Palestine Polytechnic University

College of Engineering

Treatment of Domestic Wastewater Using Duckweed

(*Lemna gibba*)

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Submitted to the College of Engineering
In partial fulfillment of the requirements for the
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Palestine Polytechnic University

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Mechanical Engineering Department

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Chair of the Department Signature

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DEDICATION

This study is dedicated to our university … our respected tutors … our parents …our Family For Constant Love and Support … Everyone who contributed to this Project … With respect and love

To all who stood by us and walked us through this long journey

No words can describe how grateful and thankful we are for God's precious gift of such a supportive families.

We dedicate this little effort.

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ABSTRACT

In Palestine, the generation of wastewater becomes a serious problem that needs to be solved in a sanitary way. Most of the generated wastewater in Palestine is either dumped in unsealed cesspits as first choice or – in “good” cases – collected then dumped in open wades. Both ways lead to many environmental problems, e.g. bad odors emissions, insects’ proliferation, pollution of both surface and groundwater bodies, diseases spreading, and soil contamination. Despite the fact that wastewater itself should be considered a source of fresh water, especially in a semi-arid country under occupation like Palestine, a one well-known method to minimize the pollutants in wastewater is to apply Duckweed treatment system. In this project indoor and outdoor containers were used, each was filled to a certain height with domestic wastewater (not sewage) from Beit Alsharq restaurant and covered with the DW species (*Lemna gibba*). This experiment was conducted to evaluate the performance of Duckweed (*Lemna gibba*) as a tool to reduce the amount of pollutants in the domestic wastewater by using low cost and simple technology and to use the harvested DW as a fodder for chicks, in order to apply minimum waste technology to reach sustainable development. The removal efficiency of indoor Duckweed container for BOD, COD and NH_4^+-N was found to be about 41.1%, 75.3%, 65.3% respectively. While the removal efficiency of outdoor Duckweed container for BOD, COD and NH_4^+-N was found to be about 80.7%, 92.4%, 98.6% respectively. It was found that the chicks fed with the mixture of dried DW and ordinary fodder (ratio 1:1) have grown faster than those fed with only ordinary fodder. Based on these simple preliminary results, it was concluded that the use of DW to treat wastewater was very effective, and breeding the chicks under ideal conditions and fed them with the mixture of harvested DW and fodder could lead to better chicks growth, and all of which achieve sustainable development.

List of Content

List of Figures

List of Tables

List of Abbreviations

BOD: biological oxygen demand

C3 plants: are those which fix and reduce inorganic CO_2 into organic compounds using only the C3 pathway in photosynthesis

Cl^-:Chloride

COD: Chemical Oxygen Demand

DO: Dissolved Oxygen

DS: dray solid

DW: duckweed

DWT : duckweed based wastewater treatment

EC: Electric Conductivity

ETE: environmental technology engineering

L. gibba:Lemna gibba

N: nitrogen

NH_4^+-N: Ammonium

PPF: µmol/m^2·sec

RGR: relative growth rate

S: Salinity

TSS : total suspended solid

TC: Total Coliforms

TDS: Total Dissolved Solids

TS: Total Solid

WW: wastewater

CHAPTER ONE

Introduction

1.1. Introduction

Wastewater (WW) is the combination of liquid and carried waste residues in the water produced from sanitary, domestic, commercial and industrial facilities [1, 2]. Untreated WW generally contains high levels of organic compound, pathogenic microorganisms, and toxic compounds. It thus entails health hazards, environmental and socioeconomic risks [2].

WW treatment and reuse represent an important option to meet the raise in water shortage around the world. In developing countries, good management for water use and handling is absent, because of limited awareness, high-cost of sophisticated treatment technologies, all this resulting to not taking advantage of WW reuse [2].

Sewage disposal is an important environmental issue, because when WW flows in wadies, its contents will accumulate in soil and create big environmental problems; especially when seeps into groundwater aquifers, which are considered the main source of freshwater supply in Palestine [2]. Methods for disposal of WW in Palestine are traditional, including mainly unsealed cesspits and random dumping it in wadies [2].

Increasing quantities of WW disposal, due to high population growth and needs, becomes a serious problem as urbanized life style demands high water consumption [2]. Globalization adds new chemicals and hazardous materials to the WW, hence increasing environmental deterioration [2].

Duckweed (DW) species *Lemna gibba* is a free-floating aquatic algae, simplest flowering plant and has one of the fastest reproduction rates that can completely cover the surface of a pond [3]. In general, it is small, green with a leaf-like frond of few millimeters wide and a short root less than one centimeter long, it can be used to treat WW under variety of climatic conditions [3].

This project was conducted to evaluate the performance of DW *Lemna gibba* as a tool to reduce the amount of pollutants in the WW and the usability of the treated effluent in irrigation, as well as to use the harvested dried DW as chicken fodder.

This project is a step forward to implement a future sustainable strategy aims to divert waste to a valued source added to the environment.

WW treatment in a semi-arid land like Palestine becomes inevitable option as water resources are nearing depletion. Random disposal of WW adds increasing negative impacts on all environmental elements (soil, air, water, biodiversity and human beings) as pollutants infiltrate into the ground are potentially damaging all earth's spheres, e.g. the atmosphere, the lithosphere, the hydrosphere and the biosphere [2].

- **Advantages**

The advantages of using DW to purify WW are many, among them are:

1) This technology proved to be inexpensive and cost - effective to construct and operate, as well as easy to implement [4].
2) DW is a prolific plant, especially in nitrogen-rich environments, and can be easily used as mulch or a natural soil organic enrichment/conditioner [5].
3) High production rate and easiness to harvest manually from the surface [4].
4) High protein content and low fiber content which makes it good as animal fodder for chicken, turkey, ducks, goose, ….. etc [4].
5) The harvested DW can be – when composted – a good fertilizer [6].
6) The purified WW is considered a surplus to the fresh water national budget and can be used in agriculture [4].
7) DW, if fermented in absence of oxygen, will produce methane [4].
8) DW can be used also as fodder for fish in aquaculture.

1.2. Problem statement:

This research project responds to the following main and sub-main questions;

1.2.1. Main questions:

1) Can a DW be used as a good method to purify WW?
2) Does the concentrations of pollutants resulting from processing through DWT matching the agricultural standards of treated WW?
3) Can the harvested DW be considered a suitable fodder for birds?

1.2.2. Sub-main questions:

1) What are the effects of DW when used to treat a wastewater?
2) What is the removal efficiency of WW treatment by using DW?
3) How do climatic fluctuations affect treatment system?
4) How efficient is the DW application in biological treatment?
5) How do WW parameters like COD, BOD, pH, EC, NH_4^+-N change after treatment?
6) What are the best factors to ensure the effectiveness of the system?
7) What is the change in the growth rate of chicks that are fed with DW over time?

1.3. Project justification:

This pilot research project is considered important to be carried out for the following reasons:

1) To find new cost-effective applications that can contribute to solve water shortage in Palestine by reusing the treated WW.

2) Replace fresh drinking water by treated WW in agriculture and industry instead of purchasing fresh drinking water, as the average water price from public network costs 5 NIS/m^3 and from water tankers 16 NIS/m^3.

3) Conserve groundwater aquifers, which are considered the main source for drinking water in Palestine. The amount of water extracted from groundwater in West Bank is about 103.8 million m^3/year [7].

4) Use cheaper, easier, and locally available device like DW to treat the WW.

5) DW application is considered a suitable alternative methodology that can easily substitute the wide-spread use of cesspits. It is a technology that can be implemented in any building or population cluster. The cesspits' option must stop as an option to dump WW for its many insanitary effects.

6) The possibility to use the harvested DW as fodder for chickens, or one component for good compost, or as a feedstock to generate biogas is seen as environmentally-sounded and economically-feasible application.

1.4. Goals and Objectives:

- ✓ The main goal is to investigate the removal of organic matter and pollutants in WW by using DW in order to contribute in reducing the environmental negative impacts produced by random dumping of the large quantities of WW available in Palestine, by applying an easy and new WW treatment technology that can be used by anyone.
- ✓ The project is to demonstrate results relating to the efficiency and capacity appropriate for treating WW using DW *Lemna gibba* lagoons.
- ✓ The treated effluent will be used for irrigation, the harvested DW as a fodder for chicks.
- ✓ Demonstrate the usability of DW fronds in the treatment of domestic wastewater under the prevailing climatic conditions in the central Highlands of West Bank.
- ✓ Demonstrate the growth rate of chicks fed by harvested DW over time and make a comparison between the growth rate of chicks that eat a mixture of DW + fodder and chicks that eat only ordinary fodder.

1.5. Literature Review

DW is one of the smallest macrophytes on the planet. It is a monocot, an angiosperm, floats on water, and has one of the fastest growth rates of any of the macrophytes, contributing to its great ability to be one of the highest accumulators of nutrients such as phosphorus, nitrogen and trace metals [8].

From literature, one of the most promising aquatic families in the phytoremediation is the DW (*Lemna gibba*) which is widely distributed in the aquatic ecosystems in Palestine [9]. The DWs are small floating aquatic macrophytes which grow on the surface of the nutrients-rich aquatic ponds and in freshwater bodies [10]. Phytoremediation of contaminated water using DW species is promising due to its ability to grow at wide ranges of temperatures, pH, and nutrients (N,P) level in areas where land is available for its application [11, 12]. DW has the ability to remediate organic compounds; uptake of these organic chemicals by DW relies on a complicated combination of abiotic and plant-driven processes [10].

1.6. Project Focus:

This project focuses on the possibility of using DW to treat the municipal WW, and the usability of the harvested DW as a fodder for chicks as well as to study its growth rate over time. Also the project focuses on finding a way to treat wastewater without producing much waste, in hope to reduce as much less as possible of waste and achieve sustainable development.

1.7. About the Project:

1.7.1. Project Location:

All tools, containers, facilities and materials specified to run the research were hosted in the building of the Environmental Lab at the Palestine Polytechnic University (PPU). The chicks, both those fed with the harvested (DW + ordinary fodder) and those fed with only ordinary fodder, were kept in areas outside the university.

1.7.2. Project Duration:

Started in Febreuary1st, 2017 and ended in May1st, 2017.

1.7.3. Implementation Phases and Progress of the Project:

- **Pre-implementation phase:**

  This phase included reviewing the literature related to DW, to get a scientific overview about the previously written articles and those already implemented projects, as well as to get to know the economic feasibility for implementing such projects.

- **Implementation phase:**

  This phase represents the main prerequisite for the project to be appropriately implemented; it included preparing all the logistics and materials needed to commence the work; e.g. the selection of the containers that will host both the WW and its DW. Two containers were used; the first was the indoor container and the second was the outdoor container. The WW from Beit Alsharq restaurant - Hebron, from the dish washing sink, and 1-week old chicks were brought. The qualities of the WW and the DW-treated water were both examined. The growth rate of chicks was monitored.

- **The post-implementation phase:**

  This phase is considered the core of the project performance process and progress as it deals with the continuous monitoring and analysis of all parameters related to this research study. It includes, among many activities, the provision of the fresh-harvested DW to the already prepared and specified WW containers. Analyses of many parameters in DW, and WW as well as the use of both, registering their successes and failures if any, also were detected.

1.8. Structure of this study

This study consists of five chapters:

Chapter1 Provides an introduction and proposal to the DW-based treatment system, the project objectives, the project significance and the action plan of the study.

Chapter2 Includes the literature review for each part in this project, e.g. biology of DW, DW processes, examples of DW treatment plants and optimum DW mechanisms.

Chapter3 Describes the project methodology and what materials and equipment used.

Chapter4 In this part of project all results of the project are illustrated and recorded.

Chapter5 Includes the final conclusions reached from the experiment and the recommendations for the future.

1.9. Research methodology:

Research methodology is illustrated in Table 1.1 below:

Table 1.1: Research methodology in successive steps

Objectives	Activities	Remarks
1. Preparation of the containers	Buying the needed containers, each with the following dimensions: Width=34 cm, Length=50cm and Height= 27 cm. Preparation of the needed quantities of DW for each container.	1. Filling each container with its specified WW up to the desired level. 2. Providing each container with approx. 30 grs of fresh healthy DW. 3. One container was kept indoor and one outdoor.
2. Collection of sufficient healthy DW from DW pond at Al-Arroub complex farm.	Going to Al-Arroub to collect DW carefully, keeping it in open clean pail and bring it to the laboratory of the PPU.	Nine kilos of wet DW at least were collected.
3. Bringing the domestic WW from Beit Alsharq restaurant	Preparing the needed concentration of the WW that will be introduced to each container.	Analysis of the WW specifications quantitatively and qualitatively before introducing it to the containers is followed.
4. Carrying out the needed tests and analysis	-BOD tests -COD tests -NH_4^+-N tests -pH tests -Conductivity -Microbiological tests	Prepare all materials, the tools, equipment, and chemicals needed for the testing procedures.

5. DW harvesting	DW grown up was Collected from the ponds' surface thoroughly. Weighing and drying it in the ovens.	The moisture content in DW was Calculated.
6. Calculate the wastewater treatment efficiency by DW	Compare values of water quality parameters before and after treatment.	Registering the results and doing the interpretations.
7. Prepare the harvested DW as fodder for chicks	Dry it completely and mix it with ordinary fodder in known percentage.	Feed the chicks and calculate their growth rate versus time.

1.10. Total project budget:

The total cost for implementing this project was estimated to be around 340$ as detailed in Table 1.2 below.

Table 1.2: The total cost for implementing the project

#	Item	Quantity	Cost
1	Transportation	Frequent	55$
2	Plastic Containers	2	35$
3	Materials for water quality testing	Multiple	190$
4	Safety requirement	Multiple	30$
5	Chicks	8	10$
6	Chicks requirement	Multiple	20$
	TOTAL =	**340$**	

1.11. The Expected Results:

The results can be summarized as follows:

1. Itself the implementation of the project taught us how to start doing a real scientific research.
2. The project introduces the biological treatment of municipal/household WW using DW as easy, cheap and effective choice.
3. Acceptable concentrations of wastewater can be treated by using DW.
4. DW is suitable and safe to be used as fodder for chicks.

1.12. Action plan:

The action plan for project is illustrated in the following Tables 1.3 and 1.4. below:

Table 1.3: Action plan for the project in first semester.

TASKS	1st Month				2nd Month				3rd Month				4th Month			
	Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4
Identification of Project Idea																
Literature Review																
Detection of DW locations																
Documentation																
Presentation in First Semester																

Table 1.4: Action plan for the project in second semester.

TASKS	1st Month				2nd Month				3rd Month				4th Month			
	Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4	Wk 1	Wk 2	Wk 3	Wk 4
Preparation of the containers																
Collection of healthy DW																
Bringing the domestic WW																
Carrying out the needed analysis																
DW harvesting																
Feeding the chicks by DW																
Obtaining final results																
Documentation																
Presentation of study																

CHAPTER TWO

Literature Review

Forward

Constructed wetlands are well known as suitable efficient systems to treat WW from different sources. This treatment system is considered a cost-effective device to be used in semi-arid areas like Palestine. A “pilot” wetland project with batch flow and free water surface using the DW plant *Lemna gibba* was constructed at the Palestine Polytechnic University (PPU). This graduation project operated on different concentrations of domestic WW influents. Water quality and system efficiency were observed during the experiment to evaluate the performance and suitability of the system to large scale applications.

2.1. project: symbioses

Wastewater handling (collection, treatment and reuse) is the most promising immediate and economically attractive option to secure more water for agriculture sector in Palestine. However, the reuse of the treated wastewater as a reliable resource is limited due to its inadequate quality, which in turn determines the quantity that can be used for irrigation purposes [10]. In Palestine, where very limited freshwater is available; DW can contribute in cleaning considerable quantities of WW types. Using this natural low cost water treatment method is not only good for the country but the environment as well [13].

Water in Palestine suffers from different sources of pollution especially those emerging from organic pollutants, including chemicals, personal care products, nitrogen, phosphorus and pesticides [14]. Therefore, good treatment of water containing chemicals is necessary for producing a quality effluent for reuse in agricultural purposes that complies with the internationally adopted standards.

Conventional chemical and biological treatment processes such as adsorption, bioremediation and coagulation can effectively remove many chemicals at high concentrations [10]. However, so far low existence of low-cost technology for removal of chemicals at low concentration [2]. Development of a cost-effective, less complex and environment friendly method to eliminate chemicals at low concentration levels from the aquatic environment is imperative [2]. Phytoremediation using floating, submerged, or emergent macrophytes is based on utilizing natural processes, and it represents an effective, low-cost technology, less energy consuming and preferred cleaner technology for the treatment of contaminated water [15].

From literature, one of the most promising aquatic families in the phytoremediation is the DW species *Lemna gibba* which is widely distributed in the aquatic ecosystems in Palestine [9]. The DW's are small floating aquatic macrophytes which grow on the surface of nutrient rich water bodies and in freshwaters [10]. Phytoremediation of contaminated water using DW species is promising due to its ability to grow at wide ranges of temperature, pH, and nutrient (N,P) level in areas where land is available for its application [11, 12]. DW has an ability to remediate organic chemicals. Uptake of organic chemicals by DW relies on a complex combination of abiotic and plant-driven processes [10].

Carbon, nitrogen and phosphorus are recycled within the system by a combination of different natural processes taking place, where the main one of them is plant uptake. Other processes are: physical sedimentation, organic matter dissolution, adsorption, filtration, burial in the sediments, combination of nitrification/denitrification processes and microbial processes [12]. There is a major necessity for the removal of nitrogen and phosphorus from WW, because in low concentrations they are a major nutrient for the plants, but in high concentrations may cause eutrophication in open water bodies [12].

The advantages of using the water "plant" DW (*Lemna gibba*) are: high production rate, easy for manual harvest from the surface, high protein content and low fiber content. Other advantages of the DW plants are: the ability to survive dry conditions, ammonia preference uptake, ability to metabolize ammonia directly from the water and high protein content [12]. All these make the DW cost-effective, for recycling as fertilizer and animal fodder. However, the DW plants grow only in the upper water surface layer where mainly pollutant removal takes place [12]. Literature recommended using a combination of several types of plants, floating and submerged to increase effluent quality. In dry lands, such as the state of Palestine, where precipitation is low and not persistent and the resources of fresh water are decreasing in quantity and quality, it is important to find additional solutions for water supply of high quality.

The water demand in Palestine increased in the last years because of the expansion of the industry, population growth and lack of adequate treatment and uncontrolled disposal of WW to the wadies. In dry regions, the only available source for spring's rehabilitation is treated effluent.

This research suggests an advanced approach to solve water shortage problems in Palestine by treating WW for agricultural reuse and spring's rehabilitation by natural processes.

Wastewater status quo in the West Bank: Only 30-35 % of the Palestinian population as a whole is connected to sewerage networks [9]. The majority of the population uses individual or communal cesspits for dumping their generated WW. Not always the cesspits are emptied by vacuum tankers that usually dump their contents onto open land, or into wadies, seldom into sewerage networks, irrigation channels, or solid waste disposal sites. In spite of the low overall percentage of access to sewerage, approximately 70 % of houses in the main West Bank cities are connected [9]. On the other hand, in refugee camps sewage flows through open drains originally constructed to convey rainwater. Most villages have no sewerage system and WW is dumped in cesspits or discharged randomly. The existing WW treatment plants in the West Bank are inadequate to serve the volume of WW being discharged [9].

2.2 What is the DW?

DW is one of the smallest macrophytes on the plant kingdom. It is a monocot, an angiosperm, and floats on water as the Figure 2.1 illustrates. DW has one of the fastest growth rates of any of the macrophytes, contributing to its ability to be high accumulator of nutrients such as phosphorus and trace elements. Its location between the air-water interface makes it simple to separate from the water and susceptible to accumulation of hydrophobic chemicals [8].

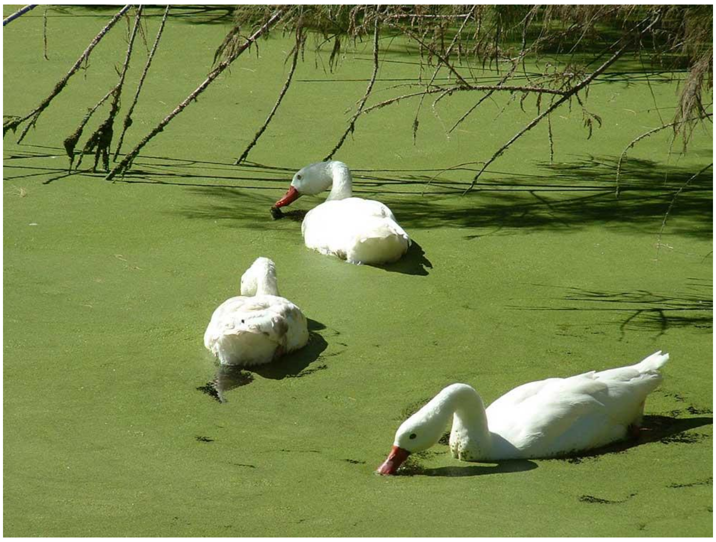

Figure 2.1: DW floating on the water surface [16]

2.3 Biology of DW

DW species are small floating aquatic plants belonging to the botanical family ***Lemnaceae***. The family consists of five genera, *Lemna, Landoltia, Spirodela, Wolffia* and *Wolffiella,* among which about 40 species have been identified worldwide [17]. Many of these species are found throughout the world, although geographical variations within species have developed in response to local conditions. They should not be considered noxious like problematic exotic species such as *Salvinia* and *Hyacinth* [18].

The natural habitat of DW is free floating on still surfaces of fresh and brackish waters (up to 4000 mg/L NaCl [18]), it prefer full sunlight but can adapt well to low light conditions. DW is reported to tolerate a wide pH range, different optimum pH conditions have been quoted that range between 4.5–8 [19]. A pH greater than 9.5 will inhibit growth. The optimum water temperature ranges for DW growth is between 17^oC and 35^oC [18].

DW occurs naturally in water with decaying organic matter that supplies it with a constant supply of growth nutrients and trace elements. Of these growth nutrients, ammonia nitrogen in its ionized form (ammonium NH_4^+) and phosphate (PO_4^{3-}) are the most critical [18].

This preference for ionized ammonium helps explain the optimum pH range, in that at alkaline pH above 8, ammonium is progressively transformed into the unionized state (NH_3). This results in the liberation of free ammonia molecules, which are toxic to DW [18]. When ammonium concentrations are limited, DW is able to utilize other forms of nitrogen especially nitrate (NO_3^-) and simple organic molecules to maintain growth [19].

DW species are capable of exploiting favorable environmental conditions by growing extremely rapidly [19]. Under ideal conditions of nutrient availability, sunlight, pH and temperature, DW plants can double their biomass every two days. This is faster than almost any other higher plants, and more closely resembles the exponential growth of unicellular algae. This ability to spreading rapidly by consuming dissolved nutrients from the water makes DW an excellent candidate for WW treatment [18].

2.4 Characteristics and Common Species in Palestine

Lemna gibba (common name DW) has its main distribution in regions with Mediterranean climate throughout the world except Australia, it also grows in countries with relatively dry, mild climate and in tropical mountainous regions [20]. It grows naturally in almost Hebron – Jerusalem – Ramallah – Nablus Heights, Dead Sea area and around with a growing season of at least 5 months. Most studies involving DW take place in climates with 9 to 10 month growing seasons.

DW is a C3 plant—which helps it to grow in the colder climates [8]. Nonetheless, since water freezes in the winter and DW floats on water, it does best in warmer climates. Of the known DW genera (*lemna gibba*) is found in Palestine; this species from these genera is reported to be cold tolerant [21]. Being a native "plant" in Palestine, its tolerance to temperate climates, and its fast growth rates, all these make it promising for nutrient removal in WW treatment applications [22].

Early in spring time as DW mat will reach approximately 7 cm thick and only the top layer of it appeared at the water surface, DW should be harvested (Figure 2.2) [8].

As plant surface density increases, frond size often decreases. Other factors contributing to an increase in frond size, including: increased light intensity; increased light duration; addition of sugar; increased nitrogen, phosphorus, potassium, calcium, and magnesium concentrations (which can also decrease frond size if too high); and increased temperature [8].

Figure 2.2: Harvested a 7 cm thick DW mat [8].

- **General view of *Lemna gibba***

Lemna gibba, the common DW, is a species of genus *Lemna* (DW) with a sub-cosmopolitan distribution, native throughout most of Africa, Asia, Europe and North America, occurring everywhere freshwater ponds and slow-moving streams occur, except for arctic and subarctic climates. It is not reported as native in Australasia or South America, though is naturalized there [3].

It is a floating freshwater aquatic plant, with one, two or three leaves each with a single root hanging in the water; as more leaves grow, the plants divide and become separate individuals. The root is 1–5 cm long. The leaves are oval, 1–8 mm long and 0.6–5 mm broad, light green, with three (rarely five) veins, and small air spaces to assist flotation (Figure 2.3, 2.4). It propagates mainly by division, and flowers are rarely produced; when produced, they are about 1 mm diameter, with a cup-shaped membranous scale containing a single ovule and two stamens. The seed is 1 mm long, ribbed with 8-15 ribs [1]. It grows in water with high nutrient levels and a pH between 5 and 9, optimally between 6.5 and 7.5, and temperatures between 6 and 33 °C [1]. Growth of colonies is rapid, and the plant frequently forms a complete carpet (matt) across still pools when conditions are suitable. In temperate regions, when temperatures drop below 6 to 7 °C it develops small, dense, starch-filled organs called 'turions', which become dormant and sink to the water bottom in winter; in the following spring, these recommence growth and float back to the surface [1, 3].

- **Characteristics of *Lemna gibba***

Flower Color for *Lemna gibba* is pale green, its flowers Bloom Period in February. The foliage duration is perennial. Its Size is under 6" tall. Its living in place having a sun to partial shade. Under cold temperature sometimes [3].

Figure 2.3: *Lemna gibba* [1]

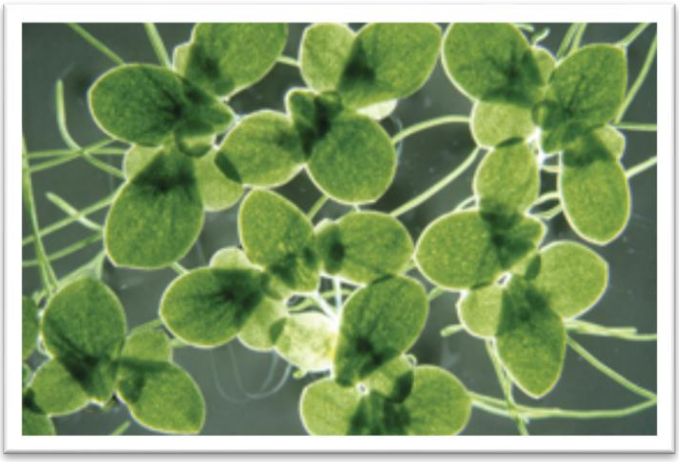

Figure 2.4: DW *Lemna gibba* under the microscope [1]

2.5 Optimum DW Mechanisms:

- **Harvesting of DW**

Harvesting is an essential component of DW nutrient removal systems because it physically removes the phosphorus from the system via the DW biomass. Without harvesting, the plant tissue would die, settle to the bottom of the lagoon, decompose and then release a significant proportion of the phosphorus and other nutrients back into the water column. This harvested biomass can be used as compost, as fodder rich in protein, or to generate fuel like methane [8].

Continuous harvesting improves nutrient removal and prevents overcrowding, biomass death, and release of nutrients back into the water column. Several studies support harvesting the entire biomass growth within a 3-week time period for improved nutrient removal. In Bangladesh, harvested every 2 to 3 days at an average of 4.5 g-dry/m^2day to obtain 74-77% total phosphorus removal (90-95% PO_4-P) [26]. In Australia harvested at 3.5, 5.5, and 10.4-day intervals and concluded that the shorter harvesting intervals correlated to increased biomass production and nutrient concentration in the biomass [8]. In Thailand, harvested every 2 to 15 days depending on whether it was the dry (warm) or wet (cool) season, respectively, noting that phosphorus concentrations below 0.3 mg-TP/L did not support normal growth [8].

Probably one of the most successful DW operations in the world is called the Agriquatics Mirzapur (Bangladesh) System that feeds DW harvested from WW to cod fish [8]. The system appears to be successful because the harvested biomass is a useful by-product used as fish food. One unsuccessful DW system in Boulder City, Nevada, used DW harvested on a 4.5 ha facultative lagoon as a compost amendment at the local landfill. The system had to be abandoned because the four person crew harvesting the lagoons 40 hours/week with two mechanical harvesters could not keep up with the biomass production [8].

In large scale frequent harvesting is recommended for increased plant production and nutrient removal, harvesting frequency also may be limited by the constraint of additional energy and labor costs [8]. With respect to the unsuccessful DW system mentioned earlier; the DW system was used for approximately 10 years before being shut down because they could not keep up with the quantity of DW produced. Hence, careful solids' management programs are necessary to guarantee sustainable and long-lasting DW systems[8].

The frequency of harvesting in natural systems and the amount of biomass removed per harvest varies from study to study. However, consistent recommendations and observations include:

1) Maintenance of 100% coverage should be used to reduce algae growth and

2) Harvesting should be carried out at least once every 20 days, and more frequently for improved nutrient removal.

➢ Growth Rates of DW

Most studies recommend starting and maintaining DW systems with a monolayer of DW sufficient to fully cover the surface area. Full coverage prevents algae proliferation that out competes the DW [23]. DW mass can double in 2 to 7 days and follows a logarithmic growth trend. Linear growth ranges occur between 10 to 120 g-dry/m^2 for *lemna gibba*. Some studies recommended 30 to 40 g-dry/m^2 where algae competition is minimized [8].

A typical relative growth rate (RGR = ln(g new/g old)/day) of DW ranges from 0.06 to 0.15, while higher growth rates from 0.24 to 0.31, even up to 0.4, are not uncommon when plant densities are low and other factors such as light, temperature, and nutrients are optimum [8]. Light intensity was the single most important variable controlling RGR, and recommended providing 200 to 300 μmol/m^2·sec (PPF) for highest growth rates indoors. Plant mat density, which is highly dependent on light intensity, is very useful for predicting growth rates and plant production [23].

Several factors inhibit DW growth rates. Growth rate decreases due to overcrowding as biomass accumulates to the point that fronds start overlapping each other [8]. Growth rate decreases with nutrient depletion [23]. DW prefers ammonium (NH_4^+) to ammonia (NH_3) and other forms of nitrogen (*e.g.*, nitrate NO_3) because the least energy is required to assimilate NH_4^+ into mobile plant amino acids and proteins [23]. Growth decreases when NH_3>NH_4^+, since NH_3 (aqueous or gas) inhibits cell metabolism and respiration via the electron transport system. Phosphorus precipitation with calcium also occurs at a pH near 9.3, which also leads to nutrient deficiencies and lower growth rates. Several studies indicated that wind or movement decreased growth [8]. Biomass started depleting at temperatures below 17°C, and completely disappeared below 5°C [24]. Growth rate also decreased due to competition between species [8].

➢ Growth Inhibitors

DW has the potential to reduce total suspended solids (TSS) in WW, primarily by shading the water column below to prevent algae growth. On the other hand, if the DW crop density is much less than 100% cover or 20 to 30 g-dry/m^2 then algae receives enough sunlight to out-compete

DW. When algae attaches to DW fronds small air bubbles appear below the frond cutting off its contact with the liquid interface [8].

Some reports recommended that in order to eliminate fungi infestations in DW, the following interventions can be applied; e.g. reduce temperatures, increase silicon in plant tissue, and to use fungicides. Lower temperatures and adding potassium silicate to the nutrient solution in the laboratory studies were not able to prevent existing fungi infestations from killing DW fronds; it took adding Ridomil Gold EC (fungicide) at a dose of 0.3 μL/L to remove the fungi [8].

Aphids living atop DW mats in some instances have been associated with decreased growth [24]. Aphids removed by occasionally disturbing the water surface. Residual chlorine has a tendency to inhibit growth of aquatic vascular plants, like DW [8].

DW grown on the non-chlorinated wastewater had a significantly higher growth rate than DW grown on the chlorinated wastewater [8]. DW growth increased with increasing light intensity and duration. DW has adapted to growing in shady and cool areas and is able to grow under reduced light intensity [8].

- **Removal/Elimination Mechanisms of Pollutant and Chemical Compounds**

The *Lemna gibba* species have stomata's that never close—even in the dark. Water loss with DW occurs at the same rate as evaporation without DW. Constantly open stomata may result in more water passing through the plant in the dark resulting in more chemical removal [25]. Vapor pressure, and certain chemicals, like trichloroethylene, will volatilize through plant leaves [25].

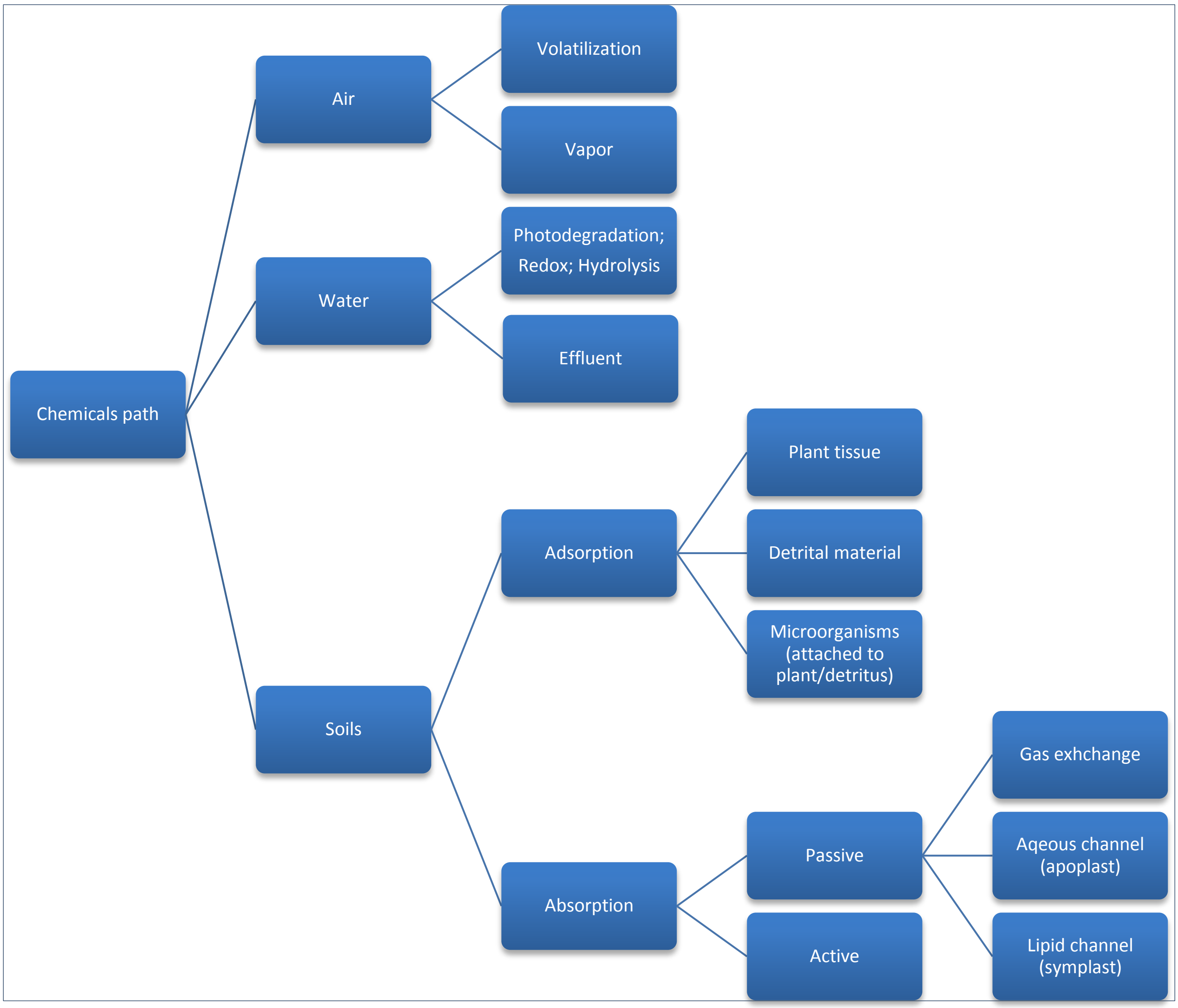

Figure 2.5: Diagram showing potential pathways for the fate of chemicals in a DW system [8].

DW can be used to remove chemical compounds. DW mats shade the water column and can limit the amount of photo-degradation in the water column. High positive redox potentials (*i.e.*, aerobic conditions) and low redox potentials (*i.e.*, less aerobic conditions) [8]. Redox potential is negative near the facultative sediment-water interface where there is an abundance of organic electron donors. The redox potential at the water surface is positive. If oxygen is transferred to the water by the plants the redox becomes more positive. If oxygen is taken up by the microorganisms attached to the DW the redox becomes more negative. pH is an important driver for the fate of chemicals because it affects their ionization which affects their solubility in water and their tendency to adsorb or absorb in DW [8].

Chemicals removed by DW plants may be transformed into nonparent compounds, *ad*sorbed/*ab*sorbed, stored/assimilated into plant tissue, and/or transpired and volatilized into the atmosphere or back into the water column (Figure 2.5) [8].

DW Phytotransformation. Transformation consists of metabolizing and/or mineralizing the parent compound. Plant metabolism occurs in three phases and is compared to a "Green Liver" because plants metabolize many compounds similar to the human liver. Phase I metabolism increases the polarity of the parent compound, which can be further metabolized by glucosidation and amino acids known as Phase II metabolism. Once the polarity is increased, the metabolites can be transferred through aqueous channels to be eliminated or assimilated into the plant, known as Phase III metabolism [8].

DW Adsorption: chemicals can adsorb to the surface of plant tissue, particulates in the water column, and microorganisms attached to the DW. Acidic compounds have less tendency to adsorb to net-negative sorbates and the opposite is true for basic compounds [8]. Several studies have used hydrophobicity alone to predict adsorption, but this method alone is unreliable based on the variability of results from several studies and adaptations that need to be made depending on the compound [8].

DW: Absorption. Chemicals can be absorbed into the DW by passive and active mechanisms. Passive mechanisms include gas exchange, aqueous channel uptake, and lipid channel uptake, and are driven by transpiration. Active mechanisms include specific enzymes or routes in the plants and require metabolic energy. Gas exchange involves fixation of gasses into the plants or volatilization of compounds already in the plant transpiration stream [8].

Some molecules may pass through plant membranes if their size is small enough. Plants take up organic chemicals with moderate hydrophobicity into their tissue. Soluble chemicals are easily transferred through plants via the xylem. The uptake of hydrophobic and hydrophilic compounds by the plant is illustrated via the root's anatomy [8].

The plant's roots can be visualized as circular layers of cells, beginning from the outermost layer: epidermis, cortex, endodermis, pericycle, and xylem. Different transport mechanisms exist to transfer chemicals through the different layers [8]. Mineral nutrients absorbed by the root are carried to the shoot by the transpiration stream moving through the xylem via the apoplast [8].

Hydrophobic compounds do not move through the apoplast; rather, they move through a network of interconnected lipid-cells known as the symplast. hydrophobic chemicals require symplastic movement through inner cells, hydrophylic chemicals require apoplastic movement through cell walls, and inorganic nutrients require “specific carrier- and channel-proteins.” Once a chemical is taken up by a plant then it can be stored, metabolized (*i.e.,* assimilated), mineralized, or volatilized [8].

Physiological characteristics of plants (*i.e.*, floating versus rooted) make specific species more adept at removing specific chemicals. In reality, ecological systems contain multiple species and chemicals of concern. Some studies have looked into using multiple types of plants, each one with a particular ability to remove a specific chemical. Other studies have observed competition among species that promote or inhibit a diversity of species that may affect the types of plants that can be used for phytoremediation in a certain environment [8].

→ Phosphorus

Nutrients such as phosphorus enter the aquatic environment by anthropogenic and natural sources. Anthropogenic sources include agricultural runoff from fertilizers and manure, phosphorus containing detergents in WW and human excreta. Non-point sources from agricultural activities and point sources like the Sewage sources. Natural, non-anthropogenic, sources originate from phosphorus containing sediment and decomposition of organic material. Naturally occurring DW has the potential to remove phosphorus from point sources [8]. DW plants typically contain more phosphorus in its tissue than other floating plants, which makes them suitable for phosphorus removal. DW systems are usually lagoon based systems that receive weak municipal WW containing 1 to 4 mg-P/L; however, DW has also been used to treat high strength WW containing 62.5 to 135 mg-P/L [8].

DW biomass as contributing 13 to 47% of the total phosphorus removal, and one account attributes all of it to DW. The phosphorus concentration in the effluent coming from DW systems almost always falls below 1 mg-TP/L and frequently less than 0.53 mg-P/L down to 0.05 mg-P/L [8]. DW growth started decreasing when phosphorus levels fall below 0.3 mg/L; however, plants may continue to survive in concentrations as low as 0.03 mg-P/L (1 μmol-P) [8].

A DW treatment system would physically remove phosphorus and other assimilated/sorbed compounds via the harvested biomass (Figure 2.6). Successful WW treatment physically removes contaminants from the water and/or converts contaminants into non-harmful constituents [8].

The 89% removal is based on the assumption in the study that flow in is 86.3 m^3/h, flow out is 0.252 (54% liquid lost due to evaporation and seepage), phosphorus is 3.88 mg-TP/L influent and 0.95 mg-TP/L effluent [8]. Sequestering of phosphorus in the sediment has the potential to re-release phosphorus into the water column as microbes decompose organic matter and as phosphates re-dissolve into the water column, as shown in the phosphorus cycle in Figure 5. 30 to 50% of the phosphorus in the plants gets released by anaerobic digestion [8].
The degree of treatment by a DW system can be measured by two ways:

1) By the composition of plant solids and

2) By the concentration of the chemical in the effluent [8].

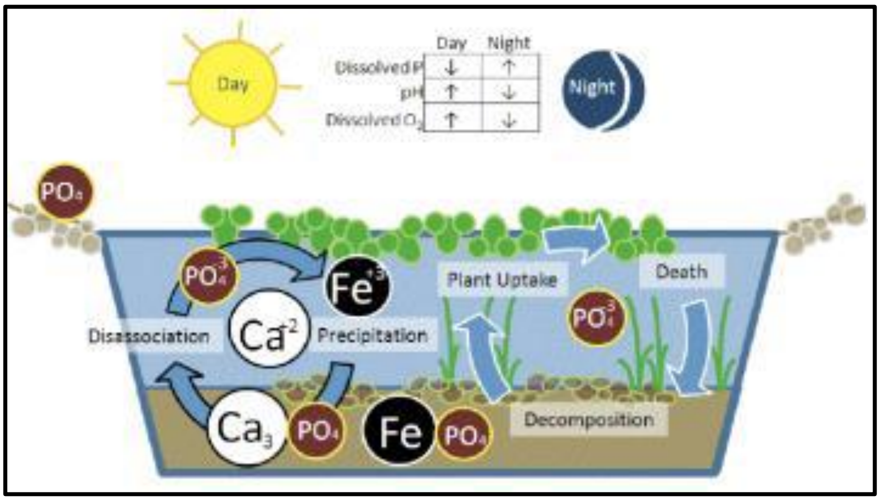

Figure 2.6: Phosphorus cycle in a typical lagoon system [8].

2.6 DW-Based Wastewater Treatment (DWT) Processes:

DWT systems operate similarly to conventional lagoon systems that can incorporate deep facultative ponds for solids removal, and stabilization and polishing ponds for further purification. However, DWT differs from conventional lagoon systems in that they work to prevent rather than encourage planktonic algal growth. This is achieved as a DW mat floating on the surface simply out-shades planktonic algae (including toxic blue-green algae/cyanobacteria). The result is cleaner discharged effluent standards in terms of reduced total suspended solids (TSS) and nutrients [18].

Nutrients contained in phytoplankton are difficult to harvest and are generally released back into the environment, whereas DW is easily harvested, which results in direct removal of nutrients from the waste stream. Inhibiting phytoplankton also regulates pH, which shows great diurnal fluctuations in traditional lagoons. In addition, evaporation from the water surface is reduced in DWT systems, and the increased efficiency of DWT over conventional lagoon systems means they can occupy less land area [19].

DW works to purify WW in collaboration with both aerobic and anaerobic bacteria [18]. Therefore, the DW plants themselves should be considered as only one component of a complete DWT system (see Figure 2.7)

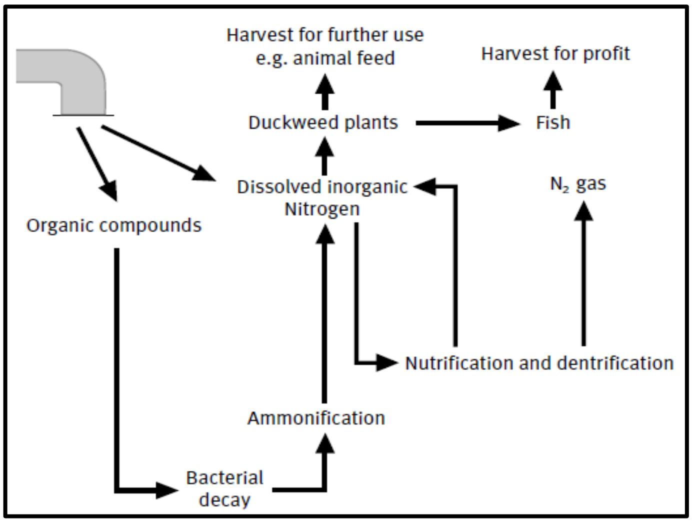

Figure 2.7: Flow of nitrogenous nutrients within a DWT system utilizing bacterial processing and uptake by DW plants [18].

Heterotrophic bacteria decompose organic waste matter into mineral components specifically forms of ammonia nitrogen and orthophosphates that are readily uptaken by the DW plants [18].

Bacterial decomposition consumes oxygen and can cause the mid-water zone to become increasingly anoxic and the bottom of the lagoon to become anaerobic, providing further zones for specialized bacterial processing of organic matter and denitrification [18].

The DW mat maintains these conditions by inhibiting atmospheric oxygen diffusion at the water surface. However, a 10cm surface layer remains aerobic due to atmospheric oxygen transferred by DW roots [18]. Bacterial oxidization of organic matter and nitrification are facilitated here, aided by the additional surface area for biofilms provided by the DW roots and fronds [18].

Harvest for further use e.g. animal feed, harvest for profit DW plants, fish, organic compounds dissolved inorganic, Nitrogen nitrification and denitrification, N gas, ammonification, bacterial decay, and other processes that aid nitrogen removal in DWT systems are sedimentation of organic matter and volatilization of ammonia. Phosphorous is normally reduced in DWT ponds by plant uptake, absorption into clay particles and organic matter, chemical precipitation and sludge removal [23]. A dense DW mat has also been reported to decrease and control mosquito larvae and odor in a WW body by providing an interface between the water and air [18].

Pathogen removal is likely to be less effective in DWT ponds than algae-based lagoons due to the absence of very alkaline conditions and less light radiation. However, this can be countered by a sufficient detention time since parasites and parasite ova precipitate with other suspended solids, and suspended pathogens simply die as a function of time [19]. A study should count fecal coliform in WW and test waste removal by DW treatment.

2.6.1 Effectiveness of DWT

DWT has great potential for renovating effluent from a wide variety of sources including municipal sewage treatment plants, intensive livestock industries (including aquaculture), abattoirs and food processing plants. The effectiveness of DWT depends on a system design that facilitates the correct combination of organic loading rate, water depth and hydraulic retention time. These will vary depending on the effluent source and the level of pre-treatment [18].

In the case where raw sewage (human or livestock waste) is to be processed, the primary treatment objective is to remove solids. This can be achieved in conventional deep anaerobic ponds that encourage the fermentation and breakdown of settled solids by bacterial processes into simple organic and inorganic molecules. DW will enhance primary treatment in these ponds by maintaining anaerobic conditions and reducing odor nuisance [19]. In addition, conventional anaerobic ponds, while effective at reducing BOD, have a negligible effect on total nutrient concentrations, so DW assimilation will enhance the nutrient removal capacity of these anaerobic systems [18].

High levels of ammonification occur in primary treatment systems. The range of DW species tested could tolerate and grow at high ammonium levels of 240 mg/L as the case in swine manure collection ponds [18]. DW may need an acclimatization period to adapt to the very high N levels in raw agricultural wastewaters [18].

Most researchers, however, suggest that efficiency gains using DWT are greater in secondary and tertiary treatment of effluent where organic sludge has already been removed or converted into simple organic and inorganic molecules that can be used directly by DW [18].

The problems encountered with municipal WW treatment include difficulties in meeting TSS and nutrient (Total N & P, ammonia) discharge regulations. Intensive livestock and other industries that release effluent into natural waterways must comply with similar regulations [18]. It is in these areas that DWT is highly competitive compared with existing treatment methods. As such, this search will concentrate on pilot-system design aspects for effective effluent treatment.

2.6.2 Nutrient uptake

Large-scale studies from both developing and western parts of the world have been conducted using various DWT system designs and effluent sources, but common recommended design features can be identified [18].

A plug-flow system is the most appropriate for secondary and tertiary effluent treatment using DWT. A system will ensure maximum contact between WW and DW, and minimize the possibility of short-circuiting [18]. This will facilitate the incremental reduction of nutrients in the WW. Plug-flow systems are also most efficient for pathogen removal [18].

The basic unit of plug-flow systems is a shallow rectangular lagoon. The system can operate singly or as a series of lagoons. The length/width ratio should be as large as possible to encourage plug-flow conditions (Fig. 2.8) [18].

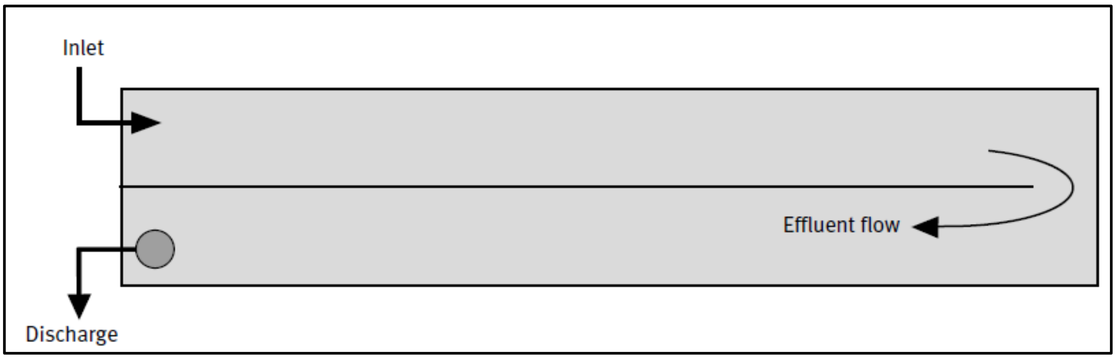

Figure 2.8: A plug-flow lagoon design, which prevents short-circuiting of flow between inlet and outlet, is most appropriate for DWT [18].

DW will be the major nutrient that uptake in these lagoons, a greater biomass will inherently result in greater nutrient uptake [18]. Greater biomass growth will occur at higher nutrient concentrations (up to a tolerance limit), but as DW incrementally gets less nutrients from the water, high biomass growth cannot be maintained. Since the ultimate object of treatment is to reduce nutrient concentration, DW starvation inevitably will occur at the latter stage in the treatment process [18].

In a plug-flow system, nutrient concentrations will be higher at the beginning of the effluent stream and lower towards the end. This will facilitate a ‘farming’ zone (high DW production/high nutrient uptake) and a ‘polishing’ zone (lower overall DW growth/lower nutrient uptake) [18].

In the farming zone, where growth nutrients (N,P) are plentiful, DW plants are predisposed to absorb them to the exclusion of other elements present in the wastewater column [19]. In the polishing zone, however, DW plants starved of N and P nutrients will scavenge for sustaining nutrients. In the process they can absorb toxins and heavy metals if present in the WW. This will have implications on the reuse or disposal of the harvested plants. However, since most agricultural or domestic WW does not contain significant concentrations of toxins or heavy metals [19], polishing zones may simply be considered to be the latter reaches of a continuous DW treatment process [18]. A longer retention period will result in a greater percentage of nutrients being removed, but create a relatively less productive ‘polishing’ zone when nutrients become limiting [18].

Overall retention time required in a DWT system depending on a range of factors including the influent nutrient levels, temperature and the discharge standards that must be met [18]. In general, 20 days hydraulic retention time would appear to be a minimum guideline for DWT to achieve acceptable discharge standards and pathogen reduction in sewage treatment [18, 19].

Retention time is in turn, a function of water depth and flow rate. Shallow ponds are better than deep ponds, but the tradeoff is the increased land area required and the lack of temperature buffering with shallow ponds. Water depths between 0.6m and 1.5m have been suggested as the most suitable for large-scale DWT systems [19].

2.6.3 Recirculating systems

For freshwater and low salinity aquaculture operations, recirculation of water through DWT offers a number of advantages. It reduces the need for recurrent extraction of water from the environment, and helps farmers meet regulated discharge quotas. Reducing or minimizing water discharge, and improving effluent quality through reduction of nutrient levels and suspended solids are now seen as priorities by the larger sectors of the aquaculture industry for maintaining their reputation as ‘clean and green’ producers as the Figure 2.9 illustrated. [18].

For aqua-culturists, managing ammonia levels is critical for maintaining cultured species in healthy condition, so DWT must be able to reduce dissolved ammonia concentration to safe levels prior to recirculation [18]. This Based on suitable retention time and daily volume of water to be treated [18].

For DWT systems that include primary treatment of raw wastes, Nitrogen levels (ammonia in particular) may be too high for DW to grow optimally at the beginning of the effluent stream [18]. This can be remedied by recirculating a portion of the final treated effluent to dilute nutrient levels and achieve greater overall nutrient recovery. This will also have the effect of further reducing pathogen counts in discharged water by dilution. Recirculation should be considered when BOD_5 is greater than 80mg/L in the first DWT lagoon [19].

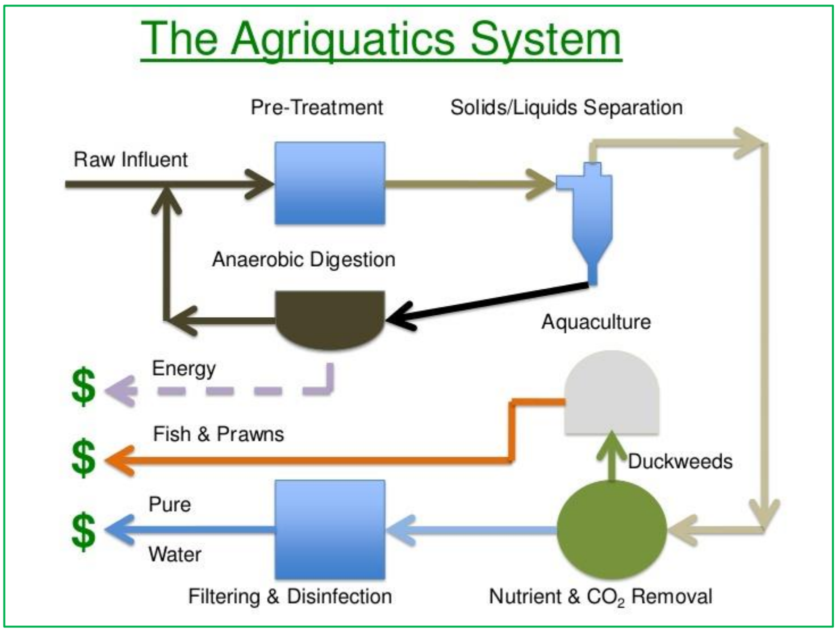

Figure 2.9: Aquaculture system [26]

For treatment systems that require recirculation, an additional design aspect is that start and end points of the plug-flow should be at a proximate location so that pumping distance is minimized (e.g. U-shaped or serpentine plug-flow. See Fig. 7) [18].

Recirculation is also recommended in systems where the primary aim of DWT is to produce DW biomass for further use. These systems would incorporate multiple inlets designed to distribute high nutrient levels along the lengths of ponds to increase the 'farming zone' area. Nutrient removal efficiencies are decreased, however, in these systems and a polishing zone would still be required if it is necessary to discharge low nutrient water. Therefore, land requirements would be greater than for a plug-flow DWT system [18].

2.6.4 Integrated Reuse Options for DWT

One of the most important benefits of DWT is that the harvested DW can be a valuable by-product. Nutritionally, DW can be substituted for soy meal and fish meal in a variety of products. DW plants grown in the 'farming' zone of a treatment lagoon will have the highest nutritional value and can contain up to 45% crude protein by dry weight, with higher concentrations of essential amino acids than most plant proteins. DW meal not only has low fiber content but high levels of vitamin A and pigment, particularly beta-carotene and xanthophyll. These make it an especially valuable and proven food source for cultured animals such as fish, ducks, chickens, and ruminants [18, 19].

While it is evident that DW has the potential to become a major protein commodity, this may not be fully realized until it can be economically reduced to a dried, easily handled product. This is because the fresh harvested material is difficult to store and transport. In addition, since fresh DW contains around 92% water, the relative concentration of nutrients available to grazers is lower than in the dried meal. If DW could be economically dried to preserve its nutritional value, and pelletized into a stable form that could be easily stored, it would offer a locally produced, environmentally friendly, fishmeal-free Ag-feed that could be applicable to a wide range of intensive livestock systems [18].

Various desiccation methods have been used on experimental scales to validate the performance of dried DW meal in livestock diets, ranging from convection and solar drying to microwave technologies [19]. However, to date no large-scale drying facility has been developed that is both economical and able to preserve the nutritional value of the DW (lengthy exposure to direct sun-drying degrades beta carotene concentration [19]).

Fresh harvested DW utilized as a livestock feed (or feed supplement), this will generally be restricted to operations in close proximity to where the DW is farmed. Such integrated operations could include dairies, cattle and sheep feedlots, and aquaculture farms [18].

Such integration may contribute to cost recovery of the wastewater treatment process or help glean environmental benefits using aquaculture. Conversely, dedicated aquaculture operations can utilize DWT to minimize the negative environmental impacts of the operation and improve profits by substituting DW for commercial diets [18].

2.7 Al-Aroub Wastewater Treatment Plant

Al-Aroub Wastewater Treatment Plant was constructed in 1997. A proper infrastructure was added to the plant such as sewer line, manholes and three small ponds with a dimension of 2, 3, 0.5 m (width, length, high respectively). Plastic sheet were installed at the bottom of the ponds to prevent seepage. DW *(Lemna gibba)* fronds were brought and installed in a mixture with wastewater and tap water for cultivation and adaptation, and then it was installed in the ponds. DW-based pond system at Al-Aroub treats 12- 15 m^3 per day of wastewater from the Al-Aroub Farm Complex [Agriculture School and College and the adjacent stable of the cows] [27].

The view of Al -Aroub treatment plant is illustrated in Figures 2.10. The system consists of settling tank, two DW ponds and storage reservoir. The wastewater coming from Al-Aroub Secondary School and Al-Aroub Technical College enters the treatment plant through the plastic pipeline and bar screen installed for this purpose to the settling tank. Then the wastewater goes to the constructed DW ponds. After that, *Lemna gibba* fronds were installed in the ponds that slowly grew and covered the whole surface. The treated wastewater is stored in a storage tank, where then taken and used for irrigation of plants in Al-Aroub Complex Farm [27].

DWT is very efficient in removing pollutants from the wastewater. One of the major advantages of this system is that it turns the waste into valuable DW meal to return a net profile against both capital and operational costs. The DW crop is enriched with nutrients during the treatment and can be used as animal fish or chicken food. DW systems are also capable to control mosquitoes and odors [27].

Figure 2.10: DW ponds system at Al-Arroub WW treatment plant adopted by Sharkh in 2007 [27].

2.8 Appropriate wastewater treatment

- WW treatment should be reliable to give an effluent fit for irrigation following the guidelines and Palestinian standards. Land treatment is not recommended due to the large area requirement and possibility of ground water contamination. Advanced and mechanized WW treatment is also not recommended due to the high investment cost of equipment's, high energy requirements and the need for skilled operators [27].

- The possibilities of using DW plants as an integrative component in constructed wetland facilities have the following advantages as compared to conventional treatment methods for WW treatment [27]:
    1) High hydraulic efficiency.
    2) Minimal operation and maintenance.
    3) Decrease in sludge formation and the problems evolving from the need to treat and dispose it.
    4) Prevention of health hazards such as fecal coliform and mosquito.
    5) Decrease in the extent of evaporation by full surface coverage of the DW plants.
    6) Higher effluent quality that fits with the international regulations and Palestinian Environmental guidelines for reuse in agriculture and water rehabilitation, especially for suspended solids and organic matter removal.
    7) Cost-effective and reliable supply of high quality water for river rehabilitation and reuse for other purposes, such as irrigation of certain crops.

Organic removal is in a linear correlation with load and the nitrogen content in the plants decreased [27]. Nutrient removal depends on water temperature and processes occurring in the water surface more than in the water column like organic matter removal [27]. The study reveals that the rural sewage is less concentrated than municipal sewage [27].

At optimal conditions, the septic tank-biofilter and DW ponds systems can ensure more than 70% COD removal which means that only 30% of the organic pollution is allowed to leave the system via the effluent. The key factors are the frequent desludging and filter backwashing; otherwise the efficiency may fall down to less than 50%. These systems are a good choice for replacing the existing cesspits and could serve a cluster of houses [27].

CHAPTER THREE

Methodology

3. Materials and methods:

3.1. Experimental Layout

A wastewater treatment experiment was conducted at laboratory in Palestine Polytechnic university by using two containers, one inside the laboratory and other outside, in order to study pollutant removal efficiencies from domestic wastewater by using *Lemna gibba*. Other experiment was also conducted to study the effect of using dried harvested DW as a fodder for chicks. Experiments were carried out during the period from the first of February 2017 until May 2017. Mean ambient temperatures ranged between 5.9 – 29.3^{o}C.

The variables examined included a single DW species (*Lemna gibba*) under a fixed depth of wastewater but with variable wastewater concentrations. The experiment was conducted in a system of two plastic containers (indoor and outdoor), each is 46L, with dimensions of 50cm, 34cm and 27cm (length, width, and high respectively). The containers were painted black for their simulations of nature. The containers filled with 30L of wastewater and fully covered by DW frond on the surface (Figure 3.1). 1L wastewater was recharged to the containers every ten days. The system was operated as a batch system by removing and adding 1L influent and effluent to keep the retention times around 10 days. Plastic containers were raised on a piece of wood to protect them from cold ground. A reference was placed inside the containers to maintain the water level. Distilled water was frequently added to compensate for evaporation until reach the reference.

Figure 3.1: Outdoor container

At last stage of study operation, eight small chicks was brought, they were placed in warm place with suitable lighting as shown in Figure 3.2. The chicks were divided into two parts, the first part was fed by a mixture composed of the harvested DW and ordinary fodder in a ratio of 1:1, and the second part was fed by 100% ordinary fodder. Their growth rate has been monitored and compared. The DW fed by chicks, was half dried by the sun and the other by oven.

Figure 3.2: Chicks prepared for the project

3.2. Wastewater Characteristics

Domestic wastewater from Beit Alsharq restaurant was discharged to the DW containers. The wastewater was diluted with different amount of tap water to change the wastewater concentrations. Grab samples of raw, treated wastewater from indoor and outdoor were collected to measure the pollutant removal efficiency. BOD, COD, ammonia, electrical conductivity, pH and microbiological test were measured for each sample.

3.3. Adaptation and Sampling of DW

The DW fronds (*Lemna gibba*) used in the study were collected from the pilot plant at Al-Arroub complex farm. It was brought and placed in plastic pail filled with small amount of wastewater. The DW was cultured and used for batch experiments. The weight for DW was measured initially before treatment, and the initial density of DW was constant for all experiments at a level of 2.5 g/m^3 (wet weight). The DW was harvested when the surface was overcrowded of DW after treatment, with maintaining that the surface was fully covered with DW. After harvesting the excess quantity of DW from two containers, it was weighted at a sensitive balance before drying to measure wet weight (Figure 3.3). Then it was dried in an oven with a temperature of 105°C for 2 hours. After drying, it was weighted to measure dry weight. During the study, wet weight, dry weight, size, color, and root length were monitored.

Figure 3.3: Harvested DW before dried

3.4. Treated Wastewater Analysis

The selected wastewater parameters were BOD, COD, NH_4^+-N, pH, conductivity and microbiological test. Effluent treated wastewater parameters were measured after 10 days retention period. All analysis were carried out at ETE’s laboratory at Palestine polytechnic university, except for the microbiological test was carried out at the Ministry of Health.

3.4.1. Chemical and Biochemical Oxygen Demand (COD and BOD)

COD and BOD tests were carried out for influent and effluent. The extent of organic matter and some inorganic contamination of water was indicated by COD test. Analysis of COD were carried out using the closed reflux method according to standard method for the examination of water and wastewater, methods: 5220 C [28]. The amount of oxygen used by bacteria in the degradation of organic matter in the wastewater is measured in the BOD test according to standard methods, Procedure 5210 B [28].

3.4.2. Ammonia NH_4^+-N

Ammonia test was carried out for influent and effluent using colorimetric nesslerization method according to the American Standard Methods (1992). After nesslerization, adding Nessler reagent, the sample absorbance was measured using a spectrophotometer at 425nm.

3.4.3. pH and Conductivity

The pH was measured for influent and effluent by using pH meter; while the conductivity was measured by using Automatic calibration conductivity meter in (μS/cm).

3.4.4. Microbiological Test

The microbiological test was measured for filtered and unfiltered effluent to assess the quality for treated wastewater. This test includes total coliforms and fecal coliforms tests. The tests were checked at the Ministry of Health.

3.5. Monitoring the growth of the chicks

The growth in weight of each chick was recorded in table versus time to give an idea about how these chicks was grow through time. A comparison was made between the chicks that ate the mixture of harvested DW with fodder (by 50% DW:50% fodder) and the chicks that ate the ordinary fodder only.

CHAPTER FOUR

Results and Discussion

4. Results and Discussion

4.1. Treated Wastewater Quality

The quality of treated wastewater was carried out after ten days detention time for many times. The following Tables 4.1, 4.2, 4.3, 4.4 and Figures 4.1, 4.2, 4.3 illustrate the result of treated wastewater analysis. From the analysis it was found that influent had a high concentration of BOD, COD and ammonia. Furthermore the analysis it was found that effluent had a good concentration of BOD, COD, pH, conductivity, ammonia and fecal coliforms tests specially in outdoor container. But it was contain a number of total coliforms bacteria.

Table 4.1: Values of wastewater analysis in 1/3/2017 (10 days detention time)

Sample	BOD mgO_2/L	COD mgO_2/L	NH_4^+-N mg/L	pH	Conductivity µS/cm
Influent	690	3070	20.33	7.5	1.6
Effluent (indoor)	427	1472	8.5	6.59	0.88
Effluent (outdoor)	99	352	0.33	7.03	0.62

Table 4.2: Values of wastewater analysis in 12/3/2017 (10 days detention time)

Sample	BOD mgO_2/L	COD mgO_2/L	NH_4^+-N mg/L	pH	Conductivity µS/cm
Influent	550	3085	20.1	7.61	1.5
Effluent (indoor)	330	320	4.9	7.27	0.84
Effluent (outdoor)	135	32	0.26	7.27	0.63

Table 4.3: Values of wastewater analysis in 23/3/2017 (10 days detention time)

Sample	BOD mgO_2/L	COD mgO_2/L	NH_4^+-N mg/L	pH	Conductivity µS/cm
Influent	545	3061	14.17	7.21	1.4
Effluent (indoor)	293	480	5.5	7.6	0.88
Effluent (outdoor)	111	320	0.13	7.56	0.58

Table 4.4: Average values of wastewater analysis (10 days detention time)

Sample	BOD mgO_2/L	COD mgO_2/L	NH_4^+-N mg/L	pH	Conductivity µS/cm	TC	FC
Influent	595	3072	18.2	7.44	1.5	--	--
Effluent (indoor)	350	757.3	6.3	7.15	0.87	60	0
Effluent (outdoor)	115	234.67	0.24	7.29	0.61	15	0

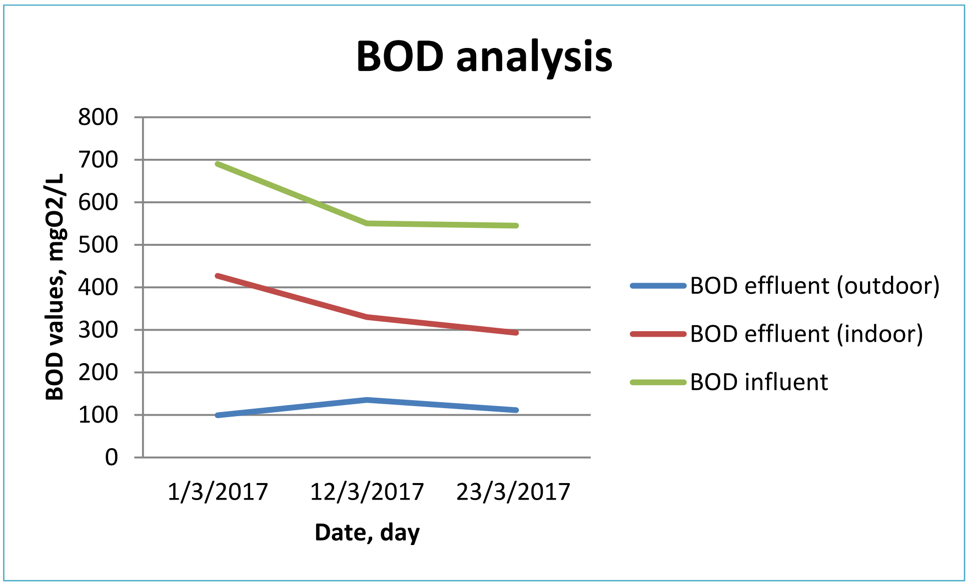

Figure 4.1: Results of BOD analysis thru time

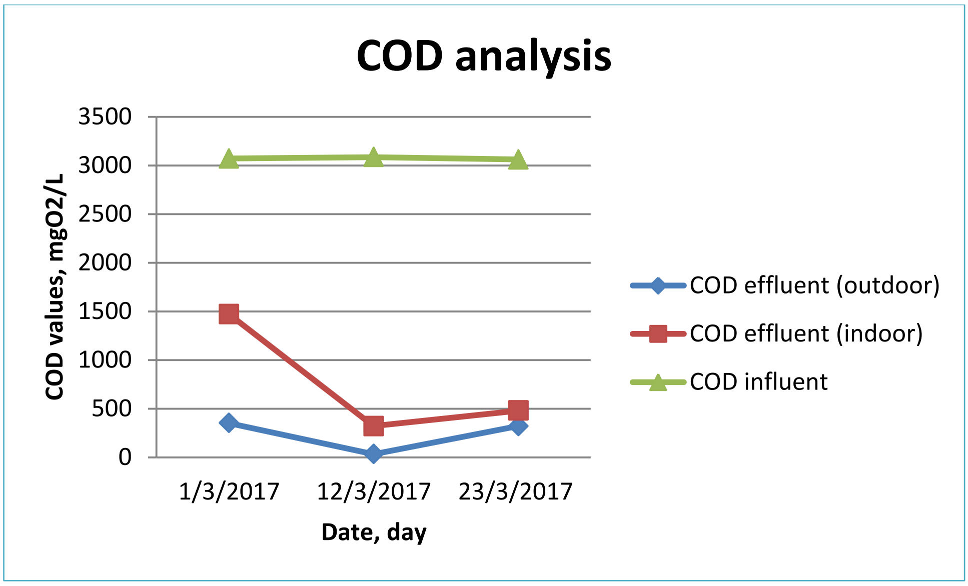

Figure 4.2: Results of COD analysis thru time

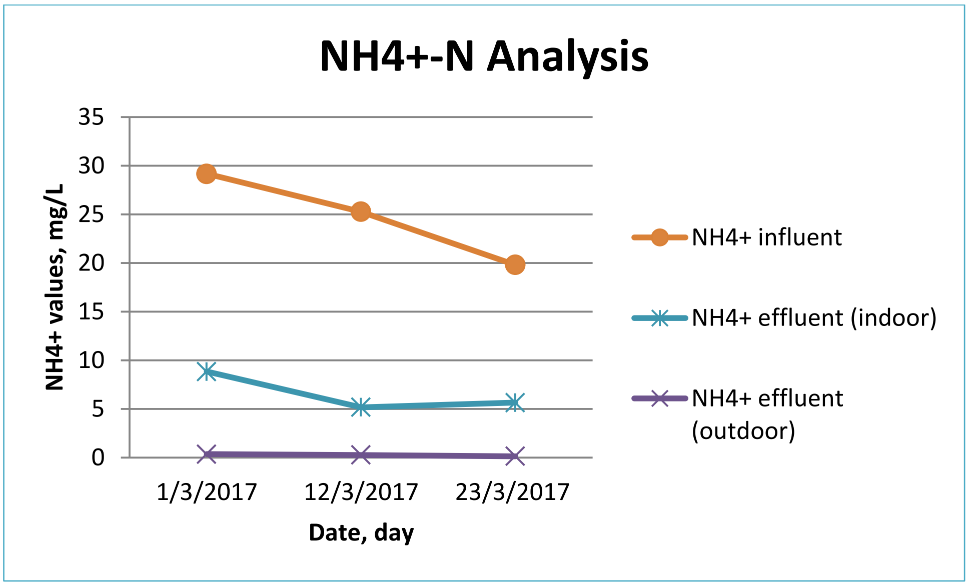

Figure 4.3: Results of NH_4^+ -N analysis thru time

4.2. COD, BOD and NH_4^+-N Removal Efficiency

COD, BOD and NH_4^+-N removal efficiency in the outdoor container was much better than that in the indoor container. So that the solar radiation, wind and other external factors are very important to achieve the desired wastewater treatment. Table 4.5. shows the removal efficiencies for COD, BOD and NH_4^+-N in two containers. DW yield is not significantly dependent on COD concentration in the effluent but on ammonium concentration and retention time [22]. In almost cases removal efficiency of COD is less for the higher ammonium concentration [22].

Table 4.5.: Pollutants removal efficiency in the two containers.

System	BOD%	COD%	NH_4^+-N	Conductivity
Effluent (indoor)	41.1%	75.3%	65.3%	42%
Effluent (outdoor)	80.7%	92.4%	98.6%	59.3%

4.3. Conductivity Reduction

In spite of the high conductivity in the influent 1.5 µS/cm, *Lemna gibba* was still able to grow. After 10 days retention time, during the experiment, it was noticed that there was a slight decrease in the EC. The conductivity reduction was about 42% at the indoor container, and about 59.3% at the outdoor container (Table 4.5).

4.4. DW Harvesting

In general, during the study, it was noticed that in sunny and warm days, DW growth was greater, so it was harvested more frequently. Unlike cold and windy days, DW growth was very slow and some of it had become yellow. During that time, the growth of DW at the indoor container was less than DW at the outdoor container. When DW was harvested regularly this helps it to grow better. As it happened at the Al-Arroub station, when the DW was harvested, it grew better as shown in Figure 4.4. Other thing noticed during the study was that when the water pollution concentration increased, the DW grew larger.

Figure 4.4: DW at Al-Arroub station after continuous harvesting

4.5. Water Content in DW

Table 4.6 shows water content in the harvested DW. The quantity of harvested DW from the outdoor container was always more than that from the indoor container, hence its growth was greater. Drying the DW in the oven had emitted bad odor, due to release of nitrogen and N oxides, so drying must be carried out in open air under sun.

Table 4.6: Water content in the indoor and outdoor harvested DW

Date	**Indoor DW**			**Outdoor DW**		
	Wet weight (g)	**Dry weight (g)**	**Water content (%)**	**Wet weight (g)**	**Dry weight (g)**	**Water content (%)**
26/2/2017	26.22	1.85	93.6	31	2.45	92.1
12/3/2017	23	1.5	93.4	29.5	2.23	92.4
20/3/2017	41	2.89	92.9	54.66	4.09	92.5
27/3/2017	20.22	1.26	93.7	60.25	4.44	92.6
Average	**93.4%**			**92.4%**		

4.6. Visual Observations

Initially, when the DW was brought, its color was almost green to black as shown in Figure 4.5. a. But after introduced to the container, its color turned clear green as shown in Figure 4.5.

b. As DW flourished and expanded in WW, it was noticed that the DW in the shade (indoor container) turns grayish.

(a) (b)

Figure 4.5: (a) Raw DW before placed in WW for the experiment, (b) DW after placed in WW for the experiment

During the whole period of the experiment, it was noticed that the root's length of the *Lemna gibba* was shorter in the indoor container than in the outdoor container as shown in Figure 4.6. it was about 2 cm in the indoor container, and about 5 cm in the outdoor container. In outdoor container roots of *Lemna gibba* were intertwined with each other, because of their excessive length, for this reason it was difficult to harvest.

(a) (b)

Figure 4.6: (a) The DW in the indoor container, (b) The DW in the outdoor container

4.7. Chicks Growth Rate

The following Table 4.7 shows the weights for chicks that were fed with a mixture of DW and fodder over the days. And Table 4.8 shows the weights for chicks that were fed only with ordinary fodder over the days. The two tables show that the growth rate of the chicks fed with the mixture is faster than those fed with the ordinary fodder (See Figure 4.7 and Figure 4.8 below). From Figure 4.9 and Figure 4.10, it was clear that the growth rate of chicks that ate the mixture of DW and fodder was more systematic than the growth rate of chicks that ate fodder only. During the study, it was observed that the chicks that ate fodder only were appetite for food was few and variable. Unlike the chicks that eat the mixture, the food was eaten regularly without change. Other things that the female chicks grow less than males chicks; male consumption of food is higher.

Figure 4.7: Chicks that fed with ordinary fodder only.

Figure 4.8: The chicks that ate mixture of DW and fodder

Table 4.7: Weights of chicks that ate DW/fodder mixture (50% DW: 50%fodder) over the days

Date	Chick #1 (g)	Chick #2 (g)	Chick #3 (g)	Chick #4 (g)	Notes
19/3/2017	47	45	48	49	Age 1 day
8/4/2017	300	310	195	270	Age 19 days
14/4/2017	620	625	410	535	Age 25 days
20/4/2017	1000	920	730	835	Age 31 days
26/4/2017	1450	1350	1200	1275	Age 37 days
28/4/2017	1730	1510	1370	1440	Age 39 days

Table 4.8: Weights of chicks that ate only ordinary fodder over the days.

Date	Chick #1 (g)	Chick #2 (g)	Chick #3 (g)	Chick #4 (g)	Notes
19/3/2017	49	47	45	45	Age 1 day
10/4/2017	372	300	295	260	Age 21 days
23/4/2017	616	566	338	400	Age 34 days
30/4/2017	785	600	495	430	Age 41 days

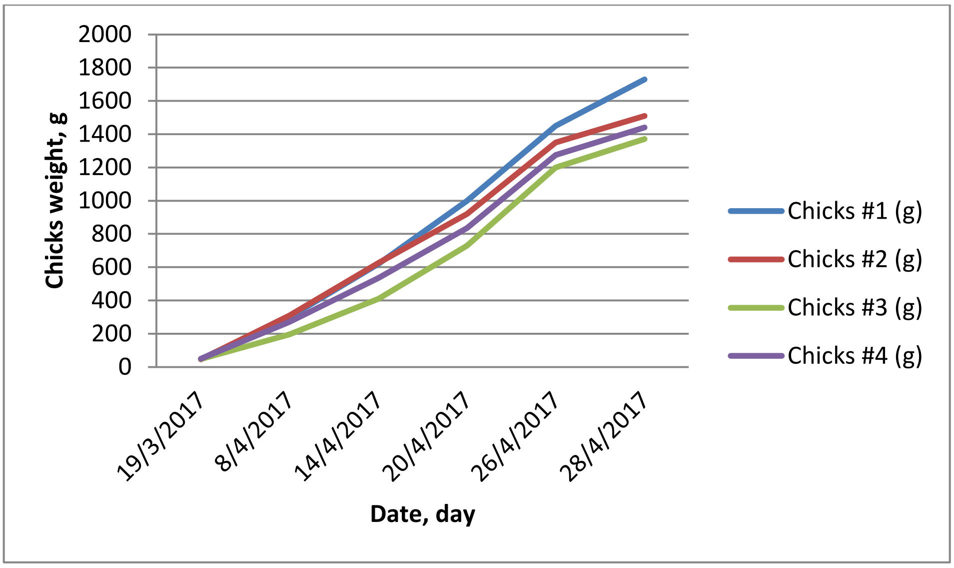

Figure 4.9: Weights' relationship between mix-fed & fodder-fed chicks thru time.

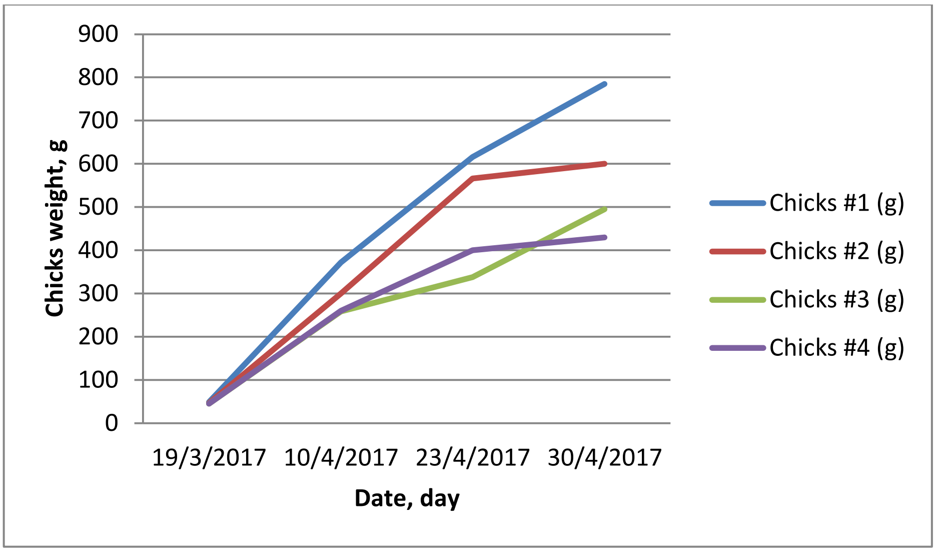

Figure 4.10: Weight growth rate for fodder-fed chicks thru time.

CHAPTER FIVE

Conclusions and Recommendations

5.1 Conclusions

This section lists the key conclusions of this research. In light of the analyses and the corresponding discussions, the followings are the conclusions:

1. DW had doubled its biomass every two days in ideal conditions. This is faster than almost any other higher plants.
2. The resulted effluent from DW treatment was cleaner with the concentration of pollutants were in allowable range (COD, BOD, ammonium, pH, conductivity and microbiological tests).
3. In general, more than 10 days hydraulic detention time would appear to be the minimum guideline for DWT to achieve acceptable discharge standards and acceptable pathogen reduction in sewage treatment.
4. Fresh DW contains around 92% of water.
5. The COD tests indicate that effluent quality meets the standards (WHO) in order to be used in agriculture, this indicates that the DW treatment system is a cost-effective system.
6. The harvested DW was used as animal fodder due to its high protein content and low fiber content.
7. The sun high radiation is very important for DW growth and dehydration.
8. It was observed that the DW flourished and expanded in wastewater and when the wastewater pollutants' concentration increased, the DW grew larger.
9. Chicks that were fed with the mixture of DW and fodder grew larger than the chicks that were fed with ordinary fodder only. This shows that the use of DW as a fodder is greatly feasible.

5.2 Recommendations:

Many recommendations can be drawn out from this research. Listed below are some of these recommendations:

1. The best way to dry the DW is under the sun.
2. Usage of a raised non-rustable mesh to dry the DW, so that the wind can circulate above and below, in order not to get rotten and allow maximum water evaporation. The mesh should not be ferrous, but be plastics, sackcloth or textile.
3. To get optimum fodder quality, it is recommended to mix the dried DW with ordinary fodder in a suitable percentage' mixture. We recommend to follow this rhythmus in feeding the chicks: For chicks from 0-2 weeks the ratio of DW: ordinary fodder is 1:1, from 2-4 weeks is 3:1, for more is 4:1.
4. The best place to build a DW treatment plant in Palestine would be Jordan Valley (e.g. Jericho area) as this area enjoys high temperature essential for better DW growth, DW drying and using it as a fodder for chicks, which in turn also needs the sun for better growth.
5. For making better fodder, we suggest to make dried DW as capsules, grinding the DW and making it as granules, then inserting it as input material into the fodder plant.
6. Lower depth is recommended more than higher depth for DW treatment pond. We think that a depth of not more than 1m is OK.
7. Building settling pond before DW pond to remove suspended solids and larger things from wastewater is a must.

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