TREATMENT AND NUTRIENT RECOVERY OF LANDFILL LEACHATE BY ALGAE AND DUCKWEED

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(2018)

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By

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A thesis submitted in partial fulfillment of

the requirement for the degree of

Doctor of Philosophy

in

Environmental Science

Institute of Environmental Sciences and Engineering (IESE)

School of Civil and Environmental Engineering (SCEE)

National University of Sciences and Technology (NUST)

(2018)

In the Name of Allāh, the Most Gracious, the Most Merciful

Dedication

This PhD work is dedicated to ***Allah Almight****y whose mercy and help brought me to this destination*

and

my ***father (Nazir Ahmad)*** *for his prayers and moral support*

Acknowledgements

Foremost, I express my gratitude to Allah Almighty who made me able to design and succesuffly complete this PhD study.

I would like to express sincere gratefulness to my supervisor Dr. Muhammad Anwar Baig, Professor, Institute of Environmental Sciences and Engineering (IESE), SCEE, NUST. His continuous support to my PhD study and research, patience, motivation, enthusiasm and immense knowledge momentously helped me to go ahead and complete the PhD requirements successfully.

Besides the supervisor, I would like to thank the rest of my Guidence and Examination Committee (GEC): Dr. Muhammad Arshad (IESE, NUST), Dr. Nasar-um-Minullah (ASAB, NUST) and Dr. Muhammad Irfan Khan (IIUI) for their guidance, encouragement and insightful comments. Also thanks to my thesis evaluators: Dr. Arshad Ali (NARC, Islamabad), Dr. Muhammad Jamal Khan (Agri Univesrsity, Peshawar), Dr. Lena Ma (University of Florida, USA) and Dr. Mohd Suffian (University of Malaysia) for their valuable comments and suggestions to improve the thesis. Also thanks to my coursework teachers Dr. Ishtiaq A Qazi, Dr. Imran Hashmi, Dr. Faheem Khokhar, Dr. Ali Awan and Dr. Muhammad Arshad for imparting valuable knowledge and techniques related to the subject.

My thanks goes to Higher Education Commision (HEC) of Pakistan for providing financial support (Indiginous PhD Fellowship) to complete this PhD study.

I feel great pleasure to express special thanks to my friend Imran Ahmad Rao for his moral support throughout the period. Thanks to my friend Dr. Muhammad Safdar and other colleagues for their continuous support and encouragement.

My special acknowledgement is to my son (Muhammad Bin Jamshaid), brother and sisters and rest of the family for their moral support.

JAMSHAID IQBAL

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LIST OF ABBREVIATIONS

$\mu S\ cm^{-1}$	Micro Siemens per Centimeter
APHA	American Public Health Association
AS	Activated sludge
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DM	Dry Mass
EC	Electrical Conductivity
GR	Growth Rate
LSD	Least Significant Difference
NEQS	National Environmental Quality Standards
NH_4^+-N	Ammonium-Nitrogen
NS	Non-significant
NUST	National University of Science and Technology
NTU	Nephelometric Turbidity Unit
SBR	Sequencing Batch Reactors
SD	Standard Deviation

TDS	Total Dissolved Solids
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus
UASB	Upflow Anaerobic Sludge Blanket
UNDP	United Nations Development Programme
UNEP	United Nations Environment Programme
VFAs	Volatile Fatty Acids
XOCs	Xenobiotic Organic Compounds
NO_3^{-1}	Nitrate
o-PO_4^{-3}-P	Orthophosphate-Phosphorus

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LIST OF PUBLICATION FROM THIS RESEARCH STUDY

Journal Publications

1. Iqbal, J. and Baig, M.A. (2017). Nitrogen and Phosphorous Removal from Leachate by Duckweed (Lemna minor). Environment Protection Engineering. 43 (4): 123-134
2. Iqbal, J., Saleem, M. and Javed, A. (2017). Effect Of Electrical Conductivity (EC) on Growth Performance of Duckweed at Dumpsite Leachate. International Journal of Science, Environment and Technology, 6 (3): 1989 – 1999
3. Iqbal, J. and Baig, M.A. (2017). Heavy Metals Removal from Dumpsite Leachate by Living Algae. Under review by International Journal of Environmental Science and Technology, Paper ID: JEST-D-17-02086

Conference Publications

1. Iqbal, J. and Baig, M.A. (2016). Effect of Nutrient Concentration and pH on Growth and Nutrient Removal Efficiency of Duckweed (Lemna Minor) From Natural Solid Waste Leachate. International Journal of Health and Medicine, 1(3): 1-7.
2. Iqbal, J. and Baig, M.A. (2017). Growth Performance of Duckweed on Leachate of Open Waste Dumps under Natural and Controlled Conditions. 14th International Phyto technologies Conference, Montreal, Canada

ABSTRACT

Currently, sustainable management of leachate produced from open waste dump sites, is one of the biggest concerns in developing countries and Pakistan is no exception. Aquatic plants and algae having potential to remove pollution and uptake nutrients from wastewater can be cost-effective and technically-feasible options for leachate treatment. Based on this fact, the overall objective of present study was to identify the optimum operational parameters (leachate concentration, pH, electrical conductivity (EC), plant density and heavy metal concentration in leachate) for algae-duckweed based leachate treatment system under climatic conditions of Islamabad, keeping in view the sustainable post usage of duckweed plants. For this purpose, series of six experiments were conducted by growing duckweed (*Lemna minor)* and mixed algae (comprising of three genera*)* on leachate. Out of this, five experiments were conducted in open environment using dumpsite leachate while, last experiment was performed under controlled conditions using synthetic leachate so as to compare the results of natural and artificial systems. Results indicated that 30 % initial leachate concentration (chemical oxygen demand (COD): about 1,700 mg L^{-1}), pH 7.1, EC 1,000 µScm^{-1}, initial duckweed density of 50% and harvesting frequency of about 2.5 days are optimum for COD reduction, nutrient removal & uptake, and growth of duckweed on leachate. Under these conditions, duckweed was able to reduce COD by 61-67% from leachate, which corresponded to removal rates of total Kjeldahl nitrogen at 152-187mg m^{-2} d^{-1}) and total phosphorous at (90-109 mg m^{-2} d^{-1}). The growth rates of duckweed were 5.5-6.8 g m^{-2} d^{-1} under optimum conditions.

Mixed algae showed maximum biosorption capacity of 5.09, 5.85, 7.03, 3.34 and 5.73 mg g^{-1} for Fe, Cu, Pb, Cr and Zn respectively at 10 mg L^{-1} initial metal concentration in leachate with algal dose of 0.8 g L^{-1}. A comparison of experiments on dumpsite and synthetic leachate revealed that duckweed decreased COD and nutrients more efficiently from dumpsite leachate under natural climatic conditions compared to grown on synthetic leachate under similar environmental conditions. However; the amount of N and P taken up by duckweed was about 14-18% and 34-36% more from synthetic leachate compared to dumpsite leachate. Duckweed growth rate (5.5 to 6.3g m^{-2} d^{-1}) was also observed high at synthetic leachate. Results of this research provide a basis to establish an algae-duckweed based leachate treatment system by presenting the optimum working conditions for such system that can be cost effective and feasible option even the landfills are put in place in near future.

Chapter 1

INTRODUCTION

1.1. BACKGROUND AND PROBLEM STATEMENT

Worldwide, untreated leachate poses serious threat to surface & ground water quality, human health, flora and fauna (Akinbile et al., 2012). At open dump sites, leachate is produced by biochemical reactions within waste stream due to interstitial water content of waste mass and percolation of rainwater through solid waste layers (Kalčíková et al., 2011). Depending on nature of solid waste, climatic conditions (temperature, sunlight and precipitation), solid waste management practices and age of dump site, leachate may contain a variety of pollutants including nitrogen, phosphorous and heavy metals (Edmundson and Wilkie, 2013).

Leachate generated from open dump sites has high pollution level and most of the parameters of leachate quality including pH, EC, biochemical oxygen demand (BOD), COD, phosphate (PO_4^{-3}), nitrate (NO_3^{-1}), chloride (Cl^-) ions and heavy metals usually exceed the permissible limits of wastewater quality standards (Iqbal et al., 2015). Heavily polluted leachate from dumping sites can mix with groundwater through surface runoff and infiltration, consequently being one of the major reasons of rapidly increasing groundwater pollution. Significant quantity of leachate from open dump sites also moves to nearby surface water bodies hence, becoming an emerging cause of surface water pollution (Akhtar and Zhonghua, 2013; Maqbool et al., 2011).

Recently, it has become major concern in the world to impose more stringent legal requirements related to management and treatment of leachate (Renou et al., 2008). Currently, in developed countries many advanced methods of leachate treatment are in practice. However, for developing countries it is required to develop more cost-effective and comprehensive solutions for leachate treatment.

Phytoremediation by aquatic plants and algae is among the least cost and efficient methods of wastewater treatment (Farrell, 2012). Growing of aquatic plants on waste media has two-fold benefits: i) treatment of polluted media, and, ii) conversion of nutrients in media into potentially useful biomass of aquatic plants (Cheng et al., 2002). Having potential of wastewater treatment, aquatic plants and algae can also be cost-effective and efficient means of leachate treatment.

1.2. AIM AND OBJECTIVES

This research aimed at providing an input for sustainable management program of leachate produced from open dumping sites. Based on natural potential of aquatic plants and algae for wastewater treatment, the core objective of this study was to identify the optimum operational parameters for algae and duckweed-based leachate treatment system.

The specific objectives of the study were as follows: -

- To investigate nutrient (nitrogen and phosphorous) removal and uptake, COD reduction and growth of duckweed on dumpsite and synthetic leachate under varying conditions of:

- Initial leachate concentration;
- pH;
- Electrical Conductivity (EC);
- Initial duckweed mat density.

- To investigate the removal dynamics of heavy metals (Cu, Zn, Cr, Pb, and Fe) from leachate by mixed algae comprising three strains under varying conditions of initial metal concentration in leachate and algal doses.
- To compare the results of experiments on natural leachate-duckweed system with those of synthetic system.

Algae in this research was used as the first step for heavy metal removal from leachate so as to ensure that duckweed grown on next steps is free from heavy metal pollution thereby, increasing the options for safe post uses of duckweed after leachate treatment. Figure 1.1 provides the overall picture of this research and research questions questions to be answered by this study.

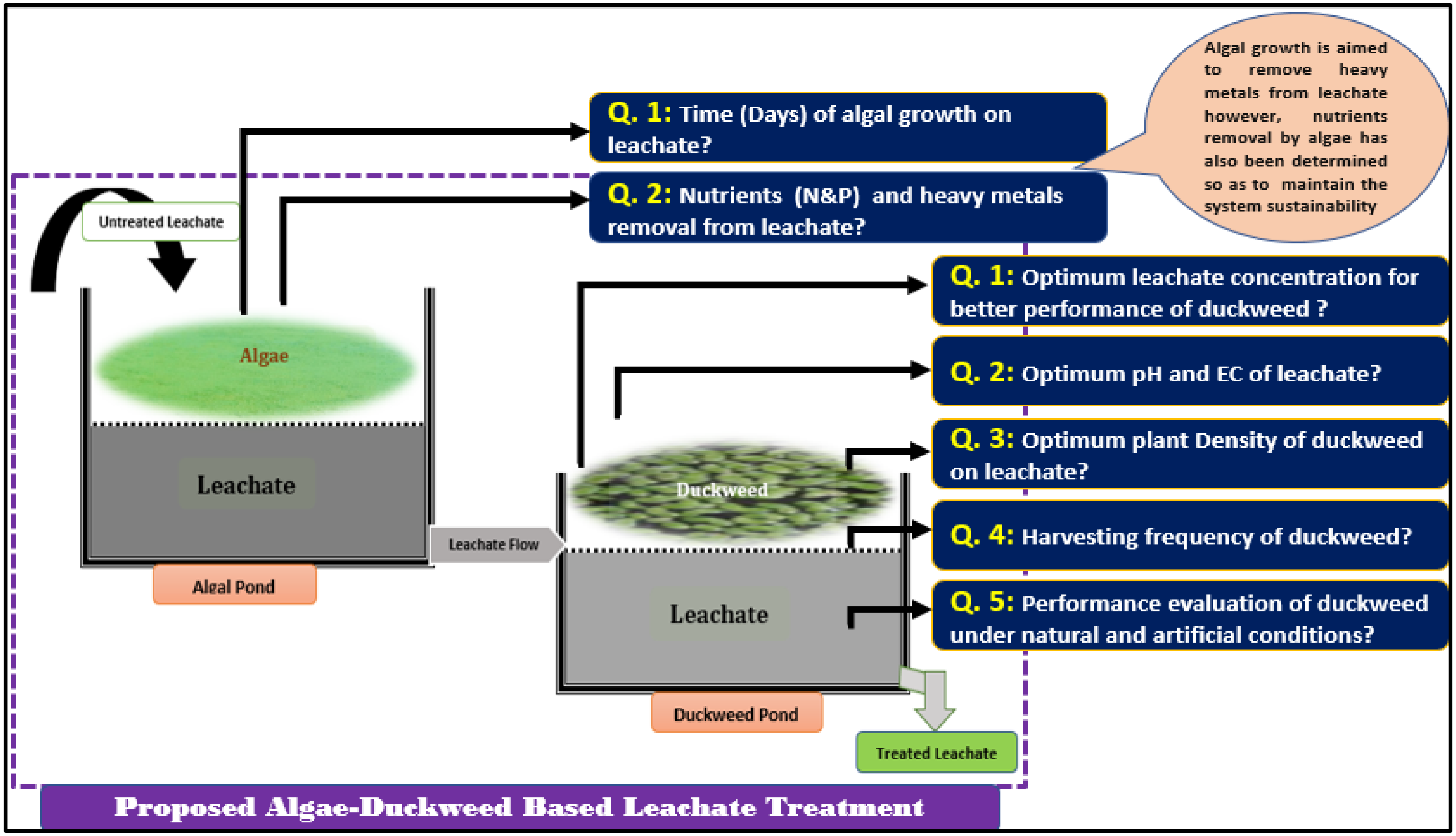

Figure 1.1: Overall picture and research questions

1.3. SIGNIFICANCE OF STUDY

Results of this study signified that a cost-effective and simple system of leachate treatment may be developed by growing duckweed and algae on leachate under optimum conditions.

So far, technical viability of such comprehensive natural system of leachate treatment has not been investigated in the world. Results of this study may be useful for developing countries where open dumping of solid waste is common along with the uncontrolled production of large amounts of leachate. Till the introduction of engineered landfills, full scale leachate treatment system at open waste dump sites can be introduced based on this study.

Chapter 2

LITERATURE REVIEW

Literature review for this study has been divided in five main parts: i) Discussion on solid waste on which the overall quantity and quality of leachate is dependent; ii) Discussion on leachate including its key characteristics and environmental threats due to leachate; iii) Commonly used methods of leachate treatment with main focus on biological treatment methods and phytoremediation, upon which this research study is based; iv) Discussion on plants and algae used in this study and; v) Synthesis of literature review is provided at the end of this chapter.

2.1. SOLID WASTE CHARACTERISTICS AND GENERATION

This section provides the following information about solid wastes:-

- Important characteristics of waste;
- Solid waste generation in the world and;
- Solid waste situation in Pakistan.

Wastes are the materials that are not prime products i.e. the products produced for market and for which the initial user has no further use in terms of his/her own purposes of production, transformation or consumption, and of which he/she wants to dispose (United Nations, 1997).

Solid waste mainly comprises of waste generated from residential, commercial and industrial facilities. (Srivastava et al., 2015). Major proportion of typical solid waste is organic component (40-80% of total waste stream). Rest includes, plastic, paper, metal, clothes, ash etc. (Dhokhikah and Trihadiningrum, 2012).

During the recent years, average rate of solid waste generation is significantly increased in the world including developing countries (Karak et al., 2012). Increase in population, economic growth, rapid urbanization and improved community living standards are the key contributors in increased amounts of solid waste in developing countries (Guerrero et al., 2013). Amount of leachate as it is directly related with the amount of solid waste has also significantly increased worldwide. Efficient management of solid waste and leachate is one of the major challenges faced by the city municipalities in developing countries mainly due to lack of planning, lack of financial resources and improper waste management organization (Burntley, 2007).

According to an estimate, average solid waste generation from major cities of Pakistan is likely to reach to 73,000 tones day^{-1} by the end of 2016 (Masood et al., 2014). In Pakistan, engineered landfill sites for solid waste disposal are non-existent except a newly operated landfill site in Lahore[1]. In the absence of scientific landfill sites, open dumping of solid waste is common practice in Pakistan (UNEP, 2004). Open waste dump sites produce large amounts of leachate particularly during the rainy seasons (Ali et al., 2014) which need proper management and treatment prior to discharge into environmental media.

Besides the leachate production, other environmental and health problems associated with open dump sites include: production of dust and odor particularly during

[1] The first ever scientific waste disposal facility (Landfill site) in Pakistan has been set up in April, 2016 in Lahore over an area of 52 hectares (Dawn, Newspaper, 19th April, 2016)

winds; breeding places for bacteria, mosquitoes, flies, viruses, rats and other vermin; release of toxic gases into the atmosphere and associated health and sanitation of the communities living in the vicinity of open dump sites (Nisar et al., 2008).

2.2. LEACHATE PRODUCTION AND CHARACTERISTICS

This section provides the relevant discussion related to leachate including: i) Process of leachate production at waste dumpsites/landfills; ii) Leachate classification and its key characteristics and composition; iii) Environmental and health issues associated with leachate; iv) Existing situation of leachate production and management in Pakistan; v) Existing methods of leachate treatment with main focus on biological methods.

Leachate is considered as high strength wastewater. At open waste dumping sites, it is produced by biochemical reactions within the waste stream, due to interstitial water content of the waste mass and percolation of rainwater through solid waste layers, (Kalčíková et al., 2011; Renou et al., 2008).

Various physico-chemical and bio-chemical processes in solid waste transfer variety of pollutants from waste streams into percolating rain water (Kjeldsen et al., 2002). A typical leachate contains four major groups of pollutants; i) dissolved organic matter, ii) xenobiotic organic compounds, iii) heavy metals and iv) inorganic macro compounds (Christensen et al., 1994).

According to the age of landfill/dump site, leachate can be categorized into young, intermediate and stabilized leachate. Young and intermediate leachate are sometimes combined to single category of young leachate. Young leachate usually has the age of 5 years. In this period leachate contains high amounts of biodegradable organic compounds including; BOD (4,000 – 13,000 mg L^{-1}), Volatile Fatty Acids (VFAs), Ammonium-Nitrogen: NH_4^+-N (2,000 – 5,000 mg L^{-1}), COD (6,000 – 60,000 mg L^{-1}), and a high ratio of BOD/COD ranging from 0.4 – 0.7 (Liu, 2013).

Stabilized leachate is formed with the increase in landfill age, when microbial activities in leachate decompose the organic materials into methane (CH_4) and carbon dioxide (CO_2). In this phase, pH of leachate increases to over 7.0 and organic compounds are no more biodegradable. Stabilized leachate phase may take over 50 years or more. Stabilized leachate typically comprises high concentration of NH_4^+-N (2,500 – 5,000 mg L^{-1}), COD (5,000 – 20,000 mg L^{-1}) and BOD/COD ratio of less than 0.1 (Liu, 2013).

Quantity and composition of leachate depends on quantity and composition of waste material, age of landfill site, availability of moisture and oxygen and hydrology of waste landfill site (Aziz et al., 2004). Nutrients content of a typical leachate may be as high as 13,000 mg L^{-1} of organic nitrogen, up to 400-3000 mg L^{-1} of NH_4^+-N and 3000 mg L^{-1} of phosphate (Akinbile et al., 2012; Robinson, 2007; Aziz et al., 2011).

Major fraction of solid waste at landfill/dump sites comprises of biodegradable waste. Naturally occurring bacteria in waste stream decompose the biodegradable waste in four phases: i) aerobic phase; ii) anaerobic acid phase; iii) initial methanogenic phase and; iv) stable methanogenic phase (Kjeldsen et al., 2002). Compaction of solid waste at open dump sites has significant effect on amount of leachate produced. The quantity of leachates in older dump sites that are properly compacted may range from 25-30% of total precipitation. However, poorly compacted dump sites produce comparatively higher quantities of leachates usually reaching up to 40% of total precipitation (Szpadt, 1998).

2.2.1. Environmental and Health Concerns of Lechate

Unattended leachate from municipal waste landfills/dumpsites may pose serious impacts on human health and surrounding eco systems including surface & ground water and soil (Salem et al., 2008). Leachate from waste landfill sites infiltrate to the soil and ground water potentially effecting the quality of ambient soil and ground water resources near waste landfill sites (Mor et al., 2006). Leachate contamination, once became the part of groundwater, may travel large distances, polluting the groundwater aquifer of the other areas as well (Saarela, 2003). From open waste dumpsites, huge quantity of leachate may enter the nearby surface water bodies including river, streams and irrigation water canals and small irrigation channels, through surface runoff during rainy seasons. Heavy metals including: Cu, Fe, Zn, Pd, Cr and Ni and xenobiotic organic compounds (XOCs) including: xylenes, ethylbenzene, benzene, toluene and tetrachloroethylene present in the leachate may cause significant long-term effects on receiving soils and surface & ground water resources. Certain pollutants in leachate have ability to bio accumulate in living tissues through food chain (Kjeldsen et al., 2002; Nagarajan et al., 2012). Health impacts of leachate on human and aquatic animals have also been demonstrated by many researchers (Aiman et al., 2016; Pande et al., 2015; Toufexi et al., 2013; Baderna et al., 2011; Longe and Balogun, 2010). Recently, it has become major concern worldwide to impose more stringent environmental requirements related to leachate management (Renou et al., 2008).

2.2.2. Existing Situation of Leachate Production and Management in Pakistan

Currently less amount of literature is available related to detailed composition and characteristics of leachate in Pakistan except some isolated work mainly describing the instantaneous characteristics of leachate at certain places. In Pakistan, engineered landfill sites for solid waste disposal are non-existent except a newly operated landfill site in Lahore.

It has been reported by few studies conducted in Pakistan, that leachate produced from open dump sites has high pollution level and most of the parameters of leachate including; pH, EC, BOD_5, COD, heavy metals and various ions including PO_4^{-3}, NO_3^{-1}, Cl^- usually exceed the acceptable limits of National Environmental Quality Standards (NEQS) (Maqbool et al., 2011; Karim, 2010).

Studies report that leachate had significant impacts on ambient soil and ground & surface quality in some areas of Pakistan (Khalid et al., 2011; Akhtar and Zhonghua, 2013; Maqbool et al., 2011). Table 2.1 provides the values of some important quality parameters of leachate produced from open dump sites in some cities of Pakistan, comparing it with NEQS.

At present, leachate produced from open dump sites in Pakistan requires efficient management and treatment so as to avoid the harmful effects of leachate on ambient soil and ground & surface water quality and human health.

Table 2.1: Average leachate composition of some cities in Pakistan

Parameters	**Range of Values (mg L^{-1})**	**NEQS (mg L^{-1})**
pH	5 - 9.1	6-9
BOD_5	114-1987	80
COD	100-10,865	150
Conductivity	531-27,440	NA
TDS	230-13.5×10^5	3,500
Lead	0.52-2.57	0.5
Cupper	0.30-2.7	1.0
Chromium	1.41-3.76	1.0
Iron	2.1-17	8.0
Total Nitrogen (TN)	400	N/A
PO_4^{-3}	79.4-92.4	N/A
NO_3^{-1}	49.3-64.2	40 (NH_3)
Cl^{-1}	268-286	1.000

Karim, 2010; Khalid et al., 2011; Akhtar and Zhonghua, 2013; Maqbool et al., 2011

2.3. LEACHATE TREATMENT METHODS

Currently many physical, chemical, biological and combination of two or more leachate treatment methods are used in the world. Recently, some advanced leachate treatment methods have also been introduced in many countries including advanced oxidation process, electrolysis-Fenton process, solar photo-Fenton processes and nanoparticle based processes (Chemlal et al., 2014; Amor et al., 2015; Wang et al., 2016). Selection of suitable leachate treatment method depends on many factors including; existing practices of waste disposal, nature and location of landfill/dump sites, local weather pattern, composition of waste and leachate and economic aspects related to leachate management and treatment (Liu, 2013). Figure 2.1 provides widely used leachate treatment methods in the world.

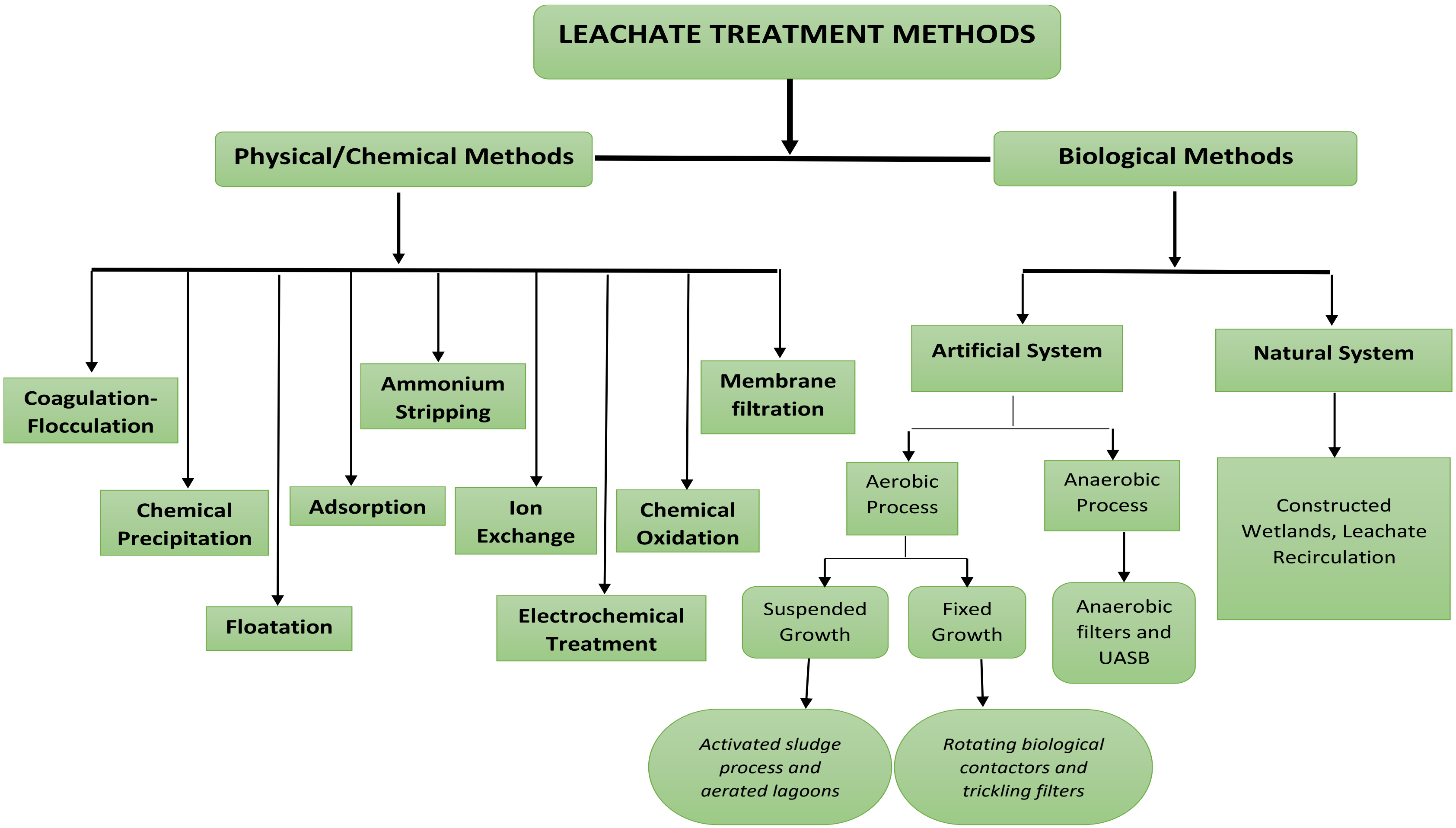

Figure 2.1: Commonly used methods of leachate treatment

Brief about existing methods of leachate treatment is provided below: -

2.3.1. Physical/Chemical Methods

Main focus of this thesis is biological method of leachate treatment however, generally used physical/chemical methods have also been described briefly.

2.3.1.1. Coagulation-flocculation

Coagulation-Flocculation is based on addition of coagulants such as; aluminum and ferrous sulphates, ferric chloride or ferric chlorosulphate to remove non-biodegradable organic compounds and heavy metals from leachate. In this method, electric repulsion effects between particles is reduced by coagulant and particles unite to facilitate precipitation (Li et al., 2010; Assou et al., 2016). By coagulation-flocculation method, about 30% and 86% of COD reduction and about 74% and 98% of heavy metal removal has been reported (Kurniawan, 2011).

2.3.1.2. Chemical precipitation

Chemical precipitation method requires the addition of some precipitation reagent into the leachate. Precipitation reagent precipitate the heavy metals and non-biodegradable organic compounds. Particles from precipitated mixture can be removed by filtration. In addition to non-biodegradable organic compounds and heavy metal removal, chemical precipitation can also be used to remove NH_4^+ -N, phosphorus and other inorganic compounds from leachate.

Metal removal efficiency by chemical precipitation methods depends on amount and concentration of precipitation reagent and metal concentration of leachate. The ammonium removal of 90% and 98% has been reported by this method when initial concentration of ammonium in leachate ranges from 1,380 mg L^{-1} to 5,618 mg L^{-1} (Kurniawan, 2011).

2.3.1.3. Floatation

In the process of Flotation, solid particles such as; ions, colloids, microorganisms, macromolecules, and fibers float over the liquid surface when gas bubbles attach to the particles in suspension (Bashir et al., 2015). Floatation is basically used for waste water treatment however, Zouboulis et al. (2003) first time used floatation method for removal of non-biodegradable compounds (humic acid) from landfill leachate and achieved about 60% removal of humic acids under optimized conditions.

2.3.1.4. Adsorption

In the process of adsorption, the dissolved material present in leachate is adsorbed on the surface of adsorbent such as, powdered and granular activated carbon etc. (Geenens et al., 2001). About 50-70% removal of ammonia nitrogen and COD from landfill leachate have been reported by activated carbon adsorption (Amokrane et al., 1997). Adsorbents other than carbon such as vermiculite, zeolite and kaolinite have also been proven to have almost similar treatment efficiency as of activated carbon (Dollar et al., 2016).

2.3.1.5. Ammonium stripping

Ammonium stripping is widely used for removal of ammonia nitrogen from landfill leachate at high pH levels (Marttinen et al., 2002). A study shows that ammonia stripping can remove about 85% to 95% of ammonia nitrogen from leachate when initial concentration of NH_4^+-N in leachate ranges from 220 mg L^{-1} to 2,215 mg L^{-1} (Kurniawan, 2011).

2.3.1.6. Ion exchange

Ion exchange involves interchange of ions between liquid and solid phases to remove metal impurities in leachate. Ion exchange is also used to remove ammonia and humic substances from leachate (Fettig, 1999). It has been reported that ion exchange can remove about 92% ammonia nitrogen and about 90% and 99% of heavy metals from leachate (Bashir et al., 2010; Majone et al., 1998). Ion exchange method has limited uses due to high operational cost and pretreatment of leachate required (Kurniawan, 2011).

2.3.1.7. Electrochemical treatment

Electrochemical treatment is based on the electronic degradation for breakdown of recalcitrant substances in leachate (Labiadh et al., 2016). This method is most commonly used in Brazil and France. The COD and NH_4^+-N removal of about 73% and 49% respectively have been reported by electrochemical treatment of leachate when initial concentrations of these pollutants in leachate were 1,855 mg L^{-1} and 1,060 mg L^{-1} respectively. Electrochemical treatment also has lesser application in leachate treatment mainly due to its high costs (Kurniawan, 2011).

2.3.1.8. Chemical oxidation

Chemical oxidation process involves the direct or indirect reaction of oxidants with the pollutant in leachate (Jung et al., 2017). Commonly used oxidants include;

Ozone, chlorine, potassium permanganate or calcium hydrochloride. Research shows that chemical oxidation can remove about 49% and 51% of COD from biologically pretreated stabilized leachate (Oulego et al., 2016).

2.3.1.9. Membrane filtration

Membrane filters can remove particulates, microorganisms and organic materials from liquid. Nano filtration (NF), reverse osmosis (RO), microfiltration (MF) and Ultrafiltration (UF) are commonly used types of membrane filtration. COD reduction of 62% to 80% have been reported by membrane filtration process (Hashemi and Khodabakhshi, 2016; Xie et al., 2014).

2.3.2. Biological Methods of Leachate Treatment

Biological methods exploit the natural potential of living organisms such as bacteria, algae, fungi and aquatic plants for degradation, absorption or adsorption of various types of organic and inorganic pollutants in liquids (leachate and wastewater etc.) (Wiszniowski et al., 2006; Xu et al., 2010). As shown in Figure 2.1 biological leachate treatment methods can be divided into two broad categories of artificial and natural treatment methods.

Artificial biological treatment methods involve some physical/chemical interventions in natural biological systems. All artificial biological treatment methods involve either aerobic or an-aerobicprocess of treatment.

Aerobic treatment requires continuous supply of oxygen. Organic material in wastewater is used as the source of energy by microorganisms. Aerobic treatment methods are widely used in the world due to cost effectiveness and high efficiency (Grady Jr et al., 2011). Suspended growth and fixed growth biomass are two major types of aerobic treatment process. Examples of suspended growth system include: activated sludge process and aerated lagoons. Fixed growth biomass is mainly used in rotating biological contactors and trickling filters (Connolly et al., 2004).

Anaerobic treatment converts the organic material in leachate to CH_4, CO_2 and other metabolites in the absence of oxygen. Anaerobic process produces comparatively less amount of sludge but generally have low rates of reactions. Optimum temperature required for anaerobic treatment is 35 °C (Renoua et al., 2008). Anaerobic treatment is most suitable for concentrated leachate. Biogas produced in this method can be reused making the process cost effective. Significant ammonia and COD reduction can be achieved by anaerobic treatment methods of leachate (Kheradmand et al., 2010; Aziz et al., 2010). Anaerobic filters and Up flow Anaerobic Sludge Blanket (UASB) are the examples of anaerobic treatment method. Main focus of this thesis is natural biological method of leachate treatment however; some commonly used artificial biological methods have also been described briefly.

2.3.2.1. Activated sludge process

Activated sludge (AS) is most commonly used method of wastewater and leachate treatment. Active bacterial floc along with oxygen is added to aeration tank in the form of activated sludge and organic matter in leachate is converted to carbon dioxide, water and new microbial biomass and sludge is separated from leachate. Some portion of separated sludge is fed back to treatment tank and remaining is disposed of (Yabroudi et al., 2013).

The COD and ammonium nitrogen removal of about 95% has been reported by this method when initial concentration of COD and NH_4^+-N ranges from 1,000 to 24,000 mg L^{-1} and 115 to 800 mg L^{-1} respectively in leachate (Kurniawan, 2011).

2.3.2.2. Sequencing batch reactors (SBR)

SBR is the form of AS process using one operation tank by sequence of stages. It is commonly used to remove organic matter and solids in leachate. SBR system is generally associated with low costs, high removal efficiencies and easy to operate. Nitrogen removal about 90-100% and COD reduction up to 45% from leachate has been reported by SBR process ((Morling, 2008; Kurniawan, 2011).

2.3.2.3. Nitrification-denitrification

Nitrification is the process through which oxidation of ammonium into nitrate occurs with the help of nitrifying bacteria. Nitrification is two-step oxidation process: i) conversion of ammonium to nitrite by ammonium oxidizer bacteria and; ii) conversion of nitrite to nitrate by nitrite oxidizer bacteria (Wang et al., 2005). Simplified equation of nitrification reaction is as below: -

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad \text{(Eq. 1)}$$

The optimum temperature for a typical nitrification process is 30 °C - 35 °C and optimum pH ranges from 7.5 to 8.5 (Kurniawan, 2011).

Denitrification process reduces the NO_3 to gaseous nitrogen (N_2) with the help of facultative heterotrophic bacteria in the absence of oxygen. Denitrification requires a carbon source to be added as food for denitrifying bacteria. Denitrification takes following chemical form: -

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad \text{(Eq. 2)}$$

Optimum pH for denitrification ranges from 6.0 to 8.0 and optimum temperature ranges from 5 °C to 60 °C (Kurniawan, 2011). A typical nitrification process can remove as much as of 90% of NH_4^+-N with an initial concentration of 270 to 535 mg L^{-1} of NH_4^+-N (Kurniawan, 2011).

2.3.2.4. Aerated lagoon

Aerated lagoons are the treatment ponds in which microorganisms decompose the organic matter in leachate aerobically and/or anaerobically in an artificial or natural system (Govahi et al., 2012). About 100% NH_4^+-N removal and more than 80% COD reduction has been reported with aerated lagoons when initial concentration of COD in the leachate is in the range of 104 mg L^{-1} to 175 mg L^{-1} (Kurniawan, 2011).

2.3.2.5. Trickling filters

Trickling filters method of leachate treatment use the filter media for biological nitrogen reduction. Filter media is usually made of bed rock slag or plastic (Naz et al., 2015). Trickling filters also have best known application in removal of turbidity, suspended solids, COD, BOD and ammonium from leachate (Aluko and Sridhar, 2013).

In some experiments, BOD and COD reduction of 79% and 75% respectively and ammonium-nitrogen removal of about 90% was achieved with the help of trickling filters (Ali et al., 2016; Jokela et al., 2002).

2.3.2.6. Rotating biological contactor

Rotating biological contactors (RBCs) process is similar to trickling filters. Wastewater comes into contact with a series of closely spaced circular disks mounted side by side as the media (Hassard et al., 2015). Biodegradation of leachate takes place with the help of microorganisms grown on circular disks. RBC process is more suitable for less concentrated leachate. About 95% of NH_4^+-N removal and 86% of COD reduction is reported to be achieved by RBC method when initial concentration of ammonium-nitrogen and COD was 400 mg L^{-1} and 9,254 mg L^{-1} respectively in leachate (Kurniawan, 2011).

2.3.2.7. Upflow anaerobic sludge blanket (UASB)

UASB is an anaerobic process of wastewater and leachate treatment in which leachate moves upward from bottom of the system through a blanket of biological granules (Govahi et al., 2012). It is suitable method for treatment of high strength leachate with COD more than 10,000 mg L^{-1}. Key advantage of the UASB process include; short hydraulic retention time and high treatment efficiency. Optimum pH and temperature for this process is 7.0 and 20-35 °C respectively. COD reduction efficiency of about 70-80 % has been reported by UASB method at temperature range of 20-35 °C (Abbas et al., 2009).

2.3.2.8. Anaerobic filter

Anaerobic filter consists of a rock filled biological bed like aerobic filters. Up flow and down flow anaerobic filters are the common types of this system. In this process, anaerobic microorganisms arrange in orders within the rock beds providing large contact area for leachate treatment (Martinez et al., 2014). COD reduction efficiency of 80-88% has been reported by anaerobic filter with 11, 000 and 16,000 mg L^{-1} of initial COD concentration of leachate and organic loading rates of 7 kg COD/m^3 d (Castrillón et al., 2010).

Natural methods of biological treatment usually work without any external physical-chemical interventions. Natural systems are more economical and environment friendly techniques of leachate treatment. Constructed wetlands, leachate circulation and phytoremediation are the common types of natural system of leachate treatment.

2.3.2.9. Constructed wetlands and leachate recirculation

In constructed wetlands, phytoremediation of liquid (wastewater) takes place through natural vegetation acting as bio filters to remove pollutants. This is considered an economical, simple and environment friendly method of leachate treatment. Constructed wetlands have a high COD reduction efficiency (Klomjek and Nitisoravut, 2005). Lavrova and Koumanova. (2010) reported a COD reduction of 96% in eight days, BOD removal of 92% in three days, ammonia removal of 100% in five days and total phosphorous removal of 100% in two days in a lab-scale vertical flow constructed wetland combined with leachate recirculation.

2.3.2.10. Phytoremediation

Phytoremediation is the use of green plants to remove, detoxify or immobilize the pollutants in environmental media (soil, water or sediments). Plants are used in several ways to clean or remove pollutants from soil, sediment or water. Plants degrade the organic contaminants and act as filters or traps for metal contaminants. Based on fate of contaminant in plant bodies, phytoremediation techniques can be classified into five major types as follows (Vidali, 2001; Shinde, 2013). The schematic of various forms of phytoremediation can be seen in Figure 2.2.

A. **Phytoextraction or phytoaccumulation** involves the accumulation of contaminants into the roots and aboveground shoots or leaves of plants.
B. **Phytotransformation or phytodegradation** involves the uptake of organic pollutants from soil, water and sediments and transformed to less toxic, stable or less mobile forms.
C. **Phytostabilization** reduces the movement and migration of pollutants in environmental media.
D. **Phytodegradation or rhizodegradation** involves the breakdown of contaminants due to proteins and enzymes produced by the plants or by soil organisms such as bacteria, fungi and yeast.
E. **Rhizofiltration** is the uptake of contaminants by plant roots in wetlands areas.

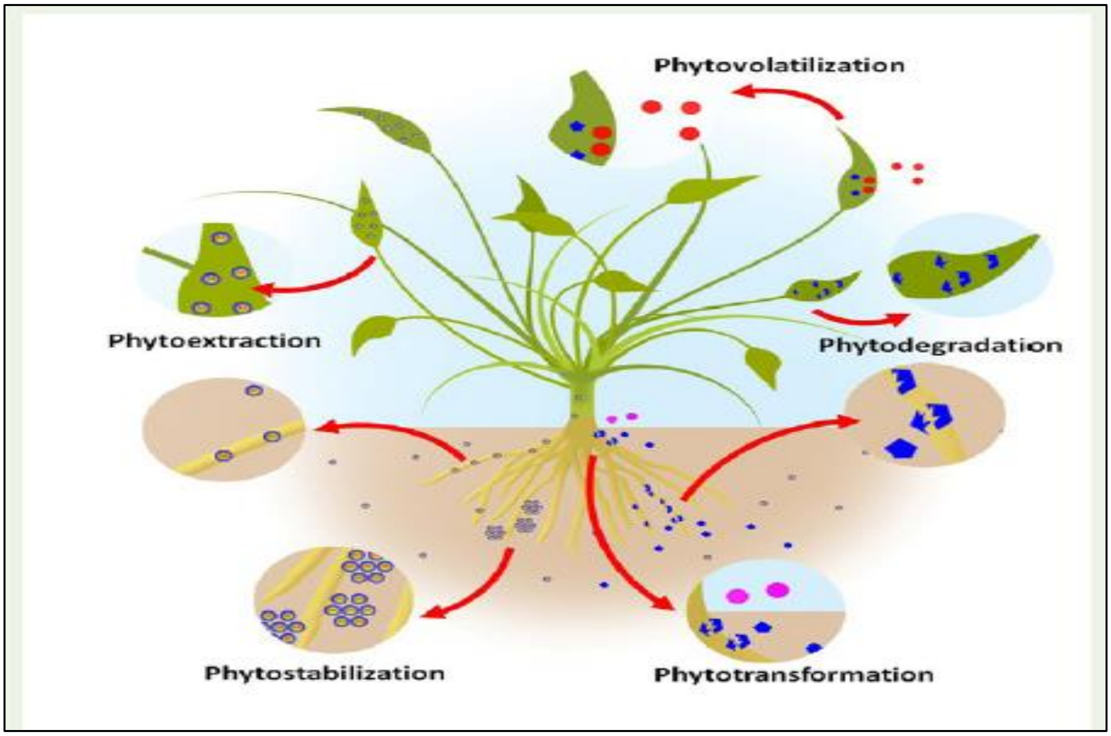

Figure 2.2: Schematic of phytoremediation process (Parmar and Singh, 2015)

2.3.2.11. Phytoremediation by aquatic plants and algae

Currently, variety of specialized plants are being used for phytoremediation of metals from soils and waters (Salt et al., 1995). Use of aquatic plants based ponds and artificial wetlands for the treatment of various types of wastewaters such as sewage, agricultural drainage water and industrial effluents is gaining interest mainly due to its cost effectiveness and ease of operations (Caicedo et al., 2000). Recently many types of aquatic plants such as duckweed, water hyacinth, water lettuce in wastewater treatment has received greater attraction (Lasfar et al., 2007; Landesman et al., 2005). Production of aquatic plants on wastewater has two-fold benefits: treatment of wastewater and, as an alternate technology, converting wastewater nutrients into potentially useful forms (Cheng et al., 2002).

Nutrient removal efficiencies and growth of plants is affected by many factors including: temperature, salinity, pH and concentration of growth media (Lu et al., 2010). In an experiment on constructed wetland using aquatic plants for synthetic leachate treatment, COD, TKN and NH4+-N removal of 66%, 67% and 72%, respectively and heavy metal removal of about 92 to 98% was achieved (Madera-Parra et al., 2015). Phytoremediation is a potential cost effective and easy to operate option for landfill leachate (Jones et al., 2006).

It is noted from literature review that currently in the world, aquatic plants are mainly used in wastewater treatment through constructed wetland (natural and artificial) system (Wojciechowska et al., 2010; Wojciechowska and Waara, 2011; Adhikari et al., 2015; Svensson et al., 2015; Dogdu and Yalcuk, 2016; Saha et al., 2017; Zhu et al., 2017).

Although, in some countries such as Bangladesh and Argentine, duckweed based farming and wastewater treatment is in progress however; the idea of integrated duckweed and algal based pond system for leachate treatment as being provided through this research, is relatively new in the world.

2.4. DUCKWEED AND ALGAE USED IN THIS STUDY

This study used the *Lemna minor* Sp of duckweed and mixed algae comprising three genera (*Ankistrodesmus, Nostoc and Anabaena)* for treatment and nutrient (N & P) recovery from leachate. Provided below is the description of duckweed and algae used in the study with particular focus on wastewater/leachate treatment and nutrient uptake potential.

2.4.1. Duckweed

Duckweed is amongst the promising aquatic plants having ability to absorb large amounts of nutrients and trace metals from eutrophicated wastewater and has high growth rates. Wastewater treatment by duckweed is owed to its high nutrients and minerals accumulation capacities into biomass and high growth rates under diverse environmental conditions. (Chaiprapat et al., 2005; Ge et al., 2012; Zhao et al., 2014).

2.4.1.1. Taxonomy and distribution of duckweed

Duckweed is a small floating macrophyte belonging to family *Lemnaceae* of monocotyledonous plants. Duckweed has 37 species belonging to 4 genera: i) *Lemna, ii) Spirodela, iii) Wolffia, iv) Wolffiella* (Cheng and Stomp, 2009). Figure 2.3 provides physical forms of duckweed plants belonging to four genera.

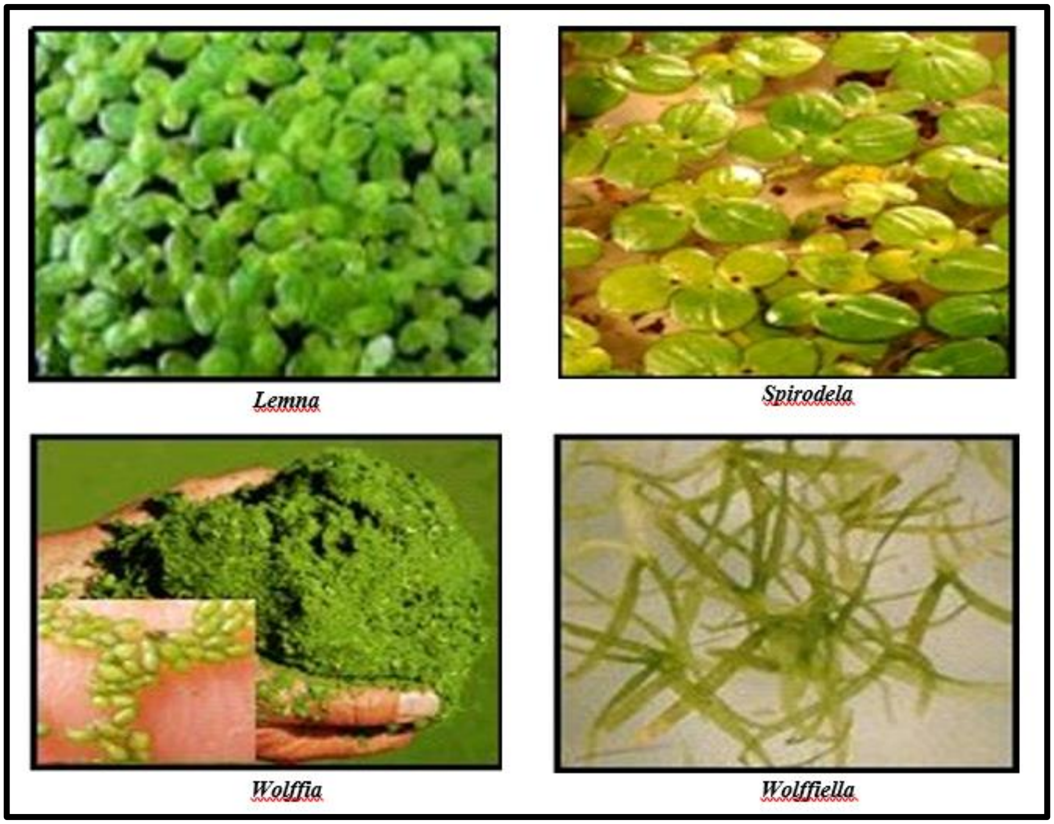

Figure 2.3: Forms of various duckweeds genera found in natural environment

Duckweed is present all over the world, however, most diverse species are found in tropical and subtropical areas. Duckweed plant is often found on wastewater ponds, swamps or ditches where abundant nutrient supply is available.

2.4.1.2. Morphology and structure

Duckweed is the simple plant having no stem or leaves. Major part of duckweed comprises a thallus called "frond" which is only a few cells thick having across length of about 1mm to less than 1cm. Frond is generally composed of chlorenchymatous cells having air pockets called aerenchyma due to which duckweed floats on Water.

Duckweed may have no root or one or more simple roots depending on duckweed species, Roots are mostly adventitious having lengths of few millimeters up to 14cm. Duckweed roots are photo synthetically active having chloroplast in it. Root hairs are not present on roots. Roots of duckweed plant help in nutrient uptake from water and also stabilize the plant (Dalu and Ndamba, 2003). Figure 2.4 provides the generalized morphology of a common duckweed belonging to *Lemna* genus.

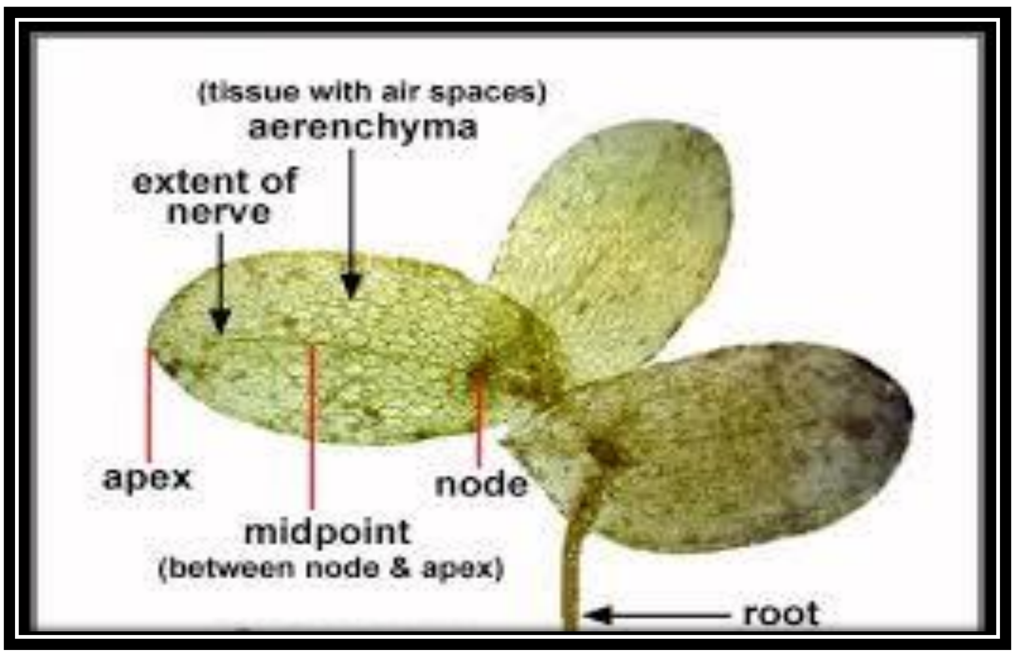

Figure 2.4: Morphology of common duckweed (Armstrong, 2001)

2.4.1.3. Duckweed plant composition

Composition of duckweed is highly variable and depends on composition of water on which it is grown. Protein constitutes the major part of duckweed biomass of most of species. Table 2.2 shows the average nutritive composition of various types of duckweed

Table 2.1: Nutritive values of various duckweed species on dry mass basis

Duckweed Type	Crude Protein %	Crude fat %	Crude Fiber %	Ash %	Sources
Duckweed (mixed/ species not mentioned by the author)	37.0	3.40	15.6	12.5	Wolverton and Mcdonald, 1979
	6.8-45.0	1.8-9.2	5.7-16.3	12.0- 27.6	Landolt and Kandeler, 1987
	35.0-45.0	- *	5.0-15.0	12.0-18.0	Mbagwu and Adeniji, 1988
	45.0**	4.0	9.0	14.0	Leng et al., 1995
	25.0-35.0***	4.4	8.0-10.0	15.0	Leng et al., 1995
	38.8	3.8	13.2	16.0	Tavares et al., 2008
Spirodela polyrrhiza	29.6	-*	-*	-*	Sutton and Ornes, 1975
	30.52	1.97	17.0	9.45	Ansal and Dhawan, 2007
Lemna minor	20.9	4.1	13.2	13.6	Tacon, 1987
	20.4	3.8	15.7	17.2	Banerjee and Matai, 1990
	28.48	4.75	10.35	-*	Ahammad et al., 2003
	18.38	2.32	-*	23.7	Yilmaz et al., 2004
	28.0	5.0	10.0	25.0	Kalita et al., 2008
Lemna spp.	38.6	9.8	18.7	19.0	Men et al., 1995
	36.0	4.5	10.7	8.46	Pedraza et al., 1996

*not reported **Grown on nutrient rich medium *** harvested from a natural lagoon

2.4.1.4. Nutrient requirements

Similar to other photosynthetic terrestrial and aquatic plants, nitrogen, phosphorus and potassium (NPK) are the main nutrients required for duckweed growth.

A. Nitrogen Requirements

Duckweeds has ability to use many nitrogenous compounds however, nitrogen is not the limiting nutrient for duckweed growth as it can grow well in the presence of even very small amounts of nitrogen in growth media when other nutrients and temperatures are favorable. The ammonium ion (NH4+) is most readily available form of nitrogen for duckweed. Nitrogen is fixed as protein in duckweed biomass. Protein content of a typical duckweed plant is highly affected by availability of nitrogen in growth media. Assimilation of nitrogen by duckweed frond and roots appears to be the primary mechanism of nitrogen fixation in this plant. However, some portion of nitrogen is also absorbed into duckweed biomass through associated N fixing cyanobacteria and algae grown in duckweed ponds (Duong and Tiedje, 1985). Duong and Tiedje. (1985) calculated that N fixation via these colonies can range from 3.7-7.5 kg N per hectare of water surface. Nitrogen requirement for active growth of typical duckweed ranges from 20-60 mg N/l however, level of nitrogen required by duckweed is highly dependent on initial composition of plant (Leng et al., 1994).

B. Phosphorous Requirements

Phosphorous constitute about 1.5% of duckweed dry mass. Duckweed has ability to grow on high phosphorous media in the presence of appropriate amount of nitrogen. Duckweed can accumulate high amount of Phosphorous (P) in its biomass due to which this plant continuous to grow in waters with less amount of P. When duckweed dies, stored P in plant biomass is readily available in the water (Campbell and Reece, 2002)

C. Potassium Requirements

Relatively low concentration of potassium in water is required for better growth of duckweed. Potassium requirements are usually met by decaying duckweed plants in growth media. Trace metals requirement of duckweed is also similar to other plants. Mineral nutrients are absorbed through all surfaces of the duckweed frond, whereas, trace elements are absorbed by specific sites on fronds (Duong and Tiedje, 1985).

2.4.1.5. Reproduction and growth of duckweed

Reproduction in duckweed takes place both by asexual and sexual methods. Flowering occurs sporadically and unpredictably. The fruit of duckweed is seed like structures which are resistant to harsh weather conditions and germinate quickly under suitable conditions.

Under uniform conditions of nutrient availability and climate, vegetative growth in duckweed exhibits cycles of senescence and rejuvenation. Each frond of duckweed produces a set number of daughter fronds during its definite life span. Daughter fronds usually have shorter life span and smaller mass (lesser number of cells) than parent fronds. Daughter fronds also produce less number of new fronds as compared to their parent fronds (Cross, 2006).

Vegetative growth in duckweed is highly dependent on nutrient availability and balance of nutrients. Growth rates are higher under optimum nutrient balance.

Duckweed has ability to grow under high concentration and high hydraulic loading of nutrients in wastewater.

Under the ideal conditions of temperature, humidity, light, pH and nutrient concentrations, duckweed can double their biomass between 16 hours to 2 days. According to a calculation, duckweed grown on surface area of 10 cm^2 may grow to cover an area of 100 million cm^2 (1 hectare) within a time of 50 days. It shows 10 million times growth in 50 days.

Under the dry conditions, duckweed produces high density starch filled structures called "turin" due to which duckweed sinks to the bottom of the water body and embedded in dried mud. Duckweed regrow at the on-set of favorable conditions.

Duckweed is the photosynthetic plant utilizing solar energy for biomass production. Duckweed also has ability to grow without sunlight by utilizing preformed organic material particularly sugars as energy source (Campbell and Reece, 2002).

2.4.1.6. Factors affecting duckweed growth

Duckweed grows well between temperature ranges of 6 to 33 °C. Growth rate increases with increase in temperature up to 30 °C after which growth starts ceasing. Duckweed can survive below freezing temperatures for many days (Cheng et al., 2002).

Optimum range of pH for duckweed growth is 6.5-7.5 however; it can grow well in pH range of 5-9. At pH range of 6.5-7.5, ammonia in growth media is present in the form of ammonium ion which is most easily absorbed form of nitrogen by duckweed (Caicedo et al., 2000).

Water depth is important factor for duckweed growth. Water depth less than 0.5 m may cause sudden fluctuations in water temperature due to high absorption of solar radiations altering the optimal temperature balance from 20-30 °C, required for duckweed growth. Water depth of 2 meters should be maintained while using duckweed for water treatment purpose. Water depth is normally adjusted according to the management requirements for wastewater treatment such as duckweed harvesting (Cross, 2006).

2.4.1.7. Uses of duckweed

Besides the wastewater treatment, duckweed also has potential applications as human and animal feeds mainly due to its high protein content up to 45% of the total dry mass of duckweed plant (Leng et al., 1995). In some countries, duckweed farming is done for use as human food or sale to poultry and pig producers. High concentration of NPK in duckweed biomass finds application as fertilizer source for crop production (Preston and Murgueitio, 1992).

2.4.2. Wastewater treatment by duckweed

Duckweed is amongst the promising aquatic plants having ability to absorb large amounts of nutrients and trace metals from eutrophicated wastewater and has high growth rates. Wastewater treatment by duckweed is owed to its high nutrients and minerals accumulation capacities into biomass and high growth rates under diverse environmental conditions (Chaiprapat et al., 2005; Ge et al., 2012; Zhao et al., 2014).Cheng et al. (2002) reported that duckweed can grow well at wastewaters with high nitrogen and phosphorus levels (240 mg NH_4-N L^{-1} and 31.0 mg PO_4-P L^{-1}). The highest nutrient uptake rate achieved was 0.995 mg N L^{-1}-h, and 0.129 mg P L^{-1}-h, and duckweed growth rate was 1.33 g dry biomass/m^2-h. Bergmann et al. (2000a) concluded that *Lemna gibba* and *Lemna minor* species of duckweed are the best for treatment of high strength swine effluent with high biomass production and nutrient removal rates. Figure 2.5 illustrates the biological process taking place in duckweed based wastewater treatment pond (Smith and Moelyowati, 2001)

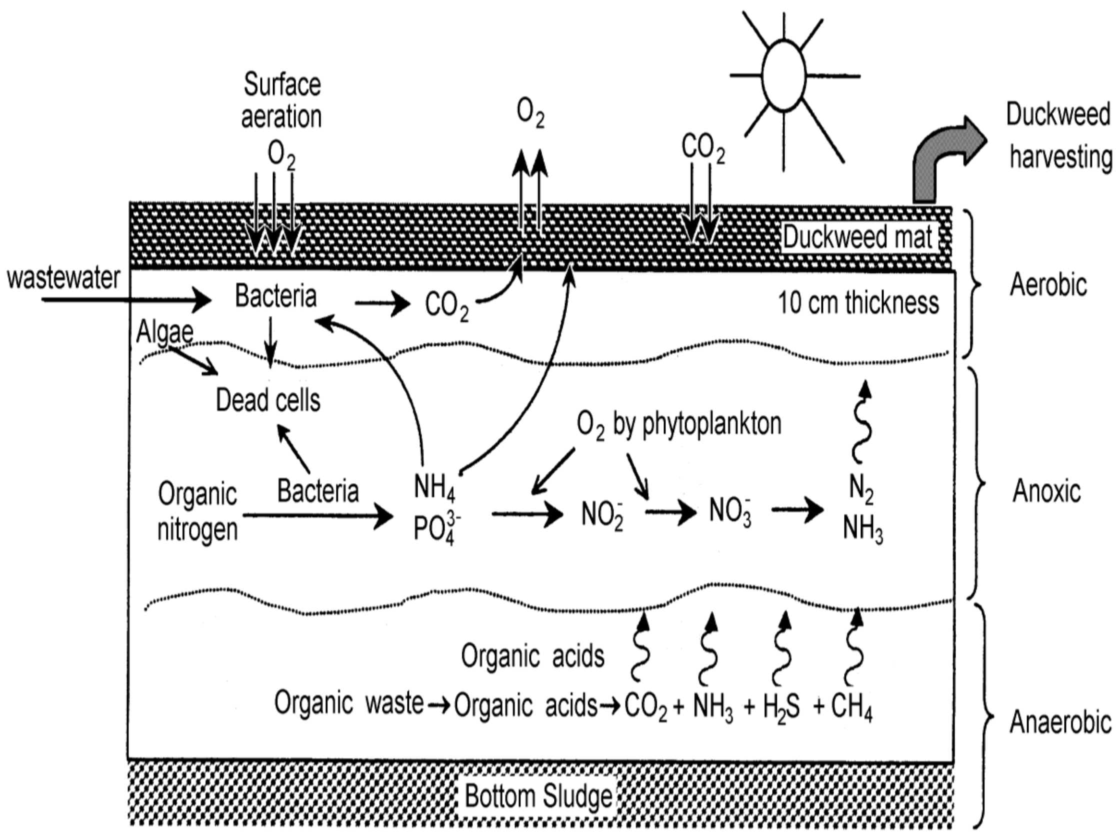

Figure 2.5: Biological processes in duckweed based wastewater treatment (Smith and Moelyowati, 2001)

2.4.3. Commercial Application of Duckweed in Wastewater Treatment

Some commercial duckweed based wastewater systems are in place worldwide. These systems are much cheaper and environment friendlier as compared to traditional chemical/physical or biochemical sewage treatment plants. Most of the duckweed based treatment systems can be incorporated or added into chemical/physical systems. However, in developing countries, where proper chemical/physical systems of wastewater treatment are rare, duckweed based ponds may be the only option for wastewater and leachate treatment (Bergmann et al., 2000).

1. In Bangladesh duckweed based wastewater treatment has been developed as the part of UNDP project. This project aims to examine the potential of duckweed-based wastewater treatment and fish production at village level. In Bangladesh, since 1989, PRISM, Foundation, has already developed duckweed based farming and tested duckweed potential for wastewater treatment and fish food (UNEP, 2004).
2. In 2015, Mama Grande[2] of Argentina launched a commercial wastewater-to-bioproduct project with 150 hectare of wastewater treatment ponds using duckweed. This project is situated in the Salta and Tucumán provinces of Northwest Argentina. Project aims to produce duckweed as starch while cleaning the wastewater.

[2] Mama Grande is a biotech social business created in 2011 to develop processes & operations to create a better, more sustainable future for everyone, specialized in water remediation coupled with renewable and biodegradable industrial products generation.

3. During a pilot scale project, duckweed based wastewater treatment systems were compared. Three duckweed based systems were constructed to investigate the effect of aeration and effluent recycling on treatment efficiency of duckweed based wastewater treatment ponds. Organic matter and nutrients were almost equally removed from three wastewater treatment plants showing that aeration has no significant effect on pollution removal efficiency of duckweed and dissolved oxygen levels (Ben-shalom et al., 2014).

2.4.4. Duckweed Used in the Study

Various species of duckweed have been studied for the pollution removal from synthetic or real wastewaters (Cheng et al., 2002; Chaiprapat et al., 2005). *Lemna minor*, belonging to genus *Lemna* of duckweed is the most widely spread specie. *Lemna minor* is sometimes labeled "common duckweed". It is among the smallest flowering plants in the world with frond size of about less than one cm across and only one root per plant. Similar to other duckweed species, *Lemna minor* under favorable conditions can double its biomass in two days forming dense mats on the surface of water body (Driever et al, 2005). *Lemna minor* has been extensively studied in wastewater treatment due to its fast growth rates, high nutritional value, and high nutrient removal efficiencies (Ozengin and Elmaci, 2007).

2.4.5. Algae-Potential Phytoremediation Plant

Algae in this research are used as the first step for heavy metal removal from leachate in algae and duckweed based leachate treatment system. Important characteristics and features of algae have been describesd below.

2.4.5.1. General characteristics of algae

Algae is the distinct group of eukaryotic organisms that are autotrophic i.e. prepare own food through the process of photosynthesis similar to green plants. Photosynthesis occurs in algae and plants due to presence of chloroplast. Chlorophyll present in the chloroplast performs the light capturing function for photosynthetic reactions. In contrast to green plants, specific types of tissues and cells are absent in most of the algae such as xylem, phloem and stomata (Brennan and Owende, 2010). Most of the algae are aquatic and simple organisms ranging in size from microalgae to large macro algae usually forming colonies. Colonies are formed when cells are arranged as aggregates. Colonies may be filamentous or non-filamentous. Filamentous occur in branched or unbranched forms. Microalgae also includes cyanobacteria, formerly called "blue-green algae" (Madigan et al., 1997; Falkowski, 1994).

Algae are worldwide used as energy sources, food sources, fertilizers and pollution control organisms. Algae are extensively used to remove organic and inorganic pollution from wastewaters (Pavasant et al., 2006).

2.4.5.2. Classification of algae

Algae has been classified into distinct groups based on cell morphology, cell wall structure, habitat, reproduction systems, life history, pigments, and reserve food material and body plan (Davis et al., 2003; Madigan et al., 2000; Prescott et al., 2002; Talaro and Talaro, 2002). Fritsch (1944) classified the algae into eleven distinct groups as provided in Table 2.3.

Green algae (Chlorophyta) are the most varied group of algae having chlorophylls a and b along with specific carotenoids. Carbohydrate in Chlophyta sp is stored as starch. Cell wall of most of them is made up of cellulose. Green algae are found both in colonial and unicellular forms (Prescott et al., 2002).

Use of algae as bio sorption material for heavy metals removal from wastewater has been investigated since 1970s (Li et al., 2015). Algal bio sorption is a cost-effective, easy-to-operate and environment-friendly method with high efficiency in detoxifying the metals.

Algae, display ideal properties for intra- and/or extra-cellular adsorption of heavy metals (Yang et al., 2015; Sheng et al., 2007). Various studies have been conducted to investigate the heavy metal removal potential of diverse algae from aqueous solutions (Martins et al., 2006; Aksu et al., 2000; Al-Rub et al., 2006; Deng et al., 2006; Vilar et al., 2007).

Table 2.2: Classification of algae (Fritsch, 1944)

S. No	Class	Key Features	Examples
1.	Myxophyceae (Cyanophyceae)	Simple plants with indefinite nucleus, commonly blue-green color and sexual reproduction is	Anabaena, Nostoc
2.	Euglenophyceae. (Flagellates)	Unicellular plants, salt water or fresh water habitat, reproduction is usually by fission	Heteronema, Euglena
3.	Chlorophyceae.	Nucleus and flagella green color, sexual reproduction present	Volvox, Spirogyra
4.	Chloromonadineae.	Bright green color, reproduction by longitudinal division of individuals	Less available information about representatives of
5.	Xanthophyceae (Heterokontae)	Yellow green chloroplast, rare sexual reproduction, cell wall divided into two halves	Tribonema, Botrydium
6.	Chrysophyceae	Old plants with brown or orange chloroplast, cell wall present in some plants, rare sexual	Chrysamoeba, Chromulina
7.	Bacillariophyceae	Symmetrical halves of cell wall, yellow or golden brown, sexual reproduction mostly absent.	Melosira, Pinnularia
8.	Cryptophyceae.	Brown shaded chloroplast, sexual reproduction by isogamy in few plants	Chilomonas, Cryptomonas
9.	Dinophyceae (Peridineae)	Mostly motile and unicellular, rare sexual reproduction	Peridinium, Heterocapsa
10.	Phaeophyceae.	Brown color and mostly marine habitat, iso-aniso-or oogamous, type of sexual reproduction	Laminaria, Ectocarpus
11.	Rhodophyceae.	Mostly marine, very few are fresh water, oogamous type of sexual reproduction	Batrachospermum, Ploysiphonia,

Polysaccharides in algal cell wall act as binding sites for metal ions. Various types of ligands including hydroxyl, carboxyl, sulfate, and amino groups in cell wall act as binding sites for metals. Metal bio sorption by algae is influenced by physical/chemical properties of metal, characteristics of specific algal biomass, and pH of the medium (Agarwal et al., 2006). Table 2.4 provides the metal affinity of various ligands present on algal cell wall.

Table 2.3: Metal affinity of various ligands present on algal cell wall

Ligand class	**Ligands**	**Metal classes**
Ligands preferred to Class A	F^-, O_2^-, OH^-, H_2O, CO_3^{2-}, SO_4^- $ROSO_3^-$ NO_3^- , HPO_4^{2-}, PO_4^{3-}, ROH, $RCOO^-$, C=O, ROR	Class A: Li, Be, Na, Mg, K, Ca, Sc, Rb, Sr, Y, Cs, Ba, La, Fr, Ra, Ac, Al, Lanthanides, Actinides
Other important ligands	Cl^-, Br^-, N_3^- , NO_2^- , SO_3^{2-}, NH_3, N_2, RNH_2, R_2NH, R_3N, =N−, –CO–N–R, O_2, O_2^- ,O_2^{2-}	Borderline ions: Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Cd, In, Sn, Sb, As
: Ligands preferred to Class B	H^-, I^-, R^-, CN^-, CO, S_2^-, RS^-, R_2S, R_3As	Class B: Rh, Pd, Ag, Lr, Pt, Au, Hg, Tl, Pb, Bi

Source: Nieboer and Richardson, 1980; Pearson, 1963; Remacle, 1990.

2.4.6. Algae used in this Study

Provided below is the description of algal species used in this research study.

Ankistrodesmus is the genus of green algae (Krienitz et al., 2001). Only few studies have been conducted so far in the world related to bio sorption capacity of *Ankistrodesmus* (Geisweid and Urbach, 1983; Maguire et al., 1984). However, *Ankistrodesmus* genus is widely studied with respect to lipid production potential (Singh et al., 2015).

Cyanobacteria; sometimes called "blue green algae" is the photosynthetic phylum of bacteria. Cyanobacteria live in a wide variety of habitats including; water and moist soils. Most of them are free living and some have symbiotic associations with plants and fungi (Chorus and Bartram, 1999)

Nostoc and *Anabaena* are important genus of cyanobacteria that are filamentous and usually develop microscopic and macroscopic colonies. *Nostoc* is commonly found in aquatic and terrestrial habitats (Dodds et al., 1995). Similar to green algae, cyanobacteria also have potential to remove pollution including heavy metals from wastewater under suitable conditions of temperature and pH of the growth medium (De Philippis et al., 2011). Heavy metal removal potential of *Cyanobacteria* has also been investigated by many researchers. Studies demonstrate that under sufficient supply of nutrients and suitable temperature the cyanobacteria have ability to remove significant amounts of copper, nickel and zinc from the media (De Philippis et al., 2007; Kumar, 2014; Gupta et al., 2006). A study indicates that heavy metals removal up to 82%, 34% and 100% of copper, cobalt and lead were removed respectively from sewage and industrial wastewater by using mixed cultures of *Nostoc* and *Anabaena* genera of Cyanobacteria (El-Sheekh et al., 2005).

2.4.7. Dead vs. Living Algae

Use of dead (dried) algal mass for heavy metal bio sorption from artificial metal solutions is widely studied throughout the world (Aksu and Acikel, 2000; Martins et al., 2006; Al-Rub et al., 2006; Vilar et al., 2007; Grimm et al., 2008; Akhtar et al., 2008). However, very small amount of research work is available related to use of living algae for heavy metal removal from natural aqueous media such as wastewater or leachate.

2.5. SYNTHESIS OF LITERATURE REVIEW

Literature reviewed during this research study can be summarized as follows: -

- Leachate produced from open dump sites is a serious threat to environment and human health.
- Leachate produced from open dump sites requires proper treatment prior to discharge into environmental media.
- Phytoremediation is an easy and cost-effective method of wastewater treatment.
- Under the suitable conditions, duckweed (*Lemna minor*) has significant potential of wastewater treatment and uptake of nutrients from leachate into its biomass.
- Living mass of mixed green algae (*Ankistrodesmus*) and *cyanobacteria* (*Nostoc* and *Anabaena*) has potential to remove heavy metals from wastewater under suitable conditions of temperature and pH and sufficient availability of nutrients in growth medium .

It is revealed from literature search during this study that duckweed and algae have extensively been studied for wastewater treatment however, very small amount of research work has been conducted on their use for leachate treatment. Therefore, the present study has been designed to explore the natural potential of duckweed and algae for leachate treatment with the ultimate objective to provide an input for sustainable leachate treatment in Pakistan and other developing countries which is cost effective and also technically more feasible. Study is intended to identify the optimum operational parameters to design an integrated algae-duckweed based leachate treatment system which is based purely on natural systems.

Chapter 3

MATERIALS AND METHODS

This chapter provides the details of methods and materials used to accomplish this study including the methodology of various steps starting from leachate production-duckweed and algal collection-experimental setups-laboratory analysis-data processing and final presentations of results.

3.1. OVERVIEW OF STUDY

This research study examines the performance of duckweed {growth and its efficiency to remove COD and uptake nutrients (nitrogen and phosphorous)} and; algae {removal of heavy metals (Cu, Zn, Cr, Pb and Fe) and nutrients (N & P)] on leachate under the natural climatic conditions; also providing the comparison of duckweed performance under the artificial conditions. Study intends to identify the optimum conditions (including: initial leachate concentration; pH and EC of the leachate; initial duckweed plant density; initial heavy metal concentration in leachate and initial dose of algae on leachate) for better performance of algae-duckweed based leachate treatment system.

3.2. STUDY METHODOLOGY

Flow chart of all procedures and methods adopted during this study are presented in Figure 3.1. Side bars in figure show the tasks associated with various main tasks.

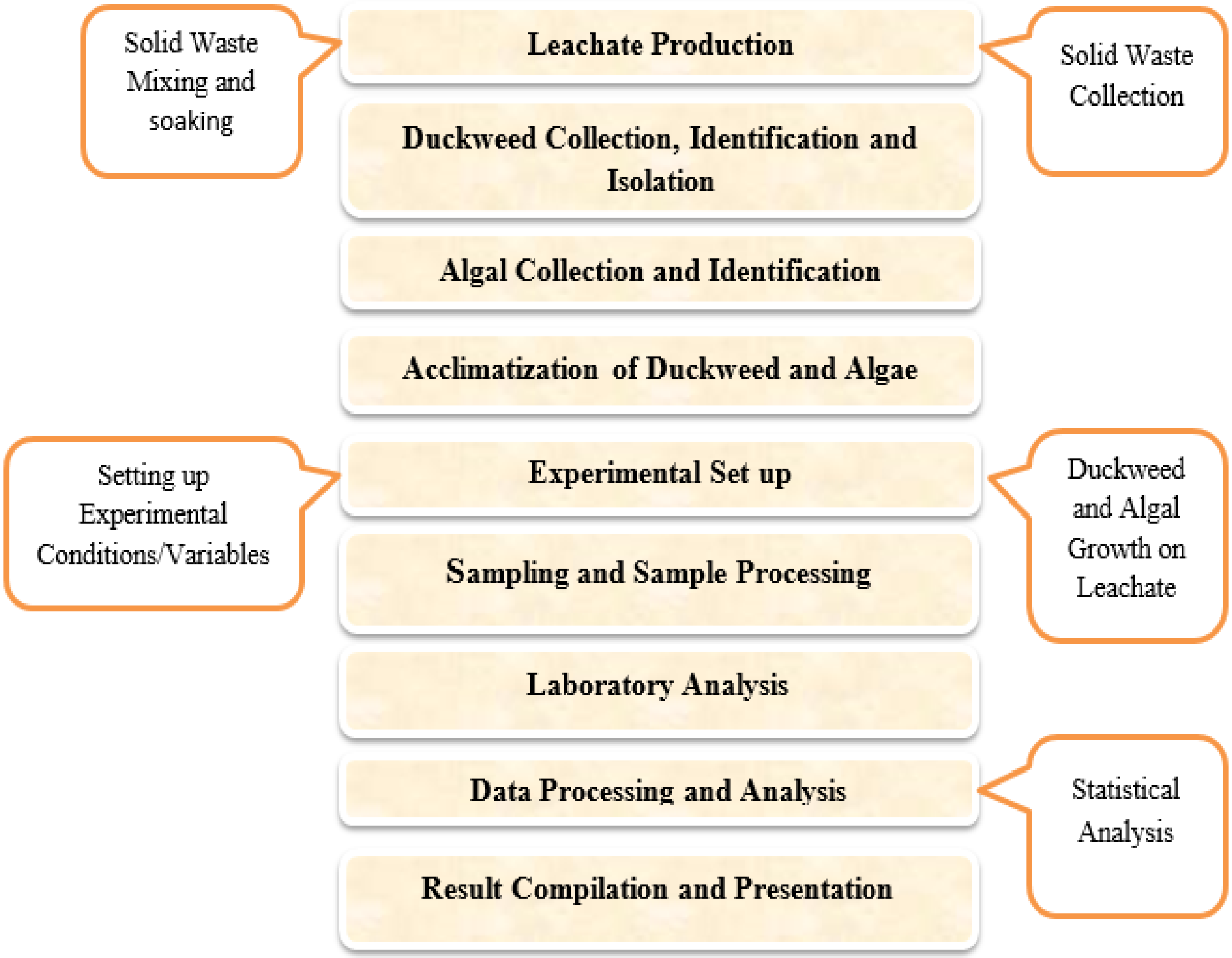

Figure 3.1: Flow chart of methodology adopted for this study

Provided below is the detail of each step mentioned in Figure 3.1.

3.2.1. Leachate Preparation

Leachate used in this research was prepared by processing the decomposed solid waste collected from municipal dump sites containing residential, commercial and industrial wastes of Islamabad and Rawalpindi, Pakistan. Solid waste was collected from following areas:-

- Municipal solid waste dump site located at Losar (Rawat), Rawalpindi;
- Dump site located at Dhamial, Rawalpindi;
- Temporary dump sites at residential areas of sectors, F-10, F-11, G-10 and G-11, Islamabad;
- Dump site located at Industrial area of sector I-10, Islamabad.

About 100 to 120 kg well decomposed solid waste was collected from each dump site. Waste was collected from pre-determined lowest points at depths of 0.5 m to 1.5 m according to the procedure adopted by Ojoawo et al., (2012). Collected wastes were mixed in plastic container having an internal diameter of about 1.5 m and height of about 1.8 m. A sieve (pore size 1mm) was fixed at an internal height of 10 cm of the plastic tank. Figure 3.2 shows the schematic of setup used for leachate production.

Thorough shaking was applied to mix the waste and achieve a homogenized sample. The homogenized waste was soaked with leaching solution (distilled water) and maintained for 30 days after which, the leachate was collected from bottom outlet. Remaining solid waste was again mixed thoroughly and soaked with distilled water. Afterwards, the leachate was collected three times at an interval of ten days. Each time the solid waste samples were thoroughly mixed and shaken. Leachate collected from various runs was mixed to form single homogenized sample to be used for this research.

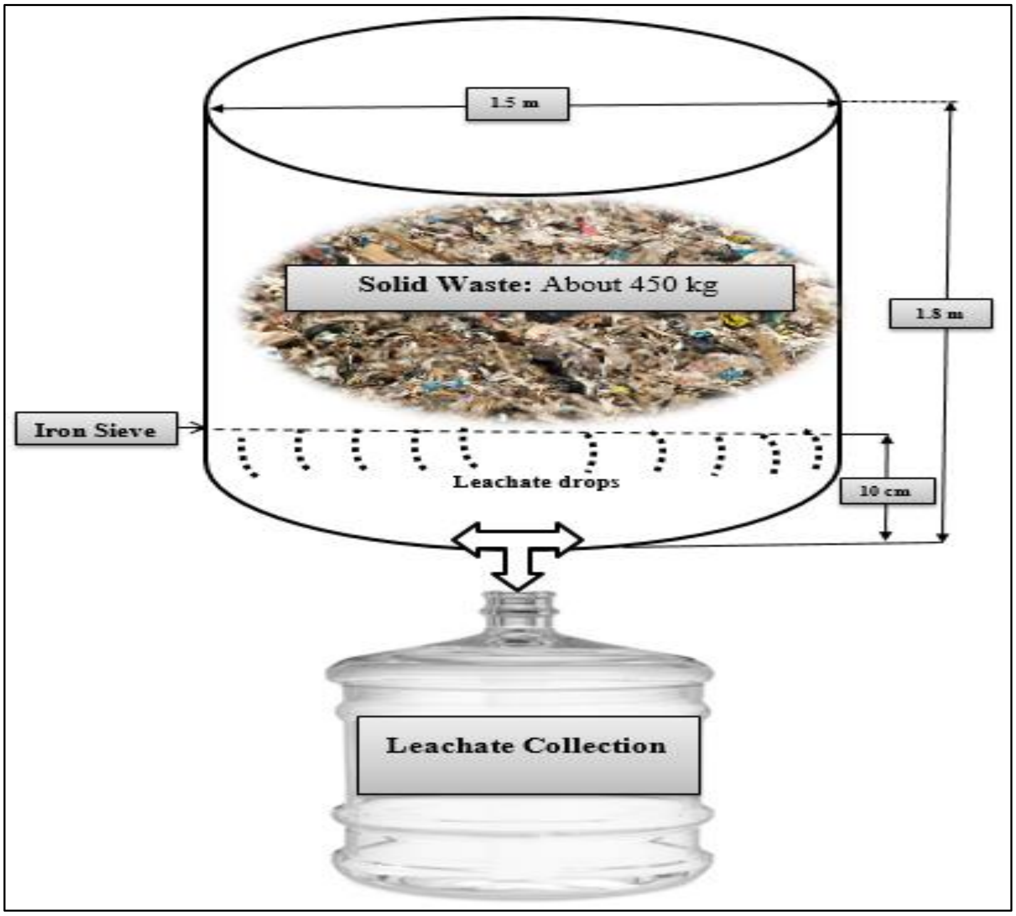

Figure 3.2: Schematic of leachate production setup from collected wastes

3.2.2. Duckweed Collection

Specimens of duckweed used in this research were collected from wastewater treatment pond located in National University of Sciences and Technology (NUST), Islamabad, Pakistan. This pond is the part of wastewater treatment plant involving a sedimentation tank and an artificially constructed wet-land divided into eight compartments towards which the wastewater from offices, hostels and residential colony at the university main campus is directed, and the treated water is used for horticulture.

Duckweed plants were harvested with the help of plastic screen. Figure 3.3 shows the duckweed collection from wastewater treatment pond.

Figure 3.3: Duckwweed collection from wastewater treatment pond at NUST

3.2.2.1. Identification and isolation of duckweed

Identification of duckweed was made by using duckweed guide of "Botanical Society of the British Isles" (BSBI), United Kingdome. This guide provides the step wise identification of duckweed species on the basis of; presence or absence of roots, size and shape of duckweed plants, and color of the duckweed fronds. On the basis of identification, Lemna minor plants were isolated from mixed duckweed.

3.2.3. Collection and Identification of Algae

Algae used in the study were collected from algal pond located at wastewater treatment pond at NUST, Islamabad, Pakistan (Figure 3.4). Identification of collected algae was made using "Algal Identification Field Guide" (Huynh and Serediak, 2006). This guide provides the genus level identification of algae. Using this guide, step wise identification of algae was made on the basis of algal habitat, odor of algae and physical appearance. Identification of algae through visual observations was verified through microscopic examination of experimental algae and comparing it with microscopic illustrations provided in the guide.

Figure 3.4: Algal collection from wastewater treatment pond at NUST

3.2.4. Acclimatization of Duckweed and Algae

Before starting the experimental work, duckweed and algae were grown on experimental leachate of various initial concentrations under the natural environment. Purpose of this work was to make the experimental plants and algae adopted to new environmental conditions as previously these organisms had different habitat i.e. the wastewater pond instead of leachate. Secondly; through this work, maximum upper concentration of leachate supporting healthy growth of duckweed and algae was determined.

During the acclimatization works, duckweed and algae was grown on various leachate dilutions starting from 10%, 20%, 30%, ------100% leachate by volume. Visual observations related to duckweed growth and frond color were recorded on daily basis. At the end of this work, in addition to acclimatization of duckweed and algae, we came up with maximum upper dilution (concentration) of leachate supporting the healthy growth of experimental algae and duckweed. This upper concentration of leachate was used as starting point for upcoming experiments during this study.

3.2.5. Experimental Setup

This research study was conducted in five (05) phases during which six (06) sets of experiments were performed by growing duckweed (*Lemna minor*) and mixed algae (*Ankistrodesmus*, *Nostoc* and *Anabaena*) on leachate. Table 3.1 provides the overall experimental framework of this study showing the phase wise detail of experimental work including the scope of each experiment in relevance to this study

Table 3.1: Framework of study on duckweed and algal performance on dumpsite leachate under natural conditions

S. No.	Research Phases	Experiment/s Conducted	Scope of Experiment	Remarks
1.	**Phase-I**	One set of experiment comprising two (02) batch experiments during the months of June and September, 2014 under the natural climatic conditions	To examine the following effects on duckweed performance (growth and its efficiency to remove COD and uptake nutrients; nitrogen and phosphorous) on leachate:- i. Seasonal effect (ambient temperature, solar intensity and day length) from June-September; ii. Effect of initial leachate concentration.	Optimum initial concentration for duckweed performance was identified during phase-I and all next experiments were conducted on this optimum concentration. Seasonal effect identified during this phase was further verified during next Phases-II and III.
2.	**Phase-II**	One set of experiment comprising two (02) batch experiments during the months of June and September, 2015 under the natural climatic conditions	To examine the following effects on removal of heavy metals (Cu, Zn, Cr, Pb and Fe), COD and nutrients (N& P) from leachate by algae:- i. Initial concentration of each metal in leachate; ii. Initial dose of algae; iii. Seasonal effect from June to September; iv. Starting from 50% leachate, determining the time (in days) during which algae could remove maximum amount of heavy metals from leachate and remaining concentration of nutrients (N &P) in leachate is nearly equal to as nutrient concentration in 30% leachate.	Purpose of this phase is to find the suitability of algae to be used as first step for heavy metal removal from leachate in integrated algae-duckweed based leachate treatment system

S. No.	Research Phases	Experiment/s Conducted	Scope of Experiment	Remarks
3.	**Phase-III**	Two sets of experiments under the natural climatic conditions as follows:-	To examine the following effects on duckweed performance on leachate:-	Purpose of phase-III experiments is to identify the optimum pH and EC of leachate for better performance of duckweed in algae-duckweed based leachate treatment system.
		Experimental Set 1: comprising two batch experiments during the months of June-July and September-October, 2015.	i. Initial pH of the leachate; ii. Seasonal effect from June to September.	
		Experimental Set 2: comprising two batch tests during the months of June-July and September-October 2015.	i. Initial Electrical Conductivity (EC) of the leachate; ii. Seasonal effect from June to September.	
4.	**Phase-IV**	One (01) experiment with single batch test during the month of June 2016 under the natural climatic conditions.	To examine the duckweed performance under various initial mat densities of duckweed plants on leachate.	Results of Phase-IV, provide the optimum conditions of; initial duckweed for healthy growth. Results of this experiment also provide the harvesting frequencies and doubling time of duckweed on leachate which is very important for the sustainable operations of duckweed based leachate treatment system.
5.	**Phase-V**	One (01) set of experiment comprising three (03) batch tests under artificial conditions of temperature, light intensity and day length.	To examine the duckweed performance on synthetic leachate at the following optimum conditions (as identified during the previous phases of this study) under controlled conditions: - i. Leachate pH; ii. Electrical Conductivity (EC); iii. Duckweed mat density and.	Results of Phase-V, provide the comparison of duckweed performance at dumpsite and synthetic leachate under the natural and artificial climatic conditions respectively.

During all experiments, duckweed and algae were grown on leachate in plastic containers as shown in Figure 3.5 From Phase I-IV, duckweed and algal containers were placed within a three-chambered meshed iron stand fixed with lock and tag and weather protection arrangements (Figure 3.5). Iron stand was placed in open environment under natural climatic conditions whereas; during the phase-V, duckweed was grown on synthetic leachate and containers were placed within the growth chamber under controlled conditions of temperature, light intensity and day length. Total leachate volume in experimental containers was maintained by using distilled water.

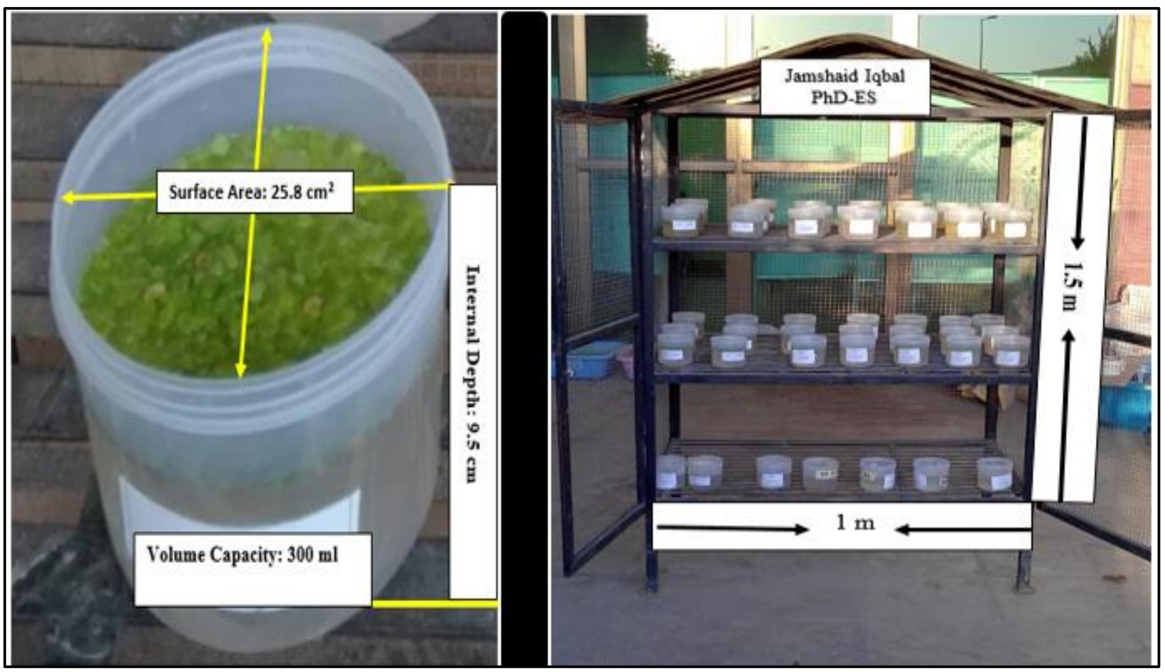

Figure 3.5: Experimental setup using plastic containers for duckweed growth on leachate under natural climatic conditions

Seasonal climatic data related to ambient air temperature and day lengths during both experiments was retrieved from website of Pakistan Metrological Department, whereas the solar radiation data during the experimental period was obtained from the web site of LEO Corporation, Pakistan. Provided below is the phase wise design/setup of experiments conducted during each phase of the study.

3.2.5.1. Phase-I

For each test, five dilutions/concentrations of leachate with leachate/water ratios of 50/50, 40/60, 30/70, 20/80 and 10/90) were prepared with distilled water and duckweed was grown separately on each dilution. Each batch test consisted of total 165 containers with 33 containing a dilution of leachate corresponding to triplicate samples of 11 time points. Each test was lasted for 22 days. Figure 3.6 provides the experimental setup of phase-I.

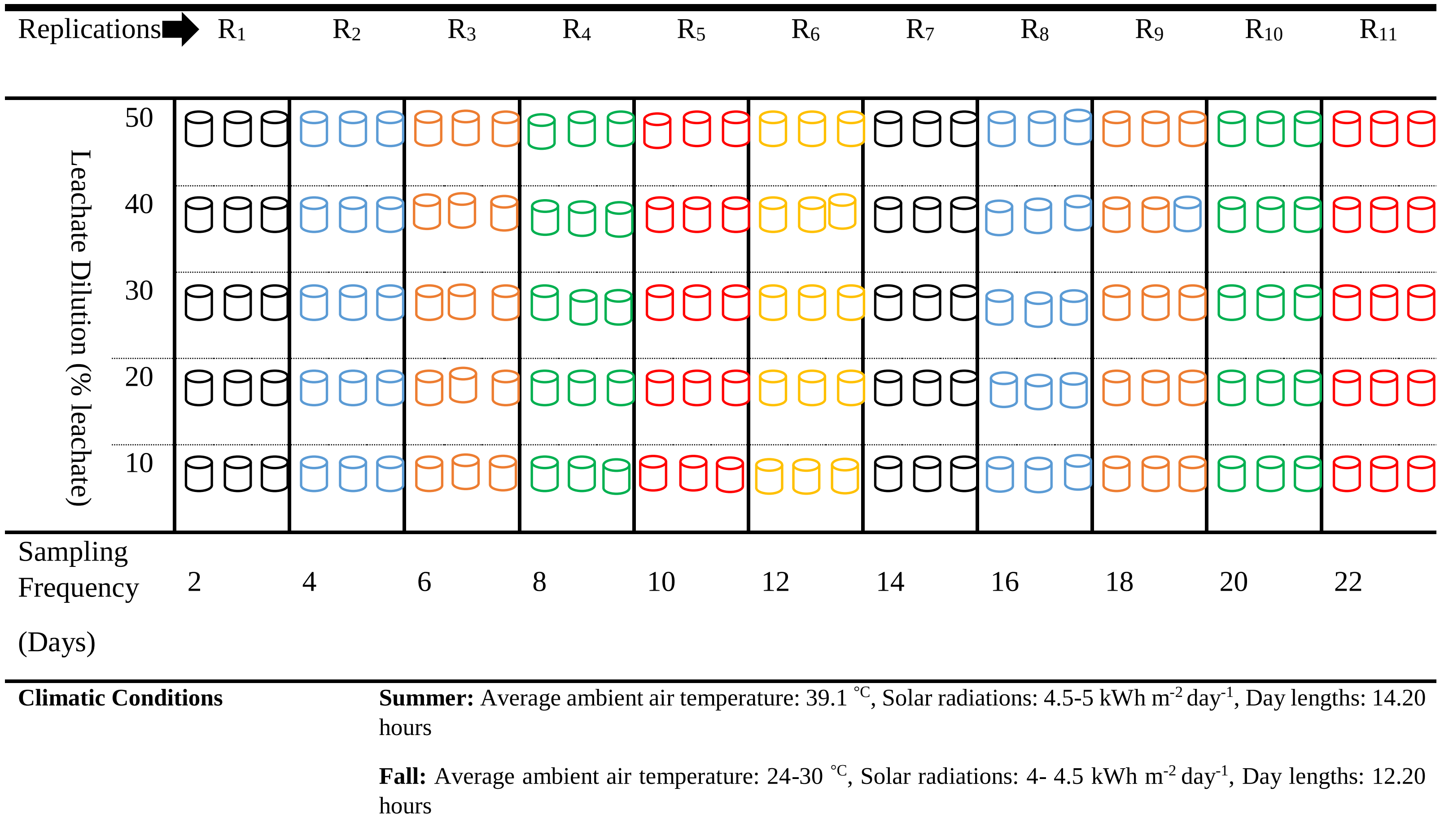

Figure 3.6: Experimental setup (phase-I) showing duckweed growth at various leachate dilutions under natural climatic conditions

3.2.5.2. Phase-II

For each test during this phase, initial metals (Fe, Cu, Pb, Cr and Zn) content of leachate were determined and measured amounts of each metal stock solutions were added to pre-tested leachate to obtain desired concentrations (5 mg L^{-1} and 10 mg L^{-1}) of each test metal in leachate. Heavy metal stock solutions for Fe, Cu, Pb, Cr and Zn were prepared in distilled water with analytical grade salts of each metal: $FeSO_4 .7H_2O$, $CuSO_4.7H_2O$, $PbNO_3$ $CrCl_3$, $ZnSO_4.7H_2O$.

For each test during this phase, leachate of 50% initial concentration was used for algal growth. Each test was lasted for eight (08) days. Throughout the experiment, all leachate containers were maintained at neutral pH of about 7 by adding 1M HCl or NaOH solution. During each test, Total 24 containers were used including three containers for each algal dose and corresponding initial metal concentration of leachate and three control containers without algae for each metal concentration and algal dose. Figure 3.7 shows the experimental setup of Phase-II.

Initial Metal Concentration →			**5**			**10**		
Algal Dose ($g L^{-1}$)	**0.8**	Algal Containers						
		Controls (without algae)						
	1.6	Algal Containers						
		Controls (without algae)						
Sampling Frequency			Daily					

Climatic Conditions	**Summer:** Average ambient air temperature: 39.6 ℃, Solar radiations: 4.7-5.1 kWh m^{-2} day^{-1}, Day lengths: 14.12 hours **Fall:** Average ambient air temperature: 25-30 ℃, Solar radiations: 4.2- 4.7 kWh m^{-2} day^{-1}, Day lengths: 12.30 hours

Figure 3.7: Experimental Setup (Phase-II) showing Algal Containers with two Initial Metal Concentration and two Algal Doses under Natural Conditions

3.2.5.3. Phase-III

Experiment-1:

Under experiment-1, two batch tests; each lasting for 25 days were conducted using the leachate of 30% initial concentration. For each test, seven initial pH levels of leachate (4, 5, 6, 7, 8, 9, and 10) were adjusted by adding 1M NaOH and HCl solutions. For each test, 28 containers were used. Out of these, 21 containers had duckweed cultures with each pH level from 4-10 in triplicates. Seven control containers at each pH were without duckweed. Experimental setup is provided in Figure 3.8 below.

pH → values	4	5	6	7	8	9	10
Duckweed Containers							
Controls (without duckweed)							
Sampling Frequency	After the completion of experiment (25 days)						
Climatic Conditions	**Summer:** Average ambient air temperature: 38.3 °C, Solar radiations: 3.8-4.9 kWh m^{-2} day^{-1}, Day lengths: 13.40 hours **Fall:** Average ambient air temperature: 26-30 °C, Solar radiations: 4.2- 4.5 kWh m^{-2} day^{-1}, Day lengths: 12.10 hours						

Figure 3.8: Experimental setup (experiment-1, phase-iii) showing duckweed containers grown at various pH levels of leachate under natural conditions

Experiment-2:

This experiment also comprises two batch tests; each lasting for 25 days. During each test, using leachate of 30% concentration, six (06) EC levels (500, 1000, 1500, 2000, 2500 and 3000 µScm^{-1}) were adjusted and duckweed was grown at leachate with each EC level. Desired EC values in leachate were achieved by adding common salt, NaCl to original leachate to increase EC level and diluting the original leachate with distilled water to decrease EC level. Each test was lasted for 25 days. For each test during summer and fall seasons, 24 containers were used. Out of these, 18 containers had duckweed cultures with each EC level from 500-3,000 µScm^{-1} in triplicates and six controls without duckweed were used for each corresponding EC level. Experimental setup of this experiment is shown in Figure 3.9 below.

EC values →	500	1,000	1,500	2,000	2,500	3,000
Duckweed Containers						
Controls (without duckweed)						
Sampling Frequency	After the completion of experiment					
Climatic Conditions	**Summer:** Average ambient air temperature: 38.3 °C, Solar radiations: 3.8-4.9 kWh m^{-2} day^{-1}, Day lengths: 13.40 hours **Fall:** Average ambient air temperature: 26-30 °C, Solar radiations: 4.2- 4.5 kWh m^{-2} day^{-1}, Day lengths: 12.10 hours					

Figure 3.9: Experimental setup (experiment-2, phase-iii) showing duckweed containers grown at various EC levels of leachate under natural conditions

3.2.5.4. Phase-IV

A single batch test was conducted using leachate of 30% initial concentration. Three different initial mat densities of duckweed (25, 50 and 100% plant cover) were grown on leachate for 25 days. For each mat density, 4 containers were used comprising three containers with duckweed and one without duckweed (control). Experimental setup of Phase-IV is shown in Figure 3.10.

<table>
<tr><th colspan="2">Duckweed Density (% Cover)</th><th colspan="3">25</th><th colspan="3">50</th><th colspan="3">100</th></tr>
<tr><td colspan="2">Duckweed Containers</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="2">Controls (without duckweed)</td><td colspan="3"></td><td colspan="3"></td><td colspan="3"></td></tr>
<tr><td>Sampling Frequency (Days)</td><td colspan="10">After completion of experiment</td></tr>
<tr><td>Climatic Conditions</td><td colspan="10">Average ambient air temperature: 34 $^{\circ}C$, Solar radiations: 3.5-3.8 kWh m^{-2} day^{-1}, Day lengths: 11.8 hours</td></tr>
</table>

Figure 3.10: Experimental setup (phase-iv) showing duckweed containers at three different densities and growth intervals of duckweed on leachate under the natural conditions

3.2.5.5. Phase-V

During this phase, three separate tests were performed (lasting for 25 days) by growing duckweed on synthetic leachate. Synthetic leachate with COD about 1527±2.42 mg L^{-1} (approximately equal to the COD of 30 % concentrated dumpsite leachate) was prepared by adding the measured quantities of $NaNO_3$, K_2HPO_3, $KHCO_3$, K_2CO_3, $NaHCO_3$, $MgCl_2.6H_2O$, $MgSO_4.7H_2O$, $CaCl_2$ and glucose powder in distilled water. Due to complex chemical composition of natural leachate, it was difficult to prepare synthetic leachate of exactly similar quality as of dumpsite leachate. However, after repeated measurements and hit and trial analysis the synthetic leachate with approximately desired COD, and nitrogen and phosphorous contents was prepared. Prepared leachate was divided into three equal portions. Three tests were conducted by growing duckweed on leachate under the following optimum conditions as identified during previous phases: -

1. Optimum pH for duckweed growth identified from phase-III
2. Optimum EC for duckweed growth as identified during Phase-III
3. Optimum duckweed density as identified from phase-IV

For each above tests, four containers were used including three duckweed containers and one control container without duckweed corresponding to each above listed parameters. Desired pH and EC of the synthetic leachate were adjusted by using 1M solutions of HCl and NaOH and NaCl salt (for adjustment of EC). Containers were placed in growth chamber maintaining approximately similar conditions of light intensity, temperature and day length as of during previous experiments on dumpsite leachate under natural conditions. Please see Figure 3.11 below for experimental setup of Phase-V.

Experimental Conditions	Optimum pH	Optimum EC	Optimum Duckweed Density
Duckweed Containers			
Control Containers			
Sampling Frequency	After the Completion of Experiment		
Climatic Conditions	Average ambient air temperature: 25 ± 2 °C, Solar radiations: 3.0-3.5 kWh m^{-2} day^{-1}, Day lengths: 10-12 hours		

Figure 3.11: Experimental setup (phase-v) showing duckweed containers at optimum conditions of pH, EC, duckweed density and growth time under controlled conditions

3.2.6. Sampling and Analysis

Throughout this study two types of sampling were involved: -

3.2.6.1. Duckweed sampling and analysis

During all experiments, duckweed was harvested from experimental containers. Harvested mass of duckweed was oven dried at 105 °C for one night and following analysis were made:-

- Duckweed Growth (dry mass);
- Total Kjeldahl Nitrogen (TKN);
- Total Phosphorous (TP).

For TKN and TP analysis, dried mass of duckweed was ground with the help of mortar and pestle and plant extract was prepared for analysis.

3.2.6.2. Leachate sampling and analysis

During this study leachate samples were analyzed for: Chemical Oxygen Demand, (COD), TKN, Ammonium nitrogen (NH_4^+-N), TP, Heavy metals (Fe, Cu, Pb, Cr and Zn), Electrical Conductivity (EC), pH.

3.2.6.3. Analytical methods and instruments used

All chemical analysis was performed using the standard methods of American Public Health Association (Federation and APH Association, 2005). Table 3.2 provides the detail of analytical instruments used for above listed analysis during this study.

Table 3.2: Analytical instruments and apparatus used during the study

S. No	Parameters	Analytical Instrument	Model
1.	Chemical Oxygen Demand (COD)	COD Reactor	Velp ECO 25
2.	Total Kjeldahl Nitrogen (TKN)	Semi-Automatic Kjeldahl Distillation System	KDN
3.	Ammonium nitrogen (NH_4^+-N)	UV Visible Spectrophotometer Portable Spectrophotometer	PG-Motel T 60 Hitachi U2800
4.	Ortho-Phosphate-Phosphorous (o-PO_4^{-3}-P)	UV Visible Spectrophotometer Portable Spectrophotometer	Hitachi U2800
5.	Total Phosphorous (TP)	UV Visible Spectrophotometer	Hitachi U2800 PG-Motel T 60
6.	Heavy Metals (Fe, Cu, Pb, Cr and Zn)	Atomic Absorption Spectrophotometer	
7.	pH	pH meter	Hanna HI 8520 Eutech pH 700 WTW 720
8.	EC	EC meter	Oakton Con 11 Series Cat WTW 720
9.	Duckweed Mass	Analytical Balance	Adam AAA 160 LE Adventure AR 3130 Phoenix, BTG-303
10.	Duckweed Drying	Oven	WTC Blinder LDO-030 N
11.	Duckweed growth under controlled conditions	Growth Chamber	Chasewood, Environmental USA

3.2.7. Data Processing and Analysis

Phase wise analytical data collected from all experiments was processed to obtain the nutrient and COD reduction rates, duckweed growth rate, percent removal of nutrients and COD, percent uptake of nutrients by duckweed biomass and heavy metal bio sorption by algae.

3.2.7.1. Mathematical calculations

For Phase-II experiment**,** following equation was used to calculate the amount of metals sorbed by algae.

$$Q = (C_0 - C_e)/W \qquad \text{(Eq. 3)}$$

Where, Q is the amount of metal sorbed at equilibrium (mg g^{-1}), C_0 is the initial concentration of metal (mg L^{-1}) in leachate, C_e is the equilibrium concentration of metal remained in leachate (mg L^{-1}), and W is algal dose (g L^{-1}).

Metal removal efficiency was calculated by following equation:

$$r = (C_0 - C_t)/C_0 \times 100 \qquad \text{(Eq. 4)}$$

Where, r is the removal percent (%), C_0 is the initial metal concentration (mg L^{-1}) and C_t is the concentration of metal remaining in solution at each testing time (mg L^{-1}).

3.2.7.2. Kinetic studies on heavy metal bio sorption by algae

Kinetic studies for bio sorption of Fe, Cu, Pb, Cr and Zn were performed at two concentrations of each metal (5 and 10 mg L^{-1}) and algal biomass used was 0.8 and 1.6 g L^{-1}. 5ml leachate sample was collected daily to analyze the residual concentration of each metal (Gupta et al. 2006). Following mathematical relation between contact time and percent removal of heavy metals has been used to find out bio sorption kinetic constants for algae:

$$R = a\,(t)^{b} \qquad \text{(Eq. 5)}$$

Where, R is percent removal, "a" and "b" are the constants, and t is the contact time in days.

The linearized relationship of Eq. (6) can be expressed as:

$$\text{Log } R = \log a + b \log t \qquad \text{(Eq. 6)}$$

3.2.7.3. Statistical analysis

All treatment in this study were performed in triplicate. Data collected on all parameters was analyzed statistically using Fisher's analysis of variance (ANOVA) techniques under completely randomized design (CRD). Difference among treatment's means were compared using Least Significant Difference (LSD) test at 5% probability level (Steel et al., 1997). Microsoft Excel and Statistix-8.1softwares were used for statistical analysis.

3.2.8. Result compilation and presentation

Processed data/results was presented in tabular and graphical forms as required according to scope of this study. Next chapter-4 provides the results and discussion in relevance to this study.

Chapter 4

RESULTS AND DISCUSSION

This chapter provides the results and relevant discussions for each phase of this study. After discussing the leachate characteristics and results of duckweed and algal identification, chapter provides the results and discussion of experiments conducted during each phase. Conclusions have also been provided at the end of results and discussion of each phase.

4.1. GENERAL CHARACTERISTICS OF LEACHATE

Leachate used in this study was prepared from solid waste according to the method provided in Section 3.2.1 of Chapter 3. Table 4.1 provides the key characteristics of pure leachate.

Table 4.1: Initial characteristics of leachate used for the growth of duckweed under natural climatic conditions.

S. No.	Parameters	Concentration	S. No.	Parameters	Concentration
1.	pH	7.55	2.	Alkalinity (mg L^{-1})	1,874
3.	Electrical Conductivity (μScm^{-1})	7,540	4.	Total Phosphorous (mg L^{-1})	142
5.	Turbidity (NTU)	4,485	6.	Ortho-phosphate (o-PO_4^{3-}) (mg L^{-1})	226
7.	Chemical Oxygen Demand (mg L^{-1})	5,912	8.	Total Kjeldahl Nitrogen (mg L^{-1})	157
9.	Biochemical Oxygen Demand (mg L^{-1})	3,727	10.	Nitrate-Nitrogen (NO_3^-) (mg L^{-1})	32.8
11.	Total Suspended Solids (mg L^{-1})	882	12.	Ammonium-Nitrogen (NH_4^+–N) (mg L^{-1})	95
13.	Hardness (mg L^{-1})	432	14.	-	-

4.2. IDENTIFICATION AND ACCLIMITIZATION OF DUCKWEED AND ALGAE

Identification and isolation of duckweed and algae was made according to the procedure mentioned in Sections 3.2.2 and 3.2.3 of Chapter 3.

A) Duckweed: Process of identification revealed that duckweed collected from wastewater pond consisted of two main species: i) *Lemna minor and;* ii) *Lemna gibba*. Both of these species belong to genus *Lemna*. The *Lemna minor* plants isolated from mixed duckweed culture were further used throughout this study.

B) Algae: Algal collection pond at wastewater treatment plant mainly holds three types of algae: i) Ankistrodesmus; ii) Nostoc and; iii) Anabaena. Mixed culture of algae containing these three genera was further used during this study.

C) Acclimatization: During the acclimatization process, it was identified that the duckweed, *Lemna minor* and mixed algae can survive on ≤ 50 % initial concentration of leachate and above this concentration, duckweed and algae showed stunted growth and death of most of the plants occurred during initial 3-5 days. All further experiments were designed using the leachate of ≤ 50 % initial concentration as explained in Table 3.1 in Chapter 3.

4.3. PERFORMANCE OF DUCKWEED AND ALGAE ON LEACHATE

As explained in Chapter 3 (Section 3.2.5), this research study was completed in five phases. Provided below is the phase wise results and discussions of experiments conducted during this research showing duckweed and algal performance on dumpsite and synthetic leachate (prepared in the laboratory). For this study "Duckweed performance" refers to growth and its efficiency to remove the COD and uptake the nutrients (nitrogen and phosphorous) from leachate whereas; the "algal performance" refers to its ability to remove heavy metals and nutrients from leachate.

4.4. RESULTS OF PHASE-I

As mentioned in Chapter 3 (Section 3.2.5.1), during Phase-I, duckweed was grown on five separate initial concentrations of dumpsite leachate (50%, 40%, 30%, 20% and 10%) during summer (June) and fall (September) seasons.

In leachate containers pH was stable within the range of 7.1 to 7.5 during summer experiments and 7.7 to 8.0 during the fall experiments. It indicates the strong buffering capacity of leachate which is slightly higher during fall season. Strong buffering capacity of leachate is very important for the growth of duckweed because in the absence of buffering, the growing duckweed plants tends to rapidly decrease the pH of growth media. During an experiment, Xu et al. (2012) observed that duckweed plants decreased the pH of media to approximately 5 from initial value of 7 within 24 hours of growth.

4.4.1. Effect of Initial Leachate Concentration

Initial concentration of nutrients and COD in diluted leachate is shown in Table 4.2.

Table 4.2: Initial concentration of nutrients and COD (mean± SD) of various leachate dilutions used as medium for duckweed growth in natural seasonal conditions.

Experiment Period	**Leachate Concentration** (Percent by volume)	**Nutrients concentration (mg L^{-1})**				**COD** (mg L^{-1})
		TKN	**NH_4^+-N**	**TP**	**o-PO_4^{-3}-P**	
Summer (June)	**50**	95±1.63	55±0.82	78±0.41	18±1.47	2,760±2.83
	40	74±0.82	40±1.63	64±3.08	14±1.22	2,248±2.16
	30	59±1.63	35±1.08	45±2.68	10±1.08	1,732±1.41
	20	37±1.78	20±1.41	28±1.41	8±0.71	1,088±2.16
	10	21±1.08	12±0.41	18±0.71	5±0.41	540±2.83
Fall (September)	**50**	102±2.16	58±2.16	82±1.63	32±1.41	2,922±7.48
	40	82±0	46±1.41	66±3.74	26±1.41	2,308±5.72
	30	61±1.41	32±0	47±0.82	22±2.94	1695±5.10
	20	40±0.82	17±2.16	33±2.83	12±1.41	1216±8.16
	10	23±0	10±1.41	19±0.82	9±0.82	522±3.74

TKN: Total Kjeldahl Nitrogen, NH_4^+-N: Ammonium Nitrogen, TP: Total Phosphorus, o-PO_4^{3-}-P: Orthophosphate and COD: Chemical Oxygen Demand

4.4.1.1. Duckweed growth

Duckweed production in terms of dry mass in grams during the experimental period is provided in Figure 4.1. It is clear from figure that during both summer and fall seasons, initial concentration of leachate has inverse effect on duckweed biomass production i.e. at high initial concentration (50%) duckweed produced less biomass as compared to the biomass produced at lower concentration (10%) of leachate.

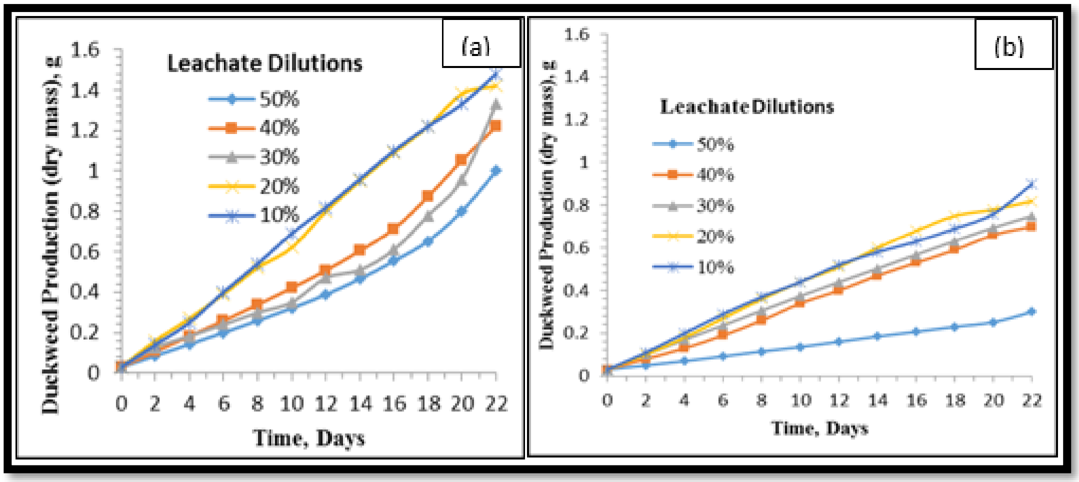

Figure 4.1: Duckweed biomass production on leachate during (a) summer and (b) fall seasons under natural conditions

Results for duckweed growth at various leachate dilutions show that during both seasons the highest duckweed growth rate (6.4 g m^{-2} day^{-1}) was achieved at 10% initial concentration of leachate whereas the lowest rate of growth (1.2 g m^{-2} day^{-1}) was observed at 50% leachate dilution (Table 4.3).

Table 4.3: Duckweed growth rates on leachate during summer and fall seasons under the natural climatic conditions.

Experiment Period	**Leachate Concentration** (Percent by volume)	**Growth rate (g m^{-2} day^{-1})**
Summer	**50**	4.3
	40	5.2
	30	5.7
	20	6.1
	10	6.4
Fall	**50**	1.2
	40	3.0
	30	3.2
	20	3.5
	10	3.8

High concentrations of nutrients particularly the ammonia nitrogen has toxic effect on duckweed growth (Clément and Bouvet, 1993; Mackenzie et al., 2003). The concentration of certain heavy metals in growth media also have been reported to have detrimental effect on duckweed growth (Clément and Merlin, 1995). Similarly Marchand et al., 2011 has reported that higher uptake of nutrients and heavy metals has negative effects on various morphological and biochemical processes of duckweed such as; photosynthesis resulting in growth reduction from 6.4 g m^{-2} day^{-1} (at 10% leachate) to 4.3 g m^{-2} day^{-1} (at 50% leachate) during summer seasons and from 3.8 g m^{-2} day^{-1} to 1.2 g m^{-2} day^{-1} respectively at 10 and 50 percent leachate during fall season.

At higher leachate concentration, the inhibitory action caused by the ammonia (NH_3) and ammonium ions (NH^{4+}) may also contribute to lesser duckweed growth. Inhibited growth of duckweed at ammonia concentration of more than 50 mg L^{-1} in domestic wastewater has also been reported by Caicedo et al. (2008).

4.4.1.2. Nutrient removal and uptake

Duckweed can efficiently remove nutrients from leachate at all initial concentrations (from 10-50 %), however, the removal dynamics is highly dependent on initial concentration of nutrients in leachate as shown in Figures 4-2 and 4-3. At high initial concentrations, relatively larger amounts of nitrogen and phosphorous was removed from the leachate during both seasons.

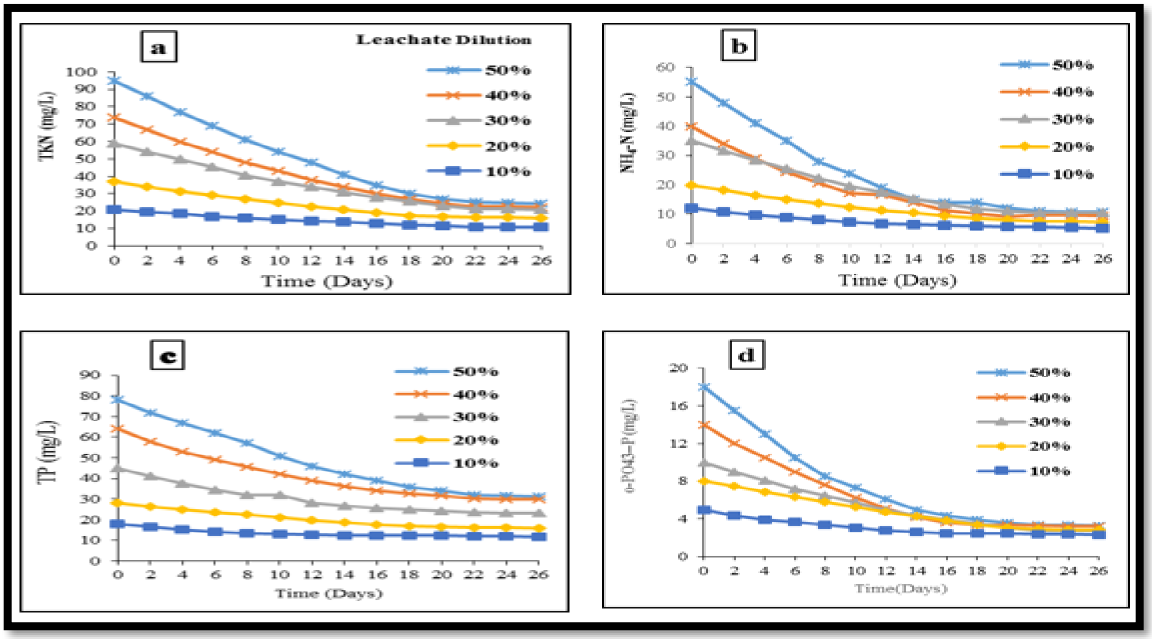

Figure 4.2: Removal of (a) total kjeldahl nitrogen (TKN), (b) ammonium-N (NH_4^+-N), (c) total phosphorus (TP) and (d) ortho-phosphate-P (o-PO_4^{-3}-P) from leachate by growing duckweed under natural seasonal conditions during summer season (June).

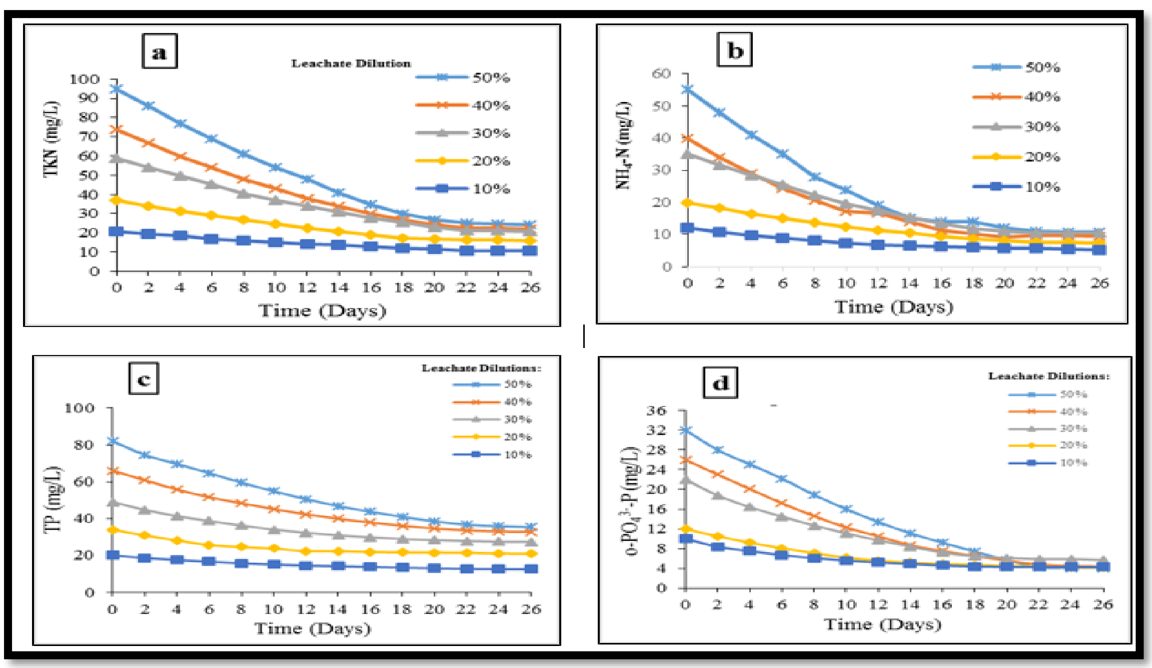

Figure 4.3: Removal of (a) total kjeldahl nitrogen (TKN), (b) ammonium-N (NH4-N), (c) total phosphorus (TP) and (d) ortho-phosphate-P (o-PO43--P) from leachate by growing duckweed under natural seasonal conditions during fall season (September).

As shown in Table 4.4, during both seasons, the overall rates of nitrogen and phosphorous removal were higher in more concentrated leachate. It indicates the high rate of microbial activity in more concentrated leachate. Cheng et al. (2002) reported that more concentrated growth media may be more favorable for microbial growth than to duckweed growth (Cheng et al., 2002). Ammonia volatilization was negligible in these experiments. It was due to the reason that average pH of the leachate was less than 8 throughout the experimental period whereas; the ammonia volatilization usually occurs at pH greater than 8 at which ammonium starts to convert into ammonia gas. Some amount of nitrogen was probably lost through algal and microbial assimilation. Similarly, the high rate of phosphorous removal at more concentrated leachate may be attributed to microbial assimilation and precipitation with minerals present in relatively larger amounts in concentrated leachate as compared to the diluted leachate media (Al-Nozaily et al., 2000).

Table 4.4: Nutrients removal rates from leachate by duckweed during summer and fall seasons under natural conditions.

Growing Season	**Leachate Concentration**	**Nutrients removal rate**			
		TKN	**NH_4-N**	**TP**	**o-PO_4^{3-}-P**
Summer	**50**	310	200	200	60
	40	230	130	150	50
	30	160	110	90	30
	20	90	50	50	20
	10	40	30	30	10
Fall	**50**	380	230	200	120
	40	280	170	140	90
	30	180	110	90	70
	20	100	60	60	30
	10	50	30	30	20

As shown in Table 4.5, significant amount of nitrogen and phosphorous could not be taken up by duckweed from more concentrated leachate during the both seasons. It is clear form table 4.4 that during summer season, duckweed absorbed only 47% and 46 % of the total removed N and P respectively from 50% leachate contrary to the 10% leachate where 95% and 90 % N and P respectively was taken up into duckweed biomass. Similar trend of nutrient removal from leachate and absorption by duckweed was observe during summer season. Less absorption of nitrogen and phosphorous by duckweed at more concentrated leachate media may be due many factors such as; presence of microorganisms, insects and undefined chemicals in field containers which caused the duckweed to take up relatively smaller percentage of total nutrients, removed from the leachate media.

Table 4.5: Mass balance of total N and P removal and uptake by duckweed at various dilutions of leachate in during summer and fall under the natural climatic conditions

Growing Season	**Leachate Concentration** (Percent by volume)	**Nutrients Removed from Leachate** **(%)**		**Nutrients Uptake by Duckweed Biomass** **(% of total removed)**	
		N	**P**	**N**	**P**
Summer	**50**	74	59	47	46
	40	69	53	56	60
	30	64	48	66	66
	20	56	42	80	83
	10	49	32	95	90
Fall	**50**	84	55	12	14
	40	78	49	51	44
	30	66	41	67	52
	20	58	35	76	70
	10	43	33	90	80

4.4.1.3. COD reduction

As shown in Figure 4.4, COD reduction from leachate also followed similar pattern as of nutrients removal. COD reduction of 46% to 79% and 44% to 67% was achieved during fall and summer experiment, respectively. The highest level of COD reduction was achieved after two days of start of each experiment. It is because of the acclimatization of duckweed which resulted into the high rate of pollution removal at the start of experiment.

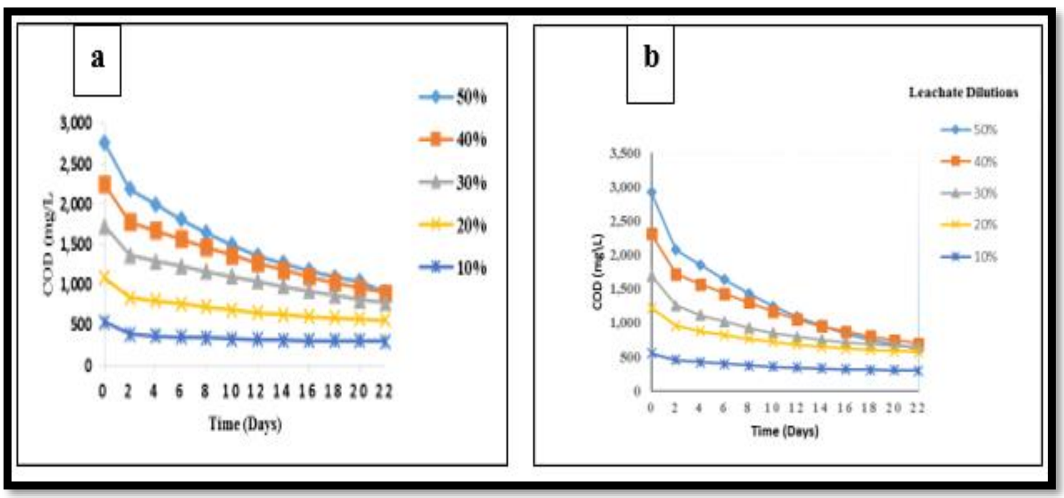

Figure 4.4: COD reduction from leachate by duckweed under natural climatic conditions during (a) summer and (b) fall seasons

It can be seen in Table 4.6 that during both seasons, COD reduction rates are significantly high at higher initial concentrations of leachate and highest removal of 6.16 g m^{-2} day^{-1} and 7.15 g m^{-2} day^{-1} of COD from 50 % leachate was achieved during summer and fall seasons respectively. It seems that in addition to removal by duckweed, high microbial activity in concentrated leachate may also be responsible for high removal of organic matted (COD).

Table 4.6: COD reduction rates from leachate by duckweed during summer and fall seasons under natural climatic conditions

Growing Season	**Leachate Concentration** (Percent by volume)	**COD** **($g\ m^{-2}\ day^{-1}$)**
Summer	**50**	6.16
	40	4.49
	30	3.17
	20	1.74
	10	0.79
Fall	**50**	7.15
	40	5.18
	30	3.62
	20	1.72
	10	0.81

4.4.2. Seasonal Effect

Table 7-7 provides the average conditions of temperature, solar radiation and day length under which, duckweed was grown on leachate during summer and fall seasons.

Table 4.7: Air temperature, solar intensity and day length for duckweed growth on leachate during summer and fall seasons.

Growing Season	**Ambient air temperature (°C)**	**Leachate temperature (°C)**	**Solar radiations ($kWh\ m^{-2}\ day^{-1}$)**	**Average day lengths (Hours)**
Summer	39.1	28-31	4.5-5	14.20
Fall	27	19-22	4- 4.5	12.20

Effect of seasonal variation on duckweed growth and its efficiency to remove nutrients and COD from leachate has been provided in Table 4.8 which shows that duckweed growth is significantly higher (6.4 g m^{-2} day^{-1}) during the summer as compared to fall season (3.8 g m^{-2} day^{-1}).

Table 4.8: Seasonal effect on duckweed growth and its efficiency to remove nutrients and COD from leachate under natural conditions.

Growing Season	**Duckweed Growth (g m^{-2} day^{-1})**	**Nutrient Removal Rates (mg m^{-2} day^{-1})**		**Nutrient Uptake by Duckweed (% of total removed)**		**COD Reduction Rate (g m^{-2} day^{-1})**
		N	P	N	P	
Summer	6.4	310	200	95	90	6.16
Fall	3.8	380	200	90	80	7.15

Temperature is one of the important parameters for plant growth and development (Yan and Hunt, 1999). Higher growth rate of duckweed in summer is due to the rate of photosynthesis which is directly proportional to temperature of growth media (Wedge and Burris, 1982). As shown in Table 4.6, at higher temperature in summer, duckweed incorporated about 95% of nitrogen and 90% of phosphorous into its biomass whereas; during fall season, relatively smaller amount of total removed N (90%) and P (80%) was taken up by the duckweed. This is consistent with high growth rate during summer season as compared to fall months.

As evident from Table 4.8, overall rate of nitrogen and COD reduction from leachate is higher during fall season when duckweed removed N at the rate of 380 mg m^{-2} day^{-1} as compared to N removal rate during summer (310 mg m^{-2} day^{-1}). This suppression in overall nitrogen removal during summer season is probably due to the factors other than duckweed such as algae and microorganisms which are more significantly affected by high temperature and solar radiations during summer season resulting in lesser absorption and decomposition of nitrogen from leachate. Algal and microbial assimilations of nutrients have been reported to play an important role in duckweed based treatment systems (Al-Nozaily et al., 2000). However, on the whole, seasonal variations in temperature, solar radiations and day length from June to September did not have any effect on phosphorous removal from leachate by duckweed.

4.4.3. Summary-Phase-I

Result of Phase-I experiment reveal that *Lemna minor* growth is high at less concentrated leachate whereas, the nutrient and COD reduction efficiency is maximum at more concentrated leachate. On the whole duckweed performs efficiently on dumpsite leachate having 20-40 % initial concentration (by volume) with an optimum concentration of 30% where duckweed maintained a good balance between growth and its efficiency to remove nutrient (N and P) and COD from leachate under the natural climatic conditions. At 30% leachate, duckweed growth rate of 5.7 and 3.2 g m^{-2} day^{-1} during summer and fall season respectively was achieved whereas, 64 & 66 N and 48 & 41% of P was removed during summer and fall seasons respectively. Out of the total removed, duckweed absorbed 66% & 67% N and 66% & 52% P respectively during summer and fall.

It is also clear that seasonal variations from June-September have indistinct effect on duckweed growth and its efficiency to remove nutrient and COD from leachate. However; the results show that relatively high temperature, sunlight and long days during summer season are more favorable for duckweed growth and its efficiency to absorb nitrogen and phosphorous from leachate in contrast to overall rates of COD and nutrients removal which are high during fall season.

4.5. RESULTS OF PHASE-II

Detail of experimental set up and treatments for Phase-II are given in Table 3.1 in Chapter-3. As mentioned in Table 3.1, during Phase-II mixed algae comprising three genera; *Ankistrodesmus, Nostoc and Anabaena* was grown on leachate with 50% initial concentration during summer and fall season under the natural climatic conditions and; removal of five heavy metals (Cu, Zn, Cr, Pb, and Fe) and nutrients (N and P) from leachate was investigated at two initial concentrations (5 and 10 mg L^{-1}) of each metal and two initial masses of algae (0.8 and 1.8 g L^{-1}). Each test during summer and fall season was run for eight (08) days.

The initial nutrients concentration (TKN, NH_4-N, TP and o-PO_4^{-3}-P) and COD of leachate is given in Table 4.9.

Table 4.9: Initial nutrients concentrations and COD of leachate (mean± SD) used as medium for growth of algae under natural conditions

Experiment Season	**Nutrients concentration (mg L^{-1})**				**COD (mg L^{-1})**
	TKN	**NH_4-N**	**TP**	**o-PO_4^{3-}-P**	
Summer	91±1.40	77±1.73	50±1.71	16±0.94	2630±6.56
Fall	99±1.64	80±1.03	54±1.49	18±0.81	2746±6.24

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophosphate, COD = Chemical Oxygen Demand

4.5.1. Nutrients Removal by Algae

During both seasons nutrients concentration in leachate was measured daily from the start of experiment till end (8 days) and results are provided in Tables 4-10 and 4-11 for summer and fall season respectively. It is clear from tables 4-10 and 4-11 that during both seasons, mixed algae performs well at 50 % leachate and significant amount of nitrogen and phosphorous was reduced during the period of eight days. It shows the high tolerance of algae for nutrients and salts present in concentrated leachate. High tolerance of algae for salts and metals have also been reported by many researchers (Gaur and Rai, 2001; Piotrowska-Niczyporuk et al., 2012; Sand-Jensen and Jespersen, 2012; Kumar et al., 2015). According to Rahman et al., 2011, this tolerance in algae may be due to genetic and/or physiological reasons. It is important to note from tables that after 5-6 days from the start of experiment during both seasons, nutrient concentration in leachate was approximately equal to the concentration of 30% leachate as given in Table 4.2 (Section 4.4.1). It suggests that starting from 50% leachate algae can be grown for 5-6 days as the pretreatment step for algae-duckweed based leachate treatment system; after which leachate would have sufficient amount of nutrients for duckweed growth after removing the heavy metals from leachate as discussed in upcoming Section 4.5.2.

Table 4.10: Daily nutrients concentration of leachate (mean± SD) with algal growth under natural conditions during summer season.

Algae	**Days**	**TKN**		**NH_4^+-N**		**TP**		**o-PO_4^{3-}-P**	
		(mg m^{-2} day^{-1})							
		5 mg L^{-1}	**10 mg L^{-1}**	**5 mg L^{-1}**	**10 mg L^{-1}**	**5 mg L^{-1}**	**10 mg L^{-1}**	**5 mg L^{-1}**	**10 mg L^{-1}**
0.8 g L-1	**1**	84.20±1.5	83.29±1.3	46.02±1.0	46.47±1.2	72.83±0.8	74.95±1.0	13.35±0.8	13.59±0.8
	2	80.12±1.2	78.08±0.9	41.84±1.1	42.25±1.1	66.22±0.9	68.14±0.9	12.96±1.0	13.20±0.8
	3	78.55±1.2	76.55±1.2	39.47±0.8	39.86±0.9	60.19±0.9	61.94±0.8	11.78±0.9	12.00±0.9
	4	71.41±1.1	70.99±0.4	39.08±0.9	39.47±0.8	54.73±1.0	56.32±0.9	10.72±1.1	10.91±1.0
	5	64.92±2.2	64.54±2.1	37.22±0.9	37.58±0.7	49.75±1.1	51.19±1.1	10.30±1.0	10.50±0.8
	6	60.88±1.1	58.29±1.9	36.49±0.8	36.85±1.1	45.23±0.8	46.54±0.7	9.36±0.9	9.53±0.9
	7	50.74±1.5	49.40±0.9	28.02±1.0	28.35±1.2	41.12±0.9	42.32±0.9	8.51±0.8	8.67±0.7
	8	42.28±2.4	44.91±1.6	23.35±0.7	23.63±1.4	38.47±0.8	37.90±1.0	6.09±1.0	6.19±0.9
1.6 g L-1	**1**	81.29±1.0	82.24±0.8	44.08±1.0	45.93±0.9	70.70±1.1	71.40±0.8	12.91±1.0	13.28±0.9
	2	75.31±1.0	76.26±1.0	40.08±0.9	40.75±0.9	64.27±1.2	64.91±0.9	12.53±0.6	12.90±0.9
	3	73.83±1.0	74.77±0.8	37.81±0.9	39.39±0.8	58.43±0.9	59.00±0.7	11.40±0.7	11.72±0.8
	4	67.97±1.3	69.59±1.9	37.44±0.6	38.99±1.0	53.11±0.9	53.63±1.1	10.36±0.9	10.67±0.8
	5	61.79±1.3	63.26±1.2	35.64±1.2	37.14±1.0	48.30±0.8	48.77±1.2	9.96±0.9	10.25±0.9
	6	57.51±2.2	59.02±1.6	34.94±1.0	36.41±1.2	43.90±0.9	44.33±0.8	9.07±0.9	9.31±0.8
	7	43.24±1.7	45.40±1.8	26.88±1.0	28.07±1.0	39.92±0.9	40.31±0.9	8.23±1.0	8.48±0.9
	8	39.31±0.7	41.27±2.5	22.40±1.2	23.39±1.0	35.59±1.2	35.59±1.2	5.88±0.9	6.05±0.9

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophospha

Table 4.11: Daily nutrients concentration of leachate (mean± SD) with algal growth under natural conditions during fall season.

Algae	**Days**	**TKN**		**NH_4^+-N**		**TP**		**o-PO_4^{3-}-P**	
		(mg m^{-2} day^{-1})[a]							
		5 mg L^{-1}	**10 mg L^{-1}**	**5 mg L^{-1}**	**10 mg L^{-1}**	**5 mg L^{-1}**	**10 mg L^{-1}**	**5 mg L^{-1}**	**10 mg L^{-1}**
0.8 g L-1	**1**	88.38±1.41	93.46±1.39	48.21±1.10	51.43±1.13	75.48±1.07	77.91±1.03	16.33±1.08	16.37±1.07
	2	84.98±0.71	89.87±1.59	46.29±1.15	48.37±1.21	68.63±0.90	70.82±1.09	15.74±1.09	15.98±1.07
	3	80.73±1.49	85.38±1.26	42.84±1.12	44.85±0.91	62.38±1.19	64.38±0.93	15.30±1.13	15.32±1.10
	4	75.08±1.09	79.40±1.51	39.85±0.97	41.68±1.13	57.71±1.15	58.54±1.20	14.83±1.09	14.91±1.09
	5	70.94±1.05	75.03±1.00	35.23±1.11	37.90±1.08	51.56±1.06	53.22±1.06	13.53±1.08	14.79±2.19
	6	57.07±0.96	61.55±1.16	32.94±0.97	33.42±1.11	46.87±0.91	48.38±0.99	12.26±1.10	12.30±1.07
	7	46.69±1.28	50.35±0.98	30.93±1.03	31.89±1.10	39.51±0.69	40.41±1.20	9.15±1.07	9.89±1.07
	8	43.61±1.24	46.13±1.05	26.75±1.13	28.46±1.06	38.74±1.08	39.61±0.95	7.63±1.12	8.24±1.08
1.6 g L-1	**1**	85.33±1.05	85.83±1.24	45.74±1.07	47.40±1.12	73.25±1.07	75.50±1.10	15.93±1.05	16.09±1.06
	2	81.40±1.20	81.57±0.72	43.91±1.07	45.50±1.16	64.60±1.15	67.73±1.13	15.53±1.09	15.61±1.10
	3	77.33±1.18	77.49±0.71	40.57±0.86	43.09±1.05	60.54±1.06	61.58±0.92	15.04±1.03	15.22±1.09
	4	71.92±0.98	72.07±0.64	37.79±0.85	39.17±0.94	53.03±1.01	55.97±0.93	14.46±1.12	14.64±1.10
	5	67.94±1.02	68.09±0.99	33.62±1.10	34.37±1.08	50.03±1.03	50.88±1.06	13.19±1.08	13.27±1.12
	6	58.41±0.96	59.12±1.02	31.24±1.09	32.37±1.12	45.49±1.17	46.27±1.03	11.18±1.07	11.98±0.98
	7	47.78±0.89	48.37±1.02	29.24±1.05	30.38±1.10	36.67±1.12	36.99±1.08	8.34±1.08	8.78±1.11
	8	41.78±1.05	41.87±0.92	24.81±1.10	25.21±0.96	35.97±1.00	36.25±1.05	6.95±1.04	7.32±1.07

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophosphate

The percent removal of nutrients from lachate during summer and fall seasons is provided in Figures 4-5 and 4-6 respectively. It is clear from figures that increase in initial concentration of each metal from 5 to 10 mg L^{-1} in leachate media has insignificant effect on nutrient removal efficiency of algae. This indicates that presence of heavy metals (Cu, Zn, Cr, Pb, and Fe) in the range of 5 to 10 mg L^{-1} in leachate has minor interference with nutrient uptake by algae. Algal tolerance for heavy metals have also been reported by Kumar et al., 2015 however; beyond this concentration heavy metals such as, Zn, Cu, Ni, and Cd in duckweed biomass may induce various enzymatic and physiological disorders resulting in stunted duckweed growth and its poor nutrient uptake efficiency as reported by many researchers (Assche and Clijsters, 1990; Prasad et al., 2001; Drost et al., 2007).

It can also be seen from Figures 4-5 and 4-6 that nutrient removal efficiency of mixed algae is slightly increased with an increase in initial algal dose from 0.8 to 1.6 g L^{-1}. It is simply because with increase in algal dose additional amounts of algal cells are available to absorb the same initial amounts of nutrients in the leachate.

A comparison of Figures 4-5 and 4-6 depicts that nutrient removal efficiency of mixed algae is less effected by seasonal variations from June to September (Figure 3.6 in Chapter 3). It is reported by Raven and Geider, 1988 that algae can survive at extreme weather conditions and can perform well in temperature range of 5-40 $^{\circ}C$.

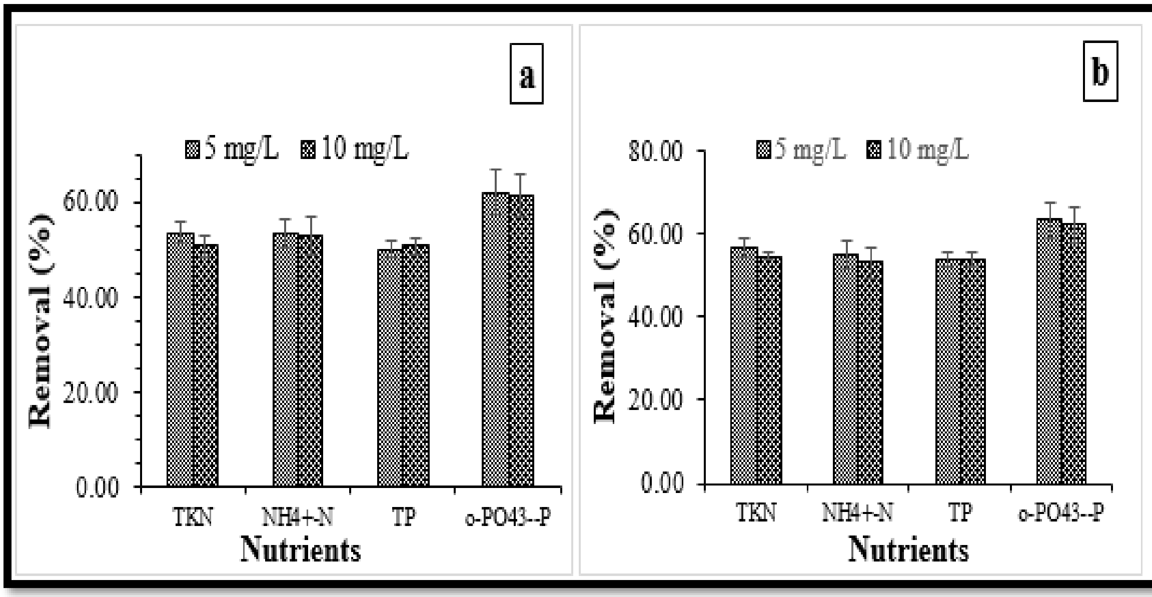

Figure 4.5: Percent of nutrients removed by algae from leachate at (a) 0.8 g L^{-1} and (b) 1.6 g L^{-1} algal dose during summer season under natural conditions

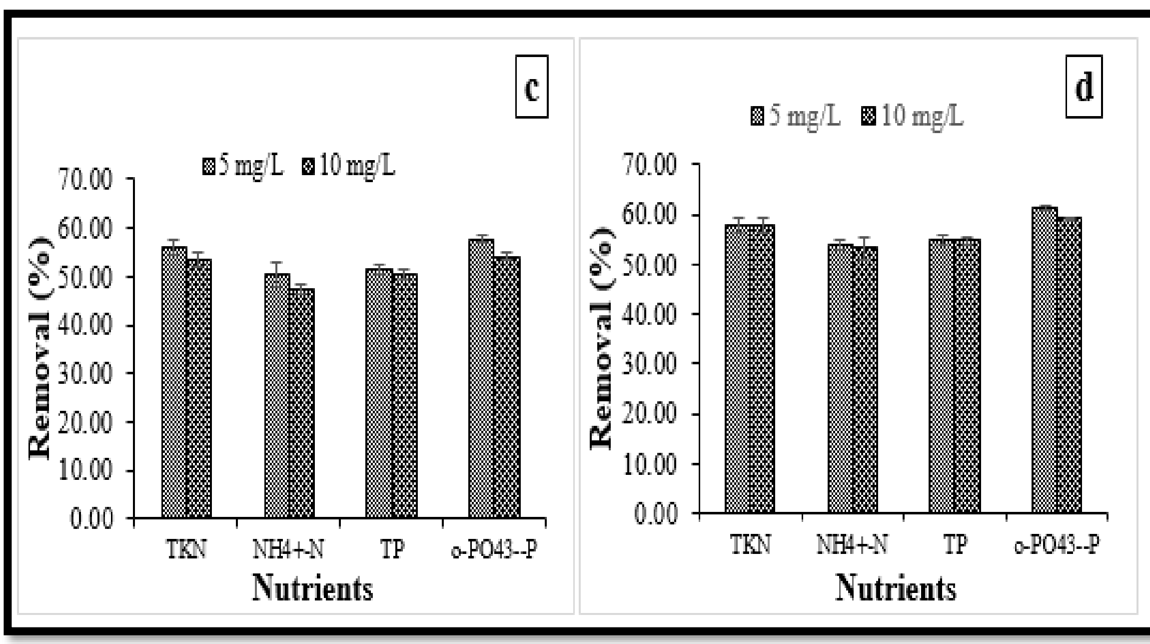

Figure 4.6: Percent of nutrients removed by algae from leachate at (c) 0.8 g L^{-1} and (d) 1.6 g L^{-1} algal dose during fall season under natural conditions

4.5.2. Heavy Metal Removal by Algae

Per gram heavy metal bio sorption capacity of mixed algae at equilibrium and its metal removal efficiency (Percent) was calculated by using the mathematical equations as explained in 3.2.7.1 in Chapter 3. Table 4.12 provides the average equilibrium bio sorption of metals for two batch tests during summer and fall seasons which indicates that mixed algae have ability to remove all test metals from leachate. Equilibrium metal bio sorption was in the order of Pb >Cu >Zn> Fe> Cr. It is clear from Table 4.12 that at both algal doses, equilibrium bio sorption capacity of algae increases with increasing initial concentration of each metal from 5 to 10 mg L^{-1}. According to Andrade et al., (2005), cell surface is the main site of metal binding in algae and heavy metal sorption by algae involves the exchange of metal ions with protons or cations bound at algal cell surface (Mehta and Gaur, 2005). At higher initial concentration of metals in solution, the chances of collusion between metal ions and available cations at bio sorbent surface increases resulting in increased bio sorption capacity of algae.

Table 4.12: Average (± SD) equilibrium bio sorption (Q) of heavy metals by algae from leachate under natural conditions

Algal Dose ($g L^{-1}$)	**Initial Concentration ($mg L^{-1}$)**	**Q (mgg^{-1})**				
		Fe	**Cu**	**Pb**	**Cr**	**Zn**
0.8	5	2.46 ±3.4	2.81 ± 2.7	3.25 ±1.9	2.08 ± 2.0	2.53 ± 3.7
	10	5.09 ±3.3	5.85 ± 2.2	7.03 ± 3.6	3.34 ± 2.3	5.73 ± 4.2
1.6	5	1.29 ± 2.5	1.46 ± 1.8	1.78 ± 1.7	0.98 ± 3.2	1.16 ± 3.1
	10	2.44 ± 1.2	2.81 ± 2.0	3.47 ± 1.8	1.63 ±2.8	2.62 ± 2.6

High sorption of Pb (7.03 mg g^{-1} at initial metal concentration of 5 mg L^{-1} and algal dose of 0.8g L^{-1}) is probably due to the presence of relatively higher amount of one or more of the preferred ligands for Pb on algal biomass including H−, I −, R −, CN −, CO, S2−, RS −, R 2S, R3As (Wang et al., 2009).

Algae exhibited least per gram bio sorption capacity for Cr metal which is attributed to the pH of leachate media which was maintained around 7 throughout the experiment. El-Sikaily et al., 2007 reported that at pH > 3 the sorbent cell wall possesses more functional group carrying net negative charges which tend to repulse the chromium anions resulting in less sorption of Pb anions at higher pH of the growth media. No significant difference in metals concentration was observed in non-algal control containers. Metal sorption amount of each control container was subtracted from amount of each metal sorbed by algae at equilibrium. In present study, high sorption of Pb (7.03 mg g^{-1} at initial metal concentration of 5 mg L^{-1} and algal dose of 0.8g L^{-1}) is probably due to the presence of relatively higher amount of one or more of the preferred ligands for Pb on algal biomass.

4.5.2.1. Kinetic studies

For kinetic studies, percent bio sorption of Fe, Cu, Pb, Cr and Zn was taken as the function of contact time in days using the mathematical relationships provided in Chapter 3 (Section 3.2.7.2). Kinetic studies were carried out at two algal doses (0.8 and 1.6 g L-1) and initial metal concentrations of 5 and 10 mg L^{-1}. Figure 4.7 shows that removal efficiency of algae for heavy metals Pb, Cu, Zn, Fe and Cr increases with an increase in contact time up to five days for different metals; after which equilibrium was achieved and removal percent of each metal becomes constant till the end of experiment at eighth days. Results of this study showed significant difference in equilibrium time which is achieved after 3-5 days in our experiment using living algal mass and dumpsite leachate under natural environment; as compared to when processed dead algal mass is used as bio sorbent for heavy metal removal from artificial metal solutions.

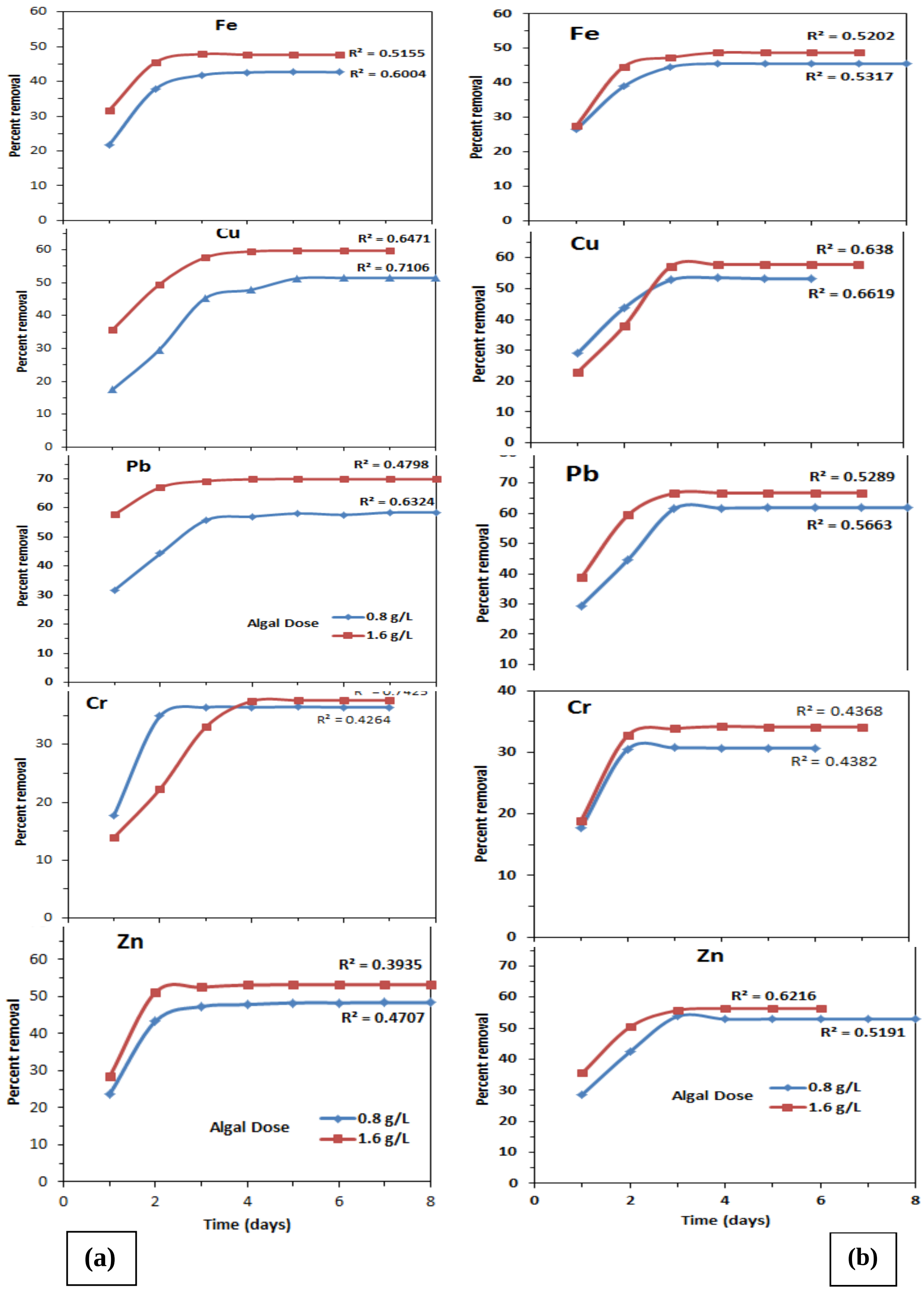

Figure 4.7: Time based removal efficiencies of heavy metals by algae from leachate at initial metal concentrations of (a) 5 mg L^{-1} and (b) 10 mg L^{-1} under natural conditions

In later cases many researchers have reported the algal equilibrium bio sorption time of 90-150 minutes for different heavy metals under various conditions of pH, algal type and dose and initial metal concentrations in sloution (Gupta et al., 2006; Prabakaran and Arivoli, 2012; Małgorzata, 2013; Gupta and Rastogi, 2008). Figure 4.7 also shows that bio sorption rate was faster during first two days and most of removal of heavy metals took place during this period.

It is evident from Figure 4.7 that a linear relationship exists between percent removal of heavy metals and contact time. Therefore, equation-4 (Please refer to Section 3.2.7.2 in Chapter 3) was fitted in experimental data and values of constants "a" and "b" were calculated and provided in Table 4.13.

Table 4.13: Values of bio sorption kinetic constants.

Metal	Initial Metal	Algal Dose	Log a	a	b
Fe	5	0.8	1.42	26.2	3.44
		1.6	1.56	36	2.47
	10	0.8	1.52	33.2	2
		1.6	1.54	34.6	2.6
Cu	5	0.8	1.37	23.4	4.4
		1.6	1.6	40.1	3.38
	10	0.8	1.52	32.7	4.25
		1.6	1.47	29.3	5.16
Pb	5	0.8	1.61	40.4	2.58
		1.6	1.8	62.5	1.19
	10	0.8	1.59	38.8	3.73
		1.6	1.68	47.6	3.52
Cr	5	0.8	1.4	25.1	2.11
		1.6	1.21	16.2	3.8
	10	0.8	1.34	21.9	1.88
		1.6	1.39	24.7	1.74
Zn	5	0.8	1.53	33.7	2.39
		1.6	1.6	39.9	2.21
	10	0.8	1.57	36.9	2.63
		1.6	1.6	39.6	3.48

It can be observed from Table 4.12 that for heavy metals; Fe, Pb and Zn, values of constant "a" increase with increase in algal dose at both initial concentrations (5 and 10 mg L^{-1}) of each metal. It suggests that metal bio sorption capacity of algae for these metals increases with increase in algal dose. However; the Copper removal efficiency of algae is slightly decreased with increasing algal dose at 10 mg L^{-1} of initial concentration of this metal in leachate suggesting the low tolerance of this algae for Cu. It can also be seen from Table 4.12 that removal efficiency of Cr also increases with increase in algal dose at initial metal concentration of 10 mg L^{-1} however, at 5 mg L^{-1} of metal concentration, the value of constant "a" indicated a decrease with increase in algal dose. This is probably due to the pH of the leachate media as discussed in Section 4.5.2 above. Vales of constant "b" ranges from 2 to 3.44 for Fe, 3.38 to 5.16 for Cu, 1.19 to 3.73 for Pb, 1.74 to 3.8 for Cr and 2.21 to 3.48 for Zn. The lower value of b shows that rate of percent removal of metal decreases with increase in contact time.

4.5.3. Conclusions-Phase-II

Mixed algae (*Ankistrodesmus, Nostoc and Anabaena)* can efficiently remove nutrients from leachate under natural climatic conditions. Nutrient removal efficiency of algae is less effected by seasonal variations from June-September in Pakistan. Variation in initial metal concentration in leachate (5 and 10 mg L^{-1}) and initial algal dose (0.8 and 1.6 g L^{-1}) also exhibited little effect on nutrient removal efficiency of algae.

Living algal mass of mixed algae can be used as an effective bio sorbent for heavy metal (Fe, Cu, Pb, Cr and Zn) removal from leachate under natural climatic conditions. Metal removal was in the order of Pb >Cu >Zn> Fe> Cr. Initial metal concentration in leachate and algal dose affected the metal bio sorption capacity and removal efficiency of algae.

Overall the results of Phase-II conclude that starting from 50% initial leachate concentration, a retention time of about 6 days is sufficient for growth of algae on leachate performing as pretreatment step for integrated algae-duckweed based leachate treatment system. During this period algae removed sufficient amount of nutrients from leachate and remaining concentration of nutrients and COD was nearly equal to the concentration of 30% leachate. Sufficient amount of heavy metals were also removed from leachate during this period ensuring the safe use of leachate for duckweed growth and its post uses as animal feed or fertilizer, etc.

4.6. RESULTS OF PHASE-III

As described in Table 3.1 (Chapter 3), Phase-III involves two sets of experiments as below: -

4.6.1. Experiment-1

This experiment was designed to investigate the effect of pH on duckweed performance grown on leachate under natural conditions. Detailed experimental design can be seen at Figure 3.7. As per details provided in Section 3.2.5.3, two batch tests were performed during summer and fall seasons using the leachate with 30% initial concentration. During each test, seven initial pH levels (4, 5, 6, 7, 8, 9 and 10) of the leachate were adjusted and duckweed was separately grown at leachate with each pH level. Table 4.14 below provides the initial concentrations of nutrients and COD in leachate during both seasons.

Table 4.14: Initial nutrients concentrations and COD (mean ± SD) of leachate used as medium for growth of *L. minor* in natural conditions.

Experiment Season	**Nutrients concentration ($mg\ L^{-1}$)**				**COD ($mg\ L^{-1}$)**
	TKN	**NH_4-N**	**TP**	**o-PO_4^{3-}-P**	
Summer	51.30±0.25	33.80±0.25	76.00±0.17	15.00±0.13	1728±3.61
Fall	58.20±0.31	30.25±0.17	44.55±0.25	17.60±0.17	1671±2.65

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophosphate, COD = Chemical oxygen demand

Considerable fluctuation in pH was observed in each container despite daily pH adjustment. Table 4.15 shows the average pH and ranges observed during the experimental period. Average pH during experimental period is calculated as average of the daily pH values before and after the adjustment and; pH range indicates the range between daily minimum and maximum average pH levels of the leachate.

Table 4.15: Average pH values (mean ± SD) of leachate and ranges observed during experimental period.

Initial pH	**Average**	**pH Range**
4	3.6±0.1	2.9-4
5	4.5±0.2	3.3-5.1
6	5.5±0.3	4.8-6
7	6.0±0.1	5.4-7
8	7.1±0.2	5.9-8
9	8.1±0.2	6.9-9
10	8.6±0.1	8.4-10

It was observed during both seasons that at pH 4 (average 3.6) almost all duckweed plants were dead during the first five to six days of planting. It is because at extremely low pH, electrochemical gradient across plasma membrane is decreased due to an increased influx of H^+ ions which is toxic for the growth of floating aquatic plants as reported by Mufarrege et al., 2011. It is also reported by Caicedo et al., 2000 that a growth media with pH less than 5 has direct detrimental effects on physiology of duckweed.

4.6.1.1. Effect of pH on duckweed growth

Results of this study show that under natural climatic conditions, duckweed; *Lemna minor* can tolerate the large fluctuations in pH of the leachate media from 4.5-8.6 without having significant impact on growth rate (Table 4.16). However, as shown in Table 4.16 pH range from about 7-8 is the most favorable for duckweed performance on leachate with an optimum value of 7.1. As reported by Caicedo et al., 2000, at pH range from 6.5 to 7.5 most of the nitrogen in growth media is present in the form of ammonium ions which is the preferred form of nitrogen for duckweed absorption. Above pH 8 ammonium in leachate starts to convert into ammonia; high concentration of which is toxic to duckweed growth as reported by Körner et al., 2001.

It can also be noted from Table 4.16 that seasonal variations from June to September have less significant effect on duckweed growth except at pH 7.1 where substantial increase in growth rates from 3.26±0.08 g m^{-2} day^{-1} to 5.51±0.16 g m^{-2} day^{-1} was observed during fall and summer seasons respectively at this pH. This may be due to combined effect of high temperature in summer and pH which seems more prominent at pH 7.1 in this experiment.

4.6.1.2. Effect of pH On nutrient and COD reduction

Removal rates of nutrients and COD at various pH level during summer and fall seasons have been provided in Table 4.16 which shows that during both seasons starting from pH 4.5, nutrients and COD reduction increases with increase in pH of the leachate until the maximum rate of removal at pH of 7.1. As pH increases above 7 the removal rates begin to decline in alkaline range but still higher when pH of the leachate is less than 7. It is evident from the results that the overall rates of nitrogen and phosphorous removal from leachate by duckweed were higher at neutral to slightly basic pH in the range of 7.1 to 8.1 during both seasons. This was found to be consistent with the duckweed growth data at this pH range.

Table 4.16: Rates of nutrients (N and P), COD reduction and duckweed *(L .minor)* growth at various pH of leachate under the natural climatic conditions of Islamabad, Pakistan.

Experiment Season	**pH**	**Nutrients removal rate (mg m^{-2} day^{-1})**				**COD (g m^{-2} day^{-1})**	**Growth rate g m^{-2} day^{-1}**
		TKN	**NH_4-N**	**TP**	**o-PO_4^{3-}-P**		
Summer	**4.5**	80.87±0.52f	67.12±0.17f	43.61±0.10f	17.52±1.15e	2.41±6.81d	2.24±0.06f
	5.5	98.41±0.65e	73.73±0.32e	63.00±0.17e	20.48±0.28d	2.47±4.58cd	2.85±0.09e
	6.0	119.69±0.79c	88.47±0.42d	71.66±0.38c	24.46±0.69c	3.12±7.94b	3.51±0.11c
	7.1	170.53±0.69a	119.25±0.37a	94.23±0.07a	31.37±1.23a	3.52±4.36a	5.51±0.16a
	8.1	143.24±0.29b	101.02±0.32b	76.80±0.99b	28.10±0.28b	3.32±6.81ab	4.47±0.13b
	8.6	115.43±0.42d	92.38±0.39c	65.07±0.31d	24.22±0.71c	2.71±5.69c	3.11±0.11d
LSD (0.05)		**1.04**	**0.61**	**0.83**	**1.45**	**0.26**	**0.20**
Fall	**4.5**	87.95±0.56f	63.74±0.17f	41.58±0.13f	34.47±0.26e	2.32±3.61d	1.94±0.08e
	5.5	106.27±0.69e	70.09±0.29e	62.01±0.17d	51.45±1.19c	2.41±4.73d	2.68±0.15d
	6.0	129.67±0.87c	84.16±0.43d	71.03±0.41c	59.89±0.19b	2.82±5.20c	2.98±0.10bc
	7.1	187.58±0.77a	113.41±0.35a	93.93±0.04a	74.59±0.97a	3.52±6.35a	3.26±0.08a
	8.1	154.37±0.31b	95.84±0.26b	76.01±0.99b	59.22±0.15b	3.21±5.51b	3.11±0.08ab
	8.6	125.54±0.45d	87.69±0.38c	58.26±0.29e	45.18±0.52d	2.68±4.04c	2.94±0.07c
LSD (0.05)		**1.13**	**0.57**	**0..82**	**1.21**	**0.18**	**0.17**

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophosphate, COD = Chemical oxygen demand
LSD = Least Significant Difference at 5% probability. Values with different letters in single column differ significantly at p = 0.05.

In addition to absorption by duckweed at pH range from 7.1 to 8.1, the process of nitrification and de-nitrification in leachate seems to have significant contribution in nitrogen removal as this pH range is most favorable for the optimum growth of microorganisms responsible for nitrification and de-nitrification (Antoniou et al., 2000). Similar results about the effect of pH on duckweed performance were reported by Körner et al. (2003).

Decomposition of organically and inorganically bounded phosphorous also increased with an increase in pH and is highest at neutral pH, resulting in highest removal rate of TP and uptake by duckweed at pH 7.1. Liu et al., 1996 during an experiment reported that biological phosphorous removal is optimum at pH range of 6.5 to 8.0. In addition to uptake by duckweed biomass, some of the P removal may also have occurred by microbial assimilation which increases with the increase in pH of the leachate media up to 7 which according to Al-Nozaily et al., 2000 is the more favorable pH for microbial growth and phosphorous assimilation.

Mass balance between total nitrogen and phosphorous removed from the leachate and amount of these nutrients absorbed by duckweed biomass is provided in Table 4.17. It is clear from the table that during both seasons, nitrogen and phosphorous uptake by duckweed shows similar pattern as of nutrient removal from the leachate i.e. the pH 7.1 was found optimum for the removal as well the uptake of nutrients from leachate by duckweed. It indicates that pH also has direct effect on nitrogen and phosphorous absorption by duckweed in addition to nutrient uptake through associated factors such as microorganisms and other organisms living in duckweed containers. It is also evident from Table 4.17 that at all pH levels, nitrogen uptake by duckweed is comparatively higher than phosphorous. Besides the direct absorption of nitrogen by duckweed fronds and roots, nitrogen fixing cyanobacteria associated with duckweed may also be accounted for high uptake of nitrogen as reported by Duong and Tiedje, 1985. According to Duong and Tiedje, 1985 heterocystous cyanobacteria are attached to the reproduction organs, lower epidermis of fronds and occasionally with the roots of duckweed. High uptake of nitrogen helps to increases the protein content of duckweed and increasing the importance of duckweed as feed resource for animals and its use as fertilizers (Leng et al., 1994).

Table 4.17: Mass balance of total N and P removal and uptake by duckweed at various pH of leachate during summer and fall seasons under the natural climatic conditions.

Experiment Season	pH	Total nutrients removed from leachate				Nutrients uptake by duckweed			
		mg L^{-1}		%		mg L^{-1}		% of total removed	
		N	P	N	P	N	P	N	P
Summer	4.5	21.35±0.14f	11.52±0.04f	41.61±0.28f	27.56±0.12f	9.35±0.35f	4.41±0.09f	43.79±1.36f	38.3±0.65f
	5.5	25.99±0.16e	16.64±0.04e	50.66±0.42e	39.80±0.13e	15.60±0.58e	7.35±0.16e	60.02±1.89e	44.2±0.91e
	6.0	31.60±0.20c	18.95±0.08c	61.60±0.61c	45.32±0.34c	20.93±0.13c	11.15±0.27c	66.24±0.06c	58.8±1.41c
	7.1	45.03±0.19a	24.88±0.07a	87.77±0.17a	59.52±0.25a	35.47±0.65a	17.37±0.50a	78.77±1.13a	69.8±2.19a
	8.1	37.81±0.09b	20.28±0.26b	73.71±0.38b	48.51±0.55b	28.35±0.09b	12.77±0.16b	74.98±0.05b	63.0±0.06b
	8.6	30.48±0.11d	17.19±0.09d	59.41±0.49d	41.11±0.14d	19.47±0.45d	8.70±0.33d	63.87±1.31d	50.6±1.66d
	LSD (0.05)	**0.27**	**0.22**	**0.74**	**0.53**	**0.77**	**0.51**	**2.11**	**2.39**
Fall	4.5	28.25±0.51f	10.97±0.02f	39.90±0.39f	24.64±0.05f	10.58±0.45e	2.96±0.20e	37.49±2.16e	26.94±1.8e
	5.5	32.89±0.58e	16.37±0.06d	48.20±0.40e	36.75±0.30d	16.40±0.53d	5.22±0.48d	49.89±2.33d	31.89±2.9d
	6.0	38.50±0.68c	18.75±0.12c	58.83±0.36c	42.09±0.48c	22.08±0.66c	8.43±0.38c	57.39±2.65c	45.00±2.3b
	7.1	51.93±0.38a	24.80±0.04a	85.10±0.79a	55.67±0.33a	37.77±0.71a	13.70±0.64a	72.74±1.80a	55.23±2.6a
	8.1	44.71±0.56b	20.08±0.25b	70.03±0.38b	45.05±0.80b	29.50±0.38b	9.72±0.20b	65.98±1.32b	48.43±1.3b
	8.6	37.38±0.65d	15.37±0.05	56.95±0.13d	34.53±0.15e	20.67±0.33c	5.55±0.18d	55.31±1.83c	36.12±1.1c
	LSD (0.05)	**1.01**	**0.21**	**0.80**	**0.76**	**0.94**	**0.69**	**3.67**	**3.71**

LSD = Least Significant Difference at 5% probability. Values with different letters in single column differ significantly at p = 0.05.

4.6.2. Conclusions-Experiment 1, Phase III

Duckweed; *Lemna minor* can tolerate slightly acidic to basic pH range of the leachate under natural climatic conditions and seasonal variations from June to September have less significant effect on duckweed performance at all pH levels. During both summer and fall seasons, the average pH range from 6-8 have been found suitable for growth and nutrient removal and uptake efficiency of duckweed with and optimum value of pH 7.1.

At this range of pH, during both seasons average duckweed growth rate was between 2.98-5.51 g $m^{-2}day^{-1}$ and duckweed was able to remove on an average about 58-87% nitrogen, 42-59% phosphorous and 61-63% COD from leachate. Out of the total removed, about 57-78% N and 45-69% P was taken up by duckweed biomass at pH range from 6-8.

Overall it can be concluded that duckweed; *Lemna minor* has high tolerance for pH changes of growth media however; an efficient pH management in the range of 6-8 can give high yield of duckweed mass and improved efficiency to remove nutrients and COD from leachate.

4.6.3. Experiment 2

As provided in Table 3.1 in Chapter 3, this experiment was performed to investigate the effect of Electrical Conductivity (EC) of leachate on duckweed performance under natural climatic conditions. Starting with leachate of 30% initial concentration, two batch tests were performed during summer (June-July) and fall (September-October) seasons. During each test duckweed was grown at six different EC levels (500, 1000, 1500, 2000, 2500 and 3000 µS cm^{-1}) of the leachate for 25 days. Please see Section 3.2.5.3 and Figure 3.8 in Chapter 3 for detailed experimental set up of experiment-2 under Phase-II. The initial concentration of nutrients and COD of leachate is provided in Table 4.18.

Table 4.18: Initial concentrations of nutrients and COD (mean ± SD) of leachate used as medium for duckweed growth under natural conditions.

Experiment Season	**Nutrients concentration (mg L^{-1})**				**COD (mg L^{-1})**
	TKN	**NH_4-N**	**TP**	**o-PO_4^{3-}-P**	
Summer	49.76±0.24	32.55±0.24	76.00±0.17	15.00±0.12	1693±3.53
Fall	56.05±0.30	29.04±0.16	43.17±0.24	16.97±0.17	1624±2.57

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophosphate, COD = Chemical oxygen demand

4.6.3.1. Effect of electrical conductivity on duckweed growth

During both summer and fall seasons, duckweed grew well at EC levels from 500 to 3,000 μScm^{-1} of the leachate as shown in Table 4.19 however; the EC value of 1, 000 μScm^{-1} was found optimal for duckweed growth during both seasons. At this EC, duckweed showed the growth rates of 5.70±0.16 and 3.21±0.08 g m^{-2} day^{-1} respectively during summer and fall seasons. It can also be seen in the table 4.19 that below and above the EC 1,000 µScm^{-1} of leachate duckweed growth was retarded. At high salinity levels, probably the osmotic effect induced by high salt concentration in leachate resulted in reduced growth of the duckweed plants as suggested by Tkalec et al., (2001) . It is generally accepted that low salinity is favorable for duckweed growth as documented by Yilmaz, (2007). But our results exhibited that salinity less than 1,000 µScm^{-1} has detrimental effect on duckweed growth. It is reported by McIlraith et al., (1989) that low salinity has positive effect on the growth of duckweed in the start of cultivation when there is less population of duckweed plants however, with the increase in population density, growth is reduced at low salinity due to increased competition for space. It is also clear from Table 4.19 that duckweed growth was less impacted by seasonal variations from June to September and during both seasons it showed minor variations in growth rates at all EC levels of leachate.

4.6.3.2. Effect of EC on nutrient and COD reduction by duckweed

It was observed that at all EC levels from 500 to 3,000 µS cm^{-1}, nutrients and COD concentration in leachate was considerably decreased during both summer and fall however; the maximum rates of nutrient and COD reduction were recorded at 1000 µS cm^{-1} EC of the leachate (Table 4.19). At this EC, duckweed exhibited the TKN removal rates of 175.64±0.72 and 182.89±0.7 mg m^{-2} day^{-1} respectively during summer and fall seasons whereas; the TP removal rates of 92.35±0.07 and 91.12±0.04 mg m^{-2} day^{-1} respectively were recorded at this EC of the leachate.

Table 4.19: Rates of nutrients and COD reduction and duckweed growth (mean ± SD) at various EC levels of leachate under the natural climatic conditions

Experiment Season	**EC μScm^{-1}**	**Nutrients removal rate**				**COD ($g\ m^{-2}\ day^{-1}$)**	**Growth rate ($g\ m^{-2}\ day^{-1}$)**
		TKN	**NH_4-N**	**TP**	**o-PO_4^{3-}-P**		
Summer	**500**	166.43±1.07c	118.80±0.37c	89.76±0.21b	30.91±0.4ab	3.46±7.21b	3.63±0.11c
	1000	175.64±0.72a	124.01±0.39a	92.35±0.07a	31.83±1.25a	3.66±9.07a	5.70±0.16a
	1500	168.45±0.34b	122.22±0.51b	90.32±1.18b	31.17±0.88a	3.46±9.37b	4.63±0.13b
	2000	164.22±1.09d	118.40±0.31c	86.44±0.24c	30.29±0.30abc	3.42±8.89bc	3.53±0.1c
	2500	154.77±1.02e	115.61±0.51d	86.09±0.41c	29.18±1.92bc	3.38±7.37c	3.47±0.10c
	3000	152.71±0.55f	115.61±0.47d	84.27±0.45d	28.49±0.83c	3.38±7.37c	3.22±0.12d
	LSD (0.05)	**1.51**	**0.78**	**0.99**	**1.92**	**0.07**	**0.22**
Fall	**500**	179.16±1.13c	114.54±0.35b	84.71±0.28c	63.19±0.16c	3.19±5.86bc	3.07±0.08ab
	1000	182.89±0.75a	117.31±0.32a	91.12±0.04a	76.86±1.77a	3.29±6.08a	3.21±0.08a
	1500	180.99±0.36b	114.83±0.50b	88.47±1.15b	75.52±0.24a	3.23±5.77b	3.17±0.18a
	2000	176.46±1.18d	111.63±0.30c	84.21±0.23c	72.35±0.94b	3.15±4.62c	2.94±0.10bc
	2500	172.86±0.62e	109.90±0.45d	82.68±0.47d	56.84±0.43d	3.14±4.73c	2.90±0.07bc
	3000	164.24±1.06f	107.54±0.55e	76.30±0.38e	52.59±0.60e	3.10±5.57c	2.87±0.12c
	LSD (0.05)	**1.60**	**0.75**	**0.97**	**1.57**	**0.06**	**0.20**

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophosphate, COD = Chemical oxygen demand

LSD = Least Significant Difference at 5% probability. Values with different letters in single column differ significantly at p = 0.05.

Percent removal of nutrients and COD from leachate by duckweed during summer and fall seasons is shown in Figures 4-8 and 4-9 respectively. It is clear from the figures that at EC 1,000 µS cm^{-1}, duckweed removed maximum amounts of N (about 86.89% & 84.83%), P (about 59.27% & 55.89% and COD (about 64.83% and 60.86%) respectively during summer and fall seasons after 25 days period of growth on leachate. Overall an EC range from 1,000 to 1,500 µS cm^{-1} was found good for better efficiency of duckweed to remove nutrients and COD from leachate during both seasons.

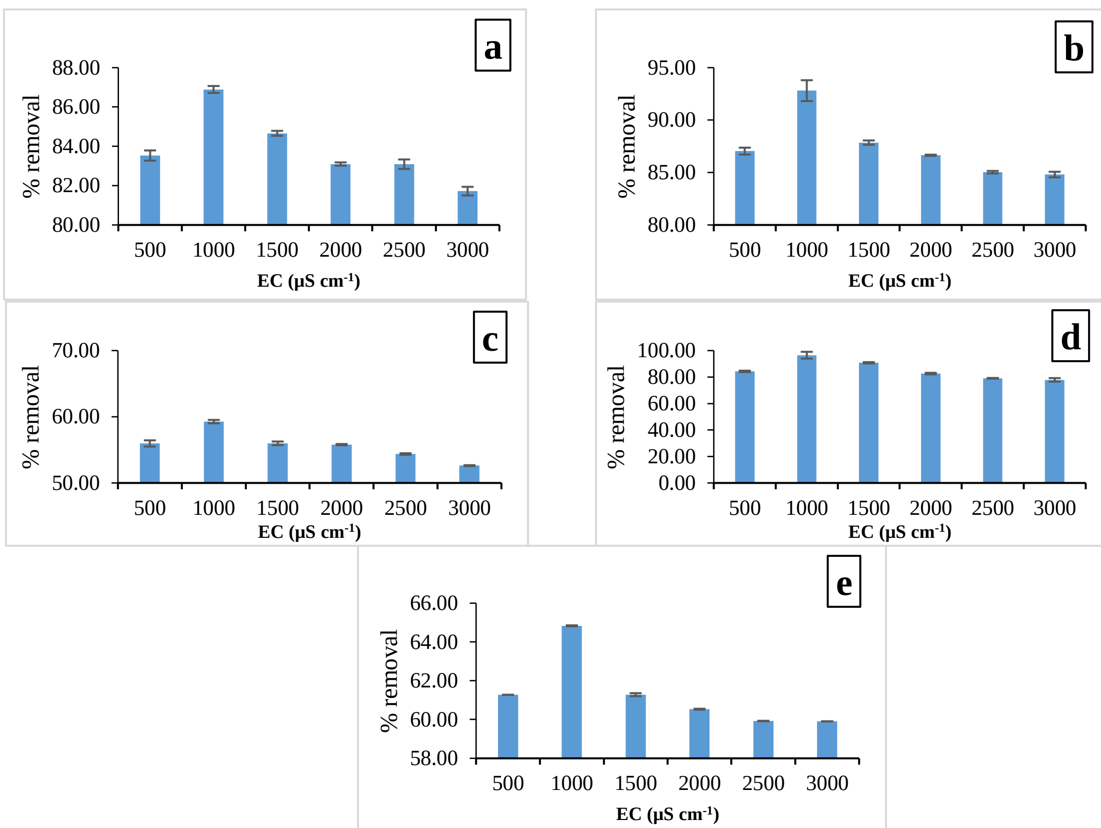

Figure 4.8: Percent removal of (a) total kjeldahl nitrogen (TKN), (b) ammonium-N (NH_4^+-N), (c) total phosphorus (TP), (d) ortho-phosphate-P (o-PO_4^{-3}-P) and (e) chemical oxygen demand (COD) at various EC levels of leachate by growing Lemna minor under natural climatic conditions during summer season.

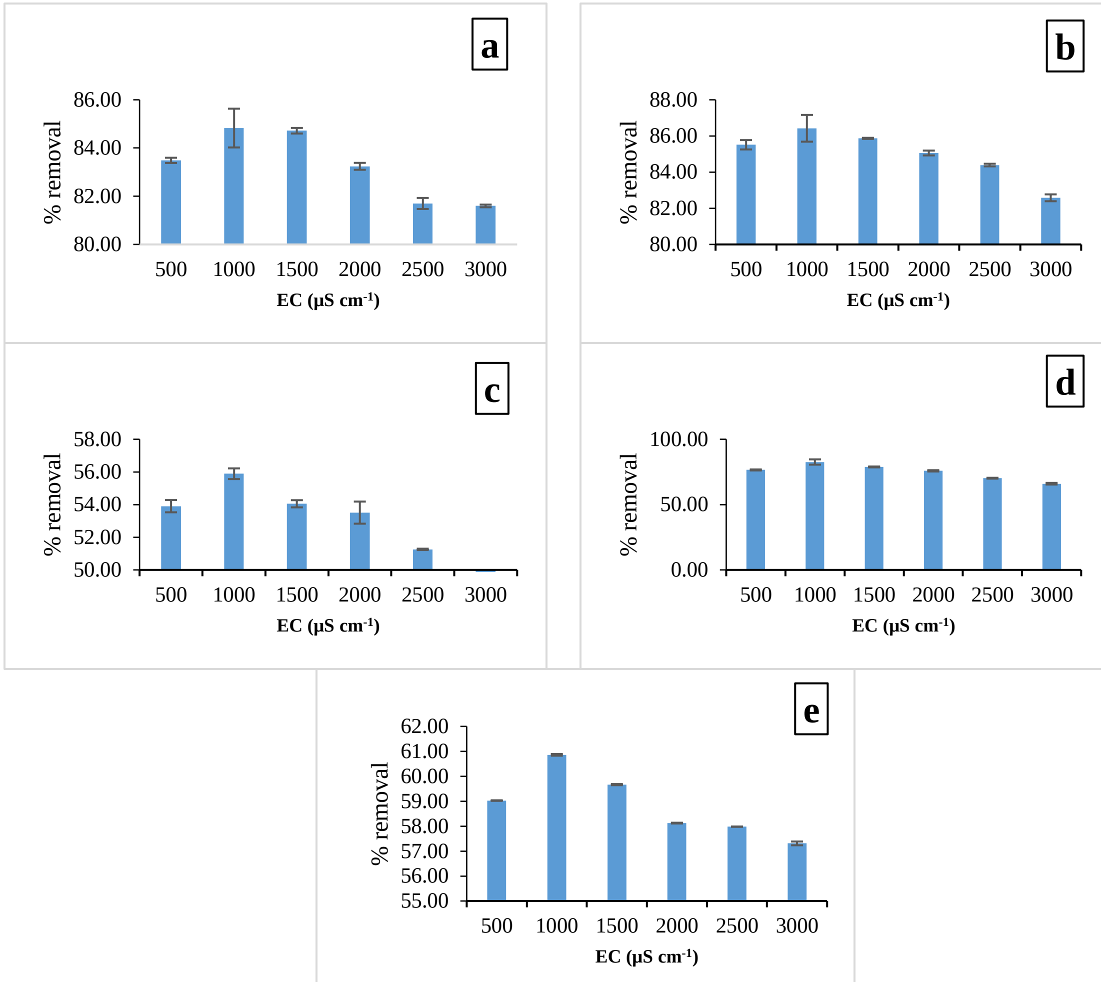

Figure 4.9: Percent removal of (a) total kjeldahl nitrogen (TKN), (b) ammonium-N (NH_4^+-N), (c) total phosphorus (TP), (d) ortho-phosphate-P (o-PO_4^{-3}-P) and (e) chemical oxygen demand (COD) at various EC levels of leachate by growing Lemna minor under natural climatic conditions during fall season.

Mostly the literature is silent for evidence concerning the effects of salinity on duckweeds except few studies conducted on synthetic leachate under artificial conditions of temperature and light intensity. Hillman. (1961) reported that duckweed is the salt sensitive plant and high electrical conductivity negatively affects the growth and nutrient removal efficiency of duckweed. Similarly, Radic and Pevalek-Kozlina. (2010) reported that salinity imposes osmotic stress which may cause damage to duckweed cells by inducing reactive oxygen species production or by disrupting detoxification mechanisms. However, the results of present study indicate that duckweed; Lemna minor has reasonable tolerance for high concentration of salts present in the leachate and an EC range of 1,000 to 1,500 µS cm-1 can be considered as optimum for growth and efficiency of duckweed to remove nutrients and COD from leachate. This is consistent with the findings of Wendeou et al. (2013) who reported that an EC range from 1,000-1,200 µScm-1 is optimum for duckweed growth on saline water.

4.6.3.3. Effect of EC on nutrient uptake by duckweed

As indicated in Table 4.20, during both summer and fall seasons, duckweed absorbed maximum amounts of nitrogen and phosphorous at 1,000 µS cm^{-1} EC of the leachate. At this value of EC, about 79.77% & 75.75% of N and 68.83% & 53.02% of P during summer and fall seasons respectively was taken up into duckweed biomass. This is consistent with the growth and nutrient removal efficiency of duckweed which is also highest at this EC value of the leachate. It is also evident from Table 4.20 that seasonal variation from have little effect on overall performance of duckweed on leachate and duckweed efficiently absorbed nitrogen and phosphorous from leachate during both seasons

Table 4.20: Mass balance of total N and P removal and uptake (mean ± SD) by duckweed at various EC levels of leachate under the natural climatic conditions during summer and fall seasons.

Experiment Season	EC (µS cm-1)	Total nutrients removed from media				Nutrients uptake by duckweed			
		mg L-1		%		mg L-1		% of total removed	
		N	P	N	P	N	P	N	P
Summer	500	41.57±0.1c	22.79±0.24b	83.53±0.26c	55.97±0.47b	31.17±0.07c	14.75±0.50b	74.98±0.05bc	64.71±1.6b
	1000	43.24±0.2a	24.13±0.01a	86.89±0.18a	59.27±0.25a	34.49±0.52a	16.61±0.42a	79.77±1.05a	68.83±1.8a
	1500	42.12±0.2b	22.80±0.05b	84.66±0.13b	55.99±0.27b	32.47±1.09b	14.83±0.22b	77.08±2.39ab	65.08±1.1b
	2000	41.35±0.2b	22.72±0.07b	83.10±0.08d	55.79±0.10b	30.68±0.16c	14.59±0.05bc	74.19±0.06c	64.21±0.1b
	2500	41.34±0.1b	22.14±0.10c	83.09±0.24d	54.38±0.11c	30.52±0.89c	14.09±0.35c	73.83±2.32c	63.63±1.3b
	3000	40.66±0.1c	21.42±0.07d	81.72±0.22e	52.62±0.07d	28.57±0.56d	13.02±0.46d	70.25±1.44d	60.76±2.0c
	LSD (0.05)	**0.25**	**0.20**	**0.35**	**0.45**	**1.17**	**0.66**	**2.75**	**2.56**
Fall	500	46.79±0.2b	23.27±0.04bc	83.49±0.11b	53.90±0.38b	33.65±0.54bc	11.50±0.30b	71.92±1.44	49.40±1.4
	1000	47.54±0.2a	24.13±0.01a	84.83±0.80a	55.89±0.33a	36.02±1.81a	12.79±0.60a	75.75±3.50	53.02±2.5
	1500	47.48±0.2a	23.33±0.04b	84.72±0.12a	54.05±0.22b	34.53±0.72ab	11.55±0.61b	72.74±1.80	49.50±2.6
	2000	46.65±0.3b	23.10±0.19c	83.24±0.14b	53.51±0.68b	33.54±0.95bc	11.20±0.70b	71.90±2.38	48.50±3.2
	2500	45.79±0.1c	22.13±0.11d	81.70±0.23c	51.26±0.04c	32.67±1.49bc	10.51±0.87bc	71.35±3.34	47.52±4.2
	3000	45.74±0.4c	21.51±0.09e	81.60±0.06c	49.84±0.12d	31.89±1.73c	10.10±0.34c	69.73±4.02	46.96±1.4
	LSD (0.05)	**0.36**	**0.17**	**0.63**	**0.64**	**2.32**	**1.07**	**NS**	**NS**

LSD = Least Significant Difference at 5% probability. Values with different letters in single column differ significantly at p = 0.05.

4.6.4. Conclusions-Experiment 2, Phase-III

Duckweed; *Lemna minor* can tolerate the wide range of leachate salinity from 5,00 to 3,000 µS cm^{-1} without having any significant impact on growth and its efficiency to remove nutrients and COD from the leachate.

An EC range from 1,000 to 1,500 µS cm^{-1} was found good for better performance of duckweed on leachate with an optimum value of 1,000 µS cm^{-1}. At this value of EC, duckweed showed maximum growth rates of 5.70±0.16 and 3.21±0.08 g m^{-2} day^{-1} respectively during summer and fall seasons. Whereas, the maximum removal of N (about 86.89% & 84.83%), P (about 59.27% & 55.89% and COD (about 64.83% and 60.86%) respectively during summer and fall seasons were observed at this EC level of leachate. Out of the total removed about 79.77% & 75.75% of N and 68.83% & 53.02% of P during summer and fall seasons respectively was taken up into duckweed biomass at EC 1,000 µS cm^{-1} of the leachate.

On the whole it can be concluded from this experiment that duckweed; *Lemna minor* is the salt tolerant aquatic plant however; an efficient management of electrical conductivity of leachate in the range of 1,000 to 1,500 µS cm^{-1} can result in better performance of duckweed under natural climatic conditions.

4.7. RESULTS OF PHASE-IV

During the Phase-IV, duckweed performance was investigated by growing three different initial plant densities i.e. 25%, 50% and 100% cover on leachate of 30 % concentration under natural climatic conditions. Purpose of this experiment was to investigate the duckweed harvesting frequency while keeping up the nutrient balance in leachate media and maintaining the health growth of duckweed plants for sustainable operations of duckweed based leachate treatment system. Please refer to Table 3.1 for the experimental conditions during Phase-IV and experimental setup can be seen in Figure 3.9. Initial concentrations of nutrients and COD of leachates are presented in Table 4.21.

Table 4.21: Initial concentrations of nutrients and COD (mean ± SD) of leachate used as medium for duckweed growth under natural conditions.

Nutrients Concentration ($mg\ L^{-1}$)				**COD ($mg\ L^{-1}$)**
TKN	**NH_4^+-N**	**TP**	**o-PO_4^{3-}-P**	
54.65±0.59	32.77±0.44	41.29±0.49	9.29±0.18	1,563±6.53

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophosphate, COD = Chemical oxygen demand

4.7.1. Effect of Duckweed Density on Growth

Duckweed growth rate at three different initial densities has been provided in Table 4.22 which shows that it achieved maximum growth rate at 50% initial mat density. At 25% initial density, although plenty of nutrients were available to plants however, at this density comparatively lesser amount of plants was present resulting in less rate of growth. At 100% density, it seems that crowding of plants retarded the overall growth rate due to increased competition among duckweed plants for light and available nutrients in leachate as reported by Weiner, 1990. Effect of density on duckweed growth has been documented by many researchers. The results of a study conducted by Frederic et al. (2006) showed that at an optimal initial mat density of 45 g-dry m^{-2} (750 g-wet m^{-2}) duckweed can achieve a maximum growth rate of 88 g-dry m^{-2} (1470 g-wet m^{-2}). Another study also found that at high initial plant densities (180 g dry weight m^{-2}) there exists a non-linear decrease in growth rate with increasing density (Driever et al., 2005). At high initial densities there is competition for light, the larger individuals may reduce light availability to smaller ones and as a result their growth is suspended. It was also noted by Driever et al. (2005) that at very low initial plant densities (<9 g m^{-2}) an inverse density dependence (Allee effect) of duckweed exists. This is probably due to high rates of fluctuations in local temeperatures within the partially filled duckweed containers at low densities. Many other studies also report that growth in *L. minor* decreases with an increase in initial plant density (Chaiprapat et al., 2005; Ziegler et al., 2015; Verma and Sutha, 2015).

Table 4.22: Growth rates of duckweed (mean ± SD) at three different initial plant densities on leachate under natural conditions.

Duckweed Density (% Cover)	**Duckweed Mass mg (g)**	**Growth Rate ($g\ m^{-2}\ day^{-1}$)**
25	30 (0.03)	5.98+0.23
50	60 (0.06)	6.84+0.13
100	120 (0.12)	6.14+0.22

4.7.2. Effect of Duckweed Density on Nutrient Removal

Rates of removal of nitrogen and phosphorous at various initial densities of duckweed been shown in Table 4.23. Maximum removal of nitrogen and phosphorous from leachate was achieved at 50 % initial density of duckweed. Whereas, at 100% density nutrients removal rate decreased as compared to removal rate at 50 % but it is higher than that of 25% duckweed density. A comparison of Table 4.22 and 4.23 reveals that nutrient removal rates are consistent with the duckweed growth rates.

Table 4.23: Rates of nutrients (N and P) and COD reduction (mean ± SD) by duckweed from leachate at different stocking densities under the natural climatic conditions

Duckweed Density (%)	**Removal rates of nutrients ($mg\ m^{-2}\ day^{-1}$)**				**COD ($g\ m^{-2}\ day^{-1}$)**
	TKN	**NH_4^+-N**	**TP**	**o-PO_4^{3-}-P**	
25	134.74±1.00	117.04±0.54	93.85±0.57	30.10±0.45	3.19±7.81
50	152.12±0.72	133.71±0.87	109.24±1.05	38.78±0.45	3.31+5.28
100	141.69±0.65	119.37±0.75	97.60±0.62	33.03±0.54	3.24+6.45

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophosphate, COD= Chemical oxygen demand

The efficient nutrient removal from wastewater is highly dependent on initial duckweed density and healthy duckweed cropping system suggested by Verma and Suthar (2015). Frederic et al. (2006) found that 45 g-dry m^{-2} (750 g-wet m^{-2}) is the optimum initial density of *Lemna minor* at which nitrogen (N) and phosphorus (P) of 483 mg-N m^{-2} d^{-1} and 128 mg-P m^{-2} d^{-1}, respectively was achieved. Xu and Shen,

(2011) during a study on Spirodela oligorrhiza, identified that at 60% initial plant densities duckweed system was capable of removing 83.7% and 89.4% of total nitrogen (TN) and total phosphorus (TP) respectively from 6% swine lagoon water in eight weeks at a harvest frequency of twice a week. It has been suggested by Liebhold and Bascompte. (2003) that the performance of duckweed based treatment system can be predicted by the very judgmental role of incubation density.Nitrogen and phosphorous removal and uptake from wastewaters by duckweed systems has strong relationship with initial density of duckweed mat on wastewater as suggested by Lasfar et al. (2007).

4.7.3. Calculations of Duckweed Doubling Time and Harvesting Frequency

Duckweed doubling time on leachate and time required to cover the entire surface of duckweed container has been calculated by following simple mathematical relationships: -

$$\text{Duckweed Doubling Time in days (DT)} = \frac{\text{Initial duckweed mass in g m}^{-2}\text{ (DM)}}{\text{Growth rate in g m}^{-2}\text{ day}^{-1}\text{ (GR)}} \qquad \text{(Eq 7)}$$

Duckweed mass in g m^{-2} can be calculated by following relationship: -

$$\text{Duckweed mass in g m}^{-2} = \frac{\text{Total mass of duckweed plants grown on leachate (g)}}{\text{Surface area of growth container (m}^2\text{)}} \qquad \text{(Eq 8)}$$

Starting from initial duckweed mat density, time required for full coverage of duckweed plants on leachate surface has been calculated by following relationship: -

Time (days) required for full coverage on duckweed container =

$$\frac{\{100/\text{Initial duckweed density (\%)} \times \text{Initial mass of duckweed (g m}^{-2})\} - \text{Initial mass (g m}^{-2})}{\text{Duckweed growth rate (g m}^{-2}\ \text{day}^{-1})} \quad \text{(Eq 9)}$$

Starting from any base density, harvesting should be done as soon as duckweed plants achieve the full coverage on growth containers. Harvesting is necessary to maintain the healthy plat growth on leachate and to avoid the crowding effect. Crowding inhibits the doubling time of duckweed and increases the competition for light and nutrients among standing plants.

4.7.4. Maintaining the Nutrient Balance in Duckweed Ponds

As shown in Table 4.21 duckweed actively removes nutrients from leachate media. It is also evident from the table that nutrient removal rates increase with increase in initial density of duckweed plants on leachate. With the passage of time, the growing plants of duckweed will deprive the leachate media from nutrients which will in turn effect the healthy growth of duckweed plants. Therefore, in addition to cropping density, maintaining a fair balance of nitrogen and phosphorous is also very important for healthy growth of the duckweed plants on leachate.

This study recommends that at the time of each harvest, loss of nutrients (N and P) from initial concentrations and remaining amount of nutrients in leachate should also be calculated to replenish the leachate media with sufficient amounts of nutrients required to maintain the healthy growth of duckweed plants.

Loss of nutrients (N and P) and their remaining amount in leachate has been be calculated by simple mathematical relationship: -

Loss of nutrients = Nutrient removal rate (g m^{-2} day^{-1}) x Time required for full coverage (Eq 10)

Remaining amount of nutrient in leachate =

{Initial concentration of nutrient (g m^{-2})} – {Nutrient removal rate (g m^{-2} day^{-1}) x Time required for full coverage} (Eq 11)

Duckweed doubling time, time required for full coverage and loss of nutrients at various initial duckweed densities on leachate has been provided in Table 4.24 below.

Table 4.24: Doubling time, time required for full coverage and loss of nutrients at various initial duckweed densities on leachate.

Duckweed Density (% Cover)	**Doubling Time** (Days)	**Time required for full coverage on duckweed containers** (Days)	**Loss of nutrients at the time of full coverage of duckweed containers** (g m^{-2})	
			N	**P**
25	1.7 ≈ 2	5	0.67	0.47
50	2.38 ≈ 2.5	2.38 ≈ 2.5	0.38	0.27
100	6.26 ≈ 6	0	0.14	0.10

As shown in Table 4.24 this study suggests that starting from 25% initial duckweed density, harvesting should be carried out after every 5 days to bring the plant density equal to the initial whereas; for 50% initial density, harvesting frequency of 2.5 days is suggested by this study. In case of 100% initial cover of duckweed on leachate, daily harvesting should be carried to maintain the healthy growth of duckweed. Nitrogen and phosphorous losses as shown in Table 4.24 are very small at the time of full plant cover on duckweed containers however, it is required to replenish the nutrients by adding sufficient quantity of leachate as required to bring the concentration of nitrogen and phosphorous equal to the initial concentration. This is very important for the sustainable operation of duckweed based leachate treatment system as proposed by this research.

4.7.5. Summary-Phase IV

Results of this experiment show that at 50 % initial cover on leachate, duckweed performed better as compared to 25% and 100 % initial density. At this density duckweed exhibited the maximum growth rate of 9.77+0.13 g m^{-2} day^{-1} and removed the TKN and TP at the rate of 152.12+0.72 and 109.24+1.05 mg m^{-2} day^{-1} from leachate.

Starting from 50 % initial density, harvesting frequency of 2.5 days is suggested by this study so as to keep the duckweed density equal to initial and to maintain the healthy growth of plants on leachate without crowding effect.

With 50 % initial density, the amount of nitrogen and phosphorous lost at the time of harvesting is very small i.e. 0.38 and 0.27 g m^{-2} N and P respectively. However, it is recommended to regularly replenish the duckweed container with these nutrients by adding sufficient amounts of leachate.

4.8. RESULTS OF PHASE-V

Phase-V was designed to investigate/compare the performance of duckweed on synthetic leachate. For this purpose, three tests were performed by growing duckweed on synthetic leachate of 30 % initial concentration under the optimum conditions of EC, pH and duckweed density as identified during the previous experiments on dumpsite leachate.

- Test 1: Conducted at optimum EC of the leachate (1000 µS cm^{-1});
- Test 2: Conducted at optimum pH level of the leachate (7.1);
- Test 3: Conducted at optimum density of duckweed on leachate (50% plant cover on leachate surface).

All tests under Phase-V were performed at similar conditions of temperature and light intensity as of the experiments on dumpsite leachate. For each test except for the test variable, all other parameters were kept same as of previous experiments. Details of experimental conditions and setup for Phase-V can be seen at Table 3.1 and Figure 3.10 respectively in Chapter 3. The initial composition of synthetic leachate used for each test is provided in Table 4.25.

Table 4.25: Initial nutrients concentrations and COD of synthetic leachate used as medium for growth of L. minor under controlled conditions.

Test Performed	**Nutrients concentration**				**COD**
	TKN	**NH_4-N**	**TP**	**o-PO_4^{3-}-P**	**(mg L^{-1})**
Test 1 (EC)	49.88±0.26	26.43±0.15	37.13±0.21	14.08±0.14	1527±2.42
Test 2 (pH)	46.56±0.24	26.32±0.15	39.65±0.22	16.02±0.16	1571±2.49
Test 3 (Density)	50.82±0.55	29.49±0.39	44.59±0.52	8.36±0.17	1517±6.33

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophosphate, COD= Chemical oxygen demand

4.8.1. Comparative Growth of Duckweed on Synthetic and Dumpsite Leachate

Comparison of duckweed growth rates under optimum conditions of EC (Test 1), pH (Test 2) and duckweed density (Test 3) is provided in Table 4.26 below.

Table 4.26: Duckweed growth rates on synthetic leachate during three tests and comparison with dumpsite leachate.

Test Performed	Leachate Type	Growth rate ($g\ m^{-2}\ day^{-1}$)
Test 1 (EC)	Dumpsite	3.07±0.07
	Synthetic	5.70±0.16
Test 2 (pH)	Dumpsite	2.97±0.06
	Synthetic	5.51±0.16
Test 3 (Density)	Dumpsite	4.06±0.18
	Synthetic	6.84+0.13

Significant difference in growth rates of duckweed can be seen at synthetic and dumpsite leachate as shown in Table 4.26. This study reveals that under the optimum condition of EC, pH and density, duckweed grows better at synthetic leachate as compared to its growth on dumpsite leachate. This is because a large proportion of nutrients from dumpsite leachate has been removed by the factors other than absorption into duckweed biomass as discussed in upcoming section; resulting in less growth at dumpsite leachate under natural conditions as compared to synthetic leachate under artificial conditions.

Although the light intensity and ambient temperature during each test were maintained in accordance with the previous experiments under natural conditions, however; it is evident from the results that duckweed showed better growth under artificial climatic conditions. It is because under the natural systems, in addition to controlled weather conditions, many other phenomenon such as ammonia volatilization, nitrification and denitrification and microbial assimilation of nitrogen and phosphorous is higher as compared to artificial system as suggested by Vermaat and Hanif. (1998). These associated factors were responsible for removal of large amounts of nitrogen and phosphorus from the dumpsite leachate without absorption by the duckweed resulting in less growth at dumpsite leachate as compared to the synthetic leachate.

4.8.2. Comparison of Nutrient and COD Reduction and Uptake by Duckweed from Synthetic and Dumpsite Leachate

Table 4.27 provides the comparison of removal rates of nutrients and COD from synthetic and dumpsite leachate for three tests.

Table 4.27: Comparison of rates of nutrients and COD reduction by duckweed from synthetic and dumpsite leachate.

Test Performed	Leachate Type	Nutrients removal rate ($mg\ m^{-2}\ day^{-1}$)				COD ($g\ m^{-2}\ day^{-1}$)
		TKN	**NH_4-N**	**TP**	**o-PO_4^{3-}-P**	
Test 1 (EC)	**Synthetic**	135.09±0.94	73.94±0.37	60.96±3.46	35.87±0.96	2.89±8.59
	Dumpsite	175.64±0.72	124.01±0.39	92.35±0.07	31.83±1.25	3.66±9.07
Test 2 (pH)	**Synthetic**	126.98±1.27	72.19±0.46	62.53±0.26	41.09±1.06	3.07±9.46
	Dumpsite	170.53±0.69	119.25±0.37	94.23±0.07	31.37±1.23	3.52±4.36
Test 3 (Density)	**Synthetic**	116.32±0.65	80.39±1.71	92.16±0.39	26.24±0.61	2.73±7.20
	Dumpsite	152.12±0.72	133.71±0.87	109.24±1.05	38.78±0.45	3.31±7.81

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophosphate, COD = Chemical oxygen demand

As shown in Table 4.27, during each test, duckweed removed larger amounts of nutrients (except ortho phosphate) and COD from dumpsite leachate as compared to the nutrients and COD removed from synthetic leachate under similar conditions of temperature and light intensity. This is due to the fact that under the natural climatic conditions many other factors such as ammonia volatilization, algal and microbial assimilation, and nitrification/denitrification also contribute to the removal of nitrogen and phosphorous from the leachate resulting in removal of higher amounts of nutrients as reported by the Oron et al. (2008). A study revealed that nitrification and denitrification process contributed for 50 % nitrogen removal from wastewater which was significantly higher in dumpsite leachate as compared to the synthetic leachate due to the presence of comparatively larger amount of nitrifying and denitrifying microorganisms in dumpsite leachate under natural conditions as compared to the the synthetic leachate under controlled conditions (Vermaat and Hanif, 1998).

Comparison of mass balance of nitrogen and phosphorous is provided in Table 4.28 showing that at optimum conditions of EC, pH and density, duckweed absorbed larger amount of nitrogen and phosphorous into its biomass from synthetic leachate as compared to the absorption of these nutrients from dumpsite leachate under the similar climatic conditions.

Table 4.28: Comparison of mass balance of total N and P removal and uptake by duckweed from synthetic and dumpsite leachate.

Test Performed	**Leachate Type**	**Total nutrients removed from media**				**Nutrients uptake by duckwee**			
		mg L^{-1}		**% removed**		**mg L^{-1}**		**% of total removed**	
		N	**P**	**N**	**P**	**N**	**P**	**N**	**P**
Test 1 (EC)	**Synthetic**	40.53±0.3	18.29±1.0	81.25±0.9	49.26±2.9	37.69±0.3	16.64±0.9	93±0.1	91±0.2
	Dumpsite	43.24±0.2	24.13±0.0	86.89±0.2	59.27±0.3	34.49±0.5	16.61±0.4	79.77±1.0	68.83±1.8
Test 2 (pH)	**Synthetic**	38.10±0.4	18.76±0.1	81.82±0.8	47.31±0.4	36.19±0.4	17.63±0.1	95±0.1	94±0.1
	Dumpsite	45.03±0.2	24.88±0.1	87.77±0.2	59.52±0.2	35.47±0.6	17.37±0.5	78.77±1.1	69.8±2.2
Test 3 (Density)	**Synthetic**	34.90±0.2	27.65±0.1	68.66±0.4	62.00±0.4	30.71±0.2	25.44±0.1	88±0.1	92±0.1
	Dumpsite	40.42±0.3	28.15±0.2	73.96±0.3	68.19±0.4	31.29±0.1	19.31±0.6	77.41±0.9	68.60±1.2

Absorption of comparatively larger amounts of nitrogen and phosphorous by duckweed from synthetic leachate is consistent with the growth which is also high at synthetic leachate as compared to dumpsite leachate. It has also been reported by Landesman et al. (2002) that many natural processes occurring in dumpsite leachate such as microbial assimilation, and nitrification/denitrification contribute to the removal of significant amounts of nitrogen and phosphorous without taken up by the duckweed plants. It was also found by Adhikari et al., (2015) that artificial constructed wetlands are the efficient means for duckweed growth and nitrogen and phosphorous recovery from high strength wastewater.

4.8.3. Summary- Phase V

From synthetic leachate under controlled conditions duckweed removed from 68% to 81% N and 47% to 63% of P respectively and out of the total removed about 88% to 95% of N and 91% to 94% of P was taken up by duckweed biomass.

A comparison of experiments on dumpsite and synthetic leachate reveals that duckweed removes COD and nutrients more efficiently from dumpsite leachate under the natural climatic conditions as compared to when grown on synthetic leachate under the artificial conditions however; the amount of N and P taken up by duckweed biomass was about 14% to 18% and 34% to 36% more at synthetic leachate as compared to dumpsite leachate. Duckweed growth rate (5.5 to 6.3g m^{-2} day^{-1}) was also observed high at synthetic leachate under artificial conditions. This suggests that under the natural climatic conditions, factors other than the absorption by duckweed such as; microbial activities, algal growth and natural decomposition also play significant role in N and P removal from leachate.

Chapter 5

CONCLUSIONS AND RECCOMENDATIONS

5.1. CONCLUSIONS

The results of this research study demonstrate that *Lemna minor* specie of duckweed is the potential aquatic plant for algae-duckweed based integrated leachate treatment system under natural climatic conditions. *Lemna minor* has enormous potential of pollution removal from dumpsite leachate while, maintaining healthy growth and nutrient (N and P) accumulation into its biomass.

As given in results it has been concluded that mixed algal genera: *Ankistrodesmus, Nostoc and Anabaena* has significant potential of heavy metal removal from dumpsite leachate under natural climatic conditions. Therefore, this algal consortium can be used as starting step for heavy metals removal from leachate during algae-duckweed based integrated leachate treatment system.

Overall this research study draws the following conclusions. Figure 5.1 at the end of this chapter provides the pictoral view of overall conclusions and results of this study with respect to proposed integrated algae and duckweed based leachate treatment system: -

1. 20-40% initial dilution of leachate (COD: 1,088 to 2,308 mg L^{-1}); optimum, 30% (COD: approximately 1,700 mg L^{-1}), maintained a good balance between

duckweed growth and its nutrient (N and P) and COD reduction efficiency from leachate under the natural climatic conditions (temperature from 27-39 ^{0}C);

2. Starting from the dumpsite leachate of about 50% initial concentration (COD: 2,630 to 2,746 mg L^{-1}) a retention time of about six days is sufficient for growth of algae on leachate performing as pretreatment step for heavy metal removal from leachate. During six days period algae also removed sufficient amount of nutrients from leachate and its remaining concentration was nearly equal to 30% leachate;
3. Duckweed performs well on dumpsite leachate at pH range from 6.5 to 8.5 with an optimum value of pH 7.1 under natural climatic conditions;
4. While considering an EC range from 700 to 1,300 was found favorable for duckweed growth however; the maximum growth and its nutrient and COD reduction efficiency at leachate was observed at EC level of 1,000 µS cm^{-1};
5. 50% starting duckweed density (Duckweed cover on leachate surface) is found optimum for algae-duckweed based leachate treatment system. At this initial density, duckweed showed maximum growth and nutrient and COD reduction from leachate under natural conditions.
6. At 50 % initial density harvesting frequency of 2.5 days is suggested by this study so as to keep the duckweed density equal to initial and to maintain the healthy growth of plants on leachate without crowding effect.
7. Weather conditions from June to October in Islamabad showed no significant differences on growth of duckweed and its efficiency to remove nutrient and COD from leachate however, the period from June-July (38-39 ^{0}C) can be

considered as optimum season for duckweed growth and its nutrient and COD reduction efficiency from leachate,

8. Comparison of results of experiments on synthetic leachate-duckweed system with the results of natural leachate-duckweed system, reveal that duckweed growth and its nutrients and COD reduction efficiency from dumpsite leachate is higher than that of synthetic leachate whereas, uptake of nutrients (N and P) into duckweed biomass was higher from synthetic leachate as compared to dumpsite leachate under the similar climatic conditions.

5.2. RECOMMENDATIONS

This research study provides suitable basis for designing an algae-duckweed based leachate treatment system under the natural climatic conditions of Islamabad, Pakistan, which is cost effective and easy to operate as well. Following recommendations and future research have been suggested to further enhance the practical viability of algal-duckweed based integrated leachate treatment system proposed by this study:-

- This research identified the operational parameters for algae-duckweed based leachate treatment system during the months of June to October. Although it is clearly established from relevant scientific literature that duckweed shows lean growth during winter months (November to March) resulting in reduced biomass production and less nutrient removal efficiency however, it is recommended that similar research study may also be conducted during remaining months (November to May) to identify the duckweed production and its nutrient and COD reduction efficiency during these months as well. This study will be useful to ensure the sustainable operations of algae-duckweed based leachate treatment system throughout the year.

- Results of this study may be extrapolated by conducting a separate research study in the scale using large sized leachate ponds for duckweed and algal growth. This large scale study should investigate the duckweed and algal performance on leachate under similar conditions of climate using the similar operational variables of leachate concentration, pH, Electrical conductivity and duckweed density as used during the present study.
- Xenobiotic organic compounds (XOCs) such as xylenes, ethylbenzene, benzene, toluene and tetrachloroethylene must be identified in leachate prior to planning an algae-duckweed based integrated leachate treatment system. Separate treatment may need to be provided for xenobiotic compounds if present in concentrations higher than acceptable national and international limits.
- Hydrogeological conditions of site should be considered while planning any algae-duckweed based leachate treatment system. It is important to minimize the harmful impacts of leachate on soil and ground & surface water quality.

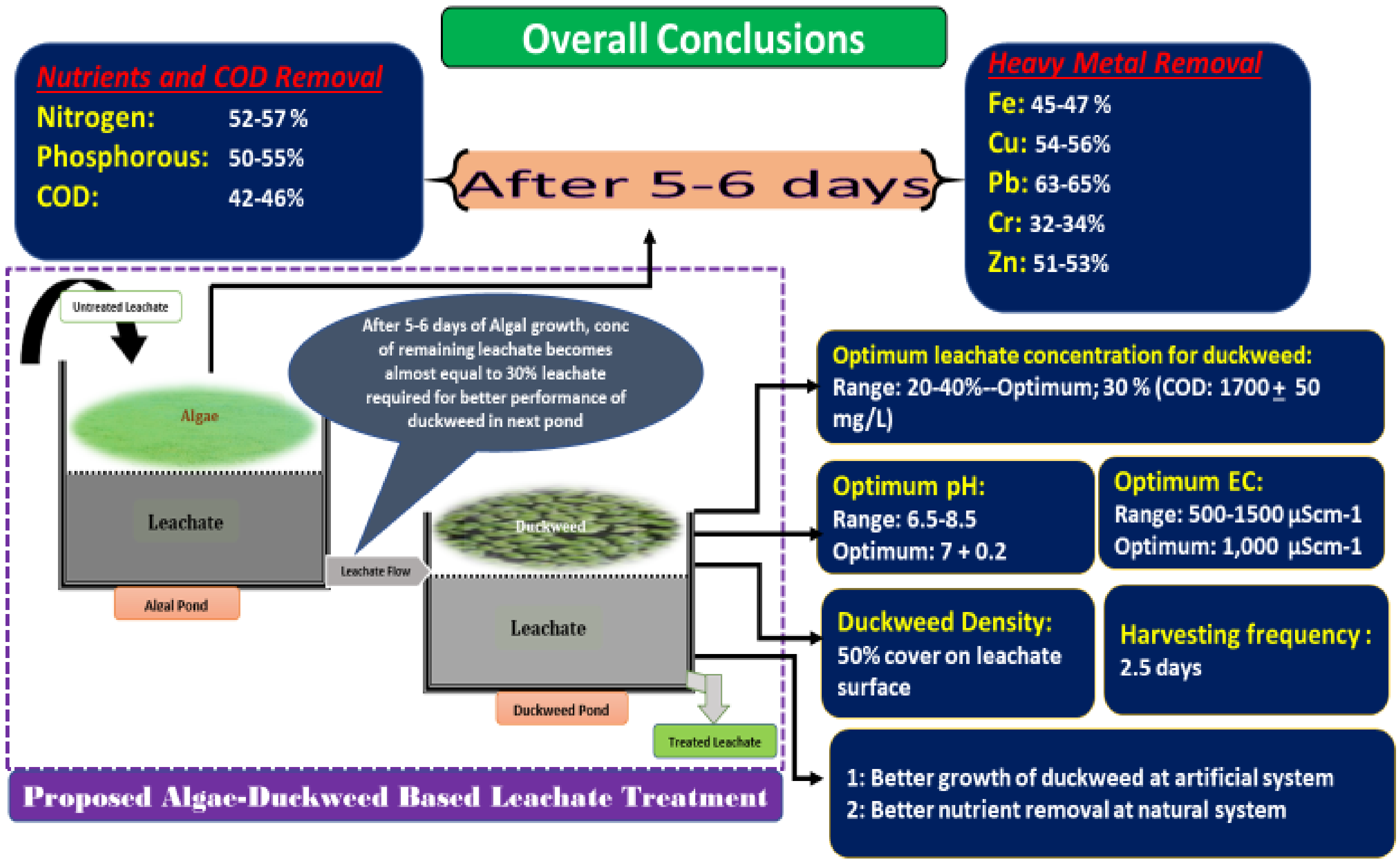

Figure 5.1: Overall conclusions and results of this study with respect to integrated algae-duckweed base leachate treatment system

REFERENCES

Abbas, A. A., Jingsong, G., Ping, L. Z., Ya, P. Y. and Al-Rekabi, W. S. (2009). Review on Land Wll Leachate Treatments. Journal of Applied Sciences Research, 5: 534-545.

Adhikari, U., Harrigan, T. and Reinhold, D. M. (2015). Use of duckweed-based constructed wetlands for nutrient recovery and pollutant reduction from dairy wastewater. Ecological Engineering, 78: 6-14.

Agarwal, S. K., Zhu, J., Kim, K., Agarwal, M., Fu, X., Huang, A. and Zhu, J. K. (2006). The plasma membrane Na^+/H^+ antiporter SOS1 interacts with RCD1 and functions in oxidative stress tolerance in Arabidopsis. Proceedings of the National Academy of Sciences USA., 103: 18816–1882

Ahammad, M. U., Swapon, M. S. R., Yeasmin, T., Rahman, M. S. and Ali, M. S. (2003). Replacement of sesame oil cake by duckweed (*Lemna minor*) in broiler diet. Pakistan Journal of Biological Science, 6(16): 1450-1453.

Aiman, U., Mahmood, A., Waheed, S. and Malik, R. N. (2016). Enrichment, geo-accumulation and risk surveillance of toxic metals for different environmental compartments from Mehmood Booti dumping site, Lahore city, Pakistan. Chemosphere, 144: 2229-2237.

Akhtar, M. M. and Zhonghua, T. (2013). A study to estimate overall environmental pollution potential in second biggest city of Pakistan. European International Journal of Science and Technology. 2: 155-163.

Akhtar, M., Ahmad, N. and Booij, M. J. (2008). The impact of climate change on the water resources of Hindukush-Karakorum Himalaya region under different glacier coverage scenarios. Journal of Hydrology, 355: 148-163.

Akinbile, C. O., Yusoff, M. S. and Zuki, A. A. (2012). Landfill leachate treatment using sub-surface flow constructed wetland by *Cyperus haspan*. Waste management, 32: 1387-1393.

Aksu, Z. (2000). Equilibrium and kinetic modeling of cadmium(II) biosorption by C. vulgaris in a batch system: effect of temperature. Separation and Purification Technology, 21: 285-294.

Aksu, Z. and Acikel, U. (2000). A single-staged bioseparation process for simultaneous removal of copper (II) and chromium (VI) by using C. vulgaris. Process Biochemistry, 34: 589-599.

Ali, I., Khan, Z. M., Sultan, M., Mahmood, M. H., Farid, H. U., Ali, M. and Nasir, A. (2016). Experimental study on maize cob trickling filter-based wastewater treatment system: design, development, and performance evaluation. Polish Journal of Environmental Studies, 25: 2265-2273.

Ali, S. M., Pervaiz, A., Afzal, B., Hamid, N. and Yasmin, A. (2014). Open dumping of municipal solid waste and its hazardous impacts on soil and vegetation diversity at waste dumping sites of Islamabad city. Journal of King Saud University-Science, 26: 59-65.

Al-Nozaily, F., Alaerts, G. and Veenstra, S. (2000). Performance of duckweed-covered sewage lagoons—II. Nitrogen and phosphorus balance and plant productivity. Water Research, 34: 2734-2741.

Al-Rub, F. A. A., El-Naas, M.H., Ashour, I. and Al Marzouqi, M. (2006). Biosorption of copper on *chlorella vulgaris* from single, binary and ternary metal aqueous solutions. Process Biochemistry, 41(2): 457-464.

Aluko, O. O. and Sridhar, M. K. (2013). Evaluation of leachate treatment by trickling filter and sequencing batch reactor processes in Ibadan, Nigeria. Waste Management and Research, 31: 700-705.

Amokrane, A., Comel, C., and Veron, J. (1997). Landfill leachates pretreatment by coagulation-flocculation. Water research, 31: 2775-2782.

Amor, C., De Torres-Socías, E., Peres, J. A., Maldonado, M. I., Oller, I., Malato, S. and Lucas, M. S. (2015). Mature landfill leachate treatment by coagulation/flocculation combined with Fenton and solar photo-Fenton processes. Journal of hazardous materials, 286: 261-268.

Andrade, A. D., Rollemberg, M. C. E. and Nobrega, J. A. (2005). Proton and metal binding capacity of the green freshwater alga *Chaetophora elegans*. Process Biochemistry, 40: 1931-1936.

Ansal, M. D. and Dhawan, A. (2007). Efficacy of Duckweed-Spirodela for low cost carp feed formulation. Indian Journal of Animal Nutrition, 26(4): 378-383.

Antoniou, P., Hamilton, J., Koopman, B., Jain, R., Holloway, B., Lyberatos, G. and Svoronos, S. A. (2000). Effect of temperature and pH on the effective maximum specific growth rate of nitrifying bacteria. Water Research, 24(1): 97-101.

Armstrong, W.P. (2001). Wayne's Word Lemnaceae On-Line: 12 May 2001. https://www2.palomar.edu/users/warmstrong/1wayindx.htm (12 June 2001).

Assche, F. V. and Clijsters, H. (1990). Effects of metals on enzyme activity in plants. Plant, Cell and Environment, 13: 195-206.

Assou, M., El Fels, L., El Asli, A., Fakidi, H., Souabi, S. and Hafidi, M. (2016). Landfill leachate treatment by a coagulation–flocculation process: effect of the introduction order of the reagents. Desalination and Water Treatment, 57(46): 21817-21826.

Aziz, H. A., Yusoff, M. S., Adlan, M. N., Adnan, N. H. and Alias, S. (2004). Physico-chemical removal of iron from semi-aerobic landfill leachate by limestone filter. Waste management, 24: 353-358.

Aziz, S. Q., Aziz, H. A., Yusoff, M. S. and Bashir, M. J. (2011). Landfill leachate treatment using powdered activated carbon augmented sequencing batch reactor (SBR) process: Optimization by response surface methodology. Journal of Hazardous Materials, 189: 404-413.

Aziz, S.Q., Aziz, H.A., Yusoff, M.S., Bashir, M.J. and Umar, M. (2010). Leachate characterization in semi-aerobic and anaerobic sanitary landfills: a comparative study. Journal of environmental management, 91(12): 2608-2614.

Baderna, D., Maggioni, S., Boriani, E., Gemma, S., Molteni, M., Lombardo, A. and Benfenati, E. (2011). A combined approach to investigate the toxicity of an industrial landfill's leachate: chemical analyses, risk assessment and in vitro assays. Environmental research, 111: 603-613.

Banerjee, A. and Matai, S. (1990). Composition of Indian Aquatic plants in relation to utilization as animal forage. Journal of Aquatic Plant Management, 28: 69-73.

Bashir, M. J., Aziz, H. A., Yusoff, M. S. and Adlan, M. N. (2010). Application of response surface methodology (RSM) for optimization of ammoniacal nitrogen removal from semi-aerobic landfill leachate using ion exchange resin. Desalination, 254: 154-161.

Bashir, M.J., Aziz, H.A., Amr, S.S.A., Sethupathi, S.A.P., Ng, C.A. and Lim, J.W. (2015). The competency of various applied strategies in treating tropical municipal landfill leachate. Desalination and Water Treatment, 54(9): 2382-2395.

Ben-shalom, M., Shandalov, S., Brenner, A. and Oron, G. (2014). The effect of aeration and effluent recycling on domestic wastewater treatment in a pilot-plant system of duckweed ponds. Water Science and Technology, 69: 350-357.

Bergmann, B. A., Cheng, J., Classen, J. and Stomp, A. M. (2000a). Nutrient removal from swine lagoon effluent by duckweed. Transactions of the ASAE. 43: 263-269.

Brennan, L. and Owende, P. (2010). Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. Renewable and Sustainable Energy Reviews, 14: 557-577.

Burntley, S. J. (2007) A review of municipal solid waste composition in the United Kingdom. Waste Management, 27(10): 1274-1285.

Caicedo, J. R., Van der Steen, N. P., Arce, O. and Gijzen, H. J. (2000). Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). Water Research, 34: 3829-3835.

Campbell, N. and J. Reece. Biology, Sixth Edition. San Francisco, CA. 2002.

Castrillón, L., Fernández-Nava, Y., Ulmanu, M., Anger, I. and Maranon, E. (2010). Physico-chemical and biological treatment of MSW landfill leachate. Waste Management, 30: 228-235.

Chaiprapat, S., Cheng, J. J., Classen, J. J. and Liehr, S. K. (2005). Role of internal nutrient storage in duckweed growth for swine wastewater treatment. Transactions of the ASAE. 48: 2247-2258.

Chemlal, R., Azzouz, L., Kernani, R., Abdi, N., Lounici, H., Grib, H. and Drouiche, N. (2014). Combination of advanced oxidation and biological processes for the landfill leachate treatment. Ecological Engineering, 73: 281-289.

Cheng, J. J. and Stomp, A. M. (2009). Growing duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. Clean–Soil, Air, Water, 37: 17-26.

Cheng, J., Landesman, L., Bergmann, B. A., Classen, J. J., Howard, J. W. and Yamamoto, Y. T. (2002). Nutrient removal from swine lagoon liquid by *Lemna minor* 8627. Transactions of the ASAE. 45: 1003-1010.

Chorus, I. and Bartram, J. (1999). Toxic Cyanobacteria in Water. A Guide to their Public Health Consequences, Monitoring and Management. Published on behalf of the World Health Organisation by E and FN Spon, London. 416 pp.

Christensen, T. H., Kjeldsen, P., Albrechtsen, H. J. R., Heron, G., Nielsen, P. H., Bjerg, P. L. and Holm, P. E. (1994). Attenuation of landfill leachate pollutants in aquifers. Critical Reviews in Environmental Science and Technology, 24: 119-202.

Clément, B. and Bouvet, Y. (1993). Assessment of landfill leachate toxicity using the duckweed *Lemna minor*. Science of the Total Environment, 134: 1179-1190.

Clément, B. and Merlin, G. (1995). The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed. Science of the Total Environment. 170: 71-79.

Connolly, R., Zhao, Y., Sun, G. and Allen, S. (2004). Removal of ammoniacal-nitrogen from an artificial landfill leachate in downflow reed beds. Process Biochemistry, 39: 1971-1976.

Cross, J.W., 2006. The charms of duckweed. Missouri Botanical Garden, 2.

Dalu, J. M. and Ndamba, J. (2003). Duckweed based wastewater stabilization ponds for wastewater treatment (a low cost technology for small urban areas in Zimbabwe). Physics and Chemistry of the Earth Parts A/B/C. 28: 1147-1160.

Davis, T. A., Volesky, B. and Mucci, A. (2003) A review of the biochemistry of heavy metal biosorption by brown algae. Water Research, 37: 4311-4330.

De Philippis, R., Colica, G. and Micheletti, E. (2011). Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process. Applied Microbiology and Biotechnology, 92: 697-708.

De Philippis, R., Paperi, R. and Sili, C. (2007). Heavy metal sorption by released polysaccharides and whole cultures of two exopolysaccharide-producing cyanobacteria. Biodegradation 18: 181-87.

Deng, L., Su, Y., Su, H., Wang, X. and Zhu, X. (2006). Biosorption of copper (II) and lead (II) fromaqueous solutions by nonliving green algae *Cladophora fascicularis*: equilibrium, kinetics and environmental effects. Adsorption, 12: 267-277.

Dhokhikah, Y. and Trihadiningrum, Y. (2012). Solid waste management in Asian developing countries: challenges and opportunities.

Dodds, W. K., Gudder, D. A. and Mollenhauer, D. (1995). The ecology of Nostoc. Journal of Phycology, 31(1): 2-18.

Dogdu, G. and Yalcuk, A. (2016). Evaluation of the treatment performance of lab-scaled vertical flow constructed wetlands in removal of organic compounds, color and nutrients in azo dye-containing wastewater. International journal of phytoremediation, 18(2): 171-183.

Dolar, D., Košutić, K. and Strmecky, T. (2016). Hybrid processes for treatment of landfill leachate: coagulation/UF/NF-RO and adsorption/UF/NF-RO. Separation and Purification Technology, 168: 39-46.

Driever, S. M., van Nes, E. H. and Roijackers, R. M. (2005). Growth limitation of *Lemna minor* due to high plant density. Aquatic Botany, 81: 245-251.

Drost, W., Matzke, M. and Backhaus, T. (2007). Heavy metal toxicity to *Lemna minor*: studies on the time dependence of growth inhibition and the recovery after exposure. Chemosphere, 67: 36-43.

Duong, T. P. and Tiedje, J. M. (1985). Nitrogen fixation by naturally occurring duckweed-cyanobacterial associations. Canadian Journal of Microbiology, 31: 327-330.

Edmundson, S. J. and Wilkie, A. C. (2013). Landfill leachate–a water and nutrient resource for algae-based biofuels. Environmental technology, 34: 1849-1857.

El-Sheekh, M. M., El-Shouny, W. A., Osman, M. E. and El-Gammal, E. W. (2005). Growth and heavy metals removal efficiency of *Nostoc muscorum* and

Anabaena subcylindrica in sewage and industrial wastewater effluents. Environmental toxicology and pharmacology, 19(2): 357-365.

El-Sikaily, A., El Nemr, A., Khaled, A. and Abdelwehab, O. (2007). Removal of toxic chromium from wastewater using green alga *Ulva lactuca* and its activated carbon. Journal of Hazardous Materials, 148: 216-228.

Falkowski, P. G. (1994). The role of phytoplankton photosynthesis in global biogeochemical cycles. Photosynthesis Research, 39(3): 235-258.

Farrell, J. B. (2012). Duckweed uptake of phosphorus and five pharmaceuticals: microcosm and wastewater lagoon studies. Utah State University

Federation W.E. and Association A.P.H. (2005). Standard Methods for the Examination of Water and Wastewater. American Public Health Association (APHA); Washington, DC, USA.

Fettig, J. (1999). Removal of humic substances by adsorption/ion exchange. Water Science and Technology, 40: 173-182.

Frederic, M., Samir, L., Louise, M. and Abdelkrim, A. (2006). Comprehensive modeling of mat density effect on duckweed (*Lemna minor*) growth under controlled eutrophication. Water research, 40(15): 2901-2910.

Gaur, J. P. and Rai, L. C. (2001). Heavy metal tolerance in algae. In Algal adaptation to environmental stresses. Springer Berlin Heidelberg. 363-388.

Ge, X., Zhang, N., Phillips, G. C. and Xu, J. (2012). Growing *Lemna minor* in agricultural wastewater and converting the duckweed biomass to ethanol. Bioresource Technology, 124: 485-488.

Geenens, D., Bixio, B. and Thoeye, C. (2001). Combined ozone-activated sludge treatment of landfill leachate. Water Science and Technology, 44: 359-365.

Geisweid, H. J. and Urbach, W. (1983). Sorption of cadmium by the green microalgae *Chlorella vulgaris*, *Ankistrodesmus braunii* and *Eremosphaera viridis*. Zeitschrift für Pflanzenphysiologie, 109(2): 127-141.

Govahi, S., Karimi-Jashni, A. and Derakhshan, M. (2012). Treatability of landfill leachate by combined upflow anaerobic sludge blanket reactor and aerated lagoon. International Journal of Environmental Science and Technology, 9(1): 145-151.

Grady Jr, C.L., Daigger, G.T., Love, N.G. and Filipe, C.D. (2011). Biological wastewater treatment. CRC press.

Grimm, N.B., Faeth, S.H. and Golubiewski, N.E. (2008). Global change and the ecology of cities. Science 319: 756–760.

Guerrero, L. A., Maas, G. and Hogland, W. (2013). Solid waste management challenges for cities in developing countries. Waste Management, 33: 220-232.

Gupta, V. K. and Rastogi, A. (2008). Biosorption of lead from aqueous solutions by green algae Spirogyra species: kinetics and equilibrium studies. Journal of Hazardous Materials. 152: 407-414.

Gupta, V. K., Rastogi, A., Saini, V. K. and Jain, N. (2006). Biosorption of copper (II) from aqueous solutions by Spirogyra species. Journal of Colloid and Interface Science, 296: 59-63.

Hashemi, H. and Khodabakhshi, A. (2016). Complete Treatment of Compost Leachate Using Integrated Biological and Membrane Filtration Processes. Iranian Journal of Chemistry and Chemical Engineering (IJCCE), 35: 81-87.

Hassard, F., Biddle, J., Cartmell, E., Jefferson, B., Tyrrel, S. and Stephenson, T. (2015). Rotating biological contactors for wastewater treatment–A review. Process Safety and Environmental Protection, 94: 285-306.

Hillman, W. S. (1961). The Lemnaceae, or duckweeds. The Botanical Review, 27: 221-287.

Huynh, M. and Serediak, N. (2006). Algae Identification field guide. Agriculture and Agri-Food Canada. 40.

Iqbal, H., Baig, M. A., Hanif, M. U., Ali, S. U. and Flury, M. (2015). Leaching of metals, organic carbon and nutrients from municipal waste under semi-arid conditions. International Journal of Environmental Research, 9: 187-196.

Jokela, J., Kettunen, R., Sormunen, K. and Rintala, J. (2002). Biological nitrogen removal from municipal landfill leachate: low-cost nitrification in biofilters and laboratory scale in-situ denitrification. Water Research, 36(16): 4079-4087.

Jones, D. L., Williamson, K. L. and Owen, A. G. (2006). Phytoremediation of landfill leachate. Waste Management. 26: 825-837.

Jung, C., Deng, Y., Zhao, R. and Torrens, K. (2017). Chemical oxidation for mitigation of UV-quenching substances (UVQS) from municipal landfill leachate: Fenton process versus ozonation. Water research, 108: 260-270.

Kalčíková, G., Vávrová, M., Zagorc-Končan, J. and Žgajnar Gotvajn, A. (2011). Seasonal variations in municipal landfill leachate quality. Management of Environmental Quality: An International Journal, 22: 612-619.

Kalita, P., Mukhopadhyay, P. K. and Mukherjee, A. K. (2008). Supplementation of four non-conventional aquatic weeds to the basal diet of Catla catla and Cirrhinus mrigala fingerlings: Effect on growth, protein utilization and body composition of fish. Acta Ichthyologica et Piscatoria, 38(1).

Karak, T., Bhagat, R. M., and Bhattacharyya, P. (2012). Municipal solid waste generation, composition, and management: the world scenario. Critical Reviews in Environmental Science and Technology, 42: 1509-1630.

Karim, S. (2010). Impacts of solid waste leachate on groundwater and surface water quality. Journal of the Chemical Society of Pakistan, 32: 606-612.

Khalid, A., Malik, A. H., Waseem, A., Zahra, S. and Murtaza, G. (2011). Qualitative and quantitative analysis of drinking water samples of different localities in Abbottabad district, Pakistan. International Journal of Physical Sciences, 6: 7480-7489.

Kheradmand, S., Karimi-Jashni, A. and Sartaj, M. (2010). Treatment of municipal landfill leachate using a combined anaerobic digester and activated sludge system. Waste Management, 30(6): 1025-1031.

Kjeldsen, P., Barlaz, M. A., Rooker, A. P., Baun, A., Ledin, A. and Christensen, T. H. (2002). Present and long-term composition of MSW landfill leachate: a review. Critical Reviews in Environmental Science and Technology, 32: 297-336.

Klomjek, P. and Nitisoravut, S. (2005). Constructed treatment wetland: a study of eight plant species under saline conditions. Chemosphere, 58: 585-593.

Körner, S., Das, S. K., Veenstra, S. and Vermaat, J. E. (2001). The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to *Lemna gibba*. Aquatic Botany, 71(1): 71-78.

Körner, S., Vermaat, J. E. and Veenstra, S. (2003). The capacity of duckweed to treat wastewater. Journal of Environmental Quality, 32(5): 1583-1590.

Krienitz, L., Ustinova, I., Friedl, T. and Huss, V. A. (2001). Traditional generic concepts versus 18S rRNA gene phylogeny in the green algal family Selenastraceae (Chlorophyceae, Chlorophyta). Journal of Phycology, 37(5): 852-865.

Kumar, D., Dhar, D.W., Pabbi, S., Kumar, N. and Walia, S. (2014): Extraction and purification of C-phycocyanin from *Spirulina platensis* (CCC540). Indian Journal of Plant Physiology, 19(2): 184-188.

Kumar, K. S., Dahms, H. U., Won, E. J., Lee, J. S. and Shin, K. H. (2015). Microalgae–A promising tool for heavy metal remediation. Ecotoxicology and Environmental Safety, 113: 329-352.

Kurniawan, T. A. (2011). Treatment of Landfill Leachate. s.l.:LAP LAMBERT Academic publishing GmbHand Co.KG.

Labiadh, L., Fernandes, A., Ciríaco, L., Pacheco, M.J., Gadri, A., Ammar, S. and Lopes, A. (2016). Electrochemical treatment of concentrate from reverse osmosis of sanitary landfill leachate. Journal of environmental management, 181: 515-521.

Landesman, L., Parker, N. C., Fedler, C. B. and Konikoff, M. (2005). Modeling duckweed growth in wastewater treatment systems. Livestock Research for Rural Development, 17: 1-8.

Landesman, L., Yang, C.J., Yamamoto, Y. and Goodwin, J. (2002). Nutritional value of wastewater-grown duckweed for fish and shrimp feed. World Aquaculture, 33(4): 39-40.

Landolt, E. and Kandeler, R. (1987). Biosystematic investigations in the family of duckweeds (Lemnaceae) (vol. 4). The family of Lemnaceae-a monographic study, 2, 211-34.

Lasfar, S., Monette, F., Millette, L. and Azzouz, A. (2007). Intrinsic growth rate: a new approach to evaluate the effects of temperature, photoperiod and phosphorus–nitrogen concentrations on duckweed growth under controlled eutrophication. Water Research, 41: 2333-2340.

Lavrova, S. and Koumanova, B. (2010). Influence of recirculation in a lab-scale vertical flow constructed wetland on the treatment efficiency of landfill leachate. Bioresource Technology, 101: 1756-1761.

Leng, R. A., Bell, R. and Stachiw, S. (1994). Unpublished observations. UNE, Armidale NSW, Australia.

Leng, R. A., Stambolie, J. H. and Bell, R. (1995). Duckweed-a potential high-protein feed resource for domestic animals and fish. Livestock Research for Rural Development, 7(1): 36.

Li, T., Lin, G., Podola, B. and Melkonian, M. (2015). Continuous removal of zinc from wastewater and mine dump leachate by a microalgal biofilm PSBR. Journal of Hazardous Materials. 297: 112-118.

Li, X., Hu, H. Y., Gan, K. and Yang, J. (2010). Growth and nutrient removal properties of a freshwater microalga Scenedesmus sp. LX1 under different kinds of nitrogen sources. Ecological Engineering, 36: 379-381.

Liebhold, A. and Bascompte, J. (2003). The Allee effect, stochastic dynamics and the eradication of alien species. Ecology Letters, 6: 133-140.

Liu, S. (2013). Landfill leachate treatment methods and evaluation of Hedeskoga and Måsalycke landfills (Doctoral dissertation, Thesis. Sweden: Luden University.[Links]).

Liu, Wen-Tso, Takashi Mino, Tomonori Matsuo, and Kazunori Nakamura. "Biological phosphorus removal processes-effect of pH on anaerobic substrate metabolism." Water Science and Technology 34, no. 1-2 (1996): 25-32.

Longe, E. O. and Balogun, M. R. (2010). Groundwater quality assessment near a municipal landfill, Lagos, Nigeria. Research Journal of Applied Sciences, Engineering and Technology, 2: 39-44.

Lu, Q., He, Z. L., Graetz, D. A., Stoffella, P. J. and Yang, X. (2010). Phytoremediation to remove nutrients and improve eutrophic storm waters using water lettuce (*Pistia stratiotes* L.). Environmental Science and Pollution Research, 17: 84-96.

Mackenzie, S. M., Waite, S., Metcalfe, D. J. and Joyce, C. B. (2003). Landfill leachate ecotoxicity experiments using *Lemna minor*. Water, Air and Soil Pollution: Focus, 3: 171-179.

Madera-Parra, C. A., Peña-Salamanca, E. J., Peña, M. R., Rousseau, D. P. L. and Lens, P. N. L. (2015). Phytoremediation of landfill leachate with *Colocasia esculenta*, *Gynerum sagittatum* and *Heliconia psittacorum* in constructed wetlands. International Journal of Phytoremediation, 17: 16-24.

Madigan MT, Martinko JM and Parker J (2000). Broc Biology of Microorganisms 9th ed. Prentice Hall International Inc New Jersey, pp. 858-907.

Maguire, R. J., Wong, P. T. S. and Rhamey, J. S. (1984). Accumulation and metabolism of tri-n-butyltin cation by a green alga, *Ankistrodesmus falcatus*. Canadian Journal of Fisheries and Aquatic Sciences, 41(3): 537-540.

Majone, M., Papini, M. P. and Rolle, E. (1998). Influence of metal speciation in landfill leachates on kaolinite sorption. Water Research, 32: 882-890.

Maqbool, F., Bhatti, Z. A., Malik, A. H., Pervez, A. and Mahmood, Q. (2011). Effect of landfill leachate on the stream water quality. International Journal of Environmental Research, 5: 491-500.

Marchand, L., Mench, M., Marchand, C., Le Coustumer, P., Kolbas, A. and Maalouf, J. P. (2011). Phytotoxicity testing of lysimeter leachates from aided phytostabilized Cu-contaminated soils using duckweed (*Lemna minor* L.). Science of the Total Environment, 410: 146-153.

Martinez, S. L., Torretta, V., Minguela, J. V., Siñeriz, F., Raboni, M., Copelli, S. and Ragazzi, M. (2014). Treatment of slaughterhouse wastewaters using anaerobic filters. Environmental Technology, 35: 322-332.

Martins, C. I. M., Eding, E. H., Verdegem, M. C. J. and Heinsbroek, L. T. N. (2006). New developments in recirculating aquaculture systems in Europe: a perspective on environmental sustainability. Aquaculture Engineering, 43: 83-93.

Marttinen, S. K., Kettunen, R. H., Sormunen, K. M., Soimasuo, R. M. and Rintala, J. A. (2002). Screening of physical–chemical methods for removal of organic material, nitrogen and toxicity from low strength landfill leachates. Chemosphere, 46: 851-858.

Masood, M., Barlow, C. Y. and Wilson, D. C. (2014). An assessment of the current municipal solid waste management system in Lahore, Pakistan. Waste Management and Research, 32: 834-847.

Mbagwu, I. G and Adeniji. (1988). The nutritional content of duckweed (*Lemna paucicostata* Heglm cx Engeim) in the Kainji Lakarea. Aquatic Botany, 29: 357-366.

Mdigan, M. T., Martinko, J.M. and Parker, J. (1997). Biology of microorganism. England: Prentice Hall; 1997.

Mehta, S.K. and Gaur, J.P. (2005). Use of alga for removing heavy metal ions from wastewater: progress and prospects. Critical Review in Biotechnology, 25: 113-152.

Men, B. X., Ogle, B. and Preston, T. R. (1995). Use of duckweed (Lemna spp) as replacement for soya bean meal in a basal diet of broken rice for fattening ducks. Livestock Research for Rural Development, 7(3): 5-8.

Mor, S., De Visscher, A., Ravindra, K., Dahiya, R. P., Chandra, A. and Van Cleemput, O. (2006). Induction of enhanced methane oxidation in compost: Temperature and moisture response. Waste Management, 26: 381-388.

Morling, S. (2008). Nitrogen removal efficiency and nitrification rates at the Sequencing Batch Reactor in Nowy Targ, Poland. Vatten, 64: 121.

Mufarrege, M. M., Di Luca, G. A., Hadad, H. R. and Maine, M. A. (2011). Adaptability of *Typha domingensis* to high pH and salinity. Ecotoxicology, 20: 457-465.

Nagarajan, R., Thirumalaisamy, S. and Lakshumanan, E. (2012). Impact of leachate on groundwater pollution due to non-engineered municipal solid waste landfill sites of erode city, Tamil Nadu, India. Iranian journal of Environmental Health Science and Engineering, 9: 35.

Naz, I., Saroj, D. P., Mumtaz, S., Ali, N. and Ahmed, S. (2015). Assessment of biological trickling filter systems with various packing materials for improved wastewater treatment. Environmental Technology, 36: 424-434.

Nieboer, E. and Richardson, D. H. S. (1980). The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. Environmental Pollution (SET.B), 1: 3-26.

Nisar, H., Ejaz, N., Naushad, Z. and Ali, Z. (2008). Impacts of solid waste management in Pakistan: a case study of Rawalpindi city. WIT Transactions on Ecology and the Environment, 109: 685-691.

Oron, G., de-Vegt, A. and Porath, D. (2008). Nitrogen removal and conversion by duckweed grown on waste-water. Water Research, 22: 179-184.

Oulego, P., Collado, S., Laca, A. and Díaz, M. (2016). Impact of leachate composition on the advanced oxidation treatment. Water Research, 88: 389-402.

Ozengin, N. and Elmaci, A. (2007). Performance of Duckweed (*Lemna minor* L.) on different types of wastewater treatment. Journal of Environmental Biology, 28: 307-314.

Pande, G., Sinha, A. and Agrawal, S. (2015). Impacts of leachate percolation on ground water quality: a case study of Dhanbad City. Global NEST Journal, 17: 162-174.

Parmar, S. and Singh, V. (2015). Phytoremediation approaches for heavy metal pollution: a review. J Plant Sci Res, 2(2):135.

Pavasant, P., Apiratikul, R., Sungkhum, V., Suthiparinyanont, P., Wattanachira, S. and Marhaba, T.F. (2006). Biosorption of Cu2+, Cd2+, Pb2+ and Zn2+ using dried marine green macroalga caulerpa lentillifera. Bioresource Technology, 97: 2321-2329.

Pearson, R.G. (1963). Hard and soft acids and bases. Journal of American Chemical Society, 85: 3533-3539.

Pedraza, G., Conde, N. and Chara, J. (1996). Evaluacion de un sistema de descontaminacion de agues a traves de la producccion de organismos y plantas acuaticas. Report CIPAV, Cali. 106 pp.

Piotrowska-Niczyporuk, A., Bajguz, A., Zambrzycka, E. and Godlewska-Żyłkiewicz, B. (2012). Phytohormones as regulators of heavy metal biosorption and toxicity in green alga Chlorella vulgaris (Chlorophyceae). Plant Physiology and Biochemistry, 52: 52-65.

Prabakaran, R. and Arivoli, S. 2012. Adsorption kinetics, equilibrium and thermodynamic studies of Nickel adsorption onto *Thespesia Populnea* bark as biosorbent from aqueous solutions. European Journal of Applied Engineering and Scientific Research, 1: 134-142.

Prasad, M. N. V., Malec, P., Waloszek, A., Bojko, M. and Strzałka, K. (2001). Physiological responses of *Lemna trisulca* L.(duckweed) to cadmium and copper bioaccumulation. Plant Science, 161: 881-889.

Prescott, M. I., Harle, J.D. and Klein, D. A. (2002). Microbiology of Food. 5th ed. McGraw-Hill Ltd, New York, USA. pp. 964-976.

Preston, T. R. and Murgueitio, E. (1992). Strategy for sustainable livestock production in the tropics. SAREC and Publishers Consultories para el Desarrollo Rural Integrado en el Tropico Ltda, Cali, Colombia.

Radic, S. and Pevalek-Kozlina, B. 2010. Effects of osmotic stress on antioxidative system of duckweed (*Lemna minor* L). Periodicum Biologorum, 112(3): 293-299.

Rahman, M. A., Soumya, K. K., Tripathi, A., Sundaram, S., Singh, S. and Gupta, A. (2011). Evaluation and sensitivity of cyanobacteria, *Nostoc muscorum* and Synechococcus PCC 7942 for heavy metals stress–a step toward biosensor. Toxicological and Environmental Chemistry, 93: 1982-1990.

Raven, J. A. and Geider, R. J. (1988). Temperature and algal growth. New Phytologist, 110(4): 441-461.

Remacle, J. (1990). The cell wall and metal binding. In: Biosorption of Heavy Metals. Volesky, B., Ed. CRC Press: Boca Raton, FL. 83-92 pp.

Renou, S., Givaudan, J. G., Poulain, S., Dirassouyan, F. and Moulin, P. (2008). Landfill leachate treatment: review and opportunity. Journal of Hazardous Materials, 150: 468-493.

Renoua, S., Givaudan, J. G., Poulain, S., Dirassouyan, F. and Moulin, P. (2008). Landfill leachate treatment: Review and opportunity. Journal of Hazardous Materials, 150(3): 468-493.

Robinson, H. (2007). The composition of leachates from very large landfills: an international review. Communications in Waste and Resource Management, 8: 19-32.

Saarela, J. (2003). Pilot investigations of surface parts of three closed landfills and factors affecting them. Environmental Monitoring and Assessment, 84: 183-192.

Saha, P., Shinde, O. and Sarkar, S. (2017). Phytoremediation of industrial mines wastewater using water hyacinth. International Journal of Phytoremediation, 19(1): 87-96.

Salem, Z., Hamouri, K., Djemaa, R. and Allia, K. (2008). Evaluation of landfill leachate pollution and treatment. Desalination, 220: 108-114.

Salt, D.E., Blaylock, M., Kumar, N.P., Dushenkov, V., Ensley, B.D., Chet, I. and Raskin, I. (1995). Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Nature biotechnology, 13(5): 468.

Sand-Jensen, K. and Jespersen, T. S. (2012). Tolerance of the widespread cyanobacterium Nostoc commune to extreme temperature variations (− 269 to 105 C), pH and salt stress. Oecologia, 169: 331-339.

Sheng, P. X., Ting, Y. P., Chen, J. P. and Hong, L. (2007). Sorption of lead, copper, cadmium, zinc, and nickel by marine algal biomass: Characterization of biosorptive heavy metal removal through bacterial biomass isolated from various contaminated sites capacity and investigation of mechanisms. Journal of Colloid and Interface Science, 275(1): 131-141.

Shinde, S. (2013). Bioremediation. An overview. Recent Research in Science and Technology, 5(5): 67-72.

Singh, P., Guldhe, A., Kumari, S., Rawat, I. and Bux, F. (2015). Investigation of combined effect of nitrogen, phosphorus and iron on lipid productivity of microalgae *Ankistrodesmus falcatus* KJ671624 using response surface methodology. Biochemical Engineering Journal, 94: 22-29.

Smith, M. D. and Moelyowati, I. (2001). Duckweed based wastewater treatment (DWWT): design guidelines for hot climates. Water Science and Technology, 43: 291-299.

Srivastava, V., Ismail, S. A., Singh, P., and Singh, R. P. (2015). Urban solid waste management in the developing world with emphasis on India: challenges and opportunities. Reviews in Environmental Science and Biotechnology, 14: 317-337.

Steel, R.G.D., Torrie, J.H. and Dickey, D. (1997). Principals and Procedures of Statistics: A Biometrical Approach. 3rd Ed. McGraw Hill Book Co. Inc. New York: 172-177.

Sutton, and Ornes, W.H. (1975). Phosphorus removal from static sewage effluent using duckweed. Journal of Environmental Quality, 4(3): 367-370.

Svensson, H., Ekstam, B., Marques, M. and Hogland, W. (2015). Removal of organic pollutants from oak leachate in pilot scale wetland systems: How efficient are aeration and vegetation treatments? Water Research, 84: 120-126.

Szpadt, R. Characteristics and treatment methods of municipal landfill leachates (In Polish). Mat. Konf. II-ej Ogólnopolskiej Konferencji Naukowo-Technicznej PZiTS nt. Rozwój technologii w ochronie wód. Międzyzdroje 1998.

Tacon, A. G. J. (1987). Nutrient sources and composition. In: The nutrition and feeding of farmed fish and shrimp-A training manual. FAO, Brasilia, Brazil. 137 pp. http://www.fao.org/docrep/field/003/ab470e/AB470E00.html

Talaro, K.P. and Talaro, A. (2002). Drugs, microbes, host-The elements of chemotherapy. In: Foundations in Microbiology. 4th ed. McGraw-Hill, New York, pp. 348-379.

Tavares, F. D. A., Rodrigues, J. B. R., Fracalossi, D. M., Esquivel, J. and Roubach, R. (2008). Dried duckweed and commercial feed promote adequate growth performance of tilapia fingerlings. Biotemas, 21(3): 91-97.

Toufexi, E., Tsarpali, V., Efthimiou, I., Vidali, M. S., Vlastos, D. and Dailianis, S. (2013). Environmental and human risk assessment of landfill leachate: an integrated approach with the use of cytotoxic and genotoxic stress indices in mussel and human cells. Journal of Hazardous Materials, 260: 593-601.

UNEP. (2004) Global Environmental Alert Service. Bulletin October 2004. United Nations Environment Programme.

United Nations. (1997). Glossary of Environment Statistics, Studies in Methods, Series F, No. 67, New York.

Verma, R. and Suthar, S. (2015). Impact of density loads on performance of duckweed bioreactor: A potential system for synchronized wastewater treatment and energy biomass production. Environmental Progress and Sustainable Energy, 34: 1596-1604.

Vermaat, J.E. and Hanif, M.K. (1998). Performance of common duckweed species (Lemnaceae) and the waterfern Azolla filiculoides on different types of waste water. Water research, 32(9): 2569-2576.

Vidali, M. (2001). Bioremediation. an overview. Pure and Applied Chemistry, 73: 1163-1172.

Vilar, V. J. P., Botelho, C. M. S. and Boaventura, R.A.R. (2007). Modeling equilibrium and kinetics of metal uptake by algal biomass in continuous stirred and packed bed adsorbers. Adsorption, 13: 587-601.

Wang, J. and Chen, C. (2009). Biosorbents for heavy metals removal and their future. Biotechnology Advances, 27: 195-226.

Wang, L., Yang, Q., Wang, D., Li, X., Zeng, G., Li, Z. and Yi, K. (2016). Advanced landfill leachate treatment using iron-carbon microelectrolysis-Fenton process: Process optimization and column experiments. Journal of Hazardous Materials, 318: 460-467.

Wang, Y., Huang, X. and Yuan, Q. (2005). Nitrogen and carbon removals from food processing wastewater by an anoxic/aerobic membrane bioreactor. Process Biochemistry, 40: 1733-1739.

Wedge, R. M. and Burris, J. E. (1982). Effects of light and temperature on duckweed photosynthesis. Aquatic Botany, 13: 133-140.

Weiner, J. (1990). Asymmetric competition in plant populations. Trends in Ecology and Evolution, 5: 360-364.

Wendeou, S. P. H., Aina, M. P., Crapper, M., Adjovi, E. and Daouda, M. (2013). Influence of salinity on duckweed growth and duckweed based wastewater treatment system. Journal of Water Resource and Protection, 5: 993-999.

Wiszniowski, J., Robert, D., Surmacz-Gorska, J., Miksch, K. and Weber, J. V. (2006). Landfill leachate treatment methods: A review. Environmental Chemistry Letters, 4: 51-61.

Wojciechowska, E. and Waara, S. (2011). Distribution and removal efficiency of heavy metals in two constructed wetlands treating landfill leachate. Water Science and Technology, 64(8): 1597-1606.

Wojciechowska, E., Gajewska, M. and Obarska-Pempkowiak, H. (2010). Treatment of landfill leachate by constructed wetlands: three case studies. Polish Journal of Environmental Studies, 19(3): 643-650.

Wolverton, B. C. (1975). Water hyacinths and alligator weeds for final filtration of sewage. NASA Technology Memorandum, TM-X-72724.

Xie, Z., Wang, Z., Wang, Q., Zhu, C. and Wu, Z. (2014). An anaerobic dynamic membrane bioreactor (AnDMBR) for landfill leachate treatment: Performance and microbial community identification. Bioresource Technology, 161: 29-39.

Xu, J. and Shen, G. (2011). Growing duckweed in swine wastewater for nutrient recovery and biomass production. Bioresource technology, 102(2): 848-853.

Xu, J., Cheng, J. J. and Stomp, A. M. (2012). Growing *Spirodela polyrrhiza* in swine wastewater for the production of animal feed and fuel ethanol: a pilot study. Clean–Soil, Air, Water, 40: 760-765.

Xu, Z.Y., Zeng, G.M., Yang, Z.H., Xiao, Y., Cao, M., Sun, H.S., Ji, L.L. and Chen, Y. (2010). Biological treatment of landfill leachate with the integration of partial nitrification, anaerobic ammonium oxidation and heterotrophic denitrification. Bioresource technology, 101(1): 79-86.

Yabroudi, S.C., Morita, D.M. and Alem, P. (2013). Landfill leachate treatment over nitritation/denitritation in an activated sludge sequencing batch reactor. APCBEE procedia, 5: 163-168.

Yan, W. and Hunt, L. A. (1999). An equation for modelling the temperature response of plants using only the cardinal temperatures. Annals of Botany, 84(5): 607-614.

Yang, T., Chen, M.L. and Wang, J.H. (2015). Genetic and chemical modifications of cells for selective separation and analysis of heavy metals of biological or environmental significance. TrAC Trends in Analytical Chemistry, 66: 96-102.

Yılmaz, E., Akyurt, İ. and Günal, G. (2004). Use of duckweed, *Lemna minor*, as a protein feedstuff in practical diets for common carp, *Cyprinus carpio*, fry. Turkish Journal of Fisheries and Aquatic Sciences, 4(2). 105-109.

Zhao, Z., Shi, H., Liu, Y., Zhao, H., Su, H., Wang, M. and Zhao, Y. (2014). The influence of duckweed species diversity on biomass productivity and nutrient removal efficiency in swine wastewater. Bioresource Technology, 167: 383-389.

Zhu, F. M., Zhu, H. G., Shen, W. Y. and Chen, T. H. (2017). Integrating a tidal flow wetland with sweet sorghum for the treatment of swine wastewater and biomass production. Ecological Engineering, 101: 145-154.

Ziegler, P., Adelmann, K., Zimmer, S., Schmidt, C. and Appenroth, K.J. (2015). Relative in vitro growth rates of duckweeds (Lemnaceae)–the most rapidly growing higher plants. Plant Biology, 17: 33-41.

Zouboulis, A. I., Jun, W. and Katsoyiannis, I. A. (2003). Removal of humic acids by flotation. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 231: 181-193.

Tkalec, M., Mlinarec, J., Vidaković-Cifrek, Ž., Jelenčić, B. and Regula, I. (2001). The effect of salinity and osmotic stress on duckweed Lemna minor L. Acta Botanica Croatica, 60(2): 237-244.

Yilmaz, D.D. (2007). Effects of salinity on growth and nickel accumulation capacity of Lemna gibba (Lemnaceae). Journal of Hazardous Materials, 147(1-2): 74-77.

McIlraith, A.L., Robinson, G.G. and Shay, J.M. (1989). A field study of competition and interaction between Lemna minor and Lemna trisulca. Canadian journal of botany, 67(10): 2904-2911.

Ojoawo, S. O., Agbede, O. A., & Sangodoyin, A. Y. (2012). Characterization of Dumpsite Leachate: Case Study of Ogbomosoland, South-Western Nigeria. Open Journal of Civil Engineering, 2(01): 33.

Published Paper

Environment Protection Engineering

Vol. 43 2017 No. 4

DOI: 10.5277/epe170410

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NITROGEN AND PHOSPHOROUS REMOVAL FROM LEACHATE BY DUCKWEED (*Lemna minor*)

Two separate experiments were conducted during the months of June and September, 2014 to investigate the nutrient (nitrogen and phosphorous) removal from leachate by growing duckweed, *Lemna minor* in various leachate dilutions under natural climatic conditions of Islamabad, Pakistan. The highest uptake of nitrogen and phosphorous by duckweed was 95% and 90%, respectively, whereas the highest growth rate of duckweed was 6.4 $g \cdot m^{-2} \cdot day^{-1}$ during both experiments. The highest rates of nitrogen and phosphorous removal from leachate media were 380 and 200 $mg \cdot m^{-2} \cdot day^{-1}$, respectively, during both experiments. Nutrient uptake by duckweed and its growth rate was rapid at more diluted leachate whereas the nutrient removal rates from leachate media were higher in more concentrated leachate. The duckweed growth and its nutrient uptake ability under natural climatic conditions were directly affected by seasonal climatic variations. Relatively higher temperature and more intense solar radiation were more favorable for the duckweed growth and its nutrient uptake ability. Both parameters can be improved by pre-acclimation of duckweed with leachate which prevents the lag phase of the duckweed growth.

1. INTRODUCTION

Sustainable management of solid waste is one of the emerging challenges faced by developing countries like Pakistan. As an existing practice, open dumping of solid waste in low lying areas is very common in Pakistan [1]. Besides other direct and indirect environmental hazards, production of large amount of leachate is one of the major problems associated with solid waste dumping.

Leachate is the concentrated wastewater. At open dump sites, it is produced by percolation of rainwater through solid waste layers [2]. Physical and bio-chemical activities in solid waste transfer variety of pollutants from waste into percolating rain water [3].

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Depending on nature of solid waste, climatic conditions (temperature, precipitation), management practices and age of dump site, leachate may contain variety of inorganic and organic pollutants [4]. Major components of general landfill leachate are: dissolved organic matter characterized as chemical oxygen demand (COD), total organic carbon, volatile fatty acids and refractory compounds; inorganic macronutrients such as calcium (Ca^{2+}), sodium (Na^{+}), potassium (K^{+}), and ammonium (NH_4^{+}) ions, sulfates (SO_4^{2-}) and hydrogen carbonates (HCO_3^{-}); heavy metal ions such as copper (Cu^{2+}), lead (Pb^{2+}), nickel (Ni^{2+}) and zinc (Zn^{2+}) and xenobiotic organic compounds [3]. Leachate poses a serious threat to surface as well as ground water quality [5]. Leachate hazards for human health, flora, fauna, and ecosystems have also been frequently investigated and documented [6]. Presently, it has become major concern worldwide to impose more stringent environmental requirements related to leachate treatment and surface and ground water quality [7].

Use of aquatic plants such as duckweed, water hyacinth, water lettuce in wastewater treatment has received greater attraction during the recent years [8–10]. Production of aquatic plants on wastewater has two fold benefits: treatment of wastewater and, as an alternate technology, converting wastewater nutrients into potentially useful forms [11].

Duckweed is a small floating macrophyte belonging to family *Lemnaceae* of monocotyledon plants. *Lemnaceae* consists of 4 genera (*Lemna*, *Spirodela*, *Wolffia* and *Wolffiella*) and 28 species [12, 13]. Duckweed has efficient ability of nutrient uptake and shows high growth rate when grown under nutrient rich wastewaters [14]. In eight week time, a duckweed based swine lagoon treatment system can remove up to 83.7% of total nitrogen (TN), and 89.4% of total phosphorus (TP) from the media when harvested twice a week [15]. High level of nutrients tolerance in duckweed is of particular importance for treatment of landfill leachate which usually has high concentrations of nitrogen and phosphorous. For example, the high concentrations of ammonia-N (3032 $mg \cdot dm^{-3}$), nitrate-N (22 $mg \cdot dm^{-3}$), and nitrite-N (120 $mg \cdot dm^{-3}$), and 3000 $mg \cdot dm^{-3}$ of phosphates have been reported in leachate samples from Hong Kong [16].

Recently, the phytoremediation of landfill leachate by aquatic plants has received growing attention [12]. Duckweed due to its high rates of growth and nutrient uptake is one of the promising aquatic plants used for leachate treatment. *L. minor* can uptake significant amount of inorganic nitrogen through roots and fronds as well [18]. Ammonium ions are the preferred forms of nitrogen uptake by duckweed [19]. Majority of total Kjeldahl nitrogen (TKN) in leachate is ammonium which is an advantage for the duckweed growth. A range of 0.2–13 000 $mg \cdot dm^{-3}$ of N has been reported in leachate samples collected from various parts of the world [6]. Duckweed has ability to grow under broad range of temperature making it an advantageous macrophyte to grow round the year in the areas where other tropical aquatic plants such as water hyacinth are unable to perform in summer [9, 11]. Duckweed can survive below freezing temperatures for many days [11]. Duckweed has high protein contents (10–40% of protein on dry mass basis). Some species of duckweed have ability to produce protein six to ten times faster than soybeans planted at an equivalent surface area [10]. A positive correlation has been reported between TN of wastewater medium and protein contents of duckweed grown on it [10]. High protein content of duckweed is an indication of its higher capacity to assimilate nitrogen. The protein content of 35% was reported in duckweed with fastest nitrogen removal rates (4.4 $g \cdot m^{-2} \cdot day$ of TKN) from swine wastes [23]. Protein content of duckweed is very useful for its end use as high protein feed for ducks, cattle, poultry and fish [24–26].

Lemna minor species of duckweed has enormous potential of nutrients removal from wastewaters of high strength such as swine lagoon wastewater [11, 13]. Under favorable climatic conditions and nutrient balance in growth media, *Lemna minor* grows well and doubles its biomass within two days [25]. The growth rate of *L. minor* close to 29 $g \cdot m^{-2} \cdot day^{-1}$ as well as TKN and TP uptake of 90% and 88.6%, respectively have been reported when grown on high strength swine lagoon under natural conditions in Raleigh, North Carolina [11].

This study has been conducted to investigate the nutrient removal dynamics from leachate by *L. minor* under the natural conditions of temperature and light intensity in Islamabad, Pakistan. The duckweed growth in relation to various nutrient concentrations in leachate and nutrient uptake rates are determined by growing duckweed on various initial concentrations of nutrients in leachate (various leachate dilutions). This study will be useful to initiate the duckweed based leachate treatment systems by providing an understanding of nutrient removal dynamics from such leachate treatment systems under natural climatic conditions.

2. MATERIALS AND METHODS

Leachate. Leachate was prepared by the processing of decomposed solid waste collected from various residential, commercial and industrial areas of Islamabad and Rawalpindi, Pakistan. 100–120 kg well decomposed solid waste was collected from each residential, commercial and industrial dump sites. Waste was collected from pre-determined lowest points at each dump site at soil depths of 0.5–1.5 m [27]. Solid waste samples were collected during both dry and wet season of the year. The samples were mixed in a large plastic water storage tank having the internal diameter of 1.5 m, 1.8 m hogh. A sieve (pore size 1 mm) was fixed at an internal height of 10 cm of the plastic container. Thorough shaking was applied to well mix the waste and obtain a homogenized sample. The homogenized waste was soaked with leaching solution (distilled water) and maintained for 30 days after which the leachate was collected from the bottom outlet in the container. Remaining solid waste was again mixed thoroughly and soaked with distilled water. Afterwards, the leachate was collected three times at an interval of ten days each, during both summer and fall. Each time the solid waste samples were thoroughly mixed and shaken. The leachate collected from various runs was mixed to form single homogenized sample to be used for this research.

Duckweed. Duckweed (*L. minor*) used in this research was collected from a wastewater pond in Islamabad, Pakistan. Prior to grow on leachate, duckweed was repeatedly washed with excess water to remove bacteria, algae and other unwanted compounds [28]. During initial experiments, duckweed was grown at various leachate dilutions in order to reach the optimal range of dilutions duckweed can tolerate. Before starting the experiments, duckweed was adopted to new environment for fifteen days by growing on experimental leachate under the same natural conditions.

Duckweed was grown in 300 cm^3 transparent plastic containers. 250 cm^3 of initial volume of leachate was used in each container at the leachate depth of 9.5 cm. Surface area of each container was 25.8 cm^2. Duckweed containers were placed within a porous iron rack having three compartments. The rack with duckweed containers was placed in an open environment at the Institute of Environmental Sciences and Engineering (IESE), National University of Sciences and Technology, Islamabad under natural climatic conditions. Seasonal effect on growth of duckweed and its nutrient removal efficiency were determined during the study. Seasonal data related to ambient air temperature and day lengths during both experiments was retrieved from the website of Pakistan Metrological Department, whereas the solar radiation data during the experimental period was obtained from the web site of LEO Corporation, Pakistan. Nutrient removal and growth of duckweed was tested at various initial concentrations of nutrients.

Experimental setup. Two batch experiments were conducted during the months of June (summer experiment) and September (fall experiment), 2014, to determine the duckweed growth and nutrient (N and P) removal rates from leachate. Five dilutions of leachate with leachate/water ratios of 50/50, 40/60 , 30/70, 20/80 and 10/90) were prepared with tap water and the duckweed was grown at each leachate dilution. About 30 mg of initial fresh mass of pre-acclimated duckweed was used during each test. Each batch test consisted of 44 containers containing a dilution of leachate corresponding to triplicate samples of 11 time points. Out of these, 33 containers contained duckweed cultures whereas 11 control containers were without duckweed. The leachate and duckweed were mixed briefly for five min every day. During each experiment, three duckweed containers and a control one were removed for destructive sampling after every 2 days to monitor the duckweed growth and nutrient levels. Each experiment lasted for 22 days. Average ambient air temperature was 39.1 °C, solar radiation 4.5–5.0 $kWh\cdot m^{-2}\cdot day^{-1}$, and day lengths during summer experiment 14.20 h. During fall it was 24–30 °C, 4–4.5 $kWh\cdot m^{-2}\cdot day^{-1}$ and 12.20 h, respectively.

Laboratory analysis. Samples of the leachate were analyzed for TKN, ammonium nitrogen (NH_4^+-N), total phosphorous (TP), *o*-phosphate-phosphorous (*o*-PO_4^{3-}-P), chemical oxygen demand (COD) and pH. Dry biomass of duckweed was analyzed for TKN and

TP contents. All chemical analyses were conducted in environmental analytical laboratories of the Institute of Environmental Sciences and Engineering (IESE), National University of Sciences and Technology, Islamabad. All chemical analyses were performed using the standard methods of American Public Health Association (APHA, 1998) [25] and US-EPA Methods (EPA, 1983) [26].

3. RESULTS AND DISCUSSION

To investigate the nutrients dynamics of duckweed based leachate treatment system, two batch experiments were performed under the natural climatic conditions of Islamabad, Pakistan during the summer and fall of 2014. Five dilutions of leachate (50%, 40%, 30%, 20% and 10%) were used as medium for the duckweed growth. Initial concentrations of average N and P and other characteristics of each leachate dilution during each season were determined (Table 1). In initial leachate medium, P:N ratio has relatively higher ranges in summer experiments (0.75–0.86) than that in the fall experiments (0.77–0.82).

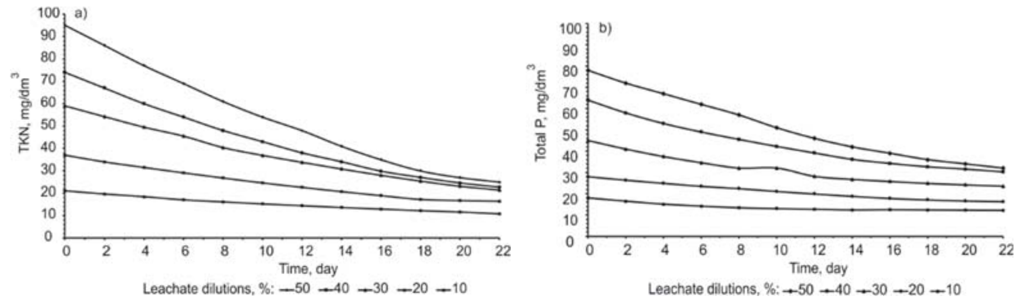
Table 1

Initial characteristics of leachate dilutions used as medium for growth of *L. minor* in natural climatic conditions of Islamabad, Pakistan in 2014

Leachate dilutions[a] [%]	Nutrient concentration[b] [mg·dm^{-3}]				COD[b] [mg·dm^{-3}]	pH[b]
	TKN	NH_4^+-N	TP	*o*-PO_4^{3-}-P		
Summer experiment						
50	95±1.63	55±0.82	78±0.41	18±1.47	2760±2.83	7.45±0.02
40	74±0.82	40±1.63	64±3.08	14±1.22	2248±2.16	7.36±0.01
30	59±1.63	35±1.08	45±2.68	10±1.08	1732±1.41	7.29±0
20	37±1.78	20±1.41	28±1.41	8±0.71	1088±2.16	7.18±0.07
10	21±1.08	12±0.41	18±0.71	5±0.41	540±2.83	7.14±0
Fall experiment						
50	102±2.16	58±2.16	82±1.63	32±1.41	2922±7.48	7.89±0.02
40	82±0	46±1.41	66±3.74	26±1.41	2308±5.72	8.00±0.08
30	61±1.41	32±0	47±0.82	22±2.94	1695±5.10	7.94±0.01
20	40±0.82	17±2.16	33±2.83	12±1.41	1216±8.16	7.82±0.01
10	23±0	10±1.41	19±0.82	9±0.82	522±3.74	7.76±0.02

[a]Initial concentrations of nutrients and COD for all leachate dilutions may not be exactly same as intended because of the deviations caused during the dilution operation.

[b]Each value is the average of those obtained from three replicate experimental containers.



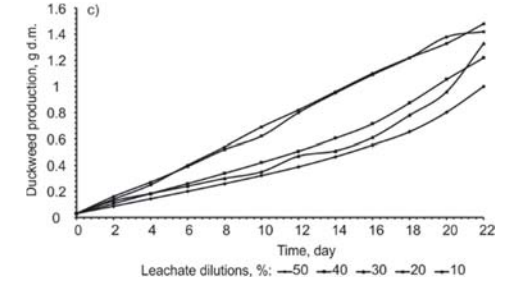
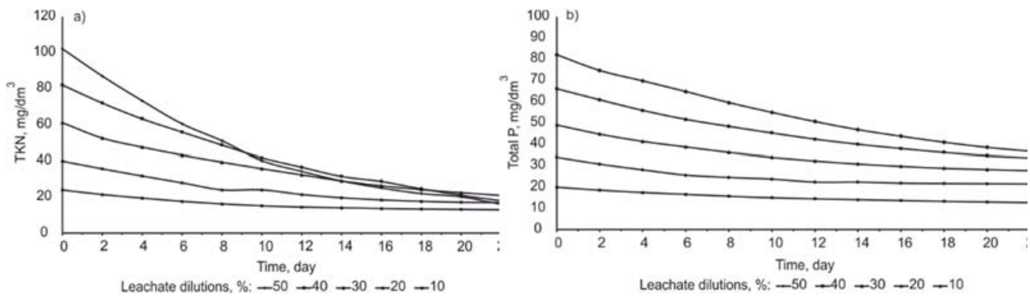


Fig. 1. Removal of a) total Kjeldahl N (TKN), b) total P from various dilutions of leachate by growing *Lemna.minor* and, c) duckweed production under natural climatic conditions of Islamabad, Pakistan in summer (June), 2014



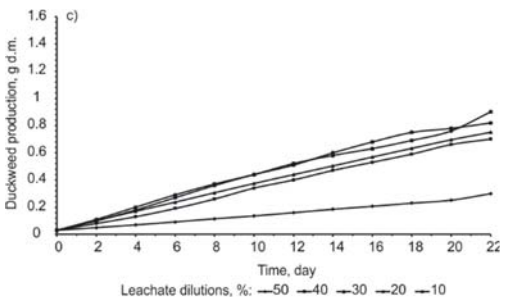


Fig. 2. Removal of a) total Kjeldahl N (TKN), b) total P from various dilutions of leachate by growing *Lemna.minor* and, c) duckweed production under natural climatic conditions of Islamabad, Pakistan in fall (September), 2014

pH in test containers remained stable within the range of 7.1–7.5 during summer experiments and 7.7–8.0 during the fall experiments. This indicates a strong buffering capacity of leachate (slightly greater during the fall experiments) which is very important for the growth of duckweed. It tends to rapidly decrease the pH of growth media without buffering (from approximately 7 to approximately 5 within 24 h) [22]. COD reduction from 46% to 79% and from 44% to 67% was achieved during fall and summer experiments, respectively, which is the indication of high microbial activity in the test containers during the experiments. The highest level of COD reduction was achieved during two first days after of each experiment. It is because of the pre-acclimation of duckweed in leachate media which resulted in high rates of pollution removal. Close to linear rates of N and P removal and duckweed growth were observed during both experiments (Figs. 1, 2). At the end of experiments, considerable amounts of nutrients were still present in more concentrated leachate.

Lag phase of the duckweed growth was not observed in both experiments (Figs. 1c, 2c). It was because, prior to start the experiments, duckweed was acclimated to the nutritional environment of leachate under natural climatic conditions. Acclimation also helps the duckweed to adjust under drastic changes within a system [27]. The highest ducked growth rate (6.4 $g\cdot m^{-2}\cdot day^{-1}$) was achieved at 10% leachate dilution in summer experiment, whereas the lowest rate of the duckweed growth (1.2 $g\cdot m^{-2}\cdot day^{-1}$) was observed at 50% leachate dilution during the fall experiment (Table 2).

Table 2

Rates of nutrient (N and P) removal and duckweed (*L. minor*) growth for various dilutions of leachate under the natural climatic conditions of Islamabad, Pakistan in 2014

Dilutions of leachate [%]	Nutrient removal rate[a] [$mg\cdot m^{-2}\cdot day^{-1}$]				Duckweed growth rate[a] [$g\cdot m^{-2}\cdot day^{-1}$]
	TKN	NH_4^+-N	TP	*o*-PO_4^{3-}-P	
Summer experiment					
50	310	200	200	60	4.3
40	230	130	150	50	5.2
30	160	110	90	30	5.7
20	90	50	50	20	6.1
10	40	30	30	10	6.4
Fall experiment					
50	380	230	200	120	1.2
40	280	170	140	90	3.0
30	180	110	90	70	3.2
20	100	60	60	30	3.5
10	50	30	30	20	3.8

[a]Each value is the average of the results obtained from three replicate experimental containers.

Overall rates of nutrient removal were higher in a more concentrated leachate, whereas the nutrient uptake by duckweed was higher in a more diluted one (Tables 2, 3). More concentrated growth media may be more favorable for microbial growth than for the duckweed growth [11]. Some nitrogen was probably lost through algal and microbial assimilation [28]. Ammonia volatilization was negligible in our experiments due to average pH of the media which was lower than 8 throughout the experiments. Large portion of N and P was not taken up by duckweed particularly in 50% leachate dilution during fall experiment which agrees with the duckweed growth (Table 3, Fig. 2).

Table 3

Mass balance of total N and P removal and nutrient uptake by duckweed (*Lemna minor*) from diluted leachate in two batch experiments under the natural climatic conditions of Islamabad, Pakistan in 2014

Leachate dilutions [%]	Total nutrients removed from media[a]				Nutrient uptake by duckweed[a]			
	[mg]		[%]		mg		[%]	
	N	P	N	P	N	P	N	P
Summer experiment								
50	20	10	16	49	9.4	4.6	47	46
40	10	8	46	50	5.6	4.8	56	60
30	9	5	39	55	5.9	3.3	66	66
20	5	3	46	58	4.0	2.5	80	83
10	2	2	62	56	1.9	1.8	95	90
Fall experiment								
50	20	10	22	52	2.4	1.4	12	14
40	20	8	03	52	10.2	3.5	51	44
30	10	5	35	58	6.7	2.6	67	52
20	6	3	40	64	4.6	2.1	76	70
10	3	2	48	58	2.7	1.6	90	80

[a]Each value is the average of those obtained from three replicate experimental container.

Overall rate of NH_4^+-N removal in the fall experiment was higher than in summer which is similar to the TKN removal pattern (Figs. 3a, 4a). Initial concentrations of *o*-PO_4^{3-}-P and its removal rates were significantly higher in the fall experiments than in summer and same was the pattern of its removal i.e. higher removal rate at higher leachate concentration (Figs. 3b and 4b). It suggests that removal rate of *o*-PO_4^{3-}-P is more dependent on its initial concentrations than on climatic conditions which were relatively less favorable for the duckweed growth during the fall experiment. Some of the *o*-PO_4^{3-}-P removal may also occur by microbial assimilation and precipitation with minerals in leachate media [28].

Duckweed production rate was smaller in the fall experiment (Table 2). This is likely due to the combination of climatic factors during this period which were less favorable for the duckweed growth such as light intensity and air temperature were comparatively lower during fall experiment and day light hours in Islamabad were also shorter in September than in June. Short term temperature fluctuations in experimental containers were observed during both seasons which are probably due to small volume of leachate (250 cm^3) in each container. Such kind of temperature fluctuations would not be expected in large ponds or lagoons for leachate treatment.

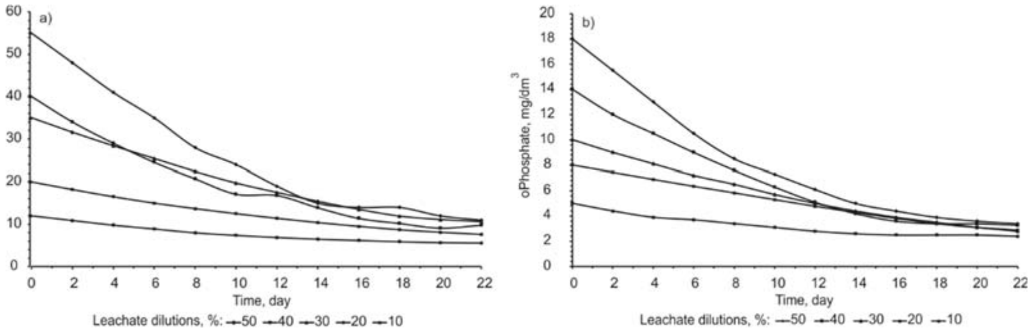


Fig. 3. Removal of a) ammonium-N, b) *o*-PO_4^{3-}-P from various leachate dilutions by growing *Lemna minor* under natural climatic conditions of Islamabad, Pakistan in summer (June), 2014

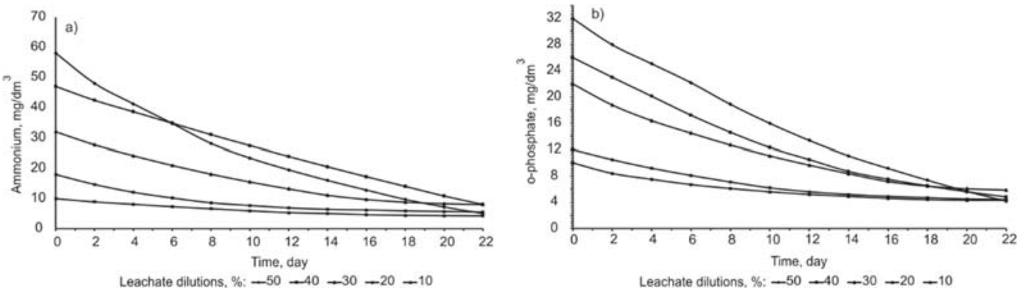


Fig. 4. Removal of a) ammonium-N, and b) *o*-PO_4^{3-}-P from various leachate dilutions by growing *Lemna minor* under natural climatic conditions of Islamabad, Pakistan in fall (September), 2014

Lower duckweed production at concentrated leachate media may be due to many factors such as presence of microorganisms, insects and undefined chemicals in field containers with more concentrated leachate which caused the duckweed to take up relatively smaller percentage of nutrients, overall removed from the media (Table 3). At higher leachate concentration, the inhibitory action caused by the ammonia (NH_3) and ammonium ions (NH_4^+) may also contribute to lower duckweed growth. It has been reported that the ammonia concentration of more than 50 $mg \cdot dm^{-3}$ in domestic wastewater is inhibitory to the duckweed growth [8]. Effect of ammonia to weaker duckweed growth seems less significant in our experiments because of the narrow fluctuations in pH of growth leachate media during both experiments (summer 7.1–7.5 and fall 7.7–8.0) as duckweed growth inhibition by ammonia is more significant at pH values above 8 [8].

Reasons for the weaker duckweed growth at more concentrated leachate need to be further investigated. Effects of high content of phosphorous and nitrogen (in more concentrated leachate) on the duckweed growth also need to be determined in future. Data reported in this paper indicate that to maintain a rapid growth and nutrient uptake by duckweed, leachate should be diluted to below 60 mg TKN·dm^{-3} and 45mg TP·dm^{-3}.

Data presented in this paper is useful to initiate duckweed based leachate treatment system. Diluted leachate should be used in such duckweed systems in order to initiate high growth and nutrient removal rates. Fresh leachate generally has high values of nutrients and other pollutants. Therefore to avoid nutrient shock loading, leachate may be treated by mixing with less concentrated wastewater. Recycling of duckweed treated leachate and mixing it into influent leachate may also reduce the nutrient loading of influent leachate [11].

Lemna minor has potential to remove N and P from landfill leachate therefore it is important to establish duckweed for in-field leachate treatment. In Pakistan, small duckweed based ponds may be established adjacent to solid waste dump sites and local strains of *Lemna minor* may be used as potential aquatic plant to treat diluted leachate under natural climatic conditions.

4. CONCLUSIONS

- *Lemna minor* maintained a healthy growth at leachate efficiently removing the nutrients (N and P) from the media. The highest duckweed growth rate of 6.4 g·m^{-2}·day^{-1} was achieved at least concentrated leachate during summer and fall experiments, whereas the nitrogen and phosphorous uptake by duckweed (95% and 90%, respectively) was also achieved at 10% leachate dilution during summer experiment.
- Highest rates of nitrogen and phosphorous removal (380 mg·m^{-2}·day^{-1} and 200 mg·m^{-2}·day^{-1}, respectively) were achieved at most concentrated leachate during summer and fall experiments.
- Lag phase of the duckweed growth can be prevented by pre-acclimation of duckweed with leachate which also improves the nutrient removal efficiency of duckweed.
- More concentrated leachate is less favorable for the duckweed growth and its nutrient uptake efficiency.
- Relatively ability of duckweed to remove nutrients from leachate media and its growth rates were affected by the climatic conditions during both experiments. Comparatively higher temperature and solar radiation during summer was more favorable for the duckweed growth and its nutrient uptake ability.

ACKNOWLEDGMENTS

The authors are grateful to the Higher Education Commission of Pakistan for financial support to accomplish this research.

REFERENCES

[1] VISVANATHAN C., GLAWE U., *Domestic solid waste management in South Asian countries. A comparative analysis*, Promoting Reduce, Reuse, and Recycle in South Asia, 2006, 27.

[2] KALCÍKOVÁ G., VÁVROVÁ M., ZAGORC-KONCAN J., ZGAJNAR G.A., *Seasonal variations in municipal landfill leachate quality*, Manage. Environ. Qual., 2011, 22 (5), 612.

[3] KJELDSEN P., BARLAZ M.A., ROOKER A.P., BAUN A., LEDIN A., CHRISTENSEN T.H., *Present and long-term composition of MSW landfill leachate. A review*, Crit. Rev. Env. Sci. Tech., 2002, 32 (4), 297.

[4] EDMUNDSON S.J., WILKIE A.C., *Landfill leachate – a water and nutrient resource for algae-based biofuels*, Environ. Technol., 2013, 34 (13–14), 1849.

[5] PABLOS M.V., MARTINI F., FERNANDEZ C., BABIN M.M., HERRAEZ I., MIRANDA J., TARAZONA J.V., *Correlation between physicochemical and ecotoxicological approaches to estimate landfill leachates toxicity*, Waste Manage., 2011, 31 (8), 1841.

[6] AKINBILE C.O., YUSOFF M.S., ZUKI A.A., *Landfill leachate treatment using sub-surface flow constructed wetland by Cyperus haspan*, Waste Manage., 2012, 32 (7), 1387.

[7] RENOU S., GIVAUDAN J.G., POULAIN S., DIRASSOUYAN F., MOULIN P., *Landfill leachate treatment. Review and opportunity*, J. Hazard. Mater., 2008, 150 (3), 468.

[8] CAICEDO J.R., VAN DER STEEN N.P., ARCE O., GIJZEN H.J., *Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (Spirodela polyrrhiza)*, Water Res., 2000, 34 (15), 3829.

[9] LASFAR S., MONETTE F., MILLETTE L., AZZOUZ A., *Intrinsic growth rate. A new approach to evaluate the effects of temperature, photoperiod and phosphorus nitrogen concentrations on duckweed growth under controlled eutrophication*, Water Res., 2007, 41 (11), 2333.

[10] LANDESMAN L., PARKER N.C., FEDLER C.B., KONIKOFF M., *Modeling duckweed growth in wastewater treatment systems*, Livest. Res. Rural Develop., 2005, 17 (6), 61.

[11] CHENG J., LANDESMAN L., BERGMANN B.A., CLASSEN J.J., HOWARD J.W., YAMAMOTO Y.T., *Nutrient removal from swine lagoon liquid by Lemna minor 8627*, Transactions of the ASAE., 2002, 45 (4), 1003.

[12] FARRELL J.B., *Duckweed uptake of phosphorus and five pharmaceuticals: microcosm and wastewater lagoon studies*, Thesis, All Graduate Thesis and Dissertation, Utah State University, 2012, Paper 1212, http://digitalcommons.usu.edu/etd/1212

[13] WENDEOU S.P.H., AINA M.P., CRAPPER M., ADJOVI E., MAMA D., *Influence of salinity on duckweed growth and duckweed based wastewater treatment system*, J. Water Resour. Protect., 2013, 5 (10), 993.

[14] FERDOUSHI Z., HAQUE F., KHAN S., HAQUE M., *The effects of two aquatic floating macrophytes (Lemna and Azolla) as biofilters of nitrogen and phosphate in fish ponds*, Turkish J. Fish. Aquatic Sci., 2008, 8 (2).

[15] XU J., SHEN G., *Growing duckweed in swine wastewater for nutrient recovery and biomass production*, Biores. Technol., 2011, 102 (2), 848.

[16] ROBINSON H., *The composition of leachates from very large landfills: an international review*, Commun. Waste Resour. Manage., 2007, 8 (1), 19.

[17] CEDERGREEN N., MADSEN T.V., *Nitrogen uptake by the floating macrophyte Lemna minor*, New Phytologist., 2002, 155 (2), 285.

[18] ORON G., DE-VEGT A., PORATH D., *Nitrogen removal and conversion by duckweed grown on wastewater*, Water Res., 1998, 22 (2), 179.

[19] MOHEDANO R.A., COSTA R.H., TAVARES F.A., BELLI FILHO P., *High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds*, Biores. Technol., 2012, 112, 98.

[20] MEN B.X., OGLE B., LINDBERG J.E., *Use of duckweed as a protein supplement for growing ducks*, Asian-Austral. J. Animal Sci., 2001, 14 (12), 1741.

[21] LENG R.A., STAMBOLIE J.H., BELL R., *Duckweed-a potential high-protein feed resource for domestic animals and fish*, Livest. Res. Rural Develop., 1995, 7 (1), 36.
[22] XU J., CHENG J.J., STOMP A.M., *Growing Spirodela polyrrhiza in swine wastewater for the production of animal feed and fuel ethanol. A pilot study*, Clean Soil, Air, Water, 2012, 40 (7), 760.
[23] OJOAWO S.O., AGBEDE O.A., SANGODOYIN A.Y., *Characterization of dumpsite leachate. Case study of Ogbomosoland South-Western Nigeria*, Open J. Civil Eng., 2012, 2, 33.
[24] ÖBEK E., HASAR H., *Role of duckweed (Lemna minor L.) harvesting in biological phosphate removal from secondary treatment effluents*, Fresen. Environ. Bull., 2002, 11 (1), 27.
[25] *Standard Methods for the Examination of Water and Wastewater*, 20th Ed., APHA, AWWA, WEF, American Public Health Association, Washington DC, USA, 1998.
[26] Methods for Chemical Analysis of Water and Waste, Environmental Protection Agency, Cincinnati, Ohio, USA, 1983.
[27] PERNIEL M., RUAN R., MARTINEZ B., *Nutrient removal from a storm water detention pond using duckweed*, Appl. Eng. Agric., 1998, 14 (6), 605.
[28] AL-NOZAILY F., ALAERTS G., VEENSTRA S., *Performance of duckweed-covered sewage lagoons. II. Nitrogen and phosphorus balance and plant productivity*, Water Res., 2000, 34 (10), 2734.

Effect of Nutrient Concentration and pH on Growth and Nutrient Removal Efficiency of Duckweed (Lemna Minor) From Natural Solid Waste Leachate

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***Abstract*— This study aims to investigate the effect of nutrient concentration and pH of leachate on growth and nutrient removal efficiency of duckweed (Lemna minor). A batch experiment was conducted using pH range of 4-10 and two initial leachate dilutions with nutrient concentrations of N (90 and 20 mg L^{-1}) and P (76 and 16 mg L^{-1}) and effect of pH and initial nitrogen (N) and phosphorous (P) contents of natural leachate was investigated on growth and nutrient removal efficiency of Lemna minor. Nutrient removal rates of duckweed increase with an increase in initial nutrient concentration of leachate at all pH levels. At both leachate dilutions, pH range of 6-8 with an optimum of 7.1 is good for nutrient removal efficiency of duckweed from leachate. The highest rates of nitrogen (1.22 g m^{-2} day^{-1}) and phosphorous (0.95 g m-2 day-1) removal were achieved from more concentrated leachate at pH 7.1. Growth rate of duckweed decreases with an increase in initial nutrient concentration of leachate at all pH levels. Maximum growth rate of duckweed (19.6 g m2 day-1) was achieved at pH 7.1 from less concentrated leachate. Nitrogen and phosphorous uptake in duckweed biomass was higher in less concentrated leachate at all pH values. Optimum pH for N and P uptake by Lemna minor is 7.1 at both leachate dilutions. At this pH duckweed showed 94 % and 91 % uptake of N and P respectively from less concentrated leachate.**

***Key Words:* Leachate, Duckweed, Lemna Minor, Nutrients, Ph, Removal Rate, Growth Rate.**

I. INTRODUCTION

Leachate is the high strength wastewater. At open waste dumping sites it is produced by percolation of rainwater through solid waste layers [1]. Various physic-chemical and bio-chemical processes in solid waste transfer variety of pollutants from waste streams into percolating rain water [2]. A typical leachate contains high amounts of recalcitrant substances, such as humic and fulvic acids, xenobiotics and pesticides, heavy metals and inorganic macro constituents (Ca, Mg, K, Na, NH4, Cl, HCO3 and SO4 etc.) [3,4]. Unattended leachate poses serious environmental problems such as contamination of surface and ground water, soil contamination and direct and indirect hazards for human health, flora and fauna and ecosystems [5,6].

Nutrients content of leachate may be as high as 13,000 mgL^{-1} of organic nitrogen [6], up to 400-3000 mgL^{-1} of ammonia-nitrogen and 3000 mg L^{-1} of phosphate [7, 8]. In Pakistan, concentrations of nitrogen and phosphorous in leachate is usually higher than national environmental standards [9]. By systematic planning and conditions optimization, the high nutrient content of leachate makes it the favored media for growth of aquatic plants. Aquatic plants convert nutrients in growth media into valuable plant biomass making full use of the postharvest biomass as animal feed, fertilizer and for production of protein rich byproducts [10].

Duckweed is amongst the promising aquatic plants having ability to absorb large amounts of nutrients from eutrophicated wastewater and has high growth rates [11,12,13]. Duckweed is a small floating macrophyte belonging to family *Lemnaceae* of monocotyledon plants. Duckweed has 37 species belonging to 4 genera (*Lemna, Spirodela, Wolffia and Wolffiella)* [14]. It is reported that duckweed based swine lagoon treatment system can remove up to 83.7% and 89.4% of total nitrogen (TN) and total phosphorus (TP) respectively from the media [15]. High level of nutrients tolerance in duckweed is of particular importance for treatment of high strength leachate. *Lemna minor* specie of duckweed has enormous potential of nutrients removal from wastewaters of high strengths [16]. Cheng et al [17] has reported a growth rate of *L. minor* close to 29 g m-2 day-1 and Total Kjeldahl Nitrogen (TKN) and Total Phosphorous (TP) uptake of 90 % and 88.6 % respectively from high strength swine lagoon.

pH is one of the important environmental factors affecting the uptake of chemicals and their distribution in living plants [18]. The pH of the growth medium determines the ratio between the $NH4^{+}$ (preferred form of nitrogen for duckweed) and un-dissociated NH3 [19]. Some researchers have investigated the effect of pH on duckweed growth using artificial growth media under artificial conditions of light intensity, temperature and humidity [20]. However, the use of natural leachate for duckweed growth under the natural climatic conditions is least explored area so far

This study investigates the effect of pH and initial concentrations of nitrogen and phosphorous on nutrient removal and growth of duckweed (*Lemna minor*) grown on natural leachate under the natural climatic conditions of Islamabad, Pakistan. Study provides an input to design the duckweed based leachate treatment system studying the nutrient removal and growth dynamics of duckweed on natural leachate under the natural climatic conditions.

II. MATERIALS AND METHODS

A. Leachate

Leachate used in this research was prepared by the processing of fresh solid waste collected from various residential, commercial and industrial areas of Islamabad and Rawalpindi, Pakistan. About 100 to 120 kg fresh solid waste from each site was collected from pre-determined lowest points at the depths of 0.5 m to 1.5 m [21]. Collected samples were mixed in a plastic tank having an internal diameter of about 1.5m and height of about 1.8 m. A sieve (pore size 1mm) was fixed at an internal height of 10 cm of the plastic tank. Thorough shaking was applied to well mix the waste and achieve a homogenized sample. The homogenized waste was showered with tap water and maintained for 60 days after which, the leachate was collected from bottom outlet. Solid waste was again mixed thoroughly and showered with water. Afterwards, the leachate was collected three times at an interval of 10 days each. Leachate collected from various runs was mixed to form single homogenized sample to be used for this research.

B. Duckweed

Duckweed (*L. minor)* was collected from wastewater pond in Islamabad, Pakistan. Prior to grow on leachate, duckweed was repeatedly washed with excess tap water to remove bacteria, algae and other unwanted compounds.

C. Experimental Setup

A batch experiment was conducted using two initial dilutions of leachate: i) leachate/water: 50/50 % (N: 90 mg L^{-1}, P: 76 mg L^{-1}, COD: 2,685 mg L^{-1}) and; ii) leachate/water: 10/90 % (N: 20 mg L^{-1}, P: 16 mg L^{-1}, COD: 510 mg L^{-1}). Seven initial pH levels of leachate (4, 5, 6, 7, 8, 9, and 10) were adjusted for each leachate dilution and duckweed growth and nutrient removal and absorption by duckweed were determined at each pH. About 50 mg initial fresh mass of duckweed was used. Duckweed was grown in plastic containers of 300 ml capacity. 250 ml of initial volume of leachate was used in each container at the depth of 9.5 cm. Surface area of each container was 25.8cm2.

For each leachate dilution, 28 containers were used. Out of these, 21 containers had duckweed cultures with each pH level from 4-10 in triplicates. Seven control containers at each pH were without duckweed. Duckweed containers were placed within an iron rack in open environment at Institute of Environmental Sciences and Engineering (IESE), National University of Sciences and Technology, Islamabad, Pakistan. Data related to ambient air temperature, solar radiations and day lengths was obtained from Pakistan Metrological Department.

An experiment lasted for 22 days, during which the nutrients concentration of leachates and duckweed and dry weight of duckweed at both leachate dilutions and each pH level was determined at the start and end of the experiment. The pH was measured every day and subsequently adjusted to the initial conditions with 1 M NaOH or HCL solutions.

The average pH during a particular day was assumed to be the average of pH measured just before the pH adjustment, and the pH that was set. The average pH for the total period was calculated by taking the average of the daily average pH values, for the triplicate containers. The pH range was defined as the range between the maximum and minimum daily average values. Average ambient air temperature, solar radiations and day lengths during the experiment was 37.1 ^{0}C, 4.3-4.9 KWh m^{-2} day^{-1} and 14 hours respectively.

D. Laboratory Analysis

Samples from the leachate were analyzed for TKN, NH4-N, TP and o-PO4-P. The dry weight of duckweed was measured after drying the plants at 70 0C until constant weight [22]. Dry biomass of duckweed was analyzed for TKN and TP contents. Chemical analyses were performed using the standard methods of American Public Health Association [23] and US-EPA [24].

E. Statistical Analysis

Measured nutrient removal rates, duckweed growth rates and nutrient uptake by duckweed were evaluated using analysis of variance. The level of statistical significance was set at $P<0.05$. Differences between groups with respect to pH values were assessed by one-way analysis of variance (ANOVA) and differences within groups were further analyzed by all pair wise comparison test at $\alpha=0.05$. All statistical analysis was performed using Statistix-8.1 software and MS excel.

III. RESULTS AND DISCUSSION

Leachate with seven initial pH levels (4, 5, 6, 7, 8, 9 and 10) was used as medium for duckweed growth. Initial concentrations of nutrients in each leachate dilution and in duckweed dry mass were determined (Table 1). Considerable fluctuation in pH was observed in each container despite daily pH adjustment. Table 2 shows the average pH and ranges observed during the experimental period.

At pH 4 (average 3.6) almost all duckweed biomass was dead in both leachate dilutions at the sixth day of experiment. At extremely low pH values electrochemical gradient across plasma membrane is decreased due to an increased influx of H+ ions which is toxic for the growth of floating aquatic plants [25]. At pH 5 (average 4.5), duckweed showed stunted growth and fronds started to become yellowish and wrinkled. Caicedo et al [19] reported that an acidic media with pH less than 5 has direct detrimental effects on physiology of duckweed.

A. Nutrient Removal

Nutrient removal rates in both leachate dilutions increase with increasing pH of the media until the maximum rate of removal at pH of 7.1; after which removal rates begin to decrease in alkaline range but still higher than acidic pH ranges of leachate (Table 3). Overall rate of nutrient removal in both leachate dilutions were higher at neutral to slightly basic pH in the range of 7.1 to 8.1. It is consistent with the duckweed growth rates and nutrient uptake by duckweed which is also higher at this pH range (Fig. 1 and 2). Overall rates of nutrient removal were higher in 50 % leachate dilution as compared to less concentrated (10 %) leachate at all pH levels. This is probably because of microbial activities which were higher in more concentrated leachate. Maximum rates of nutrients removal in 50 % leachate dilution was; TKN: 1.22 g $m^{-2}day^{-1}$, NH_4 –N: 0.79 g $m^{-2}day^{-1}$, TP: 0.95 g $m^{-2}day^{-1}$ and o-PO_4-P: 0.19 g $m^{-2}day^{-1}$. From 10 % leachate dilution, maximum TKN removal (0.27 g $m^{-2}day^{-1}$) was achieved at average pH 8.1. Whereas, the maximum removal of NH_4 –N (0.16 g $m^{-2}day^{-1}$), TP (0.21 g $m^{-2}day^{-1}$) and o-PO_4-P (0.06 g $m^{-2}day^{-1}$) from 10 % leachate was achieved at average pH of 7.1 (Table 3).

At pH range of 7.1-8.1, nitrification and de-nitrification seems to have significant contribution in nitrogen removal from more concentrated leachate media. Effect of nitrification and de-nitrification was less significant in 10 % leachate dilution, where, most of the nitrogen removed from leachate was absorbed by duckweed at same pH range of 7.1-8.1. It shows that nitrification and de-nitrification processes are more prominent in concentrated leachate media as compared to the less concentrated leachate.

NH_4-N is the preferred form of nitrogen uptake by duckweed hence maximum removal of NH_4-N was accounted for uptake into duckweed biomass which is increased with an increase in pH from acidic to more favorable neutral pH range for duckweed growth.

Decomposition of organically and inorganically bounded phosphorous increases with an increase in pH and is highest at neutral pH, resulting in highest removal rate of TP and uptake by duckweed at pH 7.1. In addition to uptake by duckweed biomass, some of the ortho-phosphate-P removal may also have occurred by microbial assimilation which increases with the increase in pH of the leachate media up to pH 7 which is more favorable pH for microbial growth and assimilation of ortho-phosphate [26].

Table I: Initial variable for experiments on nutrient removal from natural leachate by duckweed, *Lemna minor*, duckweed growth and nutrient absorption by duckweed under the natural climatic conditions in Islamabad, Pakistan

Initial pH	**Leachate Concentration**	**Nutrient concentration in leachate (mgL^{-1})**				**Nutrient concentration in duckweed ($mg\ g^{-1}$ DM)**		**Duckweed dry mass (mg)**
		TKN	NH_4-N	TP	o-PO_4-P	N	P	4.5
4, 5, 6, 7, 8, 9, 10	50 %	90	52	76	15	57.6	15	
	10 %	20	10	16	4			

Table II: Average pH values (mean ± SD, n=3) and ranges observed in the natural leachate media

Initial pH	**Average**	**Range**
4	3.6±0.1	2.9-4
5	4.5±0.2	3.3-5.1
6	5.5±0.3	4.8-6
7	6.0±0.1	5.4-7
8	7.1±0.2	5.9-8
9	8.1±0.2	6.9-9
10	8.6±0.1	8.4-10

Table III: Rate of nutrient removal from 50 % and 10 % natural leachate dilutions by duckweed, Lemna. minor (mean ± SD, n=3) at various average pH levels

Leachate dilution	**Average pH**	**Nutrient Removal Rates (g m^{-2} day^{-1})**			
		TKN	**NH4-N**	**TP**	**o-PO4-P**
50 % Leachate	4.5	0.41 ± 0.007e	0.26 ± 0.005f	0.30 ±0.007f	0.04 ± 0.002c
	5.5	0.71 ± 0.014d	0.50 ± 0.011e	0.42 ± 0.011e	0.15 ± 0.008b
	6.0	0.93 ± 0.072c	0.70 ± 0.005c	0.65 ± 0.001d	0.19 ± 0.024a
	7.1	1.22 ± 0.011a	0.79 ± 0.004a	0.95 ± 0.002a	0.19 ± 0.007a
	8.1	1.05 ± 0.007b	0.73 ± 0.028b	0.86 ± 0.024b	0.16 ± 0.009b
	8.6	0.93 ± 0.003c	0.68 ± 0.008d	0.79 ± 0.025c	0.15 ± 0.006b
	LSD value	**0.0548**	**0.0226**	**0.0241**	**0.0183**
10 % Leachate	4.5	0.03 ± 0.000e	0.02 ± 0.002d	0.01 ± 0.001e	0.01 ± 0.001d
	5.5	0.12 ± 0.001d	0.08 ± 0.001c	0.06 ± 0.001d	0.03 ± 0.003c
	6.0	0.24 ± 0.002b	0.13 ± 0.000b	0.14 ± 0.002b	0.05 ± 0.007b
	7.1	0.26 ± 0.006a	0.16 ± 0.006a	0.21 ± 0.004a	0.06 ± 0.001a
	8.1	0.27 ± 0.007a	0.15 ± 0.001a	0.14 ± 0.001b	0.05 ± 0.001b
	8.6	0.22 ± 0.001c	0.15 ± 0.001a	0.13 ± 0.003c	0.05 ± 0.002b
	LSD value	**0.0111**	**0.0073**	**0.0059**	**0.0059**

Note: Different letters in the same column indicate statistical significance at α = 0.05. TKN = Total kjeldahl nitrogen; NH4-N = Ammonium-nitrogen; TP = Total phosphorus; o-PO4-P = Orthophosphat

B. Duckweed growth

pH has significant effect on growth of duckweed at both initial concentrations of nutrients in natural leachate. Significant increase in duckweed growth rate was observed with an increase in pH up to 7.1 after which growth rate started decline. Overall, pH range of 7.1 to 8.1 was found good for duckweed growth at both leachate dilutions. Overall growth rates of *Lemna minor* were higher at less concentrated leachate at all pH levels (Fig. 1).

Overall growth rates of *Lemna minor* were higher at less concentrated leachate at all pH levels (Fig. 1). It indicates the high rate of microbial activity in more concentrated leachate which removed large amount of nutrients from leachate without duckweed uptake. Maximum growth rate of duckweed at pH 7.1 was 19.6 g m^2 day^{-1} at 10 % leachate and 13.7 g m^2 day^{-1} at 50 % leachate dilution. Many researchers have reported that most of the duckweed species including *Lemna minor* show better growth at pH 7 of the growth media which is also consistent with the findings of this study [19, 27]. At pH greater than 7 Inhibitory action of ammonia was more prominent resulting in decreased growth rate of duckweed.

C. Nitrogen and phosphorous uptake by duckweed

Figure 2 (a) and (b) show the percentage of total removed nitrogen and phosphorous absorbed by duckweed biomass at 50 % and 10 % leachate concentrations respectively. Overall nutrient uptake by duckweed is higher at less concentrated leachate at all pH levels. It agrees with duckweed growth rate which is higher at less concentrated leachate. It seems that more concentrated growth media may be more favorable for microbial growth than to duckweed growth [17]. Under the natural conditions, nitrogen assimilation by algae and microbes was also probably higher in more concentrated leachate resulting in less absorption of total nitrogen by duckweed.

At both leachate dilutions maximum amount of nitrogen was absorbed by duckweed at pH 7.1 (76 % of total removed from 50 % leachate and 94 % of total removed from 10 % leachate). At pH 7.1, relatively larger amount of NH_4–N is available for uptake by duckweed. Phosphorous uptake by duckweed followed the similar pattern as of nitrogen.

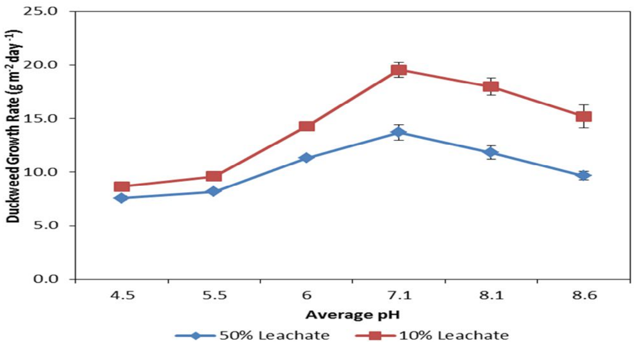
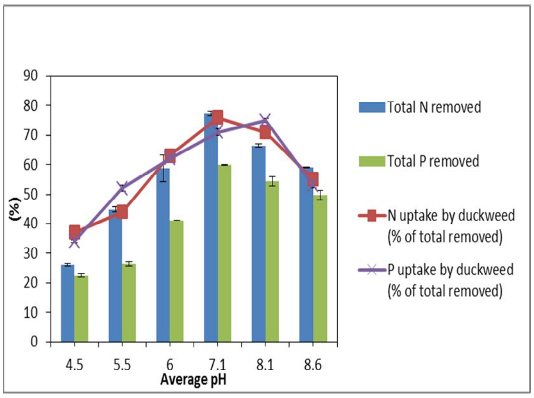


Fig. 1: Growth rates of *Lemna minor* (mean ± SD, n=3) at 50 % and 10 % natural leachate dilutions and six average pH values

Maximum P uptake at both leachate dilutions was observed at pH 7.1 (71 % at 50 % leachate and 91 % at 10 % leachate dilution) [Fig 2 (a) and (b)].

(a)



(b)

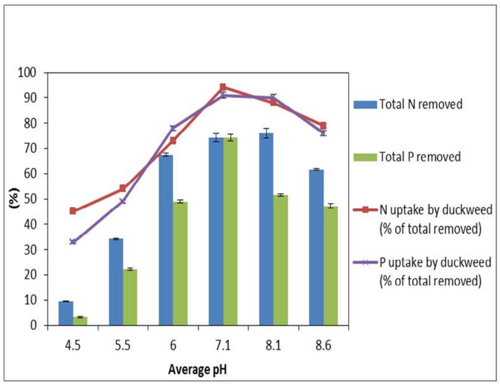


Fig. 2: Uptake of nitrogen and phosphorous (mean ± SD, n=3) by Lemna minor from (a) 50 % natural leachate an (b) 10 % leachate at various average pH levels

IV. CONCLUSIONS

Lemna minor can tolerate larger fluctuations in pH of the natural leachate under natural climatic conditions. Nutrients removal rates from natural leachate increase with increasing the initial concentrations of nutrient in leachate media at all pH levels. A pH range of 7.1-8.1 was found good for nutrient removal from natural leachate at both leachate dilutions. From 50 % leachate the highest rates of nitrogen (1.22 g m^{-2} day^{-1}) and phosphorous (0.95 g m^{-2} day^{-1}) removal was achieved at pH 7.1 while from 10 % leachate the highest rate of nitrogen removal (0.27 g m^{-2} day^{-1}) was achieved at pH 8.1 and highest rate of phosphorous removal (0.21 g m^{-2} day^{-1}) was achieved at pH 7.1.

Duckweed showed better growth at less concentrated natural leachate at all initial pH levels of leachate. A pH range of 6 to 8 is better for duckweed growth with an optimum of 7.1. Maximum growth rate of duckweed was 19.6 g m^2 day^{-1} and 13.7 g m^2 day^{-1}at 10 % and 50 % leachate dilutions respectively. Nitrogen and phosphorous uptake by duckweed was higher in less concentrated leachate at all pH values. Optimum pH for N and P uptake by duckweed is 7.1 at both leachate dilutions. At this pH duckweed uptake 76 % and 71 % of total removed N and P respectively from 50 % natural leachate dilution and 94 % and 91 % respectively from 10 % leachate dilution. On the whole, less concentrated leachate and a neutral to slightly basic pH is more favorable for duckweed growth and its efficiency to uptake nutrients from natural leachate under the natural climatic conditions.

ACKNOWLEDGMENT

Authors are grateful to Higher Education Commission of Pakistan for financial support to accomplish this research.

REFERENCES

[1] G. Kalcíková, M. Vávrová J., Zagorc-Koncan, A. Zgajnar and A. Gotvajn, "Seasonal variations in municipal landfill leachate quality", Management of Environmental Quality, vol. 22(5), pp. 612, 2011.

[2] P. Kjeldsen, M.A. Barlaz, A.P. Rooker, A. Baun, A. Ledin and T.H. Christensen, "Present and long-term composition of MSW landfill leachate: a review", Critical reviews in environmental science and technology, vol. 32(4), pp. 297. 2002.

[3] T.H. Christensen, P. Kjeldsen, P.L. Bjerg, D.L. Jensen, J.B. Christensen, A. Baun and G. Heron, "Biogeochemistry of landfill leachate plumes," Applied geochemistry, vol. 16(7), pp. 659-718, 2001.

[4] C.B. Öman, and C. Junestedt, "Chemical characterization of landfill leachates–400 parameters and compounds," Waste management, vol. 28(10), pp. 1876-1891, 2008.

[5] O. Ohwoghere–Asuma and K.E. Aweto, "Leachate characterization and assessment of groundwater and surface water qualities near Municipal solid waste dump site in Effurun, delta State, Nigeria," Journal of Environment and Earth Science, vol. 3(9), pp. 126-134, 2013.

[6] C.O. Akinbile, M.S. Yusoff and A.A. Zuki, "Landfill leachate treatment using sub surface flow constructed

wetland by Cyperus haspan," Waste management, vol. 32(7), pp. 1387-1393, 2012.
[7] [7] H. Robinson, "The composition of leachates from very large landfills: an international review," Communications in waste and resource management, vo. 8(1), pp. 19-32, 2007.
[8] S.Q. Aziz, H.A. Aziz, M.S. Yusoff and M.J. Bashir," Landfill leachate treatment using powdered activated carbon augmented sequencing batch reactor (SBR) process: Optimization by response surface methodology," Journal of Hazardous Materials, vol. 189(1), pp. 404-413, 2011.
[9] H. Iqbal, M. Anwar Baig, M. Usman Hanif, S. Usman Ali and M. Flury, "Leaching of Metals, Organic Carbon and Nutrients from Municipal Waste under Semi-Arid Conditions," International Journal of Environmental Research, vol. 9(1), pp. 187-196, 2015.
[10] M. Ahmed, C.K. Hasan, H. Rahman, M.A. Hossain and S.A. Uddin, "Prospects of Using Wastewater as a Resource-Nutrient Recovery and Energy Generation," American Journal of Environmental Sciences, vol. 11(2), pp. 99, 2015.
[11] Z. Zhao, H. Shi, Y. Liu, Y, H. Zhao, H. Su, M. Wang and Y. Zhao, "The influence of duckweed species diversity on biomass productivity and nutrient removal efficiency in swine wastewater.," Bioresource technology, vol. 167, pp. 383-389, 2014.
[12] X. Ge, N. Zhang, G.C. Phillips and J. Xu, "Growing Lemna minor in agricultural wastewater and converting the duckweed biomass to ethanol," Bioresource technology, vol. 124, pp. 485-488, 2012.
[13] S. Soda, Y. Kawahata, Y. Takai, D. Mishima, M. Fujita and M. Ike, " Kinetics of nutrient removal and biomass production by duckweed Wolffia arrhiza in continuous-flow mesocosms," Ecological Engineering, vol. 57, pp. 210-215, 2013.
[14] J.J. Cheng and A.M. Stomp, "Growing duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed," Clean–Soil, Air, Water, vol. 37(1), pp. 17-26, 2009.
[15] J. Xu and G. Shen, "Growing duckweed in swine wastewater for nutrient recovery and biomass production," Bioresource technology, vol. 102(2), pp. 848-853, 2011.
[16] S.P.H. Wendeou, M.P. Aina, M. Crapper, E. Adjovi, E and D. Mama, "Influence of Salinity on Duckweed Growth and Duckweed Based Wastewater Treatment System," Journal of Water Resource and Protection, vol. 5(10), pp. 993, 2013.
[17] J. Cheng, L. Landesman, B.A. Bergmann, J.J. Classen, J.W. Howard and Y.T. Yamamoto, "Nutrient removal from swine lagoon liquid by Lemna minor 8627," Transactions of the ASAE, vol. 45(4), pp. 1003-1010, 2002.
[18] [18] F. Duman, O. Obali and D. Demirezen, D, "Seasonal changes of metal accumulation and distribution in shining pondweed (Potamogeton lucens)," Chemosphere, vol. 65(11), pp.2145-2151, 2006.
[19] J.R. Caicedo, N.P. Van der Steen, O. Arce and H.J. Gijzen, "Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (Spirodela polyrrhiza)," Water research, vol. 34(15), pp. 3829-3835, 2000.
[20] S. Körner, S.K. Das, S. Veenstra and J.E. Vermaat, "The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to Lemna gibba," Aquatic botany, vol. 71(1), pp.71-78, 2001.
[21] S.O. Ojoawo, O.A. Agbede and A.Y. Sangodoyin,"Characterization of Dumpsite Leachate: Case Study of Ogbomosoland, South-Western Nigeria," Open Journal of Civil Engineering, vol. 2(01), pp. 33, 2012.
[22] J.E. Vermaat and K.M. Hanif, "Performance of common duckweed species (Lemnaceae) and the water fern Azolla on different types of waste water," Water Research, vol. 32(9), pp. 2576, 1998.
[23] APHA, "Standard Methods for the Examination of Water and Wastewater, 21st Edition," American Water Works Association/Environment Federation, Washington, 2005.
[24] EPA, "Methods for Chemical Analysis of Water and Waste," Environmental Protection Agency, Cincinnati, Ohio, USA.1983.
[25] M.M. Mufarrege, G.A. Di Luca, H.R. Hadad, and M.A. Maine, "Adaptability of Typha domingensis to high pH andsalinity," Ecotoxicology, vol. 20 (2), pp. 457-465, 2011.
[26] F. Al-Nozaily, G. Alaerts and S. Veenstra, " Performance of duckweed-covered sewage lagoons—II. Nitrogen and phosphorus balance and plant productivity," Water Research, vol. 34(10), pp. 2734-2741, 2000.
[27] E. Landolt and R. Kandeler, "Biosystematic investigations in the family of duckweeds (Lemnaceae), Vol. 4: The family of Lemnaceae-a monographic study, Vol. 2 1987.

International Journal of Science, Environment and Technology, Vol. 6, No 3, 2017, 1989 – 1999

ISSN 2278-3687 (O)
2277-663X (P)

EFFECT OF ELECTRICAL CONDUCTIVITY (EC) ON GROWTH PERFORMANCE OF DUCKWEED AT DUMPSITE LEACHATE

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Abstract: Two batch experiments were conducted during the months of June-July (summer) and September-October (fall) to determine the effect of Electrical Conductivity (EC) on nutrient and COD removal & uptake efficiency and growth of duckweed (*Lemna minor*) at leachate with various EC levels (500-3,000 µS cm^{-1}) under the natural climatic conditions of Islamabad, Pakistan. Maximum removal rates (mg m^{-2} day^{-1}) of TKN (175.6±0.7), NH4-N (124±0.4), TP (92.4± 0.1), o-PO_4^{3-}-P (31.8 ± 1.2) and COD (3,660±9.1) were observed at 1,000 µS cm^{-1} EC of leachate during summer while; during the fall season, these removal rates were 182.9 ± 0.7, 117.3 ± 0.3, 91.1 ± 0.04, 76.9 ± 1.8 and 3,295 ± 6.1 mg m^{-2} day^{-1} respectively. The maximum growth rate of duckweed was 5.70±0.2 g m^{-2} day^{-1} during summer and 3.21±0.08 g m^{-2} day^{-1} during fall at 1,000 µS cm^{-1} EC of leachate. Out of the total removed, about 79.77% & 68.83% of N and 75.75% & 53.02% of P was absorbed by duckweed during summer and fall seasons respectively at 1,000 µS cm-1 EC. Seasonal variations during June to September have less significant effect on duckweed growth and its nutrient & COD removal efficiency from leachate. Results of the study show that duckweed can be used as potential aquatic macrophyte for duckweed based leachate treatment under natural climatic conditions.
Keywords: Leachate, Duckweed, Lemna minor, Nutrient, Electrical Conductivity.

INTRODUCTION

Leachate is the high strength wastewater. At open waste dumping sites it is produced by biochemical reactions within the waste stream, due to interstitial water content of the waste mass and percolation of rainwater through solid waste layers [1, 2]. Various physico-chemical and bio-chemical processes in solid waste transfer variety of pollutants from waste streams into percolating rain water [3]. A typical leachate contains four major groups of pollutants; dissolved organic matter, xenobiotic organic compounds, heavy metals and inorganic macro compounds [4].

Quantity and composition of leachate depends on quantity and composition of waste material, age of landfill site, availability of moisture and oxygen and hydrology of waste landfill site

*Received May 17, 2017 * Published June 2, 2017 * www.ijset.net*

[5]. Nutrients content of a typical leachate may be as high as 13,000 mgL-1 of organic nitrogen up to 400-3000 mgL-1 of ammonia-nitrogen and 3000 mg L-1 of phosphate [6, 7].

Unattended leachate from municipal waste landfills/dumpsites may pose serious impacts on human health and surrounding eco systems including surface & ground water and soil [8]. Recently, it has become major concern worldwide to impose more stringent environmental requirements related to leachate management and treatment [2].

Currently many physical, chemical, biological and combination of two or more leachate treatment methods are used in the world [9]. Phytoremediation is the use of green plants to remove, detoxify or immobilize the pollutants in environmental media (soil, water or sediments) [10].

Use of aquatic plants such as duckweed, water hyacinth, water lettuce in wastewater treatment has received greater attraction during few years back [11-13]. Production of aquatic plants on wastewater has two fold benefits: treatment of wastewater and, as an alternate technology, converting wastewater nutrients into potentially useful forms [14].

Duckweed is a small floating macrophyte belonging to family Lemnaceae of monocotyledonous plants. Duckweed has 37 species belonging to 4 genera: i) Lemna, ii) Spirodela, iii) Wolffia and, iv) Wolffiella [15]. Composition of duckweed is highly variable and depends on composition of water on which duckweed is grown. Protein content constitutes the major part of duckweed biomass of most of duckweed species [16]. Similar to other photosynthetic terrestrial and aquatic plants, nitrogen, phosphorus and potassium (NPK) are the main nutrient required for duckweed growth [17].

Duckweed is amongst the promising aquatic plants having ability to absorb large amounts of nutrients and trace metals from eutrophicated wastewater and has high growth rates. Wastewater treatment by duckweed is owed to its high nutrients and minerals accumulation capacities into biomass and high growth rates under diverse environmental conditions [18, 19].

Cheng et al. reported that duckweed can grow well at wastewaters with high nitrogen and phosphorus levels (240 mg NH4-N/L and 31.0 mg PO_4-P/L). The highest nutrient uptake rate achieved was 0.995 mg N/L-h, and 0.129 mg P/L-h, and duckweed growth rate was 1.33 g dry biomass/m2-h [14]. Bergmann et al., 2000a concluded that *Lemna gibba* and *Lemna minor* species of duckweed are best for the treatment of high strength swine effluent with high biomass production and nutrient removal rates [20].

Duckweed grows well between temperature ranges of 6 to 33° C and can survive below freezing temperatures for many days. Optimum range of pH for duckweed growth is 6.5-7.5 however; it can grow well in pH range of 5-9. According to the management requirements, water depth of 2 meters should be maintained while using duckweed for water treatment purpose [14]. Electrical conductivity has significant effect on biomass production and growth of duckweed by effecting various biochemical and physiological processes in the plant. A study reports that growth rates and water purification efficiency of aquatic plants has inverse relationship with the dissolved salts in growth media [21].

This study has been designed to investigate the effect of Electrical Conductivity on growth and nutrient & COD removal efficiency of duckweed while grown on leachate under natural climatic conditions. So far very small amount of work have been conducted on EC and its effects on duckweed. Results of the study may be useful in designing the duckweed based leachate treatment system under natural conditions.

MATERIALS AND METHODS

Leachate used in this research was prepared by the processing of decomposed solid waste collected from various residential, commercial and industrial areas of Islamabad and Rawalpindi, Pakistan. About 100 to 120 kg well decomposed solid waste was collected from each residential, commercial and industrial dump sites. Waste was collected from pre-determined lowest points at each dump site at soil depths of 0.5 m to 1.5 m [22]. Collected samples were mixed in large plastic water storage tank having an internal diameter of about 1.5m and height of about 1.8 m. A sieve (pore size 1mm) was fixed at an internal height of 10 cm of the plastic container. Through shaking was applied to well mix the waste and achieve a homogenized sample. The homogenized waste was soaked with leaching solution (distilled water) and maintained for 30 days after which, the leachate was collected from bottom outlet in container.

Remaining solid waste was again mixed thoroughly and soaked with distilled water. Afterwards, the leachate was collected three times at an interval of ten days each. Leachate collected from various runs was mixed to form single homogenized sample to be used for this research.

Duckweed (*L. minor*) used in this research was collected from wastewater pond in Islamabad. Prior to grow on leachate, duckweed was repeatedly washed with excess water to remove bacteria, algae and other unwanted compounds [23].

Experimental Setup

Using 30% leachate, six (06) solutions were prepared with EC levels of 500, 1000, 1500, 2000, 2500 and 3000 µS/cm each. Desired EC values in leachate were achieved by adding common salt, NaCl to original leachate to increase EC level and diluting the original leachate with distilled water to decrease the EC.

Two batch experiments were conducted during summer (June-July) and fall (September-October) seasons. At each EC level, duckweed was grown in transparent plastic containers of 300 ml capacity. 250 ml of initial volume of leachate was used in each container at leachate depth of 9.5 cm. Surface area of each container was $25.8 cm^2$. For each test during summer and fall seasons, 24 containers were used. Out of these, 18 containers had duckweed cultures with each EC level from 500-3,000 µS/cm in triplicates. Six control containers having leachate without duckweed were also placed at corresponding EC levels. Values of controls were subtracted from experimental values.

Duckweed containers were placed within a porous iron rack having three compartments to place the duckweed containers. Rack with duckweed containers was placed in open environment at Institute of Environmental Sciences and Engineering (IESE), National University of Science and Technology, Islamabad under natural climatic conditions. Each experiment was lasted for 25 days.

Seasonal data related to ambient air temperature and day lengths during both experiments was retrieved from website of Pakistan Metrological Department whereas; the solar radiation data during the experimental period was obtained from the web site of LEO Corporation, Pakistan.

Average ambient air temperature, solar radiations and day lengths during summer season was 38.3 ^{0}C, 3.8-4.9 kWh m^{-2} day^{-1} and 13.40 hours, respectively and during fall season it was 26-30 ^{0}C, 4.2- 4.5 kWh m^{-2} day^{-1} and 12.10 hours, respectively.

During both tests, samples from the leachate and control containers were analyzed for TKN, NH4+-N, TP, o-PO4-3-P and COD at the start and end of experimental period. The dry weight of duckweed was measured after drying the plants at 70 0C until constant weight. Dry biomass of duckweed was analyzed for TKN and TP contents before and after the experiment. Statistical analyses during both experiments were performed using Statistix-8.1 software and MS excel.

RESULTS AND DISCUSSIONS

Duckweed was grown at six different EC levels (500, 1000, 1500, 2000, 2500 and 3000 µS cm-1) of leachate. The initial nutrients concentration (TKN, NH_4-N, TP and o-PO_4^{3-}-P) and COD of leachate is presented in Table 1.

Table 1. Initial nutrients concentrations and COD of leachate (mean± SD) used as medium for growth of *L. minor*

Experiment Season	**Nutrients concentration ($mg\ dm^{-3}$)**				**COD ($mg\ dm^{-3}$)**
	TKN	**NH_4-N**	**TP**	**o-PO_4^{3-}-P**	
Summer	49.76±0.24	32.55±0.24	76.00±0.17	15.00±0.12	1693±3.53
Fall	56.05±0.30	29.04±0.16	43.17±0.24	16.97±0.17	1624±2.57

TKN = Total kjeldahl nitrogen, NH_4-N = Ammonium nitrogen, TP = Total phosphorus, o-PO_4^{3-}-P = Orthophosphate, COD = Chemical oxygen demand

Duckweed efficiently removed the nutrients from leachate and showed healthy growth during both the summer and fall seasons. During both seasons, after 25 days of retention time of duckweed on leachate, maximum removal of nutrients and COD and duckweed growth was observed at 1,000 µS cm^{-1} EC of the leachate (Table 2.).

Table 2 shows that with an increase or decrease in EC from 1,000 µS cm^{-1}, growth rate and nutrient & COD removal efficiency is decreased. Table also shows that reduction in growth rates and removal efficiency of duckweed is more significant at higher EC levels (>1,500 µS cm^{-1}).

Maximum removal rates (mg m^{-2} day^{-1}) of TKN (175.6±0.7), NH4-N (124±0.4), TP (92.4± 0.1), o-PO_4^{3-}-P (31. 8 ± 1.2) and COD (3,660±9.1) were observed with 1,000 µS cm^{-1} EC of leachate during summer while; during the fall season, the removal rates were 182.9 ± 0.7, 117.3 ± 0.3, 91.1 ± 0.04, 76.9 ± 1.8 and 3,295 ± 6.1 mg m^{-2} day-1 for TKN, NH_4-N, TP, o-PO_4^{3-}-P and COD respectively.

Overall growth rates of duckweed were higher during the summer as compared to fall seasons. The maximum growth rate of duckweed was 5.70±0.2 g m^{-2} day^{-1} during summer and 3.21±0.08 g m^{-2} day^{-1} during fall at 1,000 µS cm^{-1} EC of leachate (Table 2.).

It is evident from table 2 that seasonal variations from June to September have little effect on nutrient removal rates of duckweed except for the ortho-phosphate which is significantly increased during fall seasons (31. 8 ± 1.2 to 76.9 ± 1.8 mg m^{-2} day^{-1}) however; the COD removal was greater during summer (3,660±9.1 mg m^{-2} day-1) as compared to fall (3,295 ± 6.1 mg m^{-2} day^{-1}).

Table 2. Nutrients and COD removal and growth rates (mean ± SD) of duckweed (*L. minor*) at various EC levels of leachate

Experiment	EC µS/cm	Nutrients removal rate ($mg\ m^{-2}\ day^{-1}$)				COD ($mg\ m^{-2}\ day^{-1}$)[a]	Growth rate ($g\ m^{-2}\ day^{-1}$)
		TKN	NH_4-N	TP	o-PO_4^{3-}-P		
Summer Experiment	500	166.43±1.07	118.80±0.37	89.76±0.21	30.91±0.42	3459±7.21	3.63±0.11
	1000	175.64±0.72	124.01±0.39	92.35±0.07	31.83±1.25	3660±9.07	5.70±0.16
	1500	168.45±0.34	122.22±0.51	90.32±1.18	31.17±0.88	3459±9.37	4.63±0.13
	2000	164.22±1.09	118.40±0.31	86.44±0.24	30.29±0.30	3417±8.89	3.53±0.11
	2500	154.77±1.02	115.61±0.51	86.09±0.41	29.18±1.92	3383±7.37	3.47±0.10
	3000	152.71±0.55	112.61±0.47	84.27±0.45	28.49±0.83	3382±7.37	3.22±0.12
Fall Experiment	500	179.16±1.13	114.54±0.35	84.71±0.28	63.19±0.16	3195±5.86	3.07±0.08
	1000	182.89±0.75	117.31±0.32	91.12±0.04	76.86±1.77	3295±6.08	3.21±0.08
	1500	180.99±0.36	114.83±0.50	88.47±1.15	75.52±0.24	3230±5.77	3.17±0.18
	2000	176.46±1.18	11.63±0.30	84.21±0.23	72.35±0.94	3147±4.62	2.94±0.10
	2500	172.86±0.62	109.90±0.45	82.68±0.47	56.84±0.43	3139±4.73	2.90±0.07
	3000	164.24±1.06	107.54±0.55	76.30±0.38	52.59±0.60	3103±5.57	2.87±0.12

At Electrical Conductivity of 1,000 µS $cm^{-}1$, maximum removal percentages of TKN, NH_4-N, TP, o-$Po^{3-}{}_4$-P and COD was 86.9 %, 92.8%, 59.3%, 96.51 % and 64.83 % during summer whereas; the removal percentages of these nutrients and COD during fall season were: 84.83%, 86.42%, 55.89%, 82.59 % and 60.86 % respectively (Fig.1& 2). It is clear from the figures 1 & 2 that removal percentage of nutrients and COD was less affected by seasonal variations from June to September.

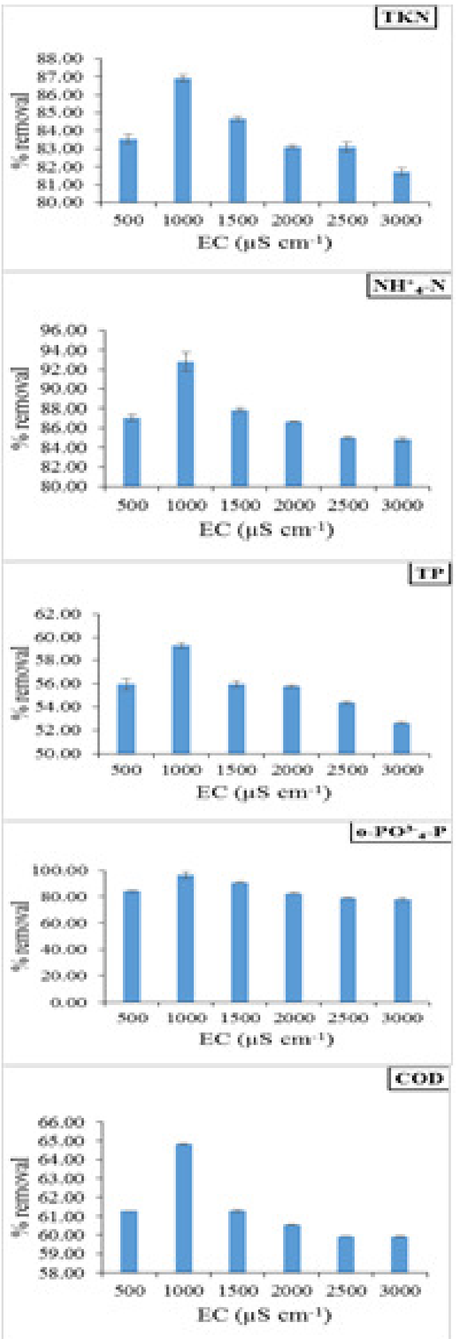

Figure 1 Percent removal of total kjeldahl nitrogen (TKN), ammonium-N (NH_4-N), total phosphorus, ortho-phosphate-P (o-PO_4^{3-}-P) and chemical oxygen demand (COD) at various EC levels of leachate by growing *Lemna minor* during summer

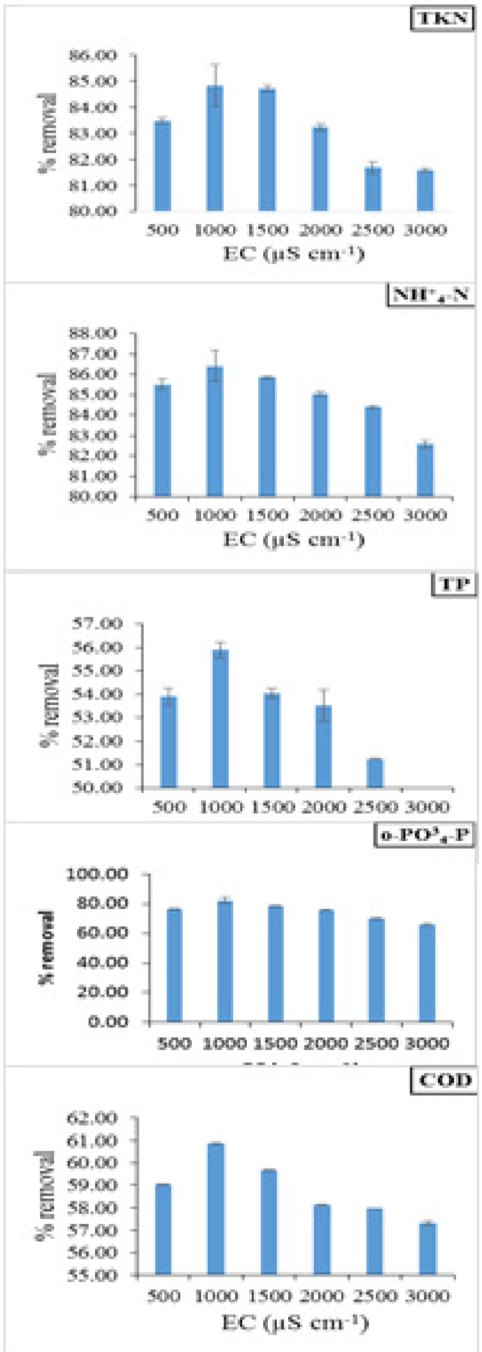

Figure 2 Percent removal of total kjeldahl nitrogen (TKN), ammonium-N (NH_4-N), total phosphorus, ortho-phosphate-P (o-PO_4^{3-}-P) and chemical oxygen demand (COD) at various EC levels of leachate by growing *Lemna minor* during fall

Table 3 shows the mass balance of nitrogen and phosphorous removed from the leachate and uptake by the duckweed into its biomass. Table shows that duckweed effectively absorbed the nitrogen and phosphorous into its biomass at all EC levels however the maximum absorption was observed at EC of 1,000 µS cm^{-1}. Out of the total removed, about 79.77% & 68.83% and 75.75% & 53.02% N and P respectively was absorbed by duckweed during summer and fall seasons at 1000 µS cm-1 EC of the leachate.

Table 3. Mass balance of total N and P removal and uptake (mean ± SD) by Lemna minor at various EC levels of leachate during summer and fall seasons.

Experiment	EC (µS cm^{-1})	Total nutrients removed from media				Nutrients uptake by duckweed			
		mg/L		%		mg/L		% of total removed	
		N	P	N	P	N	P	N	P
Summer Experiment	500	41.57±0.1	22.79±0.24	83.53±0.26	55.97±0.47	31.17±0.07	14.75±0.50	74.98±0.05	64.71±1.6
	1000	43.24±0.2	24.13±0.01	86.89±0.18	59.27±0.25	34.49±0.52	16.61±0.42	79.77±1.05	68.83±1.8
	1500	42.12±0.2	22.80±0.05	84.66±0.13	55.99±0.27	32.47±1.09	14.83±0.22	77.08±2.39	65.08±1.1
	2000	41.35±0.2	22.72±0.07	83.10±0.08	55.79±0.10	30.68±0.16	14.59±0.05	74.19±0.06	64.21±0.1
	2500	41.34±0.1	22.14±0.10	83.09±0.24	54.38±0.11	30.52±0.89	14.09±0.35	73.83±2.32	63.63±1.3
	3000	40.66±0.1	21.42±0.07	81.72±0.22	52.62±0.07	28.57±0.56	13.02±0.46	70.25±1.44	60.76±2.0
Fall Experiment	500	46.79±0.2	23.27±0.04	83.49±0.11	53.90±0.38	33.65±0.54	11.50±0.30	71.92±1.44	49.40±1.4
	1000	47.54±0.2	24.13±0.01	84.83±0.80	55.89±0.33	36.02±1.81	12.79±0.60	75.75±3.50	53.02±2.5
	1500	47.48±0.2	23.33±0.04	84.72±0.12	54.05±0.22	34.53±0.72	11.55±0.61	72.74±1.80	49.50±2.6
	2000	46.65±0.3	23.10±0.19	83.24±0.14	53.51±0.68	33.54±0.95	11.20±0.70	71.90±2.38	48.50±3.2
	2500	45.79±0.1	22.13±0.11	81.70±0.23	51.26±0.04	32.67±1.49	10.51±0.87	71.35±3.34	47.52±4.2
	3000	45.74±0.4	21.51±0.09	81.60±0.06	49.84±0.12	31.89±1.73	10.10±0.34	69.73±4.02	46.96±1.4

Little scientific information is available related to the effects of salinity on duckweed growth and its nutrient and COD removal ability. However, some studies reveal that duckweed is the salt sensitive plants and EC of the medium directly and indirectly effects the growth and physiology of duckweed [24].

High salinity imposes osmotic stress which may cause damage to duckweed cells by the production of inducing reactive oxygen species or by disrupting detoxification mechanisms in the plant [25]. Specific toxic effects of certain pollutants in leachate may also significantly affect the growth and nutrient removal efficiency of duckweed. A study found that duckweed can tolerate wide range of salinity an EC ranging from 1,000-1200 µS cm-^{1} is optimum for duckweed growth when at saline wastewaters [26].

Conclusions

Results of this study show that during both seasons duckweed (*Lemna minor*) performed well at an EC range of leachate from 500-1,500 µS cm^{-1} under natural climatic conditions whereas; the maximum growth and nutrient and COD removal efficiency of duckweed was observed at EC 1,000 µS cm^{-1}.

At this EC (1,000 µS cm^{-1}), duckweed showed the maximum removal rates (mg m^{-2} day^{-1}) of TKN (175.6±0.7), NH4-N (124±0.4), TP (92.4± 0.1), o-PO_4^{3-}-P (31. 8 ± 1.2) and COD (3,660±9.1) during summer while; during the fall season, the removal rates were 182.9 ± 0.7, 117.3 ± 0.3, 91.1 ± 0.04, 76.9 ± 1.8 and 3,295 ± 6.1 mg m^{-2} day-1 for TKN, NH_4-N, TP, o-PO_4^{3-}-P and COD respectively.

At these rates, duckweed removed about 86.9 %, 92.8%, 59.3%, 96.51 % and 64.83 % of TKN, NH_4-N, TP, o-$Po^{3-}{}_4$-P and COD respectively during the summer and 84.83%, 86.42%, 55.89%, 82.59 % and 60.86 % respectively during the fall seasons.

Out of the total removed N and P, about 79.77% & 68.83% and 75.75% & 53.02% respectively was taken up by duckweed during summer and fall seasons at the EC of 1,000 µS cm^{-1}.

The maximum growth rate of duckweed (5.70±0.2 g m^{-2} day^{-1}) was observed during summer and 3.21±0.08 g m^{-2} day^{-1} during fall at 1,000 µS cm^{-1} EC of leachate.

Seasonal variations from June to September have little effect on nutrient and COD removal rates and growth of duckweed on leachate.

Overall, the results of study show that *Lemna minor* growth and its nutrients removal efficiency is greatly influenced by presence of salts in growth media and an EC of 1,000 µS cm^{-1} can be considered as optimum for duckweed based leachate treatment system under the natural climatic conditions of Islamabad, Pakistan.

Acknowledgments

Authors are grateful to Higher Education Commission of Pakistan for financial support to accomplish this research.

References

[1] Tsarpali, V., & Dailianis, S., Investigation of landfill leachate toxic potency: An integrated approach with the use of stress indices in tissues of mussels, *Aquatic toxicology*, 124, 58-65, **2012**

[2] Renou, S., Givaudan, J.G., Poulain, S., Dirassouyan, F., Moulin, P., Landfill leachate treatment: review and opportunity, *Journal of hazardous materials*, 150, 468-493, **2008**

[3] Kjeldsen, P., Barlaz, M.A., Rooker, A.P., Baun, A., Ledin, A., Christensen, T.H., Present and long-term composition of MSW landfill leachate: a review, *Critical reviews in environmental science and technology*, 32, 297-336, **2002**

[4] Christensen, T.H., Kjeldsen, P., Albrechtsen, H.J.R., Heron, G., Nielsen, P.H., Bjerg, P. L., and Holm, P. E., Attenuation of landfill leachate pollutants in aquifers, *Critical Reviews in Environmental Science and Technology*, 24, 119-202, **1994**

[5] Aziz, H.A., Yusoff, M.S., Adlan, M.N., Adnan, N.H., and Alias, S., Physico-chemical removal of iron from semi-aerobic landfill leachate by limestone filter, *Waste management,* 24, 353-358, **2004**

[6] Akinbile, C.O., Yusoff, M. S., Zuki, A.A., Landfill leachate treatment using sub-surface flow constructed wetland by Cyperus haspan, *Waste management*, 32, 1387-1393, **2012**

[7] Robinson H., The composition of leachates from very large landfills: an international review, *Communications in waste and resource management*, 8, 1, 19, **2007**

[8] Salem, Z., Hamouri, K., Djemaa, R., Allia, K., Evaluation of landfill leachate pollution and treatment, *Desalination*, 220, 108-114, **2008**

[9] Chemlal, R., Azzouz, L., Kernani, R., Abdi, N., Lounici, H., Grib, H., Drouiche, N., Combination of advanced oxidation and biological processes for the landfill leachate treatment, *Ecological Engineering*, 73, 281-289, **2014**

[10] Vidali, M., Bioremediation. an overview, *Pure and Applied Chemistry*, 73, 1163-1172, **2001**

[11] Caicedo, J.R., Van der Steen, N.P., Arce, O., Gijzen, H.J., Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (Spirodela polyrrhiza), *Water Research*, 34, 3829-3835, **2000**

[12] Lasfar, S., Monette, F., Millette, L., and Azzouz, A., Intrinsic growth rate: a new approach to evaluate the effects of temperature, photoperiod and phosphorus–nitrogen concentrations on duckweed growth under controlled eutrophication, *Water research*, 41, 2333-2340, **2007**

[13] Landesman, L., Parker, N.C., Fedler, C.B., Konikoff, M., Modeling duckweed growth in wastewater treatment systems, *Livestock Research for Rural Development,* 17, 1-8, **2005**

[14] Cheng, J., Landesman, L., Bergmann, B.A., Classen, J.J., Howard, J.W., Yamamoto, Y. T., Nutrient removal from swine lagoon liquid by Lemna minor 8627, Transactions of the ASAE, 45, 1003-1010, **2002**

[15] Cheng, J.J., and Stomp, A.M., Growing duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed, *Clean–Soil, Air*, *Water*, 37, 17-26, **2009**

[16] Tavares, F.D.A., Rodrigues, J.B.R., Fracalossi, D.M., Esquivel, J., Roubach, R., Dried duckweed and commercial feed promote adequate growth performance of tilapia fingerlings, *Biotemas*, 21, 3, 91-97, **2008**

[17] Ansal, M.D., Dhawan, A., Efficacy of Duckweed-Spirodela for Low Cost Carp Feed Formulation, Indian Journal of Animal Nutrition, 26, 4, 378-383, **2009**

[18] Ge, X., Zhang, N., Phillips, G.C., and Xu, J., Growing Lemna minor in agricultural wastewater and converting the duckweed biomass to ethanol, *Bioresource Technology*, 124, 485-488, 2012

[19] Zhao, Z., Shi, H., Liu, Y., Zhao, H., Su, H., Wang, M., Zhao, Y., The influence of duckweed species diversity on biomass productivity and nutrient removal efficiency in swine wastewater, *Bioresource technology*, 167, 383-389, 2014

[20] Bergmann, B.A., Cheng, J., Classen, J., Stomp, A.M., Nutrient removal from swine lagoon effluent by duckweed, *Transactions of the ASAE*, 43, 263-269, 2000

[21] Bonomo, L., Pastorelli, G., Zambon, N., Advantages and limitations of duckweed-based wastewater treatment systems, *Water Science and technology*, 35, 5, 239-246, 1997

[22] Perniel M., Ruan R., Martinez B., Nutrient removal from a storm water detention pond using duckweed, *Applied engineering in agriculture,* 14, 6, 605, 1998

[23] Al-Nozaily F., Alaerts G., Veenstra S., Performance of duckweed-covered sewage lagoons—II. Nitrogen and phosphorus balance and plant productivity, *Water Research*, 34, 10, 2734, 2000

[24] Hillman, W.S., The Lemnaceae, or duckweeds, *The Botanical Review,* 27, 221-287, 1961

[25] Radić, S., Pevalek-Kozlina, B., Differential esterase activity in leaves and roots of Centaurea ragusina L. as a consequence of salinity, *Periodicum biologorum*, 112, 3, 253-258, 2010

[26] Wendeou, S.P.H., Aina, M.P., Crapper, M., Adjovi, E. Mama, D., 2013. Influence of salinity on duckweed growth and duckweed based wastewater treatment system, *Journal of Water Resource and Protection,* 5, 10, 993, 2013