USMANU DANFODIYO UNIVERSITY, SOKOTO
(POSTGRADUATE SCHOOL)

WASTEWATER TREATMENT POTENTIALSOF WATER LETTUCE (*Pistia stratiotes* L) AND WATER HYACINTH (*Eichhornia crassipes* Mart. Solms)

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BY

MUHAMMAD,Abdulrashid Haidara
ADM. NO: 14210302005
DEPARTMENT OF BIOLOGICAL SCIENCES

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DEDICATION

This dissertation is dedicated to my parents and those who believe in the oneness of one God.

CERTIFICATION

This dissertation by MUHAMMAD,Abdulrashid Haidara has met the requirements for the award of the Degree of Master ofScience (M.Sc BIOLOGY) of the Usmanu Danfodiyo University,Sokoto, and is approved for its contribution to knowledge.

………………………… Date……………………………
External Examiner

………………………… Date……………………………
Dr. A. Sanda
Major Supervisor

………………………… Date……………………………
Prof. B. L. Aliero
Co- Supervisor I

……………………… Date……………………………
Dr. A.M. Sokoto
Co- Supervisor II

……………………… Date……………………………
Prof. Q. Majeed
Head of Department Biological Sciences

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ABBREVIATIONS

ABS	Absorbance
AD	After Death
$AgSO_4$-H_2SO_4	Silver Sulphate-acid
BOD	Biological Oxygen Demand
C	Value of Water Quality Parameter
CF	Conversion Factor
COD	Chemical Oxygen Demand
CO_2	Carbon Dioxide
CRD	Completely Randomized Design
DF	Dilution Factor
DO	Dissolve Oxygen
H_2SO_4	Sulfuric Acid
$HgSO_4$	Mercury Sulphate
$K_2Cr_2O_7$	Potassium Dichromate
MgO	Magnesium Oxide
$MnSO_4$	Manganese Sulphate
NF	Nano-filtration
NH_3	Ammonia
NO_3^-	Nitrate
pH	Hydrogen Potentials
PO_4^{3-}	Phosphate
RE	Removal Efficiency
RO	Reverse Osmosis

UF	Ultra-filtration
WC	Initial Value of Water Quality Parameter
W.H.O	World Health Organization

ABSTRACT

Effluent from fish farming is increasing over the years since the reduction of capture fish.*Pistia stratiotes* and *Eichhornia crassipes* were used,with the aim of evaluating their potentials in the treatment of aquaculture wastewater. Bothplantswere grown in 5 Liters aquaculture wastewater with 21 days retention period in plastic containers.Fresh weight of *P. stratiotes* and *E. crassipes* 100 g, 150 g, 200 g and 250 g were being weight and transferred to respective containers containing aquaculture wastewater, physicochemical parameters weremeasured at interval of seven days for three weeks. Temperature Reducedue to presence of *P.stratiotes* and *E. crassipes* range from 0.24to 6.66% and 1.62 to 7.48 respectively. pHand NO_3^-reduction for *P. stratiotes* range from 0.43 to 17.54% and 26.20 to 83.93%, PO_4^{3-} and turbidity range from 0.30 to 89.93% and 28.00 to 80.00 respectively. While percentage reduction of pH and NO_3^- due to *E. crassipes* ranges from 0.00 to 27.20% and 26.20 to 87.50%, PO_4^{3-} and turbidity range from 0.30 to 81.87% and 28.00 to 84.00%. Therefore *E. crassipes*is more efficient in reducing ammonia, nitrate and phosphate than *P. stratiotes*. The plants should be compared with submerge macrophytes and usedfor treating other wastewater with longer duration.

CHAPTER ONE

1.0 INTRODUCTION

Wastewater is any water that has been adversely affected in quality by anthropogenic influence. It comprises liquid waste discharged by domestic uses, Industries and agriculture, and can encompass a wide range of potential contaminants and concentrations. In the most common usage it refers to the municipal wastewater that contains a broad spectrum of contaminants resulting from the mixing of wastewater from different sources. Urban wastewater contains 99% water, and other materials make up the remaining portion. The potential pollutants include pathogens, oil and grease, metals, organic matter, solids and nutrients such as nitrogen and phosphorous. The actual proportion of each constituent within any given wastewater varies depending on the spatial and temporal differences (IWMI 2004).

Wastewater is generated every day and this water is mostly not treated before being discharged into water bodies. Discharge of untreated or partially treated wastewater is harmful to the environment. Abdel-Halim *et al*.(2008) noted that about two-thirds of the population in developing countries have no hygienic means of disposing excreta and total wastewater which implies that inadequate sanitation are the prime causes of disease in such countries.Aquatic ecosystems are used either directly or indirectly as recipients of potentially toxic liquids from domestic uses, industries and agricultural wastes (Demirezen *et al*., 2007).

However, demand for fish has been increasingdespite declining ocean fish catches.While capture fisheries fall short of world demand, annual consumption of seafood has been rising, doubling in three decades (FAO, 2000).

Toxic threats to aquatic ecosystem suggest that we can no longer depend solely on capture fishing. Aquaculture provides over a quarter of the world's seafood supply and could rise to 50% by the year 2030 (Tidwell and Allen, 2001).

Aquaculture, the cultivation of freshwater and marine plants and animals, is one of the fastest growing segments ofagriculture. For example from 1987 to 1992, sales of farm-raised fish increased by almost 20% in the United States (Terlizzi *et al*., 1995). Aquaculture industry has grown at an average rate of 8.9% per year since 1970, compared with only 1.2% for capture fisheries and 2.8% for livestock production systems (FAO, 2004). However, the industry places great demands on water resources, and typically requires from 200-600 cm^3 of water for every kilogram of fish produced (Kioussis*et al*.,2000).

Aquaculture systems release large amounts of nutrients into the aquatic ecosystem, in the form of excretory products and excess feed (Zhou *et al*., 2006; Rodrigueza and Montano, 2007; Marinho-Soriano *et al*., 2009). Eutrophication is a general phenomenon in coastal waters, commonly attributed to the increase of shrimp, fish and shellfish aquaculture (Mao *et al*., 2005; Liu *et al*., 2010). This leads to an increase in harmful algal blooms and deterioration of water quality (Rodrigueza and Montano, 2007; Marinho-Soriano *et al*., 2009; Huo*et al*., 2011). Although some aquaculture systems (raceways and pond culture) are much more water consumptive than others (re-circulating systems), the industry generally requires more water per unit area or per unit of product than most other plant or animal production systems (Lawson, 1995). Consequently, aquaculture operations produce large quantities of effluent containing particulate and dissolved organic matter and nutrients that requires treatment and/or disposal.

The production of onetone of channel catfish releases an average of 9.2 kg of nitrogen, 0.57 kg of phosphorus, 22.5 kg of biological oxygen demand and 530 kg of settleable solids into the environment (Schwartz and Boyd, 1994). Therefore, aquaculture effluents exert adverse environmental impacts when discharged to receiving waters. It was believed thatorganic matter loading reduces dissolved oxygen (DO) levels and contributes to the buildup of bottom sediments. High nutrient loading in water stimulates excessive Phytoplankton production (Redding*et al.,* 1997; Adler *et al.,* 2000).

Three different methods have been used for aquaculture production: pond culture, flow through systems, and re-circulating systems. Pond systems, the most widely practiced form of aquaculture in the United States, have been used for the production of different species. Flow-through systems involve the continual flow of water through a tank or raceway. Re-circulating systems aresemi-closed systems, in which water flowing through a series of tanks or raceways is captured, treated, and reused. Re-circulating systems use the least amount of water, which is an advantage in areas with either limited water resources or stringent discharge standards. A high degree of management expertise is needed to manage oxygen levels and water quality in these systems.

A number of chemical, physical and biological methods applied to conventional wastewater treatment have been used in aquaculture systems. Physical and chemical remediation technologies are very expensive systems to create and control (Ruenglertpanyakul*et al.*, 2004). Numerous aquatic macrophytes have demonstrated considerable potential for nutrient removal from various types of wastewaters (Jo *et al*., 2002). The plants enhance wastewater treatment by acting as a medium for bacterial growth, by filtering/adsorbing suspended particulate matter and by removing inorganic nutrients from the wastewater (Sooknah and Wilkie, 2004).

1.1 Statement of the Research Problem

Aquaculture involving fish production has marked transition from a ‘capture’ to a ‘culture’ economy (Van-Rijn, 1996). Despite the huge economic benefits of the industry, its negative impact on the environment and natural resources needed to be managed due to its large volume of effluent that is discharged into water bodies. Deteriorationof water quality has been identified as a cause that escalates disease outbreaks and contamination of aquatic resources resulting in dramatic economic losses and depletion existing flora and fauna (Mazlin *et al*., 2009). The accumulation of feed residue and fish excreta during cultivation often cause water quality deterioration in fishponds, resulting in toxic effects on fishes (Teck *et al*., 2010). To manage these undesirable impacts on the environment there is the need to treat the wastewater scientifically to maintain sustainability becomes paramount.

1.2 Justification

Despite the nutritional and economic importance of aquaculture to humans, itswastewater cannot be allowed untreated using sustainable means. Therefore, an appropriate wastewater treatment process is required for sustaining aquaculture development. Various systems had been introduced for treatment of aquaculture wastewaters such as settling systems, centrifugal systems and mechanical filters (Evans and Furlong, 2003). However, these methods could only achieve partial performance with clear disadvantages of producing considerable sludge deposits, high energy consumption and require frequent maintenance (Nora’aini *et al*., 2005). Sand filtration is also one of the treatments techniques for aquaculture wastewater, but it is effective for suspended solid removal only (Porrello *et al*., 2003). Phytoremediation is widely viewed asecologically responsible alternative to the environmentally destructive physical remediation methods currently practiced, since plants have many endogenous genetic and physiological properties that make them ideal for soil and water remediation (Soudek *et al*., 2007). The use of Plants is considered to be a non-polluting and cost effective way of removing or stabilizing toxic chemicals that might otherwise be leached out of the soil by rain to contaminate nearby watercourses (Meagher, 2000). Hence the use *Pistia stratiotes* Land *Eichhornia crassipes*Mart Solmsto treat wastewater of aquaculture the two macrophytes are locally available.

1.3 Aim and Objectives

1.3.1 Aim

The aim of this research work was wastewater treatment potentials of*P. stratiotes*and *E. crassipes*. The specific objectives are to:

1. Determinelevel of physicochemical parameters of effluent from aquaculture.
2. Evaluate potentials of *P. stratiotes* and *E. crassipes* in moderating the physicochemical properties of wastewater from aquaculture.
3. Evaluate efficient weight of macrophytes for remediation.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Study Background

Water is our most precious natural resource. It is the foundation of rich environmental cycle that is responsible for the great abundance and diversity of lifeon earth (Preetha and Kaladevi, 2014). Water pollution is the introduction of substances whose character and quantity alter the water natural quality and impair its usefulness, and it is offensive to sight, smell, or taste (Jameel, 1998). The rapid expansion and increasing sophistication of the chemical industries in the past century and particularly over the last thirty years indicate that there has been an increase in quantity and complexity of toxic waste effluents.

Freshwaters are perhaps the most vulnerable habitats, and are often changed by the activities of man. This essential resource is becoming increasingly scarce in many parts of the world due to severe impairment of water quality. Chemical analysis of water provides a good indication of the chemical quality of the aquatic system, but does not integrate ecological factors such as altered riparian vegetation or altered flow regime and therefore, does not necessarily reflect the ecological state of the system (Karr and Benke, 2000). With population growth, concerns over providing adequate sanitation and food supply for people will also increase,as thehuman population increases; there is greater competition from domestic, industries and agricultural users for fresh water supplies. W.H.O (2006) estimated that over 40% of the world's population will live in areas where water is scarceby 2056.

Arid and semi arid regions, like the Mediterranean Basin, are struggling to keep up with the demand for fresh water (Oran *etal.,* 2007). Oran *etal.*(2007) recognized that this shortage was linked to agriculture, changes inthe environment and climate. As sources for fresh water supplies are depleting, treating wastewater is becoming necessary for domestic purposes, industries and agriculture. The agricultural industry cannot cope with the increase in demand for food. The Utilization of land and water resources for urban development is increasing and water is becoming the most limiting factor for increasing agricultural production (Angeliaskis *et al.*,1999).

In Greece, approximately 84% of fresh water is used for agricultural irrigation, while 14%is used for domestic purposes and 2% for industrial and other usage (Malaupa *et al.*, 1999). In countries with large populations, such as China fresh water resources are not distributed evenly amongst the people (Chu e*t al.*,2004). This is also the case in most developing countries where the infrastructure as well as environmental factor limits the accessibility of many people to reliable water supply (Jie *et al.*,2007). In some developed countries like Australia, the infrastructure is efficient; however potable water has been unwisely used. Treatment of wastewater is often considered to be the duty of state governments and not individual households (Colebatch, 2006). This need to be change and some ownership should be given to the people, for responsible use and management of freshwater sources,for efficient utilization of water resources (Radcliffe, 2006). Water management should include recycling of effluent. This is currently being practiced in sectors like industry and agriculture.

2.2 Sources and Effects of Water Pollution

The major and the most common source of water pollution is the discharge of effluents from Agriculture, treatment plants, drains and factories; this type of pollution is known as point source discharge. The surprising fact for UK is that more pollution is caused and distributed by agriculture rather than industry (Abel, 2002). The cause of pollution can be accidental, negligence or illegal discharge of polluted wastes to water sources.

Water pollution from such a source decreases with a distance from point of discharge and is easy to monitor and to control. On the other hand, in diffuse source pollution, pollutants enter water from multiple sources such as atmosphere, surface drainage, ground water infiltration, plant nutrients from fertilizers and pesticides. The effects of diffuse source pollution can be severe as it is difficult to control. Most methods are developed to control point source pollution. This pollution depletes the oxygen quantity and affects the temperature and pH levels which must be balanced for survival of aquatic organisms in the environment (Abel, 2002). Thus, pollution can directly influence environmental equilibrium of physical, chemical and biological phenomena. Continual changes and zonation patterns can be observed as an outcome of pollution from various sources.

2.3 Water Pollution Treatment Methods

2.3.1 Coagulation-flocculation

Coagulation and flocculation occur in sequential processes to destabilize the suspended particles, to allow particle collision and to form the growth of flocks. Both processes must be completed to achieve pollution removal (Vesilind *et al*., 2010). Coagulation occurs first to destabilize particles resulting in their sedimentation. The particle size is increased by flocculation of unstable particles into bulky floccules. The main principle in this method is preliminary adjustment of pH and addition of ferric or aluminium salts to overcome repulsion within the colloidal particles. Lime based coagulation has advantages of improved sludge settling, dewatering characteristics, capability of inactivating bacterial activities and sludge stability. The major disadvantages of this technique are high capital outlay and maintenance costs in addition to large quantity of chemicals (Duggal, 2008).

2.3.2 Flotation

This technique is applied in the liquid phase as it uses bubble attachment to separate solids. There are five different types of flotation dispersed air flotation, dissolved air flotation, vacuum air flotation, electro flotation and biological flotation. Dissolved air flotation is the one widely used to remove metals from waster waters (Vesilind *et al*., 2010).

2.3.3 Aeration

Aeration is required when water tables have anoxic conditions caused by activities such as sewage discharges, agricultural run-off or over-baiting a fishing lake. Aeration is performed by infusing air into the bottom of the water levels or by surface agitation creating a foundation device to allow air and oxygen to mix with the water which would release and remove noxious gasses such as CO_2, methane and hydrogen sulphide (Duggal, 2008). Aeration can be used to treat both wastewater and drinking water. Aeration can be categorized into natural, surface and sub-surface aeration. For example, in the time of heavy rain, the river Thames in London has large quantity of sewage inflows which reduced dissolved oxygen levels wherein species have difficulties to survive (Vesilind *et al*., 2010).

2.3.4 Membrane Filtration

This technology is advantageous in terms of no addition of chemicals with a relatively use low energy and involve easy phased conduction process. This technique can help remove dissolved inorganic contaminants such as heavy metals, suspended solids and organic compounds. Depending on the particle size to be removed, many different types of filtration techniques such as ultra-filtration (UF), nano-filtration (NF) and reverse osmosis (RO) can be utilized. UF utilizes 5 to 20 nm permeable membranes according to the compounds to be removed. NF mechanism is based on the strict (sieving) and electrical (Donnan) effects. It functions in such a way that the electrical effect between charged anions in membranes and metal co-ions in pollutants creates the rejection of metal co-ions through membranes and separate them. In RO pressure application retains the heavy metals and pure water is collected on the other side of membrane. Apart from high operating costs, RO is more effective than NF and UF techniques as it removes 97% of metal between 20 to 200 mg/l (Duggal, 2008; Wang *et al*., 2009).

2.3.5 Electrochemical Treatment

This technique utilizes the separation through membrane and ion-exchange as a combination. The main process is that ionized species in the solution are passed through an ion exchange membrane by applying an electric potential. These membranes are made of thin plastic materials and contain anionic or cationic charge. This technique is effective but the major disadvantages are high operational costs, high energy consumption, handling costs for sludge disposal and use of chemicals (Wang *et al.*, 2009).

2.4 Role of Macrophyte in WastewaterTreatment

Macrophytes play important role in balancing Lake Ecosystem. They have been recognized during 1960s and 1970s in water quality improvement (Lu, 2009). Aquatic macrophyte treatment systems for wastewater are the tools for developing countries, because they are cheaper to construct and a little skill is required to operate (Mahmood *et al*., 2005).

They improve the water quality by absorbing nutrients with their effective root system (Dhote and Dixit, 2007). Macrophyte not only retains nutrients by biomass uptake, but also increases sedimentation (Dipu *et al*., 2011). These are utilized for nutrient and metal removal from water in the form of retention pond because of their fast growth rate, simple requirements, and ability to accumulate biogenic elements and toxic substances (Lu, 2009).

However, filamentous algae or macro-algae are also considered as macrophytes since they often form masses in the water that consist of many individuals. They can be found in a wide range of permanent or seasonally permanent aquatic habitats, such as lakes, ponds, rivers, ditches, water damp, and irrigation canals except in shaded water bodies, and steep rivers and streams banks in which there were perceptible water currents. Most of the macrophytes are considered as weeds as they can cause a lot of problem; for examples, being noxious in the rice field, blocking waterways due to heavily infested weed and reducing water quality in water supply. In Nigeria, there is diversity of aquatic plants as the light intensities are high throughout the year. The development of aquatic weeds also is enhanced by eutriphication; an increase in the nutrient content of the water as a consequence of human activities (Pieterse and Murphy, 1989).

According to Pieterse and Murphy (1989), aquatic plants are commonly divided into categories according to their physiology and growth form. There are five main groups:

1) Free floating species–Most of the leaf, stem tissue and roots float above the water surface. Examples are *Eichhornia crassipes* (water hyacinth), *Salvinia molesta, Pistia stratiotes* (water lettuce) and *Azolla spp.*

2) Emergent species-Rooted plants in shallow water with most of their leaf and stem tissue emerge above the water surface. Examples are *Monocharia vaginalis, Nelumbo nucifera, Ipomoea aquatica and Limnocharis flava.*

3) Marginal species–‘Amphibious’ macrophytes. Rooted plants with most of the leaf tissue at the water surface. They can survive on land or water. Sometimes they also can be emergent. Example is *Nymphae pubescens.*

4) Submerged species-Most of the vegetative tissue beneath the water surface; they are rooted in the substrate or attached to the bottom of a water body by root-like organs. Examples are *Hydrilla verticillata* and *Elodea canadensis.*

5) Algae-Unicellular or filamentous lower plants without differentiated tissues, which grow at or below the water surface. Examples are *Spirogyra* sp.

2.5 Origin and Geographical Distribution of *Pistia stratiotes*

*P. stratiotes*commonlyknown as water lettuce is probably native to South America. It has been used in Africa as a medicine and fodder for cattle for centuries being recorded in Egypt in 77 A.D.

2.5.1 Morphology of *P. stratiotes*

P. stratiotes is a free-floating weed.It floats on the surface of the water, and its roots hanging submerged beneath floating leaves (Dipu *et al*., 2011).This plant is a stoloniferous perennial up to 15 cm tall and 30 cm wide, with long, feathery, hanging fibrous roots. Its leaves are obovate (oval); light green, velvety-hairy with many prominent longitudinal-veins (stolons). This plant is a unisexual. Under tropical conditions where plant nutrient levels are adequate, growth is rapid and many new daughter plants are produced on stolons from the parent plants. When bisexual,Inflorescence is axillary, solitary, spathulated with a single pistillate flower at base, and 2-8 staminate flowers above. Staminate flowers have two stamens, pistillate with unilocular ovary having numerous ovules, a slender style and penicillate stigma, the fruit with many thin seeds (Acevedo and Nicolson, 2005). Its seeds germinate on the hydro-soil and float to the surface within 5 days. Germination can also occur in the dark. *P. stratiotes* does not survive freezing temperatures. Germination does not occur below 20°C. It flowers in summer and give fruits at the end of hot season (Chadha, 1998).

Plate1:*Pistia stratiotes*L (Water Lettuce)

2.5.2 Taxonomic Hierarchyof *P. stratiotes*

Taxonomic hierarchy of *P. stratiotes* is similar to *E. crassipes* but they are different in the subclass in which *P. stratiotes* is Aracidae whereas *Eichhornia crassipes* is Liliidae. Nevertheless, both are perennial plants which live for more than two growing seasons (USDA, 2007).

Kingdom	Plantae (plant)
Subkingdom	tracheobionta (vascular)
Division	Magnoliophyta (angiosperm, flowering plant)
Class	Liliopsiada (monocotyledon)
Subclass	Arecidae
Order	Arales
Family	Araceae
Genus	*Pistia*

Species stratiotes

2.5.3 Growth Factors of *P. stratiotes*

P. stratiotes requires very high light intensity. It can endure the temperature of $15^{o}C$ and $35^{o}C$. The optimum temperature for this plant to grow is 22 to $30^{o}C$ (Rivers, 2002; Gopal, 1987).

2.5.4 Effect of Nutrients

According to Pieterse and Murphy (1989). Growth of this plant is fast and also viable to nutrients availability. The more nutrients available the larger individual plants will grow before they multiply.

2.5.5 Effect of pH

P.stratiotes prefers to grow in slightly acidic to neutral water and optimum productivity occurs at pH 6.5 to 7.2 (Gopal, 1987).

2.6 Origin and Geographical Distribution of *Eichhornia crassipes* Mart Solms.

It is widely reported that water hyacinth is indigenous to Brazil having first been described from wild plants collected from Francisco rive in 1824.This tropical plant spread throughout the world in late 19th and early 20^{th} century (Wilson *et al.,*2005). In Africa it was first reported in Egypt around 1879; in Asia around 1888 and about 1900 in Japan; in Australia it arrived in about 1890 (Cook, 1990). Water hyacinth originated intropical South America, but has become naturalized in many warm areas of the world: Central America,North America (California and southern states), Africa, India, Asia, Australia, and New Zealand. Water hyacinth is the most predominant, persistent and troublesome aquatic weed in India. It was first introduced as an ornamental plant in India in 1896 from Brazil (Rao, 1988). In India, water hyacinth has stretched over 2,00,000 ha of water surface in the country (Murugesan *et* *al.*, 2005) and its exuberance has been highly notice throughout the course of the river Thamirabarani, a perennial river in south India (Murugesan, 2002). Because of its beautiful blooms and foliage, water hyacinth has been carried by tourists, plant collectors and botanists to over 80 countries around the world in the last 100 years.

2.6.1 Morphology of *Eichhornia crassipes*

E. crassipes is a free-floating aquatic weed (Reddy and Sutton, 1984). One single plant consists of a rhizomatous stem, a rosette of leaves and numerous adventitious roots. The stem or the rhizome consists of an axis with several short internodes. The nodes bear the leaves, roots, offshoots, and inflorescences. The rhizomes grow up to 5-6 cm in diameter and up to 30 cm in length whilst the shoots are monopodial and whorl leaves are produced. Occasionally, internodes are produced and grow horizontally in open water condition, in contrast, in crowded condition and adequate nutrients are available; the elongated internodes grow vertically and bear offshoots at the distal end. These elongated internodes are stolons and when it holds leaves it is also called petioles.

In crowded conditions, the petioles can elongate exceeding a meter in length and the leaves become very large; on the other hand, the petioles become swollen, bulbous and spongy in the more open water. The spongy petioles act as 'floats' for this plant. The stolons are purplish violet and exhibit a wide range of size, extending up to 50 cm in length and are similarly variable in diameter. For germination, *Eichhornia crassipes* seeds require warm, shallow water and high light intensities. They have ability for rapid vegetative reproduction, which enables them to cover an available water surface in a short period of time (Dar *et al.*, 2011). As they are floating and unattached, these plants are independent of water depth. They have very short life cycle and grow up through vegetative. Their growth rates are usually very high. It has a doubling time of about 13 days to 20 days.

Plate 2:*Eichhornia crassipes* (Water hyacinth)

2.6.2 Taxonomic Hierarchy of *Eichhornia crassipes*

E. crassipes is classified as vascular and monocotyledonous plant. This plant is in the family Pontederiaceae among other species such of *Heteranthera sp., Monocharia sp*., *Pontederia sp.* (pickerelweed) and *Reussia sp.* (USDA, 2007).

Kingdom	Plantae (plant)
Subkingdom	tracheobionta (vascular)
Division	Magnoliophyta (angiosperm, flowering plant)
Class	Liliopsiada (monocotyledon)
Subclass	Liliidae
Order	Liliales
Family	pontederiaceae
Genus	*Eichhornia*
Species	*Eichhornia crassipes* Mart, Solms

2.6.3 Growth Factors of *E.crassipes*

*E. crassipes*is a heliophyte plant growing best in warm waters rich in macronutrients (Center *et al.*, 2002).The optimal water temperature for growth is 28-30°C (Center *et al.*, 2002). Temperatures above 33°C slow further growth (Center *et al.*, 2002).

2.6.4 Effect of pH

The Optimal water pH for growth of this plant is neutral but it can tolerate pH values from 4 to 10 (Center *et al.*, 2002). Due this range because inpH *Eichhornia crassipes* can be used for treatment of different types of wastewater

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The experiment was conducted at Biological Garden, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. Sokoto State is located between Latitudes 11^o 30N and 13^o 50N and Longitudes 4^o 0E and 6^o 0E it is 315m above sea level. Sokoto falls in the Sudan savanna agro-ecological zone of Nigeria (Ojanuga, 2005).Itis characterized by erratic and scanty rainfall that last for about four months (Mid June- September) and dry period (October- May). The annual rainfall of the area is highly variable over the years and averaged around 700mm (Singh, 1995) with minimum and maximum temperatures of the year fluctuating between 15 and 40^{o}C, respectively.

3.2 Collection of the Aquatic Macrophytes

The Macrophytes used in this study are *Pistia stratiotes* and *Eichhornia cressipes*. *P. stratiotes*was collected from Kware Lake,while*E. cressipes*was collected from Sokoto River, along Usmanu Danfodiyo University main campus road. The macrophytes werewashed thoroughly to remove sand and other debris, and then transported to the Biological Garden Usmanu Danfodiyo University, Sokoto. The collected samples were kept inplastic containers (30cm diameter and 40cm height) of about 30L, containing water from thenatural habitat ofthe macrophytes for one week before the commencement of the experiment. Identification of the plants wasauthenticated at the Department herbarium.

3.3 Collection of WastewaterSamples

Raw aquaculture wastewater was collected from Premier fish farm, in Wamako Local Government Area of Sokoto State.The farm is located along Usmanu DanfodiyoUniversity, Sokoto, main Campus Road, and is 350 m away from Bilya Sanda gate of the University.The farm is a commercial fish farm rearing twotypes of fish species, namely Cat fish and Tilapia fish.The fishes were reared in concrete ponds, and the ponds are of different dimensions. The raw aquaculture wastewater collected for the purpose of the experiment was stored at room temperature in accordance with the standard procedure. One hundred and fifty (150)liter aquaculture wastewater was collected from the farm pond for the purpose of the experiment.A grab sample for qualitative analysis to determine the physicochemicalparameters was used. The parameters analyzed include; Turbidity,Temperature,pH,Dissolved Oxygen (DO), Biological Oxygen Demand (BOD),Chemical Oxygen Demand (COD), Phosphate (PO_4^{3-}), Nitrate (NO_3^-),and Ammonia (NH_3). Concentrations of these parameters were determined within 24 hours after sample collection.

3.4 Experimental Design

Hydroponics (water culture) systems, consisting of plastic growth containers (10cm diameter and 16cm height) containing five (5) liter of raw wastewater was set up in a Completely Randomized Design (CRD)along with control 4×3. Healthy *Pistia stratiotes* and *Eichhornia cressipes*of size(9 cm and 20 cm) was selected for the treatment of wastewater. From the plant samples100g, 150g, 200g, 250g, of the plants material was Weighed using weighing balance(Harvard trip balance) after been blotted out using blotter (15 minutes)and transferred in the respective containers containing the aquaculture wastewater except for the control.

3.5 Determination of water parameters

The parameters mentioned (3.3) wereinvestigated in the aquaculture wastewater and the procedures were in accordance with the APHA (2005)standards methods. The analysis was carried out on weekly basis; samples from the twenty seven (27) growth containers were collected to determine their status of physicochemical parameters.

3.6 Physicochemical Analysis of Effluents

3.6.1 Determination of Temperature

Temperature was determined using mercury in glass thermometer, it was inserted into the effluent sample and the reading was recorded.

3.6.2 Determination of pH

The pH of the effluent sample was determined using a pH meter (3015 Jenway) in accordance with the manufacturer's instructions guidelines. pHvalue indicates the acidic, neutral or alkaline nature of a liquid. The electrode of the pH meter was placed into the sample. The pH was recorded when the pointer was steady.

3.6.3 Determination of Dissolved Oxygen (DO)

The amount of Dissolved Oxygen in effluent sample wasdetermined by Winkler method. Oxygen bottle was filled with 1m each of Winkler's A $MnSO_4$ and Winkler's B (Alkaline-Iodine azide).The sample bottle wasclosed carefully toexclude air bubbles and mixed by inverting bottle three times. Allow the precipitate formed to settle half way.The precipitate formed was dissolved in the laboratory using 1ml of concentrated H_2SO_4. From this solution, 20ml was titrated against 0.025M Sodium thiosulphate solution using three drops of 1% starch indicator solution. The dissolved oxygen was calculated from the formula (Ademoroti, 1996).

$$\text{Dissolved Oxygen (mg/l)} = \frac{\text{ml of titrant x Normality of titrant x 8000}}{\text{ml of sample}}$$

3.6.4 Determination of Biological Oxygen Demand (BOD)

The effluent sample was diluted with 1ml distilled water; Biological Oxygen Demand was determined using Winkler's technique also. BOD bottle was filled with samples and tied in black polythene bags and kept in the dark to prevent light penetration. Samples were fixed five (5) days after collection at 30˚C before titration. The titrimetric method was used the same as in dissolved oxygen. Biological oxygen demand was calculated from the formula (Ademoroti, 1996).

$$\text{BOD (mg/l)} = \frac{(\text{DO}_1 - \text{DO}_5)}{\text{percentdilution}}$$

Where:

DO_1 = Dissolved oxygen first day.

DO_5 = Dissolved oxygen after 5 days.

3.6.5 Determination of Chemical Oxygen Demand (COD)

The amount of Chemical Oxygen Demand was determined using Titrimetric method. 50 ml of effluent sample was measured using measuring cylinder and was transferred in a 250 ml conical flask, 10 ml of 0.00833M potassium dichromate ($K_2Cr_2O_7$) solution was added. 1 g of $HgSO_4$ and 80 ml of silver sulphate-acid ($AgSO_4$-H_2SO_4) solution was added. The sample containing the mixture was refluxed for 10 minutesand then allowed to cool rinse the condenser with 50 ml of distilled water and cool the flask under running tap water. Two drops of ferroin indicator solution was added and titrate with 0.025M ferrous ammonium sulphate solution till colour changes from blue-green to red-brown (Udo and Ogunwale, 1986).

3.6.6 Determination of Nitrate

Distillation method was used,50 ml of effluent sample was transferred in to Microkjeldh flask. 0.2 g MgO and 0.4 g Divada alloy was added and mounts on a distillation apparatus,10 ml of Boric acid indicator was placed in a conical flask under the condenser and distillation was commence. 20 ml of the distillate was titrated against 0.01M of H_2SO_4 when the colour changesfrom green to pink record the titer value. The following expression was used (Udo and Ogunwale, 1986).

NO_3^-(mg/l) = $\frac{TV \times 100}{50}$

3.6.7 Determination of Ammonia

Fifty 50 ml of effluent sample was measured using measuring cylinder and transferred into a Microkjelh flask. 0.2g of MgO was added and mountson a distillation apparatus, 10 ml of Boric acid indicator was placed in a conical flask and commence distillation. 20 ml of the distillate was titrated against 0.01M of H_2SO_4 when the colour changes from green to pink (Udo and Ogunwale, 1986).

3.6.8 Determination of Phosphate

This was determined by Olean method. 2 ml of water sample was measured and transferred into a 50 ml volumetric flask, 2 ml each phosphorus extraction solution and ammonium molybdate solution was added and the content was shaken well. The volume wasmade to about 48 ml with distilled water and 1 ml of freshly diluted stannous chloride solution was added and mixed immediately. After 5-6 minutes the colour intensity was measured using 660µmwavelength.The following expression was used to calculate phosphate (Udo and Ogunwale, 1986).

$PO_4^{3-} = (mg/l)\,\frac{ABS \times CF \times DF}{\text{atomic of P}}$

Where:

ABS= Absorbance

CF= Conversion Factor

DF= Dilution Factor

P= Atomic number of phosphorus

3.6.9 Determination of Turbidity

Turbidity was measured using digital turbidity meter model (Hach 2100p) according to manufacturers' specification.

3.7 Bioremediation Efficiency of Test Macrophyte on Nutrients Removal

Measurement of each parameter was taken at the beginning of the experiment (day 0) and at the end of the experiment. The equation by Kadlec and Knight(1996)below was used to determine the removal efficiency (%) on day based on the replicates averages of 100% of the analysis.

$$RE = \left(\frac{WC - C}{WC}\right) \times 100$$

Where:

RE = Removal efficiency (%)

WC = Initial Value of Water Quality Parameter (day 0)

C = Value of Water Quality Parameter on day 7, 14 and 21

3.8 Statistical Analysis

Data was subjected to the analysis of variance (ANOVA) usingGraph pad prism software version 5.02. Difference between means was evaluated using LSD at5%.

CHAPTER FOUR

4.0 RESULTS

4.1 Initial Values of Wastewater Quality Parameters of Aquaculture Collected

The parameters of the initial raw aquaculture wastewaternamely; Biological oxygen demand, ammonia and phosphate values were 18.20 mg/l, 5.00 mg/l and 3.31 mg/l. while the values of temperature, turbidity and pH are 29 ^oC , 25 NTU and 7.60 (Table1).

Table 1:Initial Values of Wastewater Quality Parameters of Aquaculture Collected

Parameters	Values	Unit
Temperature	29	ºC
pH	7.60	-
DO	4.10	mg/l
BOD	18.20	mg/l
COD	11.60	mg/l
Nitrate	5.60	mg/l
Ammonia	5	mg/l
Phosphate	3.31	mg/l
Turbidity	25	NTU

Note: NTU Nepholometric Turbidity Units

4.2 Physicochemical Parameters of Aquaculture Wastewater Treated with *Pistia stratiotes*

The mean values of aquaculture wastewater treated with water lettuce were presented in Table 2 and had maximum temperature of 30.00±0.00°C in control and minimum of 27.07±0.06°C in 200g. ThepH of 8.10±0.05 was the highest and 7.43±0.18 was the lowest. The value of 6.43±0.03 mg/l and 12.17±0.33 mg/l are for dissolve oxygen and biological oxygen demand. 3.40±0.06 mg/l and 0.73±0.15 mg/lare the values of nitrate and ammonia. Phosphate recorded 2.10±0.06 mg/land 14.00±0.58 NTUfor turbidity.

Table 2: Physicochemical Parameters of Aquaculture Wastewater Treated withPistia stratiotes

Parameters	Days	Treatments				
		Control	100 g	150 g	200 g	250 g
Temperature	7	30.00±0.00^a	28.93±0.06^a	28.07±0.52^b	27.77±0.62^c	27.07±0.06^d
	14	29.97±0.03^a	28.33±0.33^b	28.13±0.13^c	27.73±0.37^d	27.07±0.07^e
	21	28.37±0.12^a	28.03±0.09^a	27.77±0.28^a	27.47±0.29^a	27.40±0.31^a
pH	7	7.63±0.06^a	7.56±0.12^a	8.10±0.11^a	7.60±0.11^a	7.43±0.18^a
	14	7.37±0.19^a	7.20±0.06^a	7.07±0.07^a	7.37±0.23^a	6.s47±0.26^b
	21	7.17±0.17^a	6.37±0.09^a	6.27±0.27^a	6.97±0.54^a	6.37±0.27^a
DO	7	8.40±0.60^a	5.77±0.43^b	4.93±0.15^c	5.07±0.06^d	4.10±0.06^e
	14	6.43±0.03^a	3.20±0.12^b	3.67±0.20^c	2.73±0.37^d	2.70±0.36^e
	21	5.57±0.35^a	2.97±0.03^b	2.57±0.35^c	2.53±0.18^d	2.07±0.09^e
BOD	7	14.27±0.77^a	12.77±0.61^a	12.70±0.17^a	12.40±0.53^a	11.27±0.64^b
	14	10.23±0.15^a	12.17±0.33^b	11.73±0.27^a	10.80±0.23^a	9.33±0.67^a
	21	9.20±0.12^a	8.10±0.06^a	6.97±0.73^b	6.03±0.09^c	5.03±0.50^d
COD	7	11.23±0.50^a	10.37±0.19^a	10.07±0.07^b	9.90±0.45^c	9.20±0.15^d
	14	9.73±0.19^a	9.23±0.15^a	8.33±0.24^b	7.07±0.07^c	6.63±0.32^d
	21	9.30±0.15^a	7.90±0.21^b	6.30±0.15^c	5.70±0.25^d	4.37±0.23^e
Nitrate	7	4.13±0.24^a	3.57±0.23^a	2.27±1.05^a	3.40±0.20^a	2.90±0.60^a
	14	3.27±0.22^a	2.73±0.15^b	2.90±0.06^a	2.53±0.24^a	1.77±0.19^c
	21	3.40±0.06^a	1.57±0.23^b	1.23±0.28^c	1.17±0.03^d	0.90±0.15^e
Ammonia	7	4.60±0.16^a	2.90±0.21^b	3.40±0.23^c	2.40±0.23^d	2.13±0.13^e
	14	4.27±0.15^a	2.50±0.36^b	2.67±0.28^c	2.10±0.06^d	2.03±0.03^e
	21	4.23±0.15^a	1.80±0.35^b	1.30±0.15^c	1.77±0.12^d	0.73±0.15^e
Phosphate	7	3.18±0.09^a	3.30±0.06^a	3.00±0.03^a	2.50±0.15^b	2.10±0.06^c
	14	2.13±0.09^a	2.37±0.22^a	2.27±0.15^a	2.17±0.12^a	1.57±0.09^a
	21	2.10±0.06^a	1.57±0.09^b	1.23±0.15^c	0.80±0.12^d	0.33±0.09^e
Turbidity	7	12.00±1.16^a	9.00±.1.73^a	7.33±2.03^a	6.33±1.86^a	9.67±1.45^a
	14	18.00±2.00^a	7.00±2.00^b	6.33±0.88^c	8.00±1.53^d	8.00±1.53^e
	21	14.00±0.58^a	6.67±1.76^b	6.33±1.86^c	5.33±0.33^d	5.00±0.00^e

Note: Means followed by same superscript on same row are not significant at P<0.5. All units are in mg/l except Temperature ^oC and Turbidity NTU. DO - Dissolve Oxygen, BOD - Biological Oxygen Demand and COD - Chemical Oxygen Demand

4.3 Physicochemical Parameters of Aquaculture Wastewater Treated with*Eichhornia crassipes*

The values of aquaculture wastewater treated with water hyacinth (Table 3) from the results temperature had 30±0.00 and 27.50±0.25^{0}C as the maximum and minimum. The mean reduction of pH anddissolve oxygenhad 7.63±0.07 and 3.20±0.12 mg/l, while 8.23±0.09 and 6.03±0.33 mg/l are the values of biological oxygen demand and chemical oxygen demand. The mean value of phosphate recorded was 0.60±0.23 mg/l, while Turbidity of 4.00±0.58 NTU was obtained.

Table 3: Physicochemical Parameters of Aquaculture Wastewater Treated with *Eichhornia crassipes*

Parameters	Days	Treatments				
		Control	100 g	150 g	200 g	250 g
Temperature	7	30 ± 0.00^{a}	28.23 ± 0.28^{b}	28.53 ± 0.26^{c}	27.53 ± 0.29^{c}	27.50 ± 0.25^{e}
	14	29.97 ± 0.03^{a}	27.33 ± 0.18^{b}	27.23 ± 0.12^{c}	27.10 ± 0.10^{d}	27.00 ± 0.46^{e}
	21	28.37 ± 0.12^{a}	27.80 ± 0.61^{a}	27.17 ± 0.46^{a}	26.83 ± 0.27^{a}	26.93 ± 0.18^{a}
pH	7	7.63 ± 0.07^{a}	7.10 ± 0.06^{a}	7.60 ± 0.23^{a}	7.23 ± 0.15^{a}	7.40 ± 0.20^{a}
	14	7.37 ± 0.19^{a}	7.20 ± 0.12^{a}	7.07 ± 0.07^{a}	6.30 ± 0.15^{b}	6.10 ± 0.06^{c}
	21	7.17 ± 0.17^{a}	6.53 ± 0.24^{a}	6.93 ± 0.18^{a}	5.53 ± 0.24^{b}	6.47 ± 0.29^{a}
DO	7	8.40 ± 0.62^{a}	4.31 ± 0.25^{b}	3.57 ± 0.28^{c}	3.33 ± 0.12^{d}	3.20 ± 0.12^{e}
	14	6.43 ± 0.03^{a}	4.07 ± 0.07^{b}	3.63 ± 0.32^{c}	2.63 ± 0.22^{d}	2.03 ± 0.03^{e}
	21	5.57 ± 0.35^{a}	3.63 ± 0.32^{b}	3.13 ± 0.19^{c}	2.17 ± 0.12^{d}	1.53 ± 0.24^{e}
BOD	7	14.27 ± 0.77^{a}	11.63 ± 0.34^{b}	11.50 ± 0.29^{c}	10.73 ± 0.18^{d}	10.37 ± 0.20^{e}
	14	10.23 ± 0.15^{a}	9.73 ± 0.37^{a}	9.40 ± 0.40^{a}	9.13 ± 0.13^{a}	8.23 ± 0.09^{b}
	21	9.20 ± 0.12^{a}	8.47 ± 0.29^{a}	7.23 ± 0.15^{b}	6.07 ± 0.66^{c}	4.23 ± 0.15^{d}
COD	7	11.23 ± 0.15^{a}	10.17 ± 0.12^{a}	9.67 ± 0.24^{b}	9.83 ± 0.34^{c}	8.37 ± 0.27^{d}
	14	9.37 ± 0.19^{a}	9.10 ± 0.06^{a}	8.23 ± 0.12^{b}	6.97 ± 0.03^{c}	6.03 ± 0.33^{d}
	21	9.30 ± 0.15^{a}	7.47 ± 0.29^{b}	6.57 ± 0.35^{c}	5.80 ± 0.50^{d}	3.83 ± 0.44^{e}
Nitrate	7	4.13 ± 0.24^{a}	4.03 ± 0.09^{a}	3.07 ± 0.07^{b}	2.63 ± 0.09^{c}	2.40 ± 0.31^{d}
	14	3.27 ± 0.22^{a}	1.53 ± 0.18^{b}	1.20 ± 0.12^{c}	1.27 ± 0.18^{d}	1.20 ± 0.12^{e}
	21	3.40 ± 0.06^{a}	1.27 ± 0.18^{b}	0.63 ± 0.19^{c}	0.83 ± 0.34^{d}	0.70 ± 0.6^{e}
Ammonia	7	4.60 ± 0.16^{a}	3.23 ± 0.15^{b}	3.23 ± 0.12^{c}	2.97 ± 0.03^{d}	2.77 ± 0.09^{e}
	14	4.27 ± 0.15^{a}	2.60 ± 0.31^{b}	2.23 ± 0.12^{c}	2.33 ± 0.24^{d}	1.67 ± 0.18^{e}
	21	4.23 ± 0.15^{a}	1.50 ± 0.25^{b}	1.33 ± 0.18^{c}	1.30 ± 0.26^{d}	0.70 ± 0.15^{e}
Phosphate	7	3.18 ± 0.09^{a}	3.27 ± 0.04^{a}	3.30 ± 0.15^{a}	2.20 ± 0.12^{b}	2.00 ± 0.00^{c}
	14	2.13 ± 0.09^{a}	2.23 ± 0.04^{a}	2.27 ± 0.13^{a}	1.87 ± 0.09^{a}	1.33 ± 0.24^{b}
	21	2.10 ± 0.06^{a}	1.77 ± 0.15^{a}	1.10 ± 0.32^{b}	1.73 ± 0.09^{a}	0.60 ± 0.23^{c}
Turbidity	7	12.00 ± 1.16^{a}	6.67 ± 1.67^{a}	8.00 ± 1.53^{a}	7.67 ± 2.67^{a}	11.33 ± 3.33^{a}
	14	18.00 ± 2.00^{a}	5.33 ± 1.33^{b}	5.67 ± 0.08^{c}	7.67 ± 1.20^{d}	7.00 ± 0.00^{e}
	21	14.00 ± 0.58^{a}	4.67 ± 0.33^{b}	4.00 ± 0.58^{c}	5.00 ± 0.00^{d}	7.00 ± 1.53^{e}

Note: Means followed by same superscript on same row are not significant at $P<0.5$. All units are in mg/l except Temperature °C and Turbidity NTU. DO - Dissolve Oxygen, BOD - Biological Oxygen Demand and COD - Chemical Oxygen Demand

4.4 Removal Efficiency (%) of*Pistia stratiotes* and *Eichhornia crassipes* in Temperature, DO and BOD.

The resultanteffect of*Pistia stratiotes*and *Eichhornia crassipes* in physicochemical parameters of aquaculture wastewater over the duration of 21days was presented (Table 4). The results have revealed the changes in temperature of *P. stratiotes*and *E. crassipes* was 5.25 and 7.14 while DO was 49.29% and 62.61% respectively for *P. stratiotes*and *E. crassipes*. From the results it shows that *E. crassipes*was more efficient in reducing the parameters.Trend in pH removal after 7 days (Figure 1) *E. crassipes* performed better than *P. stratiotes.* There was increase in pH in treatment with *P. stratiotes.* At 150 g, 14 days 200 g and 250 g of *E. crassipes* has the highest removal (Figure 2). *P. stratiotes* has the lowest at 200 g of days 21 (Figure 3).

Table 4: Removal Efficiency (%) of *Pistia stratiotes* and *Eichhornia crassipes* in Temperature, Dissolve Oxygen and Biological Oxygen Demand

Parameters	Days	Plants	Removal Efficiency (%)				
			Treatments				
			Control	100 g	150 g	200 g	250 g
Temperature		*P. stratiotes*					
	7		-3.45	0.24	3.21	4.24	6.66
	14		3.34	2.31	3.00	4.38	6.66
	21		2.17	3.34	4.24	5.28	5.52
		E. crassipes					
	7		-3.45	2.66	1.62	5.07	5.17
	14		3.34	5.76	6.10	6.55	6.90
	21		2.17	4.14	6.31	7.48	7.14
Dissolve Oxygen		*P. stratiotes*					
	7		-104.8	-40.66	-20.32	-23.59	0.00
	14		-5.90	21.95	10.56	33.34	34.15
	21		-35.78	27.63	37.39	38.22	49.59
		E. crassipes					
	7		104.88	-5.05	13.00	18.71	21.95
	14		-56.90	0.80	11.39	35.78	50.41
	21		-52.02	11.39	23.59	47.15	62.61
Biological Oxygen		*P. stratiotes*					
Demand	7		21.59	29.84	30.22	31.87	38.08
	14		43.79	33.13	35.55	40.66	48.72
	21		49.45	55.49	61.72	66.85	72.35
		E. crassipes					
	7		21.59	36.10	36.81	41.04	43.02
	14		43.79	46.52	48.35	49.82	54.76
	21		49.45	53.48	60.26	66.66	76.74

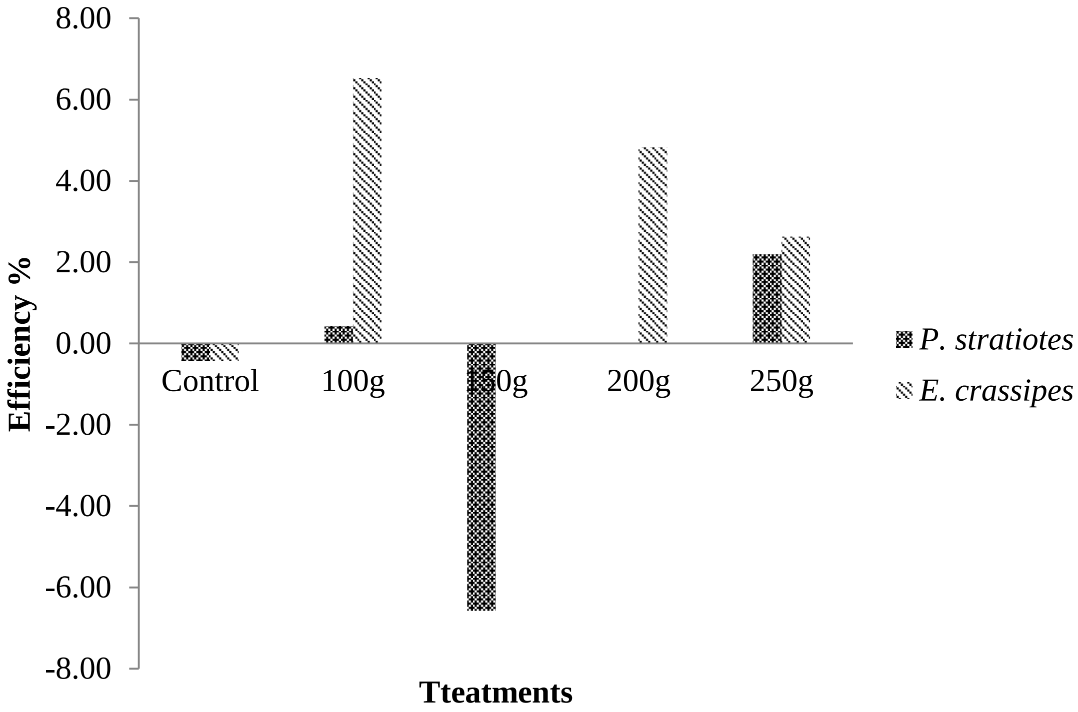

Figure 1: pH Removal (%) of *Pistia stratiotes and Eichhornia crassipes*for 7 Days.

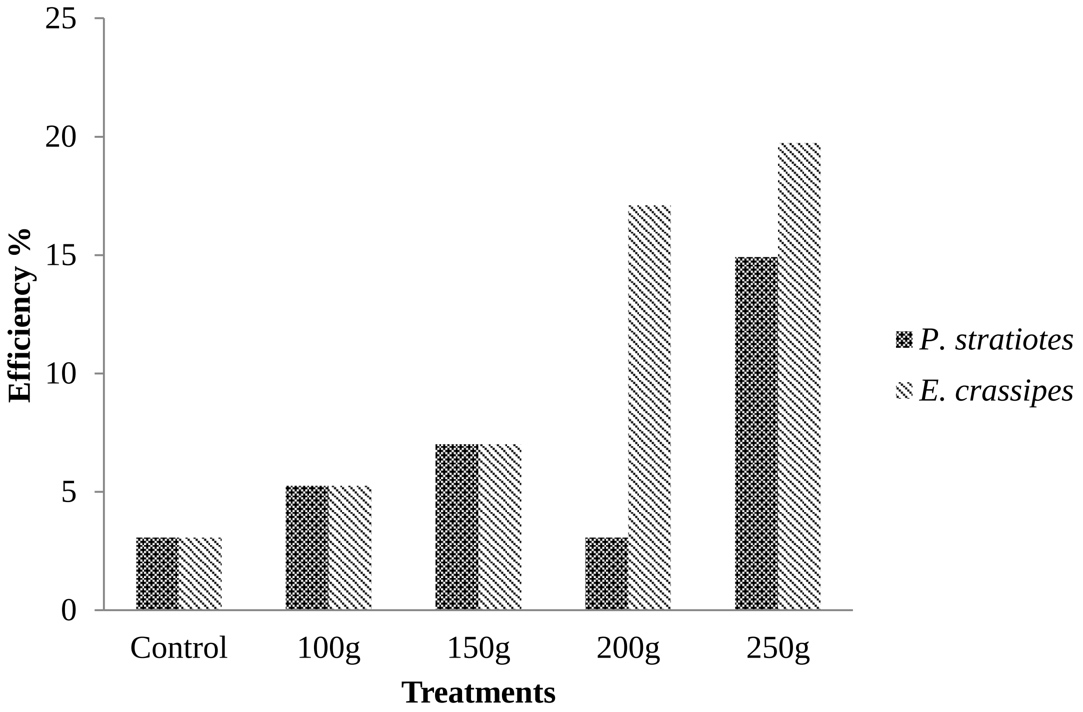

Figure 2: pH Removal (%) of *Pistia stratiotes and Eichhornia crassipes*for 14 Days.

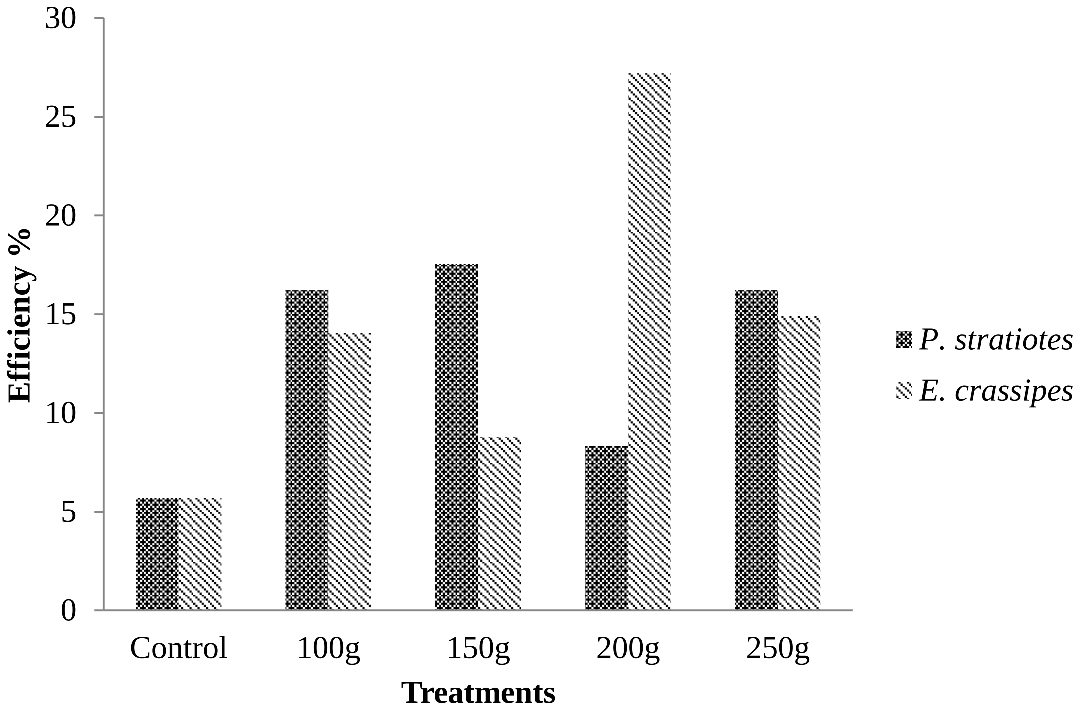

Figure 3: pH Removal (%) of *Pistia stratiotes and Eichhornia crassipes*for 21 Days.

4.5 Removal Efficiency (%) of *Pistia stratiotes* and *Eichhornia crassipes*in Chemical Oxygen Demand, Phosphate and Turbidity

The effect of *Pistia stratiotes* and *Eichhornia crassipes*in pollutant removal of aquaculture wastewater treated with*P. stratiotes* and *E. crassipes*(Table 5) from the results it has chemical oxygen demand of 31.90 and 35.63% for*P. stratiotes* and *E. crassipes*, while removal in phosphate was81.87 was the highest for*E. crassipes*. Turbidity removal was 84 and 80% respectively for *P. stratiotes* and *E. crassipes*. The removal of NO_3^- was illustrated in (Figure 4) for 7 days *P. stratiotes* has the highest. *E. crassipes* has reduced pollutant load in all the treatment (Figure 5). The removal of NO_3^- after 21 days was in (Figure 6). NH_3 removal has highest at 250 g of *P. stratiotes* flowed by 200 g, the efficiency of *P. stratiotes*and *E. crassipes*after 14 and 12 days were illustrated in Figure 8 and 9.

Table 5:Removal Efficiency (%) of *Pistia stratiotes* and *Eichhornia crassipes* in Chemical Oxygen Demand, Phosphate and Turbidity

Removal Efficiency (%) Parameters	Days	Plants	Treatments				
			Control	100 g	150 g	200 g	250 g
Chemical Oxygen Demand		*P. stratiotes*					
	7		3.19	10.60	13.19	14.66	20.69
	14		19.25	20.41	28.16	39.08	42.82
	21		19.83	31.90	45.69	50.86	62.35
		E. crassipes					
	7		3.19	12.33	16.66	15.23	27.87
	14		19.25	21.55	29.03	3.94	47.9
	21		19.83	35.63	43.30	50.00	66.96
		P. stratiotes					
Phosphate	7		3.32	0.30	9.37	24.47	36.56
	14		35.56	28.49	31.51	34.53	52.66
	21		36.56	52.66	62.75	75.83	89.93
		E. crassipes					
	7		3.84	1.21	0.30	33.53	39.58
	14		35.56	32.72	31.51	43.60	59.73
	21		36.56	46.62	66.77	47.64	81.87
Turbidity		*P. stratiotes*					
	7		52.00	64.00	70.67	74.67	61.33
	14		28.00	72.00	74.67	68.00	68.00
	21		44.00	73.33	74.67	78.67	80.00
		E. crassipes					
	7		52.00	73.33	68.00	69.33	54.68
	14		28.00	78.67	77.33	69.33	72.00
	21		44.00	81.33	84.00	80.00	72.00

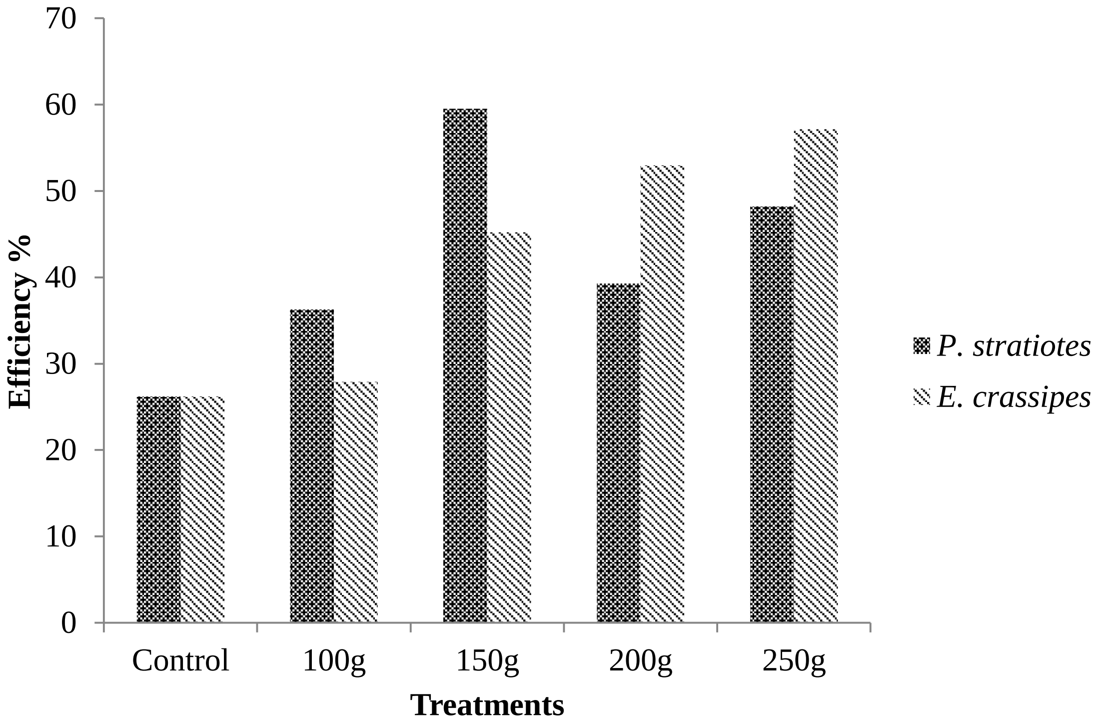

Figure 4: NO^-_3 Removal (%) of*Pistia stratiotes* and *Eichhornia crassipes*for 7 Days.

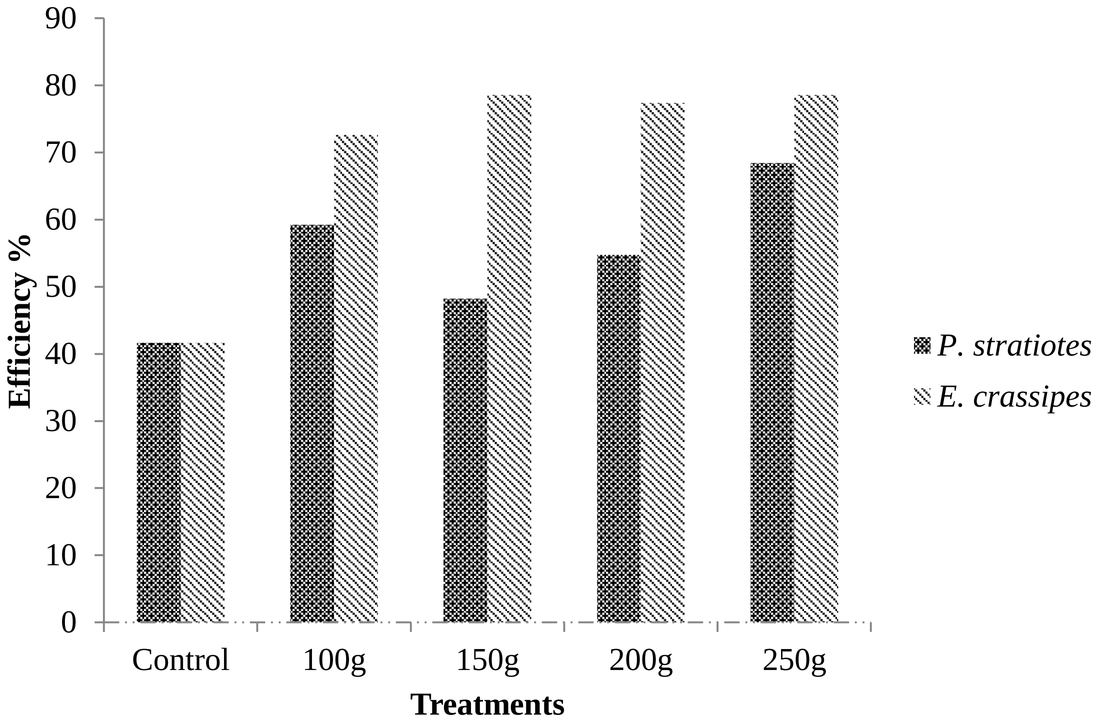

Figure 5: NO^{-}_{3} Removal (%) of *Pistia stratiotes* and *Eichhornia crassipes*for 14 Days.

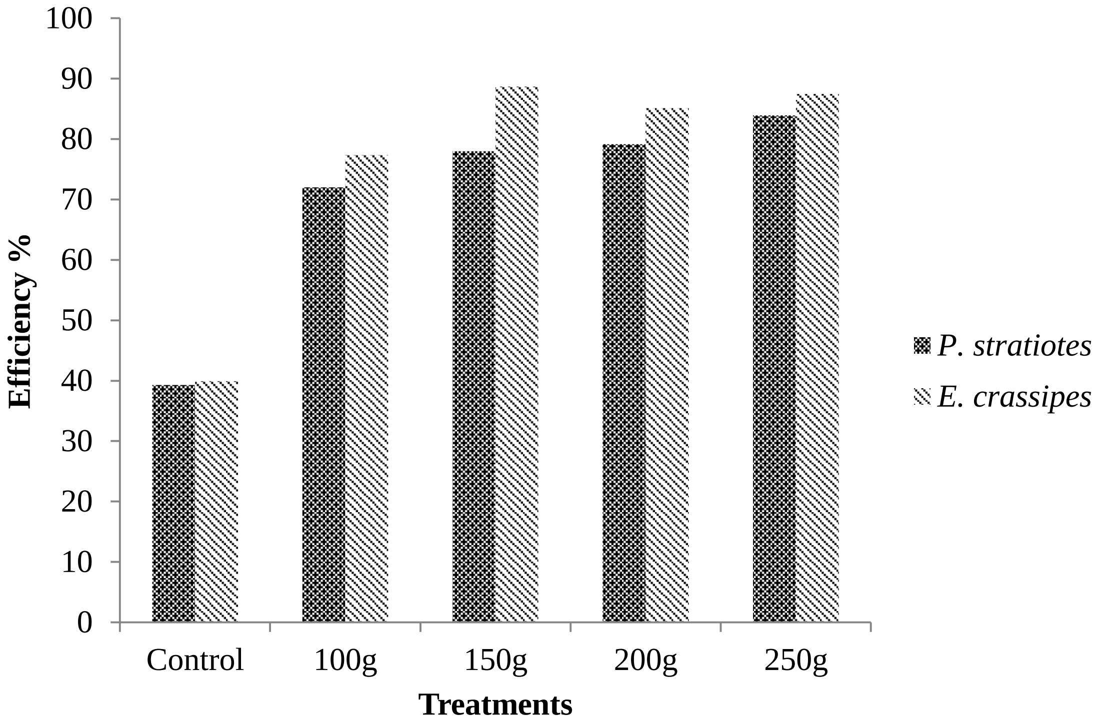

Figure 6: NO^{-}_{3} Removal (%) of *Pistia stratiotes* and *Eichhornia crassipes*for 21 Days.

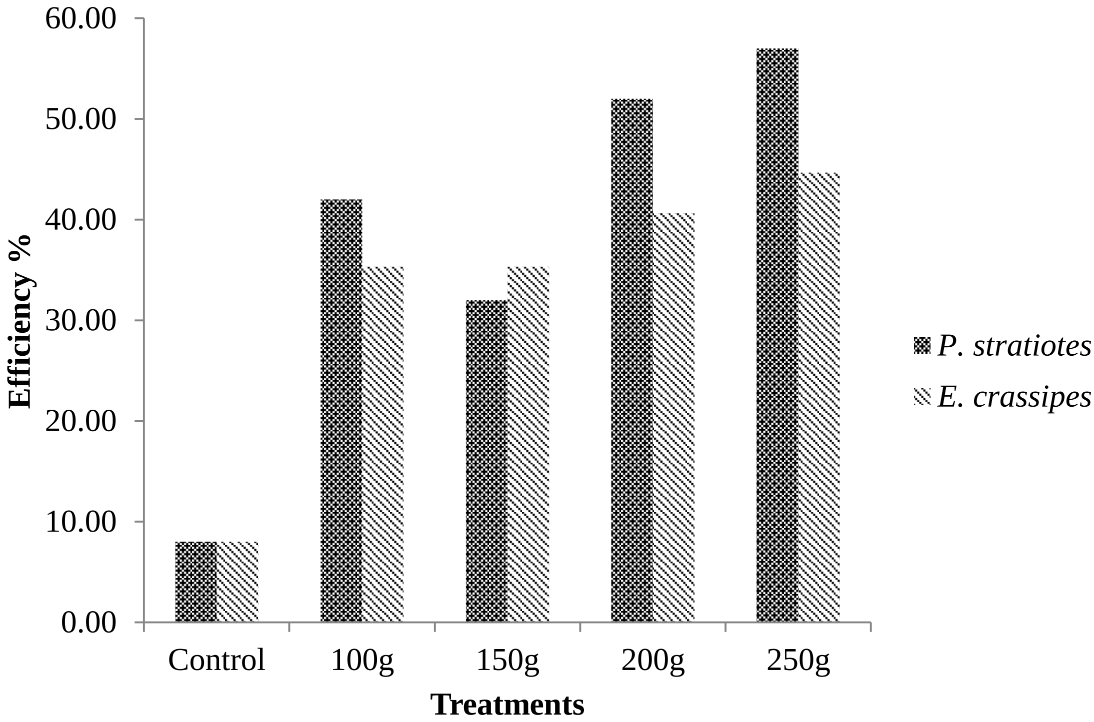

Figure 7: NH_3 Removal (%) of *Pistia stratiotes* and *Eichhornia crassipes*for 7 Days.

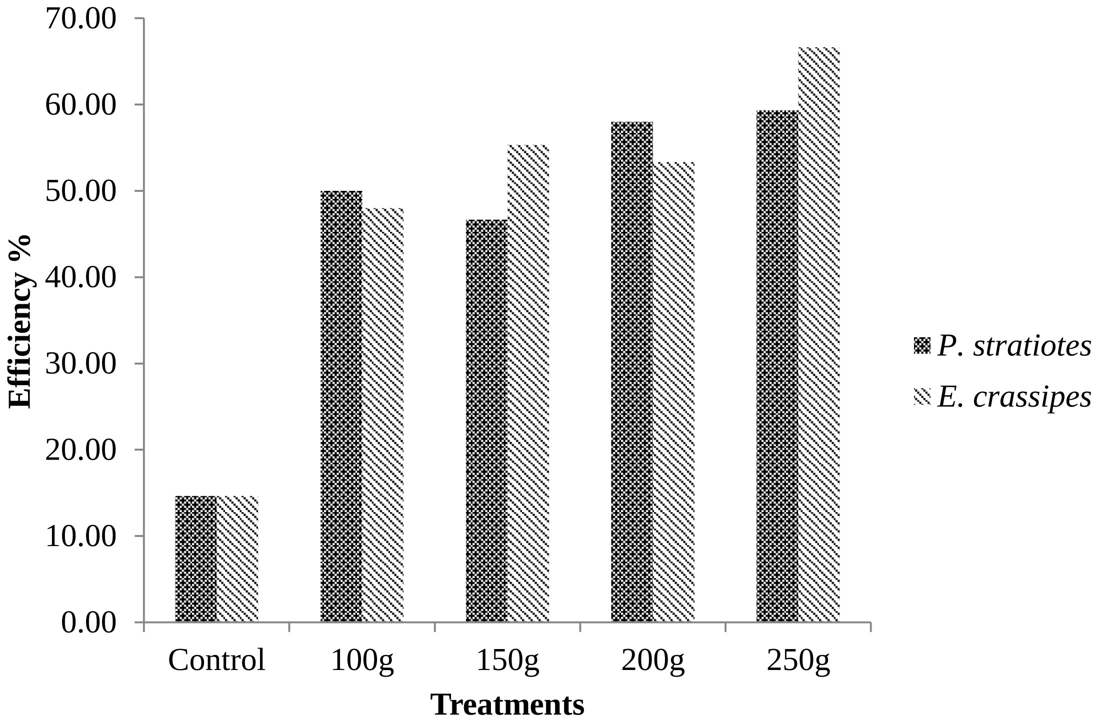

Figure 8: NH_3 Removal (%) of *Pistia stratiotes* and *Eichhornia crassipes*for 14 Days.

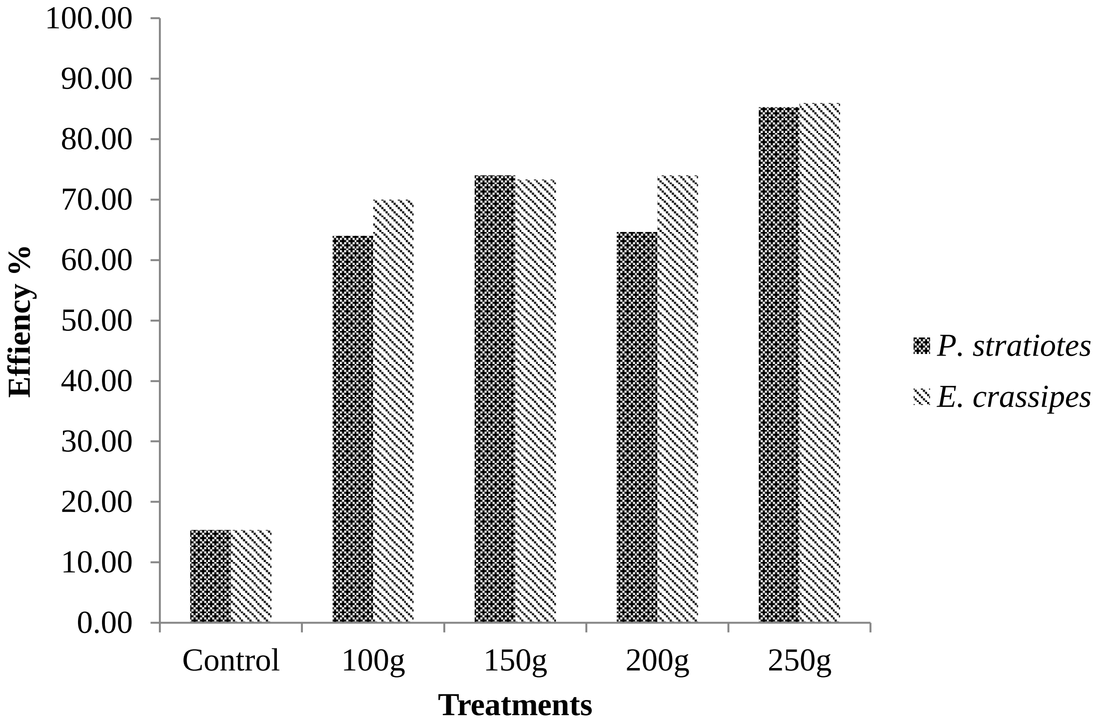

Figure 9: NH_3 Removal (%) of *Pistia stratiotes* and *Eichhornia crassipes*for 21 Days.

CHAPTER FIVE

5.0Discussion

The temperature valuesfor the wastewater treated were almost similar in both the treatments throughoutweeksand it ranged from 30.00±0.00 to 27.07±0.06^{0}Cin treatment with *Pistia stratiotes*, while the range for the treatment with *Eichhorniacrassipes*was from 30±0.00 to 27.50±0.25^{0}C.This may not be unconnected with the fact that the plant has not fully adopted in the wastewater.This was close to the finding of Akinbile and Yusoff(2012) who work on aquaculture wastewater and reported temperature range of 25.0^{0}C to 35.0^{0}C.

There was an observed increase in reduction inweight over period of time.It wasalso established that reduction tends to increase in both plants *P. stratiotes* and *E. crassipes* with increase in surface cover of the vegetative part of the plant used. Another factor may be due tofluctuation in temperature of the site. Sokoto is generally known for high temperature. The result obtained was close to that of Oladipupo *et al*.(2015) they reported percentage reduction in temperatureof 17.97%.

The pHvalue of raw aquaculture wastewater was 7.60,the mean values of 8.10±0.11and 6.47±0.26 were recorded.This result finding agrees with the work of Adeniran *et al*.(2012) whoreported a pH of 7.76 in wastewater treated with *Typha latifolia*, and that ofRabiei *et al*.(2014)whorecorded mean pH of 8.7±0.3 using *Ulva reticulata* at 12 day retention time.Fluctuation in pH of the growth medium may be caused by the uptake of cation and anions by the root system of developing plants(Kanabkaew and Puetpaiboon, 2004;Sooknah and Wilkie, 2004).The change recorded for pH in treatment with *Pistiastratiotes* and *Eichhornia crassipes*in this research coincided with that ofRuby *et al*.(2013).They noted that there was a pH reduction of 25.46% in mono culture (polluted water and*Eichhornia* species) and 29.1% in mix culture (polluted water, *Eichhornia* species and *Ceratophyllum* species).This result is contrary to that of Ugya *et al*.(2015) whotreated refinery wastewater with *Lemnaminor*, *E. crassipes* and *P. stratiotes* at differentconcentration(100and75%wastewater) and recorded 1% reduction.

The initial Dissolve Oxygen value for raw wastewater was 4.10 mg/l. The value increased in wastewater treated with *P. stratiotes* and *E. crassipes*. In the treatment with*P. stratiotes*, the DO value decreased in the second and third weeks. The increase could be attributed to the introduction of the plants that release oxygen during photosynthesis and wind that is blowing.Ugya and Imam(2015) reported increase in DO from 0.2 to 0.5 mg/l while treating refinery wastewater with *E.crassipes*. Adelere *et al*.(2014) observed mean DO of 3.14±0.19.Gupta *et al*. (2012) suggested various contaminants like TDS, EC, BOD, COD, DO have been minimized by using *E. crassipes*.

The results of efficiency in DO for*P. stratiotes*and *E.crassipes*in this research concur with that of Oladipupo*et al*. (2015) theyreported percentage reduction in DO of 57.88%.Ugya and Imam (2015) also reported 50% efficiency in point A and 10% efficiency in point B.

With regard to BOD initial value was 18.27 mg/land this has been reducedin treatment with *P. stratiotes*and *E. crassipes*. The efficiency of the plants may be as result of microbial fauna attached to the root of the plants and that of the collected wastewater which could have used it in breaking down the organic matter. The results of this finding followed the same patternwith that of Adeniran*et al*.(2012) were theyobtained mean values for Biological Oxygen Demand of 54.54mg/l and

12.80mg/l at influent and effluent respectively. The result of Pha and Tap (2016) who work on dormitory and aquaculture wastewater reported initial BOD of 302.7mg/l and 79.16 mg/l with meanreduction of 118.21±0.028and 53.74±0.06for *Eichhornia crassipes*after two week.

Furthermore, the percentage efficiency inlevel of BOD for *Pistia stratiotes*and *E. crassipes*from this work indicated that the higher the weight and duration, the higher the percentage efficiency for all the test plants.This result was similar to that of Tolu and Atoke (2012) that worked with *E. crassipes* for the treatment of different wastewater and obtained 66.98%, 73.33% and 52.94% for textile wastewater, metallurgical wastewater and pharmaceutical respectively. The result of Theophile *et al*. (2002) that work with *P. stratiotes* for treatment of domestic sewage also obtained 86.0%. While Shah *et al*. (2015) reported performance of three macrophytes 50.61%, 33.43% and 33.43% for*E. crassipes,Lemma minor and P. stratiotes*.

The initial concentration of Chemical Oxygen Demand for wastewater was 11.60mg/l. However, this finding indicated that*P. stratiotes*and *E. crassipes* hadreduced COD and the process may be as a result of the symbiotic relationship between plants roots and microorganism in the wastewater. The result of this work tally with the work done by Snow and Ghaly (2008) on comparative purification of aquaculture wastewater using three macrophytes and reportedeffluentmean reduction of 34.70±0.60, 16.60±1.0, 27.70±1.0 and 24.70mg/l at 6 day retention time for control, *E. crassipes*, *P. stratiotes* and*Myriophyllum aquaticum*. Efficiency of *P. stratiotes* and *E. crassipes*in COD for this studyagree with that of Akinbile and Yusoff (2008) that reported percentage reduction in container with *E.crassipes* (non aeration and aeration) of 59.02%and 58.86%, while that *Pistia stratiotes* (non aeration and aeration) of 53.82% and 56.91%.The Chemical Oxygen Demand decreased over time during the growth period this could be attributed to crop root that were fully developed and absorption of dissolved nutrients (Ghaly *et al*., 2005).

Concentration of nitrate was 5.60 mg/l. The mean nitrate for treatment with *P. stratiotes*and container with *Eichhornia crassipes* are reduced and may benitrite was oxidized by nitrifying bacteria. Finding of this research was close to that of Henry-Silva and Camargo (2006) that treat Niletilapia pond effluent and obtained mean reduction of21.8±10.1, 25.1±11.6 and 48.4±21.5mg/lfor*E. crassipes*,*P. stratiotes* and*Salvinia molesta*.

Efficiency of *P. stratiotes*and*E. crassipes*shows that the plants may have assimilate the nitrate for their metabolic process and this finding correspond with that of Awuah *et al*.(2004) that evaluated the potential of water lettuce for pollutant removal from a low-strength, anaerobically treated domestic sewage and reported NO_3^- reductions of 70% after 6 months of operation.

The initialconcentration of ammonia was 5.00 mg/l.*P. stratiotes*provided the highest mean reduction followed *E. crassipes*.The results of this research agrees with that of Snow and Ghaly(2008) that recorded mean reduction of 1.38±0.11, 0.54±0.06, 0.67±0.21 and 0.75±0.25mg/l for control, *E. crassipes*, *P. stratiotes*and *Myriophyllum aquaticum*at 6 day retention time respectively.

The efficiency level of ammonia in container with *P. stratiotes*and*E*. crassipes obtained conformto that of Vaillet *et al*.(2003) that evaluate effectiveness of *Datura innoxia* plant for domestic wastewater purification and reported NH_3level in effluent of 93% after 48 hours of treatment.

Aquaculture wastewatercontained 3.31 mg/lphosphate (PO_4^{3-}).The mean level of phosphate in treatment container with *Pistia stratiotes*and *Eichhornia crassipes* were reduced and this may be due to assimilation by the root of the plants.This result agrees with that ofAkinbile and Yusoff (2012) that reported reduction of phosphate for *E. crassipes* container (non aeration and aeration) reduced to8.33 and 10.67 mg/l respectively. However, Phosphate acquired by roots is rapidly loaded into the xylem and it moves upward in shoots where it is unloaded into growing sinks (Lambers and Colmer, 2005).

The reduction of phosphate in container with *P. stratiotes*and *E. crassipes*obtained in this research tally with that of Ghaly *et al*. (2005)that examined the use of hydroponically grown Barley and Oats for the removal of phosphate from aquaculture wastewater and reported phosphate reduction of 93.60% and 91.40% for compartment containing Barley and Oats after 21 days.

Turbidity was found to be 25.00 NTU from the wastewater, there was reduction inturbidity of 18.00±2.00 and 7.00±1.53 NTU for container with *P. stratiotes* and *E.crassipes*. This reduction may be as a result of the particles being attached to the root of plants and whichsettled down the container. This work conformsto that of Oladipupo*et al*.(2015) that recorded 61.00 NTU at retention time of 10 days.

The efficiency of*P. stratiotes*and*E. crassipes*from this result finding agrees with that of Dhote(2007) that recorded percentage reduction of 20 and 25% NTU for *Hydriilaverticillata*and *E. crassipes*.

5.1 Conclusion

The study provides information on use of *Pistia stratiotes*and*Eichhornia crassipes*as bio-treatment agents to reduce physicochemicalparameters of aquaculture wastewater. Their ability in reducing nutrients content in aquaculture wastewater was clearly established during the experiment. Most of the effluent quality parameters; such as temperature, pH, dissolved oxygen, biological and chemical oxygen demand, ammonia, nitrate, phosphate and turbiditywere reduced after 21 days retention period.

This study also shows that *E. crassipes* was more efficient in reduction of nutrients especially those involved in eutriphication such as ammonia, nitrateand phosphate concentration in wastewater and other parameter monitored compared to *P. stratiotes*eventhrough all the treatmentsware statistically significant at $P<0.05$. However, the presences of both macrophytes in wastewater treatment were beneficial as they can easily be set up, managed and areeconomical. However, theiruse require proper estimate of effluent concentration, volume and retention time.

5.2 Recommendations

Based on the findings from this study, the following recommendations are made:

- The plants have the potentials of treating other wastewater like domestic and effluent from industries.
- The plants should be compared withsubmerge macrophytes.
- Fast growing plant samples that allow rapid absorption of nutrients in the wastewater are preferable.
- For efficient remediation the plant should be harvested periodically to reduce the rate at which dead litter fall back and decay in the water.
- During treatment the plants should cover 70-75 percent surface area of the water.

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APPENDICES

Appendix 7.1: Summary of one way ANOVA for water lettuce according to Weeks.
Week one

Temperature

ANOVA Table	SS	df	MS	F	P-Value
Treatment	15.39	4	3.847	9.601	0.0019
Residual	4.007	10	0.4007		
Total	19.39	14			

pH

ANOVA Table	SS	df	MS	F	P-value
Treatment	0.7733	4	0.1933	4.603	0.0229
Residual	0.4200	10	0.04200		
Total	1.193	14			

DO

ANOVA Table	SS	df	MS	F	P-Value
Treatment	32.50	4	8.124	14.40	0.0004
Residual	5.640	10	0.5640		
Total	38.14	14			

BOD

ANOVA Table	SS	df	MS	F	p-Value
Treatment	13.80	4	3.451	3.437	0.0516
Residual	10.04	10	1.004		
Total	23.84	14			

COD

ANOVA Table	SS	df	MS	F	P-Value
Treatment	6.577	4	1.644	9.560	0.0019
Residual	1.720	10	0.1720		
Total	8.297	14			

Nitrate

ANOVA Tables	SS	df	MS	F	P-Value
Treatment	5.977	4	1.494	1.987	0.1726
Residual	7.520	10	0.7520		
Total	13.50	14			

Ammonia

ANOVA Table	SS	df	MS	F	P-Value
Treatment	11.41	4	2.853	24.43	< 0.0001
Residual	1.167	10	0.1167		
Total	12.58	14			

Phosphate

ANOVA Table	SS	df	MS	F	P-Value
Treatment	3.086	4	0.7715	32.37	< 0.0001
Residual	0.2383	10	0.02383		
Total	3.324	14			

Turbidity

ANOVA Table	SS	df	MS	F	P-Value
Treatment	57.73	4	14.43	1.718	0.2220
Residual	84.00	10	8.400		
Total	141.7	14			

Week two water lettuce

Temperature

ANOVA Table	SS	df	MS	F	P-Value
Treatment	13.90	4	3.476	21.28	< 0.0001
Residual	1.633	10	0.1633		
Total	15.54	14			

pH

ANOVA Table	SS	df	MS	F	P-Value
Treatment	1.663	4	0.4157	4.213	0.0296
Residual	0.9867	10	0.09867		
Total	2.649	14			

DO

ANOVA Table	SS	df	MS	F	P-Value
Treatment	28.94	4	7.234	37.29	< 0.0001
Residual	1.940	10	0.1940		
Total	30.88	14			

BOD

ANOVA Table	SS	df	MS	F	P-Value
Treatment	15.59	4	3.898	9.310	0.0021
Residual	4.187	10	0.4187		
Total	19.78	14			

COD

ANOVA Table	SS	df	MS	F	P-Value
Treatment	18.48	4	4.619	35.17	< 0.0001

Residual	1.313	10	0.1313		
Total	19.79	14			

Nitrate

ANOVA Table	SS	df	MS	F	P-Value
Treatment	3.991	4	0.9977	10.11	0.0015
Residual	0.9867	10	0.09867		
Total	4.977	14			

Ammonia

ANOVA Table	SS	df	MS	F	P-Value
Treatment	9.897	4	2.474	17.42	0.0002
Residual	1.420	10	0.1420		
Total	11.32	14			

Phosphate

ANOVA Table	SS	df	MS	F	P-Value
Treatment	1.167	4	0.2917	4.916	0.0188
Residual	0.5933	10	0.05933		
Total	1.760	14			

Turbidity

ANOVA Table	SS	df	MS	F	P-Value
Treatment	279.1	4	69.77	8.469	0.0028
Residual	80.67	10	8.067		
Total	359.7	14			

Week three water lettuce

Temperature

ANOVA Table	SS	df	MS	F	P-Value
Treatment	1.943	4	0.4857	2.879	0.0797
Residual	1.687	10	0.1687		
Total	3.629	14			

pH

ANOVA Table	SS	df	MS	F	P-Value
Treatment	2.016	4	0.5040	1.791	0.2071
Residual	2.813	10	0.2813		
Total	4.829	14			

DO

ANOVA Table	SS	df	MS	F	P-Value
Treatment	23.30	4	5.826	34.40	< 0.0001
Residual	1.693	10	0.1693		
Total	25.00	14			

BOD

ANOVA Table	SS	df	MS	F	P-Value
Treatment	32.49	4	8.123	16.65	0.0002
Residual	4.880	10	0.4880		
Total	37.37	14			

COD

ANOVA Table	SS	df	MS	F	P-Value
Treatment	44.41	4	11.10	89.06	< 0.0001
Residual	1.247	10	0.1247		
Total	45.66	14			

Nitrate

ANOVA Table	SS	df	MS	F	P-Value
Treatment	12.12	4	3.029	30.91	< 0.0001
Residual	0.9800	10	0.0980		
Total	13.10	14			

Ammonia

ANOVA Table	SS	df	MS	F	P-Value
Treatment	21.51	4	5.378	44.82	< 0.0001
Residual	1.200	10	0.1200		
Total	22.71	14			

Phosphate

ANOVA Table	SS	df	MS	F	P-Value
Treatment	5.569	4	1.392	43.51	< 0.0001
Residual	0.3200	10	0.0320		
Total	5.889	14			

Turbidity

ANOVA Table	SS	df	MS	F	P-Value
Treatment	165.7	4	41.43	9.865	0.0017
Residual	42.00	10	4.200		
Total	207.7	14			

Week one water hyacinth

Temperature

ANOVA Table	SS	df	MS	F	P-Value
Treatment	12.48	4	3.119	16.42	0.0002

Residual	1.900	10	0.1900		
Total	14.38	14			

pH

ANOVA Table	SS	df	MS	F	P-Value
Treatment	0.6360	4	0.1590	2.168	0.1464
Residual	0.7333	10	0.07333		
Total	1.369	14			

DO

ANOVA Table	SS	df	MS	F	P-Value
Treatment	57.45	4	14.36	42.68	< 0.0001
Residual	3.366	10	0.3366		
Total	60.82	14			

BOD

ANOVA Table	SS	df	MS	F	P-Value
Treatment	28.03	4	7.008	13.56	0.0005
Residual	5.167	10	0.5167		
Total	33.20	14			

COD

ANOVA Table	SS	df	MS	F	P-Value
Treatment	12.74	4	3.186	18.81	0.0001
Residual	1.693	10	0.1693		
Total	14.44	14			

Nitrate

ANOVA Table	SS	df	MS	F	P-Value
Treatment	7.591	4	1.898	18.48	0.0001
Residual	1.027	10	0.1027		
Total	8.617	14			

Ammonia

ANOVA Table	SS	df	MS	F	P-Value
Treatment	6.229	4	1.557	36.43	< 0.0001
Residual	0.4275	10	0.04275		
Total	6.657	14			

Phosphate

ANOVA Table	SS	df	MS	F	P-Value
Treatment	4.852	4	1.213	43.47	< 0.0001
Residual	0.2791	10	0.02791		
Total	5.131	14			

Turbidity

ANOVA Table	SS	df	MS	F	P-Value
Treatment	67.73	4	16.93	1.144	0.3905
Residual	148.0	10	14.80		
Total	215.7	14			

Week two water hyacinth

Temperature

ANOVA Table	SS	df	MS	F	P-Value
Treatment	19.01	4	4.752	29.34	< 0.0001
Residual	1.620	10	0.1620		
Total	20.63	14			

pH

ANOVA Table	SS	df	MS	F	P-Value
Treatment	3.876	4	0.9690	20.47	< 0.0001
Residual	0.4733	10	0.04733		
Total	4.349	14			

DO

ANOVA Table	SS	df	MS	F	P-Value
Treatment	34.52	4	8.631	92.24	< 0.0001
Residual	0.9333	10	0.09333		
Total	35.46	14			

BOD

ANOVA Table	SS	df	MS	F	P-Value
Treatment	6.671	4	1.668	8.069	0.0036
Residual	2.067	10	0.2067		
Total	8.737	14			

COD

ANOVA Table	SS	df	MS	F	P-Value
Treatment	24.15	4	6.037	62.46	< 0.0001

Residual	0.9667	10	0.09667		
Total	25.12	14			

Nitrate

ANOVA Table	SS	df	MS	F	P-Value
Treatment	9.509	4	2.377	28.99	< 0.0001
Residual	0.8200	10	0.0820		
Total	10.33	14			

Ammonia

ANOVA Table	SS	df	MS	F	P-Value
Treatment	11.56	4	2.889	22.11	< 0.0001
Residual	1.307	10	0.1307		
Total	12.86	14			

Phosphate

ANOVA Table	SS	df	MS	F	P-Value
Treatment	1.789	4	0.4474	8.062	0.0036
Residual	0.5549	10	0.05549		
Total	2.344	14			

Turbidity

ANOVA Table	SS	df	MS	F	P-Value
Treatment	332.9	4	83.23	17.34	0.0002
Residual	48.00	10	4.800		
Total	380.9	14			

Week three water hyacinth

Temperature

ANOVA Table	SS	df	MS	F	P-Value
Treatment	5.057	4	1.264	2.977	0.0737
Residual	4.247	10	0.4247		
Total	9.304	14			

pH

ANOVA Table	SS	df	MS	F	P-Value
Treatment	4.696	4	1.174	7.558	0.0045
Residual	1.553	10	0.1553		
Total	6.249	14			

DO

ANOVA Table	SS	df	MS	F	P-Value
Treatment	28.92	4	7.229	36.63	< 0.0001
Residual	1.973	10	0.1973		
Total	30.89	14			

BOD

ANOVA Table	SS	df	MS	F	P-Value
Treatment	46.69	4	11.67	34.06	< 0.0001
Residual	3.427	10	0.3427		
Total	50.12	14			

COD

ANOVA Table	SS	df	MS	F	P-Value
Treatment	49.01	4	12.25	30.18	< 0.0001
Residual	4.060	10	0.4060		
Total	53.07	14			

Nitrate

ANOVA Table	SS	df	MS	F	P-Value
Treatment	16.23	4	4.058	36.24	< 0.0001
Residual	1.120	10	0.1120		
Total	17.35	14			

Ammonia

ANOVA Table	SS	df	MS	F	P-Value
Treatment	23.06	4	5.766	46.01	< 0.0001
Residual	1.253	10	0.1253		
Total	24.32	14			

Phosphate

ANOVA Table	SS	df	MS	F	P-Value
Treatment	4.343	4	1.086	9.579	0.0019
Residual	1.133	10	0.1133		
Total	5.476	14			

Turbidity

ANOVA Table	SS	df	MS	F	P-Value
Treatment	202.3	4	50.57	27.09	< 0.0001
Residual	18.67	10	1.867		
Total	220.9	14			