EVALUATION OF COMMON DUCKWEED (*LEMNA MINOR L.)* FOR REMOVAL OF NITROGEN AND PHOSPHORUS FROM ANAEROBIC BAFFLED REACTOR EFFLUENT AND THE FERTILIZER VALUE OF THE BIOMASS ON PERENNIAL RYEGRASS

By

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- Finally, special gratitude to my husband, thank you for constantly cheering me on.

DEDICATION

To my parents, my darling husband, brothers, sisters, extended family and friends.

God bless you all.

ABSTRACT

The disposal of effluent generated from low cost sanitation technologies such as the Anaerobic Baffled Reactor (ABR) effluent, can pose challenges to the environment. Nitrogen (N) and phosphorus (P), which are essential nutrients necessary for crop production are not removed in the treatment process and these can pollute surface by erosion and ground water through leaching. There is little information in literature on the use of aquatic macrophytes, especially duckweed to remove ABR effluent nutrient (N and P) under South African climatic conditions. The study investigated the effects of loading density of common duckweed (*Lemna minor*) and ABR effluent dilutions on biomass accumulation and uptake of nitrogen and phosphorus. The fertiliser value of harvested *L. minor* biomass (dry matter) as a source of N and P for perennial ryegrass (*Lolium perenne*) was also investigated. Both experiments were conducted in a growing tunnel at Newlands Mashu Research Site in Durban, South Africa (latitude 29°58'S and longitude 30°57'E). The first experiment was laid out as a 3 x 5 factorial arrangement in split-plot design with three replications. The loading densities were 400, 600 and 800gm^{-2}. The ABR dilutions were (i) Raw ABR (ii) 75% ABR + 25% tap water (iii) 50% ABR +50 % tap water and (iv) 25% ABR +75 % tap water, with (v) Omnia® fertiliser solution as a control. Residual water mineral N (ammonium + nitrate) in the raw and 75% ABR were higher than South African disposal standards, 50% and 25% ABR dilutions, met the stipulated standard. In all ABR dilutions orthophosphate (solution P), pH and turbidity met disposal standards. Removal efficiencies ranged between 71-97%, 29 - 94%, 92-97% and 24-43.2% for mineral N, solution P, turbidity and COD, respectively. The highest biomass, N and P uptake and lowest residual water N and P were in the 50% ABR dilution with *L. minor* loading density of 600 gm^{-2}. The second study was a pot experiment with two kilograms (2kg) of soil packed in 2 litre non-draining plastic pots. The experiment was laid out in a complete randomized block design. The nutrient source used was duckweed biomass cultured in 50% ABR dilution at a loading density of 600gm^{-2}. The treatments were dried duckweed biomass applied at (1) 200kg N/ha – (from duckweed supplied as a source of nitrogen (DWN), (2) 80kg P/ha (from duckweed added at a higher rate to meet plant phosphorous requirements, which supplied higher N -DWP), and (3) 200kg N /ha – (from duckweed and from mineral P from commercial fertilizer (DWN+P)). Sodium dihydrogen phosphate (NaH_2PO_4: 25.83% P) was applied at 50kg P/ha (0.074g P/Pot), to correct for P deficiencies in DWN. The inorganic fertilizer controls were NPK, PK and K treatments applied at recommended rates. The PK and K treatments were set up as negative controls to distinguish the effects of duckweed N and NP respectively on perennial ryegrass growth. Addition of duckweed, as N and P sources, produced significant ($p<0.05$) increase in perennial ryegrass biomass, but the addition of mineral P had little to no effect on tissue uptake of all nutrients and growth characteristics. The perennial ryegrass biomass in the DWN and DWP were comparable with the NPK however, treatment K had the lowest biomass yield. The N and P uptake in the treatments were not as high in the duckweed treatments as it was in the NPK. Duckweed treatment resulted in higher plant uptake of Ca, Mg and Mn, than in the inorganic fertilizer treatments. Soil residual N and P were lower in the duckweed treatment than in the NPK commercial fertilizer treatment. The findings of this work suggest that recovery of N and P from ABR effluent using *L.minor* depends on the loading density and ABR dilution. This can lead to significant improvement in water quality coupled with providing an organic source of nutrients (duckweed biomass) for crops.

Keywords: ABR effluent, *Lemna minor*, biomass, nitrogen, orthophosphate, water quality, nutrient source, perennial ryegrass

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Chapter 1 : GENERAL INTRODUCTION

Rapid urbanisation and population growth, results in major global challenges such as environmental pollution and the depletion of natural resources, mainly in developing countries in Africa and Asia (United Nations Department of Economic and Social Affairs., 2015). Furthermore, challenges of food insecurity and water stress are urgent and are estimated to increase (Alcamo et al., 2007). The impact of these challenges are most likely to be felt among the urban poor, often living in informal settlements (United Nations Department of Economic and Social Affairs., 2015). These, among other issues, are expected to affect the ability of governments in many developing countries to provide sufficient amenities, including basic sanitation facilities. The lack of adequate sanitation facilities can be a source of environmental pollution (Abbasi et al., 2017).

The densely populated urban, peri-urban and informal communities in eThekwini Municipality are usually highly polluted as a result of lack of adequate sanitation facilities (eThekwini Municipality, 2014). Connecting these emerging communities to the existing centralized sewerage (Hudson, 2010), built purposely to treat domestic wastewater in densely populated areas (Massoud et al., 2009), is not financially feasible. Therefore, policies and practices were put in place to ensure the equitable provision of sanitation. These policies highlighted the approved on- site dry sanitation technologies such as urine diversion (UD) toilets, ventilated improved pit latrine (VIP) and the community built pit latrines (Sutherland and Lewis, 2012) for communities without centralized sewerage. Nevertheless, some households still aspire to use waterborne sanitation (Pillay et al., 2010a), which informed the evaluation of the decentralised wastewater treatment system (DEWATs).

The DEWATs was relevant for communities that were provided with limited water, too little for conventional waterborne sewerage but higher than required for use in VIP toilets (Mtembu, 2005). Results from studies conducted on the appropriateness of the DEWATs as an on-site sanitation system indicated a potential for its use within densely populated low-income communities in South Africa (Foxon et al., 2004). Adopting the decentralized approach to wastewater treatment in these emerging low income communities is essential as collection of waste is a complex process due to the geographic conditions that usually characterise these areas (Foxon et al., 2007, LI et al., 2008).

The DEWATs approach uses anaerobic baffled reactors (ABR), which have a series of hanging and standing (vertical) baffles that force wastewater to flow up and down through a series of compartments as they passed from the inlet to the outlet (Sasse, 1998, Morel and Diener, 2006). Although the ABR is efficient in wastewater treatment in terms of chemical oxygen demand (COD) and biological oxygen demand (BOD) reductions (approximately 80%), the resulting ammonium-N and orthophosphate-P are still high at 20-50 mg NH_4-N/L and 5mg PO_4-P/L (WRC Research Report No. K5/2002). Effluent disposal into rivers and estuaries could cause eutrophication. For this reason, wastewater disposal regulations have been introduced in developing countries, such as South Africa, which emphasises the removal of nutrients from wastewater intended to be discharged into rivers, water courses, and estuaries (Department of Water Affairs., 2010).

While the ABR effluent does not meet the discharge standard, it meets the standard for irrigation stipulated by the Department of Water, Forestry and Fisheries (Department of Water Affairs., 2010). Therefore, it has the potential to be used as irrigation water in agriculture. Nevertheless, without proper management and controlled irrigation, there is the possibility of over application of nutrient (especially nitrogen) during wet summers when soils are saturated, and crops do not need water. Hence, the use of cost effective, eco-friendly alternative wastewater treatment methods like aquatic plants, which accumulate plant nutrients and improve water quality standards, is advisable.

Aquatic plants that grow on nutrient rich wastewaters (Korner et al., 2003), thereby removing nutrients and heavy metals and accumulating in their plant tissue (Culley, 1973), have been studied to treat different wastewater types for many decades. Common reed (*Phragmites australis*) (Brix and Arias, 2005), water hyacinth (*Eichhornia crassipes*) (Kawai et al., 1987, Valipour et al., 2015), duckweed (Lemna species) (Ozengin and Elmaci, 2007, Verma and Suthar, 2014, Selvarani et al., 2015a), and duckweed ferns (Costa et al., 2009, Sood et al., 2012), are the most researched aquatic plants for wastewater treatment purposes. Results from these studies have shown varying efficiencies in the removal and tissue accumulation of total nitrogen and total phosphorus. Although *L minor* appeared to show greater uptake of N (0.1-2.1 g/m^2 /day) and P (0.24-0.59 g/m^2 /day) from nutrient-rich wastewater (Cheng et al., 2002c, Seidl et al., 2004).

The common duckweed (*L minor*), also known as Damslyk in South Africa, floats on wastewater absorbing nitrogen and phosphorus from the wastewater. However, the absorption is proportional to plant biomass accumulation (Al Nozaily, 2000). Nevertheless, *L.mimor* experiences toxicity effects associated with excess ammonium ion concentrations, with contradictory limits stated by different authors in literature (Caicedo, 2005, Chin et al., 2011, Seidl et al., 2004, Al Nozaily, 2000). These differences informed the need to dilute wastewater to vary ammonium ion concentration levels and loading density (mass of duckweed per surface area), most efficient for high N and P removal and biomass accumulation.

Duckweed tissue essentially accumulates high elemental/ nutrient concentration levels (Timmerman and Hoving, 2016), which makes it good for fish feed, pyrolysis gas and bio-oil production (Skillicorn et al., 1993, Muradov et al., 2010, Muradov et al., 2014b, Mohedano et al., 2012). Specifically, the high N, P, K, micronutrients, bases and perhaps, the lack of lignin and melanins in duckweed tissue (as is in vascular plants) could promote rapid decomposition (Iqbal, 1999, Leng, 1999, Verma and Suthar, 2015). This advocates for the use of duckweed biomass produced on wastewater as a source of nutrients source for crops, after wastewater purification. However, information on this reuse option and its impact on crop and residual soil is limited in the literature.

1.1. Problem Statement

The use of the DEWATS system to provide alternative sanitation solutions to communities in urban and peri-urban areas of the eThekwini Municipality poses a challenge regarding the disposal of treated effluent into the environment. The treated effluent contains high concentrations of N and P, which do not meet disposal standards and cause contamination in surface and groundwater bodies. However, the N and P in the treated effluent are important mineral elements essential for plant growth. Therefore, there is the need to devise innovative, efficient and eco-friendly means of removing N and P from the wastewater to meet disposal standards, while storing away nutrients for reuse as fertilizer when needed. The removal of these mineral elements from ABR effluent using common duckweed (*L. minor*); and processing the harvested biomass into potentially new plant nutrient sources could provide a viable option for the handling of treated effluent in a sustainable and beneficial way to communities. However, there is little information in literature on a) the efficiency of N and P removal by common duckweed from the ABR effluent and b) the use of its biomass as a nutrient source for crop production. Research is needed to understand the factors that may influence such removal and the fertiliser value of the harvested biomass.

1.2. Aims and Objectives

The aim of the study was to (i) evaluate the removal of N and P from the ABR effluent using, common duckweed (*L. minor*) and (ii) assess the fertiliser value of harvested common duckweed biomass.

Specific Objectives

1. To determine the effects of loading densities of *L. minor* and ABR effluent dilutions on biomass accumulation and the uptake of nitrogen and phosphorus
2. To determine the effect of harvested *L. minor* biomass as a source of N and P on perennial ryegrass (*Lolium perenne*) growth.

Chapter 2 : LITERATURE REVIEW

2.1 Wastewater production, treatment and quality

Contamination of ground and surface water with industrial effluents causes shortages of freshwater resources in industrialized countries. On the contrary, municipal sewage disposal seems to be the main source of water pollution, limiting freshwater resources in many developing countries (Vigneswaran and Sundaravadivel, 2004). The limited freshwater resource aggravates the competition for its allocation within sectors (municipal, industrial, and agricultural) resulting in insufficient provisions for agriculture (Qadir et al., 2010). The effluents generated from municipal and industrial use, could be processed for the reuse of wastewater in agriculture (Qadir et al., 2010). There is increase in demand to reuse wastewater for irrigation, when there is scarcity of 'good quality water' for irrigation purposes (Pescod, 1992). Although freshwater supplies are clearly limited, its scarcity for most people is caused by technological barriers that limit water access, particularly for sanitary uses, especially in developing countries (Falkenmark and Lundqvist, 1998). Therefore, since municipal wastewater could be a water resource for irrigation purposes, there is need for suitable waterborne sanitation and treatment facilities, to prevent surface (streams, lakes, estuaries, rivers) pollution, through erosion and run off and ground (aquifers) water pollution, through leaching.

There are two main types of wastewater treatments systems: (1) the centralized and (2) decentralized wastewater systems. Centralized wastewater systems, which require cistern-flush facility, a network of laid underground pipes, pumps and pump stations and a treatment water works, are common in larger urban areas. These systems are inefficient in terms of energy, water consumption, cost of construction, installation and maintenance (Pillay et al., 2010b, Selvarani et al., 2015b), and are not feasible for smaller towns or densely populated, low-income areas of cities or city-boundaries (Eales et al., 2013). The decentralized systems on the other hand uses shallower pipes without the need for pump stations and are designed to convey, treat, dispose or sometimes reuse the effluent in fairly close vicinity to its source of generation, could be suitable in these areas (Opher and Friedler, 2016). They are decentralized wastewater treatment systems (DEWATS) uses anaerobic treatment processes, such as the anaerobic baffled reactors (ABRs) and anaerobic filters, to settle sludge and degrade biosolids after which further treatment using the aerobic treatment in ponds or in constructed wetlands follows. This technology was researched and tested in South Africa where it was shown that the treatment efficiency was lower than expected Recycled resources may include nutrients (nitrogen and phosphorus mainly) and bio-energy (van Loosdrecht and Brdjanovic, 2014). Other advantages of the decentralised process include its relatively inexpensive construction, ease of building by reasonably qualified craftsmen, ease of installation, operation and maintenance. In addition, it does not use electricity and utilizes less man power (Sasse, 1998). Its appropriateness for use as a sanitation tool in rural areas, have been assessed (Foxon et al., 2007, Jamshidi et al., 2014). Providing suitable and sustainable alternative waterborne sanitation in poor communities that lack sanitation is difficult in South Africa (Parkinson and Tayler, 2003). The decentralised wastewater treatment system (DEWATS) approach could serve this purpose.

The approach, which uses anaerobic baffled reactors (ABR), is efficient in reducing chemical oxygen demand (COD) and biological oxygen demand (BOD) for various ABR design modifications in literature (Barber and Stuckey, 1999, Adnan, 2003, Bassuney et al., 2013). The ABR is an enhanced septic tank, which treats a variety of wastewater types such as grey water, blackwater and industrial wastewater. Batchmann et al. (1983) developed the ABR and described it as a series of up-flow anaerobic sludge bed blankets. The ABR has a series of hanging and standing (vertical) baffles that force the up and down flow of wastewater through a series of compartments containing the mixed anaerobes as they pass from the inlet (wastewater source) to the outlet (wetlands) (Sasse, 1998, Morel et al., 2006). The degradation of wastewater solids in the compartments is carried out by anaerobic bacteria contained in the compartments with efficient removal of total chemical oxygen demand (COD) and biochemical oxygen demand (BOD) of 76% and 55%, respectively, in an ABR treatment of domestic wastewater (Nasr et al., 2009). Similarly Hudson (2011) reported an average COD removal of 80% at Kingsburgh wastewater treatment plant in Durban, KwaZulu-Natal. Nevertheless, the generated effluent from these anaerobic treatments were not nutrient free, hence did not meet wastewater discharge standards as concentration of Total Kjeldahl Nitrogen (TKN) and phosphates in the effluent were high for direct discharge to a water resource. The Water Quality Guidelines for Agricultural Use of the Department of Water Affairs and Forestry of South Africa do not provide a limit for BOD, COD, TOC and TSS. Most importantly, no limits were set for nitrogen and phosphates, as they are important nutrients, which are utilised by crops (Table 2.1)

In general, the anaerobic treatment of wastewater has little effect on nitrogen and phosphorus removal with only partial pathogen removal (Collivignarelli et al., 1990, Foxon et al., 2004). Thus, the anaerobic treatment is only to be considered a very effective pre-treatment. Further treatment is required to reduce concentrations of nutrients and pathogens, chemical oxygen demand (COD) and total suspended solids (TSS) (Nasr et al., 2009). Cheap, and easy to maintain, non-mechanical systems are peculiarly suitable for developing countries such as South Africa and many other Sub-Saharan African countries, where money and skilled manpower, may be lacking (essentially in emerging communities). The resulting effluent from the post treatment of wastewater could nevertheless, be reused for irrigation purposes (Korner and Vermaat, 1998).

Table 2.1 Parameters used to characterise general treated wastewaters

Parameter	*Significance in wastewater reclamation	*Approximate range in treated wastewater	South African irrigation water quality [a]	Treatment goal for reclaimed wastewater	Wastewater discharge standards (SA) [b]
^ BOD	Organic substrate for microbial or algal growth	10-30 mg/L	-	<1 to10 mg/L	-
^^ COD	Measures all chemicals that can be oxidized in water	75- 100 mg/L	-		30-75 mg COD/L
^^^ TOC	Measures of organic carbon	1-20 mg/L	-	<1 to10 mg/L	-
Total suspended solids (TSS)	Measure of particles in wastewater can be related to microbial contamination, turbidity. Can interfere with disinfection effectiveness	<1 to 30 mg/L	-	<1 to 10 mg/L	18-25mg/L
Turbidity	Measure of particles in wastewater; can be correlated to TSS	1 to 30 NTU	-	0.1 to 10 NTU	-
Total Nitrogen	Nutrient source for irrigation: can also contribute to microbial growth	1 to 30 mg/L	-	<1 to 30 mg/L	1-3 mg NH_4^+/L (1.5-15 mg NO_3^-/L)
Phosphorus	Nutrient source for irrigation: can also contribute to microbial growth	0.1 to 30 mg/L	-	<1 to 20 mg/L	1-10 mg/L

*Asano (1998), [a] Department of Water Affairs and Forestry (1996), [b] Department of Water Affairs and Forestry (2010), ^BOD- Biological Oxygen Demand, ^^COD- Chemical Oxygen Demand, ^^^TOC- Total Organic Carbon

2.2 Wastewater use in irrigated agriculture

The increase in the market demand for the reuse of treated and untreated wastewater for irrigation purposes, stems from the need for irrigation water, while conserving surface and groundwater resources (Martinez and Clark, 2012). Approximately 20 million hectares of agricultural land is irrigated with wastewater, globally (Jimenez and Asano, 2008). This is mainly because domestic wastewater provides 99.9 % water and 0.1 % suspended, colloidal and dissolved solids. The suspended and dissolved solids contain plant essential nutrients, especially N and P (Mara et al., 1989).

Jiménez et al., (2010) reported that developing countries have used domestic wastewater as crop nutrient source for foods eaten raw for decades (Table 2.2). Besides possibly posing a myriad of human health problems and plant growth related problems (due to excessive nitrogen loading from untreated domestic wastewater and also the water holding capacity of the soil), there is the risk of ground and surface water pollution through nutrient leaching and runoff (Mahmood and Maqbool, 2006). The high pathogen contents in domestic wastewaters, which includes bacteria, viruses, and protozoa, pose the greatest threat to human health. The World Health Organisation (WHO) guidelines for the use of wastewater in agriculture discourages the application of effluent to vegetables eaten raw (World Health Organization., 2006). Therefore, treating the wastewater before disposal or use as irrigation water source could help mitigate these problems.

Wastewater treatment with biomass production is a unified approach to treat wastewater and reuse plant biomass. This approach uses fast-growing, high nutrient and water demanding aquatic plants, which are managed in short crop cycles, enabling sustainable nutrient recycling (United Nations Empowerment Programme., 2017). Therefore, combining wastewater treatment and aquatic plant biomass production could bring many benefits such as hygienic environment, food security and income, in many cities in Sub-Sahara Africa, facing problems of wastewater disposal. Essentially, managing water reuse for irrigation and crop nutrient supply, can be achieved with post treatment of wastewater using aquatic macrophytes (United Nations Empowerment Programme., 2017).

Table 2.2 Types of wastewater used in irrigated agriculture and their impacts on soil, crop and human health

Country	Wastewater	Crops irrigated	Irrigation types	Impact on soil/crop	Impact on health	References
India	Stabilization pond effluent	Wheat	-	Increased yields higher than irrigation with freshwater supplemented with NPK	No health impact	Shenda (1985)
Zimbabwe	Domestic and Industrial (contained heavy metals Cu, Pb, Ni > permitted limits)	Vegetables	Furrow	N, P, K, micronutrients and bases for plant growth. Increase soil heavy metal content, increases risk of heavy metal plant uptake, and in soil residue.	Heavy metal consumption by end users.	Mapanda et al. (2005)
Ghana	Polluted wells and streams (contained pathogenic microorganism)	Vegetables	Watering cans	Cheap source of nutrients especially nitrogen	Postharvest contamination (helminths eggs, total coliform and *E.Coli*), affects both farmers and end users	Amoah et al. (2007)

2.3 Macrophyte- based wastewater treatment

The use of aquatic plants has been described as an innovative practical approach for the removal of nitrogen forms and phosphorus from wastewater (Iqbal, 1999, United States Environmental Protection Agency., 2002). The different aquatic macrophytes groups remove and store essential plant nutrients, and other elements in plant tissue (Cheng et al., 2002a). Table 2.3 shows different aquatic plants used to remove N and P forms from different wastewater types with varying efficiencies, bearing in mind that the conditions of the studies varied from one experiment to the next.

Table 2.3 Types of aquatic plants used in the removal of nitrogen and phosphorus forms in different wastewater types

Group	Aquatic plants	Wastewater	Inlet (mg L^{-1})	Outlet (mg L^{-1})	Element removed (%)	Author
*Emergent	Common reed (*Phragmites australis*)	Domestic	Total P (4.5-20.6) TN (30-350)	TP (4.5-13) TN (9-190)	TP (20-30), TN (23-63)	Brix et al. (2005)
**Submerged	Coontail (*Ceratophyllum demersum L.*)	Municipal	NH_4^+ (90-135) P (4.48-13.68)	NH_4^+ (10-15) P (0.5-1.15)	NH_4^+ (66-83.3) P (77.6-91)	Foroughi et al. (2010)
***Floating – leaved	Water hyacinth (*Eichhornia crasspies*)	Industrial	TN (7-56) TP (1.93-15.4)	TN (0.0) TP (0.0)	TN (100) TP (100)	Jayaweera and Kasturiarachchi (2004)
****Floating	Duckweed (*Lemna gibba*)	Sewage lagoons	TN (29-96) TP (6.0)	TN (15-56) TP (1.32-5.2)	TN (32-86%) TP (22-87%)	Al Nozaily (2000)

*plants with large portions of the shoot above water
plants totally submerged underwater * plants with floating leaves on water surface **** non-rooted free-floating water plants

Cooperative growth of the plants and microorganisms embodies the scientific basis for wastewater treatment using vascular aquatic plant systems. A major part of the treatment process is the degradation of organics in the wastewater, which is attributed to the microorganisms living on and around the aquatic plant root systems (Wolverton, 1986). Microorganisms form a symbiotic relationship with the aquatic plant at the roots of the plants, which stimulates the degradation and removal of organic compounds from the wastewater in the immediate surrounding of the plant roots systems. The metabolites produced during degradation of the organics, are then absorbed and utilized along with nitrogen, phosphorus and other minerals by plants and as food sources (Wolverton, 1986). This use and reuse process of each other's waste enhances the rapid removal of organics from wastewater. Aquatic plants however, could produce aerobic zones around their roots (**Figure 2.1**), due to their intricate ability to translocate oxygen from the upper leaf areas into the roots; a process desirable in domestic sewage treatment process.

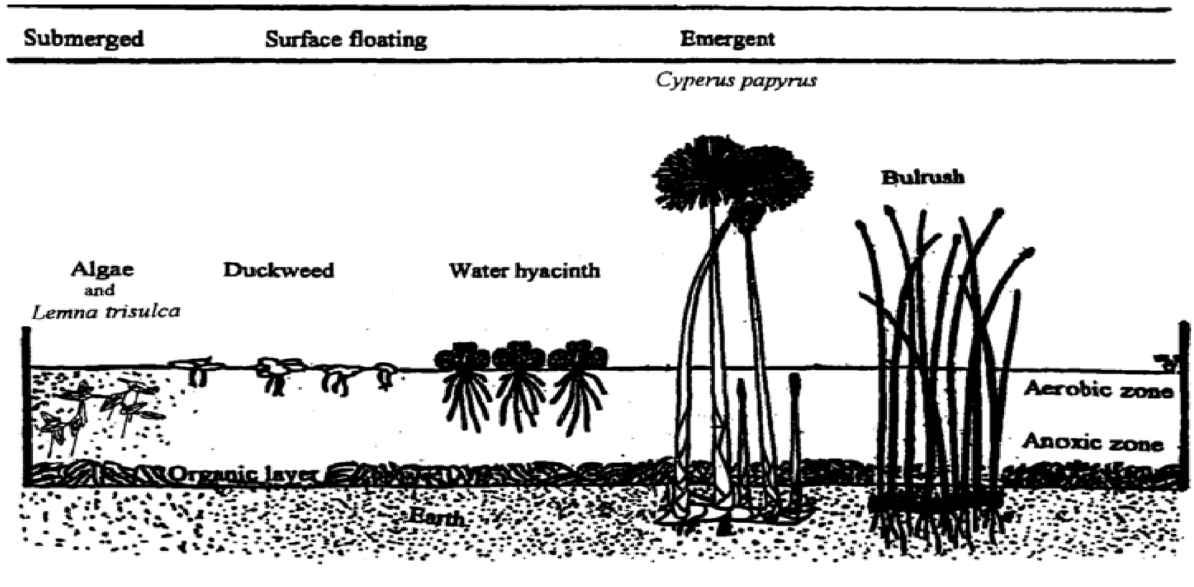

Figure 2.1: Species of macrophytes used in remediating wastewater (Source: Stowell et al., 1981 in Al Nozaily, 2005)

Aquatic macrophytes generally, improve water quality by regulating oxygen balance and by accumulating nutrients and heavy metals (Kadlec and Wallace, 2008). Due to the economic advantages accrued in the long run, considerable attention is being directed toward the wastewater treatment processes with aquatic plants. Studies conducted on the removal of total phosphorus (TP) and total nitrogen (TN) using *Phragmites australis* (Common reed) (Drizo et al., 2000), Umbrella palms (*Cyperus alternifolius*), Bulrush (Restorer) (*Scirpus californicus*), and water hyacinths have shown very wide range of removal efficiencies, but with limited or no secondary use of the harvested biomass. Zhao et al. (2014) compared the potential of duckweed and water hyacinth in the conversion of wastewater nutrients to valuable biomass. Although the biomass production by water hyacinths was high (mainly from the absorption of C), it had less resource use in comparison to duckweed which has more potential reuse options such as animal feed, for ethanol production, and potentially as a fertilizer.

A variety of duckweed species (*Lemna* spp., *Wolffia* spp., *Wolffiella* spp., *Landoltia* spp and *Spirodela* spp) have been used efficiently for the bioremediation of wastewater (Al Nozaily, 2000), due to high growth rates, ease of maintenance and biomass multiple reuse options (Journey et al., 1993). Several studies have shown 96- 99% removal of total nitrogen and total phosphorus using duckweed species to treat different types of wastewater, with varying initial concentration of both N and P (Korner et al., 1998, Korner et al., 2003, Mekdes, 2010, Mohedano et al., 2012). Also the direct conversion of ammonia in waste water into plant biomass in duckweed ponds is a “highly energy efficient process” (Smith and Moelyowati, 2001). These efficiencies in combination with multiple uses do not exist with other aquatic plants in literature. This makes duckweed preferable and economically viable than other aquatic plants for wastewater treatment.

2.4 Characteristics of Duckweed

Duckweed are macrophytes (higher plants) belonging to the family *Lemnaceae* and are the smallest floating aquatic flowering plants (Cheng et al., 2002c). Duckweeds are angiosperms and monocotyledonous plants, which float on water and have one of the fastest growth rates of macrophytes. The family consists of five genera, *Lemna, Spirodela, Landoltia, Wolffia, and Wolffiella* with about 38 species identified so far using DNA barcoding (Wang et al., 2010).

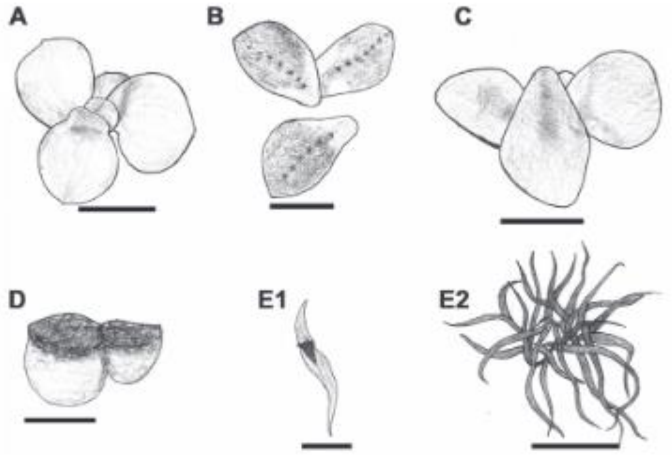

Figure 2.2 The five members of the *Lemnaecea* family. A: *Spirodela polyrhiza*, B: *Landoltia punctata*, C: *Lemna minor* D: *Wolffia arrhiza*, E: *Wolffiella gladiate* (Klaus et al., 2013).

Duckweed species are highly adaptable to various environmental and climatic zones. They adapt well to a varied range of temperature, changes in pH and nutrient compositions (Landolt and Kandeler, 1987). They have also been found in waters containing very high organic matter (Landolt, 1998). The natural habitat of duckweed is the surface of fresh or brackish water. They are suited to slow flowing water streams and water surfaces protected from wind (Skillicorn et al., 1993). However, many species have been reported to survive temperature extremes, and they grow more rapidly and favourably in tropical and temperate zones. Duckweeds can tolerate lower temperatures when compared with water hyacinth as they have a wider geographical range (Brix, 2003). They are also much easier to harvest using the surface skimming method than algae or water hyacinth (Journey et al., 1993).

The fast growth rate of duckweed is attributed to its ability to accumulate nutrients such as phosphorus, nitrogen and trace metals from wastewater or nutrient rich waters (Mkandawire et al., 2004, Odjegba and Fasidi, 2004, Mkandawire and Dudel, 2005, Olguín et al., 2005). The absorption of nutrients and water is done mainly through the lower epidermis of the fronds (Landolt et al., 1987), whereas other higher plants only use their root systems. In general, duckweed species do not grow on oligotrophic waters (waters usually poor in plant nutrients and containing abundant oxygen). They have high nutrient requirements and are resistant to relatively high salinity (Oron et al., 1985). This resistance of duckweed to high salinity could be an important factor in the application of duckweed-based systems in the reduction of conductivity to make water suitable for irrigation.

In South Africa, duckweed is also known as Damslyk (Botanical Research Institute., 1980) and they are considered an invasive plant species (Muskett et al., 2014). They are however, ever-present plants which are not endemic in South Africa (Lubke and de Moor, 1997). They are found in polluted rivers around the Eastern Cape, Gauteng, Mpumalanga and KwaZulu-Natal.

2.5 Factors determining duckweed growth

2.5.1. The effects of pH, temperature and water depth on nitrogen and phosphorus removal processes in duckweed ponds

The biomass, produced per unit of pond surface, and the effectiveness of the duckweed wastewater treatment is dependent heavily on factors such as the concentration of nutrients (N, P and others) in the solution, water pH, water temperature, sunlight and day length (Leng, 1999). Duckweed survives at a pH range of 5 - 9 with optimum growth at a range of 6.5-7.5. In this pH range ammonia is available as ammonium ion which is the most readily absorbed N form (Leng, 1999). A higher pH level, however, results in the presence of ammonia, which can be toxic, impeding duckweed growth (Zimmo et al., 2004). However, the optimum temperature range for effective nutrient removal from wastewater duckweed is 21-31^{o}C. While a minimum temperature of 7^{o}C was reported by Reed et al. (1988), severe heat stress is known to occur above 31°C (Leng, 1999, Iqbal, 1999). In a pH range between 7 – 8 and temperature range between 5 - 25 ° C, denitrifying bacteria thrive. These conditions are typically found in domestic wastewaters (Zimmo, 2003) such as ABR effluent (Hudson, 2011).

Nitrogen is a macronutrient that is vital to plant growth and it is important for plant structural and metabolic activities such as the synthesis of chlorophyll, proteins, enzymes and nucleotides (Metcalf and Eddy, 1991). Plants require larger quantities of N than other nutrients. However, its deficiency is evidenced with features such as reduced yield and chlorosis (Fageria, 2016). Phosphorus is also a vital macronutrient for energy transfer reactions, photosynthesis and respiration, development of reproductive structures, crop maturity, root growth, flower and seed development and protein synthesis. Its deficiency in plants include; wilting of leaves, purple cast on leaves, lack of fruits and flowers on the plants and delayed maturity (Uchida, 2000). Municipal and domestic wastewater usually contains organic nitrogen in the form of proteins, amino acids and other organic compounds, and inorganic nitrogen mainly as ammonium and small amounts of nitrogen oxides (Metcalf et al., 1991). Most of the treated wastewaters however, have total N concentrations of between 20 and 85 mg L^{-1} (Pescod, 1992), which implies that they are a good source of N. Due to the anaerobic conditions during wastewater treatment, ABR effluent contains nitrogen in the form of NH_4^+ (Foxon et al., 2004, Hudson, 2011, Musazura et al., 2015), which is the preferred form of nitrogen for uptake by duckweed (Cui and Cheng, 2015).

2.5.2 Nitrogen and phosphorus removal in duckweed ponds

Direct nitrogen and phosphorus uptake

Duckweeds and most aquatic plants prefer ammonium to nitrate as their source of nitrogen (Porath and Pollock, 1982). This as explained by Ferguson (1969) is due to the inhibitory effects of ammonium for plant nitrate uptake as studied in algae, and fungi. Lüönd (1980) stated that appreciably higher biomass yields (10-20%) were recorded when duckweed was grown on a medium containing NH_4^+, compared to NO_3^-. Dortch (1990) found that the uptake of nitrates by duckweed was halted when ammonium was added to growth media. Even though ammonium-N is preferred, excess ammonium concentrations and water pH, may hinder proper duckweed growth (Iqbal, 1999). In treated domestic wastewater, the presence of phosphorus is mainly from human faecal matter and cleansing agents (White and Hammond, 2008). Wastewater treatment however, does not remove P except through mechanisms such as flocculation as described by Burns et al. (2003). Studies by Hudson (2010) have shown that ABR effluent is rich in P as well as N, which may support the growth of duckweed. Optimal removal of nitrogen is achievable in duckweed treatment ponds by direct plant uptake with regular biomass harvesting (Reed et al. (1988), with volatilization of ammonia and nitrification not having major effects Zimmo (2003). Zimmo (2003) noted that denitrification is of utmost importance in the removal mechanisms of nitrogen in duckweed ponds. Therefore, nitrogen removal in duckweed totally covered systems is attributed to direct plant uptake by duckweed, uptake by the attached biofilm on duckweed and nutrient absorption by walls of the system.

The favoured form of phosphate for duckweed uptake and growth in wastewater is the ortho-phosphate (Priya et al., 2012). Phosphorus removal from duckweed ponds is mainly through direct plant uptake, adsorption by the attached biofilm, chemical precipitation and settled sludge removal. In most treated wastewaters however, the amount of P is between 6 and 20 mg L^{-1}. Culley et al. (1981), reported a considerably good growth of duckweed species within the P concentrations of 6 to 154 mg/l. While other mechanisms are negligible (Sutton and Ornes, 1977), the removal of phosphorous by uptake is enhanced by the frequent harvesting and adequate pre-treatment of raw wastewater to release organically-bound ortho-phosphates (Vermaat and Hanif, 1998, Iqbal, 1999).

Values on daily nitrogen uptake by duckweed are shown (

Table 2.4), and these values vary due to the differences in experimental procedures, climate conditions, solution pH, loading/stocking densities, duckweed species and other associated conditions. Although the experimental conditions were not the same, *L minor* appeared to show greater removal of N and P from nutrient-rich wastewater.

Table 2.4 Nitrogen and phosphorus uptake in g/m^2/day by duckweed species.

Region	Species	Wastewater	Nitrogen	Phosphorus	Reference
Florida	*Lemna obscura*	Dairy lagoon	-	0.02	DeBusk et al. (1995)
Bangladesh	*Spirodela polyrrhiza*	Municipal	0.26	0.05	Alaerts et al. (1996)
Palestine	*Lemna gibba*	Septic tank	0.2 –0.55	-	El-Shafai et al. (2013)
Egypt	*Lemna gibba*	Domestic	0.44	0.09	Zimmo (2003)
Italy	*L.gibba/L.minor*	-	0.42	0.01	Corradi et al. (1981)
Brazil	*Landoltia punctata*	Swine waste	0.44	0.47	Mohedano et al. (2012)
North Carolina	*Lemna minor*	Swine waste	2.1	0.59	Cheng et al. (2002a)
Niger	*Lemna minor*	Stabilization pond	0.1	0.24	Seidl et al. (2004)
Zimbabwe	Duckweed	Raw sewage	0.1	0.03	Nhapi (2004)

2.6 Duckweed biomass

A major feature of wastewater treatment with duckweed is the valuable biomass harvested after water purification process. The quick conversion of nutrients into biomass is an indication of the extent that duckweed can remove and accumulate macronutrients from wastewater (Ziegler et al., 2016). This is observed in literature, with varying biomass yields from different wastewater types (**Table 2.5**). Useful reuse options for the harvested biomass have also been extensively researched (**Table 2.6**).

Table 2.5 Yields of duckweeds grown on wastewaters as reported in literature.

Duckweed	Wastewater	Yield (*t* DW/ha/yr)	Authors
Landoltia punctata	Swine	68	Mohedano et al. (2012)
Spirodela polyhiza	Swine	45.2	Xu et al. (2011)
Lemna japonica	Pilot scale treatment plant	26.5	Zhao et al. (2014)
Lemna gibba	Domestic	38.3	Nasr et al. (2009)
Lemna minor	Manured pond	12.8	Ge et al. (2012)

The high nutritive value of duckweed and its low fibre content makes it a valuable feed and feed additive for animals and possibly for human consumption. In addition, the high nitrogen content makes its use as an organic fertilizer probable in agriculture either by direct soil incorporation or as compost (Iqbal, 1999). Mbagwu (1990), reported that harvested duckweed, if grown on domestic wastewater free from heavy metals and other hazardous compounds, could be used as an agricultural fertiliser and in the production of high quality compost. These makes growing duckweed to remove N and P from ABR effluent and the reuse of the harvested biomass as fertilizer, a promising concept.

Table 2.6 Products from duckweed grown in wastewater under different conditions

Products	Author
Ruminant feed	Leng (1999), Skillicorn et al. (1993)
Aquaculture	Journey et al. (1993), Leng et al. (1995), Goopy and Murray (2003)
Vermicompost	Kostecka and Kaniuczak (2008)
Biomethane	Muradov et al. (2008)
Bioethanol	Xu et al. (2011), Chen et al. (2012)
Bio-oil	Xiu et al. (2010)
Bio-char and bio-gas	Muradov et al. (2012)
Renewable fuels and petrochemicals	Muradov et al. (2014b)
Bioleum	Verma et al. (2015)
Biofuel	Cui et al. (2015)
Duckweed pellets for home heating	Hubenova and Mitov (2012)
Chinese medicine	Huang et al. (2012)
Human consumption	Porath (1993)

Soil fertilization using aquatic plants by direct incorporation of the biomass into the soil, as mulch or as compost, has been widely researched. In South Sudan, Abdalla et al. (1969) reported that water hyacinth could be effective in controlling nutsedge (*Cyperus rotundus*), substantially conserving soil moisture, and adding organic matter and nutrients when the residues were incorporated into the soil. Mbagwu and Adeniji (1988) and Mekdes (2010), stated that, *Lemna* species can act as a good fertilizer supplement for crop growth. *Lemna* plants applied to soil have been reported to contribute to improved water and cation exchange resulting in good crop harvest. Practices on the use of duckweeds in general, as organic fertilizer sources have been reported from Angola, China and Mexico (Iqbal, 1999). Therefore, incorporating dry duckweed (*L.minor*) biomass produced from ABR effluent to supply crop nutrient requirements into less fertile soils may have the potential to support growth of crops such as perennial rye grass (*Lolium perenne)*.

2.7 Conclusion

The duckweed species *L. minor* is found in abundance in South Africa, and has the most rapid growth rates and elemental uptake of all macrophytes. Several growth media such as the Hutner solutions, Hillman solution, Jacob's medium, Hoagland's medium, Gorham's medium have been used to culture different duckweed (Al Nozaily, 2000). Landolt and Kandeler (1987) noted that Hutner solution was not favourable for *Lemnaceae* species growth as it was too concentrated, containing 200 mg of ammonium nitrate (NH_4NO_3) and 400 mg of K_2HPO_4. Based on the reports by Bergmann et al. (2000a) and Cheng et al. (2002a), Caicedo (2005), however, reported optimum growth of *Spirodela polyrrhiza* in a maximum concentration of 50 mg/L NH_4 -N. whereas, Chin et al. (2011) mentioned an optimal 20-60mg/l NH_4-N for *Lemna minor* growth. Perhaps, since the ABR effluent contains more than 60mg/l, the manipulation of nutrient concentration by dilution could determine the ideal ammonium ion concentration that will result in the highest nutrient removal efficiency and duckweed biomass accumulation. This however, raised the questions;

1. Is dilution a factor that could influence growth of L minor and its capacity to remove N and P from wastewater?
2. Does the initial loading density of duckweed have any effect on the rate of nutrient removal from wastewater?

Chapter 3 : EFFECTS OF ANAEROBIC BAFFLED REACTOR EFFLUENT AND DUCKWEED LOADING DENSITIES ON BIOMASS ACCUMULATION, UPTAKE OF NITROGEN AND PHOSPHORUS AND RESIDUAL WATER QUALITY

ABSTRACT

Water quality impairment by nutrients such as nitrogen (N) and phosphorus (P) from the discharge of treated domestic wastewater such as the anaerobic baffled reactor (ABR) effluent, has been a sanitation concern, especially in the eThekwini Municipality, South Africa. Post treatment using aquatic plants such as common duckweed (*Lemna minor* L.) was evaluated for efficacy in the removal of N, P, and biomass accumulation using different N levels of ABR (dilutions). The experiment was designed as a 3 x 5 factorial arrangement with the following treatments: duckweed loading densities (3 levels – 400gm^{-2}, 600gm^{-2}, and 800gm^{-2}) and ABR effluent dilution (5 levels- Raw ABR, 75 % ABR +25% tap water, 50 % ABR+ 50% tap water, 25 % ABR+ 75 % tap water and Omnia fertilizer control) and laid out using a split-plot design (Loading densities as main plot and ABR dilutions as sub-plot) with three replications giving a total of 45 experimental units (in 5 litre plastic containers). Data collected included dry matter yield, plant uptake of N and P, pH, turbidity, electrical conductivity (EC) and residual N and P. Data was subjected to a split plot analysis of variance (ANOVA) and the means were further separated using Fisher's Unprotected Least Significant Difference Test ($p<0.05$). Statistical analyses were carried out using GenStat 17th Edition. There was an interaction effect between loading density and ABR dilutions for dry matter yield ($p<0.01$). Dry matter yield was highest in the 50% dilution and increased as loading densities increased. Loading density had no effect on pH, and EC but ABR dilutions had highest pH and EC in the raw ABR and lowest in the 25% ABR dilution which did not differ from the control. Residual mineral N (ammonium + nitrate) in the raw and 75% ABR were higher than South African disposal standard, 50% and 25% ABR, however, met the standard. Orthophosphate (OP), pH and turbidity also met the standard. Removal efficiencies of mineral N ranged from 71-97%, OP (29 - 94%), turbidity (92-97), COD (24-43.2%). Loading density 600 gm^{-2} and 50% ABR dilution had the highest biomass, N and P uptake and lowest residual water N and P. The findings suggested that duckweed treatment aids in the reduction of N and P in the ABR to below national thresholds for discharge, while the high nutrient levels in the tissue could provide a potentially be exploited as an organic fertilizer.

Keywords: Anaerobic baffled reactor effluent, *Lemna minor*, biomass, nitrogen, orthophosphate, water quality.

3.1 INTRODUCTION

The decentralized wastewater treatment system (DEWATS) is an efficient and effective low cost and low maintenance system (Naik and Stenstrom, 2016, Siegrist, 2017), which treats wastewater from domestic and commercial sewage next to the source (Omenka, 2010). It uses a range of uncomplicated technologies (United States Enviromental Protection Agency., 2005), such as the anaerobic baffled reactor (ABR), to rapidly decompose human wastes under anaerobic conditions (Barber et al., 1999). The system can serve as alternative sanitation appropriate for densely populated communities (Foxon et al., 2007, Chirisa et al., 2016). The ABR design has been improved for better effectiveness and appropriateness for a diversity of wastewater types (Barber and Stuckey, 1999). Such improved designs include that of the pilot ABR situated at Newlands East, in the eThekwini Municipality, Durban South Africa.

The current design shows a high treatment efficiency as it removes up to 80% of chemical oxygen demand (COD) from the effluent (Sasse, 1998, Reynaud and Buckley, 2016). However, the ABR is unable to remove nutrients such as nitrogen (N) and phosphorus (P) (Foxon et al., 2006), which could cause eutrophication when the wastewater is discharged into freshwater bodies. Hence, ABR effluent does not meet the discharge standards i.e. TN (1-3 mg NH_4^+/L and 1.5-15 mg NO_3^-/L) and P (1-10 mg/L) in South Africa, it meets irrigation standards as stipulated by the Department of Water, Forestry, and Fisheries (Department of Water Affairs., 2010). This implies that it has the potential to be used as irrigation water in agriculture as source of plant N and P. Investigations on the use of the ABR effluent, from the pilot plant in Durban, South Africa, have shown improved dry matter yield in maize (Bame, 2012, Bame et al., 2014). Musazura et al. (2015) also reported increased fresh biomass yields of Swiss chard irrigated with ABR effluent than with tap water and rainwater treatments. However, the long-term use of wastewater can have detrimental effects on soil physical and chemical properties. Wastewater may contain undesirable chemical constituents and pathogens that pose environmental and health risks. At the same time, several risk factors have been identified in reuse of wastewater; some of them are short term impacts (e.g., microbial pathogens) whereas others have longer-term impacts that increase with the continued use of wastewater (e.g., salinity effects on soil)

Pedrero et al. (2010), noted that high sodium concentrations in wastewater used for irrigation affected soil structure and impeded the soil-water flow. The presence of excess Na in wastewaters causes soil dispersion (Warrence et al., 2002), resulting in poor soil structure, increased surface crusting and reduced infiltration and hydraulic conductivity. In addition, continuous utilisation of wastewater for irrigation could result in pollution of surface and groundwater resources, due to surface erosion, run off and nutrient leaching.

Merghem et al. (2016), reported high nitrate and COD of shallow aquifers, while Qian and Mecham (2005) found high concentrations of Na and P contents in the surface layers of soil after continuous irrigation of a golf field with municipal wastewater. High soil P reduced the availability and/or uptake of micronutrients such as copper, zinc, manganese by plants, reducing the overall plant tissue quality Voss (1998). Post treatment of wastewater to remove COD, nitrogen and phosphorus is essential to mitigate surface or ground water pollution (Nasr et al., 2009). The use of duckweed could contribute in this regard.

The common duckweed (*Lemna minor* L.) rapidly grows on nutrient- rich waters, and is efficient in removing N and P (Korner et al., 2003, El-Shafai et al., 2013). Chaudhary and Sharma (2014), reported that treatment of domestic wastewater with common duckweed reduced COD by 73-84%, total N (83-87%), total P by 83-95% and orthophosphate P by 70-85%). However, the efficiency of this approach for post treatment of ABR effluent could depend on the loading density of the duckweed and physicochemical properties of the ABR effluent including nutrient concentrations, pH and temperature. The objective of this study was to determine effects of duckweed-loading densities and dilution of ABR effluent on biomass accumulation, uptake of N and P by common duckweed and the quality of the residual water.

3.2 MATERIALS AND METHODS

3.2.1 Experimental site

The experiments were carried out in the growing tunnel at the Newlands Mashu research site (29°58'S and 30°57'E), Durban, South Africa (Figure 3.1).

Figure 3.1 Ariel view map of Newlands Mashu experimental site

3.2.2 Experimental materials

Anaerobic Baffled Reactor (ABR) effluent

The ABR effluent used in the study was obtained from the DEWATS plant at the Newlands Mashu Research site. The plant was designed to treat domestic wastewater from 86 households (Pillay et al., 2010b) and is linked to the households by an existing trunk sewer (Mtembu, 2005). The system consists of a settler with two chambers, which serve to settle down sludge and as a biogas collection point. It has three parallel ABR streets, and two chambers each of anaerobic filters for organic polishing at the end of each ABR street (Pillay et al., 2010b). At this stage, the ABR effluent is channelled to the vertical and horizontal constructed wetlands (aerobic treatment), or stored in a 5,200 litre tank adjacent the growing tunnel. The effluent for this experiment was drawn from the storage tank.

The effluent was analysed for ammonium –N (NH_4 -N), nitrate (NO_3-N) phosphate (PO_4 -P) and chemical oxygen demand (COD) using the Merck® Spectroquant photometer (APHA, 2005). The turbidity test was carried out using a Hach® 2100Q portable turbidimeter (Hach® Company Colorado USA). Effluent pH and electrical conductivity (EC) were measured using the sensION™+ MM150 portable multi-meter (Hach® Company Colorado USA). All analyses were based on standard methods for the examination of water and wastewater (APHA, 2005).

Duckweed

The common duckweed (*Lemna minor*) was sampled from slow-flowing stream at Ashburton (29°40'S; 30°27'E) in Pietermaritzburg, KwaZulu-Natal, in April 2016. The high density of duckweed grew on water enriched with nutrients from untreated human and animal waste. The duckweed fronds were skimmed from the water, using a 2-mm size sieve, rinsed with tap water to remove extraneous materials, and re-introduced to fresh water to allow all molluscs and solids to settle. The duckweed was placed in six containers with a surface area of $0.5m^2$ and a depth of 0.6m, containing 50% ABR effluent (i.e., ABR was diluted to 50% of original concentration with water) to condition for five days, before use in the experiments.

3.2.3 Treatments and experimental design

The experiment was designed as a 3 x 5 factorial treatment structure and laid out using a split-plot design with three replications. The main plot factor was duckweed loading density (3 levels) with ABR dilution as the subplot (5 levels). The duckweed loading densities were 400, 600 and 800 g m^{-2}. The ABR dilutions were (i) Raw ABR (ii) 75% ABR + 25% tap water (iii) 50% ABR +50 % tap water and (iv) 25% ABR +75 % tap water, with (v) Omnia® fertiliser solution as a control. The characteristics of the fertiliser, ABR effluent and tap water used in this study are presented in **Table 3.1**. Except for nitrate-N, the ABR effluent had higher levels with respect to all parameters than the fertiliser solution, and tap water had the lowest. Nitrogen concentrations in the dilutions were estimated based on the initial N concentrations of the raw ABR effluent.

Table 3.1 Physico-chemical properties of ABR effluent, Omnia fertilizer solution and tap water used in the study.

Parameters	Effluent*	Fertilizer **	Tap water
pH	7.86	7.71	6.22
Electrical conductivity (µS cm^{-1})	1329	409	248
Turbidity (NTU)	127	1.35	0.20
Chemical Oxygen Demand (COD mg/L)	168	56	-
Ammonium N (mgL^{-1})	63.1	8.4	^b.d
Nitrate N (mgL^{-1})	0.3	3.6	b.d
Orthophosphate (mgL^{-1})	11.5	4.1	0.01

*Full concentration of ABR effluent ** comparable to 25% ABR dilutions in terms of N, P, and COD. ^b.d- below detection

Thirty- six litres of the different ABR dilutions and Omnia fertiliser solution were prepared and analysed (Table 3.1). Four litres of the solution were put in each plastic containers with surface area of $0.0625m^2$ and 0.012 m deep, and were used to culture duckweed at the different loading densities. The experiment was conducted for 14 days without adjusting solution pH. Effluent was sampled (20 ml) after 7 and 14 days, and replaced with 20 ml tap water each time. Additional tap water was added to address water losses through evapotranspiration and plant accumulation (decline in solution level). The solutions were analysed for ammonium-N, nitrate-N, P, pH and electrical conductivity (EC), using standard methods for the examination of water and wastewater (APHA, 2005). Nutrient removal efficiency was calculated using Equation 1.

$$\textbf{Efficiency (\%)} = \frac{C_i - C_e}{C_i} \textbf{ x 100} \qquad \textbf{Equation 3.1}$$

Where C*i* = the initial concentration of element in the ABR effluent; C*e* =the final concentration of element in the effluent after duckweed harvest. The experiment was conducted as summarised in the schematic diagram on Figure 3.2.

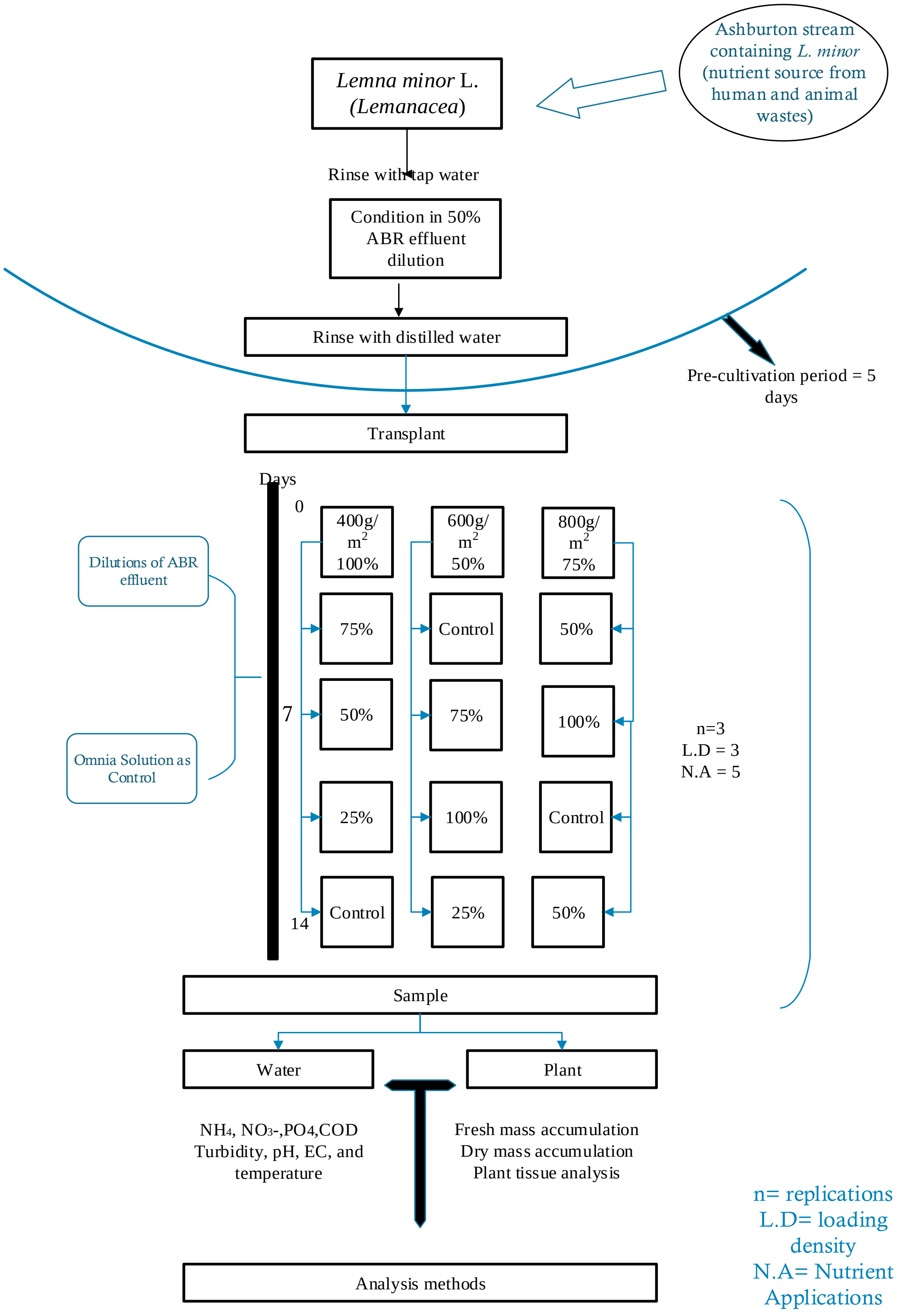

Figure 3.2 Schematic diagram of the procedures during the experiment

3.2.4 Duckweed measurements and analyses

Dry matter accumulation

At the end of the experiment (14 days), the duckweed biomass from each experimental unit was harvested, weighed and dried at 70°C for 72 hours to determine dry matter. The dry matter accumulation was determined as the difference between the initial plant material added and the total biomass. The samples were stored in Glad® zip seal airtight bags, in a cool, dark and dry environment.

Sample preparation and tissue elemental composition

A representative sample of plant tissue was dried and ground. A 5-gram vial or equivalent was used to hold a sub sample in airtight storage. After which the plant tissue analyses were done at the Soil Fertility and Analytical Service Laboratory of the KwaZulu-Natal Department of Agriculture and Environmental Affairs (CEDARA). Total N was determined using the LECO CNS 2000 autoanalyser (Leco Corporation, Michigan, USA) as described by (Matejovic, 1996). Other macronutrients and micronutrients in the tissue were determined using the Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) after dry ashing. The plant material (0.5g) was ashed in a porcelain crucible, placed in a furnace at 500°C for 4 hours. The ash was digested in 10.0 mL nitric acid and filtered, and the filtrate was diluted for elemental composition analysis using ICP-AES.

To determine the duckweed nutrient uptake, the product of the tissue nutrient content and biomass was used, rather than only the decline in the nutrient concentration of the ABR dilution. This method eliminated losses of nutrients through environmental losses and showed the uptake of nutrients by the duckweed, disregarding nitrogen and phosphorus forms.

Nitrogen and phosphorus mass balances

Nitrogen mass balances equation described by Lee et al. (2014) was used in this study. The equation is as follows:

$$Mass\ balance\ difference = N_{initial} - \left(N_{residual} + N_{uptake} \right) \qquad \textbf{Equation 3.2}$$

$N_{initial}$- the concentration of N in the nutrient solution at start of experiment

$N_{residual}$- the concentration of N in nutrient solution after plant harvest

N_{uptake}- the concentration of N removed by plant tissue as plant uptake

Phosphorus mass balance was calculated using methods described by Lee et al. (2012). The following equation was used:

$$Mass\ balance\ difference = P_{Initial\ -}(P_{\ residual} + P_{uptake}) \qquad \textbf{Equation 3.3}$$

$P_{initial}$- the concentration of P in the nutrient solution and at start of experiment

$P_{residual}$- the concentration of P in nutrient solution after plant harvest

P_{uptake}- the concentration of P removed by plant tissue as plant uptake

3.3 STATISTICAL ANALYSIS

All the data were subjected to analysis of variance (ANOVA) in a split plot design with loading density as the main plot and ABR dilutions as subplot, using the statistical software GenStat® 17th Edition (VSN International., 2014). Thereafter, the means were separated using Fisher's unprotected least significant difference (LSD) at the 5% level of significance (Fisher, 1970).

3.4 RESULTS

3.4.1 Effects of ABR effluent on duckweed biomass accumulation

3.4.1.2 Dry matter accumulation

The interaction effect of ABR dilutions and duckweed loading densities on dry matter accumulation (DM) was significant ($p<0.05$). In the 400gm^{-2} duckweed loading density, dry matter accumulated for the raw, 75% and 50% ABR dilutions were not statistically different. However, the dry matter accumulated in the 25% ABR dilution increased significantly when compared to 50% ABR, 75% ABR and the raw ABR. In the 600gm^{-2} duckweed loading density, it was observed that both the raw ABR and the 75% ABR dilution accumulated the lowest dry matter. However significant ($p<0.05$) differences in accumulated dry matter was observed when 25% ABR dilution was compared with both the raw ABR effluent and 75% ABR dilutions. Observed trends in the 800gm^{-2} duckweed loading rates was such that the 50%, and 25% ABR dilutions did not differ from the control treatment (Omnia). Nevertheless, differences were observed in the dry matter accumulation between the duckweed loading densities (400, 600 and 800m^{-2}) for the different dilutions, i.e. the dry matter accumulated in the raw ABR and the 50% ABR dilution treatments increased significantly as loading rates increased. However, in dry matter accumulated in the 75%, and 25% ABR dilutions, no differences were observed when 600 and 800gm^{-2} duckweed loading densities were compared. Comparing the three duckweed loading densities, the dry matter accumulated in the 400gm^{-2} for the dilutions were significantly ($p<0.05$) lower than in the 600 and the 800gm^{-2} duckweed loading densities. In the three duckweed loading rates, 25% ABR dilution did not differ in terms of dry matter accumulation from the Omnia (control solution) Figure 3.3.

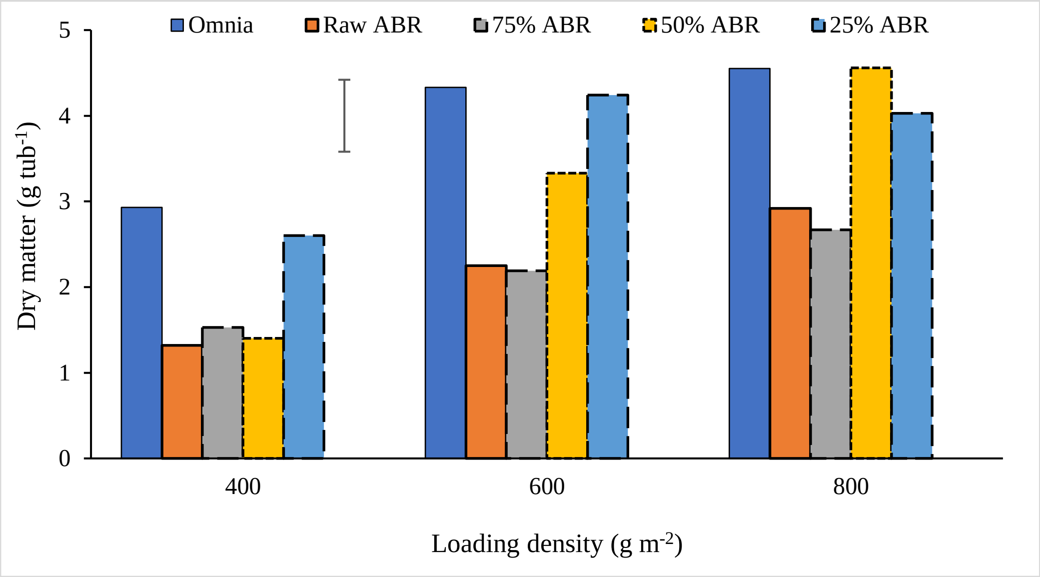

Figure 3.3**:** Duckweed dry matter accumulation as affected by loading density and ABR dilutions. The bar represents the least significant difference at $p < 0.05$.

3.4.2 Effects of ABR effluent on duckweed tissue elemental composition and uptake

Where there were no interaction effects observed, main factors were presented.

Interaction effects of duckweed loading density and ABR dilution were significant ($p<0.01$) for tissue N concentration but not in any other elementals composition. There were no interaction effects on the uptake of N, Ca, Mg, and Mn. The main factor effects of loading density were significant ($p<0.05$) for tissue P, Ca, Mg and Na composition and not on any other elements.

The main factor effects of loading density were observed in the uptake of P, K, Cu, Fe, Ca, Mg, Na, Zn, Fe and Al. The ABR dilutions had significant ($p<0.01$) effects on the plant tissue elemental compositions for all elements in the duckweed tissue (

Table **3.2**). Except for the plant uptake of both Fe and Al, the effects of ABR dilutions on the plant tissue composition and plant uptake were significant.

Table 3.2 P-values of tissue elemental composition and uptake by duckweed grown on ABR effluent

Elements	Tissue composition			Plant uptake		
	LD	NS	LD x NS	LD	NS	LD x NS
N	0.001	<0.001	<0.001	0.002	<0.001	0.187
P	0.023	<0.001	0.871	<0.001	0.003	0.570
K	0.367	<0.001	0.749	0.003	<0.001	0.850
Ca	0.030	<0.001	0.518	0.002	<0.001	0.229
Mg	0.039	0.046	0.649	0.003	<0.001	0.442
Na	0.040	<0.001	0.919	<0.001	<0.001	0.565
Zn	0.757	0.003	0.588	0.006	<0.001	0.003
Cu	0.138	<0.001	0.889	0.003	<0.001	0.663
Mn	0.187	<0.001	0.372	0.004	0.013	0.316
Fe	0.359	<0.001	0.963	0.004	0.299	0.896
Al	0.248	<0.001	0.814	0.007	0.451	0.695

LD- loading density, NS- ABR dilutions, LD x NS -interaction effects of loading density and ABR dilutions.

3.4.2.1 Tissue N concentration and uptake

The interaction effects of loading density and ABR dilutions were significant in the duckweed tissue N concentration ($p<0.01$) but the differences observed with calculated duckweed uptake of N did not differ (Table 3.2).

Tissue N in the duckweed grown using a duckweed loading density of 600gm^{-2} differed significantly, with a higher duckweed tissue N when compared with both the 400 gm^{-2} and the 800 gm^{-2} duckweed loading densities. In the raw ABR, tissue N concentration was significantly higher in the 600 (3.1%), when compared to 400 (2.5%) and 800 gm^{-2} (2.8%)

loading densities. The 600gm^{-2} duckweed loading density also had a higher tissue N in the 75% ABR dilution. In the 50 % and 25% ABR dilutions, however, 800 gm^{-2} duckweed loading density had significantly higher tissue N concentration than in the 400 gm^{-2} loading density. The Omnia fertilizer had the lowest duckweed tissue N composition at both the 400 and 600gm^{-2} duckweed loading densities (**Figure 3.4**).

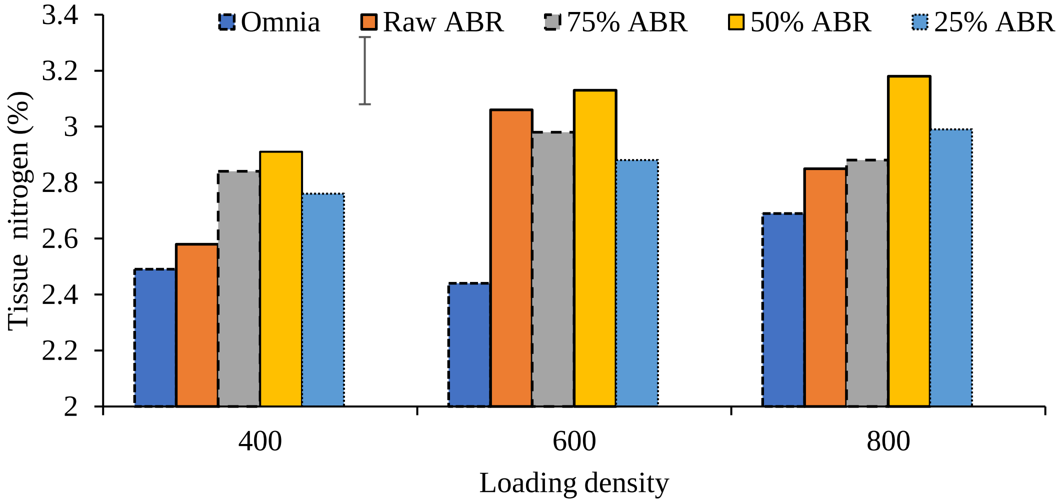

Figure 3.4 Duckweed tissue N as affected by loading density and ABR dilutions. The bar represents the least significant difference at $p< 0.05$

There was no interaction between the effects of duckweed loading density and ABR dilutions, therefore, main factor effects were reported for duckweed uptake of N.

The duckweed uptake of N increased with an increase in duckweed loading density, consequently, duckweed tissue uptake of N differed significantly ($p<0.05$) across the three loading densities. The effects of the ABR dilutions showed an increase in nitrogen uptake as dilutions increased. Nevertheless, the duckweed N uptake for the 50%, 25% ABR dilutions and the Omnia (control) did not differ (Figure 3.5).

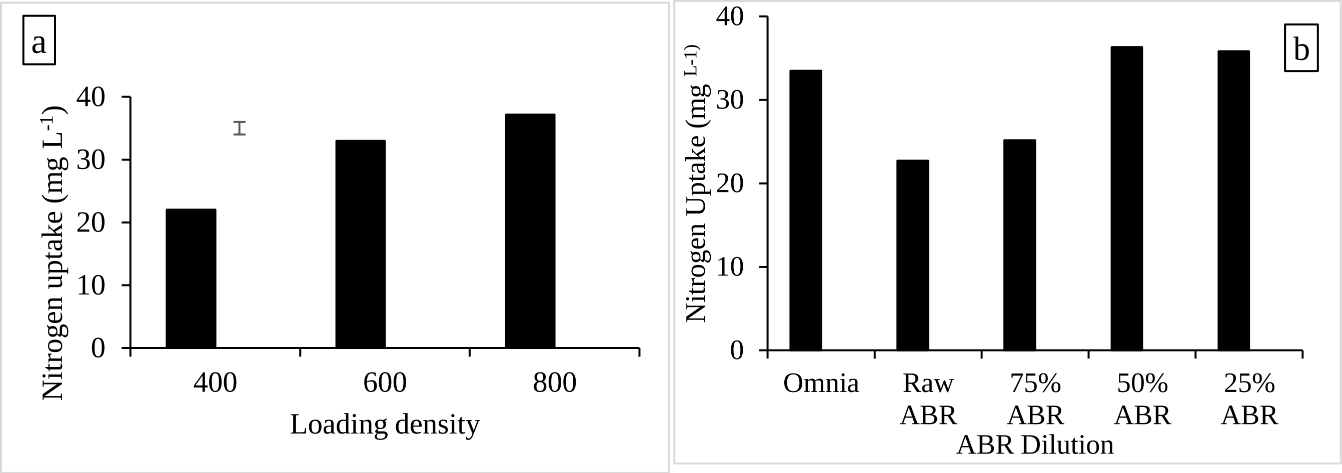

Figure 3.5 Duckweed N uptake as affected by main factors (a) loading density and (b) ABR dilutions. The bar represents the least significant difference at $p< 0.05$

3.4.2.2 Tissue phosphorus concentration and uptake

The duckweed tissue P concentration in the duckweed loading densities (400, 600 gm^{-2}and 800 gm^{-2}) differed significantly ($p<0.05$). It was observed that as duckweed loading density increased, duckweed tissue P concentration increased (**Figure 3.6**).

Tissue P concentration was significantly ($p<0.01$) affected by ABR dilutions. The 75% ABR dilution had a tissue P concentration that was not significantly different from both the raw ABR and the 50% ABR dilution. The 50% ABR dilution did not differ significantly from the Omnia fertilizer ABR dilutions. The 25% ABR dilution had the lowest tissue P concentration (**Figure 3.6**).

The duckweed uptake of P differed significantly ($p<0.01$), with the duckweed P uptake increasing as loading densities increased. The dilutions of ABR also had significant effects on the uptake of P. Omnia fertilizer and the 50% ABR dilutions had similar P uptake values which were higher than raw ABR, 75% ABR and 25% ABR. (**Figure 3.7**).

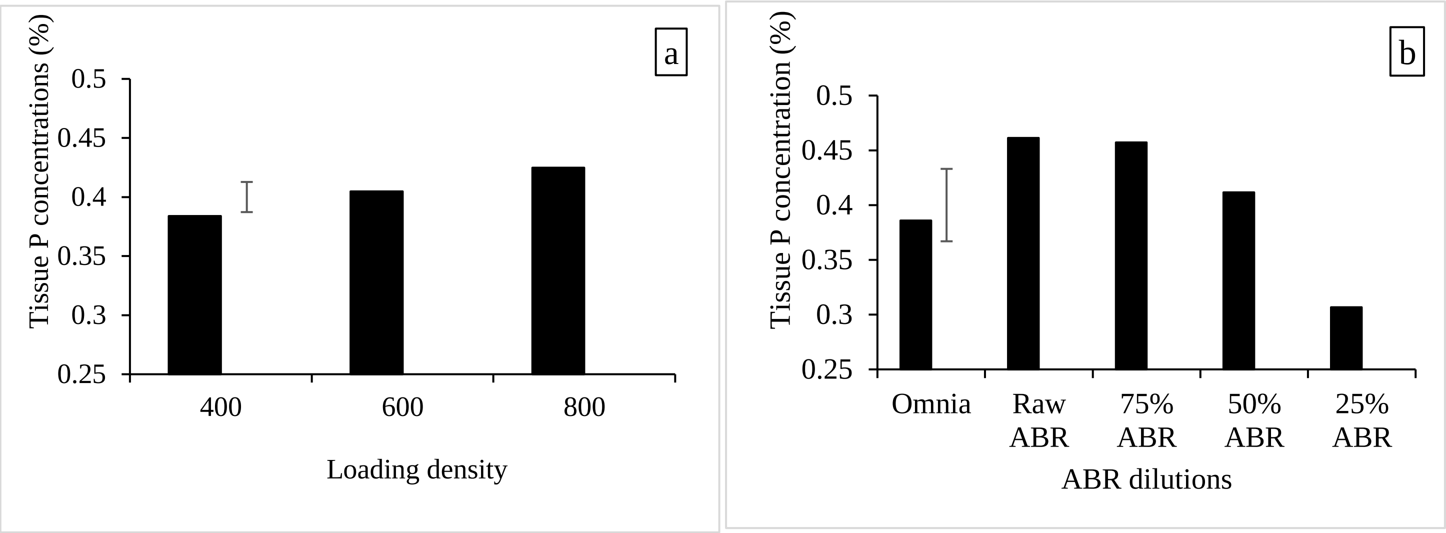

Figure 3.6 Duckweed P tissue concentration as affected by (a) loading density and (b) ABR dilutions. The bar represents the least significant difference at $p < 0.05$

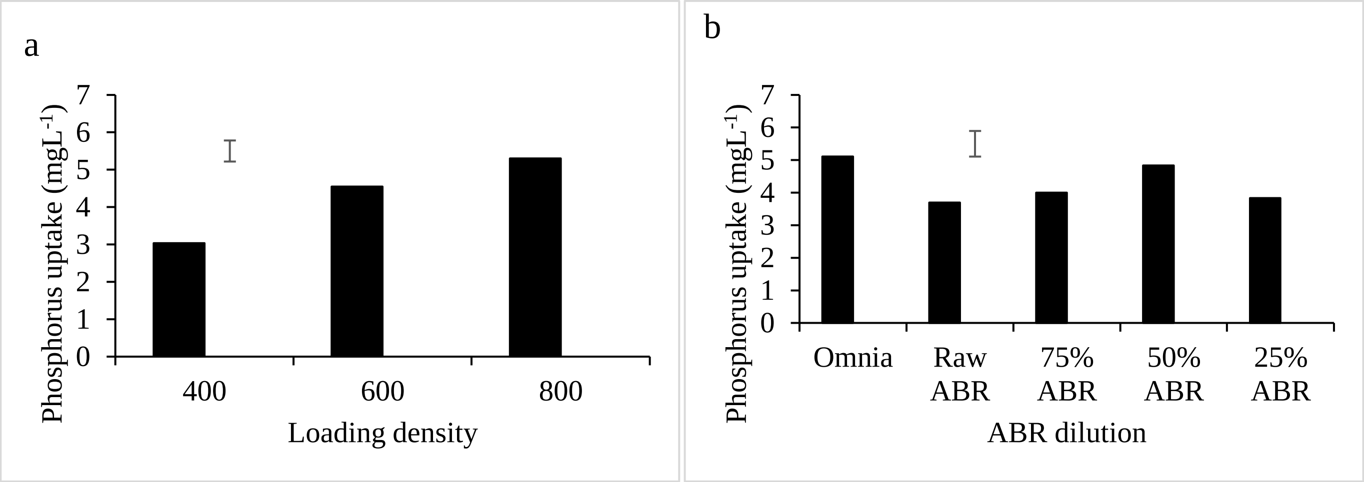

Figure 3.7 Duckweed P uptake as affected by (a) loading density and (b) ABR dilutions. The bar represents the least significant difference at $p< 0.05$

3.4.2.3. Potassium, calcium and magnesium uptake by duckweed

Loading densities and ABR dilutions had significant ($p<0.01$) effects on the uptake of K. Increasing loading densities of duckweed increased the uptake of K. Similar patterns were observed for the Ca and Mg tissue uptakes (**Table 3.3**).

The 50% ABR dilution had higher K uptake than the raw ABR and 75% ABR dilution, but lower than the Omnia. Calcium uptake in 50% and 25% ABR dilutions were not significantly different from the Omnia, but was significantly higher than in the raw and 75% ABR dilution (**Table 3.3**). The raw ABR had the lowest Mg uptake when compared with the other dilutions and Omnia control.

Table 3.3 Effects of loading densities and ABR dilutions on uptake of bases by duckweed

Factor	**Uptake of bases (mg L^{-1})**		
	K	Ca	Mg
Loading density (g/m^2)			
400	12.1 a	12.8 a	3.3 a
600	16.5 b	18.5 b	4.7 b
800	18.8 c	21.8 c	5.3 c
ABR dilution			
Omnia	23.8 c	19.5 b	5.3 b
Raw ABR	11.5 a	13.9 a	3.2 a
75% ABR	12.5 a	15.6 a	3.7 b
50% ABR	16.7 b	19.9 b	4.9 b
25% ABR	14.3 ab	19.4 b	5.0 b

Means with different letters significantly differed in each column for each factor.

3.4.2.4. Uptake of micronutrients by duckweed

There were no significant differences observed between the 600 and 800 gm^{-2} loading densities for the uptake of Zn, Cu, Mn and Fe. However, micronutrients uptake was significantly higher in the 600 and 800 gm^{-2} than in the 400 gm^{-2} loading density.

There were no significant differences observed in the uptake of Zn in all the ABR dilutions. However, higher Zn uptake was observed in the Omnia were compared with the ABR dilutions. Similar pattern was observed for the Cu uptake. The Mn uptake was highest in the 50 % ABR dilution, and no significant differences were observed comparing the 25% ABR dilution and the Omnia, raw ABR and 75% ABR dilutions. No significant differences were observed for the tissue uptake of Fe (**Table 3.4).**

Table 3.4 Effects of loading density and ABR dilutions on duckweed elemental uptake of micronutrients.

Factor	**Elemental uptake (mgL^{-1})**			
	Zn	Cu	Mn	Fe
Loading density (g/m^2)				
400	0.1 a	0.011 a	4.8 a	0.8 a
600	0.2 b	0.014 b	8.5 b	1.3 b
800	0.3 b	0.015 b	10.1 b	1.4 b
ABR dilution				
Omnia	0.3 b	0.02 b	7.7 a	1.1 a
Raw ABR	0.2 a	0.01 a	6.7 a	1.0 a
75% ABR	0.2 a	0.01 a	7.1 a	1.1 a
50% ABR	0.2 a	0.01 a	9.3 b	1.3 a
25 % ABR	0.2 a	0.01 a	8.1 ab	1.1 a

Means with different letters significantly differed in each column for each factor.

3.4.3. Effects of duckweed growth on residual water quality

3.4.3.2 **Effects of duckweed growth on water pH, and electrical conductivity**

Duckweed loading density and ABR dilution were significant ($p<0.05$) for both pH and EC. There was a slight increase in water pH values in the first week (Day 7) for 75% and 50% ABR dilutions. The Omnia solution had a decrease in pH values, which was lower than the raw ABR and ABR dilutions (Table 3.5).

Loading densities had no significant effect on the EC values of the residual water. However, raw ABR had the highest EC values in comparison to the other ABR dilutions. Nevertheless, 25% ABR did not differ from the Omnia control in both Day 7 and Day 14 sampling times. (Table 3.5).

Table 3.5 Water pH and electrical conductivity of different ABR dilutions and Omnia fertiliser solution over time of duckweed growth

ABR dilutions	Day 0	Day 7	Day 14
Water pH			
Omnia	7.71a	7.78 ab	7.60 a
Raw ABR	7.86ab	7.94 b	7.99 b
75% ABR	7.76 a	7.89 b	7.86 ab
50% ABR	7.72 a	7.82 b	7.82 ab
25% ABR	7.67 a	7.74 a	7.72 a
Electrical conductivity			
Omnia	0.41 a	0.41 a	0.34a
Raw ABR	1.33 e	1.21 e	1.05 e
75% ABR	1.01 d	0.97 d	0.84 d
50% ABR	0.77 c	0.69 c	0.64 c
25% ABR	0.54 b	0.45 b	0.39 b

Means with different letters significantly differed in each column for each parameter.

3.4.3.3 Effects of duckweed growth on water turbidity

On Day 7 of sampling, 400gm^{-2} and 600gm^{-2} turbidity values were similar. Highest initial turbidity was observed in the raw ABR effluent. On Day 14, no differences were observed in the 75% and 50% ABR dilutions, whereas in the raw ABR, increase in water turbidity was observed as loading densities increased. The Omnia and the 25% ABR were similar with the lowest turbidity values (**Figure 3.8**). **Figure 3.9**, shows the difference in raw ABR turbidity at (a) start of the experiment (Day 0) and (b) at the end of the experiment (Day 14).

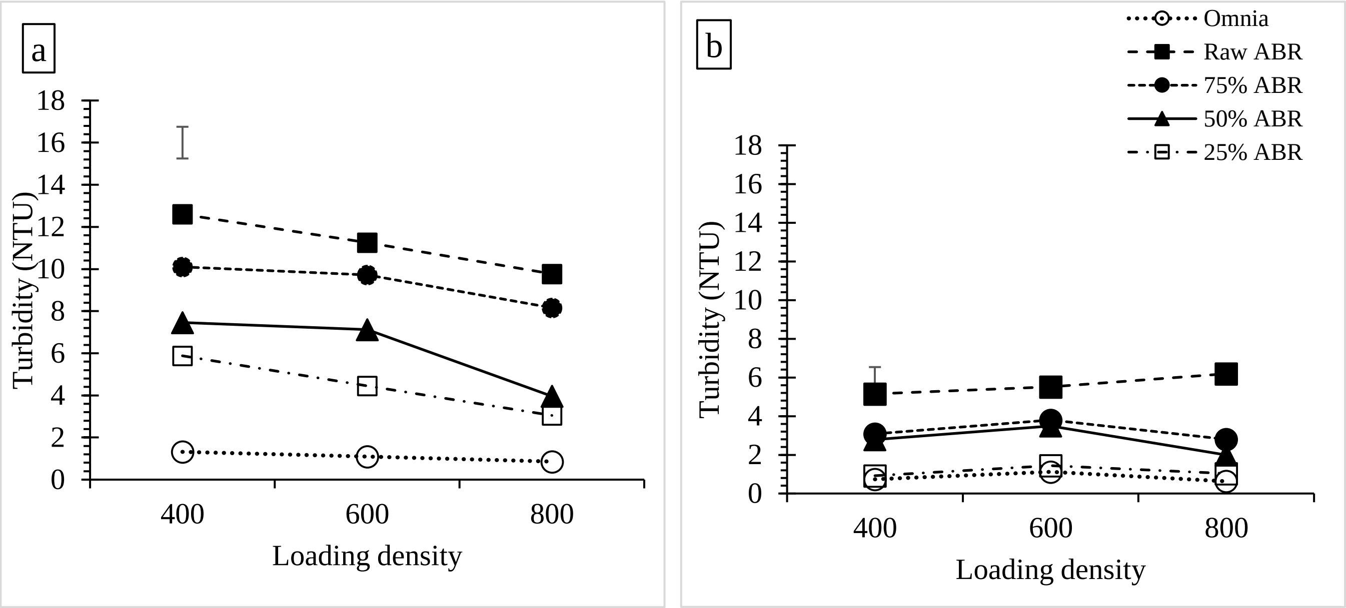

Figure 3.8 Water turbidity as affected by loading density and ABR dilutions at (a) Day 7 and (b) Day 14. The bar represents the least significant difference at $p < 0.05$

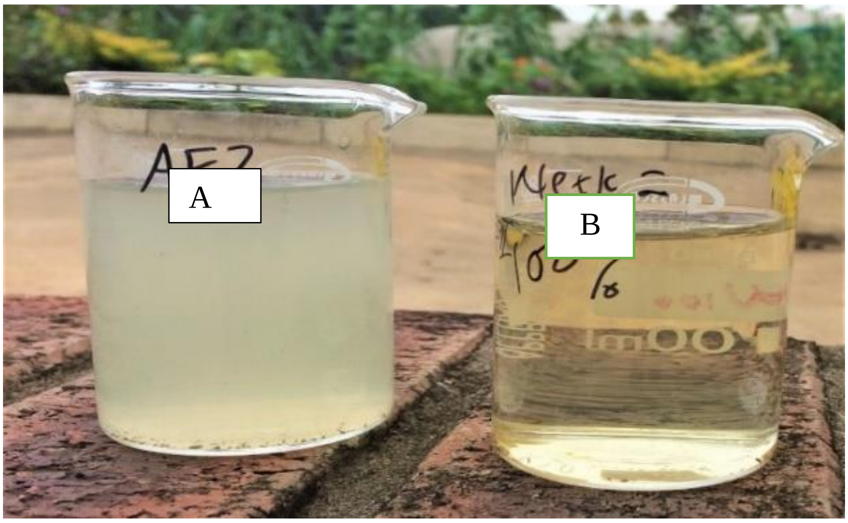

Figure 3.9 Turbidity of raw ABR (a) at Day 0 and (b) after duckweed treatment at Day 14.

3.4.3.4 Effects of duckweed growth on Chemical Oxygen Demand (COD)

Interaction effects of duckweed loading density and ABR dilutions were observed for COD. Significant decrease in COD was observed as the ABR dilutions increased (i.e. significant differences ($p<0.01$) in raw ABR, 75% ABR and 50% ABR dilutions) when compared with the Omnia. In the raw ABR, a significant reduction in COD was observed when comparing the COD concentration at Day 0 (167.3 mg COD/L) to the observed COD value at Day 14 (95 mg COD/L). Significant reductions were also observed in the 50% and 25% ABR dilutions. For the 600 gm^{-2} duckweed loading density, significant reduction in COD concentrations were observed in the 75%, 50% and 25% ABR dilutions (**Table 3.6**).

Table 3.6 Effects of duckweed growth on residual water chemical oxygen demand as affected by duckweed loading density and ABR dilutions

Loading density (gm^{-2})	Sampling day	ABR dilutions				
		Omnia	Raw ABR	75% ABR	50% ABR	25% ABR
400	Day 0	^^57.0 d	167.3 a	124.0 b	74.0 c	56.7 d
400	Day 14	40.7 fg	133 a	89.3 bc	70.6 cd	51.3 de
600	Day 0	57.0 a	167.3 a	124.0 b	74.0 c	56.7 d
600	Day 14	31.3 fgh	126.7 a	93 bc	56.3 d	37 fgh
800	Day 0	57 .0 d	167.3 a	124.0 b	74.0 c	56.7 d
800	Day 14	34.0 fgh	95.0 bc	68.0 cd	49.3 efg	42.3 fg

Means with different letters are significantly different.

3.4.4 Effects of duckweed growth on residual water nitrogen and phosphorus

Ammonium-N

The result was characterised by a high removal of ammonium- N in all the treatments (Figure 3.10). For each duckweed loading density, the initial concentration of ammonium in each ABR dilution were the same (i.e. initial concentration of raw ABR, 75% ABR, 50% ABR and 25% ABR dilutions for the three loading densities (400, 600 and 800gm^{-2}) were the same at 63.1, 49.0, 31.0, and 15.9 mgL^{-1} respectively).

At Day 7, significant differences ($p<0.01$) were observed in loading density in terms of residual NH_4^+-N, it was observed that significantly higher residual NH_4^+-N was in the 400 (41.3 mg/l) than in 600 (35.5 mg/l) and 800 gm^{-2} (32.3 mg/l) loading densities in the raw ABR effluent. The 75% ABR dilution, 400 gm^{-2} (22.2 mg/l) had significantly higher ammonium concentration than in the 800 gm^{-2} loading densities (18.9 mg/l), nevertheless, ammonium concentration was highest in the 600gm^{-2} duckweed loading density.

In all the treatments there were reduction in ammonium concentration from Day 0 (start) to Day 14 (end of experiment). However, there was no significant difference between the 600 and 800 gm^{-2} duckweed loading rates in the final ammonium concentrations of water. Nevertheless, the final (Day 14) ammonium concentrations in the raw ABR for the 400 gm^{-2} was significantly higher than the Day 14 ammonium concentrations in the 600 and 800gm^{-2} duckweed loading densities. Comparing Days 14 for 600 and 800 gm^{-2} (50% ABR dilution), no significant differences in the residual water ammonium concentration was observed, however, 400gm^{-2} had the highest residual ammonium (Figure 3.10).

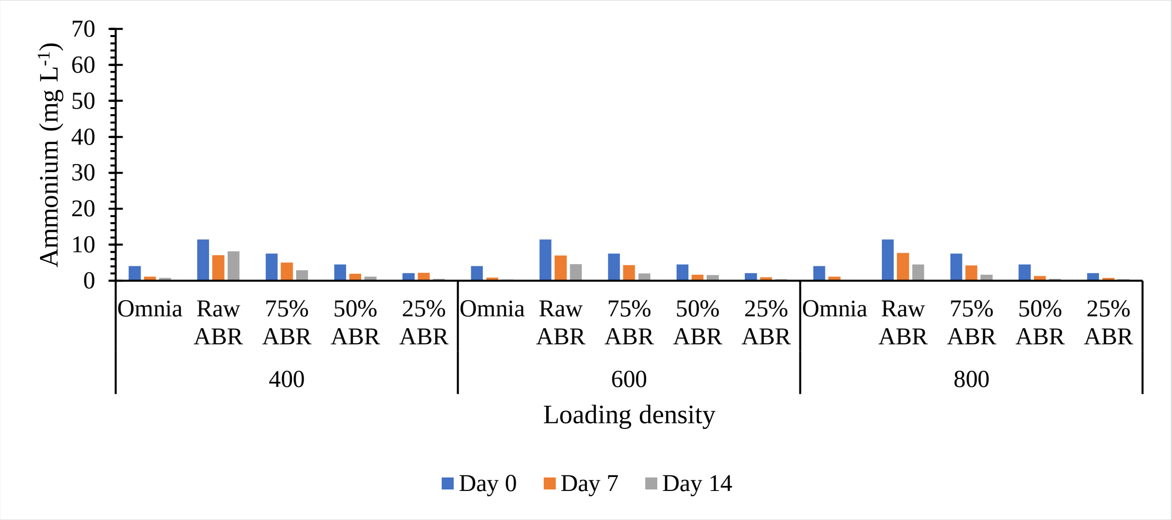

Figure 3.10 Residual water ammonium as affected by both duckweed loading densities and ABR dilutions.

Nitrate-N

The initial concentration of nitrates in the three loading densities were the same for each dilution and control (i.e. initial concentration of nitrate in the control, raw ABR, 75% ABR, 50% ABR and 25% ABR dilutions for the 400, 600 and 800gm^{-2} duckweed loading densities were the same at 9.7, 0.3, 0.6, 0.6 and 0.7 mgL^{-1} respectively). In the 400gm^{-2} duckweed loading density, raw ABR, 75% ABR and 50% ABR dilutions were observed to increase significantly from Day 0 to Day 14, nevertheless in the 25% ABR dilution, differences were not observed in the nitrate concentration when Day 0 and Day 14 were compared. In the 600gm^{-2} duckweed loading density, there was also observed a significant increase in the nitrate concentrations (Day 0 - Day 14). Nevertheless, for both 75% ABR and 25% ABR dilution, nitrate concentration was observed to decline at Day 7 and maintained at Day 14. In the 50% ABR dilution, however there was a slight increase in nitrate concentration on Day 7 and a decline at the end of the experiment (Day 14). For the 800gm^{-2} duckweed loading density, it was observed that there was a consistent reduction in water nitrate concentrations as the experiment proceeded (from Day 0 to Day 14). Consequently, the reduction of nitrate was highest in the 800gm^{-2} duckweed loading rate for the raw ABR but no significant difference in the other dilutions (comparing 600 and 800 gm^{-2} duckweed loading densities). For the 400gm^{-2} loading density, consistent increase in water nitrate was observed, except for Omnia and 25% ABR dilution (**Figure 3.11**).

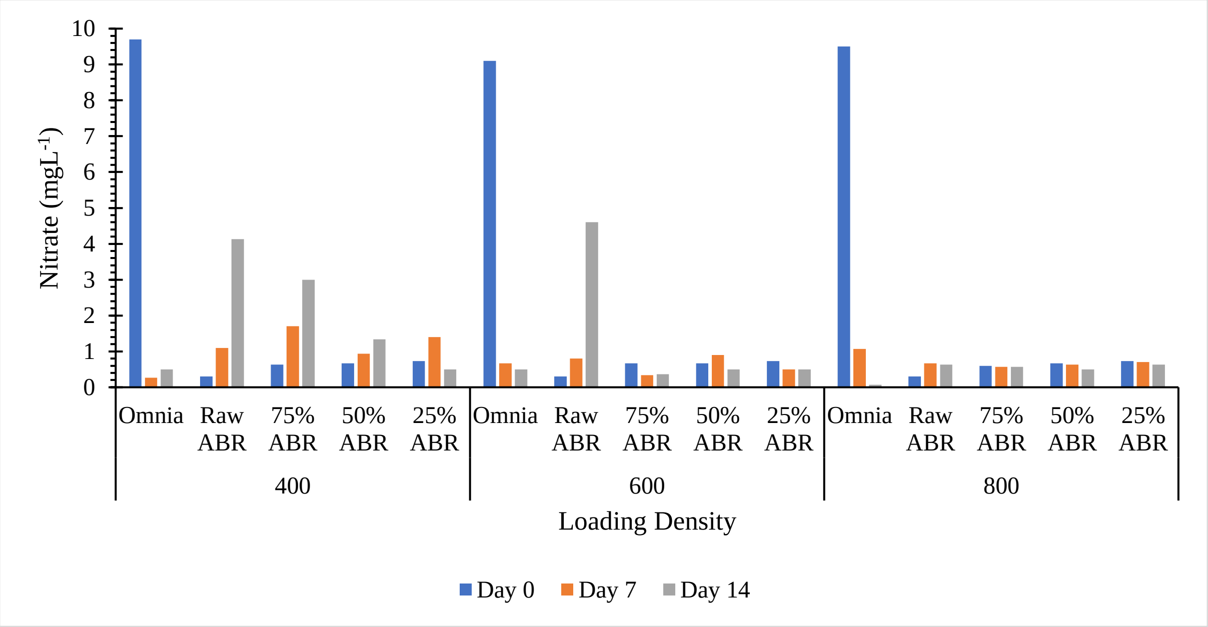

Figure 3.11 Residual water nitrate as affected by both duckweed loading densities and ABR dilutions at the three duckweed loading rates.

Orthophosphate-P

Residual orthophosphate was influenced by both loading densities and ABR dilutions. For each duckweed loading rates, the initial concentrations of orthophosphate in each ABR treatment were the same (i.e. initial concentration of orthophosphate in the control, raw ABR,75% ABR, 50% ABR and 25% ABR dilutions for the 400, 600 and 800gm^{-2} were the same at 4.1, 11.5, 7.5, 4.5 and 11 mgL^{-1} respectively).

A significant reduction in orthophosphate concentrations was observed in all the treatments except for the raw ABR in the 400 gm^{-2} duckweed loading density where the orthophosphate concentration increased when sampled on Day 14 compared to Day 7. However, significant differences were not observed in the 75% and 50% ABR dilutions for the 400, 600 and 800 gm^{-2} duckweed loading densities. Comparing the three duckweed loading densities, no significant differences were observed when the 25% ABR dilutions were compared.

Removal of OP from ABR dilutions was such that in Omnia, raw ABR, 75% and 25% ABR dilutions, for the 600 and 800 gm^{-2} loading density, were not significantly different (**Figure 3.12)**.

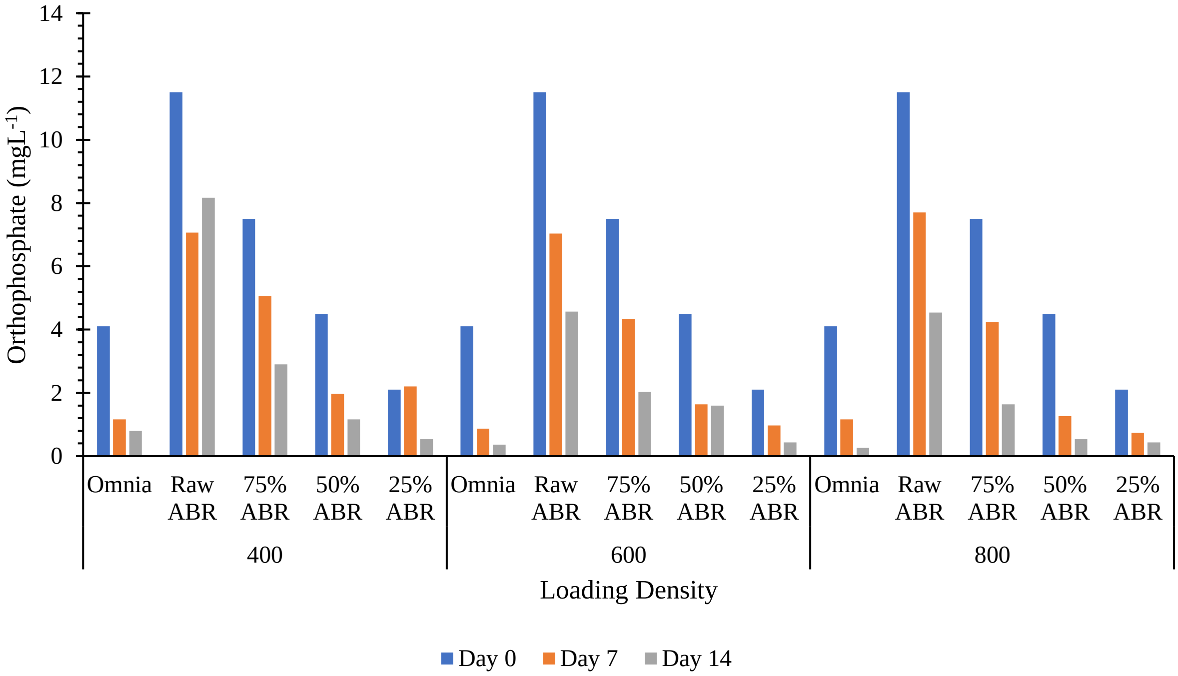

Figure 3.12 Residual water orthophosphate as affected by both duckweed loading densities and ABR dilutions

Percentage removal of Mineral-N and orthophosphate-P from water as affected by duckweed loading densities and ABR dilutions

Mineral N (ammonium and nitrate) percentage removal (equation 3.1) in the Omnia, raw ABR and 50% ABR dilution treatments for the duckweed loading density 400gm^{-2} was significantly lower than both the 600 and 800gm^{-2}. Nevertheless, the percentage removal of mineral N for 75% ABR and 25% ABR dilutions did not differ for 600 and 800gm^{-2} duckweed loading densities.

Orthophosphate P, removal was significantly lower in the 400gm^{-2} duckweed loading density (compared to both 600 and 800 gm^{-2}) for all the ABR dilutions used, except in the 25% ABR dilution which did not differ significantly in all three duckweed loading densities (Table 3.8).

Table 3.7 Percentage removal of Mineral-N and orthophosphate-P from water as affected by duckweed loading densities and ABR dilutions

Loading densities (gm^{-2})	**Removal (%)**				
	Omnia	**Raw ABR**	**75% ABR**	**50% ABR**	**25% ABR**
Mineral-N					
400	80 def	71 f	82 cde	79 ef	92 a
600	89 abc	83 bcde	89 abc	93 a	93 a
800	97 a	88 abcd	97 a	95 a	91 ab
Orthophosphate-P					
400	80 bcd	29 g	61 f	74 de	75 de
600	91 ab	60 f	73 de	64 ef	79 cd
800	94 a	61 f	78 d	88 abc	79 cd

Means with different letters significantly differed in each column and row for each parameter. Mineral N (ammonium and Nitrate)

3.4.5 Elemental mass balance

Nitrogen

The initial mineral N in the treatments were 63.4, 49.6, 31.6 and 16.5 (raw ABR, 75% ABR, 50% ABR, 25% ABR dilutions). For the 400 gm^{-2} duckweed loading density, a duckweed uptake of 24.7, 20.3, 26.1 and 16.2 mgL^{-1} respectively, was observed. Residual N, was highest in the raw ABR (18.2 mgL^{-1}) which was higher than the other dilutions (75% ABR, 50% ABR and 25% ABR (8.3, 1.9 and 1.5mgL^{-1}) respectively. The mass balance difference (equation 3.2) for N in the 400gm^{-2} duckweed loading density for raw ABR (17.7mgL^{-1}), 75% ABR dilution (17.8 mgL^{-1}), 50% ABR dilution (3.8 mgL^{-1}). In the 600 gm-2 duckweed loading density, the uptake of N in the raw ABR, 75% ABR, 50% ABR and 25% ABR dilutions were 37.4, 26.2, 34.3 and 14.2 mgL^{-1} respectively. The residual N concentration in the raw ABR, 75% ABR, 50% ABR and 25% ABR dilutions were 10.7, 4.7, 1.2, and 1.1 mgL^{-1} respectively, giving a mass balance difference of 36.7, 15.5, 1.2, and 1.2 mgL^{-1}. Nevertheless, for the 800gm^{-2} duckweed loading density, N uptake was observed to be lowest in the 25% ABR dilution (15mgL^{-1}) whereas raw ABR, 75% ABR and 50% ABR dilutions did not differ significantly (30.3, 28.9 and 30.8 mgL^{-1}). Consequently, it was observed that the uptake of N was highest at the 50% ABR dilution (34.3 mgL^{-1}) for the 600 gm^{-2} as compared with the 400 gm^{-2} (26.1 mgL^{-1}) and the 800 gm^{-2} (30.8 mgL^{-1}) (Figure 3.13).

Phosphorus

The initial orthophosphate concentrations in the raw ABR, 75% ABR, 50% ABR and 35% ABR dilutions were 11.6, 7.6, 4.2 and 2.2 mgL^{-1} respectively. The uptake of P was however, significantly higher for the raw ABR treatment in the 600gm^{-2} duckweed loading density when comparing the 400 (9.3 mgL^{-1}), 600 (9.9 mgL^{-1}) and 800gm^{-2} (7.1 mgL^{-1}) duckweed loading densities. The residual P was nevertheless lowest in the 800gm^{-2} duckweed loading density at 50% ABR dilution (Figure 3.13).

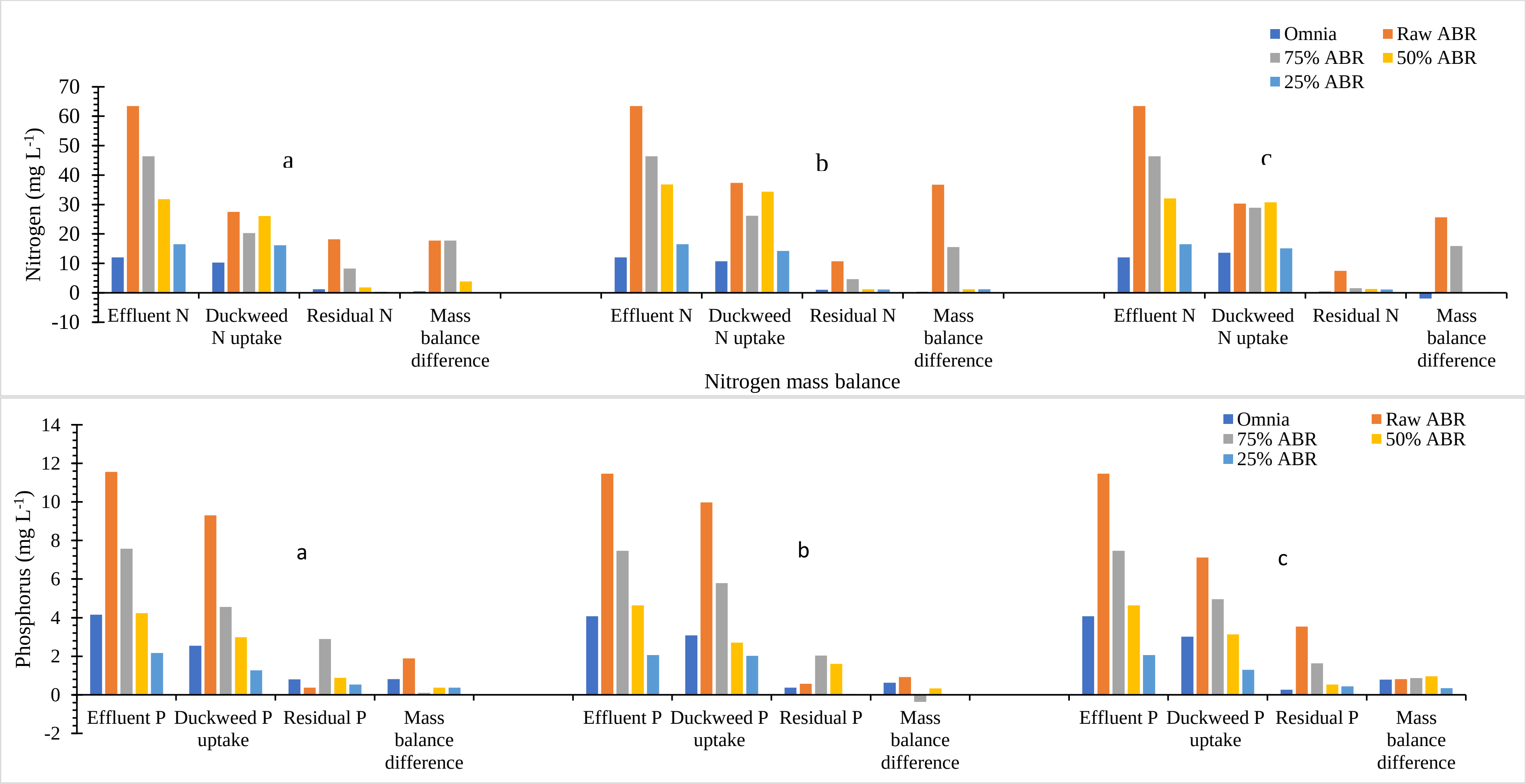

Figure 3.13 N and P mass balances for (a) loading density 1 (400 gm^{-2}), (b) loading density 2 (600 gm^{-2}) and (c) loading density 3 (800 gm ^{2})

3.5 DISCUSSION

This study investigated the effects of duckweed-loading density and dilutions of ABR effluent on biomass accumulation, uptake of N and P by common duckweed and the quality of the residual water. The results showed that common duckweed growth in the raw ABR and 75% ABR dilution was limited as a result of high levels of ammonia and salts in solution which negatively affected duckweed growth. Chin et al. (2011), reported optimum N at start, for duckweed (*Lemna* species) growth to be 20-60 mg N/l. However, the raw ABR used in this study contained higher ammonium content (63mgN/l) than the concentrations reported for optimum growth. According to Leng (1999) high free ammonia (> 60mg N/l) has toxic effects on duckweed. A distinct difference in biomass growth in the present study was, however, seen between the dilutions of ABR (50% and 25%) and the raw and 75% ABR dilution. The increase in biomass in the diluted ABR was linked to the excess root growth. When nutrients are depleted common duckweeds' response is the production of excessive roots (> half and inch long) to access nutrients (especially P) at greater depths (Ericsson et al., 1982, Barks and Laird, 2015), resulting in increased biomass (Iqbal, 1999, Chin et al., 2011). Wendeou et al. (2013) reported that electrical conductivity > 1.2 dS/cm led to a decline in relative growth rate of duckweed species. The low biomass accumulation in raw ABR and 75% ABR dilution was primarily due to the toxic effects of high N and high electrical conductivity levels which resulted in necrosis and death of the duckweed, particularly in the 400 and 800gm^{-2} duckweed loading rates. The poor growth in the 800gm^{-2}, was also as a result of high nutrient competition in the multiple layers of duckweed and poor light penetration. It was possible, that the layers formed as a result of the high loading density used, which limited nutrient availability and increased competition in the duckweed plants in the upper layers, while light (Clatworthy and HARPER, 1962) and/or CO_2 limitation (Driever et al., 2005) reduced growth in the lower layers. Nevertheless, since necrotic fronds were observed, it was not likely that nutrient limitation was a factor in growth retardation and frond death in the raw ABR and the 75% ABR dilutions, but a combination of high nutrient concentrations and overcrowding (overloading).

The higher dry matter and N and P uptake in the 600 and 800 than in the 400 gm^{-2} loading density was a result of greater number of fronds (at least 600 gm^{-2}), which reproduced and resulted in higher biomass (Chaiprapat et al., 2005). The better response in terms of dry matter accumulation to loading density in the 50% ABR dilution compared to the raw ABR suggests that the 50% dilution could be the critical concentration, such that high loading densities lowered the ammonia and salt concentration (EC), resulting in higher biomass accumulation. The reduction in water EC and pH observed in the ABR solutions may be associated with the uptake of ammonium-N and phosphate by duckweed. The highest initial N and P coupled with the least uptake of the nutrients in the raw and 75% ABR dilution than in the other treatments, may explain the higher residual ammonium-N and P levels in the water. In these treatments (raw ABR and 75%), more N and P were taken up, but less growth was observed. This may be due to the "lag period effect" (Cheng et al., 2002a, Cheng et al., 2002b) whereby duckweed, takes up N and P without associated growth. This indicated that duckweed removed nutrients and stored them in the tissue for later use (Chaudhary et al., 2014, Ruigrok, 2015).

Greater biomass accumulation and N and P uptake from the dilute ABR effluent (25 and 50%) resulted in lower residual ammonium-N and P at the end of the experiment. These findings indicate that the effluent needs to be diluted to at least 50% to reduce the negative effects of high ammonia and EC, and promote duckweed growth and N and P removal from ABR effluent.

Duckweed treatment removal efficiency for NH_4-N in raw ABR, 75%, 50% and 25% ABR dilutions were 78- 89%, 86-98%, 83%-96%, 95-96% reduction respectively. This was similar to the findings of Nhapi (2004), Chaudhary et al. (2014) and Verma et al. (2014) in separate studies. The residual water ammonium concentrations in the raw ABR (400 gm^{-2} loading density - 18.2 mg/l, 600 gm^{-2} loading density - 10.7 mg/l and 800 gm^{-2} loading density - 7.5 mg N/l), still did not meet the Department of Water Affairs disposal standards of 3mg/l (DWA, 2013). Nevertheless 50% (1.7 mg/l) and 25% (0.6 mg/l) ABR dilutions (600 gm^{-2} loading density) met the stipulated special discharge limit into water resources (DWA, 2013). Orthophosphate removal efficiencies ranged from 28% (400 gm^{-2}) to 88.2% (800 gm^{-2}) in the raw ABR dilutions. These results were comparable to findings of Mohedano et al. (2012) and Muradov et al. (2014a) in separate studies. Orthophosphates in residual water (ranged from 0.3- 8.9 mg/l) in all ABR concentrations, and met the general wastewater disposal limit (10mg/L) stipulated by the Department of Water Affairs South Africa (DWA, 2013).

The main reason for the poor uptake in the raw ABR effluent was low biomass yield. Noting that the plant nutrient uptake is dependent on both biomass yield and plant tissue elemental composition (Vymazal, 2007). The removal of nitrogen and phosphate were observed in all the experimental units. However, to determine the efficiency of duckweed nutrient uptake, which was nutrient consumption per unit of biomass was used. The nutrient uptake was derived as the product of the tissue nutrient content and biomass, rather than only the decline in the nutrient concentration of the ABR dilution. This method eliminated losses of nutrients through environmental losses and shows the uptake of nutrients by the duckweed, disregarding nitrogen and phosphorus forms. The uptake of N and P were low in the raw and 75% ABR as a result of low yield, as previously discussed. The uptake graphs (Figures 3.4 and 3.7) for N and P showed uptake of N (28-38 mg/l) and P (3.5-5 mg/l) as affected by ABR dilutions and an uptake of N (20-40mg/l) and P (3-6 mg/l) respectively, as affected by loading densities. The uptake may have been poor because nutrient uptake happens only at the water surface (Journey et al. 1999). One of the important limiting factor for the uptake of nutrients using duckweed is the slow diffusion of nutrients from the lower depths of the medium (Chaiprapat et al., 2003). Duckweed uptake of N was nevertheless, efficient in the 50%, 25% ABR dilution and Omnia fertilizer for 400 gm^{-2} (53.7, 71.5 and 90 %), for 600 gm^{-2} (43.7, 100 and 100%), but in the 800 gm^{-2} loading density, N uptake in the 50 and 25% ABR dilution and Omnia fertilizer were 50, 56.3 and 74.2 % respectively. The removal of P was through direct plant uptake. In both the 25% ABR and Omnia fertilizer, there was an addition of N (25% ABR dilution for 600 gm^{-2}) which could not be explained. Duckweed growth continued after a complete depletion of nutrients (N and P) in the ABR, which could imply that duckweed utilized its internally stored nutrients for growth (Chaiprapat et al., 2005).

In addition to improving water quality by removing N, P and salts, duckweed also reduced turbidity (92- 97%) and chemical oxygen demand in raw ABR and diluted ABR. Microbial actions within the duckweed root region, biodegrading organic particles (Iqbal, 1999), and the absorption of dissolved solids by duckweed roots (Patel and Kanungo, 2010), could have aided the reduction of water turbidity. This agreed with Lu (2009) findings, that the reduction in turbidity levels was an indication of the absence of phytoplankton and algae.

Reduction in Chemical oxygen demand (COD) was poor at 50%, which was consistent with Nhapi (2004). The COD concentration in the residual wastewater for the raw ABR did not meet standards for disposal stipulated by Department of Water Affairs. (2010). Nevertheless, the 75%, 50% and 25% ABR dilutions met the disposal standards.

The results indicated that biomass accumulation, removal of N, P, micronutrients and bases from the ABR effluent and hence the subsequent duckweed tissue nutrient concentrations was observed to be highest in the 50% ABR dilution and $600gm^{-2}$ loading density. In addition to removal of N and P, duckweed also removed K, Ca, Mg from the ABR effluent. These plant tissue concentration values (especially N and P) could be supply the essential N and P for plant growth depending on plant nutrient requirements and the soil fertility status. Applying the dry duckweed biomass provided N and P for ryegrass growth, which was utilized for biomass production. For instance, growing perennial ryegrass using duckweed as nutrient source in Cartref soil, would supply sufficient N and K but insufficient P, when applied as an N source. The insufficient P can be augmented with mineral P fertilizer or by increasing the duckweed application rate which provides excess N but sufficient P. Potassium, calcium and magnesium were also sufficient in the duckweed plant tissue. These salts are easily leached in acidic and sandy soils.

3.6 CONCLUSION AND RECOMMEMDATION

Duckweed biomass was generated which in turn removed sufficient mineral N and orthophosphate P from the ABR effluent. Duckweed growth reduced effluent turbidity and COD, to levels within the South African discharge standard limits, apart from ammonium concentrations for both the raw ABR and the 75% ABR dilution. However, the 600gm^{-2} duckweed loading density had optimum initial duckweed inoculum, which generated a biomass similar to the 800 gm^{-2} loading density. Nevertheless, N uptake, tissue nutrient compositions and low residual water N and P were consistently low in the optimal loading rate (600 gm^{-2}). This treatment combination was best since there was no overcrowding, duckweed necrosis or death.

It is however recommended that;

1. For more efficient reduction in COD and ammonium contents of the ABR effluent, the experiment could be extended.
2. Alternative uses for the harvested duckweed biomass, such as its use as fertilizer, animal feed, biooils and so on, could be explored.

Chapter 4 : EFFECTS OF DUCKWEED BIOMASS AS A PLANT NUTRIENT SOURCE ON DRY MATTER YIELD AND NUTRIENT UPTAKE BY PERENNIAL RYEGRASS (*Lolium perenne.* L)

ABSTRACT

A reduction in the use of inorganic fertilizers could be possible if nutrient rich dry duckweed biomass grown on nutrient rich ABR effluent can supply sufficient nutrients for crop growth. It was hypothesized that dry duckweed biomass would provide sufficient nutrients to meet perennial ryegrass growth requirements even with sequential cuttings. Thus, the objective of the study was to determine the effects of applying duckweed biomass from anaerobic baffled reactor (ABR) effluent, as both nitrogen and phosphorus sources for perennial ryegrass on dry matter yield, nutrient uptake of perennial ryegrass and the soil residual nutrient concentrations. Pot experiments with three duckweed treatments (i.e. duckweed used solely as a source of nitrogen -DWN, excess duckweed applied to meet ryegrass requirements for phosphorus – DWP, and duckweed applied as a source of nitrogen + mineral phosphorus – DWN+P (to augment for the low phosphorus content of the dry duckweed biomass). Also, three inorganic fertilizer treatments; which were commercial fertilizers – first, was nitrogen, phosphorus and potassium applied at ryegrass required rates (nitrogen applied at 200 kg N/ha, phosphorus applied at 80kg P/ha and potassium applied at 30kg K/ha. These brings the treatments to six. The experiment was set up in a randomised complete block design with five replicates. The pots contained two kilograms (2kg) soil packed in 2 litre non-draining plastic. The first three were duckweed- based treatments applied at (1) 200kgN/ha (duckweed as a source of N (DWN), (2) 80 kg P/ha P (duckweed as a source of P (DWP), which invariably supplied excess N, and (3) DWN augmented with mineral P (DWN+P). Sodium dihydrogen phosphate (NaH_2PO_4: 25.83% P) was applied at the rate of 50 kg P/ha, to correct for possible P deficiencies in DWN. The other three treatments were inorganic commercial fertilizers which were NPK, PK and K applied at recommended rates based on rye grass plant requirements for cartref soil. Nitrogen was applied as urea, K was applied as KCl and P was applied as NaH_2PO_4. Statistical analyses were done using GenStat 17th Edition. The data collected were subjected to analysis of variance (ANOVA) using the repeated measurements to assess the effects of nutrient sources on dry matter yield, plant parameters, tissue content/composition of the herbage at each cut. Residual soil analyses results were subjected to one-way ANOVA. Treatment means were subjected to Tukey's test and means were compared at 5% level of significance. The use of duckweed as a source of P (DWP) was not significantly different in terms of biomass yield when compared with the NPK control. The uptake results for nitrogen and phosphorus in the treatment where duckweed was applied in excess to meet phosphorus demand i.e. DWP, were comparable to the commercial fertilizer treatment applied at ryegrass recommended rates (i.e. NPK treatments). The duckweed as a source of N (DWN) had significantly lower uptake of N and P when compared with the NPK treatment. A limited N and P uptake was observed in the K treatment. The addition of mineral P to the duckweed treatment had little to no effect on tissue uptake of both N and P and growth characteristics. Significant differences ($p<0.01$) were observed between duckweed treatments and inorganic commercial fertilizer treatments in terms of plant uptake of Ca, Mg and Mn. Soil residual N and P were lower in the duckweed treatment. The findings of this work suggest that duckweed grown on ABR effluent could be a valuable organic source of N and P for crops.

Keywords: Anaerobic baffled reactor, duckweed biomass, nutrient source, perennial ryegrass

4.1 INTRODUCTION

Anaerobic baffled reactor (ABR) effluent contains high concentrations of nitrogen and phosphorus which can be removed through nutrient absorption by the aquatic plants. This provides a means for nutrient recovery, as well as providing a way of freshwater restoration, with wider ecosystem benefits (Quilliam et al., 2015).

Aquatic plants such as water lilies, water hyacinths, reeds, wood lettuce and duckweeds species have the ability (with varying efficiencies) to remove N and P, salts and heavy metals from wastewater (Rusnam and Efrizal, 2016). Even though the ABR effluent is low in heavy metals, as it originates from households, further treatment with aquatic plants such as duckweed, may be an alternative for removal of salts, N and P prior to wastewater discharge and reuse in irrigation agriculture.

In this study (Chapter 3) duckweed (*Lemna* species) have shown the ability to rapidly accumulate biomass with the highest uptake of N and P in the 50% dilution with a loading density of 600 g m^{-2} adding significant improvement in water quality. The duckweed tissue composition was 0.31% N, 0.45% P and 1.45% K. The generated duckweed biomass could be beneficial as a source of plant essential nutrients. Several articles in the literature have shown incorporation of high quality plant residue improves soil nutrient availability (Bot and Benites, 2005, Partey et al., 2013, Tully et al., 2015). These organic residues have high nitrogen and low polysaccharides, aliphatic biopolymers, tannins (KoÈgel-Knabner, 2002), lignin and polyphenols contents (Palm et al., 2001). The high quality residues can be used as nutrient sources without further addition of N fertilizers (Palm et al., 2001). The C: N ratio in aquatic plants such as duckweed (10:1) (Meyers and Doose, 1999) is lower than most terrestrial crops (higher than 20:1) (Stewart, 2010). The high N, low C:N, and lack of cellulose and lignin in aquatic plants, when compared with their terrestrial counterparts (Meyers et al., 1999), could result in rapid decomposition (Partey et al., 2014). Information on the use of duckweed dry matter as nutrient source for plants is limited in the literature. The duckweed grown on ABR effluent in Chapter 3 showed high levels of tissue N (3.1%), suggesting that it can rapidly decompose and mineralise the N. Tissue P content was 0.45%. If applied as a fertilizer based on N requirements, the duckweed biomass would not supply sufficient P. Applying the biomass as a source of P, would supply excess N. It may however, be essential to augment the P content in the duckweed by adding inorganic P fertilizer to make up for the difference in P.

It was hypothesized that duckweed biomass would provide sufficient nutrients and sustain growth of plants. The objective of this study was to evaluate the effects of applying duckweed biomass, grown on ABR effluent, as a source of N and P on dry matter yield and nutrient uptake of perennial ryegrass, and residual soil properties.

4.2 MATERIALS AND METHODS

4.2.1 Experimental site

A pot experiment was conducted in a tunnel at the Newlands Mashu Research site (29°58'S; 30°57'E) in Durban, South Africa. The tunnel had maximum and minimum air temperatures of 34^{o}C and 19^{o}C, respectively, with relative humidity ranging from 65 to 80%.

4.2.2 Soil collection and preparation

The soil used in this experiment was classified as the E horizon of a Cartref soil form (Soil Classification Working Group., 1991) (Typic Haplaquept in Soil Taxonomy). The soil was collected from 0-30 cm depth of an arable field at KwaDinababuko, close to Hillcrest in Durban, South Africa. The soil was air dried and ground to pass a 5 mm sieve (Manson and Roberts, 2000). The soil was analysed for various parameters according to methods described below, at Soil Fertility and Analytical Services Division KwaZulu-Natal Department of Agriculture, CEDARA.

Soil particle size distribution and texture

Soil samples collected were ground and passed through a series of sieves with different diameters; 2.0-0.05 mm (sand), 0.05-0.02 mm (silt) and <0.002 mm (clay). Hydrogen peroxide was used to oxidise the organic matter in a 20g soil sample (<2 mm). The sample was however, made up to 400 ml by adding de-ionized water and left to sit overnight. The clear solution above the soil was removed. Sodium hydroxide (NaOH) and sodium hexametaphosphate Na $(PO_3)_6$, dispensing agents, were added and the sample was stirred on Hamilton® Beach stirrer. The suspension is made up to 1 litre in a measuring cylinder and the clay (<0.002 mm) and fine silt (0.002-0.02 mm) fractions measured with a pipette after sedimentation. Fine silt plus clay was measured after 4-5 minutes (depending on temperature) at 100 mm, and clay was measured after 5-6 h at a depth of 75 mm. The sand fractions which included very fine sand (0.05 - 0.10 mm), fine sand (0.10 - 0.25 mm), medium sand (0.25 - 0.50 mm) and coarse sand (0.50 - 2.0 mm) were determined by sieving. Coarse silt (0.02-0.05 mm) was also estimated.

After the determination of the particle size distributions of the soil, the textural class was determined from the textural triangle, which define particle size limits of the various textural classes. The different soil compositions were expressed as percentages and compared to the USDA textural classification chart (Soil Classification Working Group., 1991).

Soil pH using KCl

In determining soil pH in KCl, where 10 g of soil sample was scooped into sample cups. Twenty-five millilitres (25 mL) of 1 M KCl solution were added to the soil, and the suspension was stirred at 400 revolutions per minute for 5 min using a multiple stirrer. The suspension was allowed to stand for about 30 minutes before pH was measured using a combination glass electrode while stirring (Manson et al., 2000).

Soil carbon and nitrogen content

Soil total C, N concentrations were analysed (Manson et al., 2000), using the Automated Dumas dry combustion method as described by Matejovic (1996), using a LECO CNS 2000 (Leco Corporation, Michigan, USA).

Ambic-2 extracting solution for extraction of P, K, Zn, Cu, Mn containing 0.25 M NH_4CO_3 + 0.01 M Na_2EDTA + 0.01 M NH_4F+ 0.05 g L^{-1} Superfloc (N100), adjusted to pH 8 with a concentrated ammonia solution, was used to extract P, K, Zn, Cu and Mn. Phosphorus was determined using a modification of the Murphy and Riley (1962) molybdenum blue procedure (Hunter, 1974). Potassium, Zn, Cu, Mn, Ca and Mg were determined by atomic absorption spectroscopy (Manson et al., 2000) (**Table 4.1**).

Table 4.1 Characteristics of Cartref Soil

Properties *	**Unit**	**Composition**
P	mg/kg	0.7
K	$Cmol_c$/kg	0.02
Ca	$Cmol_c$/kg	0.51
Mg	$Cmol_c$/kg	0.32
Exch. Acidity	$Cmol_c$/kg	0.33
Total Cations	$Cmol_c$/kg	1.19
Acid saturation	%	19.4
pH (KCl)	-	4.0
Zn	mg/kg	0.14
Mn	mg/kg	1.41
Cu	mg/kg	0.35
Organic C	%	0.5
Total N	%	0.08
Clay	%	11

Soil analysed at Soil Fertility and Analytical Services Division, KwaZulu-Natal Department of Agriculture, Cedara.

4.2.3 Duckweed biomass

Common duckweed (*Lemna minor L.*) was grown at a loading rate of 600g/m^2 in diluted anaerobic baffled reactor (ABR) effluent in a halved Jojo® tank that contained 75litres of water and 75litres of ABR effluent (50% dilution). This generated 705 g dry matter of duckweed after 14 days.

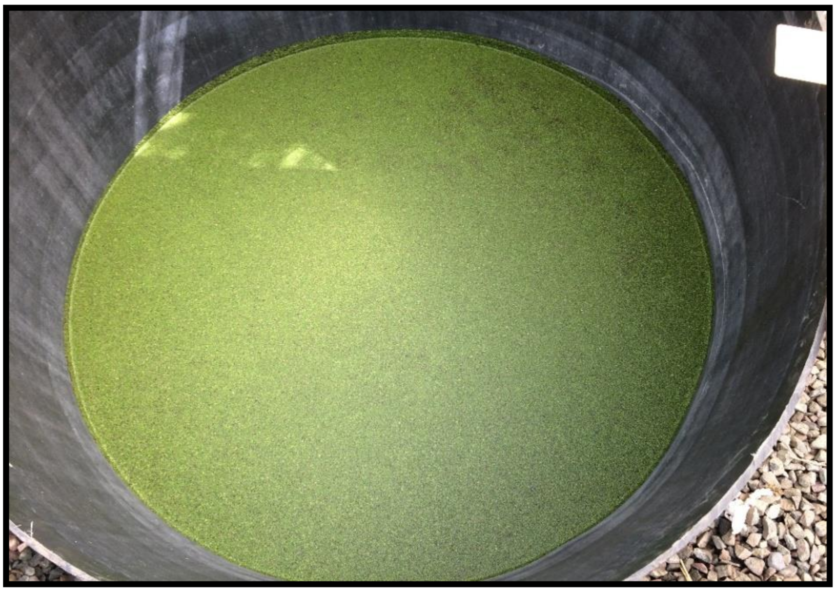

Figure 4.1: Duckweed biomass growing in 50% ABR effluent

The duckweed biomass DWN was dried at 60^{o}C for 72h and analysed for tissue N, using the Leco TruMac CNS autoanalyser. The duckweed was also analysed for P and K concentrations using an inductively coupled plasma atomic emission spectroscopy (ICP-AES) after digestion with nitric acid. The duckweed tissue contained 31.3g N/kg, 5.0 g P /kg and 14.5 g K /kg (NPK) and Ca, Mg, Mn, Zn and Cu contents were.1.67 mg Ca/kg, 0.42 mg Mg/kg, 8129 mg Mn/kg, 202.1 mg Zn/kg and 10.9 mg Cu/kg respectively. This informed the amount of N and P that were needed.

4.2.4 Experimental design

The pot experiment consisted of three duckweed treatments and three inorganic fertilizer treatments as controls. The experiment was set up in a randomised complete block design with five replicates (totalling thirty experimental units). Two kilograms (2kg) of Cartref soil was packed in

2 litre non-draining plastic pots used for the experiment. The nutrients were added based on recommendations from the Soil Fertility and Analytical Services Division KwaZulu-Natal Department of Agriculture (CEDARA) after soil tests. Organic nutrient source used in this experiment was the dry duckweed biomass. The first three treatments were duckweed- based treatments, which were (1) duckweed biomass applied at N recommended rate (200kgN/ha) - **DWN**, (2) duckweed biomass applied as a source of P (80kgP/ha), which invariably supplied excess N (**DWP**) at 417kg N/ha. (3) duckweed biomass was augmented with mineral P (**DWN+P**) using sodium dihydrogen phosphate (NaH_2PO_4: 25.83% P). This was applied at the rate of 50kg P/ha, to correct for possible P deficiencies in DWN. Phosphorus is highly deficient in the Cartref soil (Murphy, 2014) (Table 1) and limiting in duckweed biomass, the third treatment puts into consideration the inadequate P inadequacy, hence the addition of mineral P.

The three inorganic fertilizers treatments were NPK (N applied at 200kg N/ha, P applied at 80 kg P/ha and K applied at 30 kg K/ha), PK (P applied at 80 kg P/ha, K applied at 30 kgK/ha and N was not applied) and K (N and P were not applied, K was applied at 30kg K/ha) applied at recommended. Nitrogen was applied in the form of urea, K was applied as KCl and P was applied as NaH_2PO_4. The trial plan is shown in Table 4.2.

Table 4.2 Trial plan showing experimental design

BLOCK 1	DWN	DWP	DWN + P	NPK	PK	K
BLOCK 2	DWP	PK	DWN + P	DWN	NPK	K
BLOCK 3	K	DWP	NPK	DWN+P	PK	DWN
BLOCK 4	DWN	K	DWN + P	NPK	PK	DWP
BLOCK 5	K	PK	NPK	DWP	DWN+P	DWN

DWN- Duckweed biomass (N- source) – recommended N rate; DWP- Duckweed biomass (P source) - recommended P rate, DWN + P- Duckweed biomass (N source) + mineral P (applied as NaH_2PO_4); N- Nitrogen applied as urea; K- Potassium applied as KCl; P- Phosphorus applied as NaH_2PO_4

The duckweed was pre-incubated for two weeks, after which , TN (NO_3^- + NH_4^+) in soil samples from duckweed-biomass (DWN and DWP) treatments were analysed using standard methods described by Maynard et al. (1993). A preliminary study was done on mineralization rates and the results indicated that sufficient mineralization of N in the duckweed biomass occurred within two weeks.

Planting of Perennial ryegrass

Perennial ryegrass (*Lolium perenne* L., cultivar Bronsyn) was planted at double the recommended rates (40 kg ha^{-1}) in the pots on the 16th of November 2016 and the experiment was terminated on the 16th of January 2017 (after 60 days).

Fresh mass and dry mass

Ryegrass was harvested periodically when it grew to a height of 20cm and it was harvested 5 cm to the soil surface. At each harvest, fresh mass of the plants was determined by weighing the biomass on a standard laboratory scale, followed by drying at 60°C for 72 hours to obtain dry matter yield.

Soil analyses

Soil samples were collected from all pots after the last harvest of the ryegrass to determine soil chemical and physical characteristics. These analyses were carried out at the Fertilizer Advisory Service, KZN Department of Agriculture and Environmental Affairs; Soil Fertility and Analytical Service, CEDARA.

4.3 DATA ANALYSIS

All statistical analyses were carried out using GenStat 17th Edition (Payne et al., 2014). The data collected were subjected to analysis of variance (ANOVA) using the repeated measurements to assess the effects of nutrient sources on dry matter yield, plant parameters, duckweed tissue nutrient content/composition of the herbage at each cut. Residual soil analyses results were subjected to one-way ANOVA. Treatment means were subjected to Tukey’s test and means were compared at 5% level of significance.

4.4 RESULTS

4.4.1 The effects of duckweed-biomass on perennial ryegrass dry matter yield

Significant increase in ryegrass cumulative DM yield was observed when duckweed biomass was applied as a P-source (DWP) (3.2g/pot) than as an N source (2.5g/pot) (**Figure 4.2**). The cumulative DM in the NPK treatment was not significantly different when compared with DWP. Additional P in the DWN +P treatment did not result in a significant increase in DM when compared to the DWN (**Figure 4.2**). Dry matter yield in duckweed treatments were significantly higher than that observed in the controls PK and K treatments. (**Figure 4.2**).

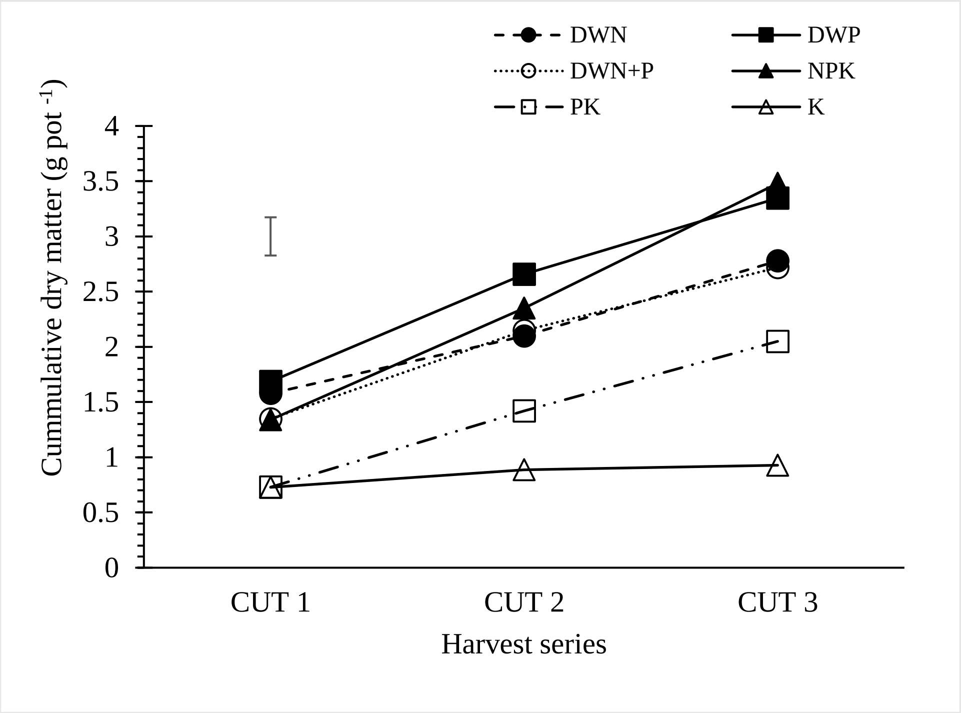

Figure 4.2 Cumulative dry matter yield of perennial ryegrass in response to duckweed as a nutrient source. DWN-Duckweed as N source, DWP- Duckweed as P source, DWN+P – DWN with mineral P. NPK, PK, and K represents inorganic nutrient sources. Error bar = $p<0.01$.

4.4.2 Effects of duckweed biomass on plant nutrient uptake of nitrogen, phosphorus, and potassium by perennial ryegrass

The least uptake of N by perennial ryegrass was recorded for the K treatment (inorganic fertilizer treatment which had no N and P) and it significantly differed from DWP (duckweed biomass as a P source) and NPK (inorganic fertilizer) treatments (

Table 4.3). In the plant uptake of P there were no significant differences in the duckweed treatments, and the inorganic fertilizer treatments, except for K treatment which had the lowest P uptake. There was no significant difference between uptake of K in the DWP treatment and the NPK treatment. However, DWP had significantly higher uptake of N, P and K than those observed for the K treatment. (Table 4.3).

Table 4.3 Effects of duckweed biomass application on N and P uptake by perennial ryegrass

Nutrient Source	N uptake (mg/pot)	P uptake (mg/pot)	K uptake (mg/pot)
^DWN	`84.2ab	7.7bc	136.1 bc
DWP	106.1 b	10.4bc	193.4 d
DWN+P	87.6 ab	12.3c	136.0 bc
NPK	115.1 b	21.4 c	161.2 cd
PK	65.1 ab	6.2ab	83.5 ab
K	39.1 a	2.1 a	64.5 a

^DWN- duckweed as N source, DWP -duckweed as P source, DWN+P -duckweed as N source + mineral P. `Means followed by different letter(s) in each column, are significantly different

4.4.3 Effects of duckweed biomass on uptake of calcium, magnesium and micronutrients by perennial ryegrass

No significant differences were observed in all the duckweed treatments and the NPK fertilizer in terms of Ca uptake (Table 4.4). No statistical differences were observed in the duckweed and fertilizer nutrient sources for Mg uptake. However, K treatment, which had the significantly lower uptake (7.1 mg/pot). The uptake of Mn was significantly higher in the duckweed treatments than in the controls (PK and K treatments). Comparing duckweed treatments (DWN and DWP), Mn uptake was significantly higher than the NPK treatment, which did not differ statistically from Mn uptake observed in the DWN+P treatment (Table 4.4). Zinc uptake did not differ significantly between duckweed treatments, but it was lowest in the K treatment. However, only in the DWP treatment was the uptake of Cu significantly higher than the K treatment (Table 4.4).

Table 4.4 Effects of duckweed biomass application on Ca, Mg and micronutrient uptake by perennial ryegrass

Treatment	Elemental uptake (mg/pot)				
	Ca	Mg	Mn	Zn	Cu
^DWN	16.5 bc	12.6 b	0.7 de	0.20 bc	0.018 ab
DWP	18.8 c	15.3 bc	0.9 e	0.23 c	0.023 b
DWN+P	16.2 bc	13.2 b	0.6 cd	0.17 bc	0.018 ab
NPK	13.8 bc	12.6 b	0.4 bc	0.20 bc	0.019 ab
PK	11.3 ab	8.8 b	0.23 ab	0.14 b	0.015 ab
K	7.1 a	4.7 a	0.12 a	0.07 a	0.008 a

^DWN- duckweed as N source, DWP -duckweed as P source, DWN+P -duckweed as N source + mineral P. ` Means followed by different letter(s) in each column, for each parameter are significantly different

4.4.4. Effects of duckweed biomass application on post-harvest soil chemical composition

Table 4.5 below shows the P values for residual soil properties after the harvest of perennial ryegrass. Residual soil pH (KCl), extractable P, exchangeable Ca, extractable Mn and Cu contents significantly decreased after harvest. Total C and N, exchangeable K, Mg and acidity, and extractable Zn were not affected by the treatments.

Table 4.5: P-values for the effects of duckweed biomass application on residual soil properties after perennial ryegrass harvest

Properties	P values
**P (mg/kg)	<0.001
K (Cmol$_c$/Kg)	0.099
*Ca (Cmol$_c$/Kg)	0.017
Mg (Cmol$_c$/Kg)	0.512
Exchangeable Acidity (cmolc/Kg)	0.925
*pH (KCl)	0.017
Zn (mg/Kg)	0.312
**Mn (mg/Kg)	<0.001
**Cu (mg/Kg)	<0.001
Organic C (%)	0.547
N (%)	0.755

*denotes $p<0.05$ level of significance, **denotes $p<0.01$ level of significance

4.4.5 Residual soil pH and nutrient composition after perennial ryegrass harvest

Among the duckweed treatments no significant differences were observed in soil pH. However, comparing the DWP treatment with the PK treatments, significant differences were observed in soil pH. (Table 4.6). Of the duckweed treatments, only the DWP had higher residual P than the negative control (K treatment). The residual soil P in the NPK and PK treatments were significantly higher when compared with the duckweed treatments (Table 4.6). Residual Ca between duckweed treatments were comparable to that for the NPK treatment. Only the DWP treatment had higher residual soil Mn than the negative controls (**Table 4.5**). Residual soil Cu was highest in DWN (0.80mg/kg), compared to all other treatments.

Table 4.6: Residual soil pH and nutrient composition after perennial ryegrass harvest

Treatment		Residual nutrient (mg/kg)			
	pH (KCl)	P	Ca	Mn	Cu
^DWN	*4.92 ab	9.81ab	1.46 ab	11.32 ab	0.80 c
DWP	5.06 b	12.31b	1.71 ab	19.45 b	0.63 b
DWN+P	4.92 ab	9.47ab	1.72 ab	6.48 a	0.55 ab
NPK	4.85 ab	49.78d	1.45 a	4.70 a	0.47 a
PK	4.76 a	20.64c	1.74 ab	3.43 a	0.54 ab
K	4.97 ab	4.23a	1.79 b	2.12 a	0.42 a

^DWN- duckweed as N source, DWP -duckweed as P source, DWN+P -duckweed as N source + mineral P. ` Means followed by different letter(s) in each column, for each parameter are significantly different

4.5 DISCUSSION

Nitrogen (N) and phosphorus (P), are limiting plant nutrients for optimal crop yield for crops grown on infertile soils (Rita et al., 2013) such as, Catref soil (Soil Classification Working Group., 1991). The duckweed amended soil surfaces were kept moist and under favourable soil temperatures, which aided plant tissue (organic matter) decomposition (Baldock and Skjemstad, 1999, Bot et al., 2005, Lamb et al., 2014). The higher dry matter yield of perennial ryegrass in the duckweed treatments, compared to the negative control (K treatment) could be explained by increased availability of N, P and K upon decomposition of the duckweed residues. The duckweed and the NPK treatments had more available nutrients which resulted in greater dry matter yield. The lower cumulative dry matter (DM) yield of ryegrass in DWN (and DWN+P) treatments than in the DWP could be explained by greater N and K availability from the decomposition of duckweed applied at a higher rate. However, K uptake appeared to explain the results more than N uptake, although both were applied at higher rates than recommended (Table 4.3). The N in the DWN and DWP treatments were applied at 200 kg N/ha and 472kg N/ha respectively, whereas P in the DWN and DWP were equivalent to 30 kg P/ha and 80kg P/ha. The similarity of dry matter yield between the DWP and the NPK treatments suggest that the decomposition of duckweed in the DWP treatment mineralised sufficient N, P and K for ryegrass growth, to achieve similar yield to the inorganic fertiliser.

The supplementation of the duckweed biomass (DWN+P) with mineral P, had no marked effects on the DM yield of perennial ryegrass, when compared to the DWN and the commercial fertilizer NPK treatments. This suggested that P was not limiting in the duckweed treatments. This was in line with the findings of Burkitt et al. (2010) and Findlay (2010), which suggests that at the lowest application levels of P, ryegrass growth was not limited, indicating that soil P was sufficient. The higher dry matter in the DWP than the DWN+P (similar P levels) could be explained by higher N and K levels, in the duckweed dry matter (DWP). Potassium uptake and not N uptake results supported this view. Although N, P and K uptake in the PK treatment were not significantly higher than in the K treatment, the presence of P explains the higher ryegrass yield compared to the K treatment.

The lower N uptake in the duckweed treatment pots compared to the NPK treatment was because duckweed decomposed quickly before ryegrass was planted. This may have led to losses of N. According to Janssen (1984) and Mary et al. (1996) plant residues decompose at varying rates. The composition of duckweed tissue (mainly of higher simple sugars, amino acids, and proteins (instead of cellulose, lignins and melanins), explains its quick decomposition rates when compared with vascular plants (Iqbal, 1999, Leng, 1999, Verma et al., 2015). The quick decomposition may have, however, lead to N losses as ammonia before planting and during the growth phase.

In addition to providing available N, P and K, decomposition of duckweed in soil also provided available Ca, Mg and micronutrients which were taken up by the ryegrass. This view was supported by the higher uptake of Ca, Mg, Mn, Zn and to some extent Cu, particularly in the DWP where the duckweed was applied at the highest rate.

The higher residual soil pH in the DWP than the PK and K controls could be a result of ammonia production during decomposition of residues applied at a high N rate (Baldock et al., 1999, Lamb et al., 2014). While nitrification could lower pH, that effect was not significant in this study. The residual soil analysis showed that P, in the duckweed treatments did not significantly differ. However, the DWP treatment retained 12.31mg/kg soil residual P, since P in the treatment became unavailable for plant uptake. The soil residual P was similar in the duckweed treatments but higher than the K control treatment. The P added by decomposition of duckweed remained in that soil while the other proportion was taken up by ryegrass. Nevertheless, the residual P could benefit the next crop. However, the subsequent crop would require additional N, P and Zn which were exhausted by the ryegrass (in all treatments) apart from DWP (where large quantities of N, P and Zn were added). The greater the uptake, the greater the likelihood of exhaustion. The same applied for Mn and Cu, where all the treatments will also require additional Mn and Cu except for DWP where large quantities of duckweed (containing excess Mn and Cu) was added (Table 4.6).

The PK treatments, which had lower ryegrass DM yield retained higher P in the soil. The poor growth ryegrass in the PK treatment could explain the poor uptake and the excess P retained in the soil. The K treatment was deficient in N and P, it markedly had the lowest DM yield and soil residual P. The higher Cu and Mn in the residual soils of DWP may be as a result of the initial high plant tissue Cu and Mn content of the biomass of DWP (Table 4.4) used for the experiment.

4.6 CONCLUSIONS AND RECOMMENDATION

Addition of duckweed biomass grown on ABR effluent, increased dry matter and uptake of a variety of mineral elements when compared to the PK and K controls, particularly when added at high levels as a P source.

Duckweed biomass can be used as an N source with mineral P fertilizer supplementation or directly as a P source. It is however not productive to use duckweed solely as a plant N source without supplementation with mineral P. If duckweed was used to meet the P requirement for ryegrass it supplies N at a much higher rate. This implies that N is likely to be lost in two main forms; as nitrates through leaching and as nitrous oxides through denitrification processes depending on the presiding soil conditions.

It is recommended that:

1. Further experimentations should be conducted on the possible use of Duckweed dry matter to grow other crop types, on different soil types, under different climatic conditions.
2. Residual soil physical and chemical properties of soils fertilized using duckweed dry matter as nutrient source should be compared with the with soil properties from commercial fertilizers for various soil types and conditions.

Chapter 5 : GENERAL DISCUSSION AND CONCLUSIONS

5.1 GENERAL DISCUSSION

This study investigated the effects of duckweed loading density and the dilutions of ABR on biomass accumulation and the uptake of nitrogen and phosphorus from ABR effluent. The study further evaluated the use of the dry duckweed biomass as an N and P nutrient source for perennial ryegrass growth. It was important, however, to factor in the chemical characteristics of the ABR effluent (in the raw ABR and the dilutions). This was because the effects of duckweed growth and N and P removal would determine the conclusions with regards to duckweed use to remove N and P from ABR effluent. Therefore, analyses of the ABR effluent was conducted to determine its physical and chemical properties. The study was conducted to determine the duckweed loading density and ABR dilution which provided the optimum biomass accumulation, tissue N and P uptake and lowest residual mineral N and orthophosphate (Chapter 3). The results showed that increased duckweed loading density increased dry matter yield, also, as ABR dilutions increased, dry matter yield also increased. This implied that an initially high duckweed loading density increased duckweed final biomass, while very high concentrations of nutrients (N especially) in the raw and 75% ABR dilutions could have impeded the growth of the duckweed (Figure 3.3). It was observed that both duckweed loading density and ABR effluent dilutions had effects of the biomass accumulated and the tissue uptake of N and P. Interestingly, the tissue uptake of N and P were comparable in all four ABR effluent treatments.

The effects of duckweed growth on water turbidity was affected both by duckweed loading density and ABR dilutions. It was observed that ABR turbidity was reduced significantly both through microbial degradations that occurred in the root zones of the duckweed plant and sedimentation (Zaidi, 2007).

Duckweed growth on the ABR effluent also reduced the electrical conductivity (EC) of the water (which is an indicator of water salinity or total salt content (Atekwanaa et al., 2004)). The reduction in EC after treatment with duckweed could be explained by the uptake of salts (Chapter 3), which occurred in ABR in ionic forms (Wendeou et al., 2013). As an example, NaCl and K_2SO_4 are not removed by the treatments of water and wastewater using conventional water and wastewater-treatment methods (Morrison et al., 2001), but were removed using duckweed.

Duckweed loading density and ABR dilutions had significant effects in the reduction of chemical oxygen demand in the effluent. In the more recent South Africa Water Quality Guidelines for domestic, recreational or aquatic ecosystem uses (DWAF, 2010) water quality discharge criteria for COD was set to 65-75 mg/l. The COD levels in water after duckweed treatment was reduced by biosorption of organic matter and consequently biodegradation process (Bassuney and Tawfik, 2017). The duckweed released oxygen into water improving water oxygen levels (Zaidi, 2007). These reduced levels improved water quality and disposal could be beneficial to the ecosystem.

Nevertheless, with ABR effluent, nutrient recovery depends on concentration. Concentrations >75% of original, limits growth possibly due to salinity (EC) and high concentration of ammonia (Chin et al., 2011), suggesting that ABR needs to be diluted to ≤ 50% of the original concentration. Loading density was also essential with an optimum of 600 g/ m^2 for *L.minor* in ABR effluent, from the results of this study. The combination accumulated highest tissue N and P concentrations and uptake. The high N and P in the duckweed tissue grown on ABR makes it a possible source of organic fertiliser material.

In chapter 4, the effects of using dry duckweed biomass (at different rates) as a nutrient source for perennial ryegrass growth was further investigated. The duckweed dry matter was used to grow perennial ryegrass which was harvested sequentially over a period. Dry duckweed biomass was applied to the soil at three different rates (as a source of N (deficient in P), as a source of P, and as a source of N+ mineral P). Planting was done in November 2016 and final harvest was done in January 2017. In the duckweed treatments biomass reduced after a few harvests, which could be as a result of nutrient depletion in the duckweed treatment (duckweed used as an N source). The results indicated significant growth in the duckweed treatments which was comparable with the control treatment (at recommended rate -NPK). The Low C: N ratio plus high nutrient composition suggested rapid decomposition. Although the nutrients were made available, it was only when the duckweed was used as a P source that it matched the NPK fertiliser, and yet soil residual N was comparable to negative control, suggesting some losses could have occurred possibly through ammonification in some loci enriched with duckweed in the soil.

The similar residual soil P in duckweed treatments to the control suggested that the available P supplied by the duckweed was exhausted but any other P remaining could have been in forms that were not extractable. The similarity in residual soil N between the NPK treatment and the duckweed treatments suggested that no organic N from duckweed remained in the soil.

A preliminary study on the sequential extraction of P from soil amended with duckweed (Chikuvire, 2017), showed an increase in Al and Fe phosphate, with all three elements originating from the duckweed. The duckweed tissue used in this study contained 8129, 1255, 1944 mg/ kg of Mn (0.81%), Al (0.13%) and Fe (0.19%) respectively. The P content was 0.4% and as such there was a high likelihood of precipitation especially in the acidic pH range. This contribution was absent where duckweed was not added. The high concentration of these basic elements (Mn, Al and Fe), bases and micronutrients (Cu and Zn) in the tissue of duckweed, indicates that duckweed removes these elements from wastewaters, including ABR effluent, improving the water quality for discharge into surface water bodies, while producing an organic fertiliser supplying multiple elements.

In addition to uptake of N and P, the high dry matter yield by the perennial ryegrass in the duckweed treatments could also be explained by high uptake of K, Ca, Mg and micronutrients (Mn, Cu, Zn). The increased uptake of these nutrients could be explained by their increased availability as a result of decomposition of duckweed residues in the soil. Although the duckweed was grown on ABR effluent to remove N and P, it also took up other elements. The higher residual soil Ca, Cu and Mn in the duckweed treatments could be explained by their high concentrations in the duckweed tissue, which provided more than enough for uptake by perennial rye grass.

5.2 CONCLUSION, RECOMMENDATION AND FUTURE STUDIES

Nitogen and P were reduced in the ABR effluent. The maximum removal of N and P, biomass accumuation, tissue concentrations and uptake of N and P and lowest residual water concentrations was in the 50% ABR dilution and 600gm^{-2} loading density combination.

The residual water quality was within the South African disposal standards for othophosphate and mineral N. Chemical oxygen demand and turbidity were substantially reduced to meet discharge standards of Department of Water Affairs and Fisheries (2010) in all dilutions but the raw ABR.

Nitrogen, phosphorus, potassium, micronutrients and bases in the duckweed tissue sustained the growth of ryegrass with or without addition of mineral P. The use of duckweed to remove N and P from ABR effluent, and the use of the duckweed dry matter as a nutrient source for ryegrass, is a promising idea. Using duckweed dry matter as a source of plant nutrient P is recommended for use because the yield was comparable to the commercial NPK fertilizer.

Futher investigations should focus on the use of duckweed to further purify ABR effluent under different field and weather conditions. It is important to investigate the effects of duckweed biomass on different soil and crop types.

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