NUTRIENT RECOVERY FROM WASTEWATER IN INTENSIVE AGRICULTURAL SYSTEMS BY DUCKWEED AND THE VALUE OF THE BIOMASS AS AN ORGANIC FERTILISER

By

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To the Infinite Intelligence.

Shrouded in profound mysteries,

Unravelling some to the appointed,

Transfixing intricacies of design and sophistication of art!

ABSTRACT

Intensive agricultural production systems in South Africa face major challenges of solid and liquid waste disposal. Leakages from these systems lead to high nutrient loads in proximate water bodies resulting in tipping of aquatic ecosystem balance. This scenario is manifested by proliferation of algal blooms and aquatic macrophytes including duckweed in the Midlands region of KwaZulu-Natal, South Africa. Nutrient recovery from water and back to land for their reuse is a critical stage of closing the loop on their transfer from anthropogenic sources. The general objective of this study was to investigate the potential of duckweed *Wolffia arrhiza* to recover nutrients from enriched water bodies and reuse the biomass as an organic source of plant nutrients. Duckweed coverage in The Midlands region was examined using Google Earth observations followed by ground-truthing where sites were randomly selected. Samples of duckweed and water were collected for species identification and laboratory analyses. Soil samples for mineralisation and green house pot experiments were collected from Baynesfield Estates (29°45'S and 30°20'E) and Ukulinga farm of the University of KwaZulu-Natal (29°39'S, 30°24'E). Effects of soil type and application rates of *W. arrhiza* biomass from Baynesfield on N and P release were determined using a completely randomised design with three replicates in a constant temperature room. Pot experiments were laid out in randomised complete block design in a glasshouse where effects of rates of application of *W. arrhiza* biomass and pre-incubation periods of *Lemna minor* and *W. arrhiza* on Swiss chard (*Beta vulgaris*) biomass yield and nutrient uptake were determined. Culturing of *Wolffia arrhiza* in swine lagoon water (SLW) to ascertain effects of nutrient concentration, its replenishment and harvest regimes for duckweed biomass quantity and quality were carried out in a growth room controlled environment. Plastic containers were laid out in a completely randomised block design with three replicates.

Water pH for all habitats was similar (pH 8.1) and N_{min} (0.85-1.98 mgL^{-1}) supported duckweed growth. Water concentration of K, Ca, Mg and P explained occurrence of duckweed genera in a habitat. Low K (<10 mgL^{-1}) favoured occurrence of duckweed species in separate habitats while generally higher levels favoured coexistence. While *Wolffia* spp. occurred on water with lower K level than sole *Lemna* spp., it had similar or higher tissue content of this element than sole *Lemna* spp. Sole *Lemna* spp. thrived in water with relatively high Ca (>12 mgL^{-1}) and Mg (> 8 mgL^{-1}) while sole *Wolffia* spp. occurred at low levels of these nutrients. Concentration of N and P in water explained tissue composition when samples of a genus were compared in sites they occurred separately. Duckweed tissue content of Cu, Zn, Fe, Mn and Al varied with habitat type while it was similar for genera that coexisted. Findings suggested that nutrients such as K, Ca and Mg in water were important in determining occurrence of a duckweed genus and that the potential to accumulate nutrients from water by the two genera maybe exploited for soil N and K improvement.

At least 5% of the total N was produced as ammonium on the first day of incubation of duckweed amended soil, with peak production within the first two weeks. Nitrate-N and mineral-N increased from days 14 to 42 with corresponding decrease in ammonium-N. Soil of relatively low inherent fertility mineralised N at a slower rate than that of higher fertility. At least 16% to about 40% of duckweed P was extractable from soil on the first day of incubation and progressively declined over the incubation period. Fractionation of P at the end of incubation period showed accumulation of Al and Fe phosphates as rates increased. Findings suggested that soil type and rate of *W. arrhiza* biomass are important determinants for N and P supply to crops.

Application of *W. arrhiza* biomass increased Swiss chard dry matter by 23-45% compared to the negative control. The positive control (urea at 100 kg N ha^{-1} rate) had highest Swiss chard biomass. Higher rates than 100 kg N ha^{-1} had no added benefit on dry matter accumulation and nitrogen

uptake of Swiss chard. Pre-incubation of duckweed for 28 days improved nutrient uptake resulting in higher dry matter than shorter periods. The Swiss chard dry matter after pre-incubation for 28 days was similar to that from urea application. Findings from this study suggested that duckweed is a resource with beneficial use for nutrient supply to vegetables especially when appropriate rates are used with pre-incubation.

Purposeful culturing of *W. arrhiza* showed that dry biomass and average growth rate of *W. arrhiza* were not affected by slurry lagoon water (SLW) replenishment periods unlike C content. Dry matter and average growth rate of *W. arrhiza* significantly decreased with increasing concentration of SLW in the order 5 >10 >15%. As SLW concentration declined from 15 to 5%, the duckweed N content declined while C and C/N content increased as period between solution replenishment increased. Frequency of harvesting did not affect the N content and uptake by duckweed and NH_4^+ -N and mineral-N of the SLW concentration over the duration of the study. Harvesting duckweed once per week generated higher duckweed growth rate and biomass, than at twice a week. Findings from the study suggested choice for optimization of growth conditions for quality or quantity of duckweed biomass at <15% SLW.

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Increased intensity of industrial and agricultural activity, in response to economic growth and changing consumption patterns, generates plenty of wastes and wastewater worldwide. The wastes can present an intractable global environmental concern through contamination of soil, air and water. Agricultural wastes include manure and other wastes from farms, poultry houses and slaughter houses (Glossary of Environmental Statistics, 1997). Harvest wastes, fertiliser runoff from fields, pesticides that enter the water bodies and salt and silt drained from fields are also included. Intensive agricultural production systems generate considerable amounts of wastes in both developing and developed countries. Most farmers in South Africa rely on the traditional disposal strategy of application of agricultural wastes on croplands as organic fertiliser (Adediran et al., 2003; Bolan et al., 2010; Lutge and Standish, 2013). Over application of nutrients on lands encourages their movement to receiving waters by runoff and leaching through permeable soils to vulnerable aquifers resulting in eutrophication. Excess nutrients movement from agricultural enterprises can also occur through leakage from poorly constructed lagoons, overflow of lagoons and runoff from farm fields during major precipitation events after recent applications of waste. In order to deal more carefully and strictly with pollution issues in South Africa, a new set of environmental legislation was created (Government Gazette, 2009). This spells out the need for a nutrient management plan that details amounts of wastes produced on daily and annual basis on a specific site. Wastewater disposal regulations were introduced which emphasize the removal of nutrients from wastewater intended to be discharged into rivers, water courses, and estuaries (Department of Water Affairs and Forestry, 1996). Despite the legislation, there is evidence of environmental

pollution, which includes eutrophication of surrounding ecosystems. Surface water bodies have increasingly been colonised by algal blooms and other obnoxious water weeds, such as hyacinth (Isikhungusethu Environmental Services, 2012), indicating compromised water quality. Of special note are aquatic macrophytes called duckweed, which elicit a controversial perspective from communities whose waterbodies they colonize.

The structural peculiarities, composition and chemical characteristics of duckweed species in addition to their direct impact on the environment, make them attract a great deal of public remonstration and conversely, interest from business and diverse researchers. Duckweed thrives in nutrient rich environments of still or slow moving water bodies, such as lagoons, ditches, canals, ponds and dams (Goopy and Murray, 2003). The growth rate of duckweed may be near exponential under ideal environments (Cheng and Stomp, 2009; Hasan and Chakrabarti, 2009). Duckweed propagates vegetatively by producing clusters of daughter fronds, from mature fronds, that push towards the open water surface. It rapidly spreads its photosynthetic mat, accumulating biomass at a rate greater than most plants including field crops (Landolt and Kandeler, 1987). Doubling times of duckweed population vary with species and environmental conditions, from as short as a day, with many species taking 2-3 days (Chang et al, 1977). Under adverse conditions, such as low temperature or desiccation, modified fronds called turions appear, which sink to the bottom of the water body and resurface when the habitat conditions are favourable. Duckweed can prevent growth of other plants by shading them with its dense mats creating anaerobic environments for rooted aquatic macrophytes as well as reducing phytoplankton abundance (Landesman et al., 2011). This also makes the habitat inhospitable for fish and other aquatic organisms. Duckweed can cause massive destruction of underwater ecosystems by adding organic matter in the water body through dead and anaerobically decomposing biomass and emission of obnoxious odours (Landesman et al., 2011). For these reasons, duckweed is discredited for general loss of

aesthetic value, recreational amenities and poor ecological water quality. However, literature demonstrates duckweed's immense capacity to accumulate nutrients, heavy metals, phenols, pesticides, dioxins and pathogens from water (Zayed et al., 1998; Iqbal, 1999; Cheng et al., 2002; Xu et al., 2012; Van der Spiegel et al., 2013). This capability opens up opportunities for exploitation of the aquatic plants by transforming what is generally considered a waste or nuisance into a valuable product. This implies that suitable use of duckweed is an incentive for its harvesting from water bodies.

The role of macrophytes for phytoremediation of domestic wastewater, as food/feed supplement and for sustainable biofuel production has been a subject of research over the years (Dalu and Ndamba, 2003; Iqbal, 1999; Verma and Suthar, 2015) with various efficiencies dependent on species, environment and experimental set ups. The most widely distributed duckweed species throughout South Africa from the commonest, are *Lemna gibba*, *Wolffia arrhiza*, *Landoltia punctata*, *Lemna aequinoctialis* and *Lemna minor* (Cholo and Foden, 2006). The KwaZulu-Natal Province has the greatest diversity of duckweed, followed by Gauteng and Free State (Cholo and Foden, 2006). The Midlands region of KwaZulu-Natal Province is one of South Africa's principal agro-ecological regions (Hitayezu et al., 2014) with anthropogenic activities, such as industrial, human settlements and intensive agricultural production systems, with capacity to pollute water bodies (Isikhungusethu Environmental Services, 2012). However, no research has been done to provide information on how the different natural habitat characteristics influence duckweed species abundance and distribution.

In South Africa, agricultural production systems involve both animal and crop husbandry (Department of Agriculture, Forestry and fisheries, 2017). Pockets of intensive production systems with an accumulation of waste could act as sources of nutrients for depleted soil. Approximately 12% of the country has soil with low fertility (Van Barmann, c2010). Most of

the soil is marginal for crop production and less than 3% of South Africa is considered as high-potential land.

Most of small-scale farmers in South Africa have poor access to credit due to lack of collateral security. This scenario places financial constraints on their acquisition of inputs such as fertilisers. According to FAO (2005) and Cedric and Nelson (2014), synthetic fertilisers are too costly to be used in large quantities for profitable field production of staple crops for own consumption in most small-scale farmer situations in South Africa. Use of duckweed from domestic and agricultural wastewater from intensive production systems to supplement synthetic fertilisers in nearby small-scale areas could be an attractive option that reduces costs, at least on small-scale practice, and serve as a waste management option with additional benefits of improving water quality. There is a paucity of information regarding the use of duckweed as a source of plant nutrients globally yet there is potential. High accumulation of N, P, K, bases and micronutrients in duckweed biomass and lack of lignin (as is in vascular plants), (Iqbal, 1999; Ge et al., 2012; Kufel et al., 2012) could promote rapid decomposition and release of nutrients for crops. However, variation in chemical composition of duckweed (Hasan and Chakrabarti, 2009) could have implications on its use as a source of nutrients. There is limited information on the use of duckweed as a source of crop nutrients and its impact on crop and residual soil.

1.2 Problem Statement

Nutrient leakages from agricultural land especially with over application of nutrients and intensive livestock production systems, sewage systems, and decomposing organic matter all enrich water bodies and encourage growth of aquatic biomass that includes duckweed.

Duckweed has potential to upset the aquatic ecological balance through aggressive colonization. As incentive to purge the enriched water bodies, duckweed has to be harvested and turned into profitable products by way of exploring its alternative uses. The macrophytes are immense accumulators of nutrients including N, P and K with potential use of their biomass as nutrient sources for crops. Whilst a multiplicity of duckweed end uses has been highlighted in literature, there is a paucity of information on duckweed use as a soil fertility amendment. Effectiveness of duckweed as a soil amendment depends on availability of biomass and its nutrient release patterns on decomposition. There is dearth of information on nutrient release patterns of duckweed species. It is important to ascertain by duckweed species the general chemical characteristics such as nutrient composition, decomposition and release patterns of important nutrients into soil. Moreover, the response of crops to duckweed amended soils is generally unknown. The rapid growth and nutrient uptake of duckweed suggest that harvesting of the different duckweed species for use as an organic fertiliser could be viable, particularly in the Midlands region of KwaZulu-Natal. There is need to understand effects of harvesting regimes, wastewater concentration and replenishment on growth and biomass quality and quantity of specific duckweed species, particularly if there is proven potential for soil fertility improvement. Research is needed to understand the fertiliser value of the harvested biomass for its effective management.

1.3 General Objective

The general objective of this study is to investigate nutrient recovery from enriched water bodies by duckweed biomass and its potential of reuse as an organic fertiliser.

1.4 Specific Objectives

1. To determine the elemental composition of prevalent duckweed species and associated water from different types of farming enterprises.
2. To determine the effect of application rate of *W. arrhiza* biomass on N and P release, and P pools in soil.
3. To determine the effects of duckweed (*W. arrhiza and L. minor*) as a nitrogen source and pre-incubation period in soil on Swiss chard (*Beta vulgaris*) biomass and nutrient uptake.
4. To assess influence of nutrient concentration of swine lagoon water (SLW), its replenishment interval and duckweed harvest frequency on *W. arrhiza* growth performance and nitrogen uptake.

1.5 Hypotheses

1. Dominant duckweed species have similar elemental composition and flourish in water bodies with similar nutrient composition regardless of types of farming enterprises.
2. Nitrogen and P release patterns of duckweed *W. arrhiza* and soil P pools are not affected by rates of application of duckweed biomass.
3. Nutrient uptake and Swiss chard dry matter are not influenced by application rates and pre-incubation of *W. arrhiza* and *L. minor* duckweed biomass.
4. Nutrient concentration and, replenishment interval of SLW and harvest regimes do not influence *W. arrhiza* growth performance and nitrogen uptake of *W. arrhiza*.

CHAPTER 2: LITERATURE REVIEW

2.1 Nutrients from Agricultural wastes

Agricultural solid and liquid waste generation has continued to grow in response to economic growth and changing food consumption patterns world over. These wastes include solid and liquid manure and other wastes from farms, livestock houses and slaughter houses (Glossary of Environmental Statistics, 1997). Harvest waste, fertiliser runoff, pesticides that enter the water bodies, salt and silt drained from fields are also included. Intensive agricultural production systems are responsible for generating most of these wastes (Zhang et al., 2006; Sarker et al., 2009). For example in South Africa, poultry production increased significantly over the last twenty years (Van Barmann, c2010). Expansion of this industry generates large quantities of wastes, including manure, hatchery debris, dead birds, bedding material, wasted feed, feathers and in some cases soil. One bird (chicken) produces approximately 1 kg of fresh manure for each kilogram of feed consumed and a commercial layer produces about 20 kg waste per year (Vest et al., 1994). A 100-sow pig production unit in South Africa, which markets about 20 piglets per sow per year, produces about 1710 tons of undiluted waste per year, which has the same pollution potential as a town with 2 800 inhabitants (Agricultural Research Council, 2006). Including waste of washing water, the total annual waste production of such a unit could exceed 6000 tons per year. It is safe to infer that millions of metric tonnes of agricultural wastes are generated in South Africa annually, taking cognisance of other types of livestock husbandry under intensive production such as dairy, beef and crocodile farming. Optimum management of these wastes to derive maximum benefits while minimizing environmental challenges requires knowledge of their composition (Bolan et al., 2010). Livestock wastes have relatively high nutrient content.

Minerals such as Ca, P, K, Mg, S, and trace minerals such as Fe, As, Se, Cr, Cu, and Zn are added to feeds to meet dietary requirements of cattle, swine, and poultry (Zhang et al., 2006). Copper and arsenic are commonly used as growth promoters in swine and poultry production. Excess minerals are excreted in urine and dung. In South Africa, poultry manure contains 1.5 - 3.7% N, 1-1.5% P, 1.5-2.0% K, 1.5-3.0% Ca and 0.56-0.9% Mg (Van Ryssen, 2001; Adediran et al., 2003; Mkhabela, 2006). Swine manure also contains a mean of 1.5% N, 0.65% P, 0.82% K, 0.42% Ca, 0.37% Mg (Bolan et al., 2010). Generally, the chemical composition of manure and wastewater varies widely, depending upon factors such as animal species, age and condition of animal, diet, and handling and storage of the manure before it is spread on land (Mkhabela, 2006). Potential benefits to agriculture and the risks to the environment depend on how the wastes are managed on the farm.

2.2 Management of agricultural wastes and impact of excess nutrients on environment

Strategies to manage wastes include closed loop systems that recycle nutrients, where outputs from one production unit become inputs for another. Some features of these loops include practices to treat wastes before disposal, using aerobic or anaerobic lagoons, dehydration, composting, vermiculture, upstream catchment management systems in crop lands and application of wastes to crop lands or pastures (Kelleher et al., 2002; Agricultural Research Council, 2006; Mkhabela, 2006). Plant species have been used for recovery of excess nutrients loads in soils by preferentially bio-accumulating nutrients such as N, P or metals (Zhang et al., 2006). A fast nutrient uptake is important and can be obtained by choosing crops that establish rapidly after harvest of the main crop and deep root development enable nutrient uptake from deeper soil layers (Schoumans et al., 2011). Cool-season grasses and some legumes have a

higher uptake of nutrients, such as N and P, and may remove other specific nutrients under optimum growing conditions and sufficient nutrient supply. As dry matter yield increases, the amount of nutrients taken up by the crop increases, and if harvested, more nutrients can be removed from fields. Nutrient removal is accomplished only by removing forage as a hay crop and transporting the nutrients elsewhere, away from the application site (Zhang et al., 2006). Land application of livestock solid or liquid wastes on croplands as organic fertiliser is generally the traditional disposal method most farmers rely on (Adediran et al., 2003; Bolan et al., 2010; Lutge and Standish, 2013). Benefits of land application are based on the ability of these resources to improve soil properties such as organic matter content, nutrient availability, soil pH, cation exchange, water holding capacity and soil structure.

Poorly managed agricultural wastes have negative impacts on the natural environments with high potential to pollute the soil, air and water bodies resulting in general loss of aesthetic value, hence the global concern on the expansion and intensification of the agricultural production systems (Xu and Shen, 2011a; Smith et al, 1999; Viguria et al., 2014). According to Mateo-Sagasta et al. (2017) agriculture enterprises predominantly pollute water world-wide. It is generally confirmed that continuous application of the wastes as a disposal strategy, irrespective of crop requirements, result in reduction in quality of soil and water bodies (Burkholder et al. 2007; Lu et al., 2012; Shabalala et al., 2013).

In South Africa, some intensive production systems apply manure or slurry application on crop lands (Lutge and Standish, 2013), and over application of the wastes can overload soils with both macronutrients, such as nitrogen and phosphorus, which are key elements in eutrophication (Smith et al., 1999) of surrounding water bodies. Conversely, in intensive crop productions systems, farmers apply large quantities of synthetic fertilisers in order to maintain or improve productivity, and this practice increases the risk of nutrient losses. As such,

agricultural fields have been non-point sources of sediments and nutrients, such as N and P (Mateo-Sagasta et al., 2017).

In South Africa, The National Environmental Management Waste Act (NEMWA) of 2008 was established to deal more carefully and strictly with pollution issues, and it requires nutrient management plans giving details of daily and annual waste generation. However, farmers focus on production, disease control and logistics of the enterprise and not on issues related to environmental compliance and studying the legislation (Van de Merwe, 2013). This mismatch of legislative requirements and farmer practice can lead to major environmental pollution problems.

Recent evidence of environmental pollution includes acidification and eutrophication of surrounding aquatic ecosystems (Isikhungusethu Environmental Services, 2012; Shabalala et al., 2013), in addition to ammonia volatilization and emission of greenhouse gases. The mechanism of pollution from arable fields and livestock wastes occurs through runoff from feedlots and recent applications of wastes on land, leakage from poorly constructed manure lagoons, or during major precipitation events resulting in either overflow of lagoons or atmospheric deposition followed by dry or wet fallout. Over application of animal wastes or application to saturated soils can also cause contaminants to move into receiving waters through soil erosion and runoff, and to leach through permeable soils to vulnerable aquifers (Withers and Lord, 2002; Lu et al., 2012). Application to environments that do not favour crop production can promote loss of these nutrients. High nutrient loads on water are responsible for eutrophication of lakes, reservoirs, ponds, and coastal waters characterised by excessive aquatic plant biomass and algal growth (Quilliam et al., 2015). Nutrient enrichment in water, in addition to favourable environmental conditions, affects the community structure of aquatic plants.

2.3 Aquatic macrophyte boom and potential of nutrient recovery

There is a spectrum of tolerance to nutrient enrichment within the aquatic plant community where species distribution and abundance is dependent on water nutrient status (Table 2.1) that increases with concentration from oligotrophic, mesotrophic to eutrophic (Thiebaut, 2008).

Table 2.1 Water status based on average summer concentrations of Inorganic N and P.

Water status	Inorganic N	Inorganic P
	mgL^{-1}	
Oligotrophic	<0.5	<5
Mesotrophic	0.5-2.5	5-25
Eutrophic	2.5-10	25-250
Hypertrophic	>10	>250

Source: Department of Water Affairs and Forestry (1996)

Floating aquatic plants, such as water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), pennwort (*Hydrocotyle umbellate*), salvinia (*Salvinia rotundifolia*), azolla (*Azolla caroliniana*), and duckweed (*Lemna minor*), were widely studied for their potential ability to remove nutrients from wastewater (Reddy and Kadlec, 1983; Zhao et al., 2014a; Valipour et al., 2015). The studies showed varying efficiencies in removal and tissue accumulation of nutrients including N and P. Besides N and P, macrophytes contained high levels of macro and micronutrients, amino acids, starch and flavonoids (Culley and Epps, 1973; Xu et al., 2012; Tao et al., 2017). The distribution and diversity of macrophyte species is an important indicator of deteriorating water quality due to nutrient loading. Assimilation of nutrients by aquatic plant biomass provides an opportunity for recovery and reuse of 'lost' nutrients that can offer an alternative approach to aquatic restoration (Quilliam et al., 2015). Recently, studies on

duckweed have increased due to a multiplicity of potential end uses of its biomass. Harvesting of duckweed that is increasingly colonising water bodies, for example, in the Midlands region of KwaZulu-Natal Province (Isikhungusethu Environmental Services, 2012) could have potential for use as source of nutrients to crops.

2.4 Duckweed characteristics and impact on aquatic ecosystems

Duckweed's chemical composition, physical characteristics and impact on the aquatic environment has drawn interest from the public, business and diverse researchers. The macrophytes occur worldwide, except in waterless deserts and permanently frozen areas, with most diverse species in tropical and subtropical areas (Leng et al., 1995). The macrophytes are the world's smallest and simplest flowering plants of the family Aracea and sub-family Lemnaceae, comprising at least 40 species of which the major ones are from the genera *Spirodela*, *Lemna*, *Wolffia* and *Wolfiella* (Figure 2.1; Leng, 1999; Les et al., 2002). The fifth genus was proposed, on the basis of biochemical and DNA studies, to be *Landoltia* with the sole species *Landoltia punctata* formerly *Spirodela punctata*. Species from the genera *Spirodela* and *Lemna* have roots while those from *Wolffia* and *Wolffiella* do not (Leng, 1999; Goopy and Murray, 2003). The thalli are free-floating aquatic angiosperms rich in macro and micronutrients and have a crude protein content that ranges from 15% to 45% DM (Landolt and Kandeler, 1987; Zhang et al., 2014). They have flattened, minute leaf-like oval to round 'fronds' from about 1 mm to less than 1 cm across (Leng, 1999). The frond embodies a fusion of leaves and stems and represents the maximum reduction of an entire vascular plant (Landolt, 1986).

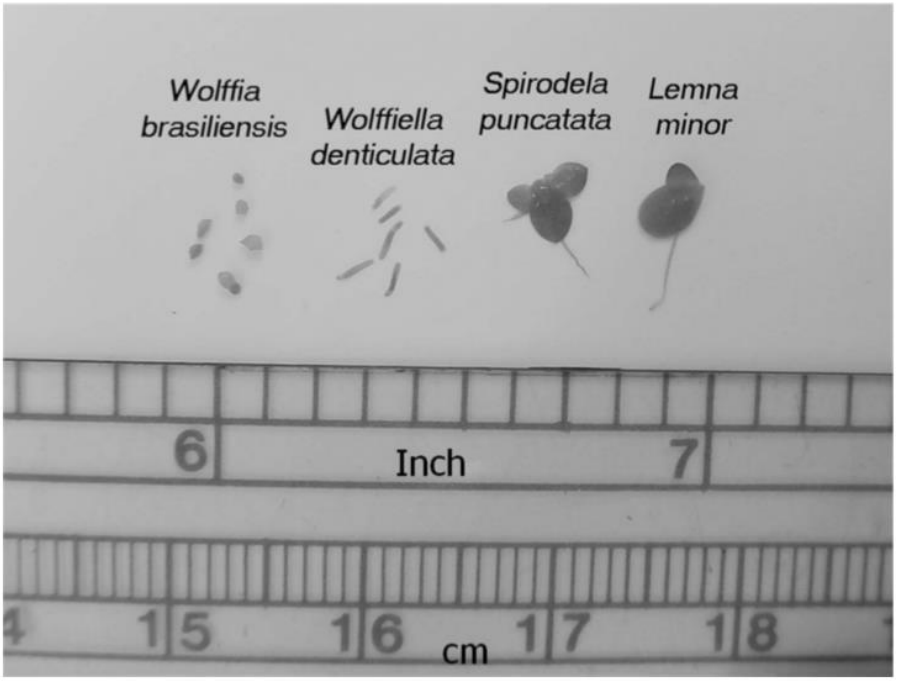

Figure 2.1 The size of duckweed species (Source: Cheng and Stomp, 2009)

Duckweed proliferates through vegetative propagation, when mature fronds produce clusters of daughter fronds that push towards the open water surface thereby spreading its photosynthetic mat. It accumulates biomass at a rate greater than most plants including field crops (Landolt and Kandeler, 1987). Duckweed's time to double its population varies with species and environmental conditions. This can be as short as 24 hrs though many species take 2-3 days (Chang et al., 1977). Modified fronds called turions, with relatively high starch content, appear for some many species under adverse conditions such as low temperature, desiccation or nutrient starvation, which sink to the bottom of the water body and resurface when the habitat conditions are favourable (Hasan and Chakrabarti, 2009).

2.4.1 Biophysical factors affecting occurrence and growth of duckweed

Duckweed thrives in nutrient rich environments of still or slow moving water bodies such as in sheltered lagoons, ditches, canals, ponds and dams (Mwale and Gwaze, 2013). The growth rate of duckweed may be near exponential if environmental conditions are satisfactory, though habitat requirements may differ with species (Cheng and Stomp, 2009; Goopy and Murray, 2003). Duckweed flourishes in a broad water temperature range of 6-33^{o}C with optimum of 25-31^{o}C (Iqbal, 1999; Leng, 1999) though Landolt (1986) reported a range of 20^{o}C and 30^{o}C and light intensity of 9000 lux. The lower and upper limit of water pH for growth of most species ranges from 3 to 10.5, with optimal values varying widely both between species and across colonies. The water pH influences nitrogen nutrition and availability of other minerals. The N, P and K concentrations of water supporting growth of Lemnaceae studied by Landolt (1996) ranged 0.003-43, 0.000-56 and 0.5-100 mgL^{-1}, respectively. Disparities in environmental conditions may affect the occurrence and distribution of duckweed. The most widely distributed species throughout South Africa from the commonest, are *Lemna gibba*, *Wolffia arrhiza*, *Landoltia punctata*, *Lemna aequinoctialis* and L*emna minor* (Cholo and Foden, 2006). The Province of KwaZulu-Natal has the greatest diversity of duckweed, followed by Gauteng and Free State. Most studies focus on culturing duckweed in laboratory and pilot scale experiments and there is scarcity of information on how natural habitat characteristics influence duckweed species abundance and distribution.

2.4.2 Impact of duckweed on aquatic ecosystems

While duckweed is influenced by habit characteristic, its presence can also alter the ecosystem function. Duckweed can prevent growth of other plants by shading them with its dense mats creating anaerobic environments for rooted aquatic macrophytes as well as reduce phytoplankton abundance (Landesman et al., 2011). The habitat becomes inhospitable for other aquatic organisms, especially fish. According to Anderson et al. (2002), duckweed can cause massive destruction of underwater ecosystems. Dead and anaerobically decomposing duckweed biomass not only adds to the organic matter in the water body but also emits obnoxious odours. Microbial decomposition depletes dissolved oxygen and this affects most organisms (Chislock et al., 2013). For these reasons, duckweed is discredited for general loss of aesthetic value, amenities for recreational purposes and poor ecological water quality. It is however important to highlight that duckweed is an important indicator of pollution and not the primary source of pollution. Farmers often consider duckweed as a nuisance on their farms and therefore make efforts to control the macrophyte using pesticides. Such efforts have not been successful, and could actually worsen the situation as it increases biological and chemical oxygen demand in the water, which is a challenge for aquatic life. Instead of viewing duckweed as a nuisance, farmers may need to view the macrophyte as a communication mechanism from the water, indicating to the farmer that the waste management strategy in use makes water eutrophic. The immense capacity of duckweed to accumulate nutrients, heavy metals, phenols, pesticides, dioxins and pathogens from water in a short period (Zayed et al., 1998; Gao et al., 2000; Cheng and Stomp, 2009; Fujisawa et al., 2010; Xu et al., 2012) opens up various opportunities for exploitation of the aquatic plants, of which selected ones will be reviewed.

2.5 Exploitation of duckweed

2.5.1 Duckweed for wastewater phytoremediation

Phytoremediation technologies are cost effective and environmentally friendly (Ali et al., 2016). In laboratory experiments, Zayed et al. (1998), measured accumulation of 13.3 g Cd kg^{-1}, 4.27 g Se kg^{-1}, 3.36g Cu kg^{-1}, 2.87g Cr kg^{-1}, 1.79 g Ni kg^{-1}, and 0.63 g Pb kg^{-1} by *L. minor* in nutrient solution. Miretzky et al. (2004) worked with *L. intermedia* and *L. minor* in laboratory experiments and measured high removal percentages of heavy metals in water except for Cr by *L. intermedia* (Table 2.2). These experiments demonstrated different capacity of duckweed species to recover specific nutrients from water, and may have potential in phytoremediation of wastewater and contaminated water.

Table 2.2 Removal (%) of metals from water by duckweed

Metal	*Spirodela intermedia*	*Lemna minor*
Fe	80.23	78.47
Zn	95.73	97.56
Mn	96.91	95.20
Cu	91.70	90.41
Cr	33.88	96.94
Pb	98.22	98.55

Source: Miretzky et al. (2004)

Conversely, hyper-accumulation of heavy metals from contaminated water by duckweed poses a potential danger (Leng, 1999) and the contaminants need strict monitoring so as not to enter the food chain. Management and disposal of heavy metal saturated duckweed from contaminated water remains a challenge. While high concentration of heavy metals in the culture solution, resulting in high tissue concentration, may militate against use of duckweed,

its susceptibility to toxic environments makes it play an important role in phytotoxicity tests (Wang, 1990; Radic´ et al., 2011).

Duckweed has shown great potential for polishing and valorization of wastewater nutrients due to its desired characteristics, such as high growth rates and multiple reuse options of biomass (Journey et al., 1993). Studies showed that duckweed genera and species or same species under different environmental conditions and experimental set ups have different capacities to remove nutrients from wastewater. Dalu and Ndamba (2003) studied the feasibility of using duckweed based wastewater stabilization ponds by introducing *L. minor* into the maturation ponds at 50% pond cover in Zimbabwe. The authors reported >60% reduction, to within permissible limits, in NO_3^-, Fe, dissolved solids and total suspended solids and conductivity of the effluent after monitoring for a period of 6-12 months. However, there were no significant reductions in phosphates, chemical oxygen demand (COD) and biochemical oxygen demand (BOD) and turbidity. In India, Priya et al. (2012) subjected domestic wastewater to treatment by *L. minor* after it initially underwent primary and secondary treatment on pilot scale. They reported orthophosphate and BOD reductions of 79.39% and 94.45%, respectively. These differences in efficiency of removal by the same species could be attributed to trials conducted without the same protocol (Goopy and Murray, 2003). A pilot scale waste treatment system with three duckweed ponds in series (5 days hydraulic retention time per pond) used by El-Shafai et al., (2007) stocked with *L. gibba* in Egypt, had removal values of 93, 96 and 91% for COD, BOD and total suspended solids (TSS), respectively in warm summer. Removal efficiencies for NH_3, total Kjedhal nitrogen (TKN) and total phosphorus (TP) were 98, 85 and 78%, respectively. However, their system was not efficient at removing nutrients during winter. In Southern Brazil, Mahedano et al. (2012) evaluated the efficiency of *L. punctata* in treating swine wastewater using two full-scale duckweed ponds in series over a period of 12 months.

The authors reported 96 and 89% removal of TKN and TP, respectively. From the above comparison, it is evident that different duckweed species under different environmental conditions have different capacities to treat wastewater. In general, though different experimental protocols were used, duckweed still showed its high efficiency in phytoremediation of wastewater, which is a cost effective technology.

As N is the major form in nutrient-rich wastewaters, duckweed has higher tolerance of NH_4^+ than other plant species making it suitable to exploit NH_4^+- N rich sources (Oron, 1994). Caicedo et al., (2000) observed that critical values for inhibition of duckweed growth depended on the combined effects of ammonia and ammonium ions on growth of *S. poylrhiza* of which the importance of one depended on the pH. At pH 5-8, maximum relative growth rate was reported at NH_4^+ concentrations of 3.5-20 mgL^{-1}. Körner et al. (2001) studied the effects of NH_3 and NH_4^+ concentrations on growth of *L. gibba* on domestic wastewater at pH 6.8-8.7. They reported a maximum tolerance level for unionized NH_3 of 8 mg NH_3-N L^{-1} but could not determine that of NH_4^+ as it required very low levels of pH to preclude the effects of NH_3. The findings above indicate that duckweed species could have different tolerance limits to NH_4^+ concentrations and more research is required. It is important to note that most documented research involved *Lemna* species, as it is reported to be the largest genus (Hasan and Chakrabarti, 2009), with less on other genera such as *Wolffia*. Possible species differences may imply that the effectiveness of *Wolffia* species in removal of nutrients, metals or other pollutants could differ from that of *Lemna* species and other genera. The duckweed biomass could have value for various uses in areas they grow or are purposely grown for removal of nutrients from wastewater due to their high nutrient composition.

2.5.2 Duckweed as feed supplement

There is extensive literature on use of duckweed as a protein feed supplement for livestock such as fish, poultry, ducks, pigs, and to some extent ruminants (Iqbal, 1999; Leng et al., 1995; Leng, 1999; Mwale and Gwaze; 2013; Gwaze and Mwale, 2015). Protein content was reported to vary between 15 and 45% depending on species and strains within species. The proteins in duckweed have a balanced amino acid profile (Table 2.3) that encourages their use as feed supplements.

Table 2.3 Amino acid composition (%) of duckweed species

Amino acid	*L. gibba*	*S. polyrhiza*	*L. punctata*	*W. columbiana*
Threonine	3.20	3.45	3.31	2.55
Serine	2.61	2.80	2.83	2.28
Proline	2.93	3.28	2.95	2.41
Glycine	3.79	3.95	3.93	2.04
Alanine	4.59	4.48	4.79	3.75
Valine	4.96	4.40	4.71	3.49
Methionine	0.83	0.83	1.07	0.87
Leucine	7.15	6.85	6.88	5.83
Lysine	4.13	4.30	4.26	3.37

Source: Rusoff et al (1980)

Suitable use of duckweed is an incentive for its harvesting from water bodies. However, characterisation of secondary metabolites in duckweed species needs more attention in animal feed trials as only oxalic acid has been the identified compound produced by duckweed that may have toxic effects to animals at high levels (Adeduntan, 2005).

In response to the Kyoto Protocol on global climate, efforts to cut down on cattle production and related meat consumption are being envisaged (Van Beukering et al., 2008). This ushers

in novel protein sources into the market like insects, algae, rapeseed and duckweed to replace the inefficiently produced animal proteins (Van der Spiegel et al., 2013). Duckweed has been used as a nutritious traditional human food in the small farm systems in South Asia. For example, *W. arrhiza* has been eaten as 'Khai-nam' in Burma, Loas and northern Thailand and has been regarded as the poor man's food (Bhanthumnavin and McGarry, 1971; Appenroth et al., 2017). Duckweed proteins have comparable amino acid composition to that of most leaf proteins (Leng et al., 1995). However, the quality of the duckweed is of paramount importance when used as animal feed or human food since duckweed contaminated with heavy metals and radionuclides poses major health risks through the food chain. More research is needed in such studies to identify ideal duckweed species and their effectiveness as feed for different types of livestock. In addition to the potential value of duckweed species as feed and/or food, other benefits can also be derived from these macrophytes.

2.5.3 Duckweed for green energy and bioplastics

The search for alternative green energy has aroused interest in biomass as new energy material. Reviews by Xu et al, (2012) and Verma and Suthar (2015) report that duckweed, especially *Spirodela* and *Lemna* spp. (Table 2.4) has been explored as novel bio-refinery feedstock for the production of bio-oils, ethanol and biogas through hydrothermal processing, thermochemical and bio-chemical conversions. Biogas production is ideal even with the worst contaminated duckweed through anaerobic digestion (Ali et al., 2016). However, there has been limited research on optimisation of environmental conditions and nutrient loads that enriches duckweed biomass with energy rich substances.

Table 2.4 Bioenergy production from different species of duckweed biomass

Species	Treatment	Product	Remarks
Lemna minor	Pyrolysis	Gas bio-oil & char	Pyrolysis temperature & residence time had minor effect on products
Lemna minor	Pyrolysis	Biochar	Catalytic activity of biochar in biogas reforming
Lemna minor	Hydrolysis & fermentation	Bioethanol	Bioethanol yield of 0.485gg^{-1} (glucose)
Lemna minor	Pre-treatment & fermentation	Bioethanol	258 mgg^{-1}Ethanol yield
Lemna gibba	Pyrolysis	Bio-oil	Components of bio-oil useful for 'green' gasoline & diesel
Lemna minuta	Photosynthetic plant fuel cell	Electricity	Current & power density up to 1.62± 0.10 A.m^{-2} and 380 ± 19 mW.m^{-2} respectively
Lemna spp.	Thermochemical liquefaction	Bio-oil	34 MJkg^{-1} average heating value
Spirodela polyrhiza	Hydrolysis & fermentation	Bioethanol	Annual starch yield 9.42 x 10^{3} kgha^{-1} & ethanol yield of 6.42 x 10^{3} L ha^{-1}
Wolffia & *Spirodela* spp.	Thermolysis	Bioleum	Bioleum with higher heating values & lower oxygenate levels

Adapted from Verma and Suthar (2015)

Further, conversion of dry duckweed proteins and starch into polymers makes duckweed potentially suitable for the bioplastics industry (Zeller et al., 2013). The authors produced biodegradable polymers from milled *Lemna* spp. where a 3:1 ratio of duckweed to glycerol produced the best polymer stability. However, the ultimate quality of the bioplastics depends on the composition of the duckweed. In addition to the potential value of duckweed biomass as a feedstock for biofuel production, there is potential for use of this biomass as an organic fertiliser for crops, because of the high nutrient content.

2.5.4 Duckweed for crop nutrient supply

As a result of the high nutrient content, duckweed biomass may have potential to be used as a natural organic fertiliser, which can be integrated with synthetic fertilisers for cost effectiveness. The elemental composition of duckweed generally depends on nutrient composition of the medium. Elemental composition of a mixture of duckweed thriving on water of variable concentrations collected from levee, highway burrow pits, backwaters of flooded streams and animal waste lagoons ranged between 1.2-4.1% N, 0.1-1% P, 1.9-3.8% K, 0.7-1.3% Ca and 0.2-0.4% Mg (Culley and Epps, 1973). However, high nutrient lagoons had duckweed tissue content as high as 7% N, 1.5-2.6% P, 2.8-4.4% K, 1.75-1.81% Ca, 0.84-0.92% Mg (Culley et al., 1981). From above, duckweed is an efficient sink for K besides hyper-accumulation of heavy metals (Ali et al., 2016).

The macrophytes have low lignin and C/N ratio content indicating a resource that can rapidly decompose (Hasan and Chakrabarti, 2009) and release nutrients when applied as organic fertiliser. According to Kumar and Goh (2000), residue decomposition processes are controlled by three main factors namely edaphic factors, kind of plant residue, and residue management. The factors are not independent of each other. Edaphic factors have much influence in areas with unfavourable conditions such as soil, rainfall and temperature. For example, edaphic factors can be influential in marginally productive areas with inherently infertile soils with sporadic rainfall patterns. Under favourable environments, the crop factors affect the process of decomposition. Plant residue particle size may also influence the decomposition of residues. Summerell and Burgess (1989) reported that small particles may readily decompose, unlike bigger ones, due to increased surface area contact with soil that enhances microbial attack. However, other studies ascribed higher microbial activity at initial phase of decomposition to a closer plant residue–soil contact only in the short term, and grinding of residues to increase

surface area was not significant for N dynamics in the long term (Ambus and Jenson, 1997). Duckweed shrinks to an even smaller size when dried and hence increases surface area that maybe favourable for its decomposition. The age of the residue and toughness were reported to be important for decomposition (Luna-Orea et al., 1996; Gallardo and Merino, 1993), since the chemical composition and silica content of most plant vary with stage of growth. Duckweed has been reported to have very low lignin content as the plants do not need mechanical support (Tao et al., 2017). This property may make it more decomposable than most plants, as lignin is known to be a recalcitrant chemical substance highly resistant to microbial degradation (Mellilo et al., 1982). The residue C/N ratio and N content are useful in predicting their decomposition rates basing on the threshold C/N ratio of 20-30 (Kumar and Goh, 2000). Decomposition is suppressed above this range. Literature widely accepts that residues with a narrow C/N ratio decompose faster than those with a wide ratio. The initial residue N content maybe important for determining the residue decomposition rate (Douglas and Rickman, 1992). For instance, high N content maintains high microbial activity due to reduced competition for available N. Duckweed N content can be high (7% N) and the C/N ratio is generally below the threshold range but their nutrient release patterns in soil are unknown as there are no studies in that area. Comparative studies with residues with a narrow C/N ratio generally agree that decomposition is not limiting. Jensen (1994) reported net immobilisation of N for 30 days with smaller particles of leaf and stem materials of pea residues (19.4 C/N and 2.29% N) in a sandy loam soil when bigger particles showed net mineralisation. From 30 to 90 days, no significant difference in net mineralisation between both sizes of residue particles was reported. The net immobilization of N with smaller particle sizes of residues was attributed to better protection of residues by clay minerals. The decomposition and mineralisation of duckweed in soil is expected to be rapid, as the biomass has fine particle size, higher N and

narrower C/N content than the leaf and stem materials of pea residues. However, this proposition needs empirical confirmation.

Though several studies have recommended duckweed biomass as organic fertiliser based on its composition (Leng, 1999; Iqbal, 1999; Kostecka and Kaniuczak, 2008) there is a paucity of literature on its use as an organic fertiliser. A few studies reported positive effects of duckweed *L. minor* on plant growth, biomass and yields of sorghum and rice (Kraider, 2015; Pulido, 2016; Ahmad et al., 1990). No literature is available to inform on the most appropriate use of duckweed as a green manure, compost or dried biomass to supply nutrients to crops. Further, while use as an organic fertiliser could be an environmentally friendly option with added benefits of improving water quality after removal from water, the suitability of duckweed genera and species to supply nutrients to various crops on different soils is a broad area of research that needs to be tackled. The potential value of duckweed as a nutrient source could be particularly important in the Midlands region of KwaZulu-Natal where the macrophytes occur on large scale commercial farms that produce large quantities of organic wastes from their intensive production systems.

2.6 Features of agricultural production systems in South Africa

South Africa has an agriculture industry consisting of well-developed commercial and subsistence oriented sectors (Shabalala et al., 2013). While a third of South Africa receives sufficient rain for crop production, only a third of this area (approximately 12% of the country) has fertile soil (Van Barmann, c2010). Less than three percent of South Africa is considered high-potential land and the rest is marginal for crop production. Common agricultural activities are intensive crop production, mixed farming in winter and summer rainfall areas, cattle

ranching in bushveld and sheep farming in more arid regions (Van Barmann, c 2010). The majority of small-scale farmers has poor access to credit, due to lack of collateral security, and cannot acquire inputs such as synthetic fertilisers. Although inorganic fertilisers can be used to replenish nutrients removed in crop harvests, they are too costly to be used in large quantities for profitable production in most small-scale farmer situations in South Africa (FSSA, 1997; FAO, 2005; Cedric and Nelson, 2014). Conversely, a study by Shabalala et al. (2013) in the catchment area of Bonsma dam in KwaZulu-Natal Province in a predominantly farming community with enterprises including beef, dairy, crop and sheep husbandry showed that agriculture is among leading causes of water quality degradation. The authors reported eutrophic inlet streams feeding the dam during the wet season. Concentration of NO_3^-, Al and Fe exceeded guidelines for irrigation and aquatic ecosystems. In the Midlands region of KwaZulu-Natal Province, there is need to understand the extent of water pollution in terms of prevalence of the different duckweed species, their nutrient composition and relationship with water quality. What beneficial options can be availed to ameliorate the situation for intensive agricultural production systems faced with challenges of water pollution due to excess nutrient losses and subsequent boom of macrophytes, yet they are in proximity to smallholder farmers' poor soils? A win-win scenario would be harvesting and exporting the aquatic biomass from intensive production systems to nearby small-scale areas with soils of low nutrient levels. Any reasonable potential of using duckweed to improve fertility of soils on smallholder farms could justify efforts to use duckweed to maximise removal of nutrients from wastewater at source, for potential use as organic fertiliser.

Effects of soil texture were reported to vary in a study to determine net mineralisation from maize, soybeans and alfalfa residues (C/N 18, 13, 26) (Pare and Gregorich, 1999). Higher N mineralisation was reported when maize residues were applied in clay than in loam and sandy soils. Conversely, alfalfa residues mineralised more rapidly in sandy soil than in clay and loam

soils. Soil texture did not affect mineralisation of soybeans until after 6 weeks of incubation, where clay and loam soils had similar but higher net N mineralisation than sandy soils. Mineralisation rates inversely varied with the magnitude of the C/N ratios. Therefore, variations in soil texture and other physico-chemical characteristics in smallholder farming systems could affect the decomposition of duckweed and consequently its effectiveness as organic fertiliser.

2.7 Purposeful culturing of duckweed

Utilisation of duckweed to recover nutrients from wastewater and converting them into beneficial products is a promising alternative technology that prevents nutrient overload in aquatic ecosystems (Cheng et al., 2002). Duckweed that recovers high nutrients levels in its tissue has potential as organic fertiliser. However, desired quality of duckweed would require manipulation of the growth medium and its harvesting regimes. Dilution of wastewater concentration could have a significant effect on the quality and quantity of duckweed. Xu et al. (2012) reported growth of *L. punctata* on anaerobically treated swine wastewater (TN 1123 mgL^{-1}, NH_4^+-N 1045 mgL^{-1} and TP 297.5 mgL^{-1}) at dilutions of 2-8% where nutrient removal and duckweed growth improved with increase in nutrient concentration. The 12% swine wastewater could not support duckweed growth. In addition, harvest regimes were reported to affect duckweed yields. For example, optimum harvest regimes that varied from twice a week, once after 4 days and once after 6 days have been reported for *Spirodela* spp. and *L. minor* (Zaki et al., 1979; Xu and Shen, 2011a; Ardiansyah and Fotedar, 2016). This indicated that specific experimental conditions, duckweed species and management of culture concentration were important factors that influenced yields. However, studies that focus at optimisation of harvest regimes, nutrient concentration, and replenishment period in order to improve the

quality and quantity of duckweed biomass for crop nutrient supply in batch systems are non-existent.

2.8 Conclusion

Fresh and wastewater bodies in intensive agricultural systems, including the Midlands region of KwaZulu-Natal Province, South Africa, continue to be colonised aggressively by macrophytes. Excessive growth of duckweed is manifestation of nutrient enrichment in aquatic systems. There is need to provide information on how different habitat characteristics influence duckweed species abundance and distribution in the natural environment. Duckweed is among macrophytes with the highest growth rates and has a unique capacity to concentrate nutrients in its tissue in a short period. Its efficiency and effectiveness in nutrient recovery and water quality improvement can be exploited for multiple end uses. Beneficial use of duckweed biomass as soil fertility amendment becomes an incentive for harvesting it from water. However, the use of duckweed as soil amendments depends on understanding its nutrient release in different soil types. Crop response to ameliorants may depend on the type of duckweed species and their nutrient composition. There is a paucity of information on the fertiliser value of duckweed, as organic nutrient source, for different crops. Effective management of duckweed cultures for quality biomass production useful for soil fertility and water quality improvement requires information that optimises harvest regimes, nutrient concentration and replacement in batch systems.

CHAPTER 3: COEXISTENCE AND TISSUE ELEMENTAL COMPOSITION OF *LEMNA MINOR* AND *WOLFFIA ARRHIZA* VARY WITH NUTRIENT CONCENTRATIONS OF WATER FROM ANTHROPOGENIC SOURCES IN THE MIDLANDS REGION OF KWAZULU-NATAL, SOUTH AFRICA.

3.1 Abstract

Anthropogenic sources of nutrient loads in water stimulated duckweed growth resulting in general loss of aesthetic value. In the quest for improving water quality and soil fertility, the study sought to determine effects of sources of nutrients and elemental composition of water on occurrence and tissue composition of duckweed species. With the aid of Google Earth, 14 sites were randomly selected followed by ground-truthing. Duckweed species were sampled and analysed for carbon (C), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) copper (Cu) and aluminium (Al). Water samples were analysed for pH, ammonium (NH_4^+), nitrate (NO_3^-), K, Ca, and Mg. Pooled data for all sampled sites showed similar water pH (8.1) and N_{min} (0.85-1.98 mgL^{-1}) supporting duckweed growth. Concentration of K, Ca, Mg and P in water explained occurrence of genera in a habitat. Low K (<10 mgL^{-1}) favoured occurrence of duckweed species in separate habitats while generally higher levels favoured coexistence. While *Wolffia* spp. occurred on water with lower K level than sole *Lemna* spp., it had similar or higher tissue content of this element than sole *Lemna* spp. Sole *Lemna* spp. thrived in water with relatively high Ca (>12 mgL^{-1}) and Mg (> 8 mgL^{-1}), while sole *Wolffia* spp. occurred at low levels of these nutrients. Concentration of N and P in water could only explain tissue composition when samples of a genus were compared across sites where they occurred separately. Duckweed tissue content of

Cu, Zn, Fe, Mn and Al varied with habitat type while, it was similar for genera that coexisted. The results suggested that nutrients, such as K, Ca and Mg, in water were important in determining occurrence of a duckweed genus and that the potential to accumulate nutrients from water by the two genera in a particular area maybe exploited for soil N and K improvement.

Keywords

Duckweed, *Lemna minor*, *Wolffia arrhiza*, habitat, tissue elemental composition

3.2 Introduction

Wastes generated in intensive agricultural production systems have a high potential to pollute soil, air and water bodies, with loss of general aesthetic value of these resources. The wastes can be treated and disposed of at landfills, dehydrated for composting (including vermiculture), stored in aerobic or anaerobic lagoons, or applied to croplands and pastures (Kelleher et al., 2002; Agricultural Research Council, 2006; Mkhabela, 2006). Leakage from poorly constructed manure lagoons and overflow during major precipitation events, and runoff from recent applications of waste to farm fields, could contaminate the environment with excess nutrients. Excessive application of fertilisers and livestock wastes on saturated soils can also contaminate surface waters and vulnerable aquifers through runoff and leaching, respectively (Fenton and Ó hUallahcháin, 2012).

High nutrient loads in surface water bodies result in eutrophication and encourage growth of macrophytes, including duckweed from the Lamnaceae family, which comprises the *Lemna*, *Spirodela*, *Landoltia*, *Wolffia* and *Wolffiella* genera, with about 40 species (Chislock et al., 2013; Verma and Suthar, 2014). The duckweed occurs in diverse geolocations with variable climatic conditions ranging from cold temperate to tropical regions, except in waterless and permanently frozen regions (Iqbal, 1999). Most of the species are prevalent in moderate climates of subtropical and tropical zones. The habitat requirements of duckweed vary with species but wind, carbon dioxide levels, light, water pH, temperature and nutrient supply (Goopy and Murray, 2003) influence them all.

Duckweed grows at water temperature range of 6-33^{o}C with optimum range of 25-31^{o}C (Iqbal, 1999; Leng, 1999). According to Landolt (1986), most species exhibit optimum growth at temperatures between 20^{o}C and 30^{o}C and light intensity of 9000 lux. The lower and upper limit

of water pH for growth of most species ranges from 3 to 10.5 with optimal values varying widely both between species and across colonies. The N, P and K concentrations of water supporting growth of Lemnaceae studied by Landolt (1996) ranged 0.003-43, 0.000-56 and 0.5-100 mgL^{-1}, respectively. These macrophytes are phenomenal absorbers of nutrients, particularly of N and P, from enriched water bodies. For example, duckweed takes up nutrients at relatively high rates and double their population in 16 to 48 hours under favourable conditions (Leng, 1999). Duckweed is able to absorb N as ammonium, nitrate, nitrite, urea and some amino acids. The main N sources are ammonium and nitrate with preference for ammonia source. When temperature is suitable, duckweed continues to grow at very low levels of N in water (Leng, 1999). It has capacity to concentrate up to 1.5% of its dry weight as P, when N is not limiting, and can continue to grow in waters devoid of P after accumulation of adequate P for biochemical activities (Leng, 1999). Duckweed is an efficient sink for K and a hyper-accumulator of heavy metals (Ali et al., 2016) but differences in its capacity across genera and species in the natural environment are not clearly understood.

The common duckweed species in South Africa are *Lemna gibba*, *Wolffia arrhiza*, *Landoltia punctata*, *Lemna aequinoctialis* and *Lemna minor* (Cholo and Foden, 2006). The Midlands region of KwaZulu-Natal Province is one of South Africa's key agro-ecological regions (Hitayezu et al., 2014) with potential to polluting water bodies with heavy nutrient loads. Anthropogenic sources of nutrient loads include human settlements and intensive agricultural production systems. While the agricultural production systems are diversified, intensive animal (poultry, piggery and dairy) and crop production pose the greatest water pollution risk. The nutrient loads in surface water bodies, and other climatic requirements could determine the duckweed species commonly found in the Midlands region, and their tissue elemental composition. While these macrophytes could be viewed as a nuisance on freshwater bodies,

their efficient nutrient uptake could improve water quality and, if they are harvested for alternative uses, could be valuable resources.

Duckweed species have been commonly used for wastewater phytoremediation or grown for human food and animal feed supplement (Gupta and Prakash, 2013; Van der Spiegel et al., 2013, Ali et al., 2016). However, there is a paucity of information on unlocking value from duckweed for soil fertility. There is need to understand the common species, occurring individually or in co-existence, and tissue elemental composition of these macrophytes in the Midlands region of Kwa-Zulu Natal, in view of possible recycling of nutrients for soil fertility improvement. The influence of nutritional composition of effluent or wastewater on the commonly occurring species and elemental composition of duckweed species, in their natural habitats, is unclear.

While Landolt (1996) provided ranges of nutrient concentration in water supporting growth of Lemnaceae, there are limited studies on elemental composition of specific duckweed species and associated water as they occur in the natural environments. Most studies involve culturing and manipulation of the medium and growing environment. Environmental characteristics, type of macrophytes and climate have utmost significance on macrophytes' potential effectiveness in nutrient recovery and production of valuable biomass (Zhao et al., 2015). A number of questions arise. Do sources of nutrients affect the prevalence of genera and species, and tissue elemental composition of duckweed? Does the elemental composition of water determine whether or not the duckweed species occur together? In view of harnessing nutrients for soil fertility improvement, the objective of this study was to determine the elemental composition of prevalent duckweed species and associated water from different types of farming enterprises.

3.3 Method and materials

3.3.1 Study Area

The study was conducted in the Midlands region of the KwaZulu-Natal Province of South Africa. The region is one of the key agro-ecological regions of South Africa consisting of an inland area stretching from the low-lying coastal strip of the Indian Ocean to the high altitude of the Drakensberg escarpment (Hitayezu et al., 2014). It has a subtropical oceanic climate with an annual rainfall range of 521-1120 mm and average maximum summer temperatures of 28^{o}C and average winter temperature of 3.2^{o}C. The region is dominated by commercial forestry in wetter mountainous areas, whilst both commercial and subsistence agriculture are prevalent in the lower lying drier areas (Hitayezu et al., 2016). Dairy, piggery and poultry dominate the animal enterprises while maize, sugarcane, soybeans and fruit trees are the commonly grown crops.

3.3.2 Sampling and analysis

With the aid of Google Earth, an aerial view from the seacoast in the east (Durban) to the Drakensburg escarpment in the west, with an altitude range of less than 100 m to close to 2000 m above sea level, revealed occurrence of duckweed on water bodies. River systems and water bodies could clearly be defined at an eye altitude of less than 2 km above the ground, with macrophytes identified with a shiny light green reflectance on water bodies (Appendix 1). The macrophytes were absent in communal and commercial forestry areas but were prevalent in commercial agricultural settings, predominantly in the low to middle altitude range (100 - 800 m). Ground-truthing, to confirm the Google Earth observations, during the rainfall season from

February to April 2016, focused on a 50 km radius from Pietermaritzburg where 14 sites were selected. The macrophytes were sampled from lagoons, ponds and fresh or wastewater reservoirs that farmers constructed by intercepting natural watercourses or streams with either dams or weirs. The farming enterprise and water type supporting duckweed growth was documented (Table 3. 1).

Table 3.1 Site activities and water types supporting duckweed

Habitat	Coordinates	Main Activity	Water type
Nottingham	29º19’50.13’’S 29º59’27.56’’E	Dairy	Wastewater
Lydgate	29º26’27.80’’S 30º12’23.61’’E	Goats	Waste water
Kildale	29º19’23.37’’S 30º02’33.87’’E	Dairy	Waste water
Thornville	29º45’06.61’’S 30º24’29.37’’E	Sugarcane, horticulture	Fresh water
Baynesfield	30º45’49.21’’S 30º20’15.02’’E	Piggery	Waste water
Ichanga	29º44’57.22’’S 30º37’05.66’’E	Poultry	Waste water
Atherstone	29º47’45.50’’S 30º22’44.58’’E	Sugarcane	Fresh water
Ashburton	29º40’34.57’’S 30º27’45.65’’E	Sewer discharge into stream	Waste water
Camperdown	29º44’06.98’’S 30º30’22.94’’E	Sugarcane, horticulture	Fresh water
Cedara	29º31’11.22’’S 30º15’48.21’’E	Dairy	Fresh water
Lynnfield	29º40’43.81’’S 30º28’04.85’’E	Settlement	Waste water
Wartburg	29º28’20.64’’S 30º28’59.26’’E	Crocodiles	Waste water
Gromo	29º43’58.61’’S 30º37’53.81’’E	Growing media (pine bark & poultry manure)	Waste water
Ukulinga	29º39’44.26’’S 30º24’11.10’’E	Piggery	Wastewater

Macrophyte and water samples replicated three times were collected from randomly selected parts of the pond at each site. Samples of approximately 1.5 kg wet mass were collected using

1 mm mesh screens. Habitat water was added to the collected macrophytes before transporting them as a dense suspension. The water samples were randomly collected as grab samples (Environmental Protection Agency, 2013), into 250 ml plastic bottles from the upper 20 cm water column. Extraneous material was removed from the macrophyte samples using a combination of distilled water and a set of screens with progressively smaller apertures. Macrophytes that shared the same habitat were separated using the 1000 (retained *Lemna* spp. fronds) and 50 µm (retained *Wolffia* spp. fronds) gauges. The macrophyte samples were rinsed with distilled water. Small portions of macrophyte samples were submitted to the School of Life Sciences at University of KwaZulu-Natal for classification and the rest were dried in an oven at 60^{o}C to constant weight. Species sharing same habitats had approximately similar dry weight.

The dry duckweed samples were ground before analysis of total C and N using the LECO Trumac CNS auto analyser version 1.1x (LECO Corporation, 2012). Phosphorus, Al, Ca, Mg, K and micronutrients Fe, Mn, Zn and Cu were analysed following the methods of The Non-Affiliated Soil Analysis Work Committee (1990). After filtering through 2.5 µm pores, water samples were analysed for pH using the radiometer PHM 210 meter. Soluble P, NH_4^+ and NO_3^- were analysed using Thermo Scientific Gallery Automated Chemistry Analyser and K, Ca, Mg by AAS following methods by Rice et al. (2012). The microelements in water could not be determined due to equipment breakdown during the time the experiment was carried out.

3.3.3 Data analysis

A one way ANOVA was used to analyse the data after tests of homoscedasticity and normality using SPSS version 18. Means were separated by the LSD at 0.05 level of significance.

3.4 Results

3.4.1 Occurrence of duckweed species

Duckweed species were found on water bodies with nutrients from piggery, poultry, dairy, crocodile, and crop production, and from sewage and composting wastes (Table 3. 1). The waters had 0.09-5.3, 0.06-7.8, 3.1-386, 7.9-46.4 and 3.5-56.9 mgL^{-1} of mineral N, soluble P, K, Ca and Mg, respectively. The duckweed was classified as species of *Wolffia* (dominated by *W. arrhiza*) and *Lemna* (dominated by *L. minor*) genera. The species of the two genera either occurred separately or co-existed.

3.4.2 Elemental composition of duckweed species and composition of water pooled across all sites

The C contents of *Wolffia* and *Lemna* spp. were not significantly different, either when they occurred alone or coexisted in habitats (Table 3.2). Where the genera occurred alone, both *Wolffia* and *Lemna* spp. had higher C content than *Wolffia* spp. in a shared habitat. The C/N ratios of duckweed were in the order: *Wolffia* spp. occurring alone (7.7) < *Wolffia* spp. in co-existence (8.7) < *Lemna* spp. occurring alone (9.1) < *Lemna* spp. in coexistence (10.4).

Table 3.2 Elemental composition of duckweed from sites with *Wolffia* spp. and *Lemna* spp. occurring individually and in co-existence

	C	N	P	K	Ca	Mg	Zn	Cu	Mn	Fe	Al
			%						$mgkg^{-1}$		
WF_a	37.8^{b}	4.9	0.6	5.3^{b}	0.6^{a}	0.33^{a}	91	11.2^{a}	192^{a}	1766^{a}	779
LM_a	36.5^{b}	4.0	0.7	2.5^{a}	1.2^{bc}	0.47^{b}	134	11.4^{ab}	670^{b}	2286^{ab}	590
WF_s	34.1^{a}	3.9	0.6	5.3^{b}	1.1^{b}	0.39^{a}	101	29.1^{bc}	495^{b}	4514^{b}	1735
LM_s	35.2^{ab}	3.4	0.7	4.9^{b}	1.7^{c}	0.47^{b}	104	32.0^{c}	586^{b}	4605^{b}	1310

Means followed by the same letter in a column are not significantly different at $p<0.05$. Means without letters in a column are not significantly different at $p<0.05$. WF_a = *Wolffia* spp. alone, LM_a= *Lemna* spp. alone, WF_s= *Wolffia* spp. in shared habitat, LM_s = *Lemna* spp. in shared habitat

Similarly to C, tissue N, P, Zn and Al in the duckweed species were similar for both *Wolffia* and *Lemna* spp., whether occurring alone or in co-existence. *Lemna* spp. occurring alone had significantly lower K content than the rest. The Ca content was similar for *Lemna* spp. occurring in different habitats. Meanwhile, the Ca content of *Wolffia* spp. occurring alone was the lowest. The Mg content of *Wolffia* spp. was lower than that of *Lemna* spp., both when they occurred separately and co-existed in habitats. *Wolffia* spp. occurring alone had lower Cu, Mn and Fe content than of co-existing species. The tissue K, Ca and Mn content of *Wolffia* spp. were different from *Lemna* spp. where the species occurred alone. *Lemna* spp. had higher Ca and Mn content than *Wolffia* spp. while the latter had higher K content. Only Ca and Mg content were different when both genera co-existed, with *Lemna* spp. having higher content than *Wolffia* spp. The *Wolffia* spp. in co-existence had lower C and higher Ca, Cu, Mn and Fe content than when the same genus occurred alone. Co-existing *Lemna* spp. had higher K and Cu content than when the same species occurred alone. Pooled data of water pH, NH_4^+, NO_3^-, and N_{min} from different types of habitats were similar (Table 3.3).

Table 3.3 Nutrient composition of water from sites with either *Wolffia* spp. or *Lemna* spp. and both species

	pH	NH_4^+	NO_3^-	N_{min}	P	K	Ca	Mg
					mgL^{-1}			
WF_a	8.2	0.54	0.31	0.85	0.14^a	8.86^a	12.89^a	6.03^a
LM_a	8.1	1.79	0.20	1.98	7.71^c	76.21^a	30.03^b	17.50^b
WF+LM	8.1	0.58	0.27	0.86	2.58^b	301.76^b	27.37^b	25.42^b

N_{min}= NH_4^+ + NO_3^-. Means followed by the same letter in a column are not significantly different at $p<0.05$. Means without letters in a column are not significantly different at $p<0.05$.WF_a = *Wolffia* spp. alone, LM_a = *Lemna* spp. alone, WF+LM = *Wolffia* and *Lemna* spp.

The soluble P concentration was lowest (significant) in water with *Wolffia* spp. existing alone and highest where *Lemna* spp. existed alone. Water in which the species co-existed had

significantly higher K levels than where the species occurred separately (alone). The Ca and Mg were low in water where *Wolffia* spp. occurred alone.

3.4.3 Tissue elemental composition of sole *Wolffi*a and sole *Lemna* species across sites

The *Wolffia* spp. occurred alone at Atherstone (sugarcane), Thornville (sugarcane & horticulture), Cedara (dairy), Nottingham (dairy) and Ukulinga (piggery). The C content of the duckweed from the dairy enterprises was significantly higher than the rest (Table 3.4).

Table 3.4 Elemental composition of *Wolffia* and *Lemna* spp. occurring separately across sites

Site	C	N	P	K	Ca	Mg	Zn	Cu	Mn	Fe	Al
				%					$mgkg^{-1}$		
Sole *Wolffia* spp.											
ATH	32.5^{a}	3.6^{a}	0.64^{b}	6.5^{bc}	0.6^{a}	0.33^{b}	135^{b}	32^{b}	388^{c}	2023^{a}	1048^{ab}
THN	32.5^{a}	5.0^{bc}	0.50^{a}	6.0^{b}	0.6^{a}	0.33^{b}	57.0^{a}	7.4^{a}	281^{bc}	2087^{a}	515^{ab}
CED	36.8^{b}	4.8^{b}	0.57^{ab}	3.4^{a}	0.8^{b}	0.35^{b}	67.0^{a}	5.7^{a}	99.0^{a}	1788^{a}	1636^{b}
NOT	35.3^{b}	5.2^{c}	0.53^{a}	3.1^{a}	0.6^{a}	0.30^{a}	68.0^{a}	3.5^{a}	43.0^{a}	2031^{a}	471^{ab}
UKU	31.7^{a}	5.8^{d}	0.95^{c}	7.4^{c}	0.6^{a}	0.34^{b}	128^{b}	6.9^{a}	151^{ab}	9055^{b}	227^{a}
Sole *Lemna* spp.											
WRT	37.6^{b}	3.1^{a}	0.46^{b}	3.0^{b}	1.2^{a}	0.4^{a}	41.0^{a}	9.9	415^{b}	2708^{b}	140^{a}
LDG	38.0^{b}	4.8^{c}	1.13^{c}	2.2^{a}	1.3^{a}	0.5^{a}	239^{c}	18.3	66^{a}	1033^{a}	424^{b}
ASH	34.0^{a}	4.1^{b}	0.40^{a}	2.3^{ab}	1.6^{b}	0.6^{b}	122^{b}	6.1	1528^{c}	3118^{c}	1207^{c}

Means followed by the same letter in a column under same category of species are not significantly different at $p<0.05$. Means without letters in a column are not significantly different at $p<0.05$. ATH = Atherstone, THN = Thornville, CED = Cedara, NOT= Nottingham, UKU = Ukulinga, WRT = Wartburg, LDG = Lydgate, ASH = Ashburton

The highest N content of *Wolffia* spp. was from the piggery, and the lowest was from the sugarcane enterprise at Atherstone. The highest P content was in *Wolffia* spp. from the piggery while the lowest was from the dairy and sugarcane & horticulture enterprises. *Wolffia* spp. from the dairy enterprises had the lowest ($p<0.05$) K content while it had the highest Ca and lowest

Mg contents, from Cedara and Nottingham, respectively. The piggery (Ukulinga) and sugarcane enterprises (Atherstone) had duckweed with the highest content of Zn, while the rest of the sites had similar concentrations. The Atherstone sugarcane enterprise had *Wolffia* spp. with the highest Cu content. Manganese content of *Wolffia* spp. from dairy enterprises was significantly lower than that from sugarcane enterprises. Duckweed from the piggery site had the highest Fe content, while that from rest of the sites was similar. *Wolffia* spp. from the dairy enterprise at Cedara had significantly higher Al than that from piggery.

Lemna spp. occurred alone in Wartburg (crocodiles), Lydgate (goats) and Ashburton (sewer discharge) habitats. The duckweed from the crocodile and goat enterprises had similar C content that was higher than that from the sewer discharge (Table 3.4). Duckweed from the goat enterprise had the highest N and P contents, while the lowest tissue N and P contents were from the crocodile enterprise and sewer discharge sites, respectively. Duckweed from crocodile enterprise had higher K content than the other two habitats. Tissue Ca and Mg were higher from sewer discharge site than from the other two habitats. There were significant differences in Zn content of *Lemna* spp., with duckweed from the goat enterprise (Lydgate) having the highest levels and that from the crocodile (Wartburg) had the lowest. The Cu content of *Lemna* spp. from all sites was similar. The Mn and Fe had a similar trend where *Lemna* spp. from Lydgate had the least levels and that from Ashburton (sewer discharge) had the highest. Aluminium content of *Lemna* spp. from Ashburton was highest while that from Wartburg was lowest.

3.4.4 Elemental composition of water where *Wolffia* and *Lemna* species occurred separately

Of the sites where *Wolffia* spp. occurred separately, water from dairy enterprises had the lowest pH (Table 3.5). The highest NH_4^+ level was in water from Nottingham (dairy), followed by those from Ukulinga (piggery) and Atherstone (sugarcane), while that from Thornville (sugarcane & horticulture) and Cedara (dairy) had the lowest. Water from Ukulinga had the highest NO_3^- level followed by that from Thornville, while that from the rest of the sites was similar.

Table 3.5 Nutrient composition of water from sites with *Wolffia* spp. and *Lemna* spp. occurring separately across sites

Site	pH	NH_4^+	NO_3^-	N_{min}	P	K	Ca	Mg
					mgL^{-1}			
				Sole Wolffia spp.				
ATH	8.5^{c}	0.55^{b}	0.04^{a}	0.59^{b}	0.09^{b}	10.9^{c}	11.7^{c}	6.6^{c}
THN	8.3^{b}	0.16^{a}	0.10^{b}	0.26^{a}	0.06^{a}	12.1^{c}	11.6^{c}	6.8^{c}
CED	7.9^{a}	0.07^{a}	0.02^{a}	0.09^{a}	0.06^{a}	6.9^{b}	10.9^{b}	5.8^{b}
NOT	8.0^{a}	1.52^{c}	0.02^{a}	1.54^{c}	0.13^{c}	3.1^{a}	7.91^{a}	3.5^{a}
UKU	8.4^{bc}	0.42^{b}	1.36^{c}	1.78^{d}	0.37^{d}	11.3^{c}	22.5^{d}	7.4^{d}
				Sole Lemna spp.				
WRT	7.7	0.17^{b}	0.03^{a}	0.19^{a}	0.10^{a}	9.11^{a}	12.13^{a}	8.59^{a}
LDG	8.3	5.19^{c}	0.06^{a}	5.25^{c}	7.76^{b}	208^{b}	46.40^{b}	21.73^{b}
ASH	8.2	0.004^{a}	0.50^{b}	0.51^{b}	0.05^{a}	10.57^{a}	31.57^{b}	22.17^{b}

Means followed by the same letter in a column under same category of species are not significantly different at $p<0.05$. Means without letters in a column are not significantly different at $p<0.05$. ATH = Atherstone, THN = Thornville, CED = Cedara, NOT = Nottingham, UKU = Ukulinga, WRT = Wartburg, LDG = Lydgate, ASH = Ashburton

The N_{min} and soluble P were highest in water from Ukulinga followed by that from Nottingham, while that from Cedara and Thornville had the lowest. The K levels in water from Nottingham and Cedara were significantly different from each other but were lower than those from the other three habitats that had similar amounts. Highest levels of Ca and Mg in water were from

the piggery (Ukulinga) enterprise. Water from the sugarcane enterprises (Atherstone and Thornville) had similar levels of K, Ca and Mg while that from dairy (Nottingham) had lowest levels of these elements.

The pH of water was similar for all sites where *Lemna* spp. occurred alone (Table 3.5). Water from the goat enterprise had the highest NH_4^+ and N_{min} levels, while the lowest N_{min} was from the crocodile enterprise. The sewer discharge water had the lowest NH_4^+ and higher NO_3^- than the other sites. Water from the goat enterprise had higher P and K levels than the other two habitats. While water from the crocodile enterprise had lowest Ca and Mg levels, that from the goat enterprise and sewer discharge had high and similar levels.

3.4.5 Elemental composition of tissue and water where *Wolffi*a and *Lemna* species co-existed

The *Wolffia* and *Lemna* spp. co-existed in habitats at Baynesfield (piggery), Camperdown (sugarcane & horticulture), Kildale (dairy), Ichanga (poultry) and Gromo (poultry litter & pine bark compost). Only tissue Ca and Mg were different between the two genera, with *Lemna* spp. accumulating more than *Wolffia* spp., where they co-existed (Table 3.6). The rest of the elements in the tissue of the two genera were similar. At habitat level, the *Wolffia* spp. from the poultry enterprise had the highest C content followed by that from the dairy enterprise while that from the piggery enterprise had the lowest (Table 3.6). *Lemna* spp. from the poultry enterprise had the highest C content while that from the piggery enterprise had the lowest.

Table 3.6 Elemental composition of *Wolffia* and *Lemna* spp. from sites they coexisted

Site	C	N	P	K	Ca	Mg	Zn	Cu	Mn	Fe	Al
				%					$mgkg^{-1}$		
					Wolffia spp.						
BF	31.0^{a}	5.4^{c}	1.01^{c}	7.4^{d}	0.97^{b}	0.39^{b}	967^{b}	12^{a}	271^{a}	980^{a}	239^{a}
CMP	32.8^{b}	3.2^{b}	0.53^{ab}	5.7^{c}	0.60^{a}	0.29^{a}	180^{a}	97^{b}	426^{b}	1849^{a}	463^{bc}
KL	35.5^{c}	5.3^{c}	0.58^{b}	3.2^{a}	0.78^{ab}	0.31^{a}	80^{a}	10^{a}	167^{a}	7020^{c}	5817^{d}
ICH	39.1^{d}	3.5^{b}	0.52^{a}	4.2^{b}	1.41^{c}	0.43^{b}	117^{a}	19^{a}	455^{b}	5193^{b}	412^{ab}
GR	33.9^{b}	2.7^{a}	0.52^{a}	6.0^{c}	1.91^{d}	0.52^{c}	75^{a}	22^{a}	1048^{c}	8666^{d}	669^{c}
					Lemna spp.						
BF	31.1^{a}	4.4^{c}	1.70^{b}	8.9^{c}	2.40^{c}	0.60^{c}	103	33^{b}	873^{c}	1465^{a}	420^{a}
CMP	36.9^{c}	2.4^{a}	0.94^{a}	5.6^{b}	1.76^{b}	0.47^{b}	132	64^{c}	634^{b}	1883^{a}	290^{a}
KL	33.3^{b}	4.4^{c}	0.59^{a}	2.4^{a}	1.01^{a}	0.37^{a}	94	10^{a}	171^{a}	7017^{b}	4952^{b}
ICH	39.6^{d}	3.2^{b}	0.54^{a}	3.4^{ab}	1.28^{a}	0.42^{a}	107	13^{a}	248^{a}	3344^{a}	269^{a}
GR	34.7^{b}	2.4^{a}	0.50^{a}	4.4^{ab}	1.98^{b}	0.49^{b}	88	41^{b}	1005^{c}	9316^{b}	617^{a}
Genera				*Wolffia*	and *Lemna* spp.						
WF	34.5	4.0	0.63	5.3	1.13^{a}	0.39^{a}	284	31	473	4742	1520
LM	35.2	3.3	0.85	4.9	1.68^{b}	0.47^{b}	104	32	586	4605	1310

Means followed by the same letter in a column under same category of species are not significantly different at $p<0.05$. Means without letters in a column are not significantly different at $p<0.05$. WF = *Wolffia* spp., LM = *Lemna* spp., BF = Baynesfield, CMP = Camperdown, KL = Kildale, ICH = Ichanga, GR = Gromo

Wolffia spp. with the highest N content was from the piggery and dairy enterprises, while that from the poultry litter & pine bark compost enterprise had the lowest. Almost similar, the piggery and dairy enterprises had *Lemna* spp. with the highest N content, whilst the poultry litter & pine bark compost and sugarcane & horticulture enterprises had the lowest levels. The *Wolffia* and *Lemna* spp. from the piggery enterprise had highest significant P and K content while the dairy enterprise was among those with lowest K content. *Wolffia* spp. from poultry litter & pine bark enterprise had the highest Ca and Mg content, while that from dairy and sugarcane enterprises had the lowest Mg content. *Lemna* spp. from the piggery enterprise had the highest Ca and Mg content, while that from the dairy and poultry enterprises had the lowest.

The *Wolffia* spp. from the piggery and sugarcane & horticulture enterprises had the highest Zn and Cu, respectively. The two enterprises had *Wolffia* spp. with the lowest Fe, which was

highest in *Wolffia* spp. from the poultry litter & pine bark compost enterprise. The Zn content of *Lemna* spp. was similar across sites. While Cu content of *Lemna* spp. from the poultry and dairy enterprises was lowest, the highest was from the sugarcane & horticulture enterprise. The *Wolffia* spp. from piggery and dairy had the lowest Mn content whilst the highest was from the poultry litter and pine bark compost enterprise. The *Lemna* spp. from the dairy and poultry enterprises had the lowest Mn content while that from the compost related enterprise (Gromo) was among those with highest Mn and Fe content. The *Wolffia* and *Lemna* spp. from the dairy enterprise had highest Al content.

The water from the poultry (Ichanga) and sugarcane & horticulture enterprises (Camperdown) had the lowest pH while that from piggery was among the highest (Table 3.7).

Table 3.7 Nutrient composition of water from habitats where *Wolffia* and *Lemna* spp. coexisted

Site	pH	NH_4^+	NO_3^-	N_{min}	P	K	Ca	Mg
					mgL^{-1}			
BF	8.6^{c}	0.48^{b}	0.80	1.23^{bc}	5.39^{c}	662^{c}	35.27^{b}	31.73^{b}
CMP	7.8^{a}	0.17^{a}	0.01	0.19^{a}	0.07^{a}	3.75^{a}	16.07^{a}	8.21^{a}
KL	8.4^{bc}	0.61^{b}	0.34	0.95^{b}	0.50^{ab}	386^{b}	36.63^{b}	34.27^{bc}
ICH	7.4^{a}	1.74^{c}	0.12	1.86^{c}	1.21^{b}	158^{ab}	14.43^{a}	13.67^{ab}
GR	8.2^{b}	0.74^{b}	0.21	0.94^{ab}	0.22^{a}	301^{b}	44.30^{b}	56.87^{c}

Means followed by the same letter in a column are not significantly different at $p<0.05$.
Means without letters in a column are not significantly different at $p<0.05$. BF = Baynesfield, CMP = Camperdown, KL = Kildale, ICH = Ichanga, GR = Gromo

Water from the poultry enterprise had the highest NH_4^+ level while that from sugarcane & horticulture enterprises had the lowest. The NO_3^- levels were similar across the habitats. The sugarcane & horticulture and poultry litter & pine bark enterprises had the lowest N_{min}. The poultry enterprise had water with the highest N_{min} level, precluding that from the piggery enterprise. Water from piggery enterprise had highest P and K levels. That from the sugarcane & horticulture and poultry enterprises had lowest Ca level. Water from poultry litter & pine

bark compost, dairy and piggery enterprises had significantly higher Mg levels than that from the sugarcane & horticulture enterprise.

3.5 Discussion

Duckweed requires relatively high nutrient levels for their growth. Non-existence of macrophyte in smallholder agricultural settings, indicated better river aquatic health due to relatively low amounts of nutrients lost to surface water bodies (Isikhungusethu Environmental Services, 2012). Thiebaut (2008) showed that with the aquatic plant community, there is a spectrum of tolerance to nutrient enrichment where *L. minor, L. gibba, L. trisulca* were found in water with nutrient status ranging from mesotrophic to eutrophic, while *W. arrhiza* was in eutrophic water. Colonisation of waterbodies by duckweed in commercial agricultural systems of the Midlands region indicated that these systems enrich the water with nutrients as supported by high concentrations of nutrients in the tissue of duckweed species. Examination of different enterprises showed inconsistent effects on duckweed elemental composition. High concentrations of N, P and basic cations in water generally indicated that commercial piggery, dairy and crop production systems were responsible for increased nutrient loads in water bodies. Nutrient composition of water, as affected by the enterprises, had pronounced and generally consistent effects on duckweed elemental composition. The high nutrient loads in water could explain the occurrence of the duckweed species in the Midlands region. Based on the results, duckweed genera grew as long as there was at least 0.09 mg N L^{-1} and pH range of 7.8-8.6. The N, P and pH values in this study were similar to those observed by other authors (McLay, 1976; Landolt, 1986; Hasan and Chakrabarti, 2009). The similarity in tissue C between the *Wolffia* and *Lemna* spp., sole or in coexistence, could be because of similar conditions such as non-limiting water N concentration, light intensity and photoperiod across

the different habitat, within 50 km of the City of Pietermaritzburg. Conditions such as temperature, pH, daily dark–light cycle, nitrogen and phosphate levels in the growth medium can limit duckweed growth resulting in increase in C content (Cui et al., 2011; Zhao et al., 2014b; Tao et al., 2017).

Existence of a genus was based on composition of elements in the waters. Relative concentrations of K, Ca, Mg and P in the waters differentiated the occurrence of a specific genus in a habitat. Generally, low K (< 20 mgL^{-1}) favoured occurrence in separate habitats, while higher levels (>150 mgL^{-1}) favoured co-existence. Sole *Lemna* spp. thrived in water with relatively high Ca, Mg and P levels (above 12, 8, 0.1 mgL^{-1} respectively), which explain the higher tissue composition, while sole *Wolffia* spp. occurred at relatively low levels. The higher Ca and Mg in *Lemna* tissue, either sole or in coexistence, suggested that this genus could accumulate a higher Ca and Mg than *Wolffia* spp. This partly agrees with a study by Culley and Epps (1973), on nutrient content of duckweed from bottom land lakes and stream in Louisiana and Arkansas, where tissue Ca of *Lemna* spp. was consistently higher than of *Wolffia* spp. but showed an opposite trend with Mg, though the author did not measure the concentrations of these cations in water. Sufficient levels of Ca and Mg, with limited competition, could explain the co-existence of the two genera in waters with high levels of these nutrient resources, though *Lemna* spp. accumulated more.

Although sole *Wolffia* spp. occurred on waters with lower K level than sole *Lemna* spp., it had higher or similar tissue content of these elements (Tables 3.2 & 3.3). This finding suggested that *Wolffia* spp. could be more efficient in the uptake of K as supported by Garbey et al. (2004) who postulated that the phenomenon may be due to physiological traits related to nutrient storage in the tissue of the species. The similar tissue P composition between the genera, either sole or in co-existence (irrespective of differences in soluble P), also supports suggestions by Thiebaut (2008) that tissue P content is generally less influenced by nutrient availability as

long as it is above critical concentrations, which have not been clearly defined. Similarity in tissue N, Zn and Al for all types of habitats grouped according to duckweed occurrence generally implied similar levels of the elements in water in different habitats. While the pooled data provided a general picture of the elemental and nutrient composition of the two duckweed genera and water from their habitats, the approach masked some significant trends. Although tissue N and P did not differ across habitats (sole or co-existence), and were not affected by their concentrations in water when the results were pooled, they varied with water composition across the sites where the genera occurred separately. This suggested that enterprises discharging high concentrations of N and P in water encouraged uptake of these elements by *Wolffia* and *Lemna* spp., when they occurred separately. The poor relationships between tissue N and P with different concentrations in water, when the genera coexisted, implied importance of other factors. A study by Kufel et al. (2012), on growth rate of duckweed in relation to the internal and ambient nutrient concentration, noted weak to no correlation between tissue N and P with water concentrations. The authors suggested that nutrient accumulation in tissue took time and was an outcome of plant growth, nutrient uptake rate and past variability of available nutrients in water. Although the pooled results showed relationship between water Ca and tissue concentrations in *Wolffia* and *Lemna* spp. across sites where they occurred alone or co-existed, there were weak relationships between water and tissue concentrations when sites with *Wolffia*, *Lemna* spp. or both were examined separately. This was also true for Mg. The Ca and Mg concentration of water in this study were within the absolute range of 0.1-365 mgL^{-1} Ca and 0.1-230 mgL^{-1} Mg proposed by Hasan and Chakrabarti (2009), suggesting that though these minerals were essential for duckweed survival, the macrophytes were not particularly sensitive to fluctuations in concentration of these nutrients once an adequate threshold was reached. Water K concentration was the main parameter that separated occurrence of the genera alone (low K) or in co-existence (high K). Water K concentrations across sites where *Wolffia*

and *Lemna* co-existed explained tissue K content, which implied importance of K when N and P critical requirements were met. While K concentration in water explained tissue K of *Wolffia* spp. across sites where the genus occurred alone, a weak relationship for sites where *Lemna* spp. occurred alone was possibly due to lower K requirements of *Lemna* spp. While Landolt (1986), provided a broad K concentration of water (0.5-100 mgL^{-1}) suitable for duckweed survival, Goopy and Murray (2003), indicated that preference for K depended on species. According to Leng (1999), only a low K concentration in water is needed to support good duckweed growth, when other minerals requirements are satisfied, though vigorous growing duckweed is a highly efficient K sink.

Although the duckweed tissue content of Cu, Zn, Fe, Mn and Al varied with site, co-existing genera showed similar tissue content (Table 3.6), influenced by inconsistent uptake by the genera under different habitats. Culley et al. (1981), observed that the mineral content in duckweed did not follow concentrations in water as closely as did N and P, suggesting other factors at play. Miretzky et al. (2004), observed the main deviation from the generally high correlation between metal concentration in water and in macrophytes as precipitation reactions. Influence of other factors such as precipitation reactions at pH > 7.5 (Brady and Weil, 2008), might explain variable nutrient uptake of the two genera at different habitats. Therefore, from observations by the above authors, distribution of the genera might not have been limited to micronutrient availability in water. Copper, Zn, Mn and Fe were important ingredients of livestock feed, whereas additional Cu was from fungicides used in horticulture and forestry. Iron and Mn levels were also influenced by surrounding soil in contact with flowing water. Dairy enterprises contributed large amounts of Al that might have been from use of Al equipment. Literature, with most studies on *Lemna minor*, shows that duckweed is an efficient

accumulator of Cu, Zn, Fe, Mn and Al (Miretzky et al., 2004; Horvat et al., 2007; Razinger et al., 2007; Kanoun-Boulé et al., 2009; Khellaf and Zerdaoui, 2009).

Duckweed's capacity to accumulate nutrients encourages phytoremediation. High concentration of nutrients in tissue of *Lemna* and *Wolffia* species indicates that the macrophytes could improve water quality especially when continuous removal of the duckweed is practised. In addition, the harvested duckweed could be a vital resource for soil improvement, which compares favourably with other organic soil amendments. Poultry manure generally contains macronutrients in the following ranges 1.5-3.7% N, 1-1.5% P, 1.5-2.0% K, 1.5-3.0% Ca and 0.56-0.9% Mg (Van Ryssen, 2001, Adediran et al., 2003; Mkhabela, 2006). Conversely, pig manure contains a mean of 1.5% N, 0.65% P, 0.82% K, 0.42% Ca, 0.37% Mg (Bolan et al., 2010). The duckweed had N and K content above that of either pig or poultry manure, with *Wolffia* spp. expected to have a better fertiliser value. Macronutrients from both genera are superior to those of pig manure, while poultry manure has relatively higher content of P, Ca and Mg. This implies an untapped soil fertility resource, particularly supplying N, K with added micronutrient benefits. The average C/N ratio of duckweed from the study was approximately 9 and implied materials with low lignin content that should readily decompose when applied to the soil. The lower C/N ratio of *Wolffia* spp. suggested that the tissue of this genus could decompose more rapidly than *Lemna* spp. with possible ammonia losses. However, accumulation of Al by duckweed may have undesired consequences when used as a soil fertility amendment as the mineralised Al would likely interfere with availability of other nutrients such as P and might have an undesired effect on the soil pH (Tisdale et al., 1993).

3.6 Conclusion

The nutrient concentrations in water had a major role in occurrence and tissue composition of *Wolffia* and *Lemna* spp. Generally, high K resulted in co-existence while low K resulted in the genera occurring separately with *Wolffia* spp. occurring on water with lower Ca and Mg than where *Lemna* spp. occurred alone. Concentration of N and P in water could only explain tissue composition of *Wolffia* and *Lemna* spp. when sites with the individual species were compared separately from where both occurred. High concentration of mineral N in the water increased tissue N of duckweed species while water P only affected tissue composition where the duckweed species occurred separately. The high nutrient composition including micronutrients, indicates the potential of these macrophytes to improve water quality and as nutrient sources for crops. There is need to understand the nutrient release patterns of these macrophytes to determine their fertiliser value.

CHAPTER 4: DECOMPOSITION OF *WOLFFIA ARRHIZA* RESIDUES RAPIDLY INCREASES MINERAL NITROGEN AND DECREASES EXTRACTABLE PHOSPHORUS IN ACIDIC SOILS

4.1 Abstract

Nutrient loads from anthropogenic sources upset aquatic ecosystems. The aquatic macrophyte *Wolffia arrhiza* (duckweed) has capacity to remediate nutrient rich water through continuous harvesting of biomass that has potential as a soil fertility amendment. The objective of this study was to determine N and P mineralization of *W. arrhiza* biomass in soil and the fate of mineralized P. An incubation experiment was conducted in a constant temperature room (25ºC) for 56 days with three soils (top soil of a Dystric Regosol and topsoil and subsoil of a Rhodic Ferralsol) amended with *W. arrhiza* biomass at rates equivalent to 501, 1002 and 1503 mg N kg^{-1} and 62, 124 and 186 mg P kg^{-1}. The experiment had 288 containers to allow for destructive sampling at 0, 3, 7, 14, 21, 28, 42 and 56 days. Ammonium and nitrate- N were extracted from a 5 g soil with 50 ml of 2M KCl. Extractable- P was extracted from 2 g soil using the ammonium bicarbonate method. The ammonium-N was analysed by the automated continuous flow injection method. The nitrate-N and extractable-P were analysed using the automated calorimetric hydrazine reduction and the molybdenum-blue methods, respectively. At least 5% of the total N was produced as ammonium on the first day of incubation, with peak production within the first two weeks. Nitrate- and mineral-N increased from days 14 to 42 with corresponding decrease in ammonium-N. Soil of relatively low inherent fertility mineralised N at a slower rate than that of higher fertility. At least 16% to about 40% of duckweed P was extractable from soil on the first day of incubation and progressively declined over the incubation period. Fractionation of P at the end of incubation showed accumulation of

Al and Fe phosphates with increasing duckweed rate. The findings suggested that soil type and rate of *W. arrhiza* biomass are important determinants for N and P supply to crops.

Keywords

Duckweed, Nitrogen mineralization, Phosphorus fractionation, *Wolffia arrhiza*

4.2 Introduction

Anthropogenic activities have resulted in decline in surface water quality, through enrichment with nutrients (Wang et al., 2007). Excessive growth of aquatic plant biomass in nutrient enriched water bodies is widely reported (Quilliam et al., 2015) with consequent upset of the aquatic ecological balance and general loss of aesthetic value. Death and decomposition of aquatic plants not only release nutrients back into water but also increase biochemical oxygen demand, turbidity, foul smell and deplete dissolved oxygen, making the environment inhospitable for fish (Smith et al., 1999; Landesman et al., 2011). Harvesting of aquatic plants could have positive effects of improving water quality through nutrient reduction in water bodies and producing a resource with alternative uses. A group of aquatic macrophytes called duckweed has potential to improve water quality and soil fertility.

Duckweed has capacity to hyper-accumulate nutrients more than other aquatic macrophytes (Chaiprapat et al., 2005). The plants can bio-accumulate as much as 99% of nutrients contained in wastewater in about 15 days (Miretzky et al., 2004) and produce protein rich biomass (Zhao et al., 2014a). A study by Culley et al. (1981) showed that a mixture of duckweed (*Spirodera polyrrhiza, Landoltia punctata, Lemna gibba* and *Wolffia columbiana*) could take up 1378 kg N (as ammonium), 347 kg P and 441 kg K from one hectare of water surface in a year. The elemental composition of duckweed generally depends on nutrient composition of the medium, with water low in nutrients generally resulting in reduced mineral content. The results in Chapter 3 (Sections 3.4.3 and 3.4.4), showed that tissue N and P in *W. arrhiza* and *L. mino*r increased with the concentration of these elements in the water of their habitats, especially where the species occurred separately. Culley and Epps (1973) reported that duckweed growing on water of different concentrations had 1.2-4.1% N, 0.1-1% P, 1.9-3.8% K, 0.7-1.3% Ca and 0.2-0.4% Mg. However, in cultured media under controlled environments, higher duckweed

elemental levels are possible. Duckweed is able to take up nutrients and double its biomass in 16-24 hrs under favourable environments (Leng, 1999). Therefore, these macrophytes could form a major part of a cost effective and environmentally sound technology, useful for phytoremediation of agricultural, industrial and municipal wastewater. Some studies showed removal efficiencies of 73-97% of total N and 63-99% total P (Journey et al., 1993; Körner and Vermaat, 1998). Constant harvesting to avoid high mat densities, that may reduce their growth, could improve the efficiency of nutrient removal from wastewater (Monette et al., 2006). Alternative uses of duckweed from water should be incentives for its harvesting.

The quest to unlock value from an apparent waste product perceives duckweed as a renewable clean energy resource, human food and animal feed supplement and potential bioplastic material (Culley et al., 1981; Zeller et al. 2013; Cui and Cheng 2014; Verma and Suthar, 2014). Whilst work on various aspects of duckweed is advancing, there is paucity on its utilisation in soil fertility, despite Culley et al. (1981) calling it 'highly efficient nutrient packets'. Conspicuously limited literature on its use as soil amendments reflects a critical gap despite proposals as potential fertiliser in combination with other materials (Lot et al. 1979; Jensen et al., 2008; Kostecka and Kaniuczak, 2008). Studies by Kostecka and Kaniuczak (2008) focused on assessing the properties of vermi-compost obtained from duckweed fed to earthworms and precluded the use of duckweed directly as soil amendment. The suitability of duckweed as soil ameliorant could be governed by its decomposition and release of nutrients. The low C/N ratio of duckweed suggests that the biomaterials could readily decompose with potential to pollute the air and water through ammonium volatilisation and nitrate leaching, respectively. Unpublished records point to use of duckweed as green manure source of N to fruit trees and vegetables. However, the N mineralisation pattern is unknown in such a scenario, which presents challenges for management that synchronises nutrient release and peak plant

requirements. Comprehensive studies on nutrient release patterns from duckweed biomass in different soil types are non-existent. Since not all duckweed genera and species are effective in recovering nutrients from wastewater (Zhao et al., 2015), their quality and mineralisation patterns are presumed different, warranting in-depth studies. The generated information is essential for management of duckweed as a soil fertility amendment.

Preliminary work done in KwaZulu-Natal (Chapter 3) showed that *Wolffia* species appears to be efficient in taking up both N and P with variable levels of other elements. The highest tissue N and P were found in *Wolffia* spp. that grew on wastewater from piggery. The effectiveness of such *Wolffia* biomass as a nutrient source could depend on tissue composition of nutrients and other elements, as well as the soil type in which the duckweed residues are incorporated. The mineralisation of N and P, during decomposition of duckweed residues needs to be understood. The objective of this study was to determine the (i) effect of application rate of *W. arrhiza* biomass on N and P release in three soil types, (ii) P pools of soils amended with *W. arrhiza*.

4.3 Materials and methods

4.3.1 Duckweed and soil sampling

The study was conducted at University of KwaZulu-Natal in Pietermaritzburg (29^{o} 37' 33.9″S; 30^{o} 24' 14″E), South Africa. About 70 kg wet mass of duckweed (*W. arrhiza*) was randomly collected from a pond that received overflow from pig slurry dams and runoff water from upland fields irrigated with the slurry at Baynesfield Estate (29^{o}45'S and 30^{o}20'E) in the Midlands region of KwaZulu-Natal Province. The duckweed was transported to the laboratory as a dense suspension, and extraneous materials such as insects, grass and small sticks were

removed by passing the suspension through a 0.5 mm gauge, with the *W. arrhiza* trapped on a 0.1 mm gauge before rinsing with distilled water. The biomass was oven dried at 60^{o}C to constant weight.

The soil samples used in the incubation study were collected from Baynesfield Estate and University of KwaZulu-Natal's research farm, Ukulinga (29^{o}39'S, 30^{o}24'E). The soils from the Baynesfield Estates, Rhodic Ferralsols (Dominy and Haynes 2002), were sampled from the 0-20 and 20-40 cm depths using augers from a field that had no history of treatment with pig slurry. The 20-40 cm soil depth was presumed to represent the relatively less fertile soil than the surface one. These soils are referred to as Baynesfield A and Baynesfield B, respectively. The land had been under maize and soyabean rotations for over 10 years. Twelve sub samples per depth were thoroughly mixed to form a composite sample, and the samples were air dried and sieved (< 2 mm) before analysis. The soil from Ukulinga, Dystric Regosols (McGranahan et al., 2016), was sampled from the 0-20 cm depth of a piece of land that was fallow for the previous five years. The sample handling and preparation was the same as for the Baynesfield soils.

4.3.2 Duckweed and soil characterisation

Carbon and N from duckweed tissue and soil were determined using the LECO Trumac CNS Auto-analyser Version 1.1x (LECO Corporation, 2012). Tissue P, K, Ca, Mg, and micronutrients Fe, Mn, Zn, Cu and basic soil fertility were determined, in triplicate, following methods of The Non-Affiliated Soil Analysis Work Committee (1990). The duckweed tissue had 42 % C, 5.01 % N, 0.62 % P, 2.81 % K, 1511mg Al kg^{-1} and 3517 mg Fe kg^{-1}. In addition, it had 0.5 % Ca, 0.49 % Mg, 277 mg Mn kg^{-1}, 61.3 mg Zn kg^{-1} and 19.1 mg Cu kg^{-1}. Soil analysis results are shown in Table 4.1.

Table 4.1 Characteristics of Ukulinga and Baynesfield soils

Parameter	Ukulinga 0 -20 cm	Baynesfield A 0- 20 cm	Baynesfield B 20 -40 cm	Standard error
Clay (%)	30.7	40.0	42.3	5.730
pH (KCl)	4.63	4.27	4.68	0.011
Total C (%)	2.28	3.40	2.80	0.042
Total N (%)	0.18	0.23	0.17	0.004
Available P ($mgkg^{-1}$)	9.70	12.2	4.73	0.785
K ($cmol_c kg^{-1}$)	0.20	0.29	0.12	0.006
Ca ($cmol_c kg^{-1}$)	6.14	4.48	4.59	0.100
Mg ($cmol_c kg^{-1}$)	3.04	1.83	1.88	0.068
Mn ($mgkg^{-1}$)	15.2	15.0	8.85	0.587
Zn ($mgkg^{-1}$)	4.12	5.01	1.45	0.415
Cu ($mgkg^{-1}$)	6.76	5.53	4.28	0.203
EA ($cmol_c kg^{-1}$)	0.04	0.23	0.04	0.017
FC moisture (%)	28	25	22	0.799

EA= Exchangeable acidity
FC= Field Capacity

4.3.3 Nitrogen and phosphorus mineralisation in soil

4.3.3.1 Incubation

An incubation study was carried out using three soils at a constant room temperature (25ºC) for 56 days, in a completely randomised design, replicated three times for each sampling period. A 100 g (oven dry equivalent) soil mass was weighed into each plastic container (500 ml) that had 8 small holes drilled below the rim to allow for gaseous exchange and a tightly fitting lid. The dried duckweed biomass was added at 1, 2 and 3% (w/w) and mixed thoroughly with a 100 g sample of each soil type per container. The rates were equivalent to 501, 1002 and 1503 mg N kg^{-1} and 62, 124 and 186 mg P kg^{-1}. Untreated soils were included as controls. Moisture was adjusted to field capacity. Throughout the incubation period, soil moisture was restored to field capacity by weight loss correction. The experiment had 288 containers to allow for destructive sampling at 0, 3, 7, 14, 21, 28, 42 and 56 days.

4.3.3.2 N and P Analyses

Ammonium and nitrate- N were extracted from a 5 g soil with 50 ml of 2M KCl (Rayment and Lyons, 2011). Extractable- P was extracted from 2 g soil using the ammonium bicarbonate method (The Non-Affiliated Soil Analysis Work Committee, 1990). The ammonium-N was analysed by the automated continuous flow injection method (The Non-Affiliated soil Analysis Work Committee, 1990). The nitrate-N and extractable-P were analysed by the Thermo Scientific Gallery Discrete Auto-analyser using the automated calorimetric hydrazine reduction (Rayment and Lyons, 2011) and the molybdenum-blue (Murphy and Riley, 1962) methods, respectively. The results were corrected for moisture content in the extracted soil. Net mineralised N (ammonium and nitrate-N) and extractable-P were obtained by subtracting values of the control. Net mineral-N was the sum of ammonium- and nitrate-N.

4.3.4 Soil P fractionation

Sequential fractionation of inorganic P was carried out using a modified method of Zhang (2009) for non-calcareous soil, using samples collected at the termination of the incubation period (56 days) for the three soil types and three duckweed treatments. The sequential fractionation was done with 1 M ammonium chloride, 1 M sodium hydroxide, citrate dithionite bicarbonate (CDB) and 0.25 M sulphuric acid extracting solutions. These solutions extracted soluble and loosely bound P, predominantly inorganic P associated with amorphous and some crystalline Al oxides and Fe, reductant soluble P in matrices of retaining aggregates/minerals and Ca associated inorganic P that is not readily available in relatively short time scales, respectively (Homyak et al., 2014). The extracted P was read on a Thermos Scientific UV vis

GENESYS 20 visible spectrophotometer using the molybdenum-blue method (Murphy and Riley, 1962). Net extractable-P was obtained by subtracting values from the control.

4.3.5 Statistical analysis

The data on mineral N, P and fractionation of inorganic P (only after 56 days) was subjected to two-way ANOVA for each sampling time. Means were separated by least significant difference (LSD) at $p<0.05$.

4.4 Results

4.4.1 Ammonium-N

At day zero, there were no differences in net ammonium-N among soil types, while duckweed rates were significantly different from each other with the lowest and highest in the 1% and 3% duckweed rates, respectively (Figure 4. 1). The lowest rate released about 5% ammonium-N on the first day of incubation. At 7-14 days of incubation, and at all rates except the 1% treated Baynesfield B soil, the ammonium-N release reached its peak. At day 7, the soil types were significantly different from each other as were duckweed rates. The ammonium-N increased with application rate. The Baynesfield A soil had the highest ammonium-N while its subsoil had the lowest. At 14 days, the 2 and 3% rates had similar ammonium-N concentrations while the 1% rate had lower. The Baynesfield B and Ukulinga soils had similar ammonium-N concentration, which were lower than the Baynesfield A soil.

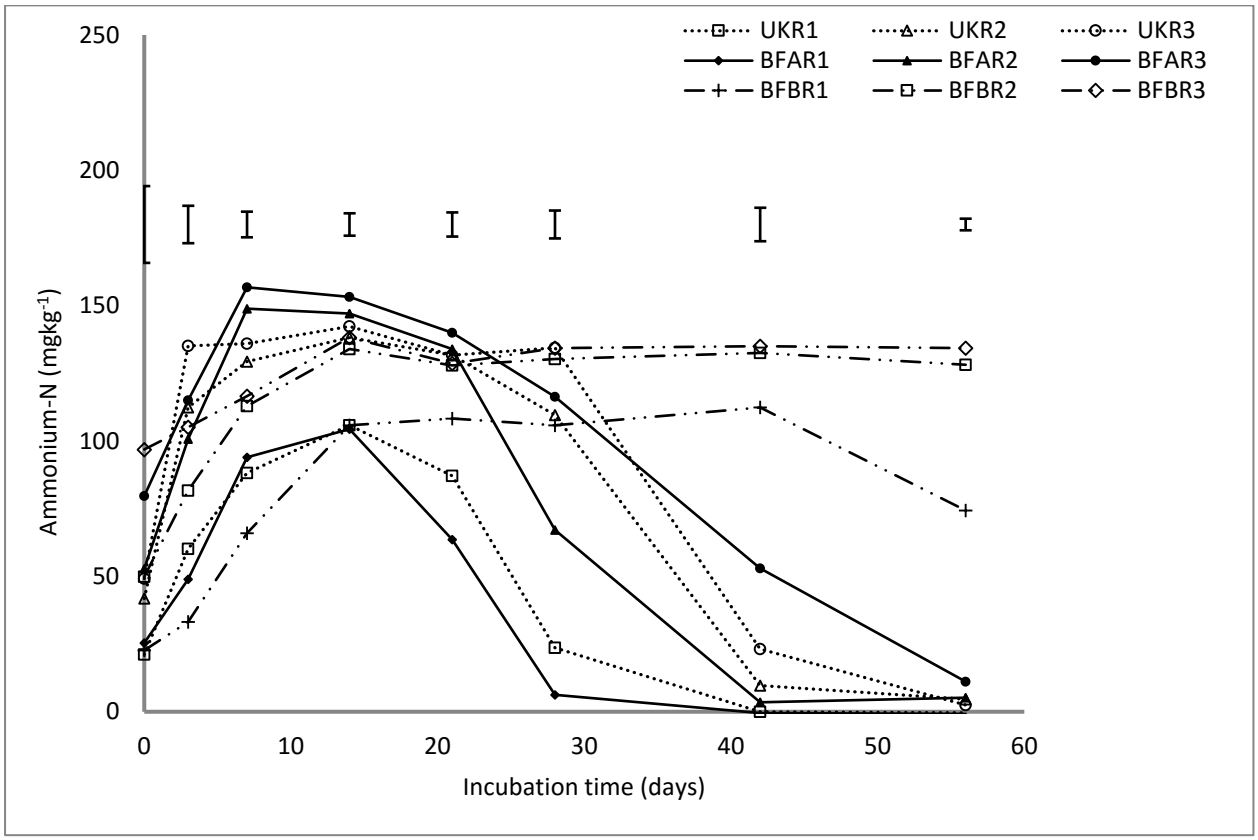

Figure 4.1 Variation of net Ammonium-N during incubation of W. *arrhiza* with three soil types. Uk = Ukulinga (0-20cm), BFA = Baynesfield A (0-20 cm), BFB = Baynesfield B (20-40 cm); R1, R2 and R3 represent *W. arrhiza* rates equivalent to 510, 1020 and 1530 mg Nkg^{-1} soil respectively. Vertical error bars indicate the LSD (p<0.05)

There was a sharp decline in ammonium-N concentration at 1% rate from 21 to 28 days for Baynesfield A and Ukulinga soils. The Baynesfield B soil had ammonium-N concentration greater than 100 $mgkg^{-1}$ from day 21 up to the end of the incubation period at all rates except at day 56, where it decreased for the 1% rate. From 28 to 42 days, a general decline in ammonium-N concentration occurred at all rates for the Baynesfield A and Ukulinga soils.

4.4.2 Nitrate-N

The net nitrate-N concentration was slightly above zero for all rates and soil types at day 0 and remained close to zero for the first 7 days for all treatments and up to 21 days for the Baynesfield B soil (Figure 4. 2). From day 14, the nitrate-N concentration started to increase at all rates for the Baynesfield A and Ukulinga soils. At day 21, and at all rates, Baynesfield A soil had nitrate-N concentration higher than that for Ukulinga. At day 28, the nitrate-N concentration had similar trends at all rates for all soils where Baynesfield A had the highest, followed by Ukulinga. At 42 days, the Baynesfield B soil had the lowest nitrate-N concentration at all rates. At this period, the soils had the same trend where nitrate-N concentration was similar for Baynesfield A and Ukulinga soils at the 1 and 2% rates. At 3% rate, Baynesfield A soil had the highest nitrate-N concentration followed by the Ukulinga soil.

At day 56, and at the 1% rate, all soils had similar nitrate-N concentration. The Baynesfield A soil had generally higher nitrate concentration than Baynesfield B. At 2 and 3% rates, soils had the same trends where the nitrate-N concentration in the Ukulinga and Baynesfield A soils were similar but higher than that of Baynesfield B soil.

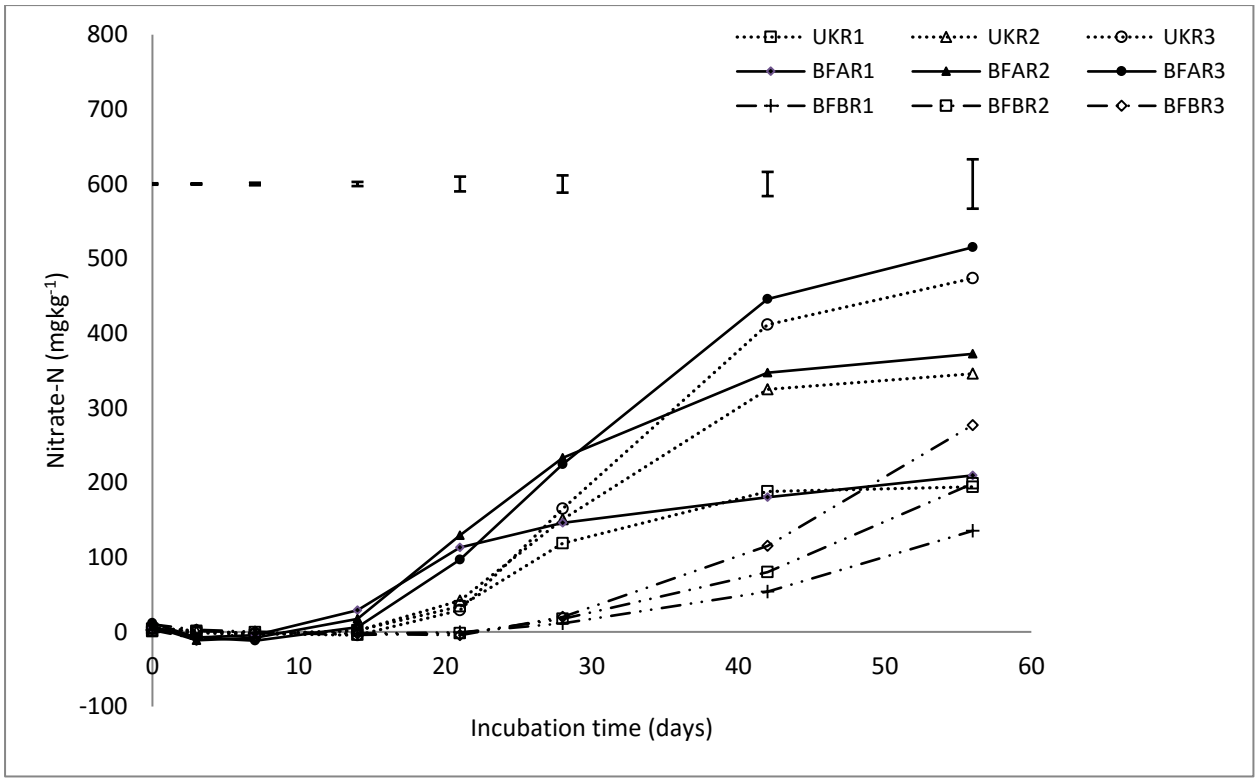

Figure 4.2 Variation of net Nitrate-N during incubation of W. *arrhiza* with three soil types. Uk = Ukulinga (0-20cm), BFA = Baynesfield A (0-20 cm), BFB = Baynesfield B (20-40 cm); R1, R2 and R3 represent *W. arrhiza* rates equivalent to 510, 1020 and 1530 mg N kg^{-1} soil respectively. Vertical error bars indicate the LSD (p<0.05)

4.4.3 Mineral-N

At day 0, the net mineral-N concentration increased with rate of application for all soils from 25.9 to 84.3 $mgkg^{-1}$ (Figure 4.3). At this period and per rate, the Baynesfield soils had similar mineral-N concentration that was higher than the Ukulinga soil. The mineral-N concentration steadily increased for all rates and soils up to day 14. Rapid increases occurred from day 14 to 21 for Baynesfield A soil at all rates while that for Ukulinga marginally increased. The mineral-N concentration of Baynesfield B soil at rates 1 and 2%, within the period from 14 to 21 days, decreased. At day 21 and at the 1% rate, the Ukulinga and Baynesfield B soils had similar mineral-N concentration that was lower than that of Baynesfield A soil. The 1 and 2% rates

had similar trends where mineral-N concentration for Baynesfield A was highest followed by Ukulinga and lastly Baynesfield B soil.

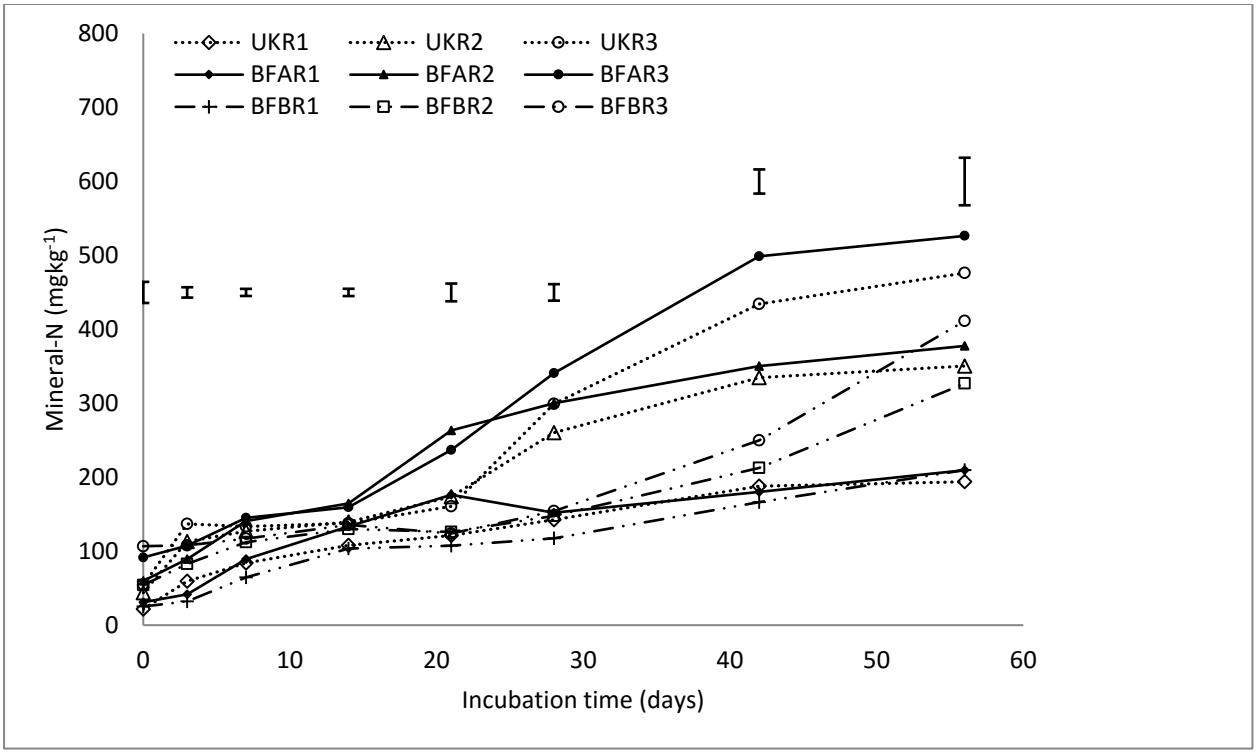

Figure 4.3 Variation of net Mineral-N during incubation of W. *arrhiza* with three soil types. Uk = Ukulinga (0-20cm), BFA = Baynesfield A (0-20 cm), BFB = Baynesfield B (20-40 cm) R1, R2 and R3 represent *W. arrhiza* rates equivalent to 510, 1020 and 1530 mg N kg^{-1} soil respectively. Vertical error bars indicate the LSD (p<0.05)

These trends for the mineral-N concentration of the two rates for all soils were similar to those at 28 days. At day 28, the 1% rate for the Ukulinga and Baynesfield A soils had similar mineral-N concentration that was higher than that of Baynesfield B. At day 42, the 1% rate had similar mineral-N concentration for all the soils, while the 2% rate had similar mineral-N concentration for Baynesfield A and Ukulinga soils that were higher than that of the Baynesfield B. At 3% rate, the Baynesfield A soil had the highest mineral-N concentration followed by Ukulinga soil. As from 42 to 56 days, the mineral-N concentration of the Baynesfield A and Ukulinga marginally increased at all rates while that of Baynesfield B at 2 and 3% rates increased rapidly.

At day 56, the mineral-N concentration increased with rate of application for all soils. At this period, the mineral-N concentration of the Ukulinga soil was similar to that of soils from Baynesfield with similar rates. The Baynesfield A soil had higher mineral-N concentration than Baynesfield B at all rates.

4.4.4 Extractable-P

Net extractable-P concentration had highest values on day zero for all soils and at all rates (Figure 4.4). At 1% rate, all soils had similar extractable-P concentration ranging from 10.2 to 13.4 mgkg^{-1}.

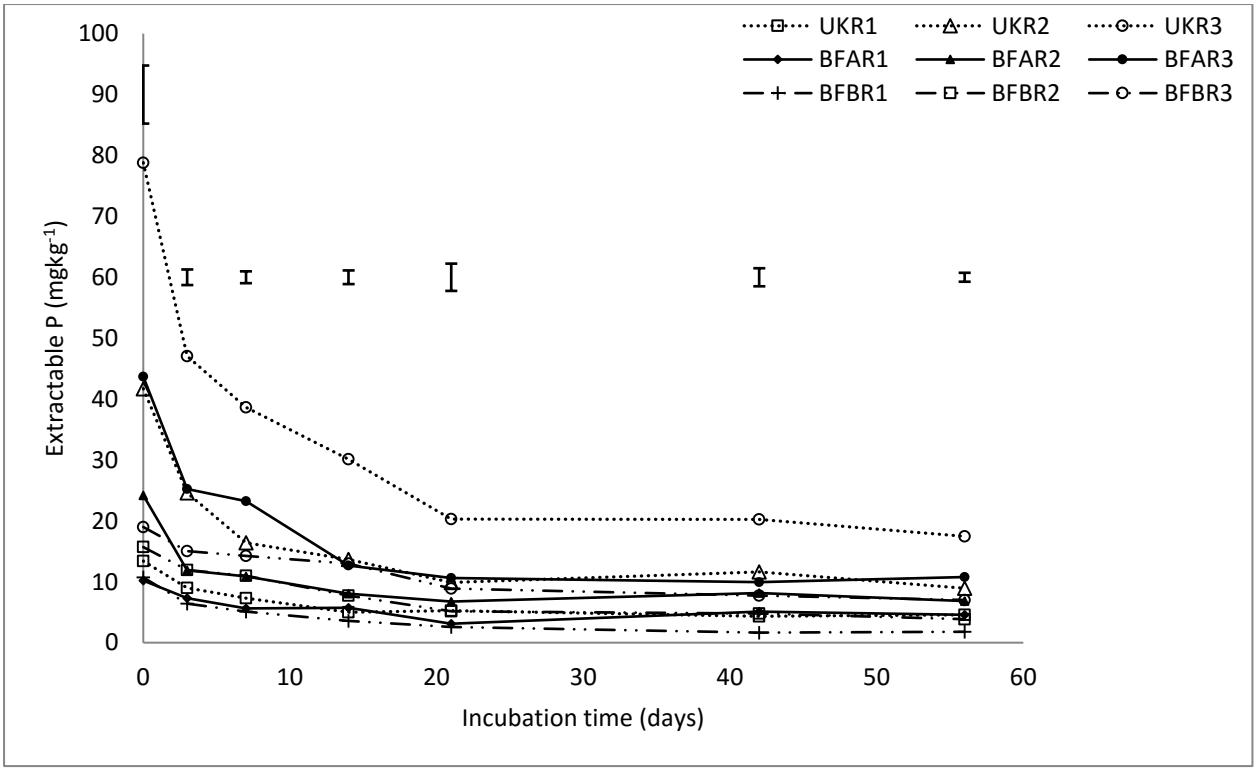

Figure 4.4 Variation of net Extractable-P during incubation of W. *arrhiza* with three soil types. Uk = Ukulinga (0-20cm), BFA = Baynesfield A (0-20 cm), BFB = Baynesfield B (20-40 cm); R1, R2 and R3 represent *W. arrhiza* rates equivalent to 62, 124 and 186 mg P/ kg soil respectively. Vertical error bars indicate the LSD (p<0.05)

At 2% rate, the Baynesfield soils had similar extractable-P concentration that was lower than that for Ukulinga soil. At 3% rate, Ukulinga soil had the highest extractable-P concentration and the Baynesfield B soil had the lowest. Rapid decrease in extractable-P concentration occurred within the period from days 0 to 3 for all soils at all rates. The general trend for all soils was a steady decrease of extractable-P concentration at all rates from 3 to 21 days. Day 3 and 7 had similar trends where Ukulinga soil had extractable-P concentration similar to the Baynesfield soils at 1% rate. Baynesfield A soil had higher extractable-P concentration than Baynesfield B at this rate. The 2% rate for Baynesfield soils had similar extractable-P concentration that was lower than that of Ukulinga soil. At 3% rate, the Ukulinga soil had the highest extractable-P concentration followed by Baynesfield A soil while Baynesfield B soil had the lowest. At day 21, the extractable-P concentration was relatively high for higher rates and low for lower rates for all soils. Baynesfield soils had similar extractable-P concentration that was lower than that of Ukulinga soil at all rates. After 21 days, the changes in extractable-P concentration, though significant, were marginal when considering the slope of the lines.

4.4.5 Fractionation of inorganic-P

Soil type had significant (p<0.05) effect on ammonium chloride extractable-P concentration where the Baynesfield soils had similar extractable-P concentrations that were lower than that of Ukulinga soil (Table 4.2). There were no significant effects of soil type on NaOH, CDB and H_2SO_4 extractable-P concentrations. The net sodium hydroxide extractable-P concentration was highest for control soil of Baynesfield A followed by Baynesfield B. The Baynesfield A control soil had higher net CDB extractable-P concentration than Ukulinga and Baynesfield B soils that had similar concentrations.

Table 4.2 Net extractable-P concentrations from three amended soil types by different extraction methods, and extractable-P concentrations from control soil

	Ammonium Chloride	**Sodium Hydroxide**	**Citrate Dithionite Bicarbonate**	**Sulphuric acid**
		$mgkg^{-1}$		
Amended soil				
Ukulinga	1.39[b]	344	56	22.8
Baynesfield A	0.74[a]	292	61	12.2
Baynesfield B	0.35[a]	279	52	27.5
Control				
Ukulinga	0.26	738[a]	462[a]	186
Baynesfield A	0.13	1722[c]	671[b]	259
Baynesfield B	0.00	906[b]	485[a]	156

Means followed by the same letter in a column are not significantly different at $p<0.05$
Means without superscripts are not significantly different at $p<0.05$

Rates of duckweed application did not significantly ($p>0.05$) affect the ammonium chloride extractable-P. The sodium hydroxide extractable-P concentration significantly increased with the rate of duckweed application for all soil types (Figure 4. 5).

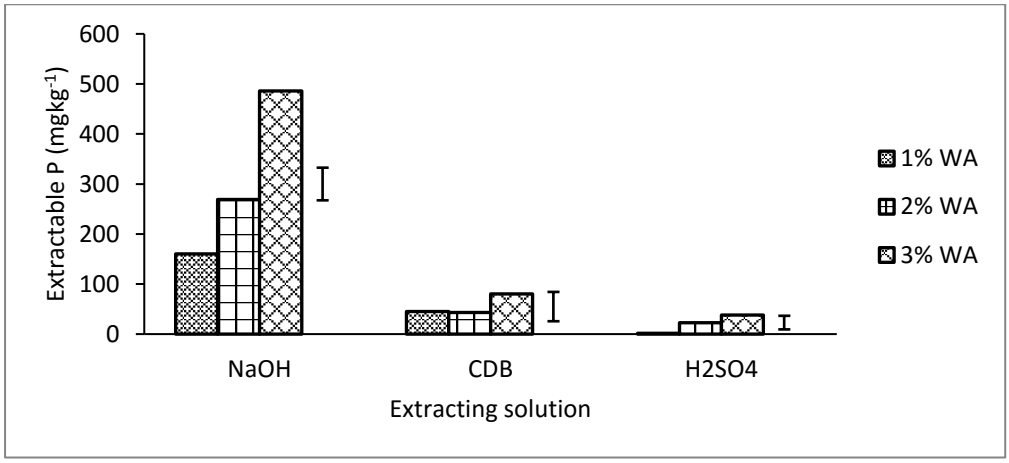

Figure 4.5 Extractable-P by Sodium hydroxide, Citrate dithionite bicarbonate (CDB) and Sulphuric acid at different duckweed rates. WA = *W. arrhiza*. Vertical error bars indicate the LSD ($p<0.05$)

The CDB extractable-P concentration was not affected by rates of duckweed application while the sulphuric acid extractable-P had the 1% rate significantly lower than the 3% rate.

4.5 Discussion

The production of at least 5% ammonium-N on the first day of incubation implied a material that readily decomposes, and this could be explained by the low C/N ratio of around eight, below the threshold value of 20-30, signifying net mineralisation (Kumar and Goh, 2000). Rapid decomposition may be facilitated by low fibre and lignin content as the macrophytes do not have upright structures that need mechanical support (Hasan and Chakrabarti, 2009; Tao et al., 2017). Generally, the peak ammonium-N production was at two weeks of incubation and deviations were due to activities influenced by rates of duckweed application and soil types. The peak ammonium-N production for Baynesfield A soil after a week at higher duckweed rates might be due to stimulation of a diversity of heterotrophs supported by the relatively more fertile soil (Table 4.1). The rapid ammonification could be accompanied by volatilisation, with possible N losses to the atmosphere. The ammonium-N concentration of lower rates of duckweed, unlike high ones, for relatively fertile soils quickly declined. The higher ammonium-N at higher duckweed rates at different incubation periods, especially within the first 28 days, could be explained by conversion of higher levels of organic-N added as a component of large quantities of duckweed. Decline in ammonium-N production after the first two weeks of incubation, for all soils except the Baynesfield B, could be explained by the inception of the nitrification phase under moist and aerated conditions (Figure 4. 2). In relation to the trends of ammonium-N, it suggests that both ammonium production and nitrification occurred at the same time up to 28 days. This is supported by the magnitude of nitrate-N produced that was much higher in soil (up to 500 $mgkg^{-1}$) than that of ammonium-N (up to 150 $mgkg^{-1}$).

The general increase in nitrate-N and mineral-N from days 14 to 42 corresponded with a decrease in ammonium-N. This explains the nitrification process engendered by a conducive

environment of soils at 25^{o}C and field capacity moisture content (Sahrawat, 2008). Whilst the Baynesfield B soil maintained a high ammonium-N concentration, its conversion to nitrate-N was sluggish, probably attributable to low initial populations of nitrifying organisms (Tisdale et al., 1993) in the subsoil that had a relatively high clay content. In addition, Brady and Weil (2008) highlight the importance of cations, phosphorus and micronutrients in stimulating nitrification and in contrast, the Baynesfield B soil had lowest amounts of these elements (Table 4.1). As such, infertile soils may result in lower nitrification of N from duckweed biomass added to soil. The higher nitrate-N at higher duckweed rates at different incubation periods could be explained by conversion of higher levels of ammonium-N from the large quantities of duckweed added.

The more rapid release of mineral-N from the Baynesfield A and Ukulinga soils, in 42 days, and the slower increase in Baynesfield B suggested that inherent soil fertility is an important factor. Soils of low fertility status would mineralise N from this amendment at a slower rate than those of higher fertility, possibly because of low initial microbial biomass. This would suggest that pre-incubation of duckweed will be necessary in order to synchronise crop nitrogen requirement and N availability (Malepfane and Muchaonyerwa, 2017) in soils of low fertility.

Mineralisation of duckweed could have been facilitated by the availability of other nutrients such as P. The C:P ratio of about 68:1 implies net mineralisation of P though extractable-P declined with duration of incubation. The net P release was consistent with the C:P ratio rule of thumb where mineralisation (C:P $\leq$ 200) prevailed during decomposition (Sharpley and Smith, 1989; Dossa et al., 2009). Depending on rate of duckweed application, at least 16% to about 40% of duckweed P extractable from the soil on the first day of incubation suggested high water soluble P in the plant material that immediately released high levels of P after

incorporation in soil as supported by Kwabiah et al. (2003). In a study on addition of organic and inorganic P sources to soil, Malik et al. (2012) reported that high P residue addition increased the concentration of fractions such as labile P within 5 hours of addition to soil, which they attributed to soluble P within the residue. Landolt and Kandeler (1987) highlight that duckweed is exceptional among macrophytes as they store P as orthophosphates within the vacuole, as condensed inorganic phosphates and in phytic acid. The rupturing of the duckweed cells on mixing with soil at field capacity moisture level could have contributed to high water soluble P at the start of the incubation. The decline of extractable-P over the incubation period for all soils was similar to trends observed by Malik et al. (2012), where their concentration of the labile resin and hydrogen carbonate inorganic P for most treatments with high P content in amendments decreased from days 0 to 56. The authors suggested transformation of inorganic P from labile into non-labile through sorption/fixation. In this study, higher concentrations of the NaOH extractable-P pool than all other pools from the sequential extraction suggested Fe and Al from both the duckweed and the acidic soils transformed most of the mineralised P from *W. arrhiza* into the non-labile form. The duckweed had high tissue Al and Fe content whose combined percentage (0.5%), was slightly less than its tissue P content. The results show that the Fe and Al content of duckweed species could have a significant influence on lability of mineralised P. This suggests need for lime addition before decomposition of high Al and Fe bearing duckweed.

Although extractable-P (available P) declined with incubation time, it was higher in the Ukulinga soil, possibly due to lower clay content and higher pH (Table 4.1), and was supported by the higher ammonium chloride-extractable pool during the fractionation (Table 4.2). The Ukulinga soil had relatively higher ammonium chloride P pool than the Baynesfield soils after 56 days due to lower clay content. The rates of duckweed application did not influence the

H_2SO_4 extractable-P since the 1% rate had almost no extractable-P, indicating little significance of Ca bound P.

4.6 Conclusion

Soil type and rate of duckweed application had an effect on decomposition of *W. arrhiza*. Application of *W. arrhiza* tissue to soil results in rapid release of mineral N with ammonium-N reaching a peak in 14 days followed by nitrification. Initial soil fertility was important on N mineralisation as relatively fertile soil stimulated faster decomposition at higher rates. Mineralised P from duckweed rich in Al and Fe is precipitated as phosphate of these elements that are from both the duckweed and the soil. The *W. arrhiza* can be utilized for crop N supply, with synchronization of N availability and crop requirement. Further research needs to ascertain the fertiliser value of duckweed to crops and to separate the effects of Al and Fe content of duckweed from the P fixing properties of the soil used. Co-application with lime on available P also needs to be studied.

CHAPTER 5: PRE-INCUBATION OF DUCKWEED BIOMASS IMPROVES NITROGEN UPTAKE AND DRY MATTER OF SWISS CHARD AND RESIDUAL SOIL NUTRIENTS

5.1 Abstract

Recovery of nutrients from water using duckweed and their reuse has significance in closing the loop on nutrient transfer from anthropogenic sources. This study investigated the effects of rates of application and pre-incubation period of duckweed on biomass and nutrient uptake of Swiss chard (Fordhook giant). Two glasshouse experiments were laid out in randomized complete block designs with three replicates. In the first experiment, Swiss chard was grown on two soils (ferralsol and regosol) amended with *Wolffia arrhiza* biomass at 0, 50, 100 and 200% of the recommended nitrogen rate. In the second experiment, the same vegetable was grown on the ferralsol amended with *W. arrhiza* and *Lemna minor* at recommended nitrogen rate, with pre-incubation periods of 0, 14 and 28 days. Application of *W. arrhiza* biomass increased Swiss chard dry matter by 23-45% compared to the negative control. The positive control (urea at 100 kg N ha^{-1} rate) had highest Swiss chard biomass. Higher rates than 100 kg N ha^{-1} had no added benefit on dry matter accumulation and nitrogen uptake of Swiss chard. Pre-incubation of duckweed for 28 days improved nutrient uptake resulting in higher dry matter than shorter periods. The Swiss chard dry matter after pre-incubation for 28 days was similar to that from urea application. Findings from this study suggest that duckweed is a resource with beneficial use for nutrient supply to vegetables especially when appropriate rates are used with pre-incubation.

Keywords

Duckweed, *Lemna minor*, Nitrogen uptake, pre-incubation, Swiss chard, *Wolffia arrhiza*

5.2 Introduction

Nutrient transfer caused by anthropogenic activities from land to aquatic systems has been reported worldwide (Smith et al., 1999; May et al., 2006; Wang et al., 2007). Excessive growth of aquatic plants on the nutrient rich water has potential to upset these ecosystems (Chislock et al., 2013). The nutrient transfer continuum model, comprising source-mobilization-delivery-impact phases, has been used to conceptualize this non-point nutrient transfer (Haygarth et al., 2005). However, Quilliam et al. (2015) criticized the model for failing to extend beyond impact (eutrophication), and advocated for the inclusion of the phase on nutrient recovery for returning to land and reuse, to be part of the model. This has significance in partially closing the loop on nutrient transfer from anthropogenic sources. Meanwhile, evolving policy and regulatory imperatives designed to ensure long-term protection of ecosystems, health and wellbeing of society, have created new challenges and opportunities for efficient and cost-effective resource recovery from a wide range of waste streams (Heathwaite, 2010; Shurin et al., 2013). While it is already common practice to harvest aquatic plant biomass in heavily impacted freshwater bodies to facilitate drainage, flood conveyance, water quality, visual appeal, navigation and recreational amenities (Quilliam et al., 2015), there are limited studies on nutrient recovery from aquatic plants and their reuse as a resource of essential plant nutrients.

The Midlands region of KwaZulu-Natal Province is one of the key agro-ecological regions of South Africa experiencing high nutrient loads in water bodies from anthropogenic activities (Isikhungusethu Environmental Services, 2012; Hitayezu et al., 2016) where a group of aquatic macrophytes called duckweed is increasingly colonizing fresh and wastewater bodies. Duckweed is among the floating aquatic macrophytes with huge capacity to absorb and even hyper-accumulate nutrients by doubling its biomass in 16-24 hrs under conducive environments (Leng, 1999; Chaiprapat et al., 2005). It belongs to the family Lemnaceae with

five genera and more than 37 species (Verma and Suthar, 2015). Due to its desirable chemical and physiological traits, duckweed has potential for phytoremediation of wastewater, energy production, feed supplement, bioplastics and phytotoxicity tests (Wang, 1990; Radic´ et al., 2011; Kufel et al., 2012; Zeller et al., 2013; Gwaze and Mwale 2015). The results in Chapter 3, Section 3.4, indicated that duckweed species of *Lemna* and *Wolffia*, with 3-5% N in the tissue, dominate on nutrient-rich water bodies from intensive agricultural systems in the Midlands region of KwaZulu-Natal. Though several studies have recommended duckweed biomass as an organic fertiliser based on its composition (Leng, 1999; Iqbal, 1999; Kostecka and Kaniuczak, 2008) there is a paucity of literature on their reuse for soil fertility improvement. The use of duckweed as a fertiliser could be an environmentally friendly option though the suitability of duckweed species to supply nutrients to various crops on different soils has not been assessed. Based on the results in Chapter 4, Section 4.4, the rapid mineralisation of N from *W. arrhiza* biomass in soil, suggested that this material could have potential to supply plant essential nutrients when directly applied to the soil.

Limited studies have been conducted to examine the potential of duckweed *L. minor* to support sorghum growth (Kraider, 2015; Pulido, 2016) and the authors concur that duckweed may be a viable source of organic fertiliser particularly supplying N and P to sorghum plants. Ahmad et al. (1990) applied duckweed *L. minor* biomass as a complementary source of N and recorded increased plant height, straw and grain yields accompanied by increase in N, P and K content of the rice plants. However, tissue elemental composition and growth of duckweed is affected by nutrient content and pH of the medium it thrive in, sunlight, temperature and species type (Landolt and Kandeler, 1987). The differences in elemental composition of duckweed could affect its effectiveness as sources of plant nutrients. Results in Chapter 3, Section 3.4, showed that the most common duckweed species found in the Midlands region of KZN Province, are *L. minor* and *W. arrhiza* with the latter having a higher N content. Various questions seek

answers, such as “Could the use of duckweed reduce the impact of non-point pollution from anthropogenic sources to adjacent water bodies and benefit agroecosystems through plant nutrient supply? Does a period of pre-incubation of biomass of different duckweed species in soil improve its effectiveness as plant nutrient source given their low C: N ratio?” The objective of this study was to determine the effects of (i) duckweed (*W. arrhiza*) biomass as a nitrogen source, (ii) pre-incubation period of *W. arrhiza* and *L. minor* in soil on Swiss chard (*Beta vulgaris*) biomass and nutrient uptake.

5.3 Methods and Materials

5.3.1 Soil and duckweed

The study was conducted at University of KwaZulu-Natal in Pietermaritzburg (29^{o} 37' 33.9″S; 30^{o} 24' 14″E), South Africa. The soil samples used for the glasshouse study were collected from the 0-20 cm depth from Baynesfield Estate (29^{o}45'S and 30^{o}20'E) and Ukulinga (29^{o}39'S, 30^{o}24'E) research farm of University of KwaZulu-Natal in the Midlands region of KwaZulu-Natal Province. Other details on these soils are as given in Section 4.3.1 of the Materials and Methods in Chapter 4. The soils of this trial were sampled at different times from those described in Chapter 4 (Table 4.1) and as such, there are slight differences in some of the characteristics. The soils were sampled from the 0-20 cm depth using picks and shovels, air dried and sieved (< 2 mm), before analysis. Soil analyses results before pot experiments are shown in Tables 5.1.

Table 5.1 Physico-chemical characteristics of soils used for pot experiments

Parameter	Regosol	Ferralsol	Standard error
Clay (%)	26.7	43.3	3.73
pH (KCl)	4.8	4.6	0.10
Total C (%)	1.69	2.88	0.27
Total N (%)	0.16	0.25	0.02
Available P ($mgkg^{-1}$)	10.6	13.7	0.85
K ($cmol_c kg^{-1}$)	0.18	0.48	0.07
Ca ($cmol_c kg^{-1}$)	7.47	4.85	0.60
Mg ($cmol_c kg^{-1}$)	3.82	1.75	0.47
Mn ($mgkg^{-1}$)	53.7	43.2	4.71
Zn ($mgkg^{-1}$)	5.07	6.53	0.34
Cu ($mgkg^{-1}$)	6.16	5.89	0.25
EA ($cmol_c kg^{-1}$)	0.07	0.19	0.05

EA= Exchangeable acidity

About 70 kg wet mass of duckweed *W. arrhiza* and *L. minor* were randomly collected from a pond that received overflow from pig slurry dams and runoff water from upland fields irrigated with the slurry at Baynesfield Estate. The duckweed was transported to the laboratory as a dense suspension, and extraneous materials such as insects, grass and small sticks were removed by passing the suspension through a set of sieves (2000 – 1000 µm), before rinsing with distilled water. Separation of *L. minor* from *W. arrhiza* was by the 1000 µm screen that only allowed the latter to pass through. The biomass was oven dried at 60^{o}C to constant weight. Duckweed analysis results before pot experiments are shown in Table 5.2.

Table 5.2 Elemental composition of duckweed species used in the study

Element	*Wolffia arrhiza*	*Lemna minor*	LSD
C/N	8.38	8.80	0.409
N (%)	5.01	4.58	0.028
Ca (%)	0.50	1.12	0.018
Mg (%)	0.49	0.50	0.019
K (%)	2.81	1.92	0.060
P (%)	0.62	0.67	0.019
Al ($mgkg^{-1}$)	1511	7394	118
Fe ($mgkg^{-1}$)	3517	6201	182
Mn ($mgkg^{-1}$)	277	237	7.23
Zn ($mgkg^{-1}$)	61	142	5.31
Cu ($mgkg^{-1}$)	19	10	4.16

5.3.2 Pot experiment 1

A 2 x 5 factorial experiment, laid out in a randomised block design was set in a glasshouse, with three replications. The blocking factor was moisture and temperature gradient, based on distance from the walls of the glasshouse humidifier. Two soils (ferralsol and regosol) were used in this experiment. The soils were weighed (3.0 kg) into pots, with inner diameter of 20 cm and height 17 cm. Biomass of *W. arrhiza* was added (treatments) at 0, 50, 100 and 200% of the N recommended rates. The Soil and Analytical Services Laboratory of the KwaZulu-Natal Department of Agriculture provided the N recommendation (100 kg N ha^{-1}) for Swiss chard. Urea fertiliser was included as a positive control at 100 Kg N ha^{-1}, split-applied with 50% application at transplanting and three weeks later. The rate per pot was converted to mass basis by adjusting the total N in duckweed or urea to the required treatment level using the recommended N rate per hectare and soil bulk density of 1.50 and 1.62 kgm^{-3} for the ferralsol and regosol respectively, for the 0-20 cm depth. The P and K for Swiss chard were supplemented as the difference between the recommended rates based on soil test values from

the ammonium bicarbonate extraction method (The Non-Affiliated Soil Analysis Work Committee, 1990) and duckweed tissue P and K content using NaH_2PO_4 and KCl.

Swiss chard (Fordhook giant) seedlings were obtained from Sunshine Seedlings Nurseries. Two seedlings (56 days old) per pot were transplanted and thinned to a single plant after two weeks. The minimum and maximum temperatures in the glasshouse were 18 and 23^{o}C, respectively. The pots were watered periodically to prevent water stress to the plants. Small amounts of leachate from the pots were returned to the soil surface, ensuring a closed watering system. Weeds were handpicked and incorporated into the soil. At 8 weeks, shoots were harvested and oven dried at 60^{o}C to constant weight and ground.

5.3.3 Pot experiment 2

The second pot experiment was set up in a randomised complete block design replicated three times in a glasshouse, with the two duckweed species at three pre-incubation times. Only the ferralsol was used in this experiment and was weighed (2 kg) into pots with inner diameter of 20 cm and height 17 cm. Duckweed *W. arrhiza* and *L. minor* treatments were added at the recommended rate of 100 kg N ha^{-1}. The amended soils were pre-incubated for 0, 14 and 28 days before planting Swiss chard seedlings while maintaining soil water content at field capacity. The periods were selected on basis of a preliminary incubation study that indicated increase in nitrate levels after 28 days. The pre-incubation was timed in such a way that all planting was done at the same time. The controls were not pre-incubated. Urea fertiliser was included as a positive control at 100 kg N ha^{-1}, split-applied with 50% application at transplanting and 3 weeks later. No N was added to the negative control. The P and K for Swiss

chard were supplemented as in pot experiment 1. Transplanting, watering, weed control, harvesting and soil and plant analyses were as in pot experiment 1.

5.3.4 Duckweed, Plant and Soil Analyses

Duckweed, soil and Swiss chard samples were analysed for C and N using the LECO Trumac CNS Auto-analyser Version 1.1x (LECO Corporation, 2012). Basic soil fertility, residual pot soil analyses and tissue P, K, Ca, Mg, Fe, Mn, Zn, Cu were determined, in triplicate, following methods of The Non-Affiliated Soil Analysis Work Committee (1990). Results of Swiss chard uptake were obtained from the product of tissue nutrient content and dry matter (g pot^{-1}).

5.3.5 Data analysis

All data were subjected to analysis of variance (ANOVA) using GenStat 14th edition. Least significant differences (LSD) (at $p<0.05$) were used to separate the treatment means of the first experiment and multiple comparison of means using Tukey's honest significance test were carried out for the second experiment where the LSD was not appropriate since the number of treatments exceeded six (Gomez and Gomez, 1984).

5.4 Results

5.4.1 Shoot dry matter and elemental uptake of Swiss chard

There were no significant interaction effects of soil type and duckweed application rate on dry matter yield and uptake of all nutrients except K and Fe. There were significant effects of rate

of application of duckweed biomass and soil type, as main factors, on Swiss chard dry matter and uptake of N, Ca, Mg, Mn, Zn and Cu (Figure 5.1, Tables 5.3 and 5.4). Application of duckweed biomass in all treatments significantly increased the Swiss chard dry matter by 23-45% compared to the negative control (Figure 5.1).

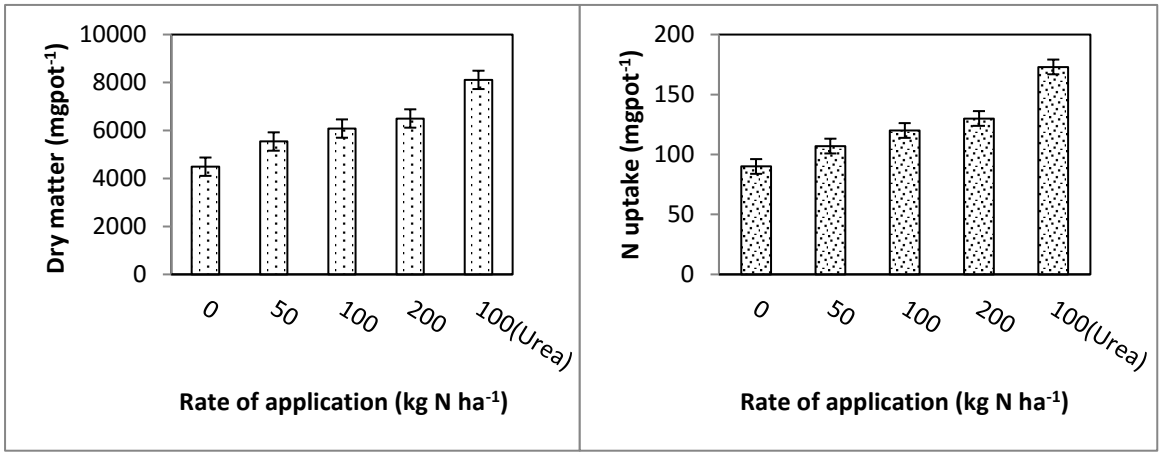

Figure 5.1 Effect of rate of duckweed application on dry matter and N uptake (mgpot^{-1}) of Swiss chard. Error bars represent the LSD at p<0.05

The positive control produced the highest Swiss chard dry matter. Application of duckweed at an equivalent rate of 200 kg N ha^{-1} and at positive control produced significantly higher dry matter than at 50 kg N ha^{-1} and negative control treatments while the recommended N rate (100 kg N ha^{-1}) had similar dry matter as the 50 and 200 kg N ha^{-1} rates. The 100 and 200 kg N ha^{-1} rate had similar N uptake by Swiss chard that was lower than of the positive control. The negative control had the lowest N uptake followed by the 50 kg N ha^{-1} rate. The 200 kg N ha^{-1} rate had higher Ca uptake than the negative control, while the positive control had higher Ca uptake by Swiss chard than the negative control and the 50 kg N ha^{-1} rates. Uptake of Mg at 200 kg N ha^{-1} rate was higher than at 50 kg N ha^{-1} and negative control (Table 5.3).

Table 5.3 Effect of rates of duckweed application on nutrient uptake (mgpot^{-1}) of Swiss chard

Rate (kgha^{-1})	P	Ca	Mg	Mn	Zn	Cu
0	31	66	54	3.85	0.94	0.09
50	36	77	65	4.40	1.15	0.11
100	35	88	76	5.18	1.27	0.12
200	36	93	80	5.20	1.34	0.12
100 (Urea)	32	97	97	5.63	1.54	0.13
LSD	5	17	14	0.62	0.13	0.02

The positive control had the highest uptake of Mg. Uptake of Mn at 50 kg N ha^{-1} rates and negative control were lower than at the rest of the treatments. Copper uptake by Swiss chard in the control was lower than at positive control, and duckweed treatments at100 and 200 kg N ha^{-1}. Rates of duckweed application did not affect P uptake of Swiss chard. The Baynesfield ferralsol had significantly higher Swiss chard dry matter and uptake of N, Ca, Mg, Mn, Zn and Cu than the Ukulinga regosol (Table 5.4). Swiss chard uptake of P was higher in the regosol.

Table 5.4 Effect of soil type on dry matter yield and uptake of N, Ca, Mg, Mn, Zn and Cu (mgpot^{-1}) by Swiss chard

Soil	DM	N	P	Ca	Mg	Mn	Zn	Cu
		uptake						
Regosol	4360	89	48	55	54	4.14	0.90	0.09
Ferralsol	7940	160	20	114	94	5.56	1.59	0.13
LSD	484	8	3	11	9	0.39	0.08	0.01

Significant interactions of rates of application of duckweed biomass and soil type existed for Swiss chard uptake of K and Fe (Figure 5.2). For the regosol, uptake of K by Swiss chard from the 200 kg N ha^{-1} rate and positive control was higher than that of the control and 50 kg N ha^{-1}. The regosol amended with duckweed at 100 kg N ha^{-1} had similar K uptake to that at 50 and 200 kg N ha^{-1}.

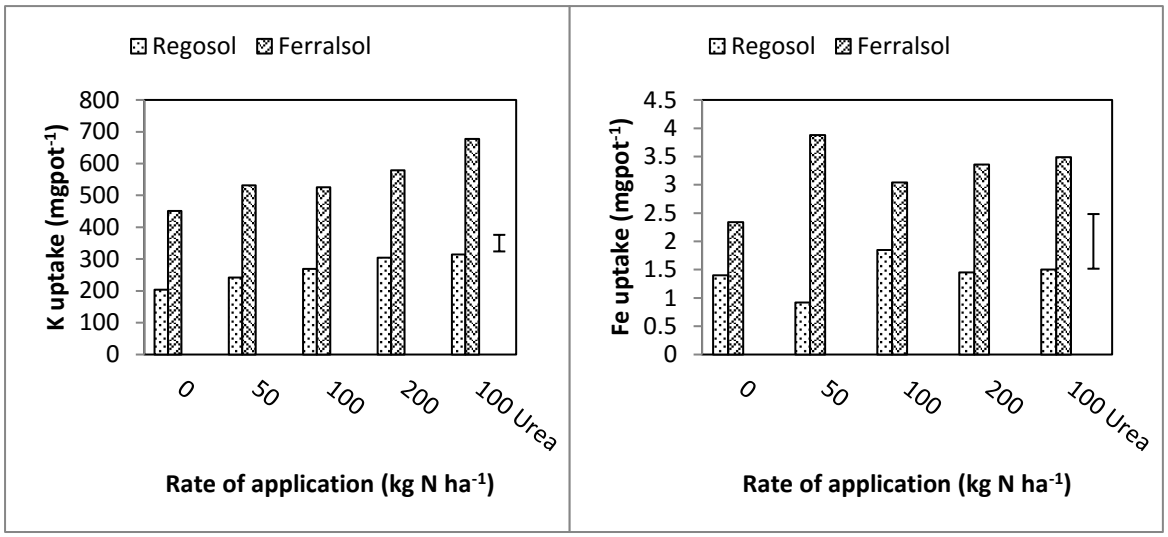

Figure 5.2 Effect of soil and rate of application of duckweed and urea on K and Fe uptake of Swiss chard. Error bars represent the LSD at $p<0.05$

For ferralsol, the highest Swiss chard uptake of K was from the positive control while the lowest was from the negative control. Uptake of K from duckweed amended ferralsol at 50 kg N ha^{-1} was similar to that at 100 and 200 kg N ha^{-1}. The uptake of Fe by Swiss chard was not significantly different at all rates on regosol. However, uptake of Fe at 50, 200 kg N ha^{-1} and positive control was higher than of the negative control on ferralsol while that at 100 kg N ha^{-1} was similar to other rates.

5.4.2 Residual soil chemical properties after growth of Swiss chard

Interactions effects of soil type and rates of application of duckweed biomass were significant on residual soil N, P, K, Ca and Cu but not on C, Zn and exchangeable acidity. The residual N content of ferralsol was higher than the regosol at all rates except the negative control, which had a similar level (Figure 5.3a). The residual soil N for the regosol was similar at all rates except for the negative control that had higher levels. The ferralsol had the highest residual N in the negative control treatment, while the 50 kg N ha^{-1}rate had the lowest, with the remaining rates having similar levels. The residual soil P was similar for both soils at all rates except at

the positive control where the regosol had higher levels than the ferralsol (Figure 5.3b). The residual soil P in the negative control of the regosol was higher than that of the positive control, 50 and 200 kg N ha^{-1}. The 100 kg N ha^{-1} rate had higher residual N than the 200 kg N ha^{-1}. In the ferralsol, the lowest residual P was in the positive control followed by the 200 kgNha^{-1} while the negative control had the highest. The residual soil K was higher for ferralsol than regosol at all rates except at the positive control, where the two soils had similar levels (Figure 5.3c). The residual soil K of the negative control of regosol was higher than that of the positive control and the 200 kg N ha^{-1} rate. For ferralsol, the residual K was similar for the treatments that incorporated duckweed while it was lowest for the positive control and highest for the negative control. The residual soil Ca at all rates was higher for the regosol than ferralsol (Figure 5.3d).

The residual Ca for the regosol at the 200 kg N ha^{-1} rate was similar to that at 100 kg N ha^{-1} and higher than that of both controls and 50 kg N ha^{-1}. Residual Ca at the 200 kg N ha^{-1} rate of the ferralsol was lower than at the positive control, 50 and 100 kg N ha^{-1} rates. The regosol had higher residual Cu than the ferralsol at all rates (Figure 5.3e). The residual soil Cu for regosol was similar at 50 and 100 kg N ha^{-1} and significantly higher than that at the 200 kg N ha^{-1} and the positive control. Residual Cu in the ferralsol at 200 kg N ha^{-1} and positive control was significantly lower than of negative control.

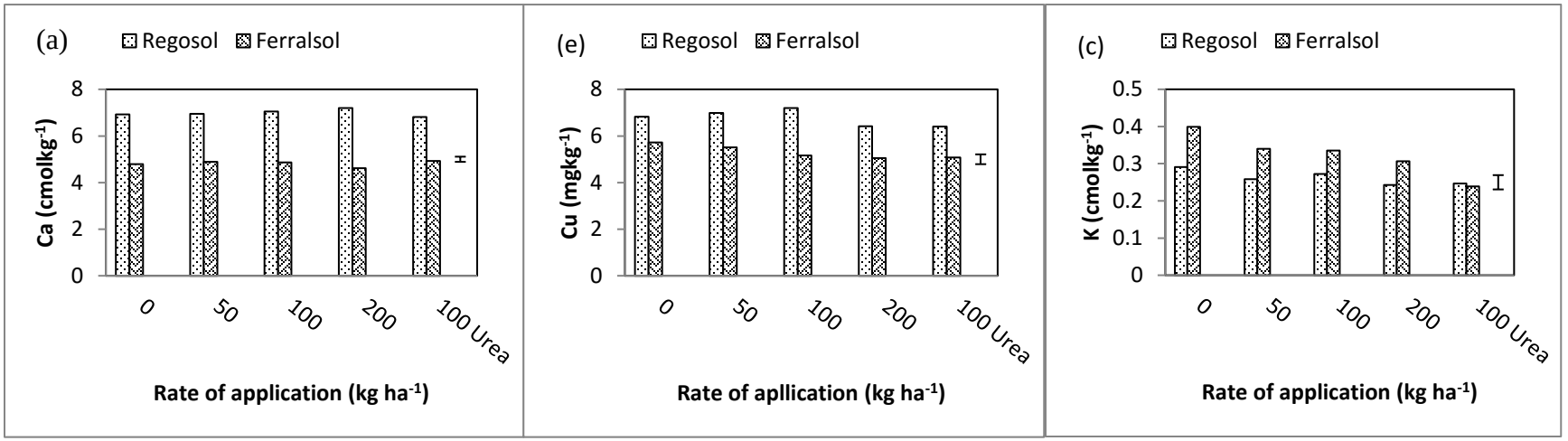

Figure 5.3 Residual N (a), extractable P (b) exchangeable K (c) and Ca (d) and extractable Cu (e) in two soils treated with increasing rates of duckweed. Error bars represent the LSD at $p<0.05$

Rates of duckweed N application had no significant effects on residual soil C content, Zn and exchangeable acidity (Table 5.5). Residual soil Mg from the negative control and 50 kg N ha^{-1} were similar and significantly higher than the 100 kg N ha^{-1} and positive control. Residual Mg at 200 kg N ha^{-1} was similar to that at 100 N ha^{-1} and positive control. Residual soil Mn at 100 kg N ha^{-1} was significantly higher than that for the positive control and 200 kg N ha^{-1}. The residual soil pH of the negative control had higher pH than soil at 100 and 200 kg N ha^{-1} rates while the positive control had the lowest. The residual soil pH values for the duckweed treatments were similar.

Table 5.5 Effect of rates of duckweed application on residual soil C, Mg, Mn, Zn, exchangeable acidity and pH

Rate ($kgha^{-1}$)	**C (%)**	**Mg ($cmolkg^{-1}$)**	**Mn ($mgkg^{-1}$)**	**Zn ($mgkg^{-1}$)**	**EA ($mgkg^{-1}$)**	**pH**
0	2.25	2.56	46.61	6.10	0.20	4.45
50	2.26	2.55	49.53	6.22	0.22	4.43
100	2.32	2.45	50.64	6.30	0.21	4.41
200	2.22	2.53	46.55	5.67	0.23	4.41
100 (Urea)	2.22	2.44	46.16	6.59	0.24	4.34
LSD	0.10	0.09	4.06	1.17	0.03	0.03

EA = Exchangeable acidity

Soil type had significant residual effects on C, Mg, Mn, exchangeable acidity and pH after Swiss chard growth (Table 5.6). The regosol had significantly higher Mg, Mn and pH than ferralsol while the later soil had significantly higher C and exchangeable acidity. The residual Zn levels were similar for both soils.

Table 5.6 Effect soil type on residual Soil C, Mg, Mn, Zn, exchangeable acidity and pH

Soil	**C (%)**	**Mg ($cmolkg^{-1}$)**	**Mn ($mgkg^{-1}$)**	**Zn ($mgkg^{-1}$)**	**EA ($mgkg^{-1}$)**	**pH**
Regosol	1.77	3.47	66.9	6.03	0.07	4.69
Ferralsol	2.74	1.55	28.9	6.33	0.37	4.14
LSD	0.50	0.07	2.57	0.74	0.02	0.02

EA = Exchangeable acidity

5.4.3 Shoot dry matter and nutrient uptake of Swiss chard after pre-incubation of duckweed

Pre-incubation of duckweed biomass had a significant effect on the dry matter of Swiss chard (Table 5.7). *W. arrhiza* and *L. minor* biomass pre-incubated for 28 days produced similar Swiss chard dry matter with that of the positive control. Pre-incubation of *W. arrhiza* for 28 days had higher Swiss chard dry matter than all other treatments besides the positive control and *L. minor* pre-incubated for the same time. Pre-incubation of *L. minor* for 28 days produced Swiss chard dry matter that was higher than the negative control and both duckweed species incorporated at transplanting (not pre-incubated). The highest Swiss chard N uptake (significant) was after pre-incubating *W. arrhiza* for 28 days followed by the positive control and *L. minor* pre-incubated for the same time. There were no significant differences in uptake of P, Fe and Al for all treatments. The uptake of K on pre-incubation of *W. arrhiza* for 28 days was higher than that for incorporation of both duckweed species, when not pre-incubated. The Ca and Mg uptake after pre-incubating *W. arrhiza* for 28 days was higher than the rest of the treatment besides the positive control and the *L. minor* pre-incubated for the same time. The *L. minor* pre-incubated for 28 days had higher Swiss chard Mg uptake than that of the control, *L. minor* pre-incubated for 14 days and both duck weed species when not pre-incubated. The uptake of Mn from *W. arrhiza* pre-incubated for 28 days was higher than that for *W. arrhiza* incorporated at transplanting and *L. minor* pre-incubated for 14 days. Pre-incubation of both duckweed species for 28 days resulted in higher Swiss chard uptake of Zn than all treatments except that of the positive control. The Cu uptake for the positive control and *W. arrhiza* pre-incubated for 28 days was higher than for *L. minor* pre-incubated for 14 days and both species when not pre-incubated.

Table 5.7 Effect of period of duckweed incorporation on dry matter yield and elemental uptake (mgpot^{-1}) by Swiss chard grown in the ferralsol

Biomass	Pre-Inc	DM	N	P	K	Ca	Mg	Mn	Zn	Cu	Fe	Al
	(days)		Uptake									
Control	0	4164^{ab}	76^{ab}	18	345^{ab}	59^{a}	56^{a}	3.95^{ab}	0.96^{a}	0.11^{ab}	3.72	4.08
+Control	0	6753^{bcd}	124^{c}	15	443^{ab}	89^{ab}	98^{abc}	5.29^{ab}	1.31^{ab}	0.15^{b}	5.25	6.16
LM	0	4164^{a}	67^{a}	17	310^{a}	55^{a}	54^{a}	3.45^{ab}	0.90^{a}	0.08^{a}	3.23	3.52
LM	14	5444^{abc}	93^{b}	16	398^{ab}	65^{a}	63^{a}	3.35^{a}	0.96^{a}	0.08^{a}	2.77	3.20
LM	28	7385^{cd}	137^{c}	15	442^{ab}	109^{ab}	120^{bc}	4.82^{ab}	1.75^{b}	0.14^{ab}	4.43	5.28
WF	0	4384^{a}	78^{ab}	15	330^{a}	54^{a}	54^{a}	3.23^{a}	0.78^{a}	0.08^{a}	3.32	3.76
WF	14	5994^{abc}	95^{b}	13	385^{ab}	75^{a}	79^{ab}	4.02^{ab}	0.90^{a}	0.11^{ab}	2.34	2.62
WF	28	8218^{d}	177^{d}	14	483^{b}	145^{b}	144^{c}	5.81^{b}	1.88^{b}	0.16^{b}	2.04	2.67

Means followed by the same letter in a column are not significantly different at $p<0.05$. Means without superscripts are not significantly different at $p<0.05$. Pre-inc = pre-incubation period, LM = *L. minor*, WF= *W. arrhiza*, +Control= Urea applied at recommended rate at transplanting and 3 weeks after transplanting

5.4.4 Residual soil chemical properties after pre-incubation of duckweed and growth of Swiss chard

Period of duckweed incorporation had no significant residual effect on soil C, N, K, Ca, Mn, Cu and exchangeable acid (Table 5.8). The residual soil P after *L. minor* pre-incubated for 14 days was higher than that of the two species pre-incubated for 28 days. Residual soil Mg for the negative control and *L. minor* incorporated at transplanting (not pre-incubated) were significantly higher than of both duckweed species pre-incubated for 28 days. The two duckweed species incorporated on the day of transplanting (not pre-incubated) had higher residual soil pH than that of the positive control and both duckweed species pre-incubated for 28 days. The residual soil Zn for *W. arrhiza* incorporated at day of transplanting was higher than all treatments except that of *L. minor* incorporated on day of transplanting and the negative control.

5.5 Discussion

The increase in Swiss chard biomass with increasing rates of duckweed application could be explained by relative increase in nutrient uptake, especially N and K, compared to the control. Nitrogen uptake of Swiss chard generally increased emphasizing the essential role of N in plant growth (Engelbrecht et al., 2010). The similarity of soil residual N at all rates for the individual soils suggested that the increase in supplied N, as a result of duckweed addition, made N more available for plant growth, with limited effects on residual levels.

Table 5.8 Effect of period of duckweed incorporation on residual soil chemical properties after Swiss chard grown in the ferralsol

Biomass	Pre-Inc (days)	C (%)	N (%)	P ($mgkg^{-1}$)	K ($cmolkg^{-1}$)	Ca ($cmolkg^{-1}$)	Mg ($cmolkg^{-1}$)	EA ($cmolkg^{-1}$)	pH	Mn ($mgkg^{-1}$)	Zn ($mgkg^{-1}$)	Cu ($mgkg^{-1}$)
Control		3.10	0.22	20.7^{ab}	0.37	4.99	1.77^{c}	0.43	4.23^{bc}	34.2	5.81^{ab}	5.29
+Control		3.31	0.27	20.9^{ab}	0.33	4.99	1.57^{abc}	0.51	4.16^{ab}	34.5	5.70^{a}	5.50
LM	0	3.22	0.25	20.2^{ab}	0.39	5.18	1.81^{c}	0.39	4.29^{c}	36.5	6.39^{ab}	6.03
LM	14	3.19	0.26	22.6^{b}	0.36	4.94	1.62^{bc}	0.37	4.22^{bc}	30.2	5.55^{a}	5.26
LM	28	3.21	0.25	18.4^{a}	0.26	5.08	1.45^{ab}	0.56	4.14^{ab}	31.2	5.70^{a}	5.61
WF	0	3.09	0.24	19.6^{ab}	0.38	4.93	1.67^{bc}	0.37	4.27^{c}	36.6	6.69^{b}	5.50
WF	14	3.06	0.23	21.5^{ab}	0.33	5.00	1.62^{bc}	0.46	4.19^{abc}	32.8	5.66^{a}	5.40
WF	28	3.20	0.25	19.1^{a}	0.22	4.81	1.32^{a}	0.53	4.10^{a}	32.9	5.67^{a}	5.00

Means followed by the same letter in a column are not significantly different at $p<0.05$. Means without superscripts are not significantly different at $p<0.05$. Pre-inc = pre-incubation period, LM = *L. minor*, WF= *W. arrhiza*, +Control= Urea applied at recommended rate at transplanting and 3 weeks after transplanting, EA= Exchangeable acidity

The highest dry matter of Swiss chard in the positive control at 100 kg N ha^{-1} and similar content in the duckweed treatments at 100 and 200 kg N ha^{-1}, were in agreement with Hammad et al. (2007) and Kołota and Czerniak (2010). Hammad et al. (2007) reported that nitrogen levels and sources influenced dry mass of spinach, while other studies generally maintained that the source of N did not influence the yield of leafy vegetables (Wang and Li, 2004; Engelbrecht et al., 2010). In this study, application of urea provided readily available N for uptake (Brito et al., 2012), resulting in higher dry matter yield in the positive control, whereas duckweed biomass decomposed with gradual N mineralization. Kołota and Czerniak (2010) reported that high N rates between 150 and 200 kg N ha^{-1} did not improve dry matter yield of Swiss chard and increment of N dose from 100 to 200 kg N ha^{-1} did not cause any substantial yield enhancement (Kołota et al., 2017). The similarity of uptake of most nutrients in this study at the 100 and 200 kg N ha^{-1} implied that N uptake generally influenced the growth of the vegetable and affected the uptake of other plant essential nutrients in a similar manner such that there was no comparative dry matter advantage at 200 kg N ha^{-1}. The increased available N from duckweed decomposition at higher rates and the positive control supported greater plant growth (Table 5.3), which facilitated greater uptake of other essential nutrients, than at lower rates where biomass accumulation was limited. This effect was evident with uptake of K, Ca, Mg, Mn, Zn and Cu. The lower uptake of these elements in treatments, where dry matter was low, was supported by the higher levels of these elements in the residual soils, including phosphorus. The higher nutrient uptake by Swiss chard on ferralsol were consistent with its relatively higher fertility status than regosol (Tables 5.1 and 5.4). Uptake trends of Fe by Swiss chard were not influenced by N rates for regosol due to soluble Fe at prevailing soil pH (<4.3), (Brady and Weil, 2008; Ranade-Malvi, 2011). This finding was supported by the relatively higher uptake of P in the regosol than ferralsol, where availability could

have been limited by fixation. The relatively lower soil pH, higher clay content and availability of soluble Fe and Al could have resulted in more P fixation (Lucas and Davis, 1961) by ferralsol than the regosol. Relative to the control, addition of higher rates of N from duckweed and urea contributed to soil acidification due to possible nitrification. Sanchez-Monedero et al. (2001) observed a decline in pH during organic waste composting due to nitrification and Turmel et al. (2015) confirmed that the overall effect of N mineralization is acidifying. In preliminary incubation experiments, mineralization of N and its subsequent nitrification resulted in pH decline in the same soils used in this study. While the increasing duckweed rate up to an equivalent of at least 100 kg N ha^{-1} increased yield, the dry matter was still lower than the positive control, which suggested that some N was yet to mineralize to be available. This view was supported by the findings of the experiment where the duckweed was pre-incubated before Swiss chard was planted.

Pre-incubation of duckweed for 28 days might have been essential in making enough N uptake and all other nutrients, resulting in similar Swiss chard dry matter with the positive control. The period is appropriate for mineralization of N in duckweed as most nutrients are initially unavailable and have to be slowly released through microbial degradation (Brito et al., 2012). This observation was confirmed by lower dry matter of Swiss chard in the negative control and both duckweed species incorporated at transplanting (not pre-incubated) than the same species pre-incubated for 28 days. This finding indicates that in order to derive maximum benefits from duckweed as a nutrient source, pre-incubation is required. Pre-incubation for 14 days (two weeks) proved to be less effective at influencing Swiss chard dry matter probably due to initially low amounts of mineralized nutrients and lack of synchrony between N crop demand and N mineralization (Sainju et al., 2006). The ferralsol in this study was highly weathered, well drained and fertile (Table 5.2) and also provided NO_3^- through nitrification as indicated by residual soil pH that mostly declined

from duckweed incorporated at transplanting to that pre-incubated for 28 days. The higher dry matter of Swiss chard at longer pre-incubation periods, in response to greater mineral N, was accompanied by great uptake of other plant essential nutrients from both the soil and incorporated duckweed biomass including K, Ca, Mg, Mn and Zn, especially for *W. arrhiza*.

Generally, high uptake of nutrients by Swiss chard after pre-incubation of *W. arrhiza* for 28 days could be explained by the amendment's elemental composition. *W. arrhiza* had a narrower C:N ratio and higher N content than *L. minor* and this could lead to the former decomposing more rapidly in soil and releasing nitrogen (Kumar and Goh, 2000; Tejada et al., 2008) that was readily taken up by the plants.

The non-response of P uptake of Swiss chard over period of pre-incubation indicated limited ability of the plant to utilize the nutrient as decomposition of duckweed proceeded. Conversely, as highlighted in Chapter 4, Section 4.4.5, high Fe and Al levels in the ferralsol at relatively low pH coupled with additions from duckweed decomposition and the acidifying nature of the nitrification process could have resulted in P fixation on Al and Fe oxyhydroxide surfaces and precipitation of Al and Fe phosphates (Lucas and Davis, 1961). This might have affected P uptake as confirmed by the inorganic fractionation of P in Chapter 4 (Section 4.4.5), and generally similar residual soil P values and even lower for the 28 day incubation period, where residual soil pH was lowest. Although all treatments were corrected for K, the pre-incubated treatments had higher K uptake by Swiss chard from *W. arrhiza* pre-incubated for 28 days than when not pre-incubated, possibly as a result of synergistic effects with the higher N released by mineralisation (Ranade-Malvi, 2011). This trend is not exhibited by *L. minor* treatments possibly due to high Ca levels, released from its biomass, that antagonized uptake of K.

5.6 Conclusion

The relatively high N content in duckweed species *W. arrhiza* makes it a suitable organic N source to improve Swiss chard yield. Higher duckweed application rate than 100 kg N ha^{-1} had no added advantages on dry matter accumulation and N uptake of Swiss chard. Dry matter, nutrient uptake by Swiss chard and residual concentrations of nutrients depended on initial soil properties, elemental composition and rate of duckweed application and pre-incubation period. Pre-incubation of duckweed biomass for at least 28 days improved nutrient availability and uptake resulting in greater dry matter of Swiss chard that was as good as urea application. Although duckweed had a low C/N ratio, synchronization of crop nutrient demand and release from duckweed is critical for use of this resource. Field experiments under different soil types need to be conducted. Strategies need to be established to purposefully culture duckweed on wastewater to maximise dry matter and tissue nutrient composition in order to take advantage of the great potential of using duckweed as a source of nutrients.

CHAPTER 6: NUTRIENT CONCENTRATION AND REPLENISHMENT OF SLURRY LAGOON WATER, AND HARVEST REGIMES ARE CRITICAL FOR NITROGEN UPTAKE AND BIOMASS PRODUCTION OF *WOLFFIA ARRHIZA*

6.1 Abstract

Biomass of the duckweed *Wolffia arrhiza* can potentially be utilized as soil fertility amendment. Purposeful culturing of the duckweed could make it possible to control improvement of water quality and biomass production and tissue nutrient composition. The study assessed the growth and nitrogen uptake of this duckweed from agricultural wastewater. Two-week experiments were designed to ascertain the effects of swine lagoon water (SLW) concentration, its replenishment and harvest regimes on dry matter, growth rate, C content and N uptake of the duckweed in a controlled environment growth room. Dry biomass and growth rate of *W. arrhiza* were not affected by SLW replenishment periods unlike C content. Dry matter and growth rate of duckweed significantly decreased with increasing concentration of SLW in the order 5 >10 >15%. As SLW concentration declined from 15 to 5%, duckweed N content declined while C and C/N content increased as period between solution replenishment increased. Frequency of harvesting did not affect the N content and uptake by duckweed and NH_4^+ -N and mineral-N of the SLW concentration over the duration of the study. Harvesting duckweed once per week generated higher growth rate and biomass, than at twice a week. Findings from the study suggested the need for optimization of growth conditions for quality or quantity of duckweed biomass at <15% SLW.

Keywords: Average growth rate, dry matter, duckweed, harvesting frequency, nitrogen uptake, swine lagoon water

6.2 Introduction

Aquatic macrophytes called duckweed have been widely used for phytoremediation of wastewater due to their desirable morphological and physiological characteristics such as rapid growth and high purification capabilities (Fugita et al., 1999; Cheng et al., 2002). The problem of duckweed disposal is a disincentive for their use for remediation purposes (Cheng and Stomp 2009). However, recent interest in duckweed has increased their utilization as bio-renewable energy sources, animal and human feed supplement, bioplastics and for phytotoxicity tests (Radic´ et al., 2011; Van der Spiegel et al., 2013; Zeller et al., 2013; Appenroth et al., 2017; Tonon et al., 2017). Duckweed's effectiveness in recovery of nutrients such as nitrogen and phosphorus from municipal and agricultural wastewater coupled with high growth rate and non-competition with soil-based crops for growth space, has potential in improving quality of effluent discharged into natural waterways (Dalu and Ndamba, 2003; Ge et al., 2012), with the use of biomass as an added benefit. There is scope for exploiting the duckweed biomass as an organic N source for crops, a use that has been largely unexplored despite recommendations. The N content of duckweed depends on species and nutrient concentration in water, with ranges of 3-9% N having been reported in literature (Timmerman and Hoving, 2016).

The utilization of duckweed biomass in an integrated system of nitrogen fertiliser management has the potential of not only reducing the effect of nitrogen leaching but the overall cost of procuring inorganic fertilisers. Therefore, a duckweed cropping system based on wastewater could transform ubiquitous and large areas of polluted water bodies into valuable biomass production systems (Cheng and Stomp 2009). To benefit from this technology, management of a duckweed cropping system for remediation of wastewater, while yielding valuable biomass for soil fertility are crucial

for long-term protection of both aquatic and terrestrial ecosystems. Purposeful culturing could make it possible to control improvement of water quality and biomass production and tissue nutrient composition of the duckweed.

Cropping conditions, such as nutrient strength and harvest frequencies, are crucial for uptake of nutrients and subsequent growth of duckweed. Regular harvesting could be important to remove the nutrients from the system as components of duckweed tissue and for maintenance of a healthy duckweed culture (Xu and Shen, 2011a). In the absence of regular harvesting, duckweed could become unhealthy, dies and releases a significant amount of nutrients into the water that in turn leads to massive growth of microalgae on the water surface (Ardiansyah and Fotedar, 2016). Adopting a harvest regime that favours development of duckweed at an appropriate density in varied concentration of medium is crucial for efficient operation of the duckweed cropping system. However, not all duckweed genera and species are effective at recovering nutrients as their growth rate and quality is both strain and climate specific (Zhao et al., 2015). Literature on duckweed cropping systems with variable concentration of media, stocking densities and harvest frequencies for nutrient recovery, biomass and starch accumulation is commonly reported for *Lemna*, *Spirodela and Landoltia* species (Sultan et al., 2000; Cheng et al., 2002; Xu and Shen 2011a; Xiao et al., 2013; Zhang et al., 2014; Zhao et al., 2014a, b). Studies focusing on combinations of nutrient replenishment, harvest frequency and nutrient concentration of *W. arrhiza* growth and nutrient composition that promotes valuable biomass for soil fertility are scarce in literature. Preliminary studies in the Midlands region of KwaZulu-Natal Province of South Africa revealed that *W. arrhiza* exclusively colonised nutrient enriched water bodies or coexisted to a smaller extent with *L. minor* and had higher N content than the latter under natural conditions. The *W. arrhiza* biomass has potential as valuable biomass for soil amendment. It is unknown how an interplay of growth

conditions and harvest regimes affects biomass production and quality of *W. arrhiza.* The objective of this study was to assess influence of nutrient concentration and replenishment interval of swine lagoon water (SLW), and harvest frequency on *W. arrhiza* growth performance and nitrogen uptake.

6.3 Materials and methods

6.3.1 Swine lagoon water sampling and duckweed conditioning

The study was conducted at University of KwaZulu-Natal in Pietermaritzburg (29º 37' 33.9″S; 30º 24' 14″E), South Africa from February to May 2016. Fifty litres of swine lagoon water (SLW), was sampled from lagoons adjacent to pig sties at Baynesfield Estate (29º45'S and 30º20'E). The SLW was collected from eight different sites of the lagoons and mixed thoroughly in 25 litre plastic containers. The SLW was screened through a 50 µm screen and stored at 4ºC in a cold room. One-litre samples of SLW were analysed for pH, electrical conductivity (EC), total Kjedhal nitrogen (TKN), total P (TP) and soluble cations by Talbot and Talbot laboratories (Table 6.1). The wastewater had high TKN and higher total P and K than FAO thresholds, while all other properties were within acceptable limits.

About 60 kg wet mass of duckweed species *W. arrhiza* were randomly collected from a pond that received pig slurry effluent at Ukulinga (29º39'S, 30º24'E) research farm of the University of KwaZulu-Natal. The duckweed was transported to the laboratory as a dense suspension. Extraneous materials such as insects, grass and small sticks were then removed by passing the suspension through a set of sieves (from 1000 to 50 µm), before rinsing with distilled water.

Table 6.1 Elemental composition of swine lagoon water passed through a 0.50 µm screen.

Parameter	Mean±se	SA Irrigation water quality [a]	FAO[b]
EC (dSm^{-1})	5.94±0.03		
pH	7.5 ±0.1	6.5-8.4	6.5-8.4
TKN (mgL^{-1})	587.5 ±13	-	-
TP (mgL^{-1})	42.3 ±3.0	-	<2
K(mgL^{-1})	381.0 ±2.0	-	<2
Ca (mgL^{-1})	57.5 ±0.5	-	<400
Mg (mgL^{-1})	27.5 ±0.5	-	<61
Al (μgL^{-1})	80.0 ±3.0	<5000	5000
Mn (μgL^{-1})	75.5 ±0.5	<200	200
Zn (μgL^{-1})	225 ±9	<1000	2000
Cu (μgL^{-1})	79.0 ±4	<200	200
Co (μgL^{-1})	7.8 ±0.2	<50	50
Cr (μgL^{-1})	<1	<100	100
Pb (μgL^{-1})	<1	<200	5000
Cd (μgL^{-1})	<1	<10	10

TKN = Total Kjedhal Nitrogen; TP = Total P; se = standard error; [a] Department of Water Affairs and Forestry (1996); [b]Food and Agriculture Organisation (Ayers and Westcot, 1985; Pescod, 1992)

A duckweed sample was oven dried at 60ºC to constant weight before elemental analysis. Characterisation of *W. arrhiza* showed that it had 32% C, 5.8% N, 0.95% P, 7.4% K, 0.6% Ca and 0.3% Mg. In addition, it had 277 mg Al kg^{-1} and 9055 mg Fe kg^{-1}, 151 mg Mn kg^{-1}, 128 mg Zn kg^{-1} and 6.9 mg Cu kg^{-1}.

The SLW (TKN 588 mgL^{-1}) was diluted to 5% using distilled water to ensure that the N content was lower than 60 mg N L^{-1} to avoid ammonia toxicity before conditioning a 100 g sample of *W. arrhiza* in a 25-litre dish for a week at room temperature of 22-25ºC.

6.3.2 Effects of replenishment of SLW and harvesting frequency on duckweed biomass and N uptake

Effect of SLW replenishment and harvest frequency on duckweed biomass was based on the 5% SLW concentration that yielded the highest duckweed biomass in earlier experiments. A 3 x 3 factorial experiment arranged in a randomised complete block design, with three replicates, was set up in the growth room. The treatments were three levels of SLW replenishment (twice a week, once a week and non-replenishment) and three levels of harvest frequency (twice a week, once a week and once after two weeks). Blocking was against airflow direction in the growth room. Plastic containers measuring 29 cm x 19cm x10 cm (surface area 545 cm^2) were filled to a depth of 5 cm with dilute solution of SLW. The containers were inoculated with 150 gm^{-2} fresh duckweed for a single layer cover. Al-Nozaily (2001) reported that duckweed growth rate decreased as biomass accumulated to the point that fronds start overlapping each other. The growth room was illuminated with fluorescence lamps at 9 000 lux on a 12 hrs light/dark cycle at 26^oC. Solution lost through evaporation was replaced using distilled water. Containers were placed on fixed platforms at a height of a metre from the ground in the growth room. Harvesting was as described in Section 6.3.5, before determination of dry matter, C and N content. The experiment lasted two weeks.

6.3.3 Effects of concentration of SLW and its replenishment on duckweed biomass and N uptake

A 3 x 3 factorial experiment arranged in a randomised complete block design, with three replicates, was set up in the growth room to determine the effects of SLW concentration and time of its

replenishment on duckweed biomass, N content and uptake. The SLW was diluted with distilled water to three treatment levels (5, 10 and 15% of the original concentrations) based on results of preliminary studies. Solution replenishment had three levels (twice a week, once a week, and non-replenishment). The diluted solutions were filled to a depth of 4.9 cm in 21cm x 14.5 cm x 6 cm (L x W x D) plastic containers (surface area 411 cm^2). The containers were inoculated with 150 gm^{-2} fresh duckweed for a single layer cover. Harvest, as detailed in Section 6.3.5, was once every week to avoid overcrowding. The experiment lasted two weeks.

6.3.4 Effects of concentration of SLW and harvesting frequency on duckweed biomass and N uptake

Effect of SLW concentration and harvesting frequency on *W. arrhiza* biomass, N content and uptake was studied by setting up a 2 x 3 factorial experiment arranged in a randomised complete block design, with three replicates in a growth room. The treatments were two levels of SLW concentration (5 and 10% dilutions with distilled water) and three levels of harvesting frequency as in Section 6.3.2. The experiment was conducted without solution replenishment. Plastic containers (surface area 545 cm^2) as in Section 6.3.2, were filled to a depth of 5 cm with dilute solution of SLW. The containers were inoculated with 150 gm^{-2} fresh duckweed. The growth room conditions and other management of the trial were as described in Section 6.3.2. Harvesting and tissue analysis were as described in Section 6.3.5, under harvesting and analyses of duckweed biomass and swine lagoon water. Samples of the solution (5 ml) were drawn and analysed for NH_4^+, NO_3^- and PO_4^{3-} at 3, 7, 10 and 14 days. The experiment lasted 14 days.

6.3.5 Harvesting and analyses of duckweed biomass and swine lagoon water

Duckweed biomass was harvested from each container using a strainer and blocker to remove fronds (combination of leaf and stem) from 50% of the surface area. Removal of 50% duckweed allowed coverage of water surface at a rate fast enough to supress algal growth. Water was allowed to drip for five minutes from duckweed, using a strainer, and wet mass was measured after lightly bloating the biomass on a paper towel. Dry weight was measured after oven drying at 60^{o}C to constant weight. The duckweed biomass was passed through a 500 µm screen and analysed for C and N by the LECO Trumac CNS auto-analyser Version 1.1x (LECO Corporation, 2012). The NH_4^+, NO_3^- and PO_4^{3-} in the water samples were analysed using the Thermo Scientific Gallery Automated Chemistry Analyser following methods by Rice et al. (2012). Mineral-N was the sum of ammonium and nitrate-N. Average growth rate over the period of culturing was determined as follows: Average growth rate = increased dry weight/ area/ time ($gm^{-2}day^{-1}$) (Zhao et al., 2014b).

6.3.6 Data analysis

All data were subjected to analysis of variance (ANOVA) using GenStat 14th edition. Least significant differences (LSD) (at $p<0.05$) were used to separate the treatment means.

6.4 Results

6.4.1 Effects of replenishment of SLW and harvesting frequency on duckweed biomass and N uptake

Replenishment of 5% SLW did not affect the fresh biomass, dry matter and average growth rate and carbon content of duckweed (Table 6.2). The N content and uptake of duckweed significantly increased with frequency of solution replacement.

Table 6.2 Effect of SLW replenishment period on properties of *W. arrhiza* cultured on 5% SLW

SLW Replacement	Fresh biomass	Dry matter	Average growth rate	N	N uptake	C
	(gm^{-2})		($gm^{-2}day^{-1}$)	(%)	(gm^{-2})	(%)
Twice /wk	690	24.0	1.32	6.98	1.67	38.8
Once /wk	661	21.1	1.15	6.23	1.30	38.9
No repl	634	24.6	1.34	3.35	0.82	38.9
LSD	137	3.70	0.283	0.431	0.245	1.47

Wk = Week, repl= replenishment, LSD at $p<0.05$ level of significance

Harvesting duckweed twice a week yielded lower fresh biomass than when harvested once per week or fortnightly at the 5% SLW concentration (Table 6.3). The dry matter content of duckweed harvested once a week was higher than that harvested twice a week and once per fortnight. This trend was similar to that of average growth rate and N uptake. Frequency of harvesting did not influence the N content of duckweed. The C content of *W. arrhiza* harvested twice a week had lower C content than that harvested once a week or once per fortnight.

Table 6.3 Effect of harvest frequency on properties of *W. arrhiza* cultured on replenished 5% SLW

Harvest Frequency	Fresh biomass	Dry matter	Average growth rate	N	N uptake	C
	(gm^{-2})		($gm^{-2}day^{-1}$)	(%)	(gm^{-2})	(%)
Twice /wk	534	19.6	0.99	5.68	1.09	37.4
Once /wk	774	27.8	1.59	5.42	1.53	38.9
Once/ 2Wks	677	22.4	1.24	5.46	1.17	40.2
LSD	137	3.70	0.283	0.431	0.245	1.47

Wk = Week, repl= replenishment, LSD at $p<0.05$ level of significance

A significant interaction ($p<0.05$) existed between the harvesting regimes and replenishment of 5% SLW on C/N ratio (Figure 6.1). At a harvest regime of twice a week and once per fortnight, the C/N ratio of duckweed was higher where the SLW was not replenished than when replenished once and twice a week. At a harvest regime of once a week, the C/N ratio of duckweed was significantly different, with the highest from treatment with no solution replenishment and lowest from the solution replenished twice a week. Harvesting regime did not have an effect on the C/N ratio of duckweed with either a solution replenishment of once or twice a week. However, with no replenishment of SLW, the C/N ratio of duckweed harvested twice a week was lower than that harvested either once a week or fortnightly.

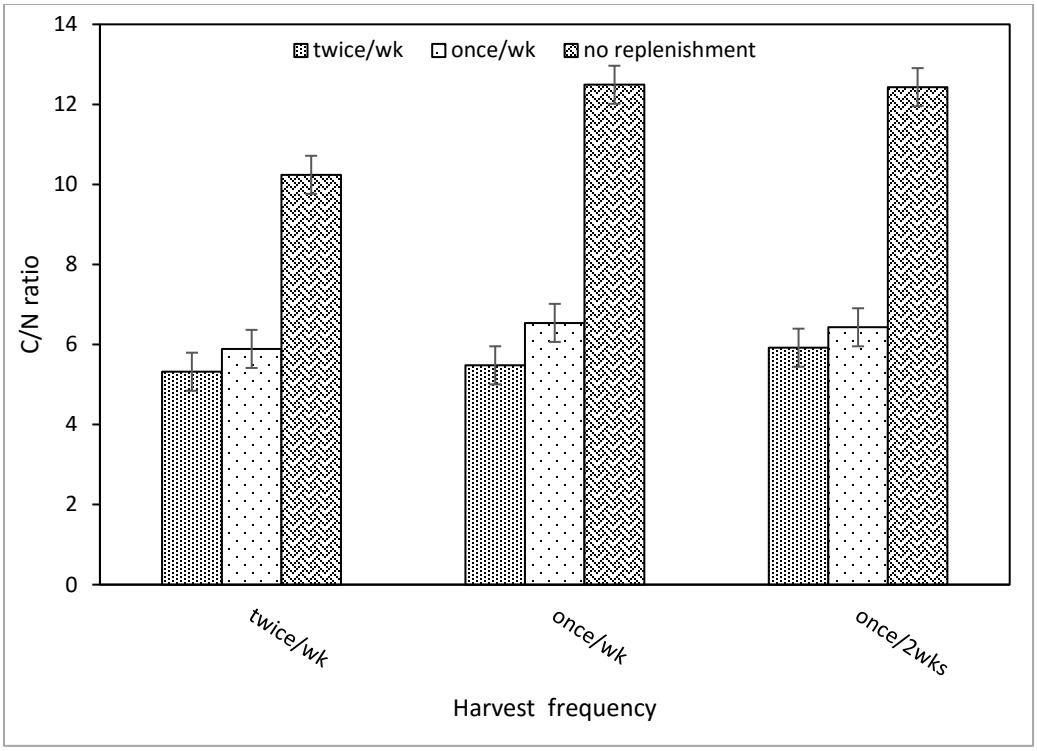

Figure 6.1 Effect of harvest frequency and SLW replenishment on the C/N ratio of *W. arrhiza* Error bars represent LSD at $p<0.05$ level of significance

6.4.2 Effects of concentration and replenishment of SLW on duckweed biomass and N uptake

There was a significant interaction ($p<0.05$) between concentration and replenishment of SLW on fresh *W. arrhiza* biomass (Figure 6.2). Replenishing a 15% SLW concentration once per week and twice per week yielded similar fresh biomass that was significantly lower than when the solution was not replenished. Frequency of replenishment of the 10% SLW concentration did not have an effect on fresh biomass. Fresh biomass harvested from the 5% SLW concentration replenished once and twice a week was similar and significantly higher than when the solution was not replenished. Reducing SLW concentration from 15 to 5% increased fresh biomass yield when the solution was replenished once or twice a week. However, when solution was not replenished, the

10 and 5% SLW concentration had similar fresh biomass that was higher than of the 15% SLW concentration.

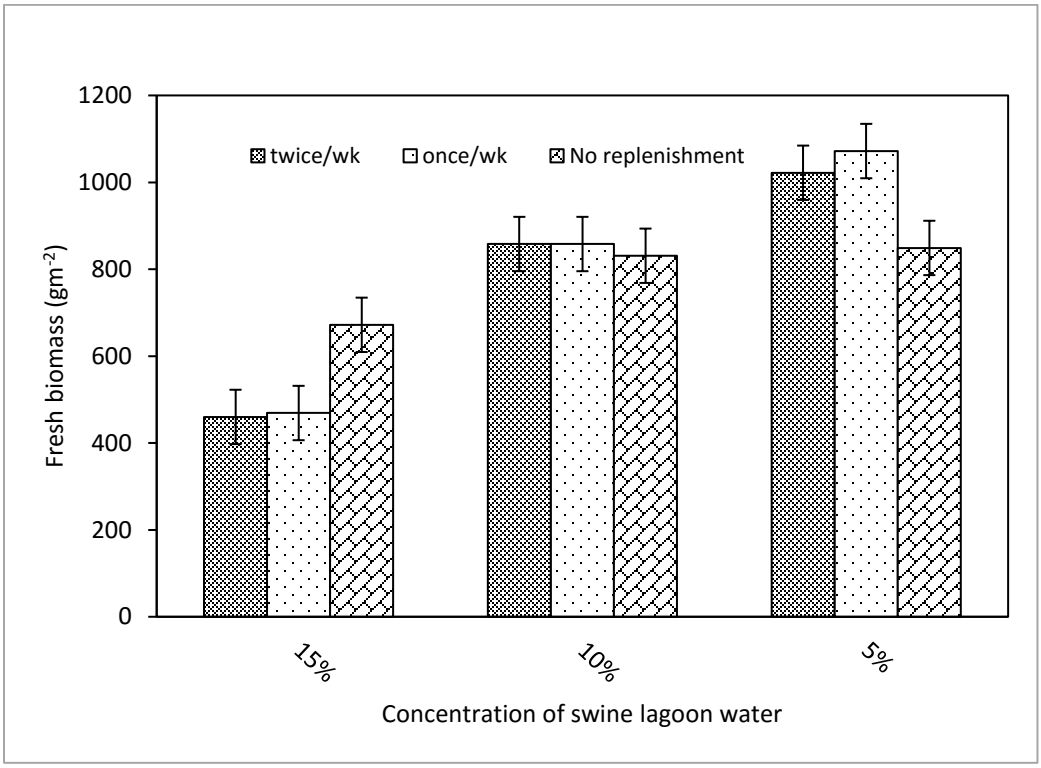

Figure 6.2 Effect of concentration of swine lagoon water and its replenishment on fresh biomass of *W. arrhiza*. Error bars represent LSD at $p<0.05$ level of significance. Error bars represent LSD at $p<0.05$ level of significance

Interaction effects of SLW concentration and replenishment were not significant for *W. arrhiza* dry matter, average growth rate and C content. Dry matter and average growth rate of *W. arrhiza* were not affected by SLW replenishment periods unlike C content (Table 6.4).

Table 6.4 Effect of SLW replenishment on *W. arrhiza* dry matter and average growth

SLW Replenishment period	Dry matter (gm^{-2})	Average growth rate ($gm^{-2}day^{-1}$)	C (%)
Twice/Wk	24.5	1.40	36.2
Once/Wk	24.0	1.38	37.3
No repl	25.8	1.50	38.00
LSD	1.85	0.138	1.27

Wk = Week, repl= replenishment, LSD at $p<0.05$ level of significance

Replenishing the SLW twice a week had duckweed with significantly lower C than when solution was not replenished. Concentration of SLW significantly affected dry matter and average growth rate of *W. arrhiza* that were in the order 5 >10 >15% (Table 6.5). The C content of duckweed from the 5% SLW was higher than that from the 15% SLW concentration.

Table 6.5 Effect of concentration of slurry lagoon water (SLW) on *W. arrhiza* dry matter and average growth

SLW Concentration (%)	Dry matter (gm^{-2})	Average growth rate ($gm^{-2}day^{-1}$)	C (%)
5	30.2	1.83	38.0
10	26.8	1.57	36.8
15	17.4	0.88	36.7
LSD	1.85	0.138	1.27

LSD at $p<0.05$ level of significance

There was significant interaction ($p<0.05$) between concentration and period of replenishment of SLW on N content and uptake (Figure 6.3). As SLW concentration declined from 15 to 5%, the duckweed N content also declined when solution was replenished either once a week or not at all. However, when the SLW was replenished twice a week, the 10 and 15% SLW concentration produced duckweed with similar N content that was higher than that at 5%. This suggested

depletion of N in solution resulting in limited uptake. Replenishment of 15% SLW once and twice a week had similar N content in *W. arrhiza* tissue, which was higher than when the solution was not replenished. At 10% concentration, replenishing the SLW twice a week had the highest duckweed N content while no replenishment had the least. At 5% SLW concentration, replenishing the solution twice a week resulted in higher duckweed N content than replenishing once or not at all. The N uptake at 15% SLW concentration was similar when the solution was replenished either once or not at all and higher than when it was replenished twice a week. This trend was similar to that for 10% SLW concentration. At 5% SLW the N uptake of duckweed was similar regardless of solution replenishment. In general, the declining duckweed N uptake trends relative to decrease in SLW concentration were similar to those of its N content.

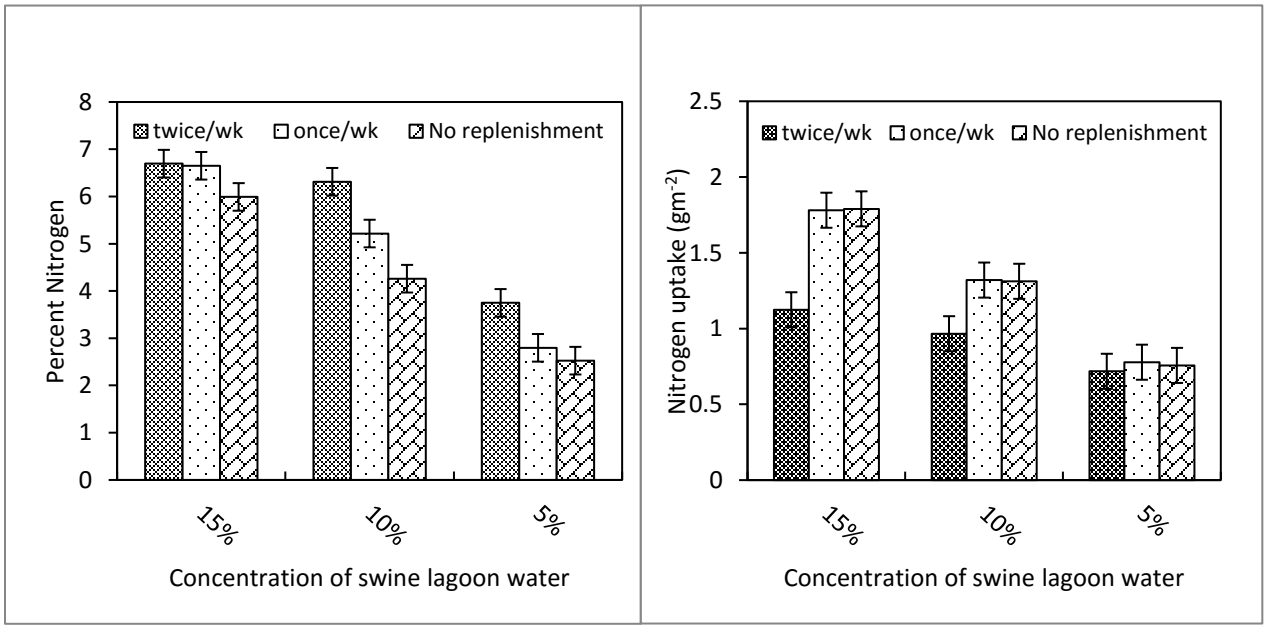

Figure 6.3 Effect of concentration and replenishment of swine lagoon water on nitrogen content and uptake by *W. arrhiza*. Error bars represent LSD at $p<0.05$ level of significance

There was a significant interaction (p<0.05) between SLW concentration and period of SLW replenishment on the C/N ratio (Figure 6.4).The duckweed C/N ratio at 15% SLW concentration was not affected by periods of solution replenishment. At 10% SLW concentration, the duckweed C/N ratio after replenishment of solution either once or twice a week was similar and lower than when solution was not replenished.

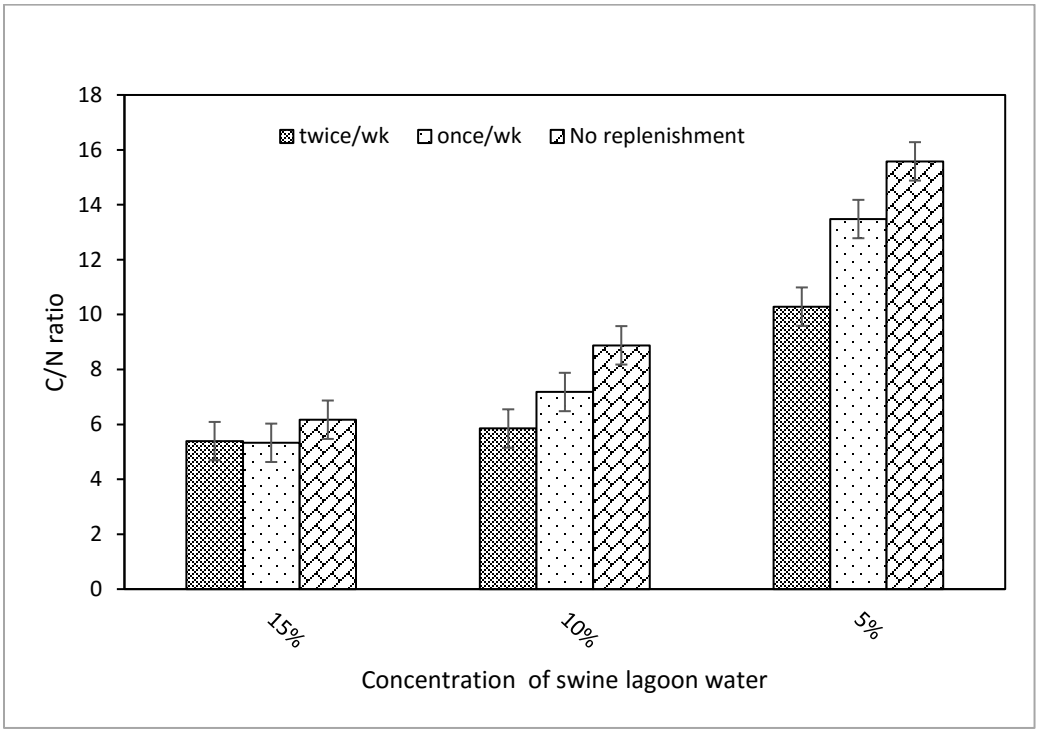

Figure 6.4 Effect of concentration and replenishment period of swine lagoon water on the C/N ratio of *W. arrhiza*. Error bars represent LSD at p<0.05 level of significance

At 5% SLW, the C/N ratio of duckweed for each of the solution replenishment periods was significantly different, where no replenishment of solution produced duckweed with highest C/N ratio while the lowest was from replenishing the solution twice a week. As SLW concentration declined from 15 to 5%, treatments with either a single or no replenishment increased their

duckweed C/N ratio. When the SLW was replenished twice a week, the duckweed C/N ratio at 5% SLW was higher than for the 10 and 15% SLW, which were similar.

6.4.3 Effects of concentration of SLW and harvesting frequency on duckweed biomass and N uptake

Interaction effects of SLW concentration and harvesting frequency on fresh and dry matter, N content and uptake, C and C/N ratio of *W. arrhiza* were not significant. There was no significant difference between fresh biomass produced at 5 and 10% SLW concentration (Table 6.6). The 5% SLW yielded higher duckweed dry matter than the 10% SLW. This trend was similar to that for average growth rate, C and C/N content. The duckweed in the 10% SLW had higher N content than the 5% SLW while the N uptake was similar at both concentrations.

Table 6.6 Effect of SLW concentration without solution replenishment on characteristics of *W. arrhiza*.

SLW concentration (%)	Fresh biomass (gm^{-2})	Dry matter (gm^{-2})	Average growth rate ($gm^{-2}day^{-1}$)	N (%)	N uptake (gm^{-2})	C (%)	C/N
5	490	22.0	1.100	3.12	0.694	38.1	12.3
10	476	18.5	0.879	3.93	0.705	36.2	9.59
LSD	39.3	1.95	0.132	0.481	0.058	0.74	1.14

LSD at $p<0.05$ level of significance

Harvesting twice a week produced lower fresh duckweed biomass than that harvested once per week or per fortnight (Table 6.7). This trend was similar for dry matter content, average growth

rate, C and C/N ratio. Frequency of harvesting did not affect the N content and uptake with no replenishment of SLW.

Table 6.7 Effect of harvest frequency and combined 5 and 10% SLW concentrations without replenishment on characteristics of *W. arrhiza*

Harvest Frequency	Fresh biomass (gm^{-2})	Dry matter (gm^{-2})	Average growth rate ($gm^{-2}day^{-1}$)	N (%)	N uptake (gm^{-2})	C (%)	C/N
Twice/ Wk	402	18.2	0.803	3.85	0.688	36.3	9.74
Once/ Wk	514	21.5	1.081	3.34	0.708	37.6	11.4
Once/ 2 Wks	533	21.1	1.084	3.38	0.688	37.7	11.6
LSD	48.09	2.39	0.162	0.589	0.071	0.910	1.40

Wk = Week, LSD at $p<0.05$ level of significance

Harvest frequency of duckweed did not have a significant effect on the NH_4^+ -N and mineral-N of the SLW concentration over the duration of the study. The NH_4^+ -N and mineral-N of the SLW rapidly declined in the first three days, beyond which the decline became slower (Fig. 6.5). The 10% SLW had higher concentration of NH_4^+ -N than the 5% SLW within the first week, after which the concentration was almost zero for the rest of the second week. The mineral-N from the 10 and 5% SLW was similar from day 10. There were significant interaction effects of SLW concentration and harvesting frequency on PO_4^{3-}-P (Figure 6.5). The PO_4^{3-}-P concentration of the 5% SLW declined by at least 72% in the first three days, while it was almost 50% for the 10% SLW. Duckweed recovered at least 95 and 75% PO_4^{3-}-P from the 5 and 10% SLW, respectively, in the first week. Harvest frequency did not affect the concentration of PO_4^{3-}-P from the 5% SLW. At day 7, the PO_4^{3-}-P concentration of the 10% SLW was significantly different from each other with harvesting regime of twice a week having the highest concentration and once a week the lowest. At day 10, harvesting duckweed twice a week from the 10% SLW resulted in higher PO_4^{3-}

-P solution concentration than the other harvesting regimes. At day 14, the harvesting regimes did not have any effect on the PO_4^{3-}-P solution concentration of the 5 and 10% SLW.

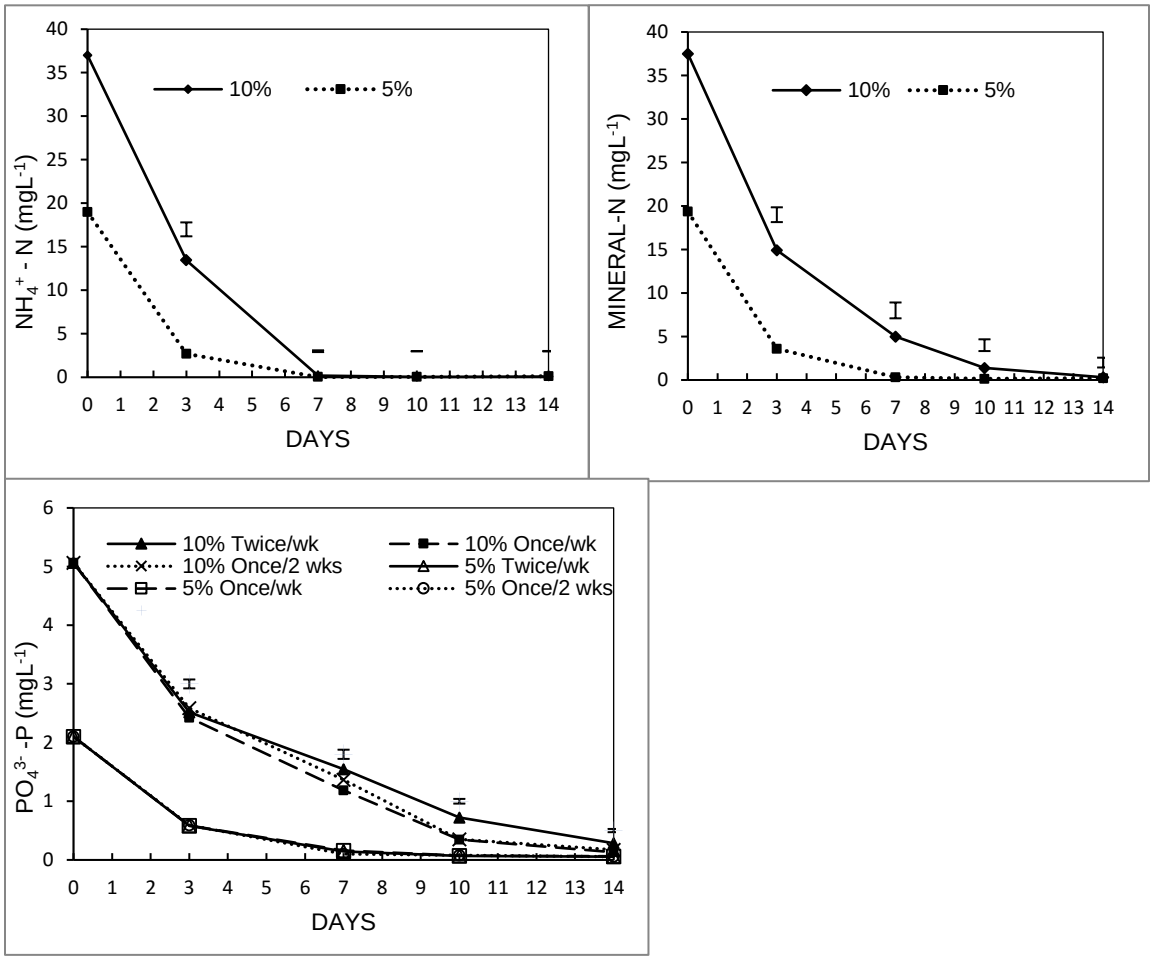

Figure 6.5 Duckweed removal of NH_4^+ -N, mineral-N from SLW and effect of harvest frequency on PO_4^{3-} removal from swine lagoon water. Error bars represent LSD at $p<0.05$ level of significance

6.5 Discussion

Fresh and dry biomass trends of *W. arrhiza* were not always highly correlated due to macrophytes' discrepancies in water uptake and disparities in water content due to techniques of measuring wet biomass. Thus, dry matter content values of duckweed were more reliable than fresh biomass. A harvesting regime of twice a week with no replenishment of solution or with replenishment of 5% SLW resulted in lower biomass and growth rate than harvesting once per week mainly due to a short harvest frequency that removed 50% of the single mat cover initially stocked at 150 gm^{-2}. This resulted in incomplete coverage of the duckweed mat on water surface that could have stimulated growth of phototrophic algae. Visual observations showed growth of algae in the treatments with the highest harvesting frequency. Consequently, a weekly harvesting frequency with replenishment of the 5% SLW yielding high biomass should be encouraged. These results contradicted the findings by Xu and Shen (2011b), who recorded highest cumulative biomass of *S. oligorrhiza* harvested twice a week, based on 20% removal of duckweed mat initially stocked at 210 gm^{-2} using 6% swine lagoon water. Differences in duckweed genera, initial stocking densities, removal rate and concentration of N and P of the culturing solution apart from other environmental variables could explain these contradictions.

The dry matter and average growth rate, in the order 5%>10%>15% SLW, showed that lower SLW concentration supported more rapid growth and biomass accumulation, with more fixed C and less of N. The low concentration of SLW directly affects the availability of N and stimulates starch accumulation in duckweed resulting in more fixed C (Xu et al., 2012). Nutrient starvation of duckweed, particularly of N, is a technique used to increase their starch content to facilitate exploitation for biofuels. Dry matter of *W. arrhiza* was in the same range as that for *W. globosa*

and *L. minor* obtained in studies by Zhao et al. (2015) and Ge et al. (2012) using dilute swine lagoon water and runoff from farmland (NH_4^+-N 18-56 mgL^{-1}, < 16 mgL^{-1} PO_4^{3-}). The ranges of growth rates in this study were lower than those obtained by Muradov et al. (2014) for *Landoltia punctata* (4.9-5.4 $gm^{-2}day^{-1}$) on 5 and 10% anaerobically digested swine wastewater (< 68 mgL^{-1} NH_4^+-N, < 15 mgL^{-1} PO_4^{3-}). The authors reported failure of *Landoltia punctata* to develop in a 15% swine wastewater. This scenario confirms findings that effective use of duckweed for nutrient uptake and biomass is dependent on species and concentration of nutrient solution (Zhao et al., 2014b).

Replenishing SLW did not influence the dry biomass and the growth rate in all experiments indicating its superfluity within a two week period. This may possibly be due to short-term availability of accumulated nutrients even when those in solution were mostly depleted after 7 days (Figure 6.5). Kufel et al. (2012) recorded a reduction of internal pool of nutrients in duckweed cultured in a nutrient depleted medium for 10 days and ascribed mobilization of internal nutrient sources a common pattern in duckweed, whenever external N and P were in short supply. While slow growth was associated with greater N accumulation at 15% SLW, the low N concentration in *W. arrhiza* from the 5% SLW was due to more rapid decline in solution. From preliminary studies, *W. arrhiza* could not survive in 100, 50 and 30% SLW (nutrient level of undiluted wastewater: TKN 588 mgL^{-1}, TP 42 mgL^{-1}). The 15% SLW, with initial TKN concentration of above 85 mg N L^{-1}, showed some inhibitory effects on growth of *W. arrhiza*. Relatively high levels of NH_4^+ in the 15% SLW (>75mgL^{-1} NH_4^+-N) explained lowest growth rate and biomass of *W. arrhiza*. Some duckweed fronds appeared chlorotic and aged prematurely attributable to relatively high NH_4^+ concentrations that induced premature senescence of fronds. Wang et al. (2014) found

out that *L. minor* could grow at NH_4^+ concentration of 7-84 $mgL^{-1}$with an optimum of 28 mgL^{-1}. For batch conditions, Caicedo et al. (2000) observed maximum relative growth rate of *S. polyrrhiza* at low concentration of NH_4^+ in the range 3-20 mgL^{-1}. In addition, Xu and Shen (2011b) observed damaged and poor growth of *S. oligorrhiza* at 10 and 12% anaerobically treated swine wastewater (Total N 101-117 mgL^{-1}, NH_4^+-N 87-103 mgL^{-1}, TP 26-36 mgL^{-1}). Relatively high NH_4^+ concentration were reported to be harmful to duckweed by means of two mechanisms involving both NH_3 and NH_4^+. The first involves lipid solubility where unionized ammonia from NH_4^+ dissociation enters through cell membranes that disturbs cell metabolism and the second involves high NH_4^+ concentration prompting depolarization of cell membranes, resulting in a general inhibition of anion transportation through the membrane (Vines and Wedding, 1960; Warren, 1962; Ingermarsson et al., 1987).

Generally, increasing the frequency of solution replenishment in this study increased the duckweed N content (Figure 6.3, Table 6.2) due to its physiological capacity to accumulate N, confirming observations by Cheng and Stomp (2009). However, no extra N uptake and content benefit accrued from replenishing the 15% SLW at highest frequency of twice a week compared to once a week due to SLW nutrient levels that remained relatively high throughout the two weeks, unlike the 5 and 10% SLW. This could be supported by results of mineral N that needed 10 days to be exhausted at 10% SLW (Figure 6.5), suggesting that 15% SLW would take longer and as such N availability would not have been a limiting factor. The higher dry matter where the 15% SLW was not replenished than with replenishment suggested that uptake of the $NH4^+$-N reduced the solution concentration into more favourable levels for growth, than when replenished. This could have resulted in more production of proteins and less of carbohydrates than with no replacement where

N could have been depleted. Therefore, culturing duckweed biomass as soil fertility amendment at concentrations below 15% requires frequent replenishment of solution to increase the N content and uptake of duckweed.

The C and C/N ratio of duckweed generally decreased as either concentration of SLW or frequency of solution replenishment increased, confirming observation by Wang et al. (2014) of decreased C and C/N ratio as NH_4^+ concentration increased. As the supply of C and N are crucial for plant growth (including duckweed), C/N balance maintains the routine and basic cellular activities (Zheng, 2009). Decrease of duckweed C content at higher concentration of SLW, with correspondingly higher NH_4^+-N concentration, could increase the risk of NH_4^+-N stress that could break the C/N balance and negatively affect the growth of the plant and inhibit biomass accumulation (Zhang et al., 2011). Solution replenishment of very dilute SLW has a crucial role in the N content and C/N ratio, hence quality, of duckweed biomass. Therefore, more frequent solution replenishment is required at low concentration while limited to no replacement is required at higher concentrations for maximum biomass production and N concentration. This ensures that there is adequate N in the solution that does not jeopardise N uptake and biomass accumulation.

Duckweed harvested from ponds not replenished with nutrients would generally have low N content that is comparable to that of chicken manure (Van Ryssen, 2001). Though the C/N ratio increased with dilution of SLW, the C/N ratio (<16) in this study would not limit decomposition of biomass when added to soil. The C/N ratio above which decomposition is suppressed is often about 20-30 (Kumar and Goh, 2000).

The NH_4^+ -N in SLW decreased rapidly in the first three days from initial concentration of 19 and 37 mgL^{-1} for the 5 and 10% SLW, respectively, due to *W. arrhiza*'s capacity to utilize NH_4^+-N, especially at lower levels for vegetative growth. Xu and Shen (2011b) reported complete removal of NH_4^+-N by *S. oligorrhiza* from 6, 4 and 2% lagoon waters after two weeks. Higher N content of duckweed from the 10% SLW (Table 6.6) even after NH_4^+-N depletion, was explained by NO_3^- -N due to nitrification that proceeded after a week and contributed to mineral-N (Figure 6.5). Other studies highlight that in the absence of NH_4^+-N, duckweed growth increased with NO_3^- concentration that accumulated in the inactive 'storage pool' in the duckweed frond (Aslam et al., 1976; Jayashree et al., 1996). This NO_3^- could be brought back to active metabolic pool for reduction under starvation condition. After a week, harvesting duckweed from the 10% SLW resulted in slow decline of solution PO_4^{3-} attributable to depleted NH_4^+ levels (Figure 6.5). Soda et al. (2013) support this suggestion that in late experimental periods, the rate limiting nutrient for *W. arrhiza* is not P but N. According to Department of Water Affairs (2010) guidelines for wastewater discharge standards [NH_4^+ 1-3 mgL^{-1}, NO_3^- 1.5-15mgL^{-1}] the NH_4^+, NO_3^- concentrations fall within range after culturing *W. arrhiza* for a week (Figure 6.5). The P concentration of the 5 and 10% SLW was within the range of the guidelines (1-10 mgL^{-1}). Harvesting without nutrient replenishment is generally ideal for phytoremediation of wastewater, especially N in batch stages before discharging into natural waterways and the duckweed biomass produced could still be an important source of N for crops.

6.6 Conclusion

Growth, dry matter, carbon content and C/N declined, while tissue N and N uptake of *W. arrhiza* increased with increase in the SLW concentration in the range of 5-15%. The NH_4^+ was depleted in the first seven days of the experiment affecting the PO_4^{3-} uptake at a concentration of 10% SLW. Solution replenishment of the 5 and 10% SLW twice a week increased the N content and lowered the C/N ratio, without increasing growth rate and dry matter yield. At low (<5%) SLW concentration, frequent replenishment is required, while at high SLW concentration limited to no replenishment is suitable for maximum duckweed biomass production and N content. Harvesting duckweed once per week generated higher duckweed growth rate and biomass, than twice a week. Findings from this study highlight that concentration and harvesting frequency have an effect on dry matter production and tissue N, while effectively remediating the water.

Further research needs to be undertaken using higher stocking densities and on a pilot scale open to other variable factors such as temperature, medium pH and sunlight, using 5 to 10% SLW, together with weekly harvesting, with or without nutrient replacement. Such studies need to be done over longer periods than the two week periods done in this study.

CHAPTER 7: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

7.1 General discussion

Waste disposal is a major challenge in intensive agricultural production systems and can lead to nutrient enrichment of proximate water bodies which is evidenced by increase in blooms of algae and aquatic macrophytes, including duckweed. This environmental scenario is prevalent in the Midlands region of the KwaZulu-Natal Province of South Africa. The rapid growth and accumulation of nutrients in duckweed tissue can contribute to purging of water bodies, especially if they are removed or harvested and not allowed to die or decompose in the water. The rapid growth rate of duckweed produces enormous quantities of biomass in a short period as duckweed can double their biomass in 24-48 hrs. This compensates their relatively low dry matter content compared to their wet mass (>94% water). The biomass accumulation and tissue composition could be affected by the species and habitat characteristics, particularly water quality. It is essential to understand the water quality characteristics that govern the occurrence of certain species of duckweed and their tissue composition, with the view of recovering the nutrients from wastewater and using the biomass as an organic fertiliser. There is scope for recovering nutrients from water bodies using duckweed and reusing the biomass as a source of plant nutrients since they have relatively high nutrient levels such as N and P. However, this potential can only be fully realised if the nutrient release patterns are clearly understood. There has been little research on the potential use of duckweed as soil ameliorant in general and none on specific genus such as *W. arrhiza*. This study therefore investigated the potential of nutrient recovery from enriched water bodies by duckweed and its use as a source of plant nutrients.

Findings from this study showed that different anthropogenic activities including livestock and crop production enterprises in the Midlands region released wastes that polluted even fresh water bodies which supported growth of two major duckweed species; *W. arrhiza* and *L. minor*. The non-occurrence of duckweed in water bodies in communal and forest areas indicated that the water in these areas had good quality, with low nutrient loads.

Stakeholders in intensive production units remonstrated duckweed's aggressive colonization of their waterbodies. Yet it is important to highlight that duckweed serves as an important indicator of water pollution. Specifically, nutrient loads in wastewater and freshwater explained the occurrence of the duckweed species in the Midlands region. High levels of K in water resulted in co-existence of the two genera *Wolffia* and *Lemna* spp., while low K resulted in the genera occurring separately, with *Wolffia* spp. dominant on water with lower Ca and Mg than where *Lemna* spp. occurred alone. The water N and P concentration differentiate tissue composition of duckweed where the genera occurred in separate sites but could not where they co-existed, indicating importance of wastewater composition in defining distribution and elemental composition of duckweed.

Preliminary experiments on culturing duckweed in this study showed that *W. arrhiza* could only survive in ≤15% SLW (<75mg NH_4^+-N L^{-1}). While water in which duckweed grew in the natural environment had 0.004-5.19 mgL^{-1} NH_4^+, 0.05-7.76 mgL^{-1} NO_3^- and 0.01- 1.36 mgL^{-1} PO_4^{3-}, the duckweed accumulated significant amounts of nutrients (Chapter 3, Section 3.4.2). Lagoons and reservoirs that continuously received agricultural wastewater but with no sign of duckweed growth in the study sites indicated toxic nutrient levels and probably other unfavourable hormonal and

antibiotic effects. The 30-100% SLW initially used in Chapter 6, Section 6.5, could not sustain the growth of the duckweed (*W. arrhiza*), mainly due to high levels of ammonium that has deleterious effects on duckweed growth. Relatively high NH_4^+ concentrations were reported to be harmful to duckweed by means of two mechanisms, which were pH dependent, involving both NH_3 and NH_4^+. These involve lipid solubility, where unionized ammonia from NH_4^+ dissociation enters through cell membranes, which disturbs cell metabolism, and high NH_4^+ concentration prompting depolarization of cell membranes resulting in a general inhibition of anion transportation through the membrane (Vines and Wedding, 1960; Warren, 1962; Ingermarsson et al., 1987). Thriving of duckweed in wastewater in the natural environment hinted at dilution of the growth medium that also influenced distribution by genera. Nutrient composition of water had pronounced and generally consistent effects on duckweed elemental composition. Based on these results, duckweed genera grew as long as there was at least 0.09 mg N L^{-1} and pH range of 7.8-8.6. However, results in Chapter 6, Section 6.4.2, show that dry matter increased while tissue N decreased with decrease in SLW between 5 and 15% of the original concentration, indicating that lower mineral N in water supports greater biomass production. The N, P and pH values of wastewater in this study were similar to those observed by other authors (McLay, 1976; Landolt, 1986; Hasan and Chakrabarti, 2009). The lack of effects of nutrients in the wastewater on tissue nutrient composition, where *W. arrhiza* and *L minor* co-existed in the natural environment, could be as a result of competition of the two species for resources with *W. arrhiza* showing greater efficiency in the uptake of P.

Generally, elemental composition of *Lemna* and *Wolffia* species biomass indicated that the macrophytes could improve water quality, especially when continuous removal of the duckweed is practised. The results of Chapter 6, Section 6.4.3, indicate that culturing *W. arrhiza* on effluent

with about 40 mg NH_4^+-N L^{-1} resulted in depletion of the mineral N in seven days, with 3.3-3.9% N accumulated in the duckweed tissue. Stakeholders in areas with plenty of duckweed in water bodies could harvest the duckweed instead of applying herbicides in an effort to eliminate them. The latter practice only results in fertilising the water bodies and exacerbates the situation. Literature confirms that continuous harvesting prevents overcrowding, biomass death, and release of nutrients back into the water system (Al-Nozaily, 2001; Nhapi, 2004). Findings from this study showed that for batch systems, harvesting regimes of duckweed, concentration of wastewater and its replenishment frequency should be optimised. Relatively high levels of NH_4^+ in the 15% SLW (>75 mgL^{-1} NH_4^+-N) limited *W. arrhiza's* growth rate and biomass accumulation. There is always need to dilute wastewater though it might mean increased pumping costs to the farmer which maybe allayed by a double benefit of duckweed biomass and improved water quality more suitable for irrigation than the wastewater. Duckweed from the 15% SLW took longer to exhaust nutrients, and improve water quality, than that at lower concentrations, suggesting that culturing duckweed biomass for water quality improvement and as soil fertility amendment at concentrations below 15% required frequent replenishment of solution to increase the N uptake and content of duckweed. Where the 15% SLW is used, the duckweed should be grown without wastewater replenishment within two week periods (the longest tested). Solution replenishment of very dilute SLW had a bearing on the N content and C/N ratio, hence quality of duckweed biomass. The study suggested that *W. arrhiza* harvested from ponds not replenished with nutrients would generally have relatively low N content around 3%, comparable to that of chicken manure (Van Ryssen, 2001). This was supported by the results in Chapter 3, Sections 3.4.3 and 3.4.4, where low *W. arriza* tissue N was measured for the samples collected from water with lower mineral N in the natural environment. However, although the water in the natural environment had lower NH_4^+-N

than the 5% SLW culturing solutions used in Chapter 6, Section 6.3, the tissue N was generally higher. This could be because the measured NH_4^+-N in the water from the natural environment was what remained after uptake by the duckweed, and not necessarily the original concentration. Harvesting of this duckweed at appropriate frequency from the natural environment could further lower the nutrient composition and improve water quality (Dalu and Ndamba, 2003). Variable harvest regimes from twice a week, once after 4 days and once after 6 days have been reported in literature (Zaki et al, 1979; Xu and Shen, 2011a; Ardiansyah and Fotedar, 2016) mainly due to differences in duckweed genera, removal percentages and stocking densities and concentration of media. Duckweed has to be harvested in such a way as to allow the remaining culture to rapidly cover much of the water surface in order to supress algal growth. In this study, a harvesting regime of once per week with no replenishment of solution yielded higher growth rate and biomass than at twice a week at 50% removal for *W. arrhiza*. However, solution replenishment was important for increased N uptake and N content. It is a common sight for *W. arrhiza* to be swept by the wind and packed into several layers to one side of the lagoon or reservoir in cases of incomplete water coverage. In such cases, a removal percentage for the harvest has to be calculated from the portion of the standing crop. As such, it is essential to determine a practical frequency of harvesting duckweed from the natural environment, considering the water surface coverage that should remain for water quality improvement without algal growth.

The nitrogen content in duckweed biomass harvested from the natural environment (Chapter 3, Section 3.4) and cultured on dilute SLW (Chapter 6, Section 6.4) is higher than commonly used organic nutrient sources like pig and poultry manure, which presents an opportunity to use the biomass as an organic N source. Macronutrient composition of poultry manure is in range 1.5-

3.7% N, 1-1.5% P, 1.5-2.0% K, 1.5-3.0% Ca and 0.56-0.9% Mg (Van Ryssen, 2001; Adediran et al., 2003; Mkhabela, 2006). Pig manure has on average, 1.5% N, 0.65% P, 0.82% K, 0.42% Ca, 0.37% Mg (Bolan et al, 2010). The N and K content of duckweed *Wolffia* and *Lemna* spp. were superior to either pig or poultry manure and are expected to have a better fertiliser value due to relatively higher N content and macronutrients. The addition of *W. arrhiza* biomass enriched the soils resulting in significant increase of Swiss chard dry matter by 23-45% compared to the soils with no amendment (Chapter 5, Section 5.4.1). Crops grown in soils of relatively low fertility under small-scale farming areas near thriving duckweed could potentially benefit from such amendments. Nitrogen uptake of Swiss chard responded to rates of application of *W. arrhiza*. In this study, application of urea, as the positive control, provided readily available N for Swiss chard uptake resulting in higher dry matter, whereas duckweed biomass additions decomposed with gradual N mineralization. Higher rates of duckweed application resulted in increased availability of N from duckweed decomposition that supported greater plant growth and was comparable to the positive control. The response of Swiss chard dry matter to addition of duckweed (*W. arrhiza* and *L. minor*) could be explained by rapid degradation of the biomass and mineralisation of N. This view was further supported by increased dry matter yields when a pre-incubation period of 28 days was used (Chapter 5, Section 5.4.3), indicating the importance of decomposition of the duckweed and mineralisation of N.

The average C/N ratio of duckweed harvested from water bodies was less than 10 and reflected a resource with low lignin content that decomposes readily when applied to soil. The C/N ratio above which decomposition is suppressed is often about 20-30 (Kumar and Goh, 2000). The low C/N ratio (<16) was also observed where *W. arrhiza* was cultured, though the ratio increased with dilution of SLW, which suggested that decomposition of the biomass and mineralisation of N

would not be limiting. As most tissue nutrients of *L. minor* and *W. arrhiza* were similar, mineralisation of duckweed was done only for the latter. The production of at least 5% ammonium-N on the first day of incubation confirmed ready decomposition of *W. arrhiza*. Rapid decomposition could be facilitated by low fibre and lignin content in their tissue (Hasan and Chakrabarti, 2009; Tao et al., 2017). Generally, the peak ammonium-N production was at two weeks of incubation and the general increase in nitrate-N and mineral-N from days 14 to 42 corresponded with a decrease in Ammonium-N indicating suitability of this technology in well-drained soils with adequate moisture levels.

Swiss chard dry matter from duckweed pre-incubation for 28 days was similar to that of the positive control indicating high N uptake. The 28 day pre-incubation period was appropriate for mineralisation of duckweed as most nutrients that were initially unavailable had to be slowly released through microbial degradation. This was supported by the mineralization experiment that showed rapid increase in mineral N content in the 16-42 day period. This finding indicates that in order to derive maximum benefits from duckweed as a nutrient source, particularly for vegetables, pre-incubation of duckweed biomass for at least 28 days improved nutrient availability and uptake resulting in greater dry matter of Swiss chard that was as good as from urea application. Similar Swiss chard dry matter was produced after incubating *L. minor* and *W. arrhiza* for 28 days, though N uptake was higher for Swiss chard with duckweed application than with urea, suggesting higher tissue N content. The relatively low C/N ratio of duckweed suggests that pre-incubation of duckweed biomass is an essential strategy for synchronizing crop nutrient demand and release from duckweed.

Mineralization of duckweed in soils with poor inherent fertility would be retarded as cations, phosphorus and micronutrients are important in stimulating nitrification (Brady and Weil, 2008).

Rates of duckweed application influenced the amount of extractable P (16- 40%) from the soil at the start of incubation of *W. arrhiza* suggesting high water soluble P in the tissue that immediately released high levels of P after incorporation. The study highlighted importance of consideration of nutritional composition of duckweed and soil characteristics before addition to soil. Duckweed with high levels of tissue Al and Fe may inhibit availability of P, added in form of duckweed, as it is transformed to non-labile Al and Fe phosphates. This was supported by the non-response of Swiss chard P uptake in Chapter 5, Section 5.4.1, that indicated limited ability of the plant to utilize the nutrient as decomposition of duckweed proceeded. Conversely, high Fe and Al levels in the ferralsol at relatively lower pH coupled with additions from duckweed decomposition and the acidifying nature of the nitrification process could have resulted in P fixation on Al and Fe oxyhydroxide surfaces and precipitation of Al and Fe phosphates. This view was supported by the increase in NaOH extractable P with increase in duckweed rate in the incubation study (Chapter 4, Section 4.4.5). This pool represents P that is fixed by amorphous and slight crystalline minerals of Al and Fe. This can be corrected by addition of lime. It is, therefore, important for enterprises to have an appraisal of nutrient loads in wastewater in line with regulations (Government Gazette, 2009; Department of Water Affairs, 2010) so as to have an appreciation of duckweed elemental composition, especially of N, Fe and Al. In the same vein, it is suggested that the Baynesfield ferralsol, that are highly weathered soils with substantial amounts of Fe and Al oxyhydroxides, also need liming to enhance P availability. It is advisable for farmers to know their soil pH so as to take corrective action if needed before use of duckweed biomass. The soil used in the experiments had lower levels of nutrients than those of duckweed biomass implying that the soils could benefit from duckweed biomass amendment.

The results of Chapter 3, Section 3.4, showed that the duckweed (*W. arrhiza* and *L. minor*) from the different sources had high levels of K, Ca, Mg, Zn, Cu, Mn and Fe, in addition to N and P. Upon decomposition of the biomass in soil, these nutrient elements become available for uptake by plants. The greater growth of Swiss chard in response to higher mineral N, especially at rates >50 kg N ha^{-1} biomass, facilitated greater uptake of other essential nutrients such as K, Ca, Mg, Mn, Zn and Cu (Chapter 5, Section 5.4.3). The low uptake levels of these elements in treatments with low Swiss chard dry matter was supported by the higher levels of these elements in the residual soils, including phosphorus. The similarity of uptake of most nutrients in this study at the 100 and 200 kg N ha^{-1} implied diminishing marginal returns at high duckweed rates. This trend needs further confirmation with other crops.

The ability of duckweed to recover nutrients from water and concentrate them in its tissue in a short period of time and release them into the soil through decomposition at a relatively fast rate make them highly efficient 'packets' of nutrients that can be used to improve soil fertility in the vicinity of intensive production units. Added benefits superior to synthetic fertilisers include addition of organic matter to soil which plays a key role in improving soil physical and chemical properties. The potential improvement of nutrient supply for crop production on marginal soils makes purposeful culturing and harvesting of duckweed from the wild worthwhile for improving water quality and recycling nutrients. Culturing of duckweed at source of the wastewater could minimise pollution of water bodies, while producing an organic fertiliser with nutrients in labile form. Where duckweed is harvested from the wild, water quality may be improved, although the frequency of harvesting could vary with the composition of nutrients in the water. Other considerations in the recycling of nutrients using duckweed relate to harvesting methods.

Manual or mechanical harvesting machines can be used to harvest duckweed from water bodies (Iqbal, 1999). Manual methods involve use of nets and boats that can be used by small-scale farmers though they are time consuming. Machine methods include use of cranes, pumping or duckweed water skimmers mainly afforded by well-endowed stakeholders. Ideally, the context demands that labour and transportation would need to be provided from public funds. This could be organised as 'Work for water' programs that bring environmental benefits (cleaner water) to society at large and economic benefits to the poor such as employment of people in the harvest, treatment and transportation of duckweed, and in the form of improved soil fertility for beneficiaries. Drying of duckweed biomass could be a challenge that can negatively influence its use, given that it has greater than 90% water. Ideally, drying of duckweed should be at site to reduce transport and energy costs. Solar drying at collection site is an attractive option that depends on weather and site availability only. Dewatering of the wet duckweed before transportation could hasten the drying process. While the quantity of harvestable dry biomass may discourage use of duckweeds to amend soil fertility, an added benefit of improved water quality is accrued.

7.2 Conclusion

Nutrient enriched water bodies from various enterprises resulted in the occurrence of *W. arrhiza* and *L. minor*, separately or in co-existence, in the Midlands region of KwaZulu-Natal. The duckweed species had high nutrient composition that was generally similar and related to water concentration. Rate of duckweed application and soil type had an effect on decomposition of *W arrhiza,* with higher rates resulting in increased rates of decomposition, particularly in initially fertile soil. Mineralised P from duckweed was precipitated as Al and Fe phosphate in the acidic

soils used. Dry matter of Swiss chard, nutrient uptake and residual concentrations in soil, increased with high duckweed nutrient content and application rates as well as favourable initial soil characteristics. Pre-incubation of duckweed biomass for at least 28 days improved nutrient availability and uptake resulting in greater dry matter of Swiss chard that was comparable with urea application. Duckweed dry matter increased while tissue N decreased with decrease in SLW concentration from 15 to 5%, as NH_4^+-N in the water was depleted. Harvesting duckweed once per week generated higher duckweed growth rate and biomass, than twice a week. Dilution of SLW to appropriate concentrations, frequency of solution replenishment and duckweed harvest regimes in a cropping system are important for high quantity and quality of biomass.

7.3 Recommendation

The following recommendations can be made from the results of this study:

1. Harvesting duckweed and not allowing it to die and decompose in water is the only sure way of improving water quality by recovery of nutrients as biomass.
2. Harvested duckweed biomass from agricultural wastewater may be utilised by smallholder farmers as an organic fertiliser to supplement synthetic fertilisers. An economic analysis of this approach is essential.
3. Pre-incubation of duckweed biomass for four weeks before planting crops is essential to synchronise periods of high nutrient demand and nutrient availability.
4. Intensive agricultural production systems may need to scale up duckweed biomass production through establishment of pilot scale culturing of *W. arrhiza,* where

manipulation of wastewater concentration, its replenishment periods and harvest regimes are carefully managed to optimise quantities and quality of biomass produced.

Recommendation for further work:

1. To ascertain the fertiliser value of duckweed to different crops. Field experiments under different soil types need to be conducted.
2. To assess the contribution of co-application of *W. arrhiza* with lime on availability of P.
3. To culture *W. arrhiza* using higher stocking densities and on a full-scale open to vagaries of weather for longer periods than those in this study.

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APPENDICES

APPENDIX 1: Duckweed in pictures

Duckweed colonising waterbodies in intensive agricultural productive systems.

Wolffia arrhiza from water bodies with different nutrient levels

Wolffia arrhiza colonising a water body in a sugarcane estate

Lemna minor flourishing in a local stream

Wolffia and *Lemna* spp. co-existing in a barricaded stream adjacent to a chicken enterprise.

APPENDIX 2: Swiss chard on duckweed amended soil

Duckweed (*Wolffia arrhiza*) application rate on growth of Swiss chard grown on Ukulinga soil.

Duckweed (*Wolffia arrhiza*) application rate on growth of Swiss chard grown on Baynesfield soil.