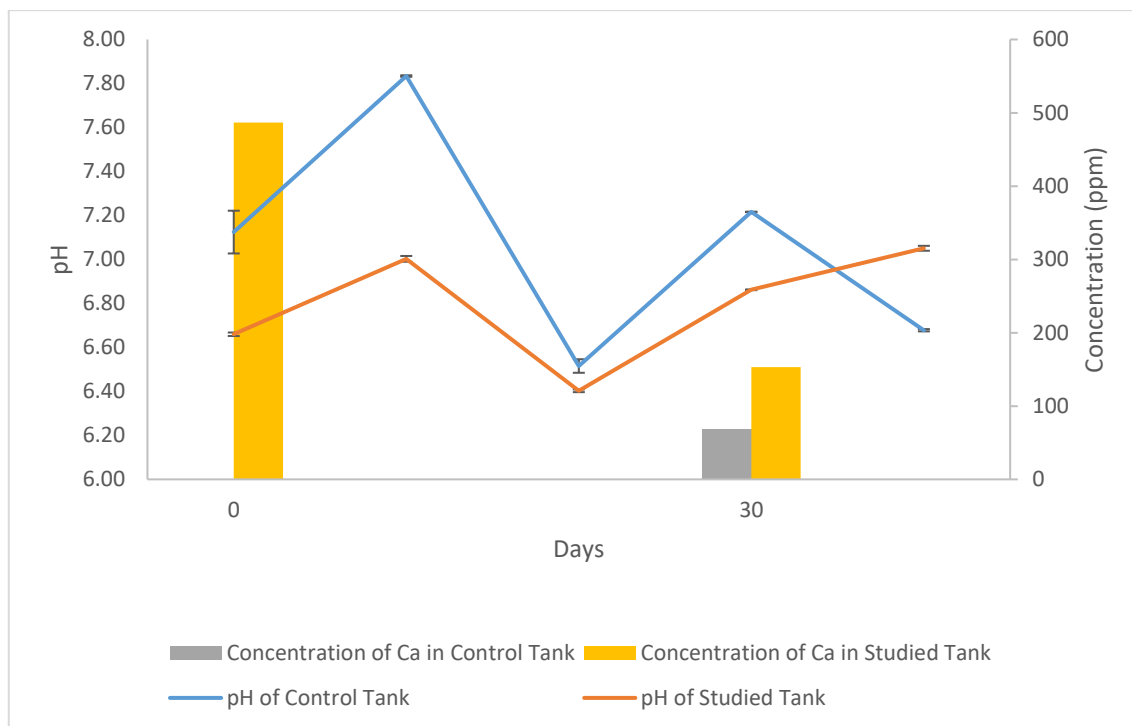
Figure 4.17 (d). The concentration of Ca and pH of the water from both aquaponics systems.

From the different graphs shown in **Figure 4.17**, the concentration of Na, Mg, K and Ca had decreased significantly at a range of pH 6.401 to 7.834. The pH readings of the water sampled from both tanks throughout the 5 weeks were within the acceptance range for pH standards set by the authority in Malaysia. *Ipomoea aquatica* might had been able to absorb Na, Mg, K, and Ca and subsequently localized the elements at its shoots and roots at optimum performance as the pH of its surrounding environment is generally neutral. The pH of the water was not consistent throughout the commence of experiment as inputs such as tap water replenishment, exposure to rain water and food pellets might have caused this fluctuation.

The electrical conductivity, total dissolved solids and salinity were closely related. Salinity was the measure of the mass of dissolved solids including ionic compounds in a known volume of solution and the common unit of expression is parts per thousand or ppt. Total dissolved solids were solids that had been dissolved in water. Total dissolved solids and salinity were said to be closely related to conductivity because the more ions that were dissolved in a solution, the higher the conductivity. Hence, electrical conductivity acted as an indicator to determine the amount of ions contained in a water

sample. The average electrical conductivity value was plotted in **Figure 4.18** along with the differences in concentration of Na, Mg, K, and Ca at Day-0 and Day-30.

Figure 4.18 (a). The concentration of Na and electrical conductivity of the water from both aquaponics systems.

Figure 4.18 (b). The concentration of Mg and electrical conductivity of the water from both aquaponics systems.

Figure 4.18 (c). The concentration of K and electrical conductivity of the water from both aquaponics systems.

Figure 4.18 (d). The concentration of Ca and electrical conductivity of the water from both aquaponics systems.

Generally, the decrease in electrical conductivity in both tanks were significant throughout the experiment for 5 weeks. There was an approximate decrease of 300 µS/cm in the studied tank from week 5 while the conductivity in the control tank 250 µS/cm from week 2 to week 4, though there was a slight increase in the value at the following week. The sudden slight increase of conductivity in the control tank from week 4 to week 5 was possibly due to the presence of Mg, K, Ca and the increase of Na in the synthetic saline water. The conductivity of the studied tank decreased consistently, which corresponded to the decrease in the concentration of Na, Mg, K and Ca. The similar trend was observed for the average value of total dissolved solids and salinity as these two parameters were directly proportional to conductivity.

The temperature of the surrounding temperature affected the uptake of metal concentration in biomass. The water temperature influenced water chemistry, metal solubility, metal uptake by plants and plant growth (Fritioff, Kautsky and Greger, 2005). Higher temperature encouraged greater biomass production and might have a greater metal uptake capacity. The average temperature readings were plotted in **Figure 4.19** along with the difference in concentration of Na, Mg, K, and Ca at Day-0 and Day-30.
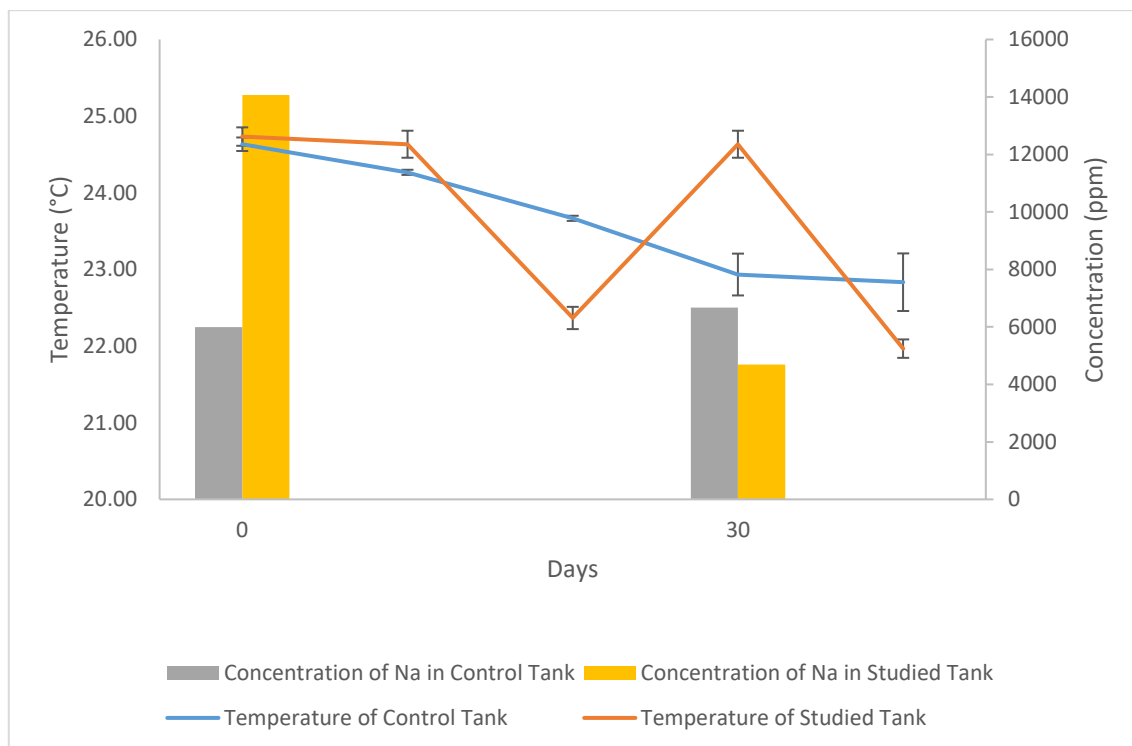
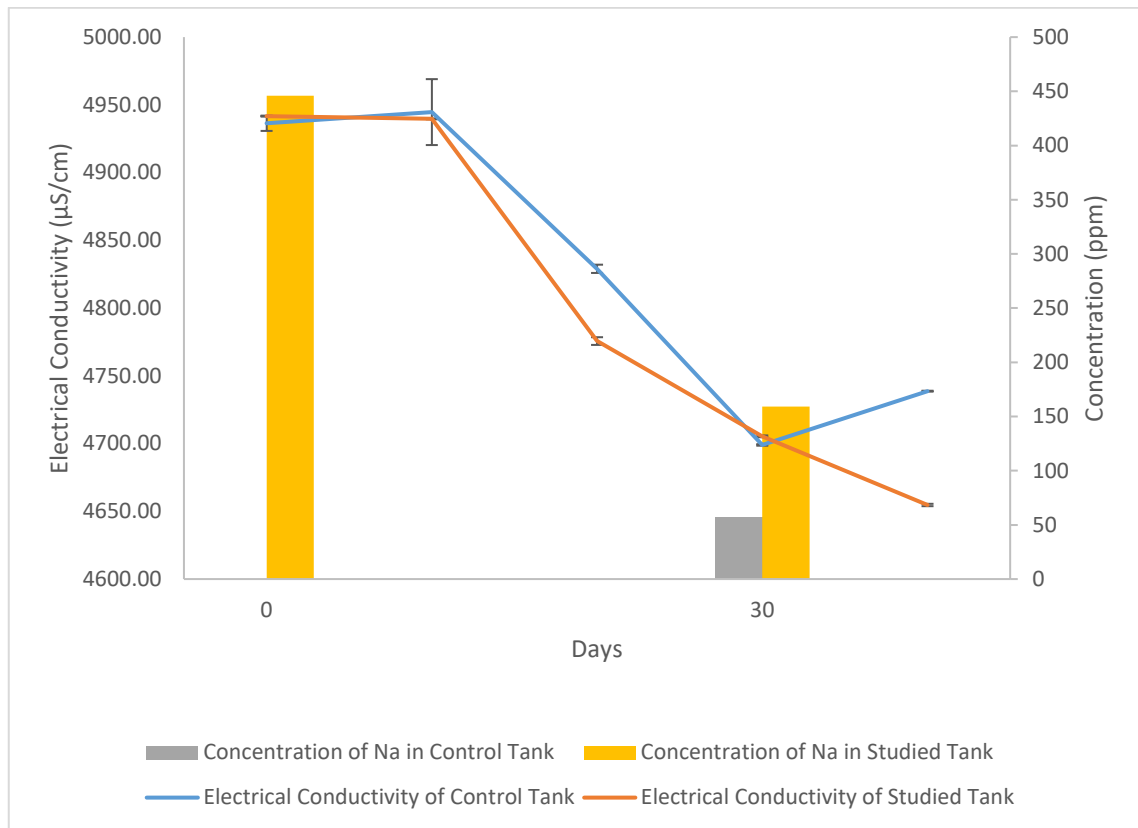**Figure 4.19 (a).** The concentration of Na and temperature of the water from both aquaponics systems.
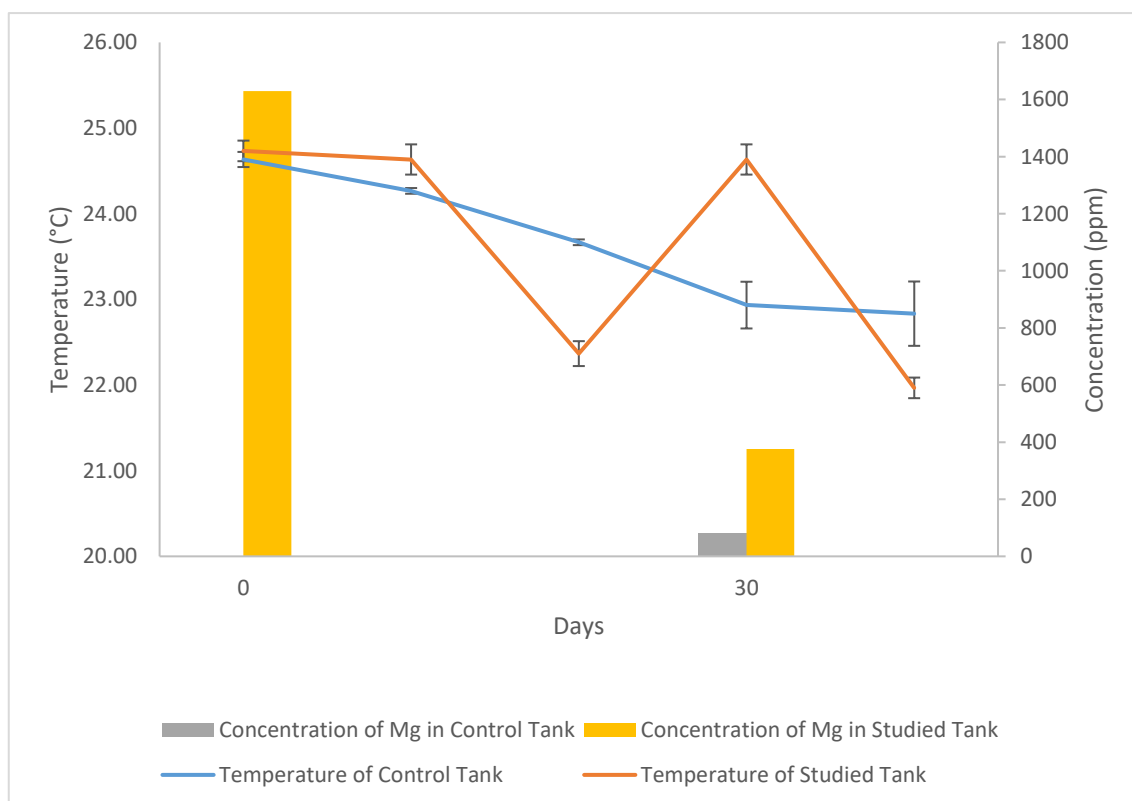
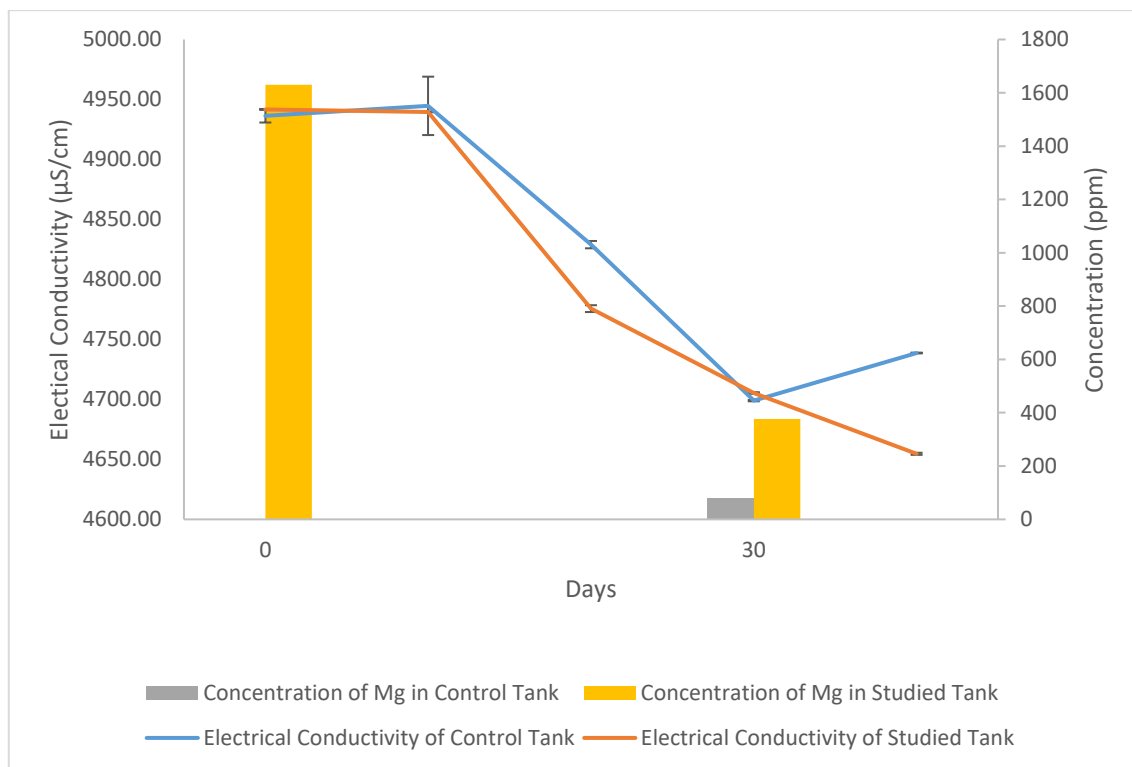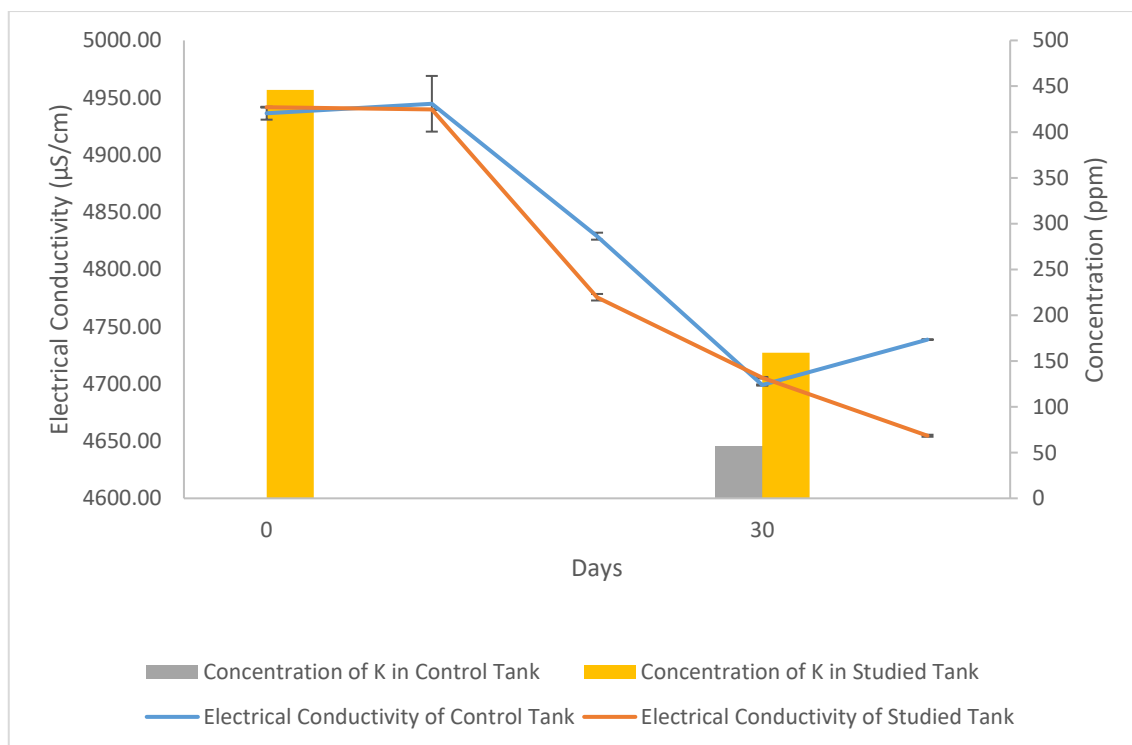**Figure 4.19 (b).** The concentration of Mg and temperature of the water from both aquaponics systems.

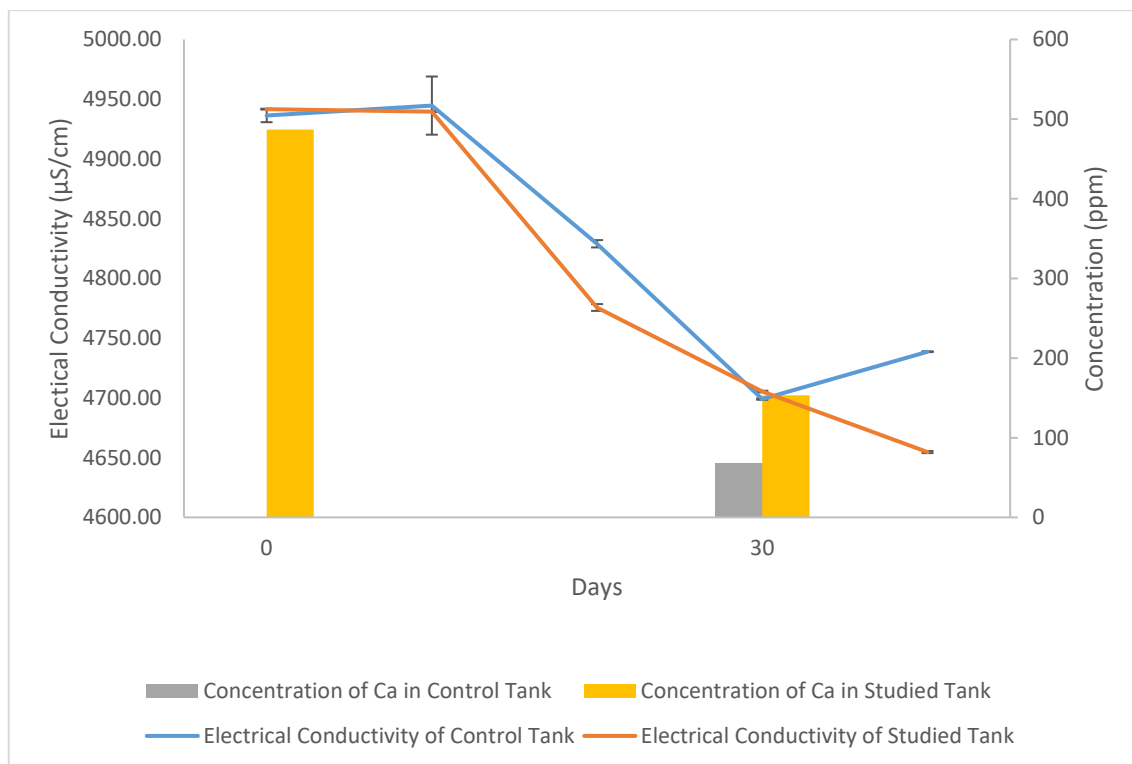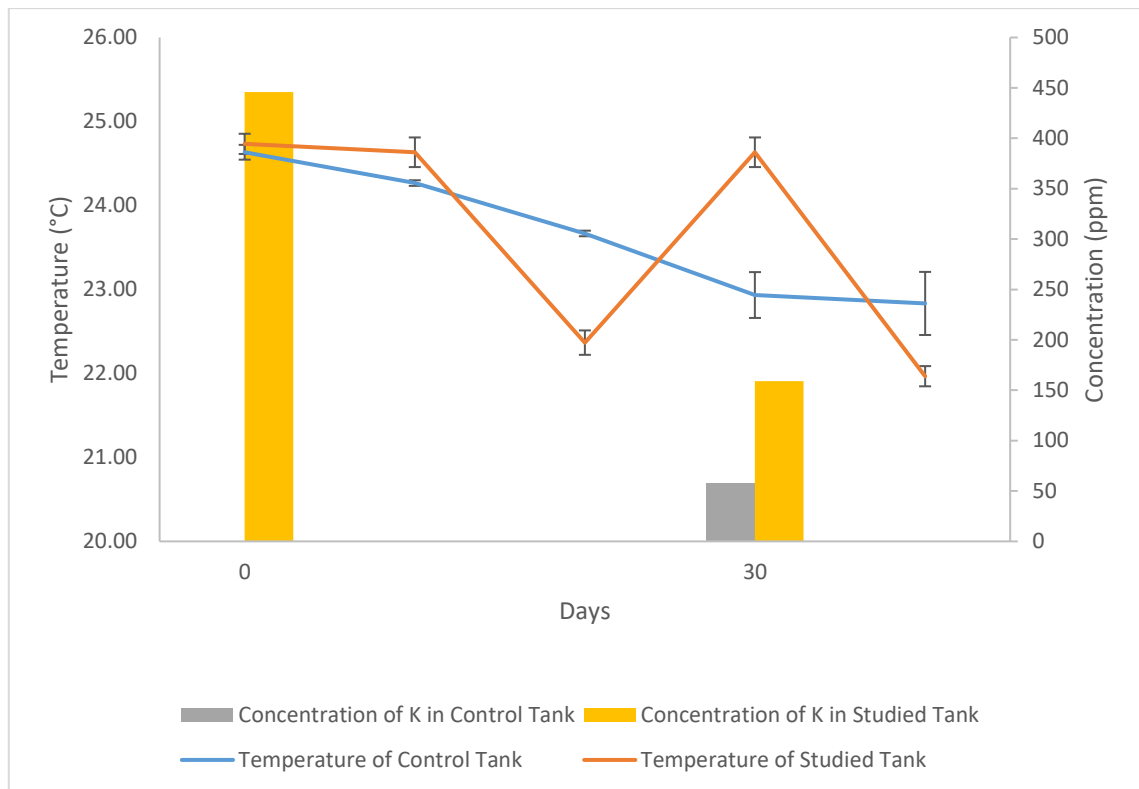***Figure 4.19 (c).*** The concentration of K and temperature of the water from both aquaponics systems.
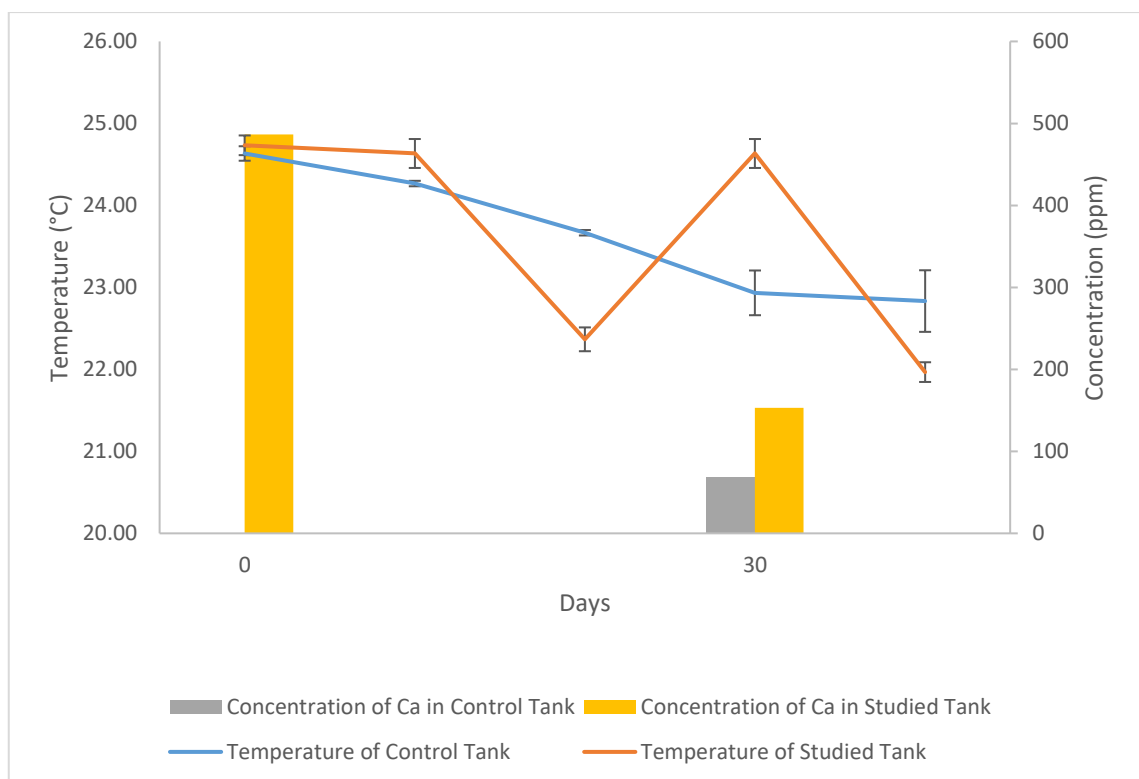
Figure 4.19 (d). The concentration of Ca and temperature of the water from both aquaponics systems.

The average temperature readings taken throughout the commence of experiment ranged from 22°C to 25°C. Within this range, Na, Mg, K and Ca in the studied tank had decreased significantly. A study had shown that dried green alga was able to uptake the highest concentration of Cd ions at 20°C when compared to higher temperatures including 30°C. 40°C and 50°C (Aksu, 2001). In this experiment, the optimum temperature at which metals were being absorbed the most by *Ipomoea aquatica* and tilapia fish could not be determined as the readings were taken as background data. The temperature of the water in the aquaculture tank was not constant throughout the experiment. However, the significant decrease of Na, Mg, K and Ca in the studied tank could have indicated that phytoremediation of metal elements performed by *Ipomoea aquatica* worked at this range of temperature at an acceptable rate.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1   CONCLUSION

At the end of the experiment, two aquaponics had been set up with *Ipomoea aquatica* in the hydroponic growth bed and tilapia in the aquaculture tank. One tank was filled with synthetic saline water which played the role as a control while the other tank was filled with field water collected from Kuantan Estuary. The magnesium uptake by the plants and the fish was determined at the end of the experiment and the phytoremediation capability of the system to reduce other metal elements was evaluated. The objectives were fulfilled.

The ICPMS analysis of the sampled field water in the studied tank clearly showed that there was a depletion for some metal elements from Day-0 to Day-30. Na was decreased from 4692.0 ppm from 14070.0 ppm, showing a percentage of 66.65%. Mg had reduced as much of 77.47%, from 1630.0 ppm to 367.2 ppm. K had a reduction rate of 64%, depleting from 446.0 ppm to 159.0 ppm while Ca had a reduction rate of 68.54% when the initial concentration of 486.7 ppm had decreased drastically to 153.1 ppm at Day-30. Cr decreased 33.33%, from 84.0 ppb to 56.0 ppb.

From the analysis of AAS, *Ipomoea aquatica* was separated into two parts, roots and shoots, for the determination of magnesium in different parts of the plant tissues and the scales of tilapia was used. *Ipomoea aquatica* and the tilapia absorbed magnesium and deposited within their tissues as the concentration of magnesium content had increased from Day-0 to Day-30.

The water quality parameters were taken for 5 consecutive weeks to monitor the quality of water in both tanks. The dissolved oxygen and pH were in the accepted range set by the Ministry of Health in Malaysia, however the electrical conductivity, total dissolved solids stated otherwise. The values of the conductivity, total dissolved solids and salinity exceeded the accepted value, thus the water in both tanks fell in the category of Class IV. Such category of water bodies requires irrigation and extensive water treatment.

The decrease in electrical conductivity, total dissolved solids and salinity implied that *Ipomoea aquatica* had phytodesalination properties. Na^+, Mg^{2+}, K^+ and Ca^{2+} which are the major constituents that contributes to the salinity had decreased significantly had shown possibility that water spinach was able to reduce salinity, thus having the potential to restore the balance of water bodies that had high salinity.

5.2   RECOMMENDATIONS

From this study, *Ipomoea aquatica* had shown positive uptake of Mg and localized it at the roots and shoots area while there was also significant trace of Mg concentration on the scales of the tilapia fish even in brackish water. The aquaponics systems integrated with *Ipomoea aquatica* and tilapia fish was able to phytoremediate Na, Mg, K, Ca and some other metal elements contained in the brackish water sampled from Kuantan Estuary.

Plant density plays an important role to improve the phytoremediation and desalination efficiency of this aquaponics system. In this experiment, an approximate wet weight of 28.79 g of *Ipomoea aquatica* was able to reduce 66.65% of Na, 77.47% of Mg, 64% of K and 68.54% of Ca and other heavy metal elements in the studied tank as mentioned in the grey-shading of Table 4.1. The conductivity of the field water had been reduced as much of 300 µS/cm from Day-0 till Day-35. Higher plant densities promote more removal of polycyclic aromatic hydrocarbons or PAHs from the surrounding environment (Ansari, Gill, Gill, Lanza and Newman, 2016). However, lower plant density promotes plant growth rates and subsequently larger plants with higher root surface area also increases the uptake of PAHs as the roots are more established. Another finding stated that the phytomass of Alnus planted at low density was twice as high compared to

the Alnus planted at higher density (Lee, Nam, Kwak, Cho and Lee, 2009). Hence the uptake rate of heavy metals was higher at low plant density and the metals absorbed by the Alnus plant at the dredging area included Pb, Zn, Cu, Cr, As and Cd.

Since there are different findings that resulted in different heavy metals uptake rate when plants are planted in different densities, further investigation can be carried out to determine the best suit plant density for *Ipomoea aquatica* in an aquaponics system to compare the phytoremediation and desalination properties. Besides that, the duration to allow the aquaponics to work should be extended for a longer period to further observe more changes in the metal elements concentration as well as the conductivity, total dissolved solids and salinity. The construction of this aquaponics system integrated with desalination technology might be used for in-situ application.

REFERENCES

Ab Razak, N.H., Praveena, S.M., Ariz, A.Z. and Hashim, Z. (2015). Drinking water studies: A review on heavy metal, application of biomarker and health risk assessment (a special focus in Malaysia). *Journal of Epidemiology and Global Health, 5*, 296-310.

Adamczyk-Szabela, D., Markiewicz, J., & Wolf, W. M. (2015). Heavy Metal Uptake by Herbs. IV. Influence of Soil pH on the Content of Heavy Metals in *Valeriana officinalis* L. *Water, Air, and Soil Pollution, 226*(4), 106.

Akan, J. and Mohmoud, S. (2012). Bioaccumulation of Some Heavy Metals in Fish Samples from River Benue in Vinikilang, Adamawa State, Nigeria. *American Journal of Analytical Chemistry*, **3**: 727-736.

Aksu, Z. (2001). Equilibrium and kinetic modelling of cadmium(II) biosorption by *C. vulgaris* in a batch system: effect of temperature. *Separation and Purification Technology, 21*(3), 285-294.

Alizadeh, A., Motamedi, J. and Erfanzadeh, F. (2014). Potential of four halophytes from the Urmia Lacke as soli phytodesalination. *Iranian Journal of Range and Desert Research, 21*(4), 663-675.

Alturiqi, A.S. and L.A. Albedair. (2012). Evaluation of some heavy metals in certain fish, meat and meat products in Saudi Arabian markets. *The Egyptian Journal of Aquatic Research*, **38**(1), 45-49.

Al-Weher, S.M. (2008). Levels of Heavy Metal Cd, Cu and Zn in Three Fish Species Collected from the Northern Jordan Valley, Jordan. *Jordan Journal of Biological Sciences*, **1**(1), 41-46.

Ashraf, M., Ozturk, M. and Ahmad, M.S.A. (2010). *Plant Adaptation and Phytoremediation*. New York: Springer.

Azrina, A., Khoo, H.E., Idris, M.A., Amin, I. and Razman, M.R. (2011). Major inorganic elements in tap water samples in Pennisular Malaysia. *Malaysia Journal of Nutrition, 17*(2), 271-276.

Bhuiyan, M.S.I., Raman, A., Hodgkins, D.S., Mitchell, D. and Nicol, H. (2015). Salt accumulation and physiology of naturally occurring grasses in saline soils in Australia. *Pedosphere, 25*(4), 501-511.

Bijvelds, M.J.C., Flik, G., Kolar, Z.I. and Wendelaar Bonga, S.W. (1996). Uptake,

distribution and excretion of magnesium in *Oreochromis mossambicus*: dependence on magnesium in diet and water. *Fish Physiology and Biochemistry,* *15*(4), 287-298.

Cai, Q.Y., Mo, C.H., Zeng, Q.Y., Wu, Q.T., Ferard, J.F. and Blanc, A.L. (2007). Potential of *Ipomoea aquatica* cultivars in phytoremediation of soils contaminated with di-*n*-butyl phthalate. *Environmental and Experimental Botany*, **62,** 205-211.

Chandra Sekhar, K., Kamala, C. T., Chary, N. S. and Anjaneyulu, Y. (2003). Removal of heavy metals using a plant biomass with reference to environmental control. *International Journal of Mineral Processing*, **68**(1-4), 37–45.

CWT. (2004). *Electrical conductivity/salinity fact sheet.* California: Clean Water Team.
.

Duman, F., Leblebici, Z. and Aksoy, A. (2009). Growth and bioaccumulation characteristics of watercress (*Nasturtium officinale R. BR*.) exposed to cadmium, cobalt and chromium. *Chemical Speciation and Bioavailability*, **21**(4), 257-265.

El Sayed, A.M. (2006). *Tilapia culture in salt water: environmental requirements, nutritional implications and economic essentials.* en Nutricion Acuicola VIII. VIII Simposium Internacional de Nutricion Acuicola. 15- 17 Noviembre. Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon, Mexico.

Enders, A. and J. Lehmann. (2012). Comparison of Wet-Digestion and Dry-Ashing Methods for Total Elemental Analysis of Biochar. *Communications in Soil Science and Plant Analysis,* **43**(7), 1042-1052.

Engineering Service Division. (2010). *Drinking water quality standard.* Malaysia: Ministry of Health.

EPA. (2001). *Parameters of water qualities – Interpretation and Standards.* Ireland: Environmental Protection Agency.

Etim, E.E. (2012). Phytoremediation and Its Mechanisms : A Review. *International Journal of Environment and Bioenergy*, **2**(3), 120–136.

FAO. (1999). *Fishery harbour manual on the prevention of pollution - Bay of Bengal Programme.* Rome: Food and Agriculture Organization of the United Nations.

Fritioff, A., Kautsky, L. and Greger, M. (2005). Influence of temperature and salinity on heavy metal uptake by submersed plants. *Environmental Pollution*, **133**(2), 265-274.

Geeganage, K.T., Mohotti, A.J., Ariyarathne, M., Mohotti, K.M. and Chandrajith, R.L.R. (2011). Phytoremediation of Metal Polluted Soils by *Ipomoea aquatica* and *Colocasia esculenta*. ***Proceedings of the Peradeniya University Research Sessions, Sri Lanka, 16***, pp. 134.

Graber, A. and Junge, R. (2009). Aquaponics system: nutrient recycling from fish wastewater by vegetable production. ***Desalination, 246,*** 147-156.

Hefni Effendi, B.A.U., Giri M. Darmawangsa. (2015). Phytoremediation of freshwater crayfish (*Cherax quadricarinatus*) culture wastewater with spinach (*Ipomoea aquatica*) in aquaponic system. ***ACCL Biofux***, **8**(3), 421-430.

Hseu, Z.Y., 2004. Evaluating heavy metal contents in nine composts using four digestion methods. ***Bioresource Technology***, **95**(1), 53–59.

Hu, Z., Lee, J.W., Chandran, K., Kim, S.P., Brotto, A.C. and Khanal, S.K. (2015). Effect of plant species on nitrogen recovery in aquaponics**. *Bioresource Technology*, 188**, 92–98.

Kamal, M., Ghaly, A.E., Mahmoud, N. and Cote, R. 2004. Phytoaccumulation of heavy metals by aquatic plants. ***Environment International***, **29**(8), 1029–1039.

Khalifa, K.M., Hamil, A.M., Al-Houni, A.Q.A and Akacha, M.A. 2010. Determination of Heavy Metals in Fish Species of the Mediterranean Sea (Libyan coastline) Using Atomic Absorption Spectrometry. ***International Journal of PharmTech Research***, **2**(2), 1350–1354.

Khan, A.G. (2005). Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. ***Journal of Trace Elements in Medicine and Biology, 18***, 355-364.

Kiraly, K.A., Pilinszky, K., Bittsanszky, A., Gyulai, G. and Komives, T. 2013. Importance of ammonia detoxification by plants in phytoremediation and aquaponics. ***Növénytermelés***, **62**, 99-102.

Komjarova, I. and Blust, R. (2009). Effect of Na, Ca and pH on simultaneous uptake of Cd, Cu, Ni, Pb, and Zn in the water flea *Daphnia magna* measured using stable isotopes. ***Aquatic Toxicology.***

Laitonjam, B.C. and Gupta, A. (2016). Phytoremediation of lead using *Ipomoea aquatica* Forsk. in hydroponic solution. ***Chemosphere, 156***, 407-411.

Lawson, O.E. and Lawson, E.O. (2011). Physico-Chemical Parameters and Heavy Metal Contents of Water from the Mangrove Swamps of Lagos Lagoon, Lagos, Nigeria. ***Advances in Biological Research***, **5**(1), 8–21.

Lee, D.B., Nam, W., Kwak, Y.S., Cho, N.H. and Lee, S.S. (2009). Phytoremediation of heavy metal contaminated soil in a reclaimed dredging area using Alnus species. *Journal of Ecology and Environment, 32*(4), 267-275.

Mazumdar, K. and Das, S. (2015). Phytoremediation of Pb, Zn, Fe and Mg with 25 wetland plant species from a paper mill contaminated site at North East India. *Environ Sci Pollut Res*, *22,* 701-710.

Mishra, V.K. and Tripathi, B.D. (2008). Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. *Bioresource Technology*, **99**(15), 7091–7097.

Othman, M.S., Ahmad, A., Mushrifah, I. and Lim, E.C. (2008). Seasonal influence on water quality and heavy metal concentrations in Tasik Chini, Peninsular Malaysia. *Proceedings of Taal2007: The 12th World Lake Conference*, 300-303.

Perkin Elmer Coorporation. (1996) . *Analytical Methods for Atomic Absorption Spectroscopy*.

Podsiki, C. (2008). *Charts of heavy metals, their salts and other compounds*.

Pouladi, S.F., Anderson, B.C., Wootton, B. and Rozema, Lloyd. (2016). Evaluation of phytodesalination potential of vegetated bioreactors treating greenhouse effluent. *Water, 8*(233).

Radosavljević, V., Ćirković, M., Ljubojević, D., Jakić-Dimić, D., Marković, Z., Zutić, J. and Milićević, V. (2014). Searching for Solutions in Aquaculture : Aquaponics. *Arhiv veterinarske medicine*, **7**(2), 71–78.

Rahman, M.A. and Hasegawa., H. (2011). Aquatic arsenic: Phytoremediation using floating macrophytes. *Chemosphere, 83*, 633-646.

Rajan, S., Mohd Firdaus, N.N, Appukutty, M. and Ramasamy, K. (2012). Effects of climate changes on dissolved heavy metal concentrations among recreational park tributaries in Pahang, Malaysia. *Biomedical Research, 23*(1), 23-30.

Rakocy, J.E., Masser, M.P., and Losordo, T.M. (2006). Recirculating aquaculture tank production systems: Aquaponics – Integrating Fish and Plant Culture. *Southern Regional Aquaculture Centre*, (454).

Rezania, S., Mat Taib, S., Md Din, M.F., Dahalan, F.A. and Kamyab, H. (2016). Comprehensive review on phytotechnology: heavy metals removal by diverse aquatic plants species from wastewater. *Journal of Hazardous Materials, 318*, 587-599.

Robinson, J.W., Frame, E.M.S. and Frame II, G.M. (2005). *Undergraduate Instrumental Analysis*. 6ᵗʰ ed. New York: Marcel Dekker.

Sturz, A., Itoh, M. and Smith, S. (1998). Mineralogy and chemical composition of clay minerals, tag hydrothermal mound. *Proceedings of The Ocean Drilling Program, Scientific Results, 158*, 277-284.

The Star. (2014, January 14).

Tüzen, M., 2003. Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry. *Microchemical Journal*, **74**(3), 289–297.

United States Protection Agency (USPA), 2000. Introduction to Phytoremediation. *U.S. Environmental Protection Agency*, (February), 1–72.

van der Velden J. A., Kolar, Z., Flik, G., de Goeij, J. J. M. and Wendelaar Bonga, S. E. (1989). Magnesium in freshwater tilapia. *Magnesium-Bulletin, 11*, 29-33.

Voica, C., Dehelean, A., Iordache, A. and Geana, I. 2012. Method validation for determination of metals in soils by ICP-MS. *Romanian Reports in Physics*, **64**(1), 221–231.

Vymazal, J. (2016). Concentration is not enough to evaluate accumulation of heavy metals and nutrient in plants. *Science of The Total Environment, 544*, 495-498.

Wang, K.S., Huang, L.C., Lee, H.S., Chen, P.Y. and Chang, S.H. (2008). Phytoextraction of cadmium by *Ipomoea aquatic* (water spinach) in hydroponic solution: effects of cadmium speciation. *Chemosphere, 72*, 666-672.

Weerasinghe, A., Ariyawnasa, S. and Weerasooriya, R. (2008). Phyto-remediation potential of *Ipomoea aquatica* for Cr(VI) mitigation. *Chemosphere, 70*, 521-524.

WEPA. (2006). *National water quality standards for Malaysia.* Japan: Water Environment Partnership in Asia.

WHO. (2009). *Calcium and magnesium in drinking water public health significance*. Geneva: World Health Organization.

Willey, N. (2007). *Phytoremediation: Methods and Review.* New Jersey: Humana Press Inc.

Yang, R.Q., Yao, T.D., Xu, B.Q., Jiang, G.B. and Xin, X.S. (2007). Accumulation

features of organochlorine pesticides and heavy metals in fish from high mountain lakes and Lhasa River in the Tibetan Plateau. *Environment International, 33*, 151-156.

Yousif, B.M, Nguyen, N.T., Fukuda, Y., Hakata, H., Okamoto, Yu., Masaoka, Y. and Saneoka, H. (2010). Effect of salinity on growth, mineral composition, photosynthesis and water relations of two vegetable crops; New Zealand spinach (*Tetragonia tetragonioides*) and water spinach (*Ipomoea aquatica*). *International Journal of Agriculture and Biology, 12*(2), 211-216.

APPENDICES

A   ICP-MS analysis for synthetic saline water and field water from Kuantan Estuary at Day-0.

CERTIFICATE OF ANALYSIS (COA)

To / Attn	FIST/Lee Pei Qi		
Address	FIST, Universiti Malaysia Pahang		
Tel No	017-6428691	Fax No	-
Sample Lab No	2016/335	No of sample	2

Sample marking   :   2016/335(13)
Sample description   :   Kuantan Estuary
Date of sample received : 18-08-2016
Date reported   :   26-08-2016

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)	14070.0	ppm	CENLAB/WI/CHEM-TM/008
3.	Magnesium (Mg)	1630.0	ppm	CENLAB/WI/CHEM-TM/008
4.	Aluminium (Al)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
5.	Pottassium (K)	446.0	ppm	CENLAB/WI/CHEM-TM/008
6.	Calsium (Ca)	486.7	ppm	CENLAB/WI/CHEM-TM/008
7.	Vanadium (V)	20.6	ppb	CENLAB/WI/CHEM-TM/008
8.	Chromium (Cr)	84.0	ppb	CENLAB/WI/CHEM-TM/008
9.	Manganese (Mn)	2.7	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)	155.7	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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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)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
22.	Lead (Pb)	3.5	ppb	CENLAB/WI/CHEM-TM/008

Sample marking : 2016/355 (14)
Sample description : Synthethic Saline Water
Date of sample received : 18-08-2016
Date reported : 26-08-2016

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)	5990.0	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)	31.4	ppb	CENLAB/WI/CHEM-TM/008
8.	Chromium (Cr)	79.7	ppb	CENLAB/WI/CHEM-TM/008
9.	Manganese (Mn)	13.8	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)	128.1	ppb	CENLAB/WI/CHEM-TM/008

14.	Zinc (Zn)	251.8	ppb	CENLAB/WI/CHEM-TM/008
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/008T
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)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
22.	Lead (Pb)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

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The above analysis is based on the sample submitted by the customer.

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MOHD RAFIE ROSLY
SCIENCE OFFICER

B   ICP-MS analysis for synthetic saline water and field water from Kuantan Estuary at Day-30.

Universiti
Malaysia
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Engineering • Technology • Creativity

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CERTIFICATE OF ANALYSIS (COA)

To / Attn	FIST/Lee Pei Qi		
Address	FIST, Universiti Malaysia Pahang		
Tel No	017-6428691	Fax No	-
Sample Lab No	2016/335	No of sample	2

Sample marking   :   2016/335(47)
Sample description   :   Kuantan River
Date of sample received :   26-10-2016
Date reported   :   04-11-2016

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)	4692.0	ppm	CENLAB/WI/CHEM-TM/008
3.	Magnesium (Mg)	376.2	ppm	CENLAB/WI/CHEM-TM/008
4.	Aluminium (Al)	101.0	ppb	CENLAB/WI/CHEM-TM/008
5.	Pottassium (K)	159.0	ppm	CENLAB/WI/CHEM-TM/008
6.	Calsium (Ca)	153.1	ppm	CENLAB/WI/CHEM-TM/008
7.	Vanadium (V)	23.5	ppb	CENLAB/WI/CHEM-TM/008
8.	Chromium (Cr)	56.0	ppb	CENLAB/WI/CHEM-TM/008
9.	Manganese (Mn)	23.4	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)	53.3	ppb	CENLAB/WI/CHEM-TM/008
13.	Copper (Cu)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
14.	Zinc (Zn)	2.2	ppm	CENLAB/WI/CHEM-TM/008

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15.	Arsenic (As)	9.8	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)	92.6	ppb	CENLAB/WI/CHEM-TM/008
22.	Lead (Pb)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008

Sample marking   : 2016/355 (48)
Sample description   : Saline Water
Date of sample received : 26-10-2016
Date reported   : 04-11-2016

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)	6674.0	ppm	CENLAB/WI/CHEM-TM/008
3.	Magnesium (Mg)	80.9	ppm	CENLAB/WI/CHEM-TM/008
4.	Aluminium (Al)	20.0	ppb	CENLAB/WI/CHEM-TM/008
5.	Pottassium (K)	57.3	ppm	CENLAB/WI/CHEM-TM/008
6.	Calsium (Ca)	68.2	ppm	CENLAB/WI/CHEM-TM/008
7.	Vanadium (V)	170.5	ppb	CENLAB/WI/CHEM-TM/008
8.	Chromium (Cr)	495.6	ppb	CENLAB/WI/CHEM-TM/008
9.	Manganese (Mn)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
10.	Iron (Fe)	2.3	ppm	CENLAB/WI/CHEM-TM/008
11.	Cobalt (Co)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
12.	Nickel (Ni)	13.8	ppb	CENLAB/WI/CHEM-TM/008
13.	Copper (Cu)	592.7	ppb	CENLAB/WI/CHEM-TM/008

14.	Zinc (Zn)	611.6	ppb	CENLAB/WI/CHEM-TM/008
15.	Arsenic (As)	153.9	ppb	CENLAB/WI/CHEM-TM/008
16.	Selenium (Se)	75.2	ppb	CENLAB/WI/CHEM-TM/008T
17.	Molybdenum (Mo)	Not Detected (Less than 0.5)	ppb	CENLAB/WI/CHEM-TM/008
18.	Silver (Ag)	9.2	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)	121.8	ppb	CENLAB/WI/CHEM-TM/008
22.	Lead (Pb)	1.1	ppb	CENLAB/WI/CHEM-TM/008

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MOHD RAFIE ROSLY
SCIENCE OFFICER

C   The comparison of metal element concentrations of the water sample from both tank taken at Day-0 and Day-30

Metal Elements	Concentration (ppm)			
	Control Tank		Studied Tank	
	Day-0	Day-30	Day-0	Day-30
Na	5990.0	6674.0	14070.0	4692.0
Mg	-	80.9	1630.0	376.2
K	-	57.3	446.0	159.0
Ca	-	68.2	486.7	153.1
Fe	-	2.3	-	-
Zn	0.252	0.612	-	2.2
Al	-	0.02	-	0.101
V	0.031	0.171	0.021	0.235
Cr	0.080	0.496	0.084	0.056
Mn	0.014	-	0.003	0.023
Ni	-	0.014	-	0.053
Cu	0.128	0.593	0.156	-
As	-	0.154	-	0.001
Se	-	0.075	-	-
Ag	-	0.009	-	-
Ba	-	0.122	-	0.093
Pb	-	0.001	0.004	-

D   The measured water quality parameters for 5 consecutive weeks. (mean ± SE)

Parameters	DO (mg/L)	pH	EC (µS/cm)	TDS (mg/L)	Salinity (%)	Temperature (°C)
Control Tank (N=3)						
Week 1	18.91 ± 0.029	7.12 ± 0.097	4936.33 ± 5.548	2661.67 ± 4.410	2.63 ± 0.003	24.63 ± 0.153
Week 2	15.56 ± 0.434	7.83 ± 0.004	4944.67 ± 24.343	2669.67 ± 13.094	2.65 ± 0.009	24.27 ± 0.058
Week 3	17.28 ± 0.223	6.53 ± 0.031	4829.00 ± 3.055	2691.00 ± 84.002	2.52 ± 0.025	23.67 ± 0.058
Week 4	16.18 ± 0.009	7.23 ± 0.001	4699.00 ± 0.577	2537.33 ± 0.333	2.50 ± 0.000	22.93 ± 0.473
Week 5	20.65 ± 0.057	6.68 ± 0.005	4738.67 ± 0.333	2558.33 ± 0.333	2.53 ± 0.000	22.83 ± 0.651
Studied Tank (N=3)						
Week 1	20.13 ± 0.140	6.66 ± 0.008	4941.67 ± 0.333	2668.00 ± 0	2.63 ± 0.007	24.73 ± 0.208
Week 2	16.57 ± 0.660	7.00 ± 0.013	4939.67 ± 0.333	2668.00 ± 0.577	2.64 ± 0.000	24.63 ± 0.306
Week 3	15.90 ± 0.116	6.40 ± 0.006	4775.67 ± 2.848	2580.33 ± 0.333	2.55 ± 0.000	22.37 ± 0.252
Week 4	16.14 ± 0.009	6.86 ± 0.003	4705.33 ± 0.667	2540.67 ± 0.333	2.51 ± 0.000	24.63 ± 0.306
Week 5	19.78 ± 0.044	7.05 ± 0.019	4654.67 ± 0.882	2512.33 ± 0.333	2.48 ± 0.000	21.97 ± 0.208

*Note: $SE = \dfrac{SD}{\sqrt{N}}$