PHYTOREMEDIATION OF DOMESTIC WASTEWATER BY AZOLLA FERN IN A CONSTRUCTED WETLAND

REDZANIELL AFIQ BIN MOHD ROSDI

FACULTY OF ENGINEERING
UNIVERSITY OF MALAYA
KUALA LUMPUR

2022

PHYTOREMEDIATION OF DOMESTIC WASTEWATER BY AZOLLA FERN IN A CONSTRUCTED WETLAND

REDZANIELL AFIQ BIN MOHD ROSDI

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE MASTER OF SAFETY, HEALTH AND ENVIRONMENTAL ENGINEERING

FACULTY OF ENGINEERING
UNIVERSITY OF MALAYA
KUALA LUMPUR

2022

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: Redzaniell Afiq Bin Mohd Rosdi (I.C/Passport No:

Matric No: 17221641

Name of Degree: Master of Safety, Health and Environmental Engineering

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

Phytoremediation Of Domestic Wastewater By Azolla Fern In A Constructed Wetland

Field of Study: Wastewater treatment

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date:

Subscribed and solemnly declared before,

Witness's Signature Date:

Name:

Designation:

PHYTOREMEDIATION OF DOMESTIC WASTEWATER BY AZOLLA FERN IN A CONSTRUCTED WETLAND

ABSTRACT

One of the major environmental problems in the present scenario is water pollution. Increased population and industrialization fasten the water pollution rate. Increase in population leads to high domestic water consumption and domestic wastewater production which plays an important role in polluting water resources. In this study an attempt is made to evaluate effectiveness of phytoremediation technique using Azolla fern (*Azolla pinnata*) for domestic wastewater by constructed wetland units. Parameters like pH, Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), Ammoniacal nitrogen (NH_3-N), Phosphate (PO_4^{3-}), were analyzed for sample after 7 days of phytoremediation within 49 days of detention period. Constructed wetlands using Azolla fern observed with a high removal efficiency in all tested parameters except pH. Concentration of BOD, COD, NH_3-N and PO_4^{3-} reduced from 27.4 mg/L, 132 mg/L, 3.28 mg/L, 3.42 mg/L to 13.8 mg/L, 9 mg/L, 0.08 mg/L, 0.09 mg/L respectively whereas the pH increase from 6.60 to 7.9. The removal efficiency % BOD (49.64%), COD (93.18%), NH_3-N (97.56%) and PO_4^{3-} (97.37%). The study conducted by Neethu & Chinamma (2017), indicated that after 28 days of treatment the removal efficiency % BOD (89.61%), COD (92.41%), NH_3-N (91.12%) and PO_4^{3-} (94.93%). For future study, it would be recommended to apply appropriate constructed wetland design, implement odour reduction strategies, select suitable plant and determine amount of dissolved oxygen.

Keywords: Phytoremediation, Azolla fern, Constructed wetland.

PHYTOREMEDIATION OF DOMESTIC WASTEWATER BY AZOLLA FERN IN A CONSTRUCTED WETLAND

ABSTRAK

Salah satu daripada masalah utama alam sekitar dalam senario ketika ini ialah pencemaran air. Peningkatan populasi dan perindustrian mengukuhkan kadar pencemaran air. Peningkatan populasi membawa kepada penggunaan air domestik yang tinggi dan pengeluaran air kumbahan domestik yang memainkan peranan penting dalam mencemari sumber air. Dalam kajian ini, percubaan dibuat untuk menilai keberkesanan teknik phytoremediasi menggunakan paku Azolla (*Azolla pinnata*) untuk air buangan domestik oleh unit lahan basah yang dibina. Parameter seperti pH, Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), Ammoniacal nitrogen (NH_3-N), Phosphate (PO_4^{3-}), dianalisis untuk sampel selepas 7 hari phytoremediasi dalam tempoh 49 hari masa tahanan. Lahan basah yang dibina menggunakan paku Azolla yang diperhatikan, mempunyai kecekapan penyingkiran yang tinggi dalam semua parameter yang diuji kecuali pH. Kepekatan BOD, COD, NH_3-N dan PO_4^{3-} dikurangkan dari 27.4 mg/L, 132 mg/L, 3.28 mg/L, 3.42 mg/L hingga 13.8 mg/L, 9 mg/L, 0.08 mg/L, 0.09 mg/L manakala pH meningkat daripada 6.60 hingga 7.9. Kecekapan penyingkiran % BOD (49.64%) COD (93.18%), NH_3-N (97.56%) dan PO_4^{3-} (97.37%). Kajian yang dijalankan oleh Neethu dan Chinamma (2017), menjelaskan selepas 28 hari rawatan kecekapan penyingkiran % BOD (89.61%), COD (92.41%), NH_3-N (91.12%) dan PO_4^{3-} (94.93%). Untuk kajian masa depan, disyorkan untuk menggunakan reka bentuk yang sesuai, melaksanakan strategi pengurangan bau, memilih pokok yang sesuai dan menentukan jumlah oksigen terlarut.

Keywords: Phytoremediasi, Paku Azolla, Lahan basah.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful

With high gratefulness to our God, Allah S.W.T, who gave me ideas and physical strength in completing this project within its expectation and time allocated. I would like to take this opportunity to express my thanks and gratitude to the following persons, who have directly and indirectly giving their generous contribution towards the completion of this project.

First and foremost, I would like to express my special thanks to my Supervisor and Coordinator, Dr Jegalakshimi A/P Jewaratnam for her guidance and comments throughout the period of this project and also for her support and knowledge regarding this topic. Her invaluable help of constructive comments and suggestions throughout the experimental and thesis works have contributed to the success of this research.

I would also give my special thanks to the laboratory assistant of the Faculty of Engineering for their information about my project. I would also like to extend my gratitude to my parents, lecturers and friends for their guidance, support and motivation. Last but not least, my deepest gratitude goes to my beloved parents Mohd Rosdi Bin Ayob and Rizawati Binti Abdul Rani for their endless love, prayers and encouragement.

Redzaniell Afiq Bin Mohd Rosdi

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

AN/NH_3N	:	Ammoniacal Nitrogen
BOD	:	Biochemical oxygen demand
COD	:	Chemical oxygen demand
DO	:	Dissolved oxygen
N	:	Nitrogen
P	:	Phosphorus
TDS	:	Total dissolved solids
TSS	:	Total suspended solids
UiTM	:	Universiti Teknologi Mara
USEPA	:	U.S. Environmental Protection Agency
STP	:	Sewage treatment plant
IWK	:	Indah Water Konsortium
H^+	:	Hydrogen ion
PO_4^{3-}	:	Phosphate ion
NaOH	:	Sodium hydroxide
H_2SO_4	:	Sulfuric acid
Cd	:	Cadmium
Cu	:	Copper
Cr	:	Chromium
Fe	:	Iron
Ni	:	Nickel
Pb	:	Lead
G	:	Gram
kg	:	Kilogram

mg/L	:	Milligram per litre
mL	:	Millilitre
Nm	:	Nanometre
µm	:	Micrometre
%	:	Percentage
°	:	Degree
°C	:	Degree Celsius

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Background

The water sources can be divided into two categories which are surface water and groundwater. Surface water is the major water source in Malaysia because of its multipurpose uses such as irrigation, transportation, power generation and food source (Al- Badaii et al., 2013; Cleophas et al, 2013; Chan, 2012). The demand for fresh water has become increasingly over the last few decades due to rapid industrialization, urbanization, and increasing population (Al-Badaii et al, 2013). The increasing of anthropogenic activities has caused the availability of fresh water becoming inadequate due to a high level of pollution resulted from anthropogenic sources. Physical, chemical and biological parameters are terms used to characterize water quality of water. In addition, dissolved oxygen, ammonia, and pH are important parameters used to describe the quality of water (Maleri, 2011).

The increasing of anthropogenic activities has caused the availability of fresh water becoming inadequate due to a high level of pollution resulted from anthropogenic sources. Physical, chemical and biological parameters are terms used to characterize water quality of water. In addition, dissolved oxygen, ammonia, and pH are important parameters used to describe the quality of water (Maleri, 2011).

1.1.1 Preventative and control measure

Preventive and control measure of water pollution is an essential requirement for effective management. Phytoremediation is one of the most effective methods to treat the contaminated water because its low-cost and eco-friendly technique. Phytoremediation is described as the removal of contaminants from the environment by using living plant (Salt et al., 1998; Valderrama et al., 2013). In defiance of its removal efficiency of pollutants, phytoremediation application has several complications whereby it is restricted by low adaptability, slow growth, low yield of plants, and short root systems.

1.1.2 Azolla fern

Azolla, a water fern, is regarded as a plant that floats on the surface of the water and normally with submerged roots. Azolla fern is a floating species which does not depend on water depth and soil. It is commonly found on calm waters such as ponds, paddy field and ditches (Kannaiyan, 2002). Azolla fern have been of particular interest to Asian agronomists and botanists due to their rapid growth in nitrogen deficient habitats (Islam & Haque, 1986). The distribution pattern of Azolla pinnata is varied at two different varieties. Azolla pinnata var. imbricata originates from tropical and subtropical Asia whereas Azolla pinnata var. pinnata originates from Africa which is recognized as Africa strain. Azolla is a good phytoremedial technology for environmental protection because of its effectiveness and cheap method. This study will investigate the suitability of Azolla in treating contaminated water.

Figure 1.1 Physical appearance *Azolla pinnata sp.* (Source: http://bios.labkit.in)

1.2 Problem statement

Recently, water pollution has become a major issue in Malaysia and pose a great threat to the sustainability of water resources. Water pollution could also affect the organisms and living plants, people's health and economy. The treatment cost of polluted water is too high and in a few cases, contaminated waters are not treatable for consumption. A large amount of water resources available in the basin, unfortunately, do not guarantee an adequate supply to all consumers because of pollution (Ling, 2010). and biological parameters are terms used to characterize water quality of water. In addition, dissolved oxygen, ammonia, and pH are important parameters used to describe the quality of water (Maleri, 2011).

1.2.1 Effect of urbanization on water quality

Development within our watersheds give rise to an increase in population growth and activities of urban life. The urbanization effect normally changed the quality of run-off in a basin, which in turn affects the quality of water of the receiving waters. Rainfall in developed areas washes down pollutants accumulated on ground surfaces into rainwater facilities.

Wastewater from commercial, industrial and residential areas causes bad smell, especially in the presence of garbage and deteriorates the quality of rainwater systems and polluted the river systems. Most sources of pollution have been caused by anthropogenic activities, although some come from natural sources. Water pollution problem is becoming more serious with reports showing a downward trend year by year.

1.2.2 Conventional wastewater treatment issues

According to Indah Water Consortium (IWK), the sewage operator in Malaysia, a few major constraints to wastewater treatment faced by Malaysia are recognized. One of it is the low sewerage tariff is not able to subsidize the high maintenance and operation costs. Moreover, the service collection of sewerage by operators is not conducive as it is unfortunate that a lot of people fail to understand the importance of sewage management with regards to a safe environment.

Figure 1.2 IWK sewage treatment plant (Source: https://www.thestar.com.my)

Furthermore, non-compliance mainly caused by high amount of influent for oil and grease discharges into sewage treatment plants serving industrial and commercial areas that do not maintained grease traps regularly. Ultimately, the dynamic of the sewerage industry where private developers constructed the sewerage infrastructures and handed over to the public operator which opens up the risk factor of quality being compromised which could give an impact to the treatment processes and operations (Shaari & How, 2015).

1.3 Significance of study

Phytoremediation remedy, based on the abilities of aquatic plants to accumulate pollutants from water, offers an effective solution for the treatment of polluted waters. Phytoremediation is emerging low-cost and feasible sustainable method for pollutants removal. In addition, it is environmentally friendly and further it does not affect people living and working in the surrounding as it uses plants for nature cleaning.

Nowadays, there is an increasing interest in using Azolla as a decontaminant plant in low-cost wastewater treatment system (Culley & Epps, 1973; Forni et al., 2001). Azolla fern is one of the fastest growing aquatic-floating ferns as it produces substantial amount of biomass when growing in natural or contaminated water. At the same time, it is proven that Azolla is a potent floating-aquatic fern for the biofiltration of various toxic metals (Xin Zhang et al., 2008). Recently, scientists have reported the ability of Azolla fern tested as biofilter to purify water and also to remove phosphorus and nitrogen elements that caused eutrophication.

Azolla fern is mostly used for animal feeding and fertilizer in Malaysia, hence phytoremediation is yet to be ventured. With outputs of this study, it would provide the knowledge on the amount of pollutants uptake mechanism, in terms of physicochemical parameters by Azolla fern. This study aims to evaluate the efficiency of Azolla fern for wastewater treatment. Up to certain extent, it could also contribute as the baseline data for the development of Azolla system in Malaysia. In addition, this study could also contribute in developing guidelines related to water quality improvement.

1.4 Objective of study

The specific objectives for this study are:

- To construct a wastewater treatment system using Azolla fern.
- To determine physicochemical parameter of wastewater collected namely pH, BOD, COD, NH_3-N and PO_4^{3-} within detention period of 49 days.
- To determine removal efficiency % of pollutants from wastewater sample after biologic treatment within detention period of 49 days.

1.5 Significance of study

There are several limitations that could be face by the researcher in carrying out this project include:

- This study must be completed in three months.
- Azolla fern will be collected and grow to certain specification before being used in wastewater treatment.
- Limitations of space to conduct the experiment.

CHAPTER 2: LITERATURE REVIEW

2.1 Water pollution

Living organisms need water as it is an essential component for life basic needs. Despite the fact that, water appears plentiful, about 2.5% of water originate on Earth is freshwater. Yet, the freshest water in the world only covers about 1% that is only 0.007% water of the planet that can be access directly by people for water sanitation. Rivers, lakes, reservoirs, ponds and underground are the sources of water. Generally, the world seems inundated by water stress but the major problem is because of poor management which causes water crisis instead of water scarcity on a global basis. (Fulazzaky et. Al., 2010; Biswas & Tortajada, 2011; Chan, 2012).

The major sources of toxic accumulation in the water are due to improper management (Roongtanakiat & Chairoj, 2001; Fulazzaky et. Al., 2010; Biswas & Tortajada, 2011) such as chemicals use for agricultural practices and off-site pollution from industrial areas. (Fulazzaky et. Al., 2010; Othman et. Al., 2012; AlBadaii et. Al., 2013). Throughout the raining season, these toxic chemicals found accumulated within the soil and can leach out and transport by the rain as surface runoff, thus come to a halt in the water bodies for instance rivers, ponds and streams. At elevated levels, these harmful chemicals can cause adverse health effects on human, animals and plants.

2.2 Water quality in Malaysia

About 97% of water supply in Malaysia comes from river, so it is essential for human and nature. (Chan, 2012; Othman et. Al., 2012). In spite of that, a lot of rivers in Malaysia, especially in developed areas are in dreadful condition because many cities have been constructed along the river. Increasing of population growth, urbanization, and water demand from industry, agricultural, recreation, tourisms and hydroelectric generation have caused pollution and flood. Reported from Department of Environment (2003) state that Malaysia's water demands intent to increase approximately 60% in year 1995 to year 2010 and increase to 113% in year 2020. Moreover, severe deterioration of river water quality is due to improper management, insufficient of funds and enforcement and low public participation.

In Malaysia, most rivers have been contaminated, thus river pollution has become such a concern. (Chan, 2012; Kusin et. Al., 2014). In 1987 to 2009, the percentage of polluted rivers have increased significantly, resulting in poor water quality which affected water supply. (Chan, 2012). Industrial development has raised the pressure in the urban areas, such as Lembah Klang which has the highest population in Malaysia. It is convinced that the degradation of water quality is regularly in Selangor river because of ineffective and improper handling of pollutant loads from industrial, agricultural and municipal wastewaters. (Fulazzaky et. Al., 2010). In 1960 to 1970, during the development of agriculture, agro-based was known to be the major water pollution sources in country, by which it has been reported that approximately 90% of the pollution is caused by insufficient provisions in regulating the flow of effluent discharges. (Othman et. Al., 2012).

Non-point source pollution which is caused by storm runoff over ground surfaces, has become a national concern. Toxic chemicals such as heavy metals (Pb, Fe, Zn, Cr) and organic materials (N, P AN-based compound) are released from the diffused sources. Non-point sources pose a threat to human health as well as environment by means of acute or chronic effects. Furthermore, this type of pollution poses a significant effect on environment in terms of biological and ecological factors. As to these issues, the government has initiated to take preventive measures so that the river can be conserved and preserved in order to keep sustaining the human needs for water and other beneficial uses.

2.3 Sewage

Sewage can be characterized in terms of physical, chemical and biological composition. Colour, odours, solids and temperature are the main physical properties of sewage. Chemical can be classified into two types, organic such as Carbohydrates, Proteins, Fats, oil and grease and inorganic such as pH, Chlorides, Phosphorus, Sulphur, Methane and Oxygen. (Indah Water Konsortium). Sewage treatment can be defined as the process of removing pollutants from household and wastewater sewage, both domestic and runoff (effluents). Physical, chemical, and biological processes is needed to remove various type of pollutants. The treatment objectives are to produce a stream of waste (treated effluent), to reuse back solid wastes and sludges into the environment and to produce suitable discharge for solid wastes and sludges. These materials are always inadvertently contaminated with numerous toxic chemical compounds. (Smith et. Al., 2009).

2.4 Wastewater

Any water that has been adversely affected in quality by anthropogenic influence is defined as wastewater. It includes liquid waste discharges by industries, household, agriculture, and commercials. It refers to the municipal wastewater that consists a broad spectrum of contaminant resulting from the heterogenous mixture of wastewater from other sources. (Salt, 2001). Generally, wastewater refers as the term of water that has been generated from industrial and household sources where throughout the global by dumping approximately 10, 000 organic compound per year. These organic compounds need a proper handling and removal if they are recognized as a potential hazardous chemical that can affect human health. Many industrial plants undergone pre-treatment of wastewater before dumping in the wastewater system. (Kulshrenta, 2004).

2.5 Water quality parameter

The restoration and maintenance of good water quality is essential for a healthy river and ecosystem. Some basic conditions must be met in order for aquatic organisms to develop well in the water. Species populations will become stressed when these conditions are not optimal. Aquatic animal and plant can die if the river conditions are poor. Therefore, several water quality parameters need to be measured so that the health of the river water can be determined, ensuring the river is safe to use for drinking and any recreational purpose. There are several parameters that need to be considered in order to determine the quality of water or river index. These parameters are classified into three groups, which are physical, chemical and biological.

2.5.1 Physical parameter

There are various types of physical parameters, some of which are temperature, turbidity, TDS, TSS and others used for water quality evaluation. These parameters have significant effect on the quality of water. The water temperature is a measure of the heat content of the water mass and affects the growth rate and aquatic life survivability. Different fish species require different needs for an optimum temperature and tolerances of extreme temperatures (Davis & McCuen, 2005). Temperature directly affect the characteristics of a river in terms of physical, chemical and biological. Most aquatic life able to survive within a definite range of temperature gradient, and some of the aquatic life able to withstand various extreme temperatures (WSDE, 2002).

Turbidity is usually referred as the total amount of fine particles suspended in water. The habitats for fish and other aquatic organisms would be damaged if the particles presence in high concentration (Said et al., 2004). In aesthetic point of view, turbidity is a major concern. High level of water turbidity shortens the filter runs. Innumerable pathogenic organisms would be encased in the particles and protected from the disinfectant (Avvannavar & Shrihari, 2007). TSS is solids that are generally larger than 0.45 µm suspended in water. A lot of contaminants like toxic heavy metals may be adhered to TSS, which is not suitable for the aquatic life. High amounts of suspended solids also limit the penetration of sunlight into the water. TDS is particles made up of dissolved materials like minerals and salts that are not able to be eliminated using conventional filtration. Synthetic organic chemicals such as fuels, solvents, paints, and detergents that are found in polluted water can cause the spread of undesirable and offensive tastes, odours and colours towards aquatic life such as fish and plants even when the concentration of chemicals are low (Avvannavar & Shrihari, 2007).

2.5.2 Chemical parameter

Chemical parameters such as pH, DO, BOD, COD, nitrates, phosphate, heavy metals, oil and grease are used to determine the quality of water. The water pH value is a parameter that determines the relative strength of acid in the water.

The concentration of the hydrogen ion is directly measured by the pH value. As the pH is lower, the concentration of hydrogen ion is higher and the water is more acidic (Davis & McCuen, 2005). The neutral pH is valued as 7.0. Dissolved oxygen is a measure of the amount of free oxygen molecules within water. It is usually expressed as a concentration in terms of percent saturation, or as a milligram per litre, which is influence by water temperature. So, cold water can hold more dissolved oxygen. (Said et al., 2004).

BOD specifies the pollutants strengths in terms of oxygen required to stabilize sewages from domestic and industrial area. A minimum of 2 to 7 mg/L of DO level need to be maintained at laboratory experimentation or should be available in the natural waters in order for oxidizable organic matter degradation to take place (Avvannavar & Shrihari, 2007). BOD also determines the amount of organic food for bacteria within the water. The BOD test delineates the amount of biodegradable waste present in the water (WSDE, 2002). The COD test is usually used to determine the amount of organic and inorganic oxidizable compounds within the water. Most COD applications measure the total amount of oxidizable contaminants originate in surface water, making COD a useful tool for water quality measurement. The concentration of COD is commonly expressed in milligrams per litre (mg/L), which indicates the mass of oxygen consumed per litre of solution.

Nitrates are a measure of the oxidized form of nitrogen and are an essential macronutrient in aquatic habitat and lives. Nitrates may be detrimental to people's health as human intestines can dissociate nitrates down into nitrites, which affect the ability of red blood cells to carry oxygen. Nitrites can also cause significant disorders in fish (Davis & McCuen, 2005). Phosphorus is vital to all living organisms. In spite of that, uncontrolled production of phosphorus may cause algae to bloom, which are harmful to most aquatic fish and plants. High Phosphorus can reduce the level of dissolved oxygen in water, and in few cases temperature increase. This can be the cause of massive fish kills and the death of many aquatic organisms (Said et al., 2004).

Metals that are introduced naturally into water bodies able to homogenize into aquatic organisms through water and food. At low concentrations, heavy metals like zinc, copper, and selenium are important components for metabolism. Nevertheless, after prolonged exposure to metal at high concentrations, metals tend to accumulate in organism body tissues and cause health problems. High concentrations of trace metals can cause negative consequences for both people and animals. Anthropogenic activities such as heavy industry and mining can result in elevated concentrations compared to those introduced naturally (Carr & Neary, 2006).

Oil within the water can be formed in four basic forms which are free oil, mechanically emulsified oil, chemically emulsified oil and dissolved oil. Free oil can be rise to the water surface in which it is contained. Mechanically emulsified oil is stabilized by forces and electrical charges agitating a free oil and water mixture to the point where it breaks up and disperse into small droplets range in size from 10 to 20 micron. Mechanically emulsified oil can be promoted by increasing water temperatures and using liquid vegetable oils. Oil and grease chemically emulsified in water, primarily through the use of surfactants, detergents, and soaps. Chemically emulsified oil particles are very small size less than 1 micron and do not rise to the surface of the water irrespective of how much time is allowed. Dissolved oil is a form of oil that is no longer exist as discrete particles. A degreasing compound can promote the dissolvability of oil in water as it is soluble in water and oil.

2.5.3 Biological parameter

Biological parameters should also be considered as important parameter to assess the water quality. Examples of biological parameters are Fecal coliform and groups of microorganisms. Fecal coliform is a type of bacteria generally originate in warmblooded animals waste.

Fecal coliform bacteria test is an indicator used to measure the level of water polluted with animal or human fecal materials. If large number of fecal coliform found in a site, there is a high probability that pathogenic organisms are exist, and this site is not suitable for bathing or any contact recreation (Said et al., 2004).

The corrosion of steel pipes is mainly caused by a few microorganisms. Drinking water with contained microorganisms is not potability or suitable for consumption because can lead to sensory defects in odour, colour and taste. Several health problems due to polluted waters like abdominal cramps, diarrhoea, and cholera is due to vibrio cholera, vomiting is due to salmonella and lungs infection is due to mycobacterium (Avvannavar & Shrihari, 2007).

2.6 Conventional wastewater treatment

In general, wastewater treatment system composed by several combination of physical, chemical and biological unit operations and processes that are selected based on the raw wastewater characteristics, wastewater flow rate and treatment goal. The wastewater treatment is classified into four main levels which are preliminary, primary, secondary and tertiary or advanced. Conventional wastewater treatment usually includes primary and secondary treatment. The primary treatment used physical unit operations and processes such as screening, grit removal and primary sedimentation which aims to remove suspended solids and organic matter from wastewater. As the suspended solids decrease, the numbers of enteric microorganisms will also decrease because most of microorganisms are attached to solid particles in wastewater (Tanji et al., 2002).

For secondary treatment, the biological unit operations and processes such as activated sludge and biofiltration are principally used to remove biodegradable organic and residual suspended solid in the wastewater (IWK). Biological wastewater treatment degrades the organic matter present in the wastewater based on the action of an active biomass. The process is affected by several factors such as the wastewater composition, pH, temperature, and oxygen concentration and retention time of the treatment process. Biological treatment process includes suspended growth and biofilm processes as well as aerobic and anaerobic processed. However, these treatment processes are very high cost because they require a lot of maintenance and inspection. Yet another technology for wastewater decontamination is Phytoremediation which is very economical, and eco-friendly technique. This technology usually employed for sediment, conservation of soil, water, and domestic wastewater treatment.

2.7 Phytoremediation

Phytoremediation can be divided into two terminologies which is phyto terms as plants and remediation terms as cleaning (Sola, 2011). Phytoremediation is the practice that eliminates or controls many types of pollutants from the environment in terms of soil, sediments or water by using living plants (Salt et al., 1998; Valderrama et. Al., 2013). Phytoremediation can be classified into several areas for instance phytodegradation, phytoextraction, phytostabilization, phytovolatilization and rhizofiltration. Phytoremediation is the effective technology in cleaning up various pollutants which are organic and inorganic. Organics can be or deteriorated in the root region or uptake by plants, followed by degradation, sequestration and volatilization. Inorganics cannot be deteriorated at the same time, it can be concentrated or neutralized in harvestable plant areas (Pilon Smits, 2005).

2.8 Plants for phytoremediation

The aquatic plants (macrophytes) play a significant role in treating wastewater by removing organic and inorganic contaminants. The aquatic plants have great economic return after being harvested. Plants constitute a very large microbial habitat as they have large surface area. The pollutants and nutrients from wastewater are removed and taken up by the aquatic plants respectively. The aquatic plants also degrade the organic and inorganic constituent within the wastewater (Kallimani & Virupakshi, 2015). The capacity of nutrients uptake by aquatic plants depend on several factors which are the plants species, the sewage quality, the growth rate and the roots depth. The development of root system is affected by water conduction of root zone and oxygen carrying capacity (Guang et al., 2009). The aquatic plants with fast developed root system and good decontamination efficiency should be those fit for local area and has economic value.

Aquatic plants are usually used as a phytoremediation agent because they capable of removing contaminants found in wastewater. Selected aquatic plants should be rapid growth, great biomass production, easy to harvest, and capable of accumulating high amount of heavy metals and nutrients over a long period of exposure (Carranza-Alvarez et al., 2008). A substantial number of aquatic plant species can be utilized in phytoremediation such as Duckweed (Lemna minor), Indian mustard (Brassica juncea (L.) Czern), Pennywort (Hydrocotyle umbellate), Sunflower (Helianthus annuus L.), Water hyacinth (Eichhornia crassipes) and Water velvet (Azolla pinnata) (Eapen et al., 2003). All of these plants use their dense roots system which has a high potential to absorb various heavy metals like Cu, Cd, Cr, Ni and Pb from wastewater (Dushenkov et al., 1995).

According to Akinbile et al., (2015), the reduction of COD concentrations is closely tied to the involvement of aquatic plants activities in the constructed wetland involving microorganisms that could further breakdown organic compounds during phytoremediation. It may also result from the oxidation of organic matter which facilitate microbial metabolism by providing energy. In other words, the substrate used for microbial metabolism usually arises from organic matter present in the wastewater and the length of culturalization time could lead to COD reduction (Akinbile et al., 2012a). According to Shah et. al., (2014), the optimum value of pH for aquatic plants performance is between 6 to 9. At pH below 5, the aquatic plants performance to remove BOD is low due to highly acidic nature of the wastewater. Under other conditions, further increases in pH start retarding aquatic plants performance in BOD removal and at pH 10 the performance of aquatic plants to remove BOD decrease to zero due to high alkalinity.

According to Hartman and Eldowney (1993), one of the unique features of aquatic plants is the transporting of oxygen from the aerial parts to the submerged parts of the plant. The water sub-canopy oxygen content increases as the aquatic plants transported the oxygen. Reddy and DeBusk (1987) claimed that transfer of oxygen by aquatic plants into the root zone plays a major role in supporting the aerobic bacteria growth and subsequent degradation of carbon within wastewater. Furthermore, the higher amount of suspended solid in wastewater can enhance microbial activity as additional substrates on the plant roots. Mahmood et al. (2005) reported that the decrease in COD and BOD concentration can result in an increase in dissolved oxygen concentration of wastewater.

Most nitrogen present in wastewater are in ammonium form and was removed within a constructed wetland where the DO concentrations high enough to support nitrification also through volatilization and fixation processes by vegetation biomass (Cronk, 1996). Processes of nitrogen removal in wetland treatment systems depends on the amount of nitrogen uptake by plant and attached microorganisms, volatilization of ammonia, sedimentation, nitrification and denitrification (Marimon et al., 2013; Korner et al., 2003).

However, the removal of nitrate through the dominant long-term nitrification and denitrification mechanism depends on the organic carbon availability (Lin et al., 2002). According to Vymazal (2007), assimilation of nitrogen refers to a variation of biological processes which often convert inorganic forms of nitrogen into organic compounds that serve as building blocks to cells and tissues. In addition, the nutrient removal efficiency depends on active plant harvesting (White and Cousins, 2013) and nutrient removal through plant uptake is dependent on the plant's growth performance. Shah et. al., (2014) reported that the nitrogen removal can occur through volatilization of NH_3-N. favoured by high pH and other several factors. In Shah et. al., study, it was observed that the nitrogen removal was occurred through NH_3-N volatilization because the pH value is greater than 6.5.

Mechanisms for removing phosphorus from wastewaters include primarily adsorption, sedimentation and filtration, besides to complexation and chemical precipitation and uptake by aquatic plants (Vymazal, 2007), algae and epiphytes, and microorganism incorporation (Gopal and Ghosh, 2008). However, Vymazal (2008), stated that the other mechanisms of phosphorus removal played insignificant role in comparison with direct plant uptake. Although phosphorus exists as PO_4^{3-} in organic and inorganic compounds in wetland, the only form of phosphorus believed to be used directly by algae and aquatic plants is free orthophosphate and therefore often represents a main link between the cycle of organic and inorganic phosphorus in wetland (Ali et al., 2013). Aquatic plants ability to assimilate phosphorus relies on their growth rates, total biomass per unit area, the season within the year, water depth, ionic composition of water, characteristics of sediment, and certain physicochemical and biochemical processes at the root-water-sediment interface.

2.9 Azolla fern

Azolla commonly known as mosquito fern, is a small floating aquatic fern that grows in freshwater habitats in subtropical, tropical, and warm-temperate areas all over the world. Prior to human intervention, Azolla pinnata distributed mostly in Asia and the coast of tropical Africa (Sculthorpe, 1967; Lumpkin & Plucknett, 1980, Watanabe, 1982; Van Hove, 1989). Three Azolla sp. i.e. Azolla caroliniana, Azolla microphylla, and Azolla pinnata are commonly found all over the Indian subcontinent. The name Azolla is derived from the two Greek words, Azo termed as to dry and Ollyo termed as to kill, thus defining that the fern can be killed by drought.

2.10 Morphology

The Azolla macrophyte length ranges from 1 cm to 2.5 cm for species such as Azolla pinnata (Raja et al., 2012). It includes of a main rhizome which branches into secondary rhizomes, all of which hold up small leaves alternately arranged. Numerous unbranched and adventitious roots hang down into the water from nodes on rhizomes ventral surfaces.

The roots also absorb nutrients directly from the water, though in extremely shallow water they may contact the soil, deriving nutrients from it. Each leaf consists of two lobes which are an aerial dorsal lobe that is chlorophyllous and a partially submerged ventral lobe, that is cup-shaped, colourless and provides buoyancy. Each dorsal lobe consists a leaf cavity which houses the symbiotic Anabaena azollae (Peters, 1977; Lumpkin & Plucknett, 1980).

Each leaf cavity interior surface is lined with an envelope (Peters, 1976) and covered by a mucilaginous layer of unknown composition which is embedded with filaments of A. azollae and permeated by multicellular transfer hairs (Shi & Hall, 1988). It has been shown that the mucilage is produced by the symbiont (Robins et al., 1986). The blue-green alga Anabaena azollae consists of unbranched trichomes containing bead-like, heavily pigmented vegetative cells, approximately 6 μm in diameter and 10 μm in length (Van Hove, 1989), and lightly pigmented, intercalary heterocysts which are slightly larger and have thicker cell walls. In very young leaves, trichomes lack heterocysts. Heterocysts gradually increase in frequency until they comprise 30-40% of the algal cells (Van Hove, 1989). According to Hill (1977), heteroeyst frequency reaches a maximum of about 30% of the cells in the 15th leaf from the apex. Mature trichomes also contain spores called akinetes. According to Peters (1975), trichomes, on average, consist of 60.9% vegetative cells, 23.1% heterocysts, and 16% akinetes.

2.11 Physiology

Azolla-Anabaena is exceptionally good because of its high productivity incorporated with its ability to fix nitrogen at substantial rates. Azolla is also capable of photosynthesizing at rates higher than most C4 plants as the variety of light-harvesting pigments contained in the two partners are complementary and can capture a broad range of light wavelengths (Shi & Hall, 1988). According to Watanabe et ai. (1977), Azolla can double its mass in three to five days, growing in nitrogen-free solution, and can accumulate 30 to 40 kg N/ha in two weeks. Five crops of Azolla were grown consecutively in a paddy field and produce about 117 kg N/ha in 106 days. The researcher also obtained doubling times of two days or less for Azolla filiculoides, Azolla caroliniana, Azolla raexicana, and Azolla pinnata under ideal conditions of light and temperature.

2.12 Azolla in phytoremediation

Several positive outcomes show that Azolla can be used to purify contaminated water. Jain et al. (1989) discovered that A. pinnata and Lemna minor (duckweed) have an ability to eliminate heavy metals like Fe and Cu from contaminated water if the concentrations of metal is low. The contaminated water can be treated by flowing it through small water bodies containing both water plants. Saxena (1995) observed that a mixed culture of Lemna and Azolla in the ratio of 2:1 have the potential to sufficiently purify highly polluted effluent from a factory to the extent that it could be used for agricultural purposes. Lately, Arora et al. (2006) reported the tolerance and phyto-accumulation of Cr by three Azolla species and also results determined by Cohen-Shoel et al. (2002) shows the ability of biofiltration of toxic elements by Azolla biomass. Hence, Azolla display a unique capability of extracting heavy metals (Cu, Cd, Cr, Ni, Pb) and nutrients directly from contaminants or wastewater.

A research conducted by Neethu & Chinnamma (2017) observed an increase in the pH within detention time of 28 days which the value reduced from 6.36 to 7.63 is due to photosynthetic activities of Azolla fern. The authors stated that BOD concentration reduced significantly during the experiment. It was reported that the initial and final BOD concentration of domestic wastewater are 77 mg/L and 8 mg/L respectively. The BOD removal efficiency within 28 days detention time is about 89.61%. Both authors also observed a significant reduction in COD concentration for domestic wastewater during the experiment. The initial concentration of COD for the sample at 0 day is 290 mg/L whereas the final concentration is 22 mg/L at 28 days. The estimated amount of COD removal efficiency for the sample is 92.41%. Neethu & Chinnamma (2017) observed a significant reduction in NH_3-N concentration within 28 days of detention time. The initial and final concentration of NH_3-N are 67.6 mg/L to 6 mg/L respectively whereas the removal efficiency of NH_3-N is 91.12%. Furthermore, the authors reported a reduction of PO_4^{3-} concentration from 45.37 mg/L to 2.3 mg/L for domestic wastewater within 28 days of detention time. The authors claimed that the PO_4^{3-} removal efficiency is approximately 94.93%.

CHAPTER 3: METHODOLOGY

3.1 Method Summary

This experiment was divided into two parts. The first part was the cultivation of Azolla fern by using constructed wetland which the plants were cultivated outside the laboratory in order to ensure adequate sunlight and cover from rain. The second part was the analysis of five physicochemical tests in which all of the parameters were conducted inside the laboratory and was carried out every week within 49 days of detention period.

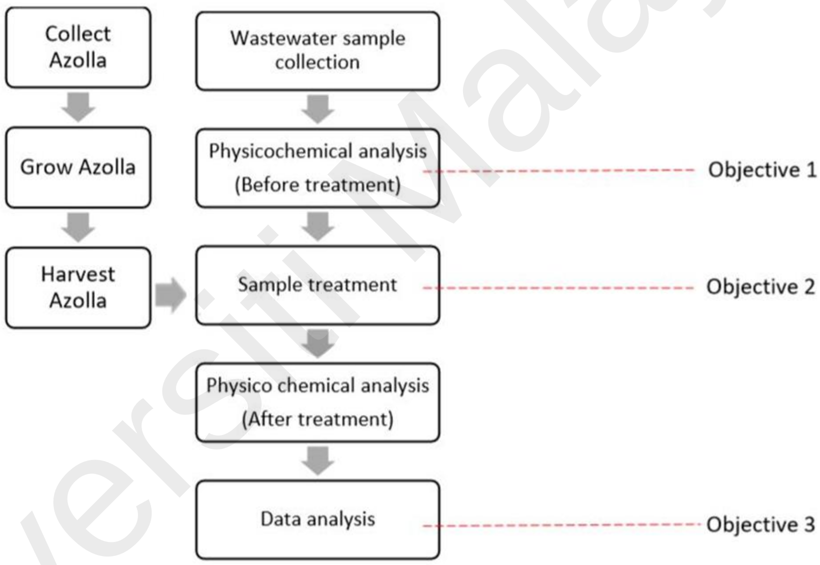

Figure 3.1 Flowchart of experimental design.

3.2 Collection of wastewater sample

The wastewater sample was collected at sewage treatment plant near Kolej Mawar at UiTM Shah Alam area and was stored in four 25 L jerry can for pre-treatment analysis. The following parameter: pH, BOD, COD, NH_3-N and PO_4^{3-} were determined using standard laboratory procedures before the treatment (HACH method).

Figure 3.2 Satellite view of Kolej Mawar sewage treatment plant.

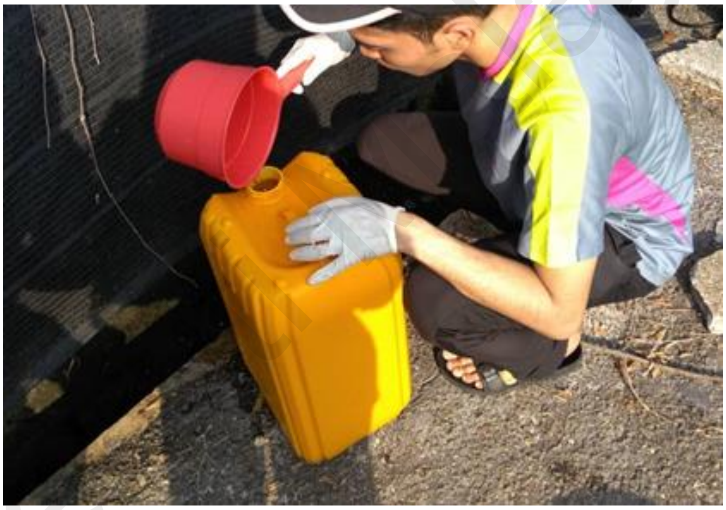

Figure 3.3 Collection of wastewater sample.

3.3 Collection of Azolla fern

Azolla fern, scientifically known as Azolla Pinnata (Figure 2) was used in this experiment. The fern was purchased from Botanical Farm near Shah Alam and transplanted on the same day in a bucket filled with water and maintained for a period of one day to remove all the previous impurities from the roots. All Azolla ranges from 1 cm to 2.5 cm.

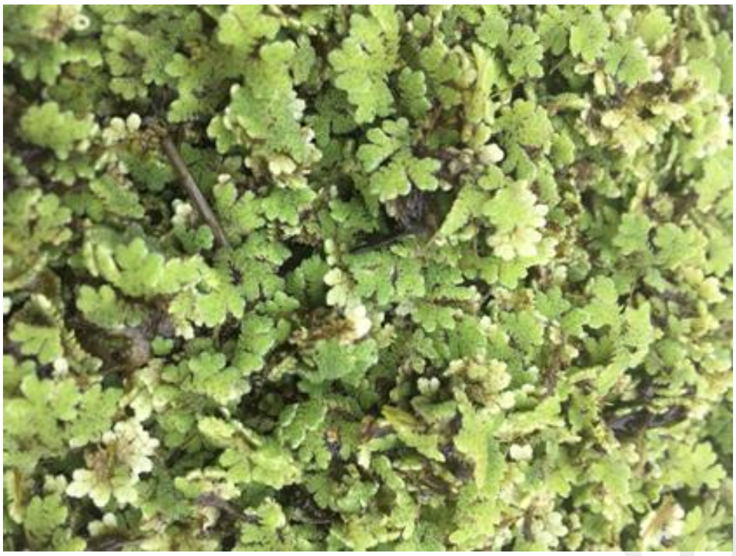

Figure 3.4: Azolla fern being rinsed and dried.

3.4 Pre-treatment of Azolla fern

The Azolla fern was placed floating in water containing nutrients in order to acclimatize the condition before the experiment as the fern treatment is going to be conducted in wastewater which has high concentration of pollutants. However, due to their high reproductive capability, the Azolla fern was harvested after several days. Healthy and matured Azolla fern was selected and rinsed with distilled water. The Azolla was blotted on the filter papers in order to remove adherent water. About 250 g of Azolla fern was laid on the water surface in the constructed wetland.

Figure 3.5 Azolla fern being rinsed and dried.

Figure 3.6 Azolla fern being weighed.

Figure 3.7 Azolla fern being laid on water surface of sample in CW.

3.5 Experimental setup

The experimental setup (Figure 10) mainly consists of two main parts which are, the constructed wet land unit and the outlet zone. A 3 x 2 feet rectangle shaped plastic container was designed and developed as constructed wetland. The constructed wetland unit was set up in a controlled atmospheric condition and integrated with other accessories such as roof on top of the container and one taps connected at the bottom of container which served as the supply for treated sample through outlet pipe.

For the constructed wetland unit, the container was placed on a slope surface in order to facilitate percolation and drainage. The drainage pipe was covered with layer of coarse gravel with a diameter 0f 3-5 mm at a height of 10 cm. The gravels act as a substrate medium for the plant. The container also served as the basin to hold wastewater samples. About 100 L of wastewater was placed into the container using four 25 L jerry can. The container was attached with a plastic UV roof to prevent rain from entering the container which may cause operational problems. The roof frame was built using several pieces of wood joint and UV plastic was placed on top of the frame using clippers.

The outlet zone consists a treated sample collecting outlet. The outlet pipe was fixed above a 1-2 centimeters from the bottom of the container. This constructed wetland system act as a free surface flow constructed wetland and the vertical flow of wastewater between constructed wetland unit and outlet zone is by gravity.

Figure 3.8 The inside view of constructed wetland (CW).

Figure 3.9 The front view of constructed wetland (CW).

Figure 3.10 The side view of constructed wetland (CW).

3.6 Qualitative analysis

Treated samples were collected from the outlet point of the constructed wetland unit every week within detention time of 49 days to undergo physicochemical test for the following parameter: pH, COD, BOD, NH_3-N and PO_4^{3-} by using standard laboratory procedures (HACH method). Hach method represent the best current practice of both water and wastewater analysis as this method provides a lot of EPA-approved laboratory tests for quality and properties of water and wastewater. The laboratory manual of water and wastewater analysis mainly refers to Hach method.

$$Removal\ efficiency\ (\%) = \frac{Influent\ (mg/L) - Effluent\ (mg/L)}{Influent\ (mg/L)} \times 100$$

Equation 3.1. Formula for removal efficiency (%) of pollutants

3.7 Determination of pH

The pH is a measure of acidity or alkalinity of a substance based on a pH scale range from 1.0 to 14.0. The strength of wastewater acidity or alkalinity affects both environment and treatment. The pH is actually the measure of the inverse concentration of hydrogen ions (H+) and is a logarithmic scale. Lower pH value means high acidity, whereas higher pH value indicates high alkalinity. The wastewater pH needs to maintain between 6.0 and 9.0 in order to protect beneficial organisms. The inactivation of treatment process is due to alteration of pH level by acids, cleaning agents and other substances which introduced into wastewater.

3.7.1 Apparatus and reagents required

The apparatus used in this analysis are pH meter with electrode, beakers whereas the reagents used are Buffers Solution; pH 4, pH 7 and pH 9.

3.7.2 Procedure

The pH meter was calibrated using three standard buffer solutions. The water sample was poured into a 100 mL beaker. The electrode was placed into the beaker containing the water sample and the reading was taken after it has remained constant for approximately one minute. The electrode was removed from the water sample and washed with distilled water. The electrode was wiped gently with soft tissue.

3.8 Determination of Biochemical oxygen demand (BOD)

The BOD test measures the ability of aerobic microorganisms to break down organic matter, generally incubated for five days at 20 °C by analyzing the amount of oxygen depleted. BOD refers as a common parameter used in determining oxygen demand on the receiving water of an industrial or municipal discharge. BOD could also be used for evaluation of treatment processes efficiency and measure the level of biodegradable organic material in water.

3.8.1 Apparatus and reagents required

The apparatus used in BOD analysis are BOD TrakTM apparatus (BOD bottles, magnetic stir bar, seal cups, analytical funnel), BOD incubator, beaker (500 mL), magnetic plate and stirrer and graduated measuring cylinder (500 mL) while the reagents used in this analysis are BOD nutrient buffer pillow, nutrient buffer pillow, glucose and glutamic acid standard (3000 mg/L), lithium hydroxide powder pillow and seed.

3.8.2 Procedure

The sample will be heated or cooled to within 2 °C of its incubation temperature (20 °C). To prepare the BOD nutrient buffer solution, the plastic container was filled with 3 L of water and was placed with one BOD nutrient buffer pillow. To prepare the BOD seed, the bottle was filled with 1L of BOD nutrient buffer solution. Two inoculum capsules of seed were inserted into the bottle and aerated for 30-40 min. The bottle was inserted into the incubator and waited for next day. Based on Hach, the BOD range selected was 0-70 mg/L, the sample volume for BOD analysis was 355 mL.

The 355 mg/L sample volume was poured into a BOD Trak sample bottle by using a clean graduated cylinder. A 1.5 inches magnetic stir bar was placed in each sample bottle. The content of the BOD nutrient buffer pillow was added to each bottle for optimum bacteria growth. Stopcock grease was applied to the seal lip of each bottle and to the top of each seal. A seal cup was placed in the neck of each bottle connected the bottle to the pressure sensor of instrument via connecting the caps and tubing seal cup (of lithium hydroxide crystal).

The contents of one lithium hydroxide powder pillow was added to each seal cup by using a funnel. The bottles were placed on the base of the BOD Trak apparatus. The appropriate tube was connected to the sample bottle and the cap was tightened firmly. Each tube was tagged with the channel number. The channel number displayed on the control panel. The instrument was placed into the incubator. The electrical plug was connected and the instrument was turned on. All stir bars were ensured to rotate.

The left keys were pressed simultaneously and held until the time menu appear to select a test duration. The channel 6 key was pressed to activate the test length parameter. The arrow keys used to choose 5 days test. The “OFF” key was pressed to save selection and exit the menu. The channel number that corresponds to the sample bottle was pressed to start a test. Each channel (1-6) was started individually. The “ON” key was pressed.

The menu of BOD selection was displayed. The left arrow key was pressed for two times to select the BOD range of 0-70 mg/L. The “ON” key was pressed and held to start a test. The graph was appeared. The “OFF” key was pressed to cancel a test. The key that corresponds to each sample channel was pressed to display BOD results and the results were observed. A brush and hot, soapy water were used to clean all bottles, stir bars and seal cups. Thoroughly, rinsed with distilled water.

3.9 Determination of Chemical oxygen demand (COD)

The COD is used as an indicator to measure the amount of oxygen required by a water sample to oxidized organic substances. COD tests are carried out by using a strong chemical oxidant such as potassium dichromate. The dichromate is favoured over other oxidants such as potassium permanganate because it is a strong oxidising agent and very applicable to variety of samples and ease of manipulation. Dichromate has an ability to oxidize about 95-100% of organic compounds.

3.9.1 Apparatus and reagents required

The apparatus used in COD analysis are COD reactor, Spectrophotometer (HACH DR2800), pipette (2.0 mL), COD vials rack while the reagent used for COD analysis are COD digestion reagent vials (low range: 3-150 mg/L).

3.9.2 Procedure

For Digestion of sample the COD reactor was turned on and preheated to 150 °C. The safety shield was placed in front of the reactor. The caps of a COD digestion reagent vials were removed for appropriate range. This analysis used a low range (LR) COD digestion reagent vial type to determine sample size of COD range from 3-150 mg/L.

The vial was held at 45° angle. About 2.0 mL (low range) of was pipetted into the vial. The vials were capped tightly. The exterior of the COD vial was rinsed with deionized water and the vial was wiped with paper towel to clean. The vial was held by the cap and over a sink. The vial was inverted gently several times to mix the content. The vial was placed in the preheated COD reactor. A blank was prepared by repeating the same steps, the 2.0 mL of deionised water was substituted for the sample.

The exterior wall of all the vials were wiped until clean and heated simultaneously for two hours in the digestion reactor. The reactor was turned off and the vials were cooled to about 120 °C. Each vial was inverted several times while still warm. The vials were placed into a rack and cooled to room temperature. The colorimetric determination was conducted to measure the COD concentrations.

For Colorimetric determination the programme 430 COD LR was selected. START key was pressed. The exterior of the vial was cleaned with a damp towel followed by a dry one to remove fingerprints or other marks. The blank was placed first into the cell holder. The window "ZERO" was pressed. The display showed 0 mg/L COD. The sample vial was placed into the cell holder. The window "READ" was pressed and the result was read in mg/L.

3.10 Determination of Ammoniacal nitrogen (NH_3-N)

Nitrogen comes in several forms; ammonia, nitrate, nitrite and organic nitrogen. For wastewater analysis, HACH offers the USEPA-accepted Nessler's method for reporting of ammonia. This method causes the conversion of ammonia to ammonium. The mineral stabiliser complexes hardness in the sample. The polyvinyl alcohol dispersing agent aids the colour formation in the reaction of Nessler's reagent with ammonium ions. A yellow colour is formed proportional to the ammonia/ammonium concentration. Should there be cloudiness, it is due to iron or sulphide contamination.

3.10.1 Apparatus and reagents required

The apparatus used in this analysis are spectrophotometers (HACH DR4000), sample cells (25 mL) with appropriate stoppers, graduated cylinder (25 mL) and pipette (1.0 mL). The reagents used in this experiment are mineral stabiliser, Nessler reagent, Polyvinyl alcohol dispersing agent and distilled water.

3.10.2 Procedure

The soft key under HACH PROGRAM was pressed for HACH DR4000. The stored program for low range ammonia (NH3-N) was selected by pressing 2400 with the numeric keys. The Enter key was pressed, ascorbic acid method, powder pillows will be pressed with the numeric key. The display showed: HACH PROGRAM: 2400 N Ammonia Nessler. The 425 nm of wavelength is going to be selected automatically.

A 25 mL mixing graduated cylinder (prepared sample) was filled to the 25 mL mark with standard. Another 25 mL mixing graduated cylinder (blank) was filled with deionized water. Three drops of mineral stabiliser were added to each cylinder. The reagent bottle was held vertically and inverted several times to mix. Three drops of polyvinyl alcohol dispersing agent were added to each cylinder by holding the dropping bottle vertically. The reagent bottle again was held vertically and inverted several times to mix. A 1.0 mL of Nessler reagent was pipetted into each cylinder. The cylinders are was stoppered and inverted several times to mix.

The soft key under START TIMER was pressed. The instrument timer was started. A one-minute reaction reactions period has begun. While timer is running each solution was poured into a 10 mL sample cell. When the timer expires, the blank was placed into the cell holder and the light shield was closed. The soft key under ZERO was pressed. The display showed 0.00 mg/L N NH3. The prepared sample was placed into the cell holder and the light shield was closed. The result was read in mg/L ammonia expressed as nitrogen (NH_3-N).

3.11 Determination of Phosphate (PO_4^{3-})

Phosphorus occurs in wastewaters almost solely as phosphates. Phosphorus can be categorized as orthophosphate, organically bound phosphate or condensed phosphate. The only form of phosphate determined directly is orthophosphate. Reactive phosphorus is a measure of orthophosphate.

In this experiment, the reaction between orthophosphates and molybdate in an acid medium produce the end product such as phosphomolybdate complex. The complex is reduced by ascorbic acid, forming into an intense blue colour of molybdenum.

High amount of turbidity can cause unreliable results in the phosphate tests because of the presence of acid within the powder pillow may dissolve some of the suspended particles and also due to variable desorption of orthophosphate from the particles.

3.11.1 Apparatus and reagents required

The apparatus used in this analysis are spectrophotometer (HACH DR4000), pH meter, sample cells (10 mL), volumetric flask (1000 mL), volumetric flask (50 mL) and beakers whereas the chemical reagents used in this analysis is PhosVer 3 powder pillows.

3.11.2 Apparatus and reagents required

The soft key under HACH PROGRAM was pressed for HACH DR4000. The stored program number for phosphorus, ascorbic acid method, powder pillows was pressed with the numeric key. The display showed: HACH PROGRAM: 3025 P React. AS. LR. The 890 nm of wavelength was selected automatically.

A 10 mL cell riser was inserted into the cell compartment. A 10 mL sample cell was filled with 10 mL of sample. The contents of one PhosVer 3 phosphate powder pillow was added into the 10 mL sample cell (prepared sample). The cell was stoppered immediately and shake vigorously for 30 seconds.

The soft key under START TIMER was pressed. The instrument timer was started. A two minutes reaction period has begun. When the timer expires, another sample cell (blank) was filled with 10 mL of sample. The blank was wiped and placed into the cell holder. The light shield was closed. The soft key under Zero was pressed when the timer beeps. The display showed 0.00 mg/L PO_4^{3-} . The prepared sample was placed into the cell holder and the light shield was closed. The result was read in mg/L PO_4^{3-}.

3.12 Percentage removal efficiency

The removal efficiency (%) was calculated for all the parameter except for pH to determine the potential uptake of pollutants by Azolla fern from the wastewater sample.

CHAPTER 4: RESULT AND DISCUSSION

4.1 Constructed wetland using Azolla fern

The wetland was initially constructed and placed with wastewater sample which parameter within the limits provided by Department of Environment (DOE). Then, the Azolla fern was introduced to treat the sample. The outcome was positive as the Azolla grew very well in the constructed wetland and keep growing week after week (Figure 4.1 & 4.2). The constructed wetland units produced good water quality and remarkable yield of Azolla after 49 days of treatment.

Figure 4.1 The aerial view of CW by using Azolla fern.

Figure 4.2 The inside view of CW by using Azolla fern.

Table 4.1 Physicochemical parameter of wastewater sample collected.

Parameter	Detention time (days)							
	0	7	14	21	28	35	42	49
pH	6.60	7.08	7.24	7.43	7.59	7.61	7.74	7.79
BOD (mg/L)	27.4	ND	ND	ND	22.6	16.4	15.4	13.8
COD (mg/L)	132	72	54	35	27	16	12	9
$NH\text{-}N_3$ (mg/L)	3.28	0.73	0.40	0.27	0.20	0.17	0.13	0.08
PO_4^{3-} (mg/L)	3.42	0.62	0.33	0.14	0.13	0.11	0.10	0.09

Table 4.2 Removal efficiency % of pollutants from sample.

Parameter	Detention time (days)						
	7	14	21	28	35	42	49
	Removal efficiency %						
BOD	ND	ND	ND	17.52	40.15	43.80	49.64
COD	45.45	59.09	73.48	79.55	87.88	90.91	93.18
NH_3-N	77.74	87.80	91.77	93.90	94.82	96.04	97.56
PO43-	81.87	90.35	95.91	96.20	96.78	96.78	97.37

4.2 Determination of pH

Based on figure 4.3, the pH value of sample within 49 days of detention time were 6.60 (0 day), 7.08 (7 days), 7.24 (14 days), 7.43 (21 days), 7.59 (28 days), 7.61 (35 days), 7.74 (42 days), 7.79 (49 days) respectively. Based on the graph, the pH increased gradually between 0 day and 28 days. After that, the pH rose slowly from 28 days to 49 days. The results of present study are in agreement with Neethu & Chinnamma (2017) who observed an increase in pH value of domestic wastewater after 28 days of phytoremediation which the value reduced from 6.36 to 7.63. The increase of pH after phytoremediation might be due to photosynthetic activities of Azolla fern (Neethu & Chinnamma, 2017).

According to Shah et. al., (2014), the optimum value of pH for aquatic plants performance is between 6 to 9. At pH below 5, the aquatic plants performance to remove BOD is low due to highly acidic nature of the wastewater. Under other conditions, further increases in pH start retarding aquatic plants performance in BOD removal and at pH of 10 the performance of aquatic plants to remove BOD decrease to zero due to high alkalinity. So, the pH of sample affects the performance of aquatic plants. Furthermore, high pH can affect the nitrogen removal by plant. The authors reported that the nitrogen removal can occur through volatilization of NH_3-N favoured by high pH and other several factors. Based on previous study, the authors observed that the nitrogen removal was occurred through NH_3-N volatilization because the pH value is greater than 6.5.

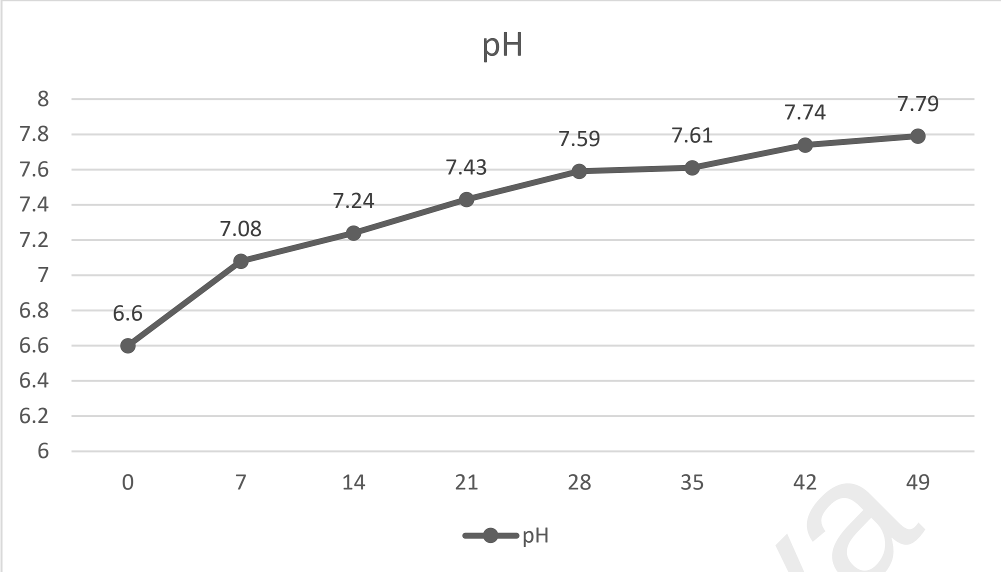

Figure 4.3 Value of pH against detention time (days)

4.3 Determination of Biochemical oxygen demand (BOD)

Based on figure 4.4, the BOD concentration of sample for 0, 28, 35, 42 and 49 days were 27.4 mg/L, 22.6 mg/L, 16.4 mg/L, 15.4 mg/L and 13.8 mg/L respectively. The BOD concentration sample are not detectable for 7 days, 14 days and 21 days because the incubator is under maintenance on that time. From figure 4.5, the removal efficiency of BOD after 28, 35, 42 and 49 days of phytoremediation were 17.52%, 40.15%, 43.8% and 49.64% respectively. The BOD concentration declined gradually between 0 day and 35 days, then decreased slowly between 35 days and 49 days whereas the BOD removal efficiency increased significantly between 28 days and 35 days, then rose slowly from 35 days to 49 days. However, these results can be contended by Neethu & Chinnamma (2017), who indicated that BOD concentration reduced significantly after 28 days of biologic treatment. It was reported that the initial and final BOD concentration of domestic wastewater are 77 mg/L and 8 mg/L respectively. The BOD removal efficiency of the author's experiment is about 89.61% which is higher compared to BOD removal efficiency of present study which is 17.52% (28 days).

Azolla fern role in BOD removal are not well established because there were many proven mechanisms which could lead to a significant reduction in BOD concentration, but with longer operating time, might be in hours or days. According to Hartman and Eldowney (1993), one of the unique features of aquatic plants is the transporting of oxygen from the aerial parts to the submerged parts of the plant. The water sub-canopy oxygen content increases as the aquatic plants transported the oxygen. Reddy and DeBusk (1987) claimed that transfer of oxygen by aquatic plants into the root zone plays a major role in supporting the aerobic bacteria growth and subsequent degradation of carbon within wastewater. Furthermore, the higher amount of suspended solid in wastewater can enhance microbial activity as additional substrates on the plant roots. Mahmood et al. (2005) reported that the decrease in BOD and COD concentration can result in an increase in dissolved oxygen concentration of wastewater.

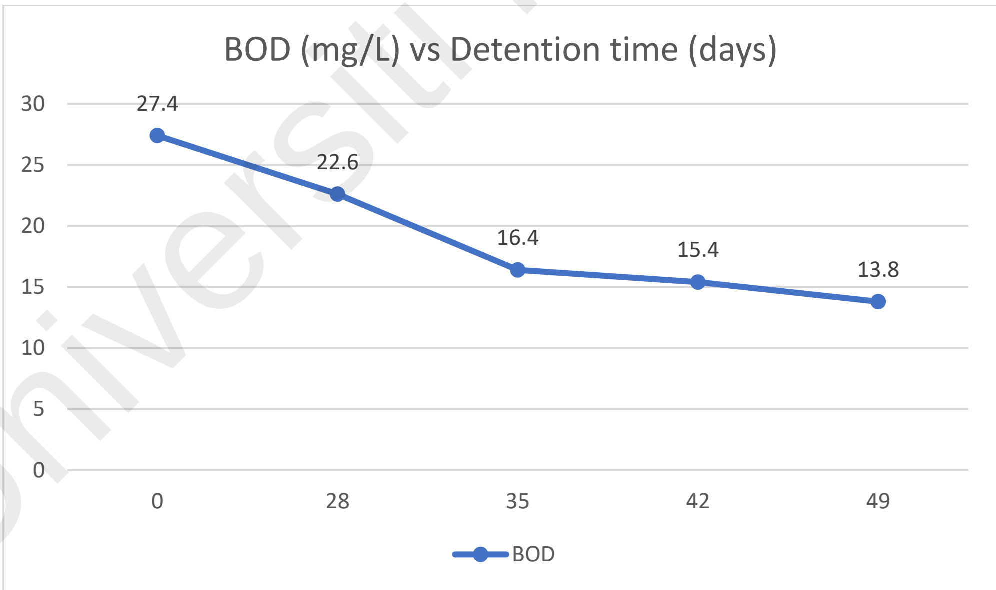

Figure 4.4 BOD (mg/L) against detention time (days)

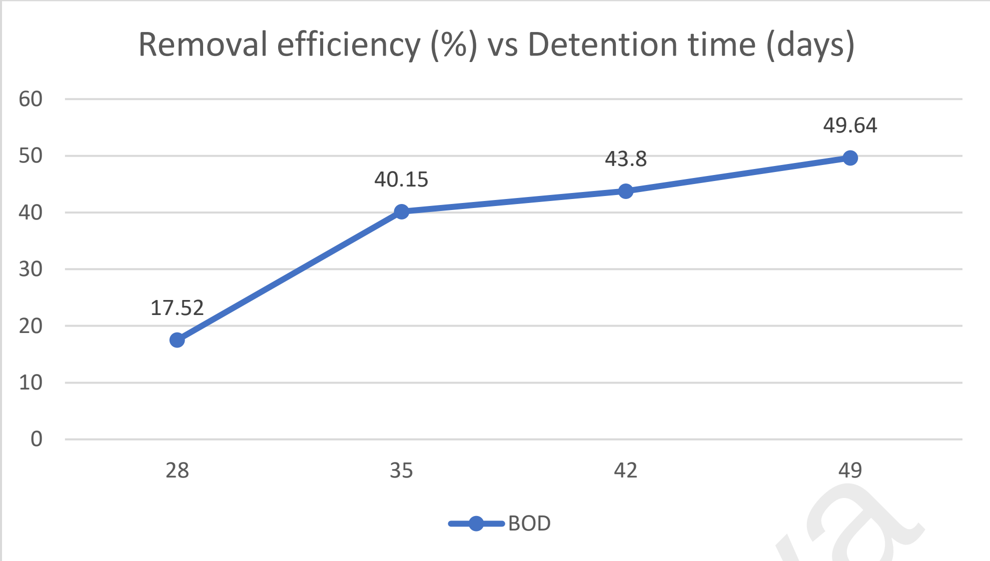

Figure 4.5 BOD removal efficiency (%) against detention time (days)

4.4 Determination of Chemical oxygen demand (COD)

Based on figure 4.6, the COD concentration of sample within 49 days of detention time were 132 mg/L (0 day), 72 mg/L (7 days), 54 mg/L (14 days), 35 mg/L (21 days), 27 mg/L (28 days), 16 mg/L (35 days), 12 mg/L (42 days), 9 mg/L (49 days). The COD concentration of sample decreased significantly between 0 day to 49 days because of organic matter degradation. From figure 4.7, the removal efficiency of COD after 7, 14, 21, 28, 35, 42 and 49 days of phytoremediation were 45.45%, 59.09%, 73.48%, 79.55%, 87.88%, 90.91% and 93.18% respectively. The removal efficiency of COD gradually increases as the COD concentration gradually decreases. This can be supported by findings from Neethu & Chinnamma (2017), who observed significant reduction in COD concentration of the domestic wastewater during the experiment. The authors stated that the COD concentration of sample reduced from 290 mg/L to 22 mg/L after 28 days of phytoremediation has a removal efficiency about 92.41% which is higher compared to COD removal efficiency of present study which is 79.55% (28 days).

The findings of Akinbile et al., (2012) observed that the reduction of COD concentrations is closely tied to the involvement of aquatic plant, Azolla activities in the constructed wetland involving microorganisms that could further breakdown organic compounds during phytoremediation. It may also result from the oxidation of organic matter which facilitate microbial metabolism by providing energy. In other words, the substrate used for microbial metabolism usually arises from organic matter present in the wastewater and the length of culturalization time could lead to COD reduction.

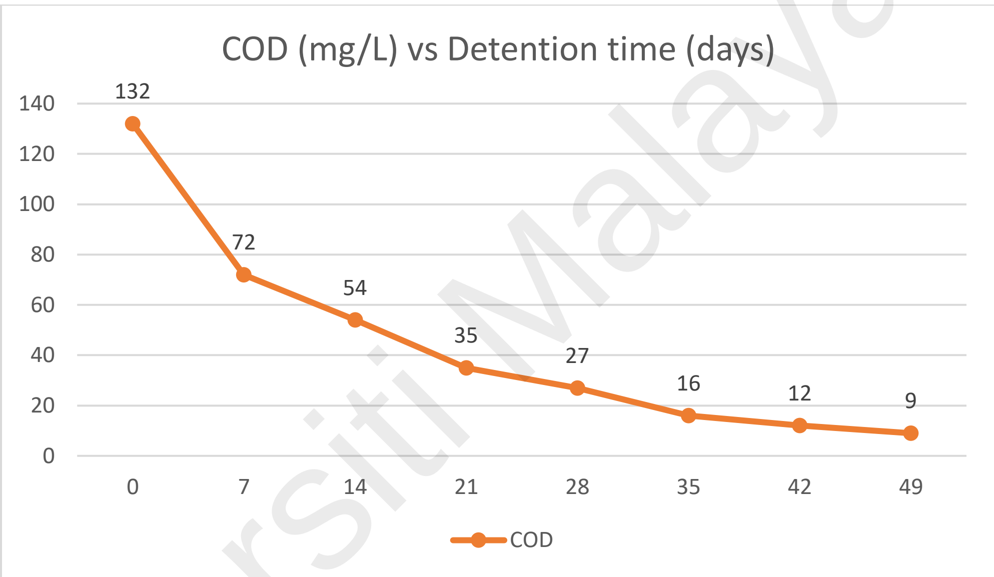

Figure 4.6 COD (mg/L) against detention time (days)

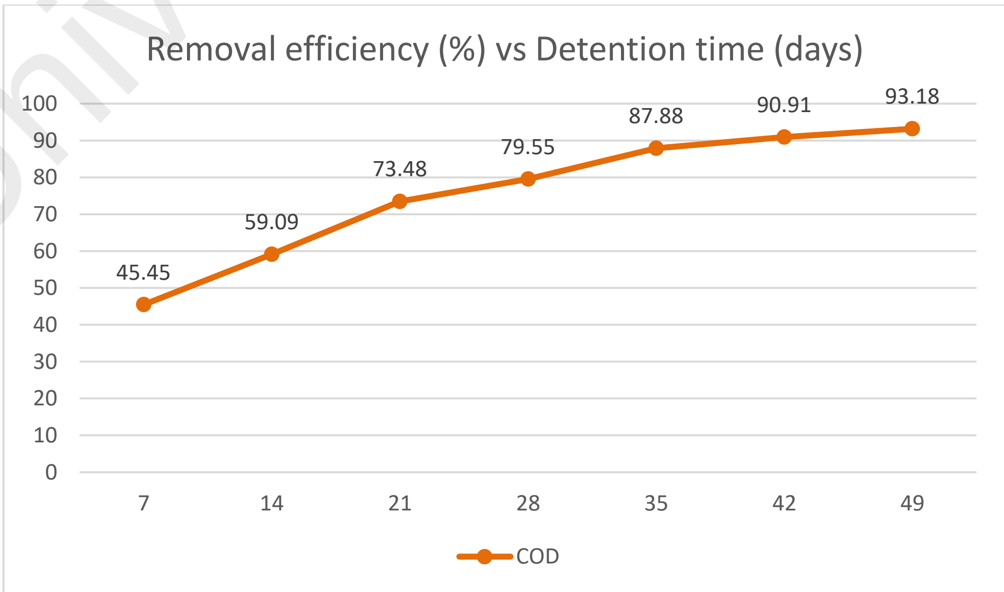

Figure 4.7 COD removal efficiency (%) against detention time (days)

4.5 Determination of Ammoniacal nitrogen (NH_3-N)

Based on figure 4.8, the NH_3-N concentration of sample within 49 days of detention time were 3.28 mg/L (0 day), 0.73 mg/L (7 days), 0.40 mg/L (14 days), 0.27 mg/L (21 days), 0.20 mg/L (28 days), 0.17 mg/L (35 days), 0.13 mg/L (42 days) and 0.08 mg/L (49 days) respectively. From figure 4.9 the removal efficiency of NH_3-N after 7, 14, 21, 28, 35, 42 and 49 days of treatment were 77.74%, 87.8%, 91.77%, 93.9%, 94.82%, 96.04%, 97.56% respectively. Based on the graph, NH_3-N concentration dropped sharply between 0 day and 7 days, then decreased slowly between 7 days and 49 days whereas the NH_3-N removal efficiency increased slightly between 7 days and 49 days. This result was almost identical to experiment conducted by Neethu & Chinnamma (2017), which observed a significant decrease in NH_3-N concentration within detention time of 28 days. According to the authors, the concentration of NH_3-N decreased from 67.6 mg/L to 6 mg/L after 28 days of treatment has the removal efficiency about 91.12% which is slightly lower compared to NH_3-N removal efficiency of present study which is 93.9% (28 days).

Most nitrogen present in wastewater are in ammonium form and was removed within a constructed wetland where the DO concentrations high enough to support nitrification also through volatilization and fixation processes by vegetation biomass (Cronk, 1996). Processes of nitrogen removal in wetland treatment systems depends on the amount of nitrogen uptake by plant and attached microorganisms, volatilization of ammonia, sedimentation, nitrification and denitrification (Marimon et al., 2013; Korner et al., 2003). However, the removal of nitrate through the dominant long-term nitrification and denitrification mechanism depends on the organic carbon availability (Lin et al., 2002). According to Vymazal (2007), assimilation of nitrogen refers to a variation of biological processes which often convert inorganic forms of nitrogen into organic compounds that serve as building blocks to cells and tissues. In addition, the nutrient removal efficiency depends on active plant harvesting (White and Cousins, 2013) and nutrient removal through plant uptake is dependent on the plant's growth performance.

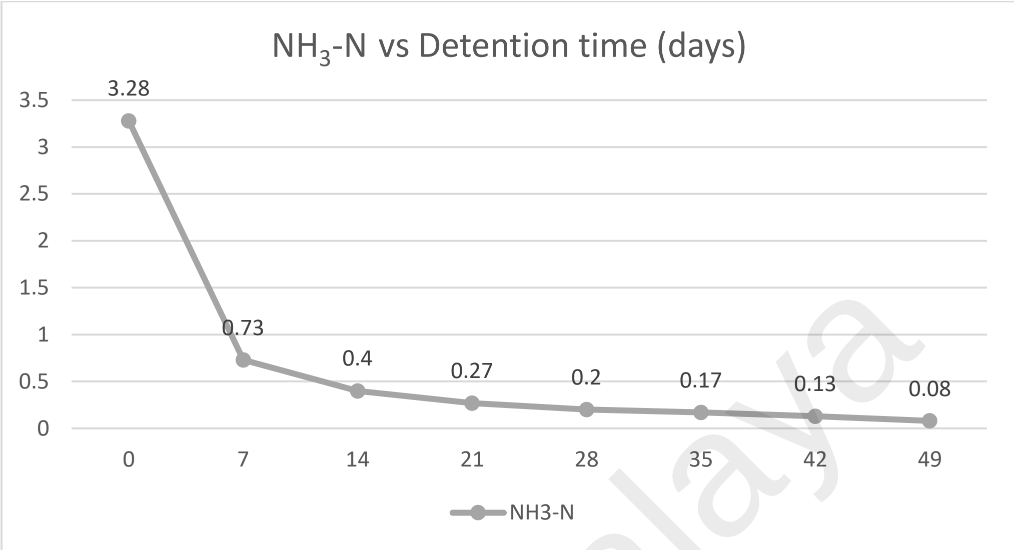

Figure 4.8 NH_3-N (mg/L) against detention time (days)

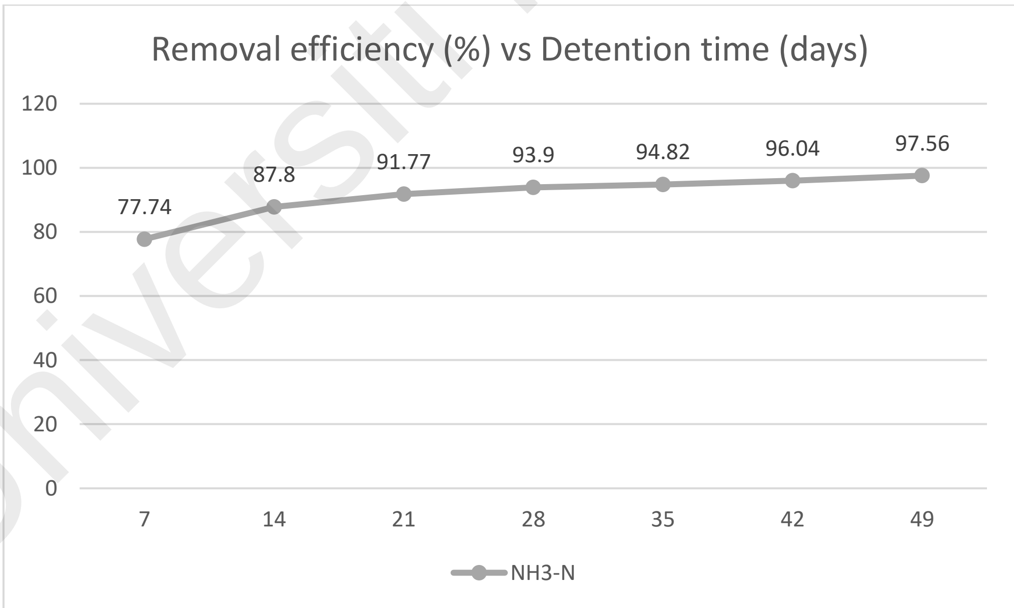

Figure 4.9 NH_3-N removal efficiency (%) against detention time (days)

4.6 Determination of Phosphate (PO_4^{3-})

Based on figure 4.10, the PO_4^{3-} concentration of sample within 49 days of detention time were 3.42 mg/L (0 day), 0.62 mg/L (7 days), 0.33 mg/L (14 days), 0.14 mg/L (21 days), 0.13 mg/L (28 days), 0.11 mg/L (35 days), 0.11 mg/L (42 days) and 0.09 mg/L (49 days) respectively. From figure 4.11, the removal efficiency of PO_4^{3-} for 7, 14, 21, 28, 35, 42 and 49 days of phytoremediation were 81.87%, 90.35%, 95.91%, 96.2%, 96.78%, 96.78% and 97.37% respectively. The PO_4^{3-} concentration dropped rapidly between 0 day and 7 days, then decreased slowly between 7 days and 49 days whereas the PO_4^{3-} removal efficiency increased significantly between 7 days and 21 days, then rose slowly from 21 days to 49 days. These results can also be supported by similar research done by Neethu & Chinnamma (2017), which observed reduction of PO_4^{3-} concentration from 45.37 mg/L to 2.3 mg/L for domestic wastewater within 28 days detention time. The authors claimed that the PO_4^{3-} removal efficiency is approximately 94.93% which is slightly lower compared to PO_4^{3-} removal efficiency of present study which is 96.2% (28 days).

Mechanisms for removing phosphorus from wastewaters include primarily adsorption, sedimentation and filtration, besides to complexation and chemical precipitation and uptake by aquatic plants (Vymazal, 2007), algae and epiphytes, and microorganism incorporation (Gopal and Ghosh, 2008). However, Vymazal (2008), stated that the other mechanisms of phosphorus removal played insignificant role in comparison with direct plant uptake. Although phosphorus exists as phosphate in organic and inorganic compounds in wetland, the only form of phosphorus believed to be used directly by algae and aquatic plants is free orthophosphate and therefore often represents a main link between the cycle of organic and inorganic phosphorus in wetland (Ali et al., 2013). Aquatic plants ability to assimilate phosphorus relies on their growth rates, total biomass per unit area, the season within the year, water depth, ionic composition of water, characteristics of sediment, and certain physicochemical and biochemical processes at the root-water-sediment interface.

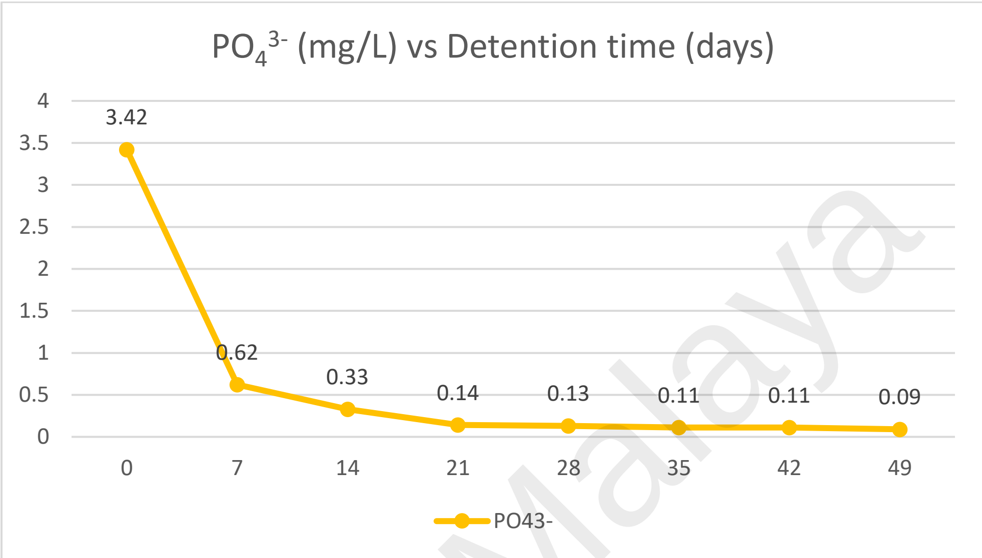

Figure 4.10 PO_4^{3-} (mg/L) against detention time (days)

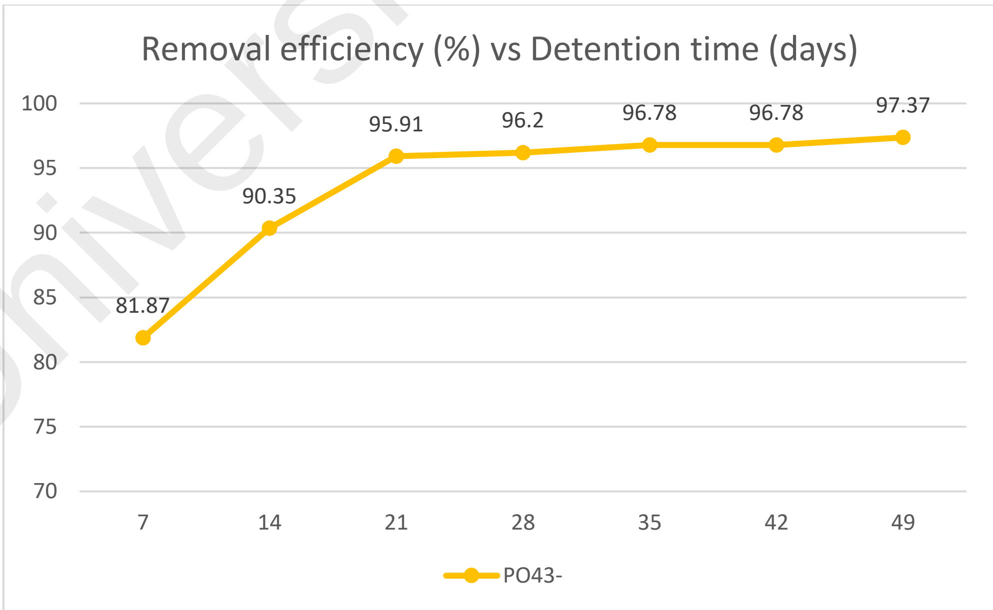

Figure 4.11 PO_4^{3-} removal efficiency (%) against detention time (days)

CHAPTER 5: CONCLUSION AND RECOMMENDATION

5.1 Introduction

This chapter looks at a summary for the whole research and objectives. It was an important part of the research progress. Based on the objectives that being establish in earlier chapter, this chapter will be discuss in two parts. In the first part, it will cover research analysis process and findings of research objectives. The second part comprises of recommendation regarding future study that relate to phytoremediation of wastewater.

5.2 Conclusion

The results obtained from the study proved that Azolla fern had a great potential for phytoremediation agent because of its ability in treating wastewater. It was observed that, the wastewater treatment system using Azolla fern was successfully constructed as the system was efficient in reducing all parameters studied except in pH. The physicochemical parameter for wastewater collected within detention period of 49 days are pH (6.60, 7.08, 7.24, 7.43, 7.59, 7.61, 7.74, 7.79), BOD (27.4 mg/L, 22.6 mg/L, 16.4 mg/L, 15.4 mg/L, 13.8 mg/L), COD (132 mg/L, 72 mg/L, 54 mg/L, 35 mg/L, 27 mg/L, 16 mg/L, 12 mg/L, 9mg/L), NH_3-N (3.28 mg/L, 0.73 mg/L, 0.40 mg/L, 0.27 mg/L, 0.20 mg/L, 0.17 mg/L, 0.13 mg/L, 0.08 mg/L) and PO_4^{3-} (3.42 mg/L, 0.62 mg/L, 0.33 mg/L, 0.14 mg/L, 0.13 mg/L, 0.11 mg/L, 0.11 mg/L, 0.09 mg/L). The removal efficiency % of pollutants from wastewater sample after biologic treatment within detention period of 49 days are BOD (17.52%, 40.15%, 43.80%, 49.64%), COD (45.45%, 59.09%, 73.48%, 79.55%, 87.88%, 90.91%, 93.18%), NH_3-N (77.74%, 87.80%, 91.77%, 93.90%, 94.82%, 96.04%, 97.56%) and PO_4^{3-} (81.87%, 90.35%, 95.91%, 96.20%, 96.78%, 96.78%, 97.37%).

5.3 Recommendation

The availability of water to maintain the required regime is important as the water within constructed wetland may loss due to evapotranspiration. After several weeks, the water level significantly reduced and caused operational problems. Therefore, it is crucial to apply appropriate design models to predict wetland hydraulics.

It is of great importance to choose appropriate species adapted to tropical environments. In tropics where growth rates are high, it is necessary to consider the frequency and harvesting cost. It is not likely to be feasible to use fast growing plants like Azolla fern, which requires frequent harvesting. Before choosing such a plant, economic utilization of excess biomass and frequent harvesting costs should be well evaluated.

Bad odours are another potential problem that might be associated with using constructed wetland to treat wastewater. All wetlands whether it is natural or artificial have their own characteristic odours. The odour levels vary depending on the quality of the influent wastewater and dissolved oxygen. Odour-producing compounds are produced under anaerobic conditions and may be obnoxious. Nuisance odours can be reduced by keeping low levels of BOD. Therefore, odour reduction strategies should be carefully considered for constructed wetlands located on near residential areas or non-remote public land. Wetland designers should be aware of potential pollutant transfer and toxicity in the system and take actions to eliminate or reduce the risk.

Dissolved oxygen (DO) refers to amount of oxygen that dissolved in water. DO is measured in ppm or mg/L. DO should be determine in this study to show the relation response with other parameters. The determination of DO should be before and after 7 days of treatment within 49 days of detention period. In this study, BOD and COD are directly affected by DO. For instance, DO value increase as BOD decrease because DO is not totally consumed by aerobic microbes during degradation of organic matter. COD also decrease when DO is high because the amount of organic matter to be oxidize is lesser. DO should be higher because low level of DO produce anaerobic conditions which affects the aquatic life.

REFERENCES

Akinbile C.O., Yusoff MS, Ahmad Zuki AZ. (2012). Landfill leachate treatment using sub surface flow constructed wetland by Cyperus haspan, Waste Manage, 32: 1387– 1393.

Akinbile, C. O., Ogunrinde, T. A., Che bt Man, H., & Aziz, H. A. (2015). Phytoremediation of domestic wastewaters in free water surface constructed wetlands using *Azolla pinnata*. *International Journal of Phytoremediation*, *18*(1), 54–61. https://doi.org/10.1080/15226514.2015.1058330

Ali, H., Khan, E., Sajad, M.A., (2013). Phytoremediation of heavy metals: concepts and applications. Chemosphere 91, 869–881.

Al-Badaii, F., Othman, M.S. & Gasim, M.B. (2013). Water Quality Assessment of the Semenyih River, Selangor, Malaysia. Hindawi Publishing Corporation, Journal of Chemistry, Article ID 871056. Retrieved from http://dx.doi.org/10.1155/2013/871056

Arora A, Saxen S, Sharma DK (2006). Tolerance and Phytoaccumulation of Chromium by three Azolla species. World Journal of Microbiology and Biotechnology. 22: 97-100.

Avvannavar, S. M., and Shrihari, S. (2007). Evaluation of water quality index for drinking purposes for river Netravathi, Mangalore, South India. Environmental Monitoring and Assessment.

Biswas, A. K. & Tortajada, C. (2011) Water Quality Management: An Introductory Framework. Int. J. Water Resources Development, 27(1), 5-11, doi: 10.1080/07900627.2010.547979

Carr, G. M., & Neary, J. P. (2006). Water quality for ecosystem and human health. Canada: United Nations Environment Programme Global Environment Monitoring System (GEMS).

Carranza-Álvarez, C., Alonso-Castro, A.J., Alfaro-De La Torre, M.C., García-De La Cruz, R.F. (2008). Accumulation and Distribution of Heavy Metals in *Scirpus americanus* and *Typha latifolia* from an Artificial Lagoon in San Luis Potosí, México. Water, Air, and Soil Pollution, 188(4), 297-309.

Chan, N. W. (2012). Managing urban rivers and water quality in Malaysia for sustainable water resources. Wat. Res. Dev., 28(2), 343-354. doi: 10.1080/07900627.2012.668643

Chandra, B.J & Kulshrenta, S. (2004). Wastewater. Production, Treatment and Use in Nepal. Renewable Energy, 263-268

Cleophas, F. N., Isidore, F., Lee, K.H. & Bidin, K. (2013). Water quality status of Liwagu River, Tambunan, Sabah, Malaysia. Journal Of Tropical Biology And Conservation, 10: 67-73

Cohen-Shoel N., Barkay Z., Ilzycer D., Gilath L., Tel-Or E., (2002). Biofiltration of toxic elements by Azolla biomass. Water, Air, Soil Pollut., 135, 93–104.

Cronk, J.K., (1996). Constructed wetlands to treat wastewater from dairy and swine operations: A review. Agriculture Ecosystems and Environment, 58, pp. 97–114.

Culley, D.D., amd E.A. Epps. (1973). Use of duckweed for waste water treatment and animal feed. WPEF. J 45:337-347

Davis, A. P. & McCuen, R. H. (2005). Storm water management for smart growth. 1st edition. Springer Science and Business Media

DeBusk, T.A., Reddy, K.R., (1987). Wastewater treatment using floating aquatic macrophytes: management strategies. In: Reddy, K.R., Smith, W.H. (Eds.), Aquatic Plants for Water Treatment and Resource Recovery. Magnolia Publishing, Orlando, FL, pp. 643–656.

Dushenkov, V., Kumar, P.B.A.N., Motto, H., Raskin, I. (1995). Rhizofiltration: The Use of Plants to Remove Heavy Metals from Aqueous Streams. Environmental Science and Technology, 29.

Eapen, S., Suseelan, K.N., Tivarekar, S., Kotwal, S.A., Mitra, R. (2003). Potential for rhizofiltration of uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor*. Environmental Research, 91(2), 127-133.

Engku Azman Tuan Mat, Jamil Shaari, & Voon Kok How (2012). Wastewater Production, Treatment, and Use in Malaysia. Retrieved from https://doi.org/10.1016/j.watres.2014.11.002

EPA, (2012). Water monitoring and Assessment: conductivity, United States Environmental Protection Agency.

Forni, C., Chen, J., Tancioni, L., Caiola, M.G., (2001). Evaluation offern Azolla for growth, nitrogen and phosphorus removal from wastewater. Water Research 35 (6), 1592–1598

Fulazzaky, M.A., Seong, T.W., & Masrin, M.I.M. (2010). Assessment of Water Quality Status for the Selangor River in Malaysia. Water Air Soil Pollut., 205, 63–77. doi 10.1007/s11270-009-0056-2

Gopal, B., Ghosh, D., (2008). Natural wetlands. In: Jørgensen, S.E. (Ed.), Applications in Ecological Engineering, first ed. Elsevier B.V., Amsterdam, The Netherlands.

Guang Sun, Yongshen Ma, Ran Zhao, (2009) "Study on Purification in Constructed Wetlands with Different Plants", World Rural Observations, vol 1(2), pp 35-3.

Hartman MC, Eldowney W (1993) Pollution: ecology and biotechnology. Wiley, New York, pp 174–18

Indah Water Konsortium Sdn Bhd (2016). Sewage Treatment Plant. Retrieved from https://www.iwk.com.my/do-you-know/sewage-treatment-methods

Islam, A.B.M.S. & Haque, M.Z. (1986). Growth of Azolla in association with rice crop culture and its contribution to soil fertility. Bangladesh Journal of Agriculture, 11: 87-90.

Jain, S. K., P. Vasudevan & N. K. Jha. (1989). Removal of some heavy metals from polluted water by aquatic plants: Studies on duckweed and water velvet. Biol. Wastes 28:115 126.

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

Kavya S. Kallimani and Arjun S. Virupakshi. (2015) "A Review on Removal Efficiency of Pollutants in Subsurface flow Systems", ISSN;0976-1353 volume 14 Issue 2.

Korner, S., Vermaat, J.E., Veenstra, S., (2003). The capacity of duckweed to treat wastewater: ecological considerations for a sound design. J. Environ. Q. 32, 1583–1590.

Lin, Y.F., Jing, S.R., Wang, T.W., et al., (2002). Effects of macrophytes and external carbon sources on nitrate removal from groundwater in constructed wetlands. Environ. Pollut. 119, 413–420.

Lim, C.Y. (2015), IWK sewage treatment plant. [Photograph]. Retrieved from https://www.thestar.com.my/lifestyle/features/2015/01/29/heres-why-you-should-pay-your-iwk-bill/

Ling JKB (2010). Water quality study and its relationship with high tide and low tide at Kuantan river. Ph.D. Dissertation, University Malaysia Pahang, Pahang, Malaysia

Lumpkin, T.A., and D.L. Plucknett. (1982). Azolla: Botany, physiology and use as a green manure. Econ. Bot. 34: 111-153.

Mahmood Q, Zheng P, Islam E, Hayat Y, Hassan MJ, Jilani G, Jin RC (2005) Lab scale studies on water Hyacinth (*Eichhornia crassipes Marts Solms*) for bio treatment of textile wastewater. Caspian J Environ Sci 3(2):83–88

Maleri, M. (2011). Effects of rainbow trout (*Oncorhynchus mykiss*) cage culture on Western Cape irrigation reservoirs., (March). Retrieved from http://scholar.sun.ac.za/handle/10019.1/6680

Marimon, Z.A., Xuan, Z., Chang, N.-B., (2013). System dynamics modelling with sensitivity analysis for floating treatment wetlands in a stormwater wet pond. Ecol. Modell. 267, 66–79.

Neethu V.C., & Chinnamma, M.A., (2017). Phytoremediation Effect in Dairy Waste Water and Domestic Waste Water Using Constructed Wetlands of *Azolla Pinnata*. International Journal for Research in Science Engineering and Technology, 4(5), 9–17.

Othman, F., Eldin, M. E. A., & Mohamed, I. (2012). Trend analysis of a tropical urban river water quality in Malaysia. J. Environ. Monit., 14, 3164-3173. doi: 10.1039/c2em30676j

Peters, G. A. (1975). The *Azolla-Anabaena* azollae relationship III. Studies on metabolic capacities and a further characterization of the symbiont. Arch. Microbiol. 103:113-122.

Peters, G. A. (1976). Studies on the *Azolla-Anabaena* azollae symbiosis. Pages 592-610 in W. E. Newton & C. L Nyman (ed.), Proceedings of the First International Symposium on Nitrogen Fixation. Vol. 2. Washington State University Press, Pullman.

Peters, G. A. (1977). The *Azolla-Anabaena* azollae symbiosis. Pages 231-258 in A. Hollaender (ed.), Genetic engineering for nitrogen fixation. Plenum Press, New York.

Physical appearance of *Azolla pinnata sp*. [Photograph]. (2017). Retrieved from http://bios.labkit.in/2017/02/amazing-azolla.html

Pilon-Smits, E. (2005) Phytoremediation. Annual Review of Plant Biology, 56, 15-39. http://dx.doi.org/10.1146/annurev.arplant.56.032604.144214.

Raja W.; Rathaur P.; John, S. A. and Ramteke, R.W. (2012). Azolla: an aquatic *P teridophyte* with great potential International Journal of Research in Biological Sciences2(2): 68 72

Robins, R. J., D. O. Hall, D. J. Shi, R. J. Turner & M. J. C. Rhodes. (1986). Mucilage acts to adhere cyanobacteria and cultured plant cells to biological and inert surfaces. FEMS Microbiol. Lett. 34: 155-160.

Roongtanakiat, N. & Chairoj, P. (2001). Uptake Potential of Some Heavy Metals by Vetiver Grass. Kasersart J. (Nat. Sci.), 35, 46 – 50.

Said, A., Stevens, D. K., and Sehlke, G. (2004). An innovative index for evaluating water quality in streams. Environmental Management. 34(3), 406–414.

Salt, D.E., Smith, R.D., & Raskin, I. (1998). Phytoremediation. Annu. Rev. Plant. Physiol Plant Mol. Biol., 49, 643-668.

Seulthorpe, C. D. (1967). The biology of aquatic vascular plants. Edward Arnold, London.

Shah, M., Hashmi, H. N., Ali, A., & Ghumman, A. R. (2014). Performance assessment of aquatic macrophytes for treatment of municipal wastewater. Journal of Environmental Health Science and Engineering, 12(1), 1–12. https://doi.org/10.1186/2052-336X-12-106

Shi, D. J. & D. O. Hall. 1988. The Azolla-Anabaena association: Historical perspective, symbiosis and energy metabolism. Bet. Rev. (Lancaster) 54: 353-386.

Smith, K.M., Fowler, G.D., Pullket, S., Graham, N.J.D. (2009). Sewage sludge-based adsorbents: A review of their production properties and use in water treatment applications. Water Research, 43, 2569-2594.

Tanji, Y., Mizoguchi, K., Akitsu, T., Morita, M., Hori, K., Unno, H. (2002). Fate of coliphage in waste water treatment process and detection of phages carrying the Shiga toxin type 2 gene. Water Science and Technology, 46 (11-12), 285-289.

Valderrama, A., Tapia, J., Peñailillo, P., & Carvajal, D.E. (2013). Water phytoremediation of cadmium and copper using *Azolla filiculoides* Lam. in a hydroponic system. Water and Environment Journal, 27, 293-300.

Van Hove, C. (1989). Azolla and its multiple uses with emphasis on Africa. Food and Agriculture Organization, Rome.

Vymazal, J., (2007). Removal of nutrients in various types of constructed wetlands. Sci. Total Environ. 380, 48–65.

Vymazal, J., (2008). Constructed wetlands, surface flow. In: Jørgensen, S.E. (Ed.), Applications in ecological engineering, first ed. Elsevier B.V., Amsterdam, The Netherlands.

Washington State Department of Ecology (2002). Introduction to water quality index. Retrieved from http://www.fotsch.org/WQI.htm

Watanabe, I, C. R. Espinas, N. S. Berja & B. V. Ailmagno. (1977). Utilization of the *Azolla-Anabaena* complex as a nitrogen fertilizer for rice. Intl. Rice Res. Inst. Res. Pap. Ser. 11:1-15.

Watanabe, I, (1982). Azolla-Anabaena symbiosis--Its physiology and use in tropical agriculture. Pages 169-185 in Y. R. Dommergues & H. G. Diem (eds.), Microbiology of tropical soils and plant productivity. Martinus Nijhoff/W. Junk, The Hague.

White, S.A., Cousins, M.M., (2013). Floating treatment wetland aided remediation of nitrogen and phosphorus from simulated stormwater runoff. Ecol. Eng. 61, 207–215.

Xin Zhang, Ai-Jun Lin, Fang-Jie Zhao, Guo-Zhong Xu, Gui-Lan Duan & Yong-Guan Zhu Arsenic accumulation by the aquatic fern Azolla: Comparison of arsenate uptake, speciation and efflux by *A. caroliniana* and *A. filiculoides*. Environ.Poll 156 (2008) 1149– 1155.