

**NITROGEN AND PHOSPHORUS RELEASE IN SOIL AND  
FERTILISER VALUE OF *LEMNA MINOR* BIOMASS RELATIVE TO  
CHICKEN LITTER COMPOST**

Kanya Jilimane (BSc Honours- Soil Science)

Submitted in fulfilment of the academic requirements for the degree of Masters  
of Science in Soil Science

School of Agricultural, Earth and Environmental Sciences,  
University of KwaZulu-Natal

Pietermaritzburg

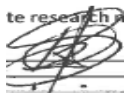
2019

## DECLARATION

I, Kanya Jilimane, hereby declare that:

1. The study described in this dissertation, except where specified, is my own original work.
2. This work has not been submitted to any other institution for any degree or diploma.
3. This dissertation does not comprise other individuals' data, pictures, graphs or other information, unless where referenced and acknowledged for the owner of that particular work.
4. This dissertation does not comprise other individuals' writing, unless specifically acknowledged as being found from other researchers. Where other written sources have been quoted, then:
  - a. Their words have been re-written but the general information attributed to them has been referenced
  - b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed



\_\_\_\_\_ Date 21/08/2019\_\_\_\_\_

Candidate's signature



\_\_\_\_\_ Date 21/08/2019\_\_\_\_\_

Supervisor's signature

As the supervisor candidate's I have/ have not agreed to the submission of this dissertation.

## ABSTRACT

Intensive agricultural production systems produce nutrient-rich wastewaters, which may pollute the environment. High nutrient concentrations on surface water bodies encourages the growth of aquatic plants, and harvesting of these plants could improve water quality and produce an organic fertiliser. The fertiliser value of duckweed may depend on the effluent on which it grows, since it affects its nutrient composition. The aim of this study was to determine the effects of effluent types on duckweeds (*lemna species*) tissue composition and its influence on (i) mineral nitrogen (N) and phosphorus (P) release in the soil during an incubation and (ii) nutrient uptake and dry-matter yield of spinach (*Spinacia oleracea*) under greenhouse and field conditions, relative to chicken litter. Duckweeds were sampled from surface water at Ashburton (Lemna AB) enriched with effluent from sewage and cattle manure, at Baynesfield (Lemna BF) on effluent from a piggery, at Wartburg (Lemna WB) enriched with crocodile wastewater (crocodile farm) and chicken litter compost from RGS Drumnadrochit farm, all in the Midland region of Kwa-Zulu Natal. A loam soil was amended with dried Lemna AB, Lemna BF, Lemna WB or chicken litter at a rate of one percent (w/w) in one incubation, while in another incubation 2 and 4% rates were used with an un-amended soil as a control in both. The treatments were adjusted to field capacity moisture and incubated at 25°C for 28 days. Destructive sampling was done after 0, 7, 14, and 28 days of incubation, and analysed for pH, ammonium and nitrate-N, and extractable-P. A leaching experiment was also conducted where 2.0 g of dry matter Lemna AB, Lemna BF and Lemna WB were leached over a period of 0, 6, 12 and 24 hours on sandy soil using deionize water (25 ml). The leachates were analysed for ammonium and nitrate-N and extractable-P. A pot trial in the glasshouse was conducted with the same treatments used in the incubation experiment, at recommended rate of 100 kg N ha<sup>-1</sup> replicated three times. Spinach (*Spinacia oleracea*) seedling were grown for six (6) weeks, and harvested before the determination of dry matter (DM), tissue composition and nutrient uptake.

The experiment was repeated under field conditions with Lemna BF, Lemna WB, chicken litter compost, all applied at 100 kg N ha<sup>-1</sup>, and a negative control (no added N), after a two-week pre-incubation. In the first incubation ammonium-N was higher in the Lemna WB treatments, while nitrate-N was highest on the Lemna AB treatments with the highest peak observed on day 14. In the case of Lemna BF treatments had the highest amount of extractable-P, with the control having less of all determined parameters. Lemna WB rapidly leached higher nutrients (ammonium-N and exchangeable-P) at about 26.47 mg N/kg and 25.59 mg P/kg respectively, while Lemna AB (69.42 mg/kg) was high in nitrate-N within 24 hours in comparison to the other treatments on the leaching experiment. In the second incubation Lemna BF showed higher amounts of ammonium-N (230 mg/kg), nitrate-N (140.83 mg/kg) and extractable-P (10.66 mg/kg), throughout the incubation period than all other treatments, while the control had the least of all determined parameters. Ammonium-N was highest after 7 days of the incubation and declined thereafter while nitrate-N increased. Soil pH was highest in the chicken litter compost treatment, and it declined with incubation period. Spinach dry matter was similar for all duckweed treatments, while the negative control had lower levels in the pot experiment. However, under field conditions the Lemna BF treatment (74.2 g/plant) had higher spinach dry matter (DM) yield than all the other treatments which were similar in DM. The results suggested that duckweed N mineralises rapidly in soil and also has a significant value on spinach yield, both depending on the initial elemental composition of duckweed, which is affected by the effluent on which it grew.

**Key words:** Duckweed, chicken litter compost, *Lemna species*, nutrient release, nutrient recovery and dry matter.

## **ACKNOWLEDGEMENTS**

First and foremost, I thank God Almighty for his strength, care and protection, on a daily basis, throughout the duration of the study. Many thanks to the National Research Foundation (NRF) for funding the research through their generous bursary. I extend my gratitude to my supervisor Professor Pardon Muchaonyerwa for his patience, support, guidance, assistance and sharing his knowledge.

I would like to extend my outmost appreciation to Mr K. Mbambo for his assistance in the collection of the different duckweed materials for the research, and to the University of KwaZulu-Natal for allowing me to use their facilities. Special thanks to the Discipline of Soil Science technical staff, Mr J. S. Buthelezi, Mr J. Buthelezi, and Mrs T. Nala, for going all out to make sure all the analyses are done timeously. My appreciation also goes to my colleagues Miss N. M. Malepfane, Miss N.P. Mkonza and Mr. N. Dlamini for their help in various ways to guarantee the smooth running of my work. Thanks to my friends for their moral support. Finally, I would like to give my gratitude to my awesome extended family as whole, for their understanding, support and love during my absence.

## TABLE OF CONTENTS

DECLARATION .....	i
ABSTRACT.....	ii
ACKNOWLEDGEMENTS .....	iv
LIST OF TABLES .....	viii
LIST OF FIGURES .....	ix
LIST OF APPENDICES .....	x
CHAPTER 1: GENERAL INTRODUCTION .....	1
1.1 Background .....	1
1.2 Objectives and Hypothesis .....	6
CHAPTER 2: LITERATURE REVIEW .....	7
2.1 Introduction .....	7
2.2 Quantity and quality of wastewater produced globally and in South Africa .....	8
2.3 Effects of nutrient loads from waste water on aquatic life and the environment.....	12
2.4 Different duckweeds species and their effectiveness on taking up nutrients.....	17
2.5 Effects of wastewater type and nutrient composition on the tissue composition of individual species of duckweed.....	20
2.6 Uses of duckweed biomass .....	22
2.7 Different duckweeds species used as an organic fertiliser of nutrient source N, P and other nutrient concentration and uptake .....	23
2.8 Chicken compost composition .....	25
2.9 Conclusion.....	26

CHAPTER 3: CHANGES IN NITROGEN AND PHOSPHORUS CONCENTRATION DURING A DUCKWEED INCUBATION IN SOIL AND ITS FERTILISER VALUE FOR SPINACH GROWTH IN A POT TRIAL STUDY .....	27
3.1 Introduction .....	27
3.2 Methods and Materials .....	29
3.2.1 Duckweed .....	29
3.2.2 Soil .....	31
3.2.3 Mineralisation of nitrogen and phosphorus from duckweed incubated in soil.....	31
3.2.4 Leaching of nitrogen and phosphorus from duckweed samples .....	32
3.2.5 Pot trial.....	33
3.2.6 Statistics .....	34
3.3 Results .....	35
3.3.1 Tissue composition .....	35
3.3.2 Nitrogen and phosphorus release of duckweeds during incubation.....	36
3.3.3 Leaching results .....	39
3.3.4 The spinach dry matter, concentration, uptake of C, N and P .....	40
3.3.5 Residual soil nutrient concentrations .....	42
3.6 Discussion .....	43
3.7 Conclusion.....	50
 CHAPTER 4: FERTILISER VALUE OF DUCKWEED AND CHICKEN LITTER COMPOST IN TERMS OF THEIR RATE OF NUTRIENT RELEASE OF NITROGEN FOR SPINACH GROWTH : A FIELD TRIAL .....	 51

4.1 Introduction .....	51
4.2 Method and material.....	52
4.2.1 Nitrogen and phosphorus release from duckweed and compost during incubation	52
4.2.2 Nitrogen fertiliser value of duckweed relative to chicken litter compost on spinach .....	53
4.2.3 Experimental layout, harvesting and after harvesting handling.....	54
4.2.4 Analyses .....	55
4.2.5 Statistical analysis .....	56
4.3 Results .....	56
4.3.1 Nitrogen and phosphorus mineralisation from duckweed and compost during incubation.....	56
4.3.2 Leaf area index and dry matter of spinach.....	59
4.3.3 Concentration and uptake of N and P in spinach.....	60
4.3.4 Residual soil C, N, P, pH and EC .....	61
4.4 Discussion .....	61
4.5 Conclusion.....	64
<b>CHAPTER 5: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS</b>	<b>65</b>
5.1 General discussion.....	65
5.2 Conclusion.....	69
5.3 Recommendations .....	69
<b>REFERENCES .....</b>	<b>71</b>
<b>LIST OF APPENDICES.....</b>	<b>79</b>



## LIST OF TABLES

<u>Table</u>	<u>Page</u>
<b>Table 3.1:</b> The study was carried out throughout the Midland region of Pietermaritzburg....	30
<b>Table 3.2:</b> Chemical composition of water from sites where <i>Lemna</i> species were found as reported by (Chikuvire, 2018).....	30
<b>Table 3.3:</b> Composition (%) of macronutrients in duckweed tissue from different sources .	35
<b>Table 3.4:</b> Micronutrients composition (mg/kg) in tissue of duckweed from different sources .....	36
<b>Table 3.5:</b> Determination of dry matter (DM), Leaf area index (LAI), P uptake, C, N and P content of spinach treated with pre-incubated and non-incubated <i>L. minor</i> dry matter. ....	41
<b>Table 3.6:</b> Nitrogen uptake of spinach dry matter amended with pre-incubated and non-incubated <i>Lemna</i> species.....	42
<b>Table 3.7:</b> Characteristics of residual soil after spinach harvesting.....	43
<b>Table 4.1:</b> Chemical composition of duckweed ( <i>L. minor</i> ) tissue and chicken litter compost. ....	53
<b>Table 4.2:</b> Concentrations of nutrients, uptake of N and P in spinach tissue fertilised with duckweed and compost .....	60
<b>Table 4.3:</b> Characteristics of residual field soil after spinach fertilised with duckweed and compost.....	61

## LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
<b>Figure 3. 1:</b> Ammonium-N concentration during incubation of duckweed species in loam soil.....	36
<b>Figure 3. 2:</b> Nitrate-N concentration during an incubation of duckweed species in loam soil. .....	37
<b>Figure 3. 3:</b> Extractable-P concentration during an incubation experiment in loam soil. Ash = Lemna from Ashburton (Lemna AB), Bayn = Lemna from Baynesfield (Lemna BF) and Wart = Lemna from Wartburg (Lemna WB). Error bars denote the LSD at $p < 0.05$ .....	38
<b>Figure 3. 4:</b> Changes in pH of incubated soil throughout the experiment. Ash = Lemna from Ashburton (Lemna AB), Bayn = Lemna from Baynesfield (Lemna BF) and Wart = Lemna from Wartburg (Lemna WB). Error bars denote the LSD at $p < 0.05$ .....	38
<b>Figure 3. 5:</b> Leachate ammonium-N concentration during leaching duckweed tissue (Lemna AB, Lemna BF and Lemna WB) with de-ionised water. Error bar denote the LSD at $p < 0.05$ .....	39
<b>Figure 3. 6:</b> Leachate nitrate-N concentration during leaching duckweed tissue (Lemna AB, Lemna BF and Lemna WB) with de-ionised water. Error bar denote the LSD at $p < 0.05$ .....	40
<b>Figure 3. 7:</b> Leachate P during leaching duckweed tissue (Lemna AB, Lemna BF and Lemna WB) with de-ionised water. Error bar denote the LSD at $p < 0.05$ .....	40
<b>Figure 4. 1:</b> Treatment arrangement in the field experiment .....	54
<b>Figure 4. 2:</b> Ammonium-N concentration during incubation of duckweed (Lemna BF and Lemna WB) and compost in loam soil .....	56
<b>Figure 4. 3:</b> Nitrate-N concentration during incubation of duckweed (Lemna BF and Lemna WB) and compost in loam soil.....	57
<b>Figure 4. 4:</b> Extractable P concentration during incubation of duckweed (Lemna BF and WB) and compost in loam soil.....	58
<b>Figure 4. 5:</b> Changes in soil pH during incubation of duckweed (Lemna BF and Lemna WB) and compost in loam soil. ....	58
<b>Figure 4. 6:</b> Leaf area index (LAI) of spinach fertilised with duckweed and compost in the field. Means with the same letter are not significantly different. ....	59
<b>Figure 4. 7:</b> Spinach dry matter as affected by duckweed and compost treatments. Means with the same letter are not significantly different. ....	60

## LIST OF APPENDICES

<b>Appendix 1:</b> Statistical output for analysis of variance (ANOVA) for duckweed tissue analysis.....	78
<b>Appendix 2:</b> Statistical output for analysis of variance (ANOVA) for the incubation study...	79
<b>Appendix 3:</b> Statistical output for analysis of variance (ANOVA) for the leaching study.....	80
<b>Appendix 4:</b> Statistical output for analysis of variance (ANOVA) for the pot trial.....	80
<b>Appendix 5:</b> Statistical output for analysis of variance (ANOVA) for the field trial.....	83

## CHAPTER 1: GENERAL INTRODUCTION

### 1.1 Background

Intensive agricultural systems, like piggery, dairy, poultry and crocodile farming continuously produce nutrient rich organic wastes, and their disposal to ponds, rivers and lakes results in pollution of water bodies by nutrients, particularly nitrogen and phosphorus (Qu and Fan, 2010). In addition, poorly functioning sewage systems also release nutrient rich organic wastes into water bodies. The pollution of surface water bodies by these nutrient rich organic effluents results in eutrophication, where algae and macrophytes, including duckweed, grow (World Health Organization, 2002). Eutrophication is regarded as an activity where a high volume of nutrients dominate a water source resulting in a high bloom of macrophytes (Van Ginkel, 2011). The growth of these organisms, particularly duckweed is testimony that such nutrient enrichment is occurring in these water systems.

Duckweed is from a family of floating plants (Lemnaceae), which consists of five genera, *Landoltia*, *Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella* (Klaus et al., 2013, Tang et al., 2015). These five genera of duckweed, can be morphologically described in terms of their fronds (leaf like appendages in duckweed) and diameters. *Spirodela* have five fronds with a diameter of 10mm, *Landoltia* have three fronds with 5mm diameter, *Lemna* have three to four fronds with diameter of 2mm, while *Wolffia* is granular shaped with a 1mm diameter, and *Wolffiella* ranges from a single or cluster of fronds with a diameter ranging from 1 to 5mm (Klaus et al., 2013). These plants have the ability to multiply rapidly, doubling their numbers in a short period of time within a matter of hours (16 to 48) to a number of days (2 to 3) (Khellaf and Zerdaoui, 2009a, Chikuvire, 2018). They rapidly take up nutrients, particularly nitrogen (N), phosphorus (P) and potassium (K) from waste water. When the duckweed is fully developed it is able to

decrease the levels of carbon dioxide (CO<sub>2</sub>) in the air and also reduces the N and P in the water, thereby improving the water quality (Stomp, 2005).

A study conducted by Ozengin and Elmaci (2007), in Turkey for about three weeks, showed that 34 - 99% N and 14 - 92% P were recovered from wastewater by duckweed (*Lemna minor*). Another study conducted in Bangladesh by Alaerts et al. (1996), revealed that a duckweed *L. minor*, was able to remove 74% (from 10.5 to 2.7 mg N/L) of total N and 77% (1.95 to 0.4 mg P/L) total P from pig effluent at a rate of 0.26 g N m<sup>-2</sup> day<sup>-1</sup> and 0.05 g P m<sup>-2</sup> day<sup>-1</sup> over a period of four weeks. Patel and Kanungo (2010) reported that *L. minor* was able to reduce total organic C from 51.86 to 9.57 mg/L, total N from 118.52 to 29.8 mg/L and P from 37.14 mg/L to 16.42 mg/L in domestic wastewater in a period of about seven days. In addition to taking up macronutrients, *L. minor* was also reported to accumulate 11.38 mg Cu /kg and 8000 mg Zn /kg within a period of 8 days (Sasmaz et al., 2015). A study conducted in Egypt showed that *L. gibba* was also able to improve water quality in a primary treated sewerage plant (El-Kheir et al., 2007). The analysis of literature shows that there is variation in the effectiveness of nutrient removal amongst the duckweed genera.

A study by Toyama et al. (2018) of *Spirodela polyrhiza*, *Lemna minor*, *Lemna gibba*, and *Landoltia punctata* grown on municipal wastewater, swine and anaerobic digested effluents, showed that *S. polyrhiza* had the highest rate of removal of N in all of these effluents, than the other species. In addition to species differences, the effectiveness of duckweed to remove nutrients from waste water depends on the conditions of where they are grown. Total N in water ranging from 5 to 15 mg/L resulted in growth of duckweed (*Lemna* species) dry matter of about 10 to 30 tonnes/ha/yr (Anh et al., 1997). Other abiotic factors for the growth of duckweed besides nutrient composition include pH, light and temperature (Bornette and Puijalon, 2011).

Different duckweed species prefer different light intensities and temperatures. *Lemna aequinoctialis* grows optimally at a temperature of 23°C and light intensity of 110  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Yin et al., 2015). In another study, *L. minor* growth was influenced by a light intensity of 200 to 250  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , while 250 to 400  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  limited growth from 0.19 to 0.14 mg/day and 450  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  light intensity further inhibited growth at a temperature of 21°C (Tabou et al., 2014). Water bodies with temperatures ranging from 6 to 34°C encourages higher duckweed growth. The rise in temperature increases duckweed growth rate, 34°C is the upper limit at which growth rate becomes slower and greater temperatures halts growth. Duckweed tolerates a range of temperature conditions which makes it much ideal for wastewater treatment, since it can be present in wastewater all year around while other macrophytes only grow in the summer months (El-Kheir et al., 2007). However some winter seasons result in duckweed becoming dormant (Farrell, 2012). Farmers generally view duckweeds as a nuisance which needs to be controlled, although they may need to consider it as a communication mechanism between man and the water environment. The accumulation of nutrients in duckweed suggests that the biomass may be of value.

Duckweed has mostly been used as animal feed due to its high nutrient content (protein of 20 to 35%) as well as the accumulation of large biomass at a fast rate (Leng et al., 1995, Appenroth et al., 2017). Tissue composition of duckweed depends on the water on which it grows. A study by Chikuvire et al. (2018a), showed that swine lagoon water with 29 mg N/L and 60 mg N/L resulted in a fresh weight of 490 and 476  $\text{g}/\text{m}^2$  of *Wolffia arrhiza* with N uptake of 0.694 and 0.705  $\text{g}/\text{m}^2$  respectively, in a period of a week. The *W. arrhiza* showed a tissue composition to be 3.12 to 3.93% total N, 38.1 to 36.2% total C and C/N ratio of 12.3 to 9.59, respectively. The differences in C/N ratio suggests that the duckweed grown on wastewater with different

concentrations of N could vary in the rate of decomposition when added to soil. The high concentration of N in duckweed, makes it a potential organic fertiliser. For example, harvested *L. minor*, having recovered nutrients from polluted water could be used as a fertiliser source according to Crites et al. (2006). However, there are limited studies on duckweed value as an organic fertiliser. The recycling of nutrients from wastewaters, through the use of duckweed could contribute to the reduction of fertiliser requirements especially in low input agriculture. A study by Chikuvire et al. (2018b), showed that high N content in *W. arrhiza* is suitable for facilitating growth of Swiss chard in a pot trial when used as a soil amendment. A 28-day period of pre-incubation of duckweed tissue in soil ensured a higher dry matter of Swiss chard as a result of increased availability and uptake of nutrients in comparison with the application of an inorganic fertiliser. Species of *Wolffia* and *Lemna* commonly occur on nutrient rich water bodies in South Africa, with tissue nutrients being lower for *Lemna* species (Chikuvire, 2018).

The Midlands region of KwaZulu-Natal has large scale intensive animal production systems which include dairy, piggery, crocodile and poultry. Waste in these farms is mostly deposited into water lagoons, while there are other avenues which are viable for nutrient rich waste such as composting. Composting stabilises the nutrients in the waste by product, making it easier to handle and reduces the odours unlike in lagoons. On the other-hand small-holder farmers in peri-urban areas face challenges of having to purchase fertilisers. Harvesting of duckweed could be cost effective, more especially in small holder farmers due to the duckweed ponds being near their premises of commercial farm lands.

A survey conducted in the Midlands region of KwaZulu-Natal showed that the dominant duckweed genera were *Wolffia* and *Lemna* with *W. arrhiza* and *L. minor* as the prominent species (Chikuvire, 2018). These species occur separately and in co-existence depending on

nutrient composition of the effluent. Elemental composition, including N, P, bases and micro-nutrients in the tissue were related to the composition of the water on which they grew (Chikuvire, 2018). This variation in elemental composition could affect the quality of the duckweed as a nutrient resource. Effluent sources differ in their nutrient composition, resulting in duckweed that vary in tissue composition. Since *L. minor* is one of the major duckweed species commonly occurring in South Africa (Chikuvire, 2018), there is need to understand its potential to recycle nutrients from waters polluted with different types of organic waste. It is therefore essential to understand the effects of effluent types on nutrients and tissue composition of individual duckweed species. The value of duckweed biomass as a nutrient source could be affected by the rate of nutrient release in the soil. There is need to understand the N and P release in soil amended with a specific duckweed species from different effluents.

In fresh poultry litter, proteins and uric acid are the two sources of N and they influence the mineralization of N, nitrification and potential losses through leaching of nitrate-N. The application of fresh chicken litter in excess on agricultural lands results in environmental issues such as ammonia volatilization, leaching and runoff (Tiquia and Tam, 2002). Therefore, composting poultry litter is beneficial in reducing pathogen, stabilizing nutrients such as N amongst others, resulting in gradual release of nutrients as compared to the rapid release of nutrients from non-composted litter. Chicken litter compost general has a nutrient range of 1.1 to 2.5% N, 0.75 to 2% P and 1.1 to 3% K (Maynard, 1994). The fertiliser value of duckweed grown on different types of effluent may need to be tested against rich organic solid wastes commonly used as an organic fertiliser, which include poultry litter.



## **1.2 Objectives and Hypothesis**

The main objective of this study is to determine variations in nutrient composition of *L. minor* from different effluents around the Midlands region of KZN and its potential as nutrient source for vegetable production. The specific objectives are to:

- (i) Determine the effect of effluent type on N and P composition of *L. minor*;
- (ii) Determine the effect of *L. minor* from different sources and their rates on nitrogen and phosphorus release in soil;
- (iii) Determine the effect of *L. minor* biomass from different sources, as N source, on dry matter yield and nutrient composition of spinach.

The alternative hypotheses were:

- (i) Duckweed from different effluent types differ in N and P composition.
- (ii) Nutrient release of *L. minor* depends on the effluent type and application rate.
- (iii) Effluent type affects the N fertiliser value of *L. minor* biomass for spinach.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 Introduction

Anthropogenic activities have been reported to result in the accumulation of nitrogen (N) and phosphorus (P) in water bodies (Huang et al., 2003). Wastes from domestic sewage, industrial waste water, agriculture fertilizer amongst others, contain high nutrient content, which end up in surface waterbodies or leached to underground water. The high nutrients, especially N and P result in eutrophication in freshwater bodies (Morrison et al., 2001). Eutrophication results in an uncontrollable growth of plant biomass in lakes, rivers as well as coastal waters as induced by high levels of N and P (Nyenje et al., 2010). The high potential of aquatic macrophytes to recover nutrients from waste water, results in macrophytes such as duckweed being widely preferred for nutrient (N and P) recovery from domestic waste and agricultural waste amongst others sources of waste water (Sooknah and Wilkie, 2004). The interest in using duckweed for nutrient recovery is due to its ability to accumulate large amounts of nutrients in comparison to other macrophytes such as water hyacinth or *Salvinia* as reported by Cheng et al. (2002b).

The growth of duckweed is induced by the availability of nutrients such as N, P, and K in large amounts (Leng, 1999a), in addition to Ca, Na, Mg Zn, Cu and Mn which are also important for duckweed growth. The ability of duckweed to accumulate large protein content ranging from 15 to 40 % depending on where is being grown, as well as decreased amounts of fibre (Landesman et al., 2002), makes duckweeds to have a good potential for recycling nutrients. There are a wide range of uses for the dried biomass of duckweed in the agriculture sector including feed for cattle, pigs, poultry and fish (Yilmaz et al., 2004b). The duckweed dry matter could also be considered as an organic fertiliser. This chapter is a review of literature on duckweeds and their potential use as sources of nitrogen (N) and phosphorus (P) for growth of plants.

## **2.2 Quantity and quality of wastewater produced globally and in South Africa**

Worldwide there are approximately about 133 million dairy farms with an average of about 90 to 300 dairy cows depending on the region (Lowder et al., 2016), while South Africa has over 4000 dairy farms (Esterhuizen et al., 2015). These dairy farms produce an average of 15 to 20 L/cow per day volume of waste water which contributes total N, P and K at about 60, 50, and 80 kg/ha/ y respectively. These wastewater from dairy farms are produced at rate 20 to 40 L/m<sup>2</sup>/day, with nutrients ranging, from 6 to 183 mg/L for total P, 0.3 to 6.5 mg/L for nitrate-N, and 5 to 625 mg/L for ammonium-N (Morin et al., 2008).

On the other hand a number of pig farms worldwide were estimated to be 941 million in the year 2009 and continue to increase at a steady rate (Kemp et al., 2011). In South Africa commercial pig farms are approximately 400 and small holder pig farms are estimated to be about 4000 (Roelofse, 2013). A medium size (intermediate farm between commercial and smallholder) pig farm produces approximately 30 and 35 m<sup>3</sup>/day of wastewater depending on its size (Saucedo Terán et al., 2017). A study conducted in South Africa on four pig farms showed that wastewater from these farms contained 55 to 1680 mg/L phosphate-P, 37.5 to 2730 mg/L nitrate-N and 50 to 1427 mg/L nitrogen dioxide-N (Mofokeng et al., 2016). The wastewater usually contains high concentration of nutrients such as organic nitrogen (N), phosphorous (P), sulphur (S), bases, macro nutrients and in some case, heavy metals, which together cause pollution which devastate aquatic environments (Musfique et al., 2015).

A study conducted in the Eastern Cape province of South Africa showed that the final effluents from treatment plants were still compromised in terms of quality of physico-chemical component of wastewater from sewage discharge (Igbiosa and Okoh, 2009). The effluent had pH 7.03, 133.26 mg/L total solids, 8.73 mg/L nitrate-N, 0.08 mg/L nitrite-N, and 4.81 mg/L

orthophosphate after treatment during the summer season. While Popa et al. (2012), reported that orthophosphate was more prevalent in domestic wastewater than in industrial wastewater due to foods and dish washing detergents which contain phosphorous compounds as one of their constituents ranging from 1 and 6 mg P/L. Industrial waste water had high amounts of N-nitrate (2 to 5 mg/L) as opposed to domestic waste water having (0 to 1.5 mg/L). While Romanian and European law tolerates a maximum threshold of about 2 mg P/L (Popa et al., 2012). A study conducted in an estuary in Pearl River in South China coast, showed high concentrations of inorganic nitrogen (0.30 to 1.6 mg/L) and phosphate (0.015 to 0.030 mg/L), which are responsible for eutrophication. This is a result of an increase in the economic and urbanization, which resulted in increased waste water deposition into rivers (Huang et al., 2003).

There are a variety of other contributors which produce waste water that contain high amounts of nutrients such as poultry, crocodile and domestic water production. In the case of poultry waste produced in industries results in nutrient rich effluents with nutrients ranging between 100 to 250 mg/L for both total nitrogen and phosphorus (Molapo, 2009). Average of total nitrogen and phosphorus concentrations were found to be 230 and 22 mg/L for crocodile effluent and 30 and 8 mg/L for domestic waste respectively (Sudha, 2008). The various studies showed that the composition of the effluents vary across different production systems and across different regions. Disposal of the different wastewaters will therefore have different effects on surface and underground water quality.

### **Effects of nutrient rich waste water on water quality: Global and South African trends**

Fresh water bodies contain diverse amounts of aquatic species including amphipods, crabs, crayfish, midges and water fleas to name a few depending on the regions where they are found.

Good quality water is required to maintain and protect these aquatic species found in fresh water ecosystem. A number of studies have shown that pH between 6.5 to 9 and 5 to 9.5 mg/L dissolved oxygen are ideal for the survival of fishes, regardless of the water temperature preferred by aquatic organisms as mentioned by Enderlein et al. (1997). Depending on the species that is found in the water, the ideal biochemical oxygen demand (BOD) are 0 to 6 mg O<sub>2</sub>/L for salmonid, 3 and 6 mg O<sub>2</sub>/L for cyprinid. Phosphorus and its compounds are acceptable at levels of 0.05 and 0.5 mg/L as long as they do not allow growth of algae (Kotoski, 1997). In the case of nitrate-N, levels considered ideal for aquatic organisms to thrive are  $\leq 2$  mg/L (Camargo et al., 2005). The survival of aquatic organisms in water with ammonia-N is pH and temperature dependent. Ammonium-N is toxic at temperature  $> 30^{\circ}\text{C}$  especially at lower water pH values (World Health Organization, 1989).

The effects of eutrophication on aquatic systems is devastating since it affects the fauna and flora components of water by increasing its toxicity, as a result of over loading of nutrients, which result in cyanobacteria (blue-green algae) colonisation of the water sources and their production of metabolites which are harmful to biota. Eutrophication threatens plankton ecosystems and larger aquatic animals, as well as the functioning of a water sources (Chen et al., 2014b). Reduction of oxygen levels in water polluted by wastewater from sewage plants that are inefficient at removing high nutrients from wastewater, is a major problem in sub-Saharan African countries (Nyenje et al., 2010). Accumulation of N and P in water from lakes, rivers and coasts resulting in bloom of macrophytes.

On a global scale, the quality of water affected by eutrophication 53, 48, 41, and 28%, in Europe, North America, South America and Africa respectively according to Nyenje et al. (2010). The main contributing sources of nutrient include domestic and agricultural

wastewater, raw sewerage and industrial discharges. Domestic waste water from Johannesburg, in South Africa, as well as areas surrounding this city was observed to be the main source of nutrient enrichment of Hartbeespoort dam. The dam is non-functional due to high concentration of nutrients such as total P and N, at amounts of 20 and 128 g/m<sup>2</sup>/y respectively (Scott et al., 1980). For example, another study showed that high nutrient loads are disposed annually in this dam at ranges between 80 to 300 tonnes of P and over the years the dam has been receiving quite high inflows of total P equivalent to 700 mg/L/day (Harding et al., 2004).

Pig lagoons are usually found to have high amounts of nutrients ranging from 200 to 800 mg N/L and 30 to 100 mg P/L, and these are within tolerable limits of duckweed unlike other macrophytes (Cheng et al., 2002b). The Modder River in Bloemfontein is one of the rivers that are eutrophic due to high nutrients from domestically treated and untreated industrial wastewater. The water quality parameters ranged 10 - 650 NTU for turbidity, 10 - 67 mS/m for conductivity, 20 - 816 mg/L for nitrate-N, 4 - 64 mg/L for phosphate-P, with bacterial counts (*Escherichia coli*) of  $3,96 \times 10^3$  /100ml, which exceeded recreational water limits of 150N/100ml (Koning et al., 2000).

A major challenge in South Africa is the health risk posed by sewage seepage which results in contamination of fresh and clean waters. This is more frequently in developing countries with a larger portion of rural areas adversely affected, as the urban settling increases geometrically (Igbinosa and Okoh, 2009). The contamination of the water sources comes from a variety of sources for instances sewage seepages, industrial waste, and as well as domestic waste from the villages. This results in concentration of nutrients such as nitrate which compromises the water quality of surface water bodies at amounts greater than 25 mg/L and in ground water at amounts greater than 100 mg/L (Camargo et al., 2005).

In a study conducted by Fatoki et al. (2003), it was shown that Keiskamma river in the Eastern cape contains higher amounts of nitrate (2.2 mg/L), orthophosphate (0.08 mg/L), as well electrical conductivity which varied between 150 to 350 mS/m, these were within the Southern African law limits as reported by Nyenje et al. (2010). The permissible limits under the South African guidelines regarding the nutrient concentration in domestic water for nitrate-N (6 mg/L), phosphate (5µg/L), electro-conductivity 70mS/m and pH 6 to 9 (Fatoki et al., 2003). In the past decades duckweed plants have shown to be more suitable for treating wastewaters due to their ability of gemmation (asexual reproduction) and being able to uptake large amounts of nutrients in water bodies which are slow moving (Frédéric et al., 2006).

### **2.3 Effects of nutrient loads from waste water on aquatic life and the environment**

In Africa about 28% of natural water sources are affected by eutrophication in a study by Nyenje et al. (2010) reported by the Water Research Commission of South Africa. Khan and Ansari (2005) reported that high amounts of nutrients such as phosphorus (80 tons/day) in Lake Erie, resulted in high accumulation of blue-green and green algae (*Cladophora spp*) in amounts above of 350 tons from a P content of about 400g/L. In turn there is a reduction in light penetration in water, oxygen availability and reduced growth of phytoplanktons. Wastewater from industrial sectors is shown to be rich in heavy metals, which in turn devastates the aquatic ecosystems. However, there are aquatic plants that are able to tolerate the high amounts of nutrients in water bodies such as water hyacinth, *salvinia* and duckweed (*L. minor*). As such they are used to recycle nutrients from a number of water bodies (Dhote and Dixit, 2009, Khellaf and Zerdaoui, 2009b).

In South Africa, there are concerns about high amounts of cyano-bacterial blooms, which release cyanotoxins, are a sign of eutrophic water bodies and a threat to the water supply. The most

prevalent species of cyanobacteria are *Microcystis spp*, *Oscillatoria spp*, and *Cylindrospermopsis*. The most documented exotic macrophytes in South Africa are water hyacinth, red water fern (*Azolla spp.*), water lettuce (*Pistia stratiotes*), Kariba weed (*Salvinia molesta*), *Hydrilla (Hydrilla verticillata)* and parrots feather (*Myriophyllum aquaticum*) (Van Ginkel, 2011). Life (fish and shellfish) where eutrophication occurs is eliminated due to the limitation of oxygen availability, as a result of abundance of macrophytes such as free-floating algae and phytoplankton which distort the light penetration and water transparency to the bottom of the water body (World Health Organization, 2002, Srivastava et al., 2008). Macrophytes are divided into three categories emergent, free floating (*Eicchornia crassipes*, *Lemna* species etc.) and submerged (*Hydrilla verticillata*). A high concentration of biomass of macrophytes, as influenced by the nutrient content of water (Srivastava et al., 2008, DeBusk et al., 1995), could be harvested to be utilize as an energy source, composted and used as animal feed which would be beneficial as they nutrient recover, resulting in improving water quality (Brix and Schierup, 1989, Dhote and Dixit, 2007).

In an experiment duckweed was shown to be able to decrease microscopic parasites which can cause illnesses and bacteria in ponds used to treat domestic waste water, such as faecal coliform (bacteria), *Giardia*, *Cryptosporidium*, and coliphage by about 98%, 89%, 62% and 40% respectively. Duckweed species (*Lemna obscura*, *Lemna minor*, *Lemna majus*, and *Lemna gibba. Lemna spp.*) are amongst the macrophyte that accumulate large amounts of nutrients from wastewater into their tissue more especially N and P (El-Kheir et al., 2007).

### **Classification of duckweed and factors affecting their growth**

Over the previous years the plants taxonomy has seen some changes from having been classified under Lamnaceae family, to being classified under Araceae family in subfamily of



Lemnoideae. Duckweed however has roughly about 40 species consisting of 5 genera (Mohedano et al., 2012), which are *Landoltia*, *Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella* species (Khellaf and Zerdaoui, 2009a). *Landoltia* are tiny aquatic plants that grow in stagnant water sources like many other duckweed genera. However, what sets them apart from other genera's is their almost kidney shape, resembling *Lemna* species with 2 to 5 roots descending from their fronds. The other genera are distinguishable through their fronds with *Spirodela* and *Lemna* having flat fronds, oval and leaf like. *Spirodela* has two or more thread-like roots on each frond while *Lemna* having only one. *Wolffiella* and *Wolffia* are rootless; they are much smaller than *Spirodela* or *Lemna*. *Wolffia* fronds are usually sickle shaped whereas *Wolffiella* are boat shaped and neither has roots. These characteristics are well defined depending on where the duckweed species was grown. The different duckweed species nutrient recovery through absorption, more especial in presences of ammonium in slightly acidic effluents (Leng, 1999b, Hillman, 1961, Grippo et al., 2017).

As such these duckweed genera differ in the amounts and factors which induce their optimum growth such as pH, nutrient levels, light, speed of water movement and CO<sub>2</sub> levels. In the case of *Lemna* species for them to grow at optimum growth, factors such as temperature and pH should be kept at the following ranges 6 to 30°C and 6.9 to 7.8 respectively, while a temperature of 30°C is ideal for *Spirodela* species (Srivastava et al., 2008). A study by Mohedano et al. (2016), showed that *Landoltia punctata* grown at high concentration of carbon dioxide of about 100 000 ppm, resulted in an increased amounts of starch content from about 9.6 to 24.7% which was a 150% increase, as well as high uptake of nitrate and phosphate of about 82 and 79% from concentrations of 308 mg NO<sub>3</sub><sup>-</sup>/L and 28 mg PO<sub>4</sub>/L respectively grown in an artificial medium. A Low carbon dioxide concentration of 380 ppm results in a low biomass of about 1 g/m<sup>2</sup>/day, while 100 000 ppm carbon dioxide concentration results in high biomass of 53.5 g/m<sup>2</sup>/day.

Another study conducted by Andersen et al. (1985), showed *Lemna gibba* to have an optimum growth rate of 46% at 6,000 ppm concentration of carbon dioxide as opposed to 350 ppm concentration. *Lemna minor* has an optimum pH level of 6.2, in terms of carbon dioxide concentration a lower concentration of 65 ppm propagates growth in *L. minor*, while 330 ppm results in much increased growth rate, and 9,000 ppm concentration increases only the fronds size. Therefore it is safe to assume that the different duckweed genera's respond to different carbon dioxide concentrations (Ruigrok, 2015).

*Lemna minor*'s growth rate as influenced by light intensity showed that 200 to 250  $\mu\text{mol}/\text{m}^2/\text{s}$  light intensity allows for optimal growth while a light intensity of 250 to 400  $\mu\text{mol}/\text{m}^2/\text{s}$  limits growth from 0.19 to 0.14/day and 450  $\mu\text{mol}/\text{m}^2/\text{s}$  light intensity inhibits growth to as little as 0.07g/day (Tangou Tabou et al., 2014). Ammonium is preferred by duckweed as an N source even though at higher amounts it restricts duckweed growth. The different genera of duckweed prefer nutrients at different amounts. This was shown to be the case in the studies reviewed by Caicedo et al. (2000a), where *Spirodela polyrrhiza* seized growth with ammonia amounts greater than 46 mg/L at pH between 5 to 8. *Spirodela polyrrhiza* at about 3.5 to 20 mg/L concentration of ammonium-N grows at its optimum growth. While another study reported 375 mg/L as a high limit that restricts *S. polyrrhiza* growth. In the case of *Lemna gibba* 200 mg/L ammonium-N at a pH of 7 restricted its growth. While in the case *Lemna minor* an ammonia amount of 7.2 mg/L halved the biomass yields.

To ensure an optimum growth of duckweed ammonia and pH levels should be kept less than 50 mg/L and 8 respectively. Levels of nutrients need to sustain the different duckweed species vary from minimum, optimum and maximum, as such minimum nitrogen levels needed by *L.*

*miniscula* and *Lemna* species are 0.0016 mg/L and 0.08 mg/L respectively, while an optimum requirement for *W. colombia* and *S. polyrriza* is 0.01 and 30 mg/L respectively, and the maximum range tolerance for *L. miniscula* and *L. aequinoctialis* is from 30 to 450 mg/L. The pH amounts that allow for optimum growth of duckweed between species vary from species to species. In the case of *Spirodella* and *Lemna* species 7 is the optimal pH for growth with 3 to 5 being the minimum level and the maximum pH level being 10.5. Most species have an optimal temperature range of about 12°C to 30°C. A depth of 0.5 is considered ideal for growth of duckweed, since deeper water columns result in inaccessible nutrient sources (Goopy and Murray, 2003). Regardless of the differences in nutrient preferences amongst the different genera's, they all find refuge in stagnant water sources.

Abundance of nutrients in wastewater, standing water and favourable condition (pH and light intensity) are responsible for the well establishment along with growth of duckweed species in effluent rich waste water bodies. Though the rate of growth depends on the particular species of duckweed as well as the environment of growth. In suitable conditions duckweed doubles its biomass within a period of 16 to 48 hours (Goopy and Murray, 2003) in a study of Leng (1999b). *Landoltia punctata* (duckweed ) grown on piggery effluent in a study conducted in Brazil (Santa Catarina state) by Mohedano et al. (2012) was able to yield up to 68.8 ton/ha dry weight (DW) in a year. Other duckweed species like *L. minor* and *L. gibba* grown in up-flow anaerobic sludge blanket (UASB) reactor waste water for a period of 8 months yielded 33 tons/ha, and *Lemna valdiviana* grown on domestic effluent yielded 50 tons/ha (Mohedano et al., 2012). The growth of duckweed is induced by the availability of nutrients such as N which is converted to protein, P and K in large amounts are assimilated into the plant tissue (Leng, 1999a).

The micro and macro-nutrients such as zinc (Zn), copper (Cu), molybdate (Mo) and calcium (Ca), sodium (Na), magnesium (Mg) respectively are also important for duckweed growth. *Lemna minor*'s ability to grow in a variety of waste waters is due to its tolerance and its ability to adapt. *Lemna minor* is able to tolerate lower rates of heavy metals such as Cu and Ni in industrial waste water ranging from 0.2 and 0.5 mg/L respectively, which results in frond growth, while higher rates of 1.29 mg Ni/L and 0.47 mg Cu/L limit its growth (Khellaf and Zerdaoui, 2009b).

#### **2.4 Different duckweeds species and their effectiveness on taking up nutrients**

Water conditions play a huge role on the amounts and nutrient recovery rates by duckweed, as they influence pH and light intensity which in turn determine nitrate/ ammonium ratio during the growth of duckweed species (Goopy and Murray, 2003). The most limiting macronutrients on the growth of any duckweed species are the availability of N, P and K (Culley Jr and Epps, 1973). Duckweed is able to utilize nutrients from a number of effluent sources from dairy wastewaters, raw and diluted domestic sewage, waste stabilization ponds and as well as fish culture systems, through the production of large amounts of biomass (Selvarani et al., 2015). Lemnaceae species absorb nitrogen either as nitrate, ammonium, nitrite, and even as amino acids amongst others (Goopy and Murray, 2003).

A study conducted in South Africa by Chikuvire et al. (2018a), of duckweed (*Wolffia arrhiza*) grown in diluted (5%) swine lagoon with an N content less than 60 mg/L, showed that *W. arrhiza* is effective at recovering nutrients from pig waste water. Harvesting of *W. arrhiza* once a week resulted in fresh and dry biomass of 774 and 27.8 g/m<sup>2</sup> respectively. *Wolffia arrhiza* in this study had a growth rate of 1.59 g/m<sup>2</sup>.day which resulted in a dry matter with a nitrogen content and N uptake of 5.42% and 1.53 g/m<sup>2</sup> respectively.

Another specie of duckweed *Spirodela punctata* was able to take up nutrients like nitrogen (N) and phosphorus (P) in a synthesized medium similar to swine waste water. This duckweed at high concentrations of N (240 mg NH<sub>4</sub><sup>+</sup>/L) and P (31 mg PO<sub>4</sub><sup>-</sup>/L) thrived and produced 1.33 g dry biomass/m<sup>2</sup>/hr amounting to 31.92 g/m<sup>2</sup> per day. In this study *S. punctata* was able to recover nutrients at a rate of 0.995 mg N/L/hr and 0.129 mg P/L/hr (Cheng et al., 2002a). These nutrients are stored in the duckweed tissue, the total nitrogen, total phosphorous and ammonium-N are preferred to be take up since they are much easier to assimilate. These were recovered at rates of about 70%, 94% and 100% respectively by *L. minor* grown and harvested from human urine in a study of Cheng et al. (2002b).

Xu and Shen (2011a), reported that *Spirodela oligarrhiza* in 6% diluted swine lagoon was able to extracted more than 83.7 and 89.4 % total nitrogen (TN) and phosphorus (TP) when duckweed was harvested twice a week. Nutrient concentration in the 6% dilution were as follows 52.1, 58.4 and 15.9 mg/L of ammonium-N, total nitrogen and total phosphorus respectively. Frequently harvesting duckweed twice weekly resulted in a growth rate of 0.065 g/g per day with a fresh weight biomass of 106.1 g/day. While when the *S. oligarrhiza* is covering a swine waste water surface of about 80 to 20% amounted to a 210.8 to 52.7 g/m<sup>2</sup> biomass. This resulted in increased recovery rates of ammonium-N being 80%.

A study conducted in the United States of America by Bergmann et al. (2000b), showed that three duckweed species (*lemna gibba*, *Lemna minor* and *Spirodela punctata*) grown in dilutions of 50 and 25% of swine lagoon effluent, had nutrient recovery rates ranges of 67.7 to 71%, 82.8 to 89.4% and 71 to 85.3% respectively for total nitrogen. After a 12 day growing period for *L. gibba*, *L. minor* and *S. punctata* resulted in a removal rate of total nitrogen 68.8, 90.2 and 75.9%, while for total phosphorus 36.1, 60.3 and 28.5% respectively from the swine lagoon. Nutrient recovery by these duckweed species resulted in variation in fresh weight and dry

matter accumulation from *L. gibba* being high 611.2 and 26.6 g, followed by *L. minor* 499.5 and 23.3 g, and *S. punctata* having the least 365.5 and 18.6 g respectively. *L. minor* showed its ability to remove nutrients at much higher rate in comparison to other duckweed species.

A study conducted in Utah by Farrell (2012), showed that two duckweed species (*Lemna turionifera* and *Wolffia borealis*) were able to remove P from municipality wastewater. Phosphorus removed was about 113 mg P/m<sup>2</sup> day in a light intensity of 200  $\mu\text{mol/m}^2$  from a concentration of 387 mg P/L, resulting in biomass ranging from 0.5 to 1.5 kg dry duckweed/m<sup>2</sup>/day over a 90-day period. Harvesting of duckweed biomass recovered 30 to 90% of phosphorus in the waste water. An effectiveness of these duckweeds to reduce effluent concentration with P ranging from 3.22 to 5.2 mg TP/L to lower amount of 0.88 mg P/L within a period of 3 days was realised.

The ability of duckweed to nutrient recover does not only depend on the species, it is also influenced by wastewater source. This is revealed in a number of studies conducted with *L. minor*. One of those studies was conducted in Pakistan for 22 day were *L. minor* growth and nutrient removal efficiency from combination of wastes from residential, commercial and industrial sites was reported by Iqbal and Baig (2016), in order to show pH (6-8) as one of the parameters that influences the efficiency at which nutrients are removed. Nutrient concentration on the growth medium from which *L. minor* was grown of N and P ranged from 90 to 20 mg N/L and 76 to 16 mg P/L. The rates of nutrient removal by *L. minor* for both N and P were 1.22 g/m<sup>2</sup>/day and 0.95 g/m<sup>2</sup>/day respectively. An efficiency of 94% and 91% for nutrient uptake by duckweed was achieved for N and P respectively.

A synthesized growth medium with a nutrient concentration of 740 mg/m<sup>2</sup>/day nitrate -N and 73 mg/m<sup>2</sup>/day phosphate amongst other nutrients, *L. minor* was able to recover these nutrients at a rate of 75.3% and 50% respectively (Uysal and Zeren, 2004). *Spirodela polyrhiza* grown in three effluent types (municipal, swine and anaerobic digestion wastewater) over a 7 day period within an effluent with a total nitrogen content range of 20 to 50 mg/L. Total nitrogen (TN) was removed at range of 2 to 10.8 mg TN/L/day from the growth mediums which resulted in accumulation of biomass ranging from 52.6 to 70.3 mg DW /L/day (Toyama et al., 2018). The difference in duckweed species results in difference in nutrient removal efficiency as influenced by a number of factors such as waste water composition, this clearly shows that *L. minor* in a number of effluent types is able to effectively nutrient recover at much higher rates.

### **2.5 Effects of wastewater type and nutrient composition on the tissue composition of individual species of duckweed**

The type of wastewater and its nutrient composition influences the duckweeds tissue composition. A study by Appenroth et al. (2017), showed that duckweeds grown in the same medium (schenk-hildebrandt solution) resulted in difference in tissue composition. Five duckweed species (*S. polyrhiza*, *L. punctata*, *L. minor*, *L. gibba*, *W. hyalina* and *W. microscopica*) were compared in terms of tissue composition. The total protein, starch, fat and dry weight content were determined to range between 18 to 36%, 3 to 9%, 3.9 to 6.5 and 4 to 7.9 respectively. *L. punctata* (20%) had the least protein and *W. hyalina* (35%) had the greatest amount. While *L. minor* (4%) had the lowest starch content with *L. punctata* (10%) having the highest.

In another study by Zhao et al. (2014), conducted in Norwich where *L. minor* was grown in a fish pond, it was observed that its tissue composition had a total protein (12%), starch (19.9%) and dry matter (3% to 14%) as influence by the water source. Hanczakowski et al. (1995),

reported that *L. minor* grown in sewerage wastewater had a tissue composition high in protein content (30%) and a dry matter of 947 g DM/Kg as well as micro nutrients (Zn, Cu and Cd) at amounts of 98, 4.7 and 0.09 mg/kg DM respectively. The difference in protein content of *L. minor* grown in different sources of water shows that the wastewater composition plays a role in duckweed tissue composition.

In a study by Chikuvire et al. (2018a), of duckweed *Wolffia arrhiza* species grown in pig effluent wastewater at different concentrations of 5 and 10%, showed a tissue composition of total N to be 3.12 and 3.93%, total carbon being 38.1 and 36.2%, and C/N ratio being 12.3 and 9.59 respectively to the concentrations. While *Wolffia arrhiza* grown in a synthetic medium (Hutner solution) by Fujita et al. (1999), was shown to have a total nitrogen and total phosphorus of 6 to 7% and 1 to 2% respectively in its tissue. Total nitrogen and total phosphorus in the solution were found to be 13.3 and 7.1 mg/L respectively. These nutrients were recovered at a rate of 126 mg P/m<sup>2</sup>/d and 38 mg P/m<sup>2</sup>/d. Biomass obtained from the experiment was found to be 2.4 g DW/m<sup>2</sup>/d.

Growing *Spirodela polyrhiza* in domestic wastewater resulted in higher carbohydrate (45.5%), starch (40.8%), lipid (46.4%) and, protein (56.4%) contents on its dry matter tissue (Gaur and Suthar, 2017). According to Mohedano et al. (2012), not all duckweeds species are efficient and ideal for effluent remediation and protein accumulation, however *Landoltia punctata* is considered to be ideally good in protein accumulation as well as being good in remediating piggery waste water. When grown in a pig effluent its total nitrogen and crude protein were observed to be 6.6% and 35% respectively.



There are limited studies on tissue composition for certain duckweed genera and also for other growth mediums besides swine effluent and a few other growth mediums for duckweed growth with tissue composition as main focus. However, other duckweed species such as *Lemna turionifera* and *Wolffia borealis* grown in pharmaceutical waste water were observed to contain a protein content ranging from 21-38% (Farrell, 2012). A study conducted by Hanczakowski et al. (1995), showed the effect of sewage wastewater on the protein composition of harvested duckweed *Lemna minor* which showed a difference of about 30% in comparison to a duckweed coming from a sewerage inlet and in comparison with duckweed from purified water.

The protein in the duckweed tissue is highly induced by the concentration of N in the effluent water as reported by Li et al. (2017), for duckweed *spriodela polyrrhiza* grown in high concentration of inorganic nitrogen in swine lagoons which resulted in difference of crude protein with one farm having 18.3% while the other had 20.8%. The amount of nutrients in the water are a determining factor for duckweed tissue composition hence availability of nutrients is able to influence duckweed crude protein to amount to 40%. The presence of nitrogen in wastewater stimulates increased amounts of carotenoids in the duckweed tissue (Tu, 2012).

## **2.6 Uses of duckweed biomass**

Duckweed species have a variety of uses in the agricultural industry for instance they are used to mop up nutrients in nutrient recovery system such as secondary sewage effluent, domestic and animal effluents (Iqbal, 1999). Their use extends as far as being feed for animal due to their high biomass, protein and ability to be an energy source. The large amounts of protein content (15 to 40 %) and low amount of fibre in duckweed, depending on where it has been grown (Landesman et al., 2002). This results in the dried biomass being useful feed for cattle, pigs, poultry, and fish. Freshly harvested duckweed has essential amino acids and up to 43%

of protein, which could make it a complete feed for fish (Yılmaz et al., 2004a). The high protein content ranging from 15 to 45% of duckweed species, make it a good feed for cattle, poultry, fish and ducks (Mohedano et al., 2012, Bergmann et al., 2000a). Other animal feed contain protein contents at about 49.9% (soybean meal), 24.4% (whole cottonseed) and 42.8% (whole soybean, roasted) (Hall et al., 2005).

According to Toyama et al. (2018), duckweed has a potential to be used as feedstock for production of biofuel such as ethanol. Mohedano et al. (2012), reported high amounts of starch (3 to 75%) are produced by duckweed which can then be turned to bioethanol which is a biofuel made from waste wastewater. Bioethanol is created through fermentation of sugars. There are quite a number of plant components from where bioethanol could be produced from, such as sugar, starch and cellulose. Amongst the commonly used components for production of bioethanol is starch. Production of biofuel from duckweed occurs through a process of saccharification where starch and carbohydrates are converted to sugars and these sugars are fermented to ethanol in an eight hour process (Cui and Cheng, 2015).

### **2.7 Different duckweeds species used as an organic fertiliser of nutrient source N, P and other nutrient concentration and uptake**

The loss of nitrogen in high amounts from planting areas makes it difficult to sustain growth of crops while also contributing to environmental pollution and the lost N needs to be replenished, since they result in high amounts of nitrogen application. Therefore, a way to mitigate the N losses in agriculture, which is a high contributor of nitrogen emission, is required. The N losses result in a number of environmental effects such as eutrophication in water and soil acidity.

There are Limited studies of duckweed as nutrient source. However, duckweeds ability to nutrient recover at high rates make it ideal for use as organic fertilisation. In most studies regarding duckweed species the main focus is on the nutrient recovery ability regarding high accumulation and tolerance of nitrogen and phosphorous from a wide range of effluents. Two studies showed duckweed as a green manure that's able to reduce ammonia loses from rice fields.

A study in China by Li et al. (2009), of *Lemna minor* applied in coexistence with urea in six treatments of 0, 90 and 180 kg N/ha with and without duckweed in rice fields, showed that the presence of duckweed was able to increase yields by about 9.4 to 9.8%. While on the other hand this duckweed was able to decrease ammonia loses by rate of 19 to 53.7%. Duckweed cover on the rice farms was shown to reduces temperature and pH in water, which in terms restricts the volatilization of ammonia, thereby reducing its loses. This study clearly showed the combination of urea and duckweed increased rice biomass, rice grain yield and rice N uptake in all treatment rates with the highest rate of 180 kg N/ha having 47.9 mg/ha, 8.6 mg/ha and 146.1 kg/ha respectively.

In another study from China duckweed (*Spirodela polyrhiza*) was used as green manure in the growth of rice and was applied in coexistence with urea it was then determined that there was no need to reapply N fertilizer due to the duckweeds ability to recover nutrients. The use of duckweed and urea resulted in decreasing loses of ammonia by a rate 36 to 52% resulting in increased rice yields by 9 to 10% (Yao et al., 2017). Ammonia losses were reduced through duckweed ability recover ammonium and also shield the water surface. The potential of duckweed from different sources as organic fertilizer may need to be evaluated against commonly used organic fertilizers.

A study conducted by Chikuvire (2018), on the other hand, in South Africa of *W. arrhiza* and *L. minor* were used as soil amendments for the growth of Swiss chard. This showed that pre-incubating of duckweeds (*W. arrhiza* and *L. minor*) for a 28-day period before planting Swiss chard resulted in identical yield between duckweed and positive control of urea treatments when applied as soil amendments. On the other hand, pre-incubating *W. arrhiza* for 28 days resulted in higher yields of Swiss chard in comparison to all the other treatments in the study. Nitrogen uptake was shown to be high in Swiss chard amended with *W. arrhiza* (177 mg/plot), followed by *L. minor* (137 mg/plot) when incubated for 28 days and the least being urea (124 mg/plot). Nitrogen uptake facilitates the intake of other nutrients in the growth of vegetation regardless of rate.

## **2.8 Chicken compost composition**

Most organic wastes have an abundance of essential nutrients like N, P, and K, their use reduces the uses of fertilizers. A study by Maynard (1994), concluded that chicken manure is likely able to supplement for fertilizer in terms of its N content, and is suitable for growth of a wide variety of vegetables. However, environmental issues arise as this nutrients leach and runoff as result of over application (Bolan et al., 2010, Tiquia and Tam, 2002). Poultry litter is highly volatile due to the combination of solid waste and urine, as a result it has an unreliable N content ranging from 2.3 to 6% (Whitmore, 2007). Amending soil with manure adds nutrients, elevates organic matter and physical conditions of the soil. However, this has a down side of adding heavy metals, pathogens and also pollutes the environment. A more suitable process of mitigating the environmental impact of raw manure application, is composting and is regarded as a treatment for organic waste to be more suitable to use as a fertilizer. However during composting ammonium decreases as nitrate increases (Huang et al., 2017). A study by Dikinya and Mufwanzala (2010), showed that the addition of humified chicken manure resulted in

higher N than P, even though it decreased with the increase in rates of application than P (5, 10, 20 and 40%). The availability of nutrients in chicken litter compost and its stability makes it more appropriate as a reference organic material when evaluating N and P release and fertiliser value of duckweed from different effluents.

## **2.9 Conclusion**

Duckweed species vary in their ability to take up nutrients from wastewaters and polluted surface water bodies as well as their tissue N and P composition. These variations are a result of variation in duckweed species, waste water qualities and environmental factors. Due to its high growth and nutrient recovery rates of duckweed biomass, it has been used as animal feed and has potential to be green manure. Commonly occurring duckweed species found in South Africa such as *Wolffia* species and *Lemna minor* are effective in taking up N and P, thereby improving water quality and if harvested, could provide a potential nutrient source for plant growth. There are no documented studies on the nutrient release patterns from *L. minor*, together with its fertiliser value, particularly when harvested from different effluents, which are commonly occurring in the Midlands region of KwaZulu-Natal.

# **CHAPTER 3: CHANGES IN NITROGEN AND PHOSPHORUS CONCENTRATION DURING A DUCKWEED INCUBATION IN SOIL AND ITS FERTILISER VALUE FOR SPINACH GROWTH IN A POT TRIAL STUDY**

## **3.1 Introduction**

Large amounts of solid or liquid organic wastes from anthropogenic activities are high in nutrients, heavy metals and pathogens (Huang et al., 2017), and may result in water pollution, a major challenge facing intensive agricultural systems (Mallin and Cahoon, 2003). Direct land application of the organic waste is widely practiced globally, as it replenishes nutrients (N, P, and K), elevates organic matter and improves physical conditions of the soil. Furthermore, it may cause pollution of the environment through addition of heavy metals and pathogens (Tiquia and Tam, 2002). Poor waste management resulting in excessive nutrient additions to soils and leaching of nutrients from the agricultural systems, this leads to enrichment of water bodies with nutrients.

Over application of fertilizer in commercial crop productions, wastes from intensive animal productions and human waste discharges are documented as causes of water pollution (Huang et al., 2003). For example, application of poultry litter on basis's of N crop requirement, leaves P being excessive to crops, and with potential for leached (Preusch et al., 2002), while excessive additions results in nutrient enriched run-off and subsurface flow into water bodies. Eutrophic water bodies, are highly enriched with nutrients such as N and P, result in the growth of algae and macrophytes (floating water plants) (Dalu and Ndamba, 2003).

The most important macrophytes that grow on nutrient rich waters include species of duckweed from genera including *Spirodela*, *Lemna* and *Wolffia* (Rahman and Hasegawa, 2011). These different species of macrophytes are found in wide variety of nutrient-rich water bodies (Leng,

1999a). They take up the nutrients from water, particularly N and P. The growth of duckweed is induced by the availability of nutrients such as N, P, and K in large amounts in water bodies and, these nutrients are assimilated into the plant to form organic compounds including proteins (Leng, 1999a). A study by Mohedano et al. (2012), revealed that *L. punctata* tissue is able to recover 98% total nitrogen and 98.8% phosphorus within 30 days. As a result of the high composition of nutrients, the biomass of these macrophytes could be used to recover nutrients from wastewaters. The recovery efficiency depends on the duckweed species, and the environmental conditions.

*Wolffia* and *Lemna* species are among the most commonly occurring duckweeds in South Africa and their composition appears to depend on the quality of the water on which they grow (Chikuvire, 2018). Harvesting of duckweed and applying it to the soil could improve water quality and produce an organic fertiliser. Chikuvire et al. (2018a), reported that N uptake and its concentration on *Wolffia arrhiza* tissue depended on the composition of swine lagoon water. There is need to understand the effect of type of polluted water (source) on nutrient composition of tissue of *Lemna* species and their rate of nutrient release during decomposes in soil.

Nutrient composition of plant matter determines the patterns of nutrient release of an organic amendment in the soil. The rate of decomposition and availability of nutrients is influenced by C/N (< 20), C/P (<200) and N/P (>78) ratios present in plant material (Kirkby et al., 2011). Succulent plant materials have low lignin and polyphenol contents, which limit the rate of decomposition, while the low C/N, C/P and N/P facilitates rapid degradation and possibly leaching of nutrients from the material in the first few hours of incorporation (Masunga et al., 2016, Nguyen and Marschner, 2017). These nutrients are in organic form. A pre-incubation period may be required for them to mineralise and be available.

Chikuvire et al. (2018b) reported that N in *W. arrhiza* tissue rapidly mineralised particularly in the first 28 days of incubation. Where mineral nutrients are high at the initial stages of incubation, there is need to understand whether they leach directly from the biomass. The nutrient release of the *L. minor* tissue and its fertiliser value for crops need to be understood if it is to be used as a nutrient source. The objective of this study were to determine the effects of water (i) on nutrient composition of *L. minor* (ii) N and P release patterns of *L. minor* (ii) its fertiliser value under controlled condition.

### **3.2 Methods and Materials**

#### **3.2.1 Duckweed**

The duckweed (*Lemna* species) samples used in this study were collected from Ashburton (*Lemna* AB), Baynesfield (*Lemna* BF) and Wartburg (*Lemna* WB) in the Midland region of KwaZulu-Natal. Table 3.1 shows the sites and the water bodies on which the duckweed grew. A 1mm sieve was used to collect the duckweed from the water surface bodies, large scale harvesting may present challenges, and harvesting strategies need to be developed if the practice is to have practical applications. The duckweed samples used in this study were transported to the laboratory while fresh with large quantities of water and dried in an oven. However, solar drying approaches closer to the harvesting site could be more practical on a large scale. Duckweed samples were rinsed with distilled water, and any extraneous materials were physically removed. The duckweed samples were then dried at 60°C for 48 - 72 h. The dry duckweed samples were grinded before analysing for micro and macronutrients.



**Table 3.1:** Sampling for duckweed and their respective source on nutrients

Site	Coordinates	Source of nutrients
Ashburton (Lemna AB)	29.403398°S 30.274579°E	Stream receiving sewage effluent and cattle manure.
Baynesfield (Lemna BF)	29.454998°S 30.201506°E	Pond receiving pig effluent
Wartburg (Lemna WB)	29.281933°S 30.285996°E	Pond on a crocodile farm.

The chemical composition of water from the different sites showed that pH of the treatments was not significantly different to each other. Lemna BF showed a significant difference in ammonium-N, P and K to the other treatment while nitrate-N, N<sub>min</sub>, Ca and Mg were similar for both treatments (Lemna BF and AB). In the case of Lemna WB it had the least of all determined parameters (Table 3.2).

**Table 3.2:** Chemical composition of water from sites where *Lemna* species were found as reported by (Chikuvire, 2018).

Site	pH	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	N <sub>min</sub>	P	K	Ca	Mg
		mg/L						
Lemna AB	8.2	0.004a	0.50b	0.51b	0.05a	10.6a	31.6b	22.2b
Lemna BF	7.8	0.48c	0.80b	1.23b	5.39b	662b	35.3b	31.7b
Lemna WB	7.7	0.17b	0.03a	0.19a	0.10a	9.11a	12.1a	8.59a

The different letters down the columns denote a significant difference between the means at  $p < 0.05$ , while the same letter show no significant difference between treatments. Those means without a letter show that there is no significant difference between the treatments at  $p < 0.05$ . N<sub>min</sub> is the sum of ammonium and nitrate-N

### 3.2.2 Soil

The loam soil collected from the 0-20 cm depth at Ukulinga (Glenrosa soil form), the research farm of the University of KwaZulu-Natal, had pH (KCl) 5.9, 2.1% organic C, 0.24% total N, 13.7 mg P/kg and 22.0% clay. The performance of duckweed as a source of nutrients in a variety of soils including young, moderately weathered and highly weathered ones needs to be determined to understand soil type effects.

### 3.2.3 Mineralisation of nitrogen and phosphorus from duckweed incubated in soil

#### Experimental procedure

The incubation study was conducted in a constant temperature room at 25°C for a 28-day period. A completely randomised design was used with three replicates of the amendments. Plastic containers (48) of about 500 ml volume were used, with holes drilled on the edges to allow respiration. Duckweed biomass (Lemna AB, Lemna BF and Lemna WT) was added at rate of 0, 2 and 4% (w/w) ratio on 100g soil container mixed properly. An untreated soil was added as a control. Moisture was kept at field capacity (determined through a pressure plate) throughout the experiment adjusted for after every 3-4 days. Destructive sampling was done after 0, 7, 14 and 28.

#### Analyses

Soil moisture was determined by oven drying (10g) an aliquot of each sample and used for moisture correction in the calculation of ammonium-N and nitrate-N, which were extracted by shaking 2.0 g of soil in 20 ml of 2M KCl (2:20 ratio) in a centrifuge tube at 200 rpm for 30 minutes on a rotary shaker. The suspension was filtered through Whatman no. 1 filter paper (Okalebo et al., 2002) and analysed using the Gallery discrete analyser. Orthophosphate P was extracted using Ambic-2 method as described by the Non-Affiliated Soil Analysis Work

Committee (1990). The soil (2.5 g) was weighed into a centrifuge tube and 25ml of Ambic-2 ( $0.25 \text{ mol dm}^{-3} \text{ NH}_4\text{CO}_3 + 0.01 \text{ mol dm}^{-3} \text{ Na}_2\text{EDTA} + 0.01 \text{ mol dm}^{-3} \text{ NH}_4\text{F} + \text{Superflock}$ ) added. The suspension was shaken for 30 minutes and filtered with Whatman no. 1 filter paper and analysed with the Gallery discrete auto-analyser.

### 3.2.4 Leaching of nitrogen and phosphorus from duckweed samples

The incubation study showed relatively high initial mineral N and P on the first day of duckweed incorporation into the soil. A study was conducted to determine possible leaching of N and P from dried duckweed biomass when added to soil and moistened. An acid washed sand, with low N and P and minimal nutrient retention, was used in the leaching study (Wang et al., 2014). The treatments were replicated three times in a complete randomized design and the fourth duckweed was *Wolffia* which was excluded since the study is focusing more on *Lemna* species. Dried biomass (2.0 g) of *Lemna* AB, *Lemna* BF and *Lemna* WB was mixed with 100g of ground (<500  $\mu\text{m}$ ) acid washed sand and leached with 25ml of deionized water after 0, 6, 12 and 24 hours. The leachates were filtered through Whatman no. 1 filter paper (Okalebo et al., 2002) and analysed for ammonium and nitrate-N and extractable-P. Mineral-N was determined using UV/VIS spectrophotometer as reported by (Okalebo et al., 2002). Where a mixture of two reagents was used to determine ammonium-N with the first consisting of sodium salicylate, sodium citrate, sodium tartrate and nitroprusside and the second consisted of sodium hydroxide and sodium hypochloride. While nitrate-N was determined using a mixture of reagents which consisted of sodium hydroxide, salicylic acid, sulphuric acid, and potassium nitrate. The absorbance values were read at wavelengths of 655 nm for ammonium-N and 410 nm for nitrate-N on a Thermo Scientific UV-Vis GENESY 20 spectrophotometer after colour development (Cataldo et al., 1975). For analysis of leachate P, a 2 ml aliquot of the extract was mixed with 8ml of deionized water and 10 ml diluted ascorbic colour reagent

was added. After 40 minutes the absorbance was read from a spectrophotometer set at 670nm (Hunter, 1974).

### 3.2.5 Pot trial

The soil and duckweed biomass used in the incubation study were also used in the pot trial. Two kg soil was placed in pots and amended with Lemna AB (2.24 g/pot), Lemna BF (2.08 g/pot) and Lemna WB (2.96 g/pot) and the rates were equivalent to 100 kg N/ ha. A soil without duckweed was included as a negative control. The treatments were replicated three times in a RCBD. Phosphorus and K in the treatments were corrected to levels equivalent to 74.4 kg P/ha and 188 kg K/ha (recommended rates for spinach) using  $\text{NaH}_2\text{PO}_4$  and potassium chloride (KCl), respectively. Before planting 21 pots out of 42 were pre-incubation for 14 days where the treatments were mixed into the soil without planting and sufficient moisture provided. Spinach seedlings were grown in each pot for six weeks, with no moisture limitations in the glasshouse at a temperature range of 25 to 27°C (Chikuvire, 2018).

At harvest, shoots were cut approximately 1 cm above the soil surface and the soil emptied from the pots to separate the roots. The leaf area index (LAI) was calculated using the measured length and width of six leaves from each pot. The leaf width was measured from one end of the leaf to the other end on the middle part of the leaf and was divided by sum of plants measured for each pot as reported by Msibi et al. (2014). The shoots were washed with tap water, to remove soil particles, and bloated with a paper towel to remove excess water, before they were weighed for fresh weight and then oven dried at  $\pm 70^\circ\text{C}$  for 72 h. The oven-dried samples were weighed for dry matter and ground using a mortar and pestle to pass through a 1 mm sieve.

### Analyses

The plant dried tissue was analysed for total C and N, using the Leco TruMac CNS/NS Carbon/Nitrogen/Sulfur Determinator (LECO Corporation, 2012), based on dry combustion of samples (0.2 g) in crucibles at a furnace temperature of 1450°C for about 6 minutes. Total P was analysed with the ICP-OES after microwave digestion. Plant samples were digested in aqua regia solution (mixture of nitric acid and hydrochloric acid in a ratio of 1:3). A 5 ml of the clear supernatant solution was pipetted into a 50 ml volumetric flask where 20 ml of distilled water was added as well as 10 ml of the ascorbic acid colour reagent. After 1 hour of blue colour development the samples were analysed at a wavelength of 880 nm (Okalebo et al., 2002).

Residual soil was analysed for mineral N and P as described in section 3.2.3, while pH and EC were determined as described below. Soil pH and electrical conductivity (EC) for them to be determined, the samples were stirred in 1M KCl at a ratio of 1:2.5 (Soil: KCl) for 5 seconds and allowed to stand for 30 minutes before stirring again and allowing to stand for ten minutes, and PHM 210 standard meter used to determined pH. The same procedure was also followed to determine EC in 1.0 M KCl as describe above for pH analysis, instead a CDM 210 conductivity meter was used to determine EC as described by Rayment and Lyons (2011).

### 3.2.6 Statistics

Analysis of variance (ANOVA) was performed for each week of data collection to determine the effect of treatments and rate, including their interactions, using GenStat Statistical package for Windows, version 18. Mean separation was done using least significant difference (LSD) and Tukey's test 5% level (Payne et al., 2011).

### **3.3 Results**

#### **3.3.1 Tissue composition**

Tissue elemental composition of *L. minor* varied across the different duckweed sources (Table 3.2). Tissue C ranged from 31-38% and was in the order Lemna BF < Lemna AB < Lemna WB (Table 3.3). Tissue N was  $\geq 4.0\%$  for Lemna BF and Lemna AB, and 3.1% for Lemna WB. The C/N ratio ranged from 6.9 to 12 and followed the same trend as tissue C. While the C/P and N/P ratio followed a trend of Lemna AB > Lemna WB > Lemna BF. Lemna BF tissue had 1.7% P while Lemna AB and Lemna WB had < 0.5% (Table 3.3). Lemna BF had higher tissue K (8.79%) than the others, which had < 3%. Samples from Lemna AB and Lemna BF had higher Ca than Lemna WB, while Mg ranged from 0.4-0.6% for duckweed from all sources.

**Table 3. 3:** Composition (%) of macronutrients and ratios in duckweed tissue from different sources

<b>Duckweed</b>	<b>N</b>	<b>C/N</b>	<b>C/P</b>	<b>N/P</b>	<b>C</b>	<b>P</b>	<b>Ca</b>	<b>Mg</b>	<b>K</b>	<b>Na</b>
Lemna AB	4.14b	8.2	85	10.4a	34.0	0.4a	1.6b	0.6b	2.35a	0.8b
Lemna BF	4.47b	6.9	18.2	2.6b	31.0	1.7b	2.4c	0.6b	8.79b	0.5a
Lemna WB	3.14a	12	82	6.8b	37.7	0.46a	1.2a	0.4a	2.97a	0.4a

The different letters down the columns denote a significant difference between the means at  $p < 0.05$ , while the same letter show no significant difference between treatments. Those means without a letter show that there is no significant difference between the treatments at  $p < 0.05$ .

Tissue Zn, Mn and Al were in the order Lemna AB > Lemna BF > Lemna WB, while Lemna BF had higher Cu and lower Fe than the other two (Table 3.4). Tissue Zn ranged from 41 – 122 mg/kg, while tissue Cu ranged 6.1 to 32.2 mg/kg. Tissue Mn ranged from 4000 to 15000 mg/kg, Fe from 1400 to 3000 mg/kg while tissue Al ranged 140 to 1200 mg/kg (Table 3.4).

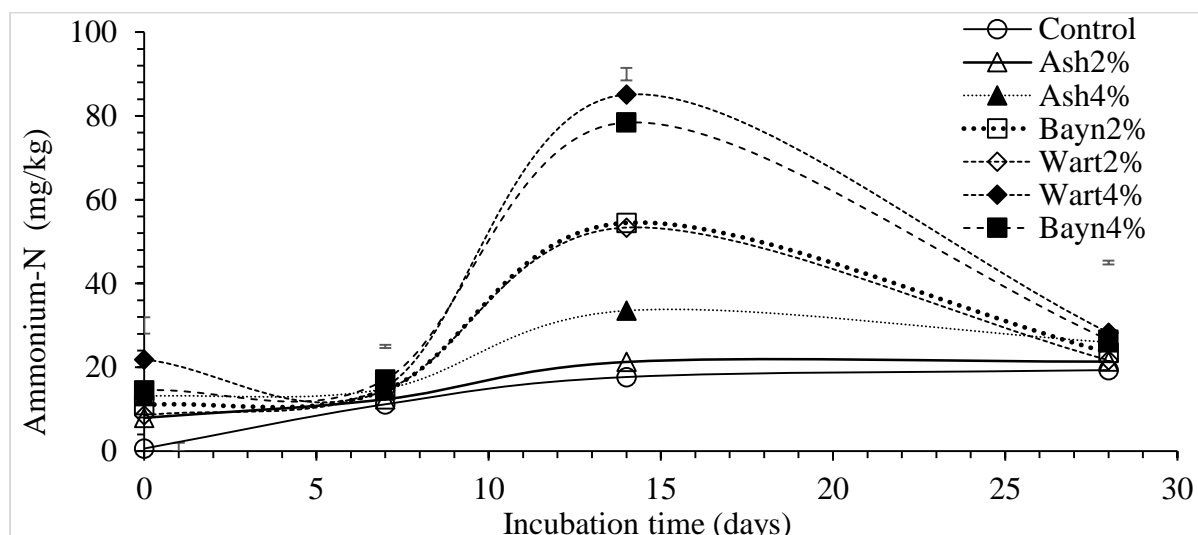
**Table 3. 4:** Micronutrients composition (mg/kg) in tissue of duckweed from different sources

Sites	Zn	Cu	Mn	Fe	Al
Lemna AB	122c	6.1a	15276b	3117b	1207c
Lemna BF	102b	32.6a	8731a	1465a	420b
Lemna WB	41a	9.9a	4153a	2708b	140a

The different letters down the columns denote a significant difference between the means at  $p < 0.05$ , while the same letter show no significant difference between treatments. Those means without a letter show that there is no significant difference between the treatments at  $p < 0.05$ .

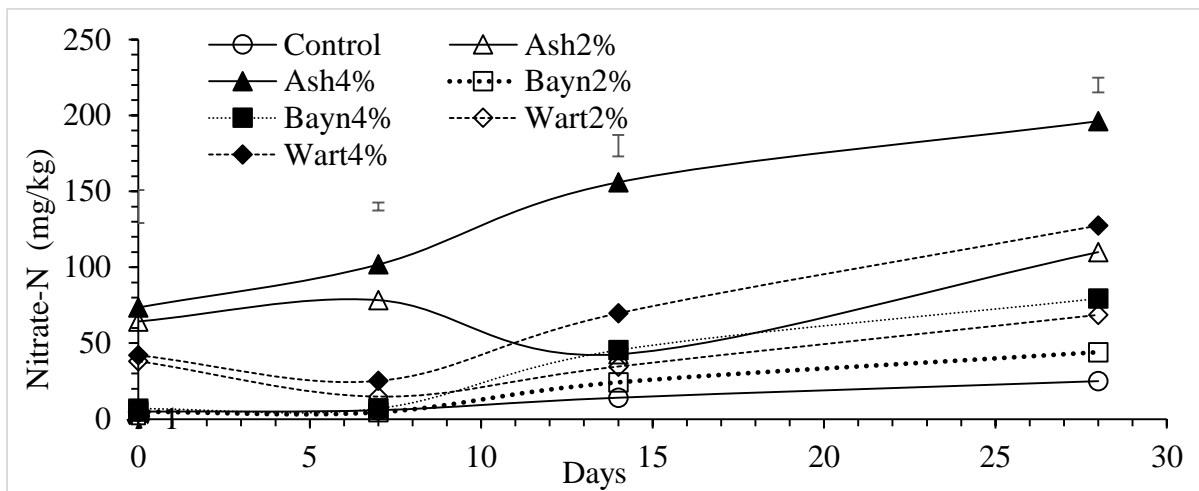
### 3.3.2 Nitrogen and phosphorus release of duckweeds during incubation

The highest ammonium-N was released after 14 days of incubation for all the duckweed species. At each rate Lemna WB and Lemna BF did not differ significantly in ammonium-N released at each incubation period (Figure 3.1). Lemna AB, at 4% rate, released less ammonium-N than the other two duckweeds even at 2% rate. The lowest ammonium-N released was in the control, which was not significantly different from Lemna AB at 2% rate.



**Figure 3. 1:** Ammonium-N concentration during incubation of duckweed species in loam soil. Ash = Lemna from Ashburton (Lemna AB), Bayn = Lemna from Baynesfield (Lemna BF) and Wart = Lemna from Wartburg (Lemna WB). Error bars denote the LSD at  $p < 0.05$

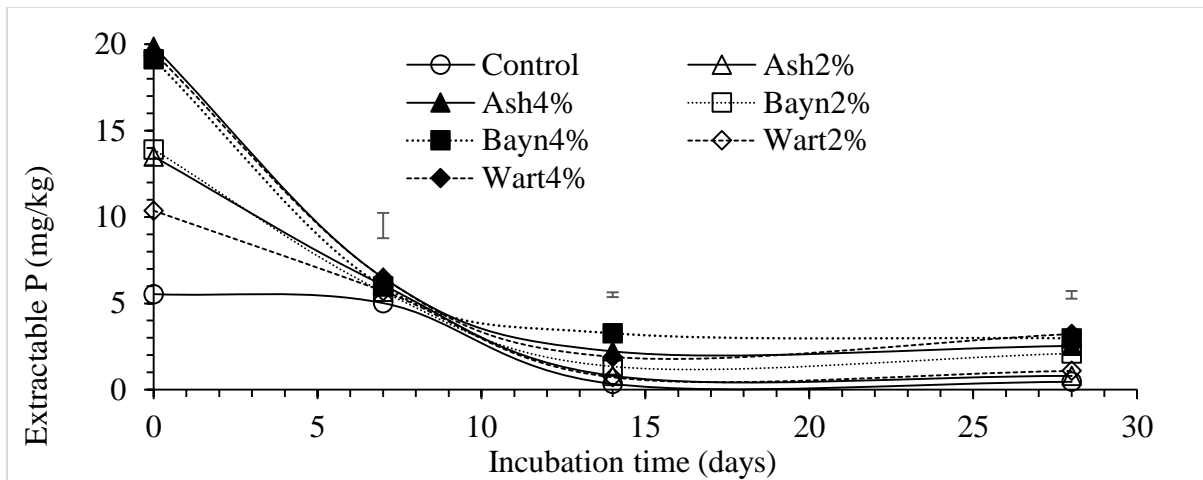
Nitrate-N increased with increase in incubation time particularly between days 14 and 28. The 4% rate was higher than the 2% for all duckweed treatments and the control throughout the experiment (Figure 3.2). At each rate nitrate-N was in the order Lemna AB > Lemna WB > Lemna BF > control. The Lemna AB had higher nitrate-N than Lemna BF throughout the experiment and was higher than Lemna WB up to day 7.



**Figure 3. 2:** Nitrate-N concentration during an incubation of duckweed species in loam soil. Ash = Lemna from Ashburton (Lemna AB), Bayn = Lemna from Baynesfield (Lemna BF) and Wart = Lemna from Wartburg (Lemna WB). Error bars denote the LSD at  $p < 0.05$

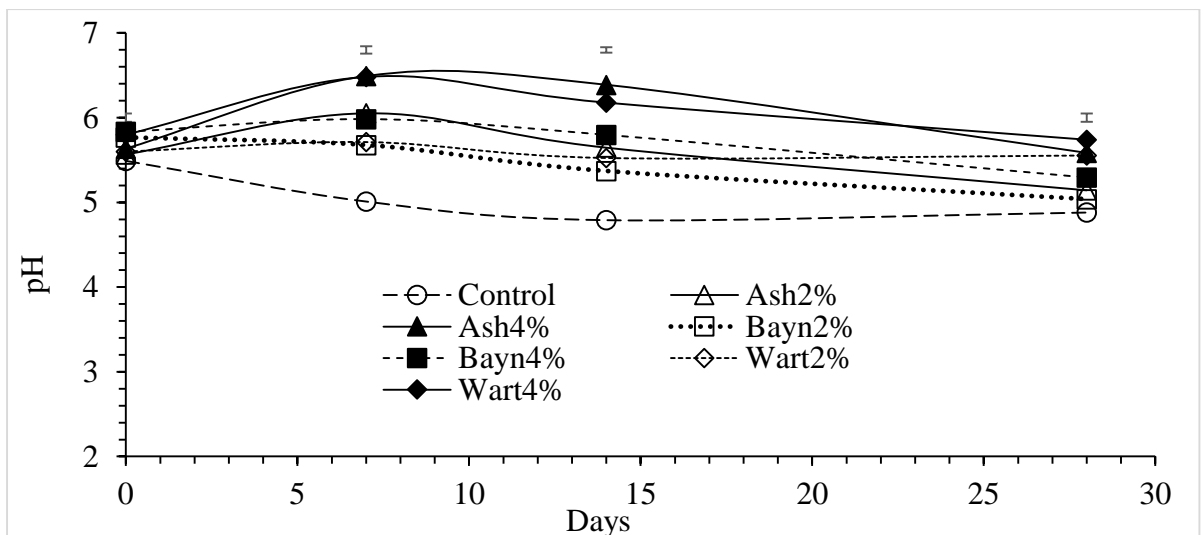
Extractable-P declined with incubation time during the first 14 days. The higher application rate (4%) had higher extractable P than the 2% rate and the control, throughout the experiment except after 7 days (Figure 3.3). Although there were no differences in extractable P among the duckweed treatments at the 4% rate, Lemna BF and Lemna AB had higher concentrations than Lemna WB at 2% rate at the beginning of the incubation. After 14 days Lemna BF had higher extractable P than the other treatments, while on 28<sup>th</sup> day all treatments were similar. The least extractable-P was observed in the control throughout the incubation period.





**Figure 3. 3:** Extractable-P concentration during an incubation experiment in loam soil. Ash = Lemna from Ashburton (Lemna AB), Bayn = Lemna from Baynesfield (Lemna BF) and Wart = Lemna from Wartburg (Lemna WB). Error bars denote the LSD at  $p < 0.05$

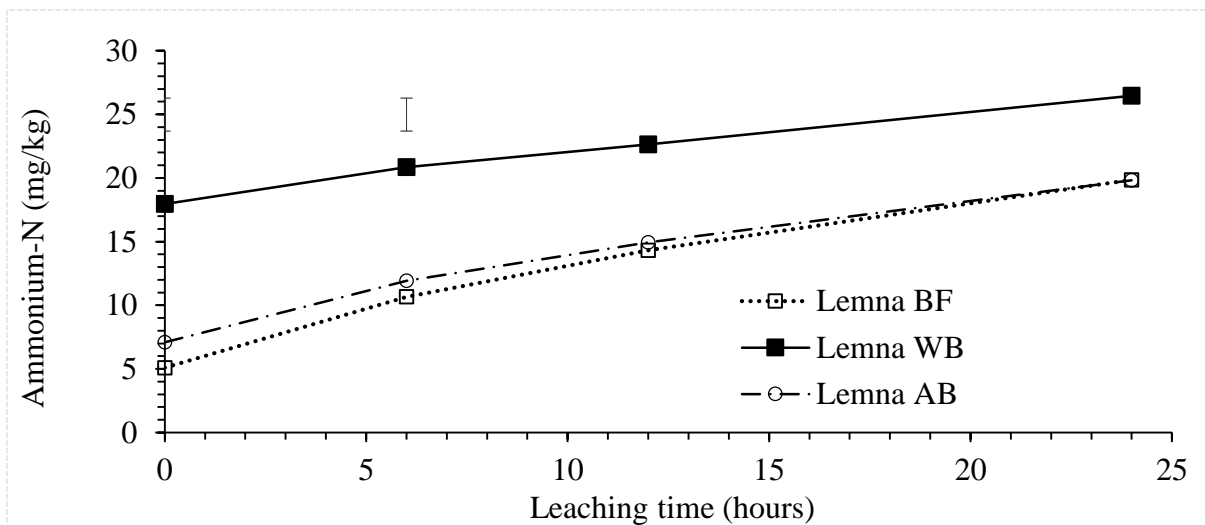
Soil pH increased in the first 7 days and declined thereafter at the 4% rates, with greater pH in Lemna AB and Lemna WB throughout the incubation (Figure 3.4). At the 2% rate, Lemna AB treatment had higher soil pH than the other two throughout the incubation period. The control had the least soil pH throughout the incubation period.



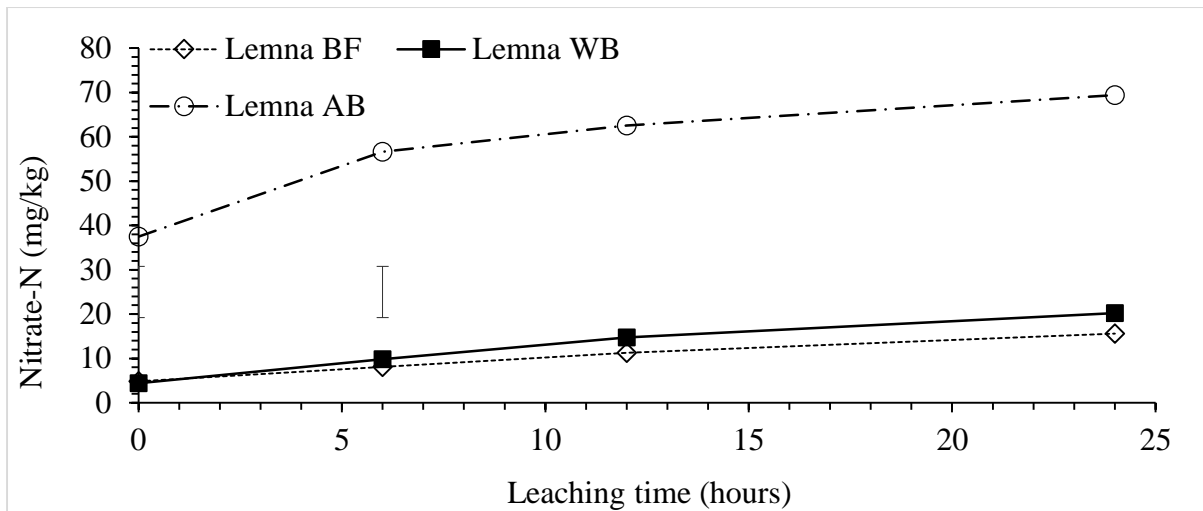
**Figure 3. 4:** Changes in pH of incubated soil throughout the experiment. Ash = Lemna from Ashburton (Lemna AB), Bayn = Lemna from Baynesfield (Lemna BF) and Wart = Lemna from Wartburg (Lemna WB). Error bars denote the LSD at  $p < 0.05$

### 3.3.3 Leaching results

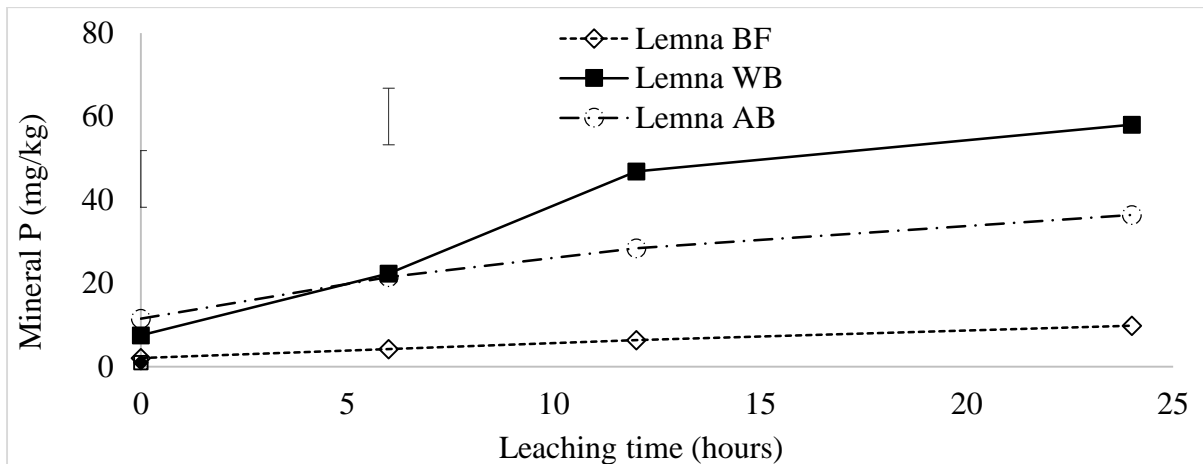
Ammonium-N in the leachate increased throughout the 24-hour period, with higher concentrations being leached from Lemna WB than the other two throughout the 24-hour period (Figure 3.5). Lemna AB had higher nitrate-N than the other duckweed treatments throughout the 24-hour period. Nitrate-N increased between 0 and 6 hours and remained constant thereafter for the Lemna AB treatment while there were no changes in the other two duckweed treatments (Figure 3.6). Leachate P was the same for all treatments at 0 hours, and increased with leaching times for Lemna AB and Lemna WB but not Lemna BF (Figure 3.7). In the leachates collected after 12 and 24 hours, the P concentration was in the order Lemna WB > Lemna AB > Lemna BF.



**Figure 3. 5:** Leachate ammonium-N concentration during leaching duckweed tissue (Lemna AB, Lemna BF and Lemna WB) with de-ionised water. Error bar denote the LSD at  $p < 0.05$



**Figure 3. 6:** Leachate nitrate-N concentration during leaching duckweed tissue (Lemna AB, Lemna BF and Lemna WB) with de-ionised water. Error bar denote the LSD at  $p < 0.05$



**Figure 3. 7:** Leachate P during leaching duckweed tissue (Lemna AB, Lemna BF and Lemna WB) with de-ionised water. Error bar denote the LSD at  $p < 0.05$

### 3.3.4 The spinach dry matter, concentration, uptake of C, N and P

Duckweed treatments had a significant difference amongst each other except with the control in spinach dry matter. There was no significant difference between treatments except with the control in terms of dry matter yield (Table 3.5). Leaf area index (LAI) of Lemna BF was significantly different from the other treatments, while Lemna AB = Lemna WB  $\geq$  control. The N content significantly differed in the spinach tissue and followed the trend Lemna BF > Lemna AB > Lemna WB > control. Lemna BF and Lemna WB had significantly higher P uptake than

the control. There were no significant differences observed as a result of pre-incubation for spinach DM, LAI, C, P content and uptake. Tissue N content in spinach was significantly increased by pre-incubating the amendment treatments, in comparison to the non-incubated treatments (Table 3.5).

**Table 3. 5:** Effect of *L. minor* source and pre-incubation on dry matter (DM), Leaf area index (LAI), P uptake, C, N and P content of spinach.

<b>Factor</b>	<b>DM</b>	<b>LAI</b>	<b>C</b>	<b>N</b>	<b>P</b>	<b>P uptake</b>
<b>Duckweed</b>	(g/pot)	(cm <sup>2</sup> pot)		(%)	(mg/kg)	(mg/pot)
Control (-)	0.492a	270a	35.5	1.46a	0.49	2.25a
Lemna AB	10.9b	598bc	35.7	1.93c	0.43	47.0ab
Lemna BF	11.1b	713c	35.3	2.23d	0.70	77.9b
Lemna WB	10.5b	437ab	36.3	1.68b	0.57	58.9b
<b>Incubation</b>						
Pre-incubated	8.41	513	36.0	1.95b	0.65	0.06
Non-incubated	8.13	495	35.5	1.70a	0.40	0.04

The different letters down the columns denote a significant difference between the means at  $p < 0.05$ , while the same letter show no significant difference between treatments. Those means without a letter show that there is no significant difference between the treatments at  $p < 0.05$ .

There were significant interaction effects of incubation type and duckweed type on N uptake by the spinach dry matter. Uptake of N by spinach was higher in pre-incubated duckweed treatments than non-incubated ones, while there was no effect of pre-incubation on the control. The N uptake was in the order Lemna BF > Lemna AB > Lemna WB > control, the same trend as tissue N (Table 3.6).

**Table 3. 6:** Nitrogen uptake of spinach dry matter amended with pre-incubated and non-incubated *Lemna* species.

Duckweed type	N uptake (mg/pot)	
	Pre-incubation	Non-incubation
Control (-)	7.80a	8.70a
Lemna AB	226c	195c
Lemna BF	276d	222d
Lemna WB	172b	135b

The different letters down the columns denote a significant difference between the means at  $p < 0.05$ .

### 3.3.5 Residual soil nutrient concentrations

In the residual soil after spinach harvesting, there were significant differences between pre-incubated treatments and those that were not incubated for total C, N, extractable-P and EC but not for pH. The Lemna BF treated soil was high in amounts of N, P and EC than the other treatments in pre and non-incubation treatments. However, the Lemna WB treated soil had higher total C than all other treatments. The availability of nutrients in the residual soil followed the following patterns Lemna BF > Lemna AB > Lemna WB > controls (Table 3.7).

**Table 3. 7:** Characteristics of residual soil after spinach harvesting

Treatments	Pre-incubation	Total N	Total C	Extractable P	pH	EC
		(%)	(%)	(mg/kg)		(dS/cm)
Lemna AB	Pre-Incubated	0.85d	2.43e	8.38bc	4.89	0.82d
	Non-Incubated	0.29b	1.83d	7.50b	5.38	0.44c
Lemna BF	Pre-Incubated	0.95d	1.13ab	13.5g	4.93	0.96e
	Non-Incubated	0.53c	1.0a	14.7g	5.67	0.71d
Lemna WB	Pre-Incubated	0.62c	2.85f	9.15cd	4.87	0.77d
	Non-Incubated	0.17ab	2.32e	8.35bc	5.49	0.33bc
Control (-)	Pre-Incubated	0.50c	1.52c	0.17a	4.80	0.28ab
	Non-Incubated	0.13a	1.19b	0.12a	5.25	0.19a

The different letters down the columns denote a significant difference between the means at  $p < 0.05$ , while the same letter show no significant difference between treatments. Those means without a letter show that there is no significant difference between the treatments at  $p < 0.05$ .

### **3.6 Discussion**

#### *Incubation and leaching discussion*

The tissue N, P, K, Ca could be explained by the composition of water from which the duckweeds were collected. The higher water concentration of these elements results in greater uptake of the element elevating the concentrations particularly for the Lemna BF which grew on pig effluent. As such lower concentration of the elements in the crocodile effluent, resulted in lower uptake and concentration and greater growth of fibrous roots increasing total C. A

number of studies have reported the chemical composition found in the duckweed tissue is a result of the waste water from which it grows (Landesman et al., 2002, Cheng and Stomp, 2009). Although concentrations of micronutrients were not part of the measured parameters of the study, they may have been released during decomposition of duckweed (Table 3.2). The availability of ammonium and nitrate-N and extractable P at initial stages of the experiment prove the ability of duckweed to accumulate these elements in mineral form (Figure 3.1 to 3.3). This view is supported by the results of leaching. The higher nitrate-N from Lemna AB than the others corresponds with the higher nitrate-N leached for that duckweed tissue (Figure 3.6), indicating that this duckweed had its N mainly in nitrate form. Further mineralisation and nitrification resulted in increase in nitrate-N. This was in agreement with the studies of Dossa et al. (2009) and Cookson et al. (2002) that observed nitrate-N as the most prevalent form of mineral N in an incubation study of organic residues.

The greater P after 14 to 28 days of incubation could also be explained by tissue P which was higher in Lemna BF. Also a study by Lupwayi et al. (2007), determined that the tissue composition of organic residue affects the amount of P released in the soil. Although Lemna AB and Lemna WB leached higher P than Lemna BF in 24hours, extractable P in the Lemna BF was higher than Lemna WB in the incubation study. The  $\text{pH} > 6.5$  suggests that higher Ca could precipitate with P in Lemna AB and WB. A study by Wang et al. (2011), reported similar findings with addition of organic residues in soil, a  $\text{pH} > 6.5$  resulted in precipitation of P with Ca. Generally, organic material mineralization in the soil is influenced by the soil properties and the biochemical composition of plant matter such as the types of soils, depth of soil, temperature, soil moisture, pH, C/N ratio and lignin content respectively (Roy and Kashem, 2014a).

The difference in C/N ratio plays an important role in the decomposition of organic matter added in the soil, Abbasi et al. (2015), reported that an incorporation of organic matter with high C/N (>20) causes a net N immobilization, whilst lower C/N (<20) results in a net N mineralization in soil, which reveals that the chemical difference in chemical components has an influence on the mineralization- immobilization relationship. According to Johnson et al. (2005), the high C/N stimulates biological activity which results in a huge demand for nitrogen, causing immobilization temporally as the microbes die organic N is released in their bodies which is then nitrified to plant available form which is nitrate-N and becomes available to the soil. Treatments used in the experiment showed difference in C/N ratio with Lemna WB having a higher C/N of 12 as compared Lemna BF with 6.9, and Lemna AB with 8.2 (Table 3.3). Therefore, the difference in N mineralization is attributed to the difference in C/N ratio in this incubation study amongst other factors.

According to Murugan and Swarnam (2013), a difference in chemical composition of treatments results in the difference in nitrogen release and availability. For organic matter to nutrient release mineral N (ammonium and nitrate-N) the soil should be favourable to allow their availability. The composition of organic matter plays a role in N availability. Plant residues as well as legumes and other plant litter are a good comparison to duckweed due to their ability to accumulate large amounts of nitrogen (N) and phosphorus (P). Therefore, a difference in the medium of growth of treatments results in the difference in nitrogen release. For example an incubation study by Nezomba et al. (2009), of indigenous legumes incubated for 155 days, a 50% mineralization of N was observed within 30 day period while the highest peak of mineralisation of N was observed in 55 days. This N mineralization was influenced by the microbial activity due to moisture availability. However, our incubation study, showed a higher mineralization of N within 14-day period (Figure 3.1 and 3.2). Lemna WB had a highest



ammonium-N mineralisation at a rate of 4% on day 14, which was in agreement with the study by Yan et al. (2006).

Immobilization and mineralization occur interchangeable with one another the most prevailing of the two depends upon the residue chemical composition (Dossa et al., 2009). This could be realized, with the decrease in ammonium-N as nitrate-N increased (nitrification) in this incubation study (Figures 3.1 and 3.2). However, there is scarcity of literature on the nutrient release of duckweed on soil incubation, as such its potential nutrient release could be realised when observing the nutrient release of legumes and other organic material. A study conducted by Yan et al. (2006), reported that a decline in soil pH at the later stages of an incubation is a result of nitrification of N mineralization (ammonium-N) from the incorporation of plant matter in the soil.

The rate of application of duckweed also plays a role on the mineralisation of plant matter in the soil, this was observed in treatments Lemna AB and BF at high rate of 4% having a higher ammonium-N release in the first two weeks (Figure 3.1). In the case of nitrate-N treatments Lemna AB and WB had the highest at the rate of 4% at the last two weeks of the experiment (Figure 3.2). The increase in nitrate-N is a result of nitrification of ammonium-N. Breaking down of high amounts of organic-N results in high rates having high mineral-N (Table 3.3). The higher ammonium-N in Lemna WB and BF than Lemna AB, suggested that more mineralisation occurred, while Lemna AB already had high nitrate N, in the tissue with lower proportion of organic N. There were higher nitrate-N in Lemna WB than Lemna BF, even though ammonium-N suggests that the conditions (high pH) in the Lemna WB treatment supported greater nitrification. Therefore, the availability of nitrate-N is a result of high presence of ammonium-N from the duckweed tissue used as an amendment (Chikuvire, 2018).

The quantity of decomposition and availability of nutrients is influenced by C/N (< 20), C/P (<200) and N/P (>78) ratios present in plant material. Early mineralization of nutrient is a result of lower C/N and C/P ratios as influenced by microbial action demand since they possess a low (<20) C/N ratio (Masunga et al., 2016, Nguyen and Marschner, 2017). The C/N and C/P ratios of all three duckweed samples were lower than the thresholds such that they would not limit their degradation. Although Lemna BF had lower C/N than the Lemna WB, there were no differences in ammonium-N. The lower C/P ratio in Lemna BF (18:1) explains the higher P mineralisation than in Lemna WB (82:1) and Lemna AB (85:1).

The extractable-P was highly available at the initial stages of the incubation for all the treatments (Figure 3.3). Which is in agreement with a number of studies which were observed by Kaloi et al. (2011) and Lupwayi et al. (2007), of high P availability and released upon plant material incorporation into the soil and decrease of P availability is observed towards the end of the incubation studies. According to Dossa et al. (2009), plant matter release of mineral P right after being incorporated into the soil is due to higher water soluble P present in the plant matter. As incubation time increases, P became immobilised through P fixation as result of slightly acid soil. Under acidic conditions, there are  $Al_3^+$  and  $Fe_3^+$  ions in soil solutions which precipitate with phosphorus as Al- and Fe-phosphates. This could be attributed to the availability Al and Fe oxides which fix P making it unavailable. In addition, the oxides of Fe and Al have positive charges under acidic conditions resulting in fixation of phosphorus.

The pH declined with the progression of the experiment with higher rates of Lemna AB and WB still had the highest pH than the other treatments. The Control, Lemna BF and WB showed a decline in pH in the initial stages of the experiment and remained constant from day 7 up to the end of the experiment (Figure 3.4) which was in agreement with the study conducted by

Paul et al. (2001). Plant matter used as an amendment source results in an increase in soil pH as influenced by microbial oxidation and net N immobilization, in the case of the decrease in pH is attributed to net N mineralization accompanied by nitrification (Butterly et al., 2013).

### Pot trial discussion

There was higher N content on spinach dry matter in the pre-incubated treatments (Table 3.5 and 3.6). This could be attributed to the mineralisation of organic N in the duckweed tissue with its incorporation to the soil (Figure 3.1 to 3.2). Chikuvire (2018), reported that a 14 day pre-incubation period of *Wolffia* tissue was ineffective for growth of Swiss chard, even though such a pre-incubation resulted in increase in the spinach parameters measured, compared to the control. However, a contrary study by Fosu et al. (2004), reported that two weeks of pre-incubation of devil bean increases cereal yields. While another study by Malepfane and Muchaonyerwa (2017), reported that a 28 day period of pre-incubation of human hair was essential to increase nutrient availability in order to increase crop yields. The higher DM in duckweed treatments than the control could be a result of increase in nutrient availability from the duckweed particularly N. Higher LAI in Lemna AB and BF than the other treatments could be explained by N uptake which encouraged leaf growth. Amending soil with nutrient rich material, increases the soil nutrient status (Rengel, 2007).

The main mineral-N preferred by crops depends on the particular plant species. Some plants prefer ammonium-N form to nitrate-N for growth. However the availability of ammonium and nitrate-N in the soil improves growth of plants (Neal Jr, 2009). Matsumoto et al. (1999), discussed a difference in the growth of spinach from a number of studies were some concluded that N uptake is a result of high availability of nitrate-N which was in agreement with this study (Figure 3.2). Therefore, it is safe to assume that species of plant being grown and the

medium of growth are the main influence behind the nutrient uptake of spinach. Matsumoto et al. (1999), also reported that nutrient uptake of vegetation, such as N uptake was depended on N content of the organic source used as a soil amendment. Uptake of nutrients is highly depended on their availability in the soil. Therefore, uptake of N was higher in treatments with high N content. Generally nutrient release is dependent and influenced by the chemical composition of the plant tissue used as a soil amendment (Yan et al., 2006).

Properties of the organic material determine the patterns of nutrient release, as influenced by the C/N ratio of the organic matter. An adjustment of treatments ensures a balance of C/N and C/P (Nguyen and Marschner, 2017). This could be attributed to the decreased in pH and P availability to some extent. Incorporation of plant matter increases the growth while decreasing inorganic fertiliser application resulting in a reduction of costs to farmer's inputs. On the other hand, C and P uptake showed no signs of change with the use of amendments in spinach growth in this study. Even though a change in N uptake was observed for spinach dry matter amended with Lemna BF. This could be attributed to correcting for P for these treatments to levels equivalent to 74.4 kg P/ha a recommended rate of spinach.

The reason for the greater total N and C in the pre-incubated treatments of the residual soil was not clear. However, the effects of duckweed treatments on the soil P are as shown in the residual soils where extractable-P was in the order of Lemna BF > Lemna WB = Lemna AB > control. This followed the trend of tissue P in the residues added. The similarity in residual soil pH suggests high nitrification was not high enough to lower soil pH. This was similar to a study of Samuel and Ebenezer (2014), where pH did not change much with addition of an organo-mineral fertilizer, as result of insufficient occurrence of nitrification.

### **3.7 Conclusion**

Different source of *L. minor* result in differences in the nitrogen and phosphorus release patterns, particularly when high rates are used. The incorporation of *L. minor* tissue in the soil resulted in a rapid release of ammonium-N within 14 days followed by nitrification in the 14 to 28-day period. Some duckweed tissue (Lemna AB and BF) had high concentration of mineral N and P which leached out upon addition to moist soil. This resulted in the leaching experiment showing a rapid release of ammonium, nitrate and phosphate on the initial stages. The difference in the nutrient content of the duckweeds as well as the internal factors of duckweed (low lignin and polyphenol contents) played a role in difference of the decomposition rates. Pre-incubation of *L. minor* tissue in soil resulted in an increased N concentration, especially for duckweed with the highest N and P content. These effects need to be tested under field conditions relative to commonly used organic nutrient sources.

**CHAPTER 4: FERTILISER VALUE OF DUCKWEED AND CHICKEN LITTER  
COMPOST IN TERMS OF THEIR RATE OF NUTRIENT RELEASE OF  
NITROGEN FOR SPINACH GROWTH: A FIELD TRIAL**

**4.1 Introduction**

Intensive agriculture may have a harmful effect on the environment as a result of high nutrient release of waste which leaches to water bodies resulting high accumulation of organic waste. Use of these organic wastes in agriculture could recover and recycle these nutrients not only from agricultural based waste but also from anthropogenic activities. Therefore, direct application of these organic wastes on agricultural lands could be more feasible. However excessive application results in eutrophication due to the presence of high amounts of N and P which accumulate in aquatic environment through leaching and runoff (Preusch et al., 2002). On the other hand they can be composted, since it is regarded as a process that stabilize nutrients found in manure, including chicken litter resulting in the reduction of their environmental impact (Tiquia and Tam, 2002). During composting ammonium decreases as nitrate increases (Tiquia and Tam, 2002, Huang et al., 2017).

Preusch et al. (2002), observed that mineral N in fresh chicken litter ranged between 42 to 64% in comparison with composted chicken litter which had 1 to 9%. The handling of fresh chicken litter has an influence on mineral N, while composted litter is not affected. This is because composting provides a more dependable source of mineral N in comparison to the fresh poultry litter. The quantities of organic wastes may be too large to be feasible handled by composting so they are applied to the soil as fertiliser for nutrient recycling. The ability of aquatic plants to recover high amounts of N and P from effluent rich waterbodies, also mitigates the environmental impact of leached nutrients in water bodies. More particularly duckweed growth

as induced by the enrichment of water bodies by nutrients (Dalu and Ndamba, 2003). Therefore, the growth of duckweed results in its amount of N and P being determined by the amount of nutrients present on wastewater from which it is grown (Hanczakowski et al., 1995).

The results of the incubation studies and pot trial indicated that the N and P release, and the fertiliser value of *L. minor* from different sources is dependent on the nutrient composition of the water sources. It is essential to establish how the nutrient release and fertiliser value of this duckweed compares with a commonly used organic fertiliser especially under field conditions. A field study was conducted to determine the potential of duckweed, from two water sources, and chicken litter compost used as a source of N and P for growth of spinach. The aim in this chapter is to compare the N and P release and N fertiliser value of duckweed (*L. minor*) relative to chicken litter compost. The specific objectives were to:

- i. Determine the nutrient release of duckweed relative to chicken litter compost under uncontrolled conditions.
- ii. Determine N fertiliser value of duckweed to spinach when compared with chicken litter compost under field conditions.

## **4.2 Method and material**

### **4.2.1 Nitrogen and phosphorus release from duckweed and compost during incubation**

A second incubation had to be done to compare the chicken litter compost used in the field with duckweed in terms of their nutrient release patterns. The soil used in chapter 3 was also used in this incubation study. The incubation experiment was conducted with Lemna BF, Lemna WB and chicken litter compost, and the characteristics of the soil and Lemna BF and Lemna WB are given in Chapter 3. The chicken litter compost used in this study was produced

with the Biomax Rapid Thermophilic Digestion Technology from RGS Drumnadrochit farm in the midland region of Kwa-Zulu Natal (Mawonga, 2016). A mixture of chicken litter, eggshells, feed mill, wood chips, paper and grass were digested with body mass index (BMI) enzyme at 70 to 80°C for 24 hours. The treatments had different N contents of 447 (Lemna BF), 314 (Lemna WB) and 300 (compost) mg N/kg. The application rates were added at amounts of 1 g/pot (Lemna BF), 1.38 g/pot (Lemna WB) and 1.49 g/pot (compost) to supply the same amount of N. The rest of the management, sampling and analyses are as detailed in Chapter 3. Composition of duckweed and chicken litter compost used in the incubation and field study varied amongst treatments resulting in a trend of Lemna BF > Lemna WB > Compost for nutrients N, P, C, Ca, Mg, K and Cu, while Lemna AB had higher Fe, Mn and Zn than all other treatments (Table 4.1).

**Table 4. 1:** Chemical composition of duckweed (*L. minor*) tissue and chicken litter compost.

Samples	N	P	C	Ca	Mg	K				
							%			mg/kg
Lemna BF	4.47b	1.70b	31.0	2.40c	0.60b	8.79b	1465b	8731c	33c	102b
Lemna WB	3.14a	0.46a	37.7	1.2b	0.40a	2.97a	2708c	4153b	9.9b	41a
Compost	3.0a	0.95a	30.3	0.78a	0.42a	1.79a	171a	144a	3.7a	120c

The different letters down the columns denote a significant difference between the means at  $p < 0.05$ , while the same letter show no significant difference between treatments.

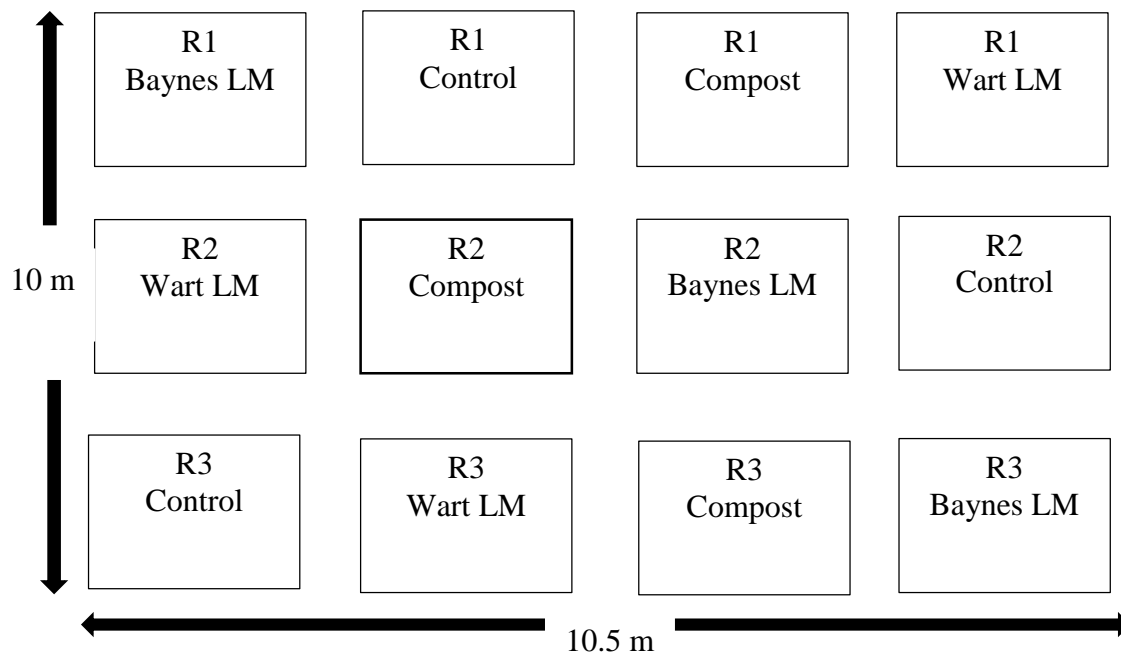
#### 4.2.2 Nitrogen fertiliser value of duckweed relative to chicken litter compost on spinach

The field experiment was conducted at the Ukulinga Research Farm of the University of KwaZulu-Natal. The soil was similar to the one used in the incubation studies and the pot trial (Chapter 3).



The experiment was set up as a randomised complete block design with four treatments which included Compost, Lemna BF and Lemna WB applied at 100 kg N/ha, recommended for spinach, and an un-amended control. The treatments were randomly applied in the trial (Figure 4.1). Phosphorus was adjusted using  $\text{NaH}_2\text{PO}_4$  (74.4 Kg P/ha). These treatments were replicated three times under the three blocks with a blocking factor against the terrain which had three strata; higher, middle and lower (Figure 4.1). A 14-day pre-incubation was done where duckweed and compost were buried in the planting holes before planting and irrigated. In total there were 12 plots with a total of 12 plants per plot. Five-week old spinach seedlings were planted on the 25<sup>th</sup> of October 2017 and harvested on 13<sup>th</sup> of December 2017. Supplemental irrigation was added to sure that water was not limiting.

4.2.3 Experimental layout, harvesting and after harvesting handling



**Figure 4. 1:** Treatment arrangement in the field experiment

The plots had dimensions of 2m x 1.5m, with a plant spacing of 75cm between the 12 plants in each plot. The treatments were added at 28g duckweed/plant (Lemna BF), 40g duckweed/plant

(Lemna WB) and 52g compost/plant (chicken litter compost). Harvesting was done using a scissors, placing each plant harvested in properly labelled paper bags. After harvesting the plants were washed to ensure the removal of soils from the leaves. Wet plant matter after drying with a paper towel was weighed before drying at 65°C for 24 hours then dry weight determined before analysis. The soils in plots after spinach harvest (residual soil) were sampled at a 20cm depth, air-dried and sieved (<2 mm) before analysis.

#### 4.2.4 Analyses

The tissue were analysed for total C and N using the Leco TruMac CNS/NS Carbon/Nitrogen/Sulfur Determinator (LECO Corporation, 2012), based on dry combustion of air dried samples of about (0.2 g) at a furnace temperature of 1450° C for about 6 minutes per sample.

Tissue P was analysed using the spectrophotometer after acid digestion of plant samples (0.5g) in a microwave digester using aqua regia solution (a mixture of nitric acid and hydrochloric acid in a ratio of 1:3). The supernatant clear solution (5ml) was pipetted into a 50 ml volumetric flask, a 20 ml distilled water and 10 ml of the ascorbic acid added to each flask. Phosphorus standards were also included. The solutions were made up to 50 ml with distilled water stoppered, shaken well and let stand for 1 hour for colour development. The absorbance (blue colour) of the standards and samples were measured at 880 nm wavelength (Okalebo et al., 2002).

Residual soil was analysed for total N and C and extractable P, pH and EC. Total N was analysed with the Leco TruMac and extractable P was measured with UV-Vis GENESY 20 spectrophotometer after extraction as described in Section 3.2.3 of Chapter 3. Soil pH and EC were also measured as detailed in Section 3.2.5 of Chapter 3.

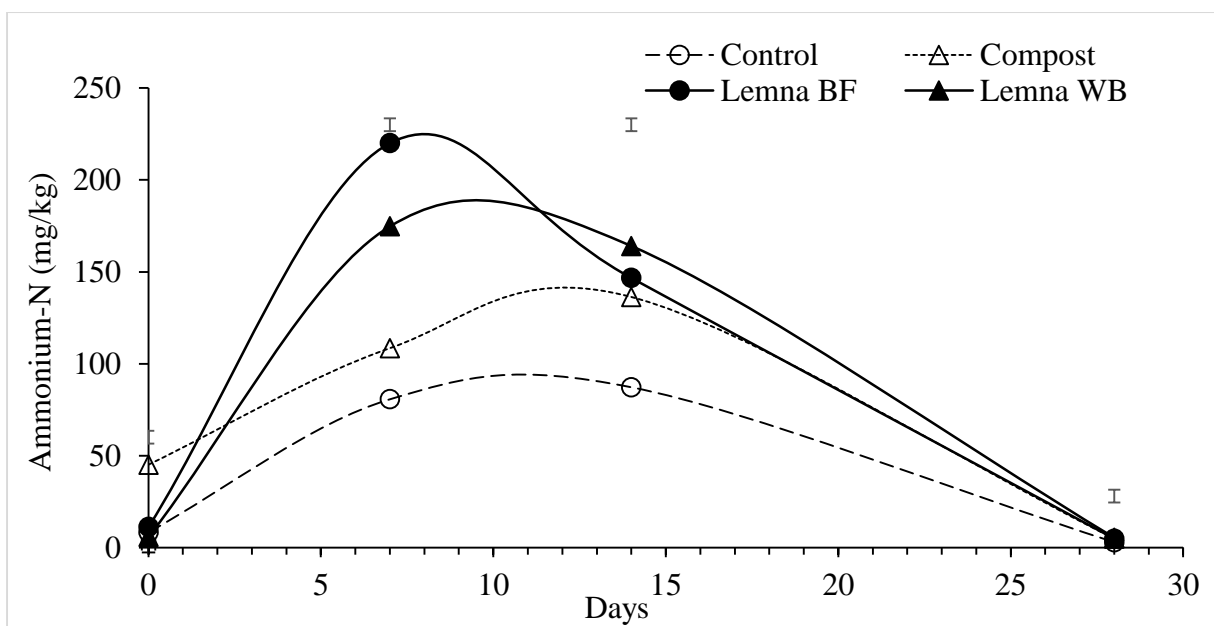
#### 4.2.5 Statistical analysis

Analysis of variance (ANOVA) was performed using GenStat Statistics for Windows, version 18. Mean separation was done using both least significant difference (LSD) and Tukey test at  $P < 0.05$  for all means (Payne et al., 2011). The analysis of variance was carried out for time of field data collection.

### **4.3 Results**

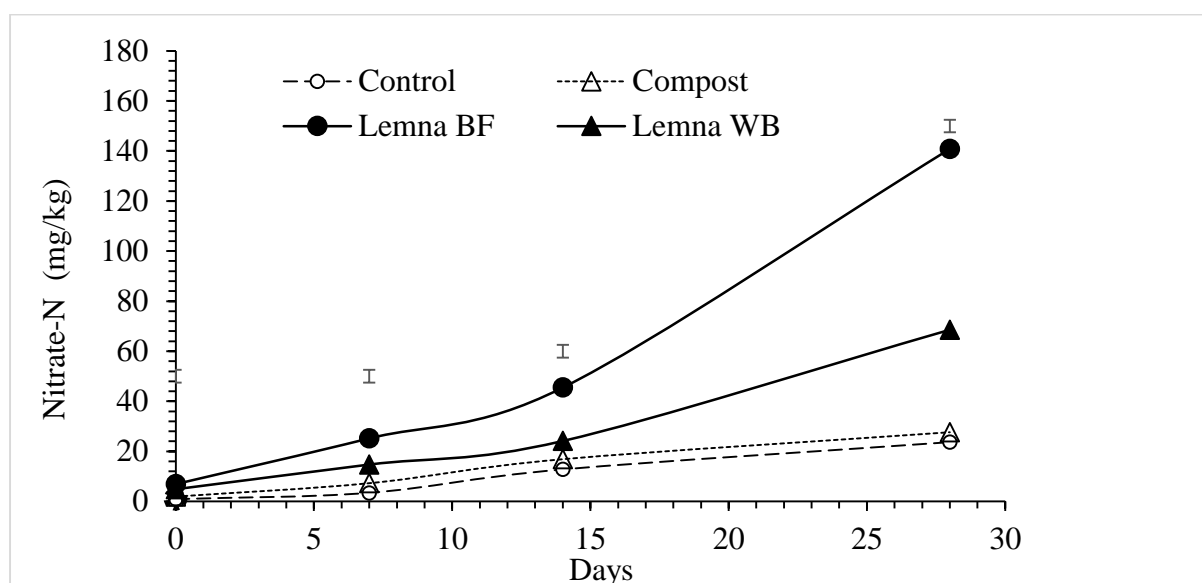
#### 4.3.1 Nitrogen and phosphorus mineralisation from duckweed and compost during incubation

The levels of ammonium-N increased rapidly up to day 7 for all treatments (Figure 4.2). The compost treatment had higher ammonium-N than all other treatments at the beginning of the incubation, while after 7 days the concentrations were in the order Lemna BF > Lemna WB > compost > control. The ammonium-N declined between days 7 and 28 (Lemna BF and WB), while for the compost and the control, it declined between days 14 and 28. There were no differences among the treatments after 28 days of incubation, where the concentration was low.



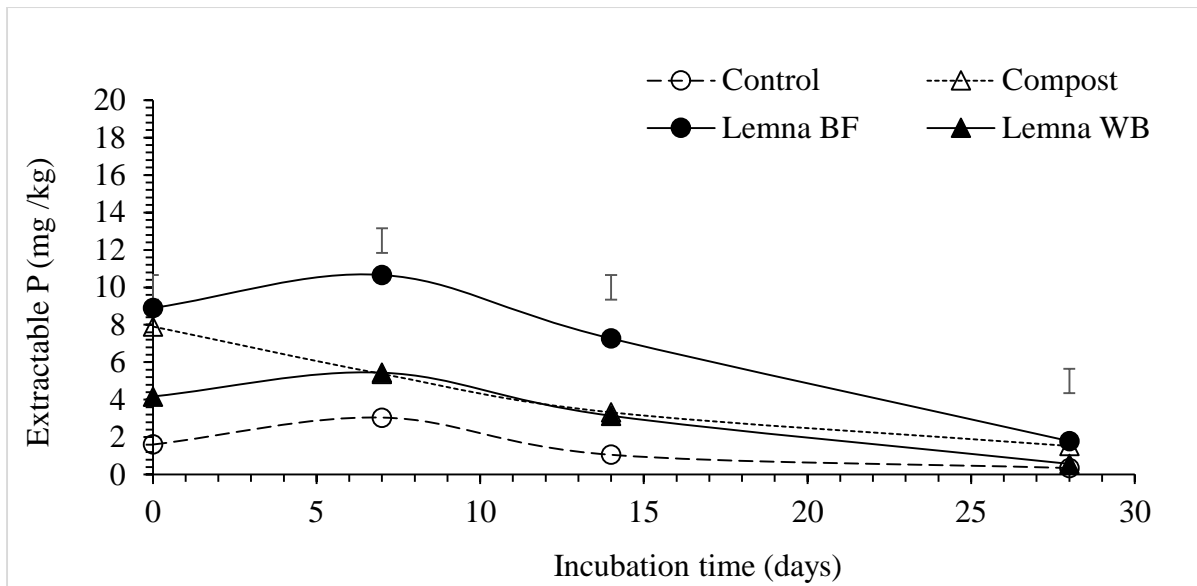
**Figure 4. 2:** Ammonium-N concentration during incubation of duckweed (Lemna BF and Lemna WB) and compost in loam soil. Error bar denote the LSD at  $p < 0.05$

The concentration of nitrate-N increased with increase in incubation time for all the treatments (Figure 4.3). The nitrate-N was in the order Lemna BF > Lemna WB > compost = control (Figure 4.3) throughout the incubation period. The highest nitrate-N in the compost and the control was less than 30 mg/kg, while those of Lemna BF and Lemna WB were 140 and 67 mg/kg after 28 days.

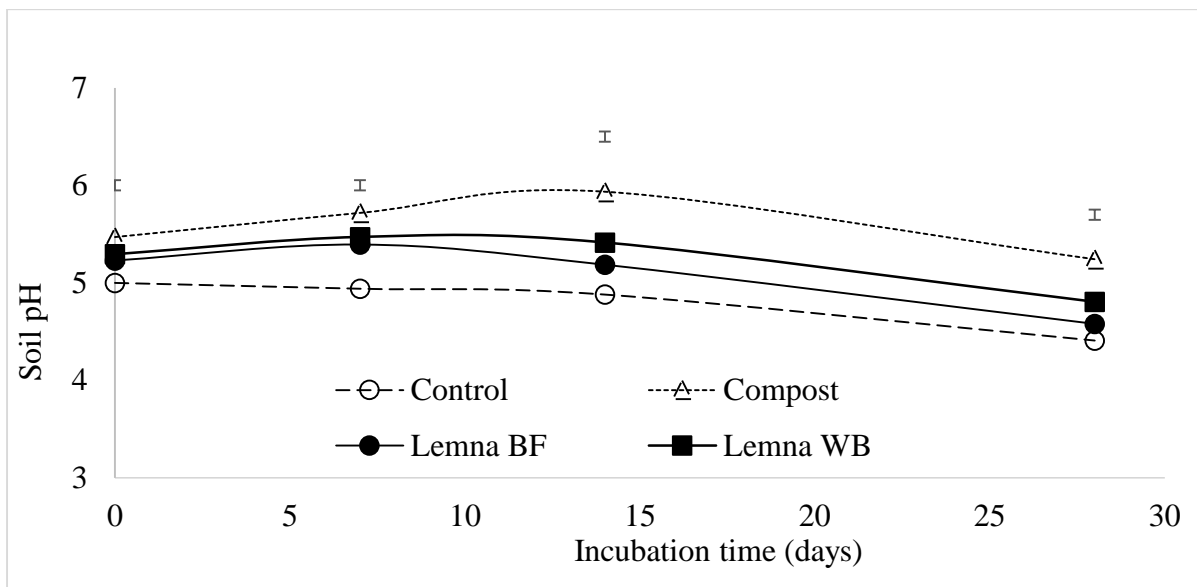


**Figure 4. 3:** Nitrate-N concentration during incubation of duckweed (Lemna BF and Lemna WB) and compost in loam soil. Error bar denote the LSD at  $p < 0.05$

Extractable P was higher in the Lemna BF and compost than the Lemna WB and the control. Extractable P declined with incubation time from day 0, for compost, and from day 7 for the duckweed treatments and the control (Figure 4.4). The concentrations were in the order Lemna BF > Lemna WB = Compost > control after 7 and 14 days of incubation, with no differences among the treatments on 28 days of incubation (Figure 4.4). The compost treatment had higher pH while the control had lower than the other treatments throughout the experiment. The pH generally declined from day 7. Treatment of Lemna WB only had higher pH than the Lemna BF after 14 and 28 days of incubation (Figure 4.5).



**Figure 4. 4:** Extractable P concentration during incubation of duckweed (Lemna BF and WB) and compost in loam soil. Error bar denote the LSD at  $p<0.05$

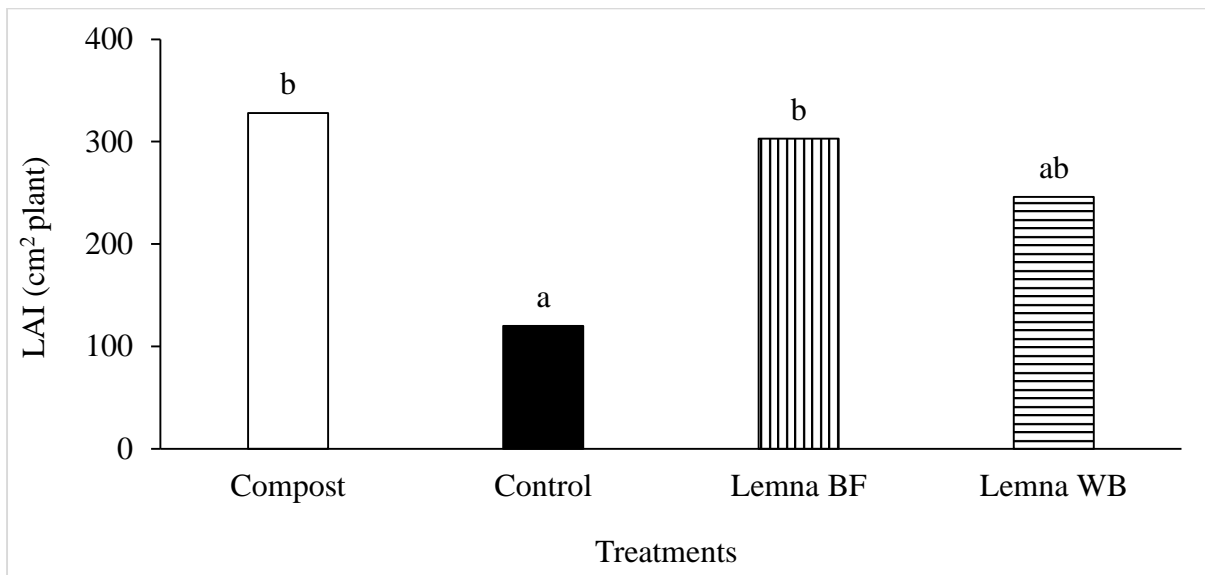


**Figure 4. 5:** Changes in soil pH during incubation of duckweed (Lemna BF and Lemna WB) and compost in loam soil. Error bar denote the LSD at  $p<0.05$

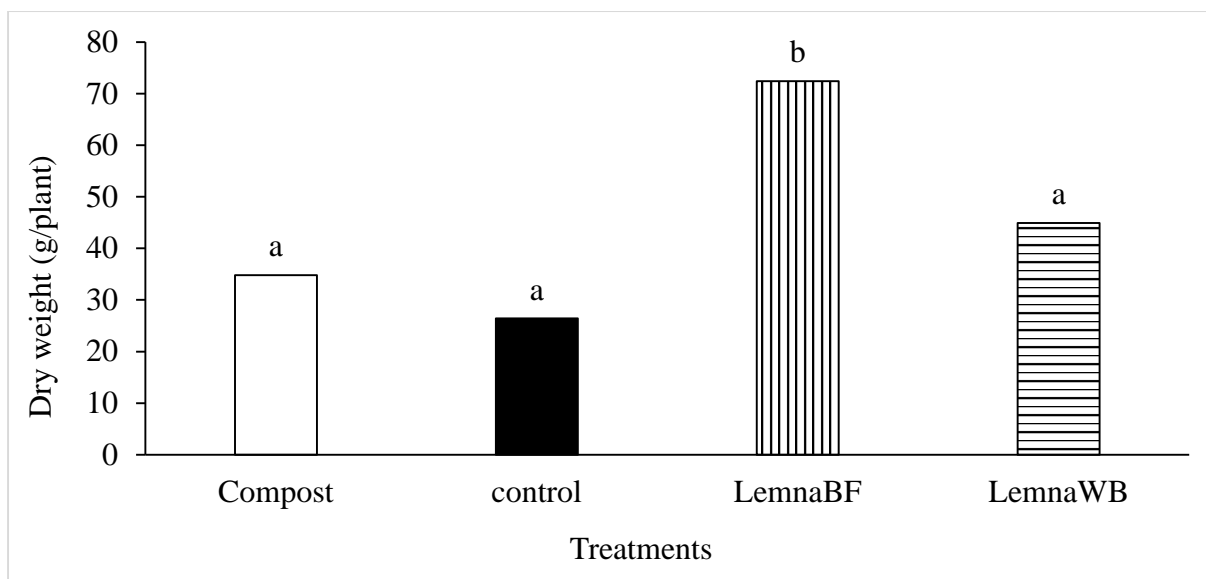
## *Fertiliser value of duckweed and chicken litter compost on spinach*

### 4.3.2 Leaf area index and dry matter of spinach

The control had lower leaf area index (LAI) than the compost and Lemna BF treatments (Figure 4.6). The LAI in Lemna WB treatment was not significantly higher than the control. Only the Lemna BF had higher spinach dry matter than all other treatments (Figure 4.7). The rest of the treatments (control, compost and Lemna WB) were not significantly different to each other (Figure 4.7).



**Figure 4. 6:** Leaf area index (LAI) of spinach fertilised with duckweed and compost in the field. Means with the same letter are not significantly different. Error bar denote the LSD at  $p < 0.05$



**Figure 4. 7:** Spinach dry matter as affected by duckweed and compost treatments. Means with the same letter are not significantly different. Letters denote the LSD at  $p < 0.05$  between treatments

#### 4.3.3 Concentration and uptake of N and P in spinach

Spinach tissue N composition and uptake were higher in the Lemna BF than all other treatments. While there were no significant differences among the treatments in tissue P concentration, P uptake in the Lemna BF was higher than the control and the compost treatment, with no difference in P uptake between the two duckweed treatments (Table 4.2).

**Table 4. 2:** Concentrations of nutrients, uptake of N and P in spinach tissue fertilised with duckweed and compost

Treatments	Tissue N (%)	Tissue P (%)	Uptake	
			N uptake mg/plot	P uptake mg/plot
Control (-)	2.36a	0.37	626a	99a
Lemna BF	3.56b	0.38	2560b	272b
Lemna WB	2.65a	0.38	1226a	171ab
Compost	2.60a	0.37	737a	112a

The different letters down the columns denote a significant difference between the means at  $p < 0.05$ , while the same letter show no significant difference between treatments. Those means without a letter show that there is no significant difference between the treatments at  $p < 0.05$ .

#### 4.3.4 Residual soil C, N, P, pH and EC

There were no significant differences in total C and N among the soils under the different treatments after spinach harvesting. Only the compost treatment had higher extractable P than the control after spinach harvest. The EC values of soils from the duckweed treatments were higher than the control soil. The pH of soil treated with Lemna species and compost were similar (Table 4.3).

**Table 4. 3:** Characteristics of residual field soil after spinach fertilised with duckweed and compost

<b>Treatments</b>	<b>Total N</b> (%)	<b>Extractable P</b> (mg/kg)	<b>Total C</b> (%)	<b>pH</b>	<b>EC</b> (dS/m)
Compost	0.20	9.49b	2.39	4.57ab	0.28ab
Control	0.20	2.24a	2.48	4.43a	0.16a
Lemna BF	0.21	8.07ab	2.49	4.87ab	0.81c
Lemna WB	0.20	6.68ab	2.40	4.69a	0.53b

The different letters down the columns denote a significant difference between the means at  $p < 0.05$ , while the same letter show no significant difference between treatments. Those means without a letter show that there is no significant difference between the treatments at  $p < 0.05$ .

#### **4.4 Discussion**

Rapid increase in ammonium-N within the 7 days was explained by rapid decomposition and mineralisation of N particularly from duckweed treatments. The higher ammonium-N in Lemna BF was due to higher N than in Lemna WB. A study by Masunga et al. (2016), agreed



with this study, where white clover with a high N content was shown to have a greater N mineralisation than the other organic amendments. Higher initial ammonium-N in the compost treatment was because of mineralisation during the composting process. The decline in ammonium-N from 14 to 28 days of incubation was a result of nitrification. This was supported by the results of pH which declined during the same period (Figure 4.5). This is similar to study by Vanzolini et al. (2017), where lower soil pH was influenced by N mineralisation which resulted in nitrification. There was higher nitrate-N in Lemna BF treatment followed by Lemna WB than the compost and the control, this was due to the nitrification of higher ammonium-N in the duckweed treatments. The higher mineral N in the Lemna BF suggests that addition of this duckweed supply more mineral N for crops if applied at appropriate rates, than chicken litter compost. This was similar to a studies by Badr et al. (2016) and Courtney and Mullen (2008), which reported that a high N content in an organic material leads to great supply of N content in soil, which is good for crop growth. As well as a study by Cooperband et al. (2002), of fresh poultry litter as an amendment source with a high N content which resulted in higher yields of maize than composted chicken litter (had lower N) due to their difference in N contents. The decline in extractable P during the incubation period could be a result of immobilisation by microbes and fixation by oxides of Al and Fe.

The higher LAI and dry matter yield in Lemna BF treatment could be explained by uptake of N and P which followed the same trend. Nitrogen and phosphorus are among the nutrients that are required in large quantities by plants and yet they occur in lower amounts in soil. Courtney and Mullen (2008), reported organic material (mushroom compost) applied at required rates (0, 25, 50 and 100 t/ha) elevated the availability of N and P which are essential for increasing barley yields. The mineralisation of N and P in duckweed and compost made the nutrients available for uptake, resulting in increased dry matter. This view is supported by the results of

mineral N which were made available by both treatments (Lemna BF and compost) (Figure 4.3). A pre-incubation period of two weeks was done with irrigation in the field to allow for decomposition and mineralisation of nutrients. However, contrary to that N and P mineralization increases spinach yields, more especially when ammonium-N is more dominantly available than nitrate in the soil in the case of N content (Wang and Li, 2004, Spiegel et al., 2018).

The mineralised N resulted in greater N uptake and growth of spinach which is attributed to high N content in duckweed and compost treatments. Badr et al. (2016), reported that a high supply of N results in higher uptake of N by the plants. High accumulation of N content in spinach tissue proves that a source of amended to be a good supplier of N in the case of Lemna BF (Maftoun et al., 2005). While soil P declined with incubation period, possibly due to immobilisation and mineralisation by microbes and fixation by oxides, considering the relatively low soil pH, uptake of P in the Lemna BF was higher than the control and the compost treatments. A greater P uptake from the Lemna BF treatment could have also contributed to greater dry matter yield of spinach. The Lemna BF had higher total N in its tissue and the treatment had higher extractable P in soil especially in the early stages of mineralisation. The higher P uptake in the Lemna BF explains the lack of significant differences in extractable P in the residual soil compared to the control. According to Maftoun et al. (2005), spinach dry weight is influenced by the P availability with insufficient P availability resulting in lower yields. The lower pH value in the residual soil of the Lemna BF treatment than the control was a result of greater nitrification resulting in acidification of the soil as supported by results of the incubation study. Soil acidification is a result of a presence of  $H^+$  ions influenced by nitrification occurrence (Malepfane and Muchaonyerwa, 2017). The lower pH value could also explain lower residual P, higher electric conductivity (EC) in the residual soil of the two

duckweed treatments could be a result of other nutrients such as basic cations that could have been released during duckweed decomposition in the soil. Higher soil EC is a result of addition of organic residues with high cation content according to Courtney and Mullen (2008), as observed in the duckweed treatments.

#### **4.5 Conclusion**

The source of duckweed affects N and P release and fertiliser value for spinach compared to chicken litter compost. Greater N (ammonium and nitrate-N) were mineralised from duckweed tissue, particularly duckweed grown from piggery effluent, than the chicken litter compost. The Lemna BF also had higher extractable P than the compost, which had similar levels of P than Lemna WB. The *Lemna minor* tissue from piggery effluent dam (Lemna BF) had a greater fertiliser value than the duckweed from crocodile effluent (Lemna WB) and chicken litter compost, particularly after a two-week pre-incubation. Greater mineral N caused a greater N uptake by spinach in the Lemna BF treatment than the Lemna WB and compost. A higher P uptake from Lemna BF and WB treatments resulted in lower extractable P in the residual soil after spinach harvesting.

## CHAPTER 5: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

### **5.1 General discussion**

Eutrophication is a problem globally that does not only affect the domestic and fresh water, but also coastal areas fed by rivers which become rich in nutrients due to pollution (Huang et al., 2003). Aquatic plants, including duckweeds often grow on the polluted waters and take up these nutrients. These aquatic plants decompose in water which results in awful odours, reduced oxygen demands and death of aquatic species. The nutrient uptake and rapid biomass accumulation by these aquatic plants could mitigate water pollution, if harvested, and produce a potential organic fertiliser source. Nutrient composition of duckweed tissue from a variety of effluent sources vary in ranges of 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 (Chikuvire et al., 2018b). The potential of a species of duckweed as an organic fertiliser could depend on the nutrient composition in the tissue, which may be affected by source and the rate of mineralisation of nutrients in the soil. The fertiliser value may therefore be affected by the source of duckweed from which it has grown and needs to be evaluated against commonly used organic fertilisers. The objective of the study was to determine the N and P mineralisation patterns of *L. minor* biomass as affected by source, and when compared to chicken litter compost.

The N results of the study shows that the composition of water affected the nitrogen composition of *Lemna minor*. The duckweed tissue N composition varied from 3.14 to 4.47%, with the highest being Lemna BF followed by Lemna AB. The trend in tissue N followed the same trend as mineral N in the water from which they were collected. Higher mineral N in the pond receiving piggery effluent resulted in greater accumulation of N in the duckweed tissue than from the crocodile effluent. The medium of growth of duckweed affects its nutrient

composition according to Chaiprapat et al. (2005) and Singh and Singh (2006). Lemna WB leached higher ammonium-N than the other two treatments within 24h suggesting that its biomass accumulated ammonium-N in the tissue. This higher uptake also explains the higher ammonium-N in the incubation study, than Lemna AB (Figure 3.1). While leachate ammonium-N were lower in Lemna BF, there were differences observed in the incubation study probably due to high total N. The relative concentration of ammonium-N was high in Lemna BF, while nitrate-N was similar for Lemna AB and BF in the water source where *Lemna* species were found. A high ammonium-N/nitrate-N ratio promoted ammonium-N leaching while a low ratio favoured nitrate-N. On the other hand, Lemna AB leached more nitrate-N than Lemna BF, which was similar to incubation study to the leaching (Figure 3.2), implying a greater nitrate-N tissue accumulation, even though water did not differ in nitrate-N content. The higher accumulated nitrate-N also showed a higher nitrate-N for Lemna AB in the incubation experiment (Figure 3.2). A higher leachate amount of ammonium-N than nitrate-N in the leachate of Lemna BF suggests that this duckweed accumulates more ammonium-N than nitrate-N while the levels in water were similar. A number of studies have reported that duckweed mostly favours mineral N in the form of ammonium-N (Caicedo et al., 2000b, Xu and Shen, 2011b).

The higher ammonium-N in Lemna WB in comparison with Lemna BF, which had higher tissue total N, could be because of greater leaching of ammonium-N from the tissue. Although ammonium-N levels, were highest after 7 days of incubation in chapter 4, it was low in chapter 3 for all treatments with the highest being after 14 days. Moreover, the maximum ammonium-N in chapter 3 (80mg/kg) was lower than in chapter 4. However, after 14 days of incubation the trends between Lemna BF and Lemna WB remained the same across the two chapters. There was higher nitrate-N in treatment Lemna AB than the other treatments during the

incubation as a result of leaching from the biomass than the other two (Figure 3.2). Highest nitrate-N (excluding Lemna AB) was (equivalent to 100 mg/kg) in chapter 3 and 140 mg/kg in chapter 4 also suggesting more N mineralisation in chapter 4 possible because of better management of moisture. Nitrate-N was higher in chapter 3 and lower in chapter 4 for Lemna WB when compared with Lemna BF. The higher nitrate-N in chapter 4 could be because of difference in soil pH which increased mineralisation and nitrification from Lemna BF which had higher total N. The higher ammonium and nitrate-N from mineralisation explains the higher spinach N composition, uptake and dry matter yield in duckweed treatments than the control (chapter 3 and chapter 4). Other nutrients that could be contributing to higher yield are Ca, Mg, K, Mn and Fe which are higher in duckweed than compost. The growth of duckweed is induced by N, P and macronutrients in the water source from which it is grown, as such the N content in duckweed tissue is similar to inorganic fertilisers which leads to a belief of having a high potential fertiliser value (Chaiprapat et al., 2005). In addition to N the spinach dry matter yield could be explained by P uptake, which was higher in Lemna BF treatments than the Lemna WB, compost and the control in chapter 4. The higher P uptake was due to greater growth in response to N source, since the P and K were added and corrected for. A study by Citak and Sonmez (2010), concluded that N application has an influence on the other essential nutrients such as P in terms of their plant availability.

Although mineral P declined with the incubation period, higher extractable-P occurred in the Lemna BF (and Lemna AB in chapter 3) which makes greater contribution to plant available P than Lemna WB. There were lower initial P in chapter 4 than chapter 3 because of the differences in rate of application, equivalent to about 1% in chapter 4 (100 kg N/ha) and 2 to 4% in chapter 3. The difference in initial pH may have resulted in the controls having a difference in the initial P of the two chapters. Even then the duckweed treatments and compost

had higher extractable P in chapter 4 with a slower decline at day 7, 14 and 28 days of incubation, suggesting better incubation conditions possibly moisture. Conducive amounts of favourable conditions such as temperature and moisture allow for rapid decomposition, particularly for the succulent materials (such as duckweed) (Roy and Kashem, 2014b, Lupwayi et al., 2007). The high extractable P in Lemna BF for both incubation studies than Lemna WB, could be explained by mineral tissue P of the duckweed, which was higher in Lemna BF (1.7%) than Lemna WB (0.46%) while the compost had 0.95% P. The initial tissue P was also explained by mineral P content in the water on which the duckweed grew. Cover crops with a high P content result in a greater P soil release than those with a lower P content, for example vetch residues with (3.2 g P/kg) release more P than oats (2.7 g P/kg) residues in a study of Vanzolini et al. (2017).

The, harvesting of duckweed to amend soil would not only be beneficial in mitigating the challenge of re-deposition of recovered nutrients back to the water (Chikuvire, 2018), but also to ameliorate the soil quality. The use of plant material as a soil amendment has been shown to be beneficial to soil quality with soil properties also playing a huge role in the nutrients released by plant matter (Moreno-Cornejo et al., 2014, Mafongoya et al., 2000), chemical and physical properties and also nutrient content is increased (Chen et al., 2014a). The incubation studies revealed *L. minor* as being full of potential for nutrient release into the soil with treatments varying in their nutrient release rates with higher rates having higher nutrient release (Section 3.3.2) and those treatments with high N and P content having high mineralisation rate (Section 4.3.1).

Higher amounts of micro elements Ca, K, Fe, Mn in duckweed (*L. minor*), are attributed to the sources of wastewater from which the duckweed species are grown, this suggests that

decomposition of *L. minor* would release more of these elements. Although our study did not focus more on these macro and micro-nutrients, the results of the pot and field trial showed no indication of their effects on the growth of spinach even though they are likely mineralized and taken up by plants. The similarity of heavy metals in the different duckweed species suggested that the addition of *L. minor* to soils may result in differences in the levels of metals released in the soil. Therefore, faster decomposition of *L. minor* as observed in the leaching experiment could result in greater accumulation of these metals at least in the short-term. However, this aspect were not studied.

## **5.2 Conclusion**

Polluted water with higher N and P produces *Lemna minor* with higher N and P, increasing their mineral forms in soil during incubation than those produced on water with lower nutrient composition. The *L. minor* tissue with > 4% N has greater N fertiliser value than those chicken litter compost (with 3% N) even when applied at the same N rate.

## **5.3 Recommendations**

Despite a huge potential of duckweed as source of nutrient for plant growth. There are quite a number of aspects about this aquatic plant that are not fully understood, due to less research on its potential as soil amendment type for plant growth. A number of factors have to be considered before its full-blown use as an amendment source, such as where it has been harvested, the quality and quantity of essential nutrients present in its tissue amongst other aspects. Other studies should focus on the cost-effective ways of harvesting the duckweed from the waste waters from which they grow. Even though duckweed (*L. minor*) showed an immediate release of nutrients when applied in the soil. It is recommended that further research on making those nutrients essential for plant to be readily available throughout the growing



season of a crop of interest. Further, research is needed on the effects of the other nutrients present in duckweed tissue, like micronutrients, on plant growth.

## REFERENCES

- ABBASI, M. K., TAHIR, M. M., SABIR, N. & KHURSHID, M. 2015. Impact of the addition of different plant residues on nitrogen mineralization-immobilization turnover and carbon content of a soil incubated under laboratory conditions. *Solid Earth*, 6, 197.
- ALAERTS, G., MAHBUBAR, R. & KELDERMAN, P. 1996. Performance analysis of a full-scale duckweed-covered sewage lagoon. *Water Research*, 30, 843-852.
- ANDERSEN, I., DONS, C., NILSEN, S. & HAUGSTAD, M. 1985. Growth, photosynthesis and photorespiration of *Lemna gibba*: response to variations in CO<sub>2</sub> and O<sub>2</sub> concentrations and photon flux density. *Photosynthesis research*, 6, 87-96.
- ANH, N. D., TAO, H. & PRESTON, T. 1997. Effect of management practices and fertilization with biogas effluent on biomass yield and composition of duckweed. *Livestock Research for Rural Development*, 9, 46-51.
- APPENROTH, K.-J., SREE, K. S., BÖHM, V., HAMMANN, S., VETTER, W., LEITERER, M. & JAHREIS, G. 2017. Nutritional value of duckweeds (Lemnaceae) as human food. *Food Chemistry*, 217, 266-273.
- BADR, M., ABOU-HUSSEIN, S. & EL-TOHAMY, W. 2016. Tomato yield, nitrogen uptake and water use efficiency as affected by planting geometry and level of nitrogen in an arid region. *Agricultural Water Management*, 169, 90-97.
- BERGMANN, B., CHENG, J., CLASSEN, J. & STOMP, A.-M. 2000a. In vitro selection of duckweed geographical isolates for potential use in swine lagoon effluent renovation. *Bioresource Technology*, 73, 13-20.
- BERGMANN, B., CHENG, J., CLASSEN, J. & STOMP, A.-M. 2000b. Nutrient removal from swine lagoon effluent by duckweed. *Transactions of the ASAE*, 43, 263.
- BOLAN, N. S., SZOGI, A., CHUASAVATHI, T., SESHADRI, B., ROTHROCK, M. J. & PANNEERSELVAM, P. 2010. Uses and management of poultry litter. *World's Poultry Science Journal*, 66, 673-698.
- BORNETTE, G. & PUIJALON, S. 2011. Response of aquatic plants to abiotic factors: a review. *Aquatic Sciences*, 73, 1-14.
- BRIX, H. & SCHIERUP, H.-H. 1989. The use of aquatic macrophytes in water-pollution control. *Ambio. Stockholm*, 18, 100-107.
- BUTTERLY, C., BALDOCK, J. A. & TANG, C. 2013. The contribution of crop residues to changes in soil pH under field conditions. *Plant and soil*, 366, 185-198.
- CAICEDO, J., VAN DER STEEN, N., ARCE, O. & GIJZEN, H. 2000a. Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). *Water research*, 34, 3829-3835.
- CAICEDO, J. R., VAN DER STEEN, N. P., ARCE, O. & GIJZEN, H. J. 2000b. Effect of total ammonia nitrogen concentration and pH on growth rates of duckweed (*Spirodela polyrrhiza*). *Water Research*, 34, 3829-3835.
- CAMARGO, J. A., ALONSO, A. & SALAMANCA, A. 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere*, 58, 1255-1267.
- CATALDO, D., MAROON, M., SCHRADER, L. & YOUNGS, V. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in soil science and plant analysis*, 6, 71-80.
- CHAIPRAPAT, S., CHENG, J., CLASSEN, J. & LIEHR, S. 2005. ROLE OF INTERNAL NUTRIENT STORAGE IN DUCKWEED GROWTH FOR SWINE WASTEWATER TREATMENT. *Transactions of the ASAE*, 48, 2247-2258.
- CHEN, B., LIU, E., TIAN, Q., YAN, C. & ZHANG, Y. 2014a. Soil nitrogen dynamics and crop residues. A review. *Agronomy for Sustainable Development*, 34, 429-442.
- CHEN, M., YE, T.-R., KRUMHOLZ, L. R. & JIANG, H.-L. 2014b. Temperature and cyanobacterial bloom biomass influence phosphorous cycling in eutrophic lake sediments. *PLoS one*, 9, e93130.

- CHENG, J., BERGMANN, B. A., CLASSEN, J. J., STOMP, A. M. & HOWARD, J. W. 2002a. Nutrient recovery from swine lagoon water by *Spirodela punctata*. *Bioresource Technology*, 81, 81-85.
- CHENG, J., LANDESMAN, L., BERGMANN, B., CLASSEN, J. J., HOWARD, J. & YAMAMOTO, Y. 2002b. Nutrient removal from swine lagoon liquid by *Lemna minor* 8627. *Transactions of the ASAE*, 45, 1003.
- CHENG, J. J. & STOMP, A. M. 2009. Growing duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. *Clean–Soil, Air, Water*, 37, 17-26.
- CHIKUVIRE, T. J. 2018. *Nutrient recovery from wastewater in intensive agricultural systems by duckweed and the value of the biomass as an organic fertiliser*. Doctor of Philosophy Soil Science University of KwaZulu-Natal.
- CHIKUVIRE, T. J., MUCHAONYERWA, P. & ZENGENI, R. 2018a. Biomass, Nitrogen Uptake and Content of *Wolffia arrhiza* Depends on Strength of Swine Lagoon Water. *Water Environment Research*, 90, 2066-2074.
- CHIKUVIRE, T. J., MUCHAONYERWA, P. & ZENGENI, R. 2018b. Decomposition of *Wolffia arrhiza* residues rapidly increases mineral nitrogen and decreases extractable phosphorus in acidic soils. *Environmental monitoring and assessment*, 190, 510.
- CITAK, S. & SONMEZ, S. 2010. Effects of conventional and organic fertilization on spinach (*Spinacea oleracea* L.) growth, yield, vitamin C and nitrate concentration during two successive seasons. *Scientia horticulturae*, 126, 415-420.
- COOKSON, W. R., CORNFORTH, I. S. & ROWARTH, J. S. 2002. Winter soil temperature (2–15°C) effects on nitrogen transformations in clover green manure amended or unamended soils; a laboratory and field study. *Soil Biology and Biochemistry*, 34, 1401-1415.
- COOPERBAND, L., BOLLERO, G. & COALE, F. 2002. Effect of poultry litter and composts on soil nitrogen and phosphorus availability and corn production. *Nutrient Cycling in Agroecosystems*, 62, 185-194.
- COURTNEY, R. G. & MULLEN, G. J. 2008. Soil quality and barley growth as influenced by the land application of two compost types. *Bioresource Technology*, 99, 2913-2918.
- CRITES, R., MIDDLEBROOKS, E. & REED, S. 2006. *Natural Wastewater Treatment Systems*. Taylor & Francis Group, 552.
- CUI, W. & CHENG, J. 2015. Growing duckweed for biofuel production: a review. *Plant biology*, 17, 16-23.
- CULLEY JR, D. D. & EPPS, E. A. 1973. Use of duckweed for waste treatment and animal feed. *Journal (Water Pollution Control Federation)*, 337-347.
- DALU, J. & NDAMBA, J. 2003. Duckweed based wastewater stabilization ponds for wastewater treatment (a low cost technology for small urban areas in Zimbabwe). *Physics and Chemistry of the Earth, Parts A/B/C*, 28, 1147-1160.
- DEBUSK, T. A., PETERSON, J. E. & REDDY, K. R. 1995. Use of aquatic and terrestrial plants for removing phosphorus from dairy wastewaters. *Ecological Engineering*, 5, 371-390.
- DHOTE, S. & DIXIT, S. 2007. Water quality improvement through macrophytes: a case study. *Asian J. Exp. Sci*, 21, 427-430.
- DHOTE, S. & DIXIT, S. 2009. Water quality improvement through macrophytes—a review. *Environmental Monitoring and Assessment*, 152, 149-153.
- DIKINYA, O. & MUFWANZALA, N. 2010. Chicken manure-enhanced soil fertility and productivity: Effects of application rates. *Journal of Soil Science and Environmental Management*, 1, 46-54.
- DOSSA, E., KHOUMA, M., DIEDHIOU, I., SENE, M., KIZITO, F., BADIANE, A., SAMBA, S. & DICK, R. 2009. Carbon, nitrogen and phosphorus mineralization potential of semiarid Sahelian soils amended with native shrub residues. *Geoderma*, 148, 251-260.
- EL-KHEIR, W. A., ISMAIL, G., EL-NOUR, F. A., TAWFIK, T. & HAMMAD, D. 2007. Assessment of the efficiency of duckweed (*Lemna gibba*) in wastewater treatment. *International Journal of Agriculture and Biology (Pakistan)*.

- ENDERLEIN, S., ENDERLEIN, R. E. & PETER, W. 1997. Water Quality Requirements. *Water Pollution Control. A Guide to the Use of Water Quality Management Principles*, 1.
- ESTERHUIZEN, L., FOSSEY, A. & POTGIETER, E. 2015. Groundwater quality on dairy farms in central South Africa. *Water SA*, 41, 194-198.
- FARRELL, J. B. 2012. *Duckweed uptake of phosphorus and five pharmaceuticals: microcosm and wastewater lagoon studies*, Utah State University.
- FATOKI, O., GOGWANA, P. & OGUNFOWOKAN, A. 2003. Pollution assessment in the Keiskamma River and in the impoundment downstream. *Water SA*, 29, 183-188.
- FOSU, M., KÜHNE, R. F. & VLEK, P. L. 2004. Improving maize yield in the Guinea savannah zone of Ghana with leguminous cover crops and PK fertilization. *Journal of Agronomy*, 3, 115-121.
- FRÉDÉRIC, M., SAMIR, L., LOUISE, M. & ABDELKRIM, A. 2006. Comprehensive modeling of mat density effect on duckweed (*Lemna minor*) growth under controlled eutrophication. *Water research*, 40, 2901-2910.
- FUJITA, M., MORI, K. & KODERA, T. 1999. Nutrient removal and starch production through cultivation of *Wolffia arrhiza*. *Journal of Bioscience and bioengineering*, 87, 194-198.
- GAUR, R. Z. & SUTHAR, S. 2017. Nutrient scaling of duckweed (*Spirodela polyrhiza*) biomass in urban wastewater and its utility in anaerobic co-digestion. *Process Safety and Environmental Protection*, 107, 138-146.
- GOOPY, J. & MURRAY, P. 2003. A review on the role of duckweed in nutrient reclamation and as a source of animal feed. *Asian-australasian journal of animal sciences*, 16, 297-305.
- GRIPPO, M. A., HLOHOWSKYJ, I., FOX, L., HERMAN, B., POTHOFF, J., YOE, C. & HAYSE, J. 2017. Aquatic nuisance species in the great lakes and Mississippi river basin—a risk assessment in support of GLMRIS. *Environmental management*, 59, 154-173.
- HALL, J. B., SEAY, W. W. & BAKER, S. M. 2005. Nutrition and feeding of the cow-calf herd: essential nutrients, feed classification and nutrient content of feeds.
- HANCZAKOWSKI, P., SZYMCZYK, B. & WAWRZYŃSKI, M. 1995. Composition and nutritive value of sewage-grown duckweed (*Lemna minor* L.) for rats. *Animal Feed Science and Technology*, 52, 339-343.
- HARDING, W., THORNTON, J., STEYN, G., PANUSKA, J. & MORRISON, I. 2004. Hartbeespoort Dam remediation project (phase 1). *Final report*, 1, 166.
- HILLMAN, W. S. 1961. The Lemnaceae, or duckweeds. *The Botanical Review*, 27, 221-287.
- HUANG, J., YU, Z., GAO, H., YAN, X., CHANG, J., WANG, C., HU, J. & ZHANG, L. 2017. Chemical structures and characteristics of animal manures and composts during composting and assessment of maturity indices. *PloS one*, 12, e0178110.
- HUANG, X., HUANG, L. & YUE, W. 2003. The characteristics of nutrients and eutrophication in the Pearl River estuary, South China. *Marine Pollution Bulletin*, 47, 30-36.
- HUNTER, A. 1974. Tentative ISFEI soil extraction procedure. *International Soil Fertility Evaluation and Improvement Project, North Carolina State University, Raleigh, North Carolina, USA*.
- IGBINOSA, E. & OKOH, A. 2009. Impact of discharge wastewater effluents on the physico-chemical qualities of a receiving watershed in a typical rural community. *International Journal of Environmental Science & Technology*, 6, 175-182.
- IQBAL, J. & BAIG, M. A. 2016. Effect of Nutrient Concentration and pH on Growth and Nutrient Removal Efficiency of Duckweed (*Lemna Minor*) From Natural Solid Waste Leachate. *International Journal of Health and Medicine*, 1, 1-7.
- IQBAL, S. 1999. Duckweed aquaculture. *Potentials, possibilities and limitations for combined wastewater treatment and animal feed production in developing countries. SAN-DEC Report*.
- JOHNSON, C., ALBRECHT, G., KETTERINGS, Q., BECKMAN, J. & STOCKIN, K. 2005. Nitrogen basics—the nitrogen cycle. *Agronomy Fact Sheet Series, Fact Sheet*, 2.
- KALOI, G., BHUGHIO, N., PANHWAR, R., JUNEJO, S., MARI, A. & BHUTTO, M. 2011. Influence of incubation period on phosphate release in two soils of district Hyderabad. *J. Anim. Plant Sci*, 21, 665-670.

- KEMP, B., SOEDE, N. & DEN HARTOG, L. Pig production in The Netherlands: analyses and trends. Proceedings of the 2011 Manitoba Swine Seminar, 2-3 February 2011, Manitoba, Canada, 2011. 161-167.
- KHAN, F. A. & ANSARI, A. A. 2005. Eutrophication: an ecological vision. *The botanical review*, 71, 449-482.
- KHELLAF, N. & ZERDAOUI, M. 2009a. Growth response of the duckweed *Lemna minor* to heavy metal pollution. *Journal of Environmental Health Science & Engineering*, 6, 161-166.
- KHELLAF, N. & ZERDAOUI, M. 2009b. Growth response of the duckweed *Lemna minor* to heavy metal pollution. *Iran J Environ Health Sci Eng*, 6, 161-166.
- KIRKBY, C., KIRKEGAARD, J., RICHARDSON, A., WADE, L., BLANCHARD, C. & BATTEN, G. 2011. Stable soil organic matter: a comparison of C: N: P: S ratios in Australian and other world soils. *Geoderma*, 163, 197-208.
- KLAUS, J., NIKOLAI, B. & ERIC, L. 2013. Telling duckweed apart: genotyping technologies for the Lemnaceae. *应用与环境生物学报*, 19, 1-10.
- KONING, N., ROOS, J. & GROBBELAAR, J. 2000. Water quality of the Modder River, South Africa. *Southern African Journal of Aquatic Sciences*, 25, 202-210.
- KOTOSKI, J. 1997. Black earth creek and limnology manifests and analysis. *Spring Harbor Environmental Magnet Middle School*, 1-2.
- LANDESMAN, L., CHANG, J., YAMAMOTO, Y. & GOODWIN, J. 2002. Nutritional value of wastewater-grown duckweed for fish and shrimp feed. *World Aquaculture*, 33, 39-40.
- LECO CORPORATION 2012. Leco Trumac CNS/NS Carbon/Nitrogen/Sulfur Determinators.
- LENG, R. 1999a. Duckweed. a tiny aquatic plant with enormous potential for agricultures and environment. *FAO Animal Production and Health Paper*.
- LENG, R. 1999b. Duckweed: A tiny aquatic plant with enormous potential for agriculture and environment.
- LENG, R., STAMBOLIE, J. & BELL, R. 1995. Duckweed-a potential high-protein feed resource for domestic animals and fish. *Livestock Research for Rural Development*, 7, 36.
- LI, H., LIANG, X., LIAN, Y., XU, L. & CHEN, Y. 2009. Reduction of ammonia volatilization from urea by a floating duckweed in flooded rice fields. *Soil Science Society of America Journal*, 73, 1890-1895.
- LI, L., LIU, M., WU, M., JIANG, C., CHEN, X., MA, X., LIU, J., LI, W., TANG, X. & LI, Z. 2017. Effects of duckweed (*Spirodela polyrrhiza*) remediation on the composition of dissolved organic matter in effluent of scale pig farms. *Journal of Environmental Sciences*, 55, 247-256.
- LOWDER, S. K., SKOET, J. & RANEY, T. 2016. The number, size, and distribution of farms, smallholder farms, and family farms worldwide. *World Development*, 87, 16-29.
- LUPWAYI, N., CLAYTON, G., O'DONOVAN, J., HARKER, K., TURKINGTON, T. & SOON, Y. 2007. Phosphorus release during decomposition of crop residues under conventional and zero tillage. *Soil and Tillage Research*, 95, 231-239.
- MAFONGOYA, P., BARAK, P. & REED, J. 2000. Carbon, nitrogen and phosphorus mineralization of tree leaves and manure. *Biology and Fertility of soils*, 30, 298-305.
- MAFTOUN, M., MOSHIRI, F., KARIMIAN, N. & RONAGHI, A. 2005. Effects of two organic wastes in combination with phosphorus on growth and chemical composition of spinach and soil properties. *Journal of plant nutrition*, 27, 1635-1651.
- MALEPFANE, N. & MUCHAONYERWA, P. 2017. Hair from different ethnic groups vary in elemental composition and nitrogen and phosphorus mineralisation in soil. *Environmental monitoring and assessment*, 189, 76.
- MALLIN, M. A. & CAHOON, L. B. 2003. Industrialized animal production—a major source of nutrient and microbial pollution to aquatic ecosystems. *Population and Environment*, 24, 369-385.
- MASUNGA, R. H., UZOKWE, V. N., MLAY, P. D., ODEH, I., SINGH, A., BUCHAN, D. & DE NEVE, S. 2016. Nitrogen mineralization dynamics of different valuable organic amendments commonly used in agriculture. *Applied Soil Ecology*, 101, 185-193.

- MATSUMOTO, S., AE, N. & YAMAGATA, M. 1999. Nitrogen uptake response of vegetable crops to organic materials. *Soil science and plant nutrition*, 45, 269-278.
- MAWONGA, T. 2016. *Rapid biomax thermophilic composting effects on quality, nutrient release and fertiliser value of chicken litter composts*.
- MAYNARD, A. A. 1994. Sustained vegetable production for three years using composted animal manures. *Compost Science & Utilization*, 2, 88-96.
- MOFOKENG, D. S., ADELEKE, R. & AIYEGORO, O. A. 2016. The analysis of physicochemical characteristics of pig farm seepage and its possible impact on the receiving natural environment. *African Journal of Environmental Science and Technology*, 10, 242-252.
- MOHEDANO, R. A., COSTA, R. H. & BELLI FILHO, P. 2016. Effects of CO<sub>2</sub> concentration on nutrient uptake and starch accumulation by duckweed used for wastewater treatment and bioethanol production. *Revista Latinoamericana de Biotecnología Ambiental y Algal*, 7, 1-12.
- MOHEDANO, R. A., COSTA, R. H., TAVARES, F. A. & BELLI FILHO, P. 2012. High nutrient removal rate from swine wastes and protein biomass production by full-scale duckweed ponds. *Bioresource Technology*, 112, 98-104.
- MOLAPO, N. A. 2009. *Waste handling practices in the South African high-throughput poultry abattoirs*. Bloemfontein: Central University of Technology, Free State.
- MORENO-CORNEJO, J., ZORNOZA, R. & FAZ, A. 2014. Carbon and nitrogen mineralization during decomposition of crop residues in a calcareous soil. *Geoderma*, 230, 58-63.
- MORIN, S., BARRINGTON, S., WHALEN, J. & MARTINEZ, J. 2008. A modified septic system for the treatment of dairy farm milk house wastewaters. *Canadian Biosystems Engineering*, 50.
- MORRISON, G., FATOKI, O., PERSSON, L. & EKBERG, A. 2001. Assessment of the impact of point source pollution from the Keiskammahoek Sewage Treatment Plant on the Keiskamma River-pH, electrical conductivity, oxygen-demanding substance (COD) and nutrients. *Water Sa*, 27, 475-480.
- MSIBI, S., KIHUPI, N., TARIMO, A. & MANYATSI, A. 2014. Technical performance evaluation of centre pivot sprinkler irrigation system at Ubombo Sugar estate, Swaziland. *International Journal of Agricultural Science and Bioresource Engineering Research*, 3, 23-38.
- MURUGAN, A. V. & SWARNAM, T. 2013. Nitrogen release pattern from organic manures applied to an acid soil. *Journal of Agricultural Science*, 5, 174.
- MUSFIQUE, A., HASAN, C. K., HAFIZUR, R., HOSSAIN, M. A. & UDDIN, S. A. 2015. Prospects of using wastewater as a resource-nutrient recovery and energy generation. *American Journal of Environmental Sciences*, 11, 99-114.
- NEAL JR, J. A. 2009. *The effects of electron beam irradiation and sanitizers in the reduction of pathogens and attachment prevention on spinach*, Texas A&M University.
- NEZOMBA, H., TAURO, T., MTAMBANENGWE, F. & MAPFUMO, P. 2009. Indigenous legumes biomass quality and influence on C and N mineralization under indigenous legume fallow systems. *Symbiosis*, 48, 78-91.
- NGUYEN, T. T. & MARSCHNER, P. 2017. Soil respiration, microbial biomass and nutrient availability in soil after addition of residues with adjusted N and P concentrations. *Pedosphere*, 27, 76-85.
- NON-AFFILIATED SOIL ANALYSIS WORK COMMITTEE 1990. Handbook of standard soil testing methods for advisory purposes. *Soil Science Society of South Africa, Pretoria*, 1, 1-35.
- NYENJE, P., FOPPEN, J., UHLENBROOK, S., KULABAKO, R. & MUWANGA, A. 2010. Eutrophication and nutrient release in urban areas of sub-Saharan Africa—a review. *Science of the Total Environment*, 408, 447-455.
- OKALEBO, J. R., GATHUA, K. W. & WOOMER, P. L. 2002. Laboratory methods of soil and plant analysis: a working manual second edition. *TSBFCIAT and SACRED Africa. Nairobi, Kenya*.
- OZENGIN, N. & ELMACI, A. 2007. Performance of Duckweed (*Lemna minor* L.) on different types of wastewater treatment. *Journal of Environmental Biology*, 28(2), 307-314.

- PATEL, D. & KANUNGO, V. 2010. PHYTOREMEDIATION POTENTIAL OF DUCKWEED(LEMNAMINOR L: A TINY AQUATIC PLANT) IN THE REMOVAL OF POLLUTANTS FROM DOMESTIC WASTEWATER WITH SPECIAL REFERENCE TO NUTRIENTS. *Bioscan*, 5, 355-358.
- PAUL, K., BLACK, A. & CONYERS, M. 2001. Effect of plant residue return on the development of surface soil pH gradients. *Biology and Fertility of Soils*, 33, 75-82.
- PAYNE, R., HARDING, S., MURRAY, D., SOUTAR, D., BAIRD, D., GLASER, A., WELHAM, S., GILMOUR, A. & R, T. 2011. GenStat 14th edition. *VSN International, United Kingdom*.
- POPA, P., TIMOFTI, M., VOICULESCU, M., DRAGAN, S., TRIF, C. & GEORGESCU, L. P. 2012. Study of physico-chemical characteristics of wastewater in an urban agglomeration in Romania. *The scientific world journal*, 2012.
- PREUSCH, P., ADLER, P., SIKORA, L. & TWORKOSKI, T. 2002. Nitrogen and phosphorus availability in composted and uncomposted poultry litter. *Journal of environmental quality*, 31, 2051-2057.
- QU, J. & FAN, M. 2010. The current state of water quality and technology development for water pollution control in China. *Critical Reviews in Environmental Science and Technology*, 40, 519-560.
- RAHMAN, M. A. & HASEGAWA, H. 2011. Aquatic arsenic: phytoremediation using floating macrophytes. *Chemosphere*, 83, 633-646.
- RAYMENT, G. E. & LYONS, D. J. 2011. *Soil chemical methods: Australasia*, CSIRO publishing.
- RENGEL, Z. 2007. The role of crop residues in improving soil fertility. *Nutrient cycling in terrestrial ecosystems*. Springer.
- ROELOFSE, J. J. H. 2013. *Economic feasibility study of the establishment of smallholder pig farmers for the commercial market: Empolweni case study*. Stellenbosch: Stellenbosch University.
- ROY, S. & KASHEM, M. A. 2014a. Effects of Organic Manures in Changes of Some Soil Properties at Different Incubation Periods. *Open Journal of Soil Science*, 2014.
- ROY, S. & KASHEM, M. A. 2014b. Effects of organic manures in changes of some soil properties at different incubation periods. *Open Journal of Soil Science*, 4, 81.
- RUIGROK, T. 2015. *Temperature response of duckweed growth at the Ecoferm greenhouse*.
- SAMUEL, A. L. & EBENEZER, A. O. 2014. Mineralization rates of soil forms of nitrogen, phosphorus, and potassium as affected by organomineral fertilizer in sandy loam. *Advances in Agriculture*, 5.
- SASMAZ, M., TOPAL, E. I. A., OBEK, E. & SASMAZ, A. 2015. The potential of Lemna gibba L. and Lemna minor L. to remove Cu, Pb, Zn, and As in gallery water in a mining area in Keban, Turkey. *Journal of environmental management*, 163, 246-253.
- SAUCEDO TERÁN, R. A., DE LA MORA OROZCO, C., GONZÁLEZ ACUÑA, I. J., GÓMEZ ROSALES, S., DOMÍNGUEZ ARAUJO, G. & RUBIO ARIAS, H. O. 2017. Removing Organic Matter and Nutrients from Swine Wastewater after Anaerobic–Aerobic Treatment. *Water*, 9, 726.
- SCOTT, W., ASHTON, P., WALMSLEY, R. & SEAMAN, M. 1980. Hartbeespoort Dam: a case study of a hypertrophic, warm, monomictic impoundment. *Hypertrophic ecosystems*. Springer.
- SELVARANI, A. J., PADMAVATHY, P., SRINIVASAN, A. & JAWAHAR, P. 2015. Performance of Duckweed (Lemna minor) on different types of wastewater treatment.
- SINGH, V. K. & SINGH, J. J. J. O. E. B. 2006. Toxicity of industrial wastewater to the aquatic plant Lemna minor L. 37, 385-390.
- SOOKNAH, R. D. & WILKIE, A. C. 2004. Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater. *Ecological Engineering*, 22, 27-42.
- SPIEGEL, H., SANDÉN, T., DERSCH, G., BAUMGARTEN, A., GRÜNDLING, R. & FRANKO, U. 2018. Soil Organic Matter and Nutrient Dynamics Following Different Management of Crop Residues at Two Sites in Austria. *Soil Management and Climate Change*. Elsevier.
- SRIVASTAVA, J., GUPTA, A. & CHANDRA, H. 2008. Managing water quality with aquatic macrophytes. *Reviews in Environmental Science and Bio/Technology*, 7, 255-266.
- STOMP, A.-M. 2005. The duckweeds: a valuable plant for biomanufacturing. *Biotechnology Annual Review*, 11, 69-99.

- SUDHA, S. 2008. Treatment of saltwater crocodile Pond wastewater using constructed Wetland system.
- TABOU, T. T., BAYA, D., EYUL'ANKI, D. M. & VASEL, J. 2014. Monitoring the influence of light intensity on the growth and mortality of duckweed (*Lemna minor*) through digital images processing. *Biotechnology, Agronomy, Social, Environmental*, 18, 37-48.
- TANG, J., LI, Y., MA, J. & CHENG, J. 2015. Survey of duckweed diversity in Lake Chao and total fatty acid, triacylglycerol, profiles of representative strains. *Plant Biology*, 17, 1066-1072.
- TANGOU TABOU, T., BAYA1, D. T., D., M. E. A. & VASEL, J.-L. 2014. Monitoring the influence of light intensity on the growth and mortality of duckweed (*Lemna minor*) through digital images processing. *Sanitation and Environment Laboratory, University of Liege, Faculty of Science*.
- TIQUIA, S. M. & TAM, N. F. 2002. Characterization and composting of poultry litter in forced-aeration piles. *Process Biochemistry*, 37, 869-880.
- TOYAMA, T., HANAOKA, T., TANAKA, Y., MORIKAWA, M. & MORI, K. 2018. Comprehensive evaluation of nitrogen removal rate and biomass, ethanol, and methane production yields by combination of four major duckweeds and three types of wastewater effluent. *Bioresource technology*, 250, 464-473.
- TU, D. T. M. 2012. *Manipulation of the nutritive value of duckweed (Lemna minor) as a feed resource for local Muscovy ducks*. MSc. Thesis in Agricultural Sciences Animal Husbandry, Cantho University.
- UYSAL, Y. & ZEREN, O. 2004. Removal efficiencies of nutrients from wastewater treated with duckweed(*Lemna minor* L.). *Fresenius Environmental Bulletin*, 13, 1016-1019.
- VAN GINKEL, C. 2011. Eutrophication: Present reality and future challenges for South Africa. *Water SA*, 37, 693-702.
- VANZOLINI, J. I., GALANTINI, J. A., MARTÍNEZ, J. M. & SUÑER, L. 2017. Changes in soil pH and phosphorus availability during decomposition of cover crop residues. *Archives of Agronomy and Soil Science*, 63, 1864-1874.
- WANG, J., CHEN, Z., CHEN, L., ZHU, A. & WU, Z. 2011. Surface soil phosphorus and phosphatase activities affected by tillage and crop residue input amounts. *Plant, Soil and Environment*, 57, 251-257.
- WANG, L., TONG, Z., LIU, G. & LI, Y. 2014. Characterization of biomass residues and their amendment effects on water sorption and nutrient leaching in sandy soil. *Chemosphere*, 107, 354-359.
- WANG, Z. & LI, S. 2004. Effects of nitrogen and phosphorus fertilization on plant growth and nitrate accumulation in vegetables. *Journal of plant nutrition*, 27, 539-556.
- WHITMORE, A. 2007. Determination of the mineralization of nitrogen from composted chicken manure as affected by temperature. *Nutrient cycling in agroecosystems*, 77, 225-232.
- WORLD HEALTH ORGANIZATION 1989. Health guidelines for the use of wastewater in agriculture and aquaculture: report of a WHO scientific group [meeting held in Geneva from 18 to 23 November 1987].
- WORLD HEALTH ORGANIZATION, W. 2002. Eutrophication and health. *Luxembourg: Office for Official Publications of the European Communities. Geneva: WHO*.
- XU, J. & SHEN, G. 2011a. Growing duckweed in swine wastewater for nutrient recovery and biomass production. *Bioresource technology*, 102, 848-853.
- XU, J. & SHEN, G. J. B. T. 2011b. Growing duckweed in swine wastewater for nutrient recovery and biomass production. 102, 848-853.
- YAN, F., HÜTSCH, B. W. & SCHUBERT, S. 2006. Soil-pH dynamics after incorporation of fresh and oven-dried plant shoot materials of faba bean and wheat. *Journal of plant nutrition and soil science*, 169, 506-508.



- YAO, Y., ZHANG, M., TIAN, Y., ZHAO, M., ZHANG, B., ZHAO, M., ZENG, K. & YIN, B. 2017. Duckweed (*Spirodela polyrhiza*) as green manure for increasing yield and reducing nitrogen loss in rice production. *Field Crops Research*, 214, 273-282.
- YILMAZ, E., AKYURT, I. & GÜNAL, G. 2004a. Use of duckweed, *Lemna minor*, as a protein feedstuff in practical diets for common carp, *Cyprinus carpio*, fry. *Turkish journal of fisheries and aquatic sciences*, 4.
- YILMAZ, E., AKYURT, İ. & GÜNAL, G. 2004b. Use of duckweed, *Lemna minor*, as a protein feedstuff in practical diets for common carp, *Cyprinus carpio*, fry. *Turkish Journal of Fisheries and Aquatic Sciences*, 4, 105-109.
- YIN, Y., YU, C., YU, L., ZHAO, J., SUN, C., MA, Y. & ZHOU, G. 2015. The influence of light intensity and photoperiod on duckweed biomass and starch accumulation for bioethanol production. *Bioresource technology*, 187, 84-90.
- ZHAO, X., MOATES, G., WELLNER, N., COLLINS, S., COLEMAN, M. & WALDRON, K. 2014. Chemical characterisation and analysis of the cell wall polysaccharides of duckweed (*Lemna minor*). *Carbohydrate polymers*, 111, 410-418.

## LIST OF APPENDICES

### Appendix 1: Statistical output for analysis of variance (ANOVA) for duckweed tissue analysis

#### Analysis of variance

Variate: Al

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	1	15071.	15071.	11.77	
Replicate.*Units* stratum					
DW_TYPE	2	1223434.	611717.	477.62	0.002
Residual	2	2562.	1281.		
Total	5	1241067.			

Variate: Zn

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	1	847.761	847.761	311.28	
Replicate.*Units* stratum					
DW_TYPE	2	7149.314	3574.657	1312.53	<.001
Residual	2	5.447	2.723		
Total	5	8002.522			

Variate: N

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	1	0.066137	0.066137	18.08	
Replicate.*Units* stratum					
DW_TYPE	2	1.937002	0.968501	264.71	0.004
Residual	2	0.007318	0.003659		
Total	5	2.010457			

Variate: P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	1	0.00763	0.00763	0.41	
Replicate.*Units* stratum					
DW_TYPE	2	2.15383	1.07691	58.19	0.017
Residual	2	0.03702	0.01851		
Total	5	2.19847			

Variate: N\_P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate stratum	1	0.6833	0.6833	1.41	
Replicate.*Units* stratum					
DW_TYPE	2	61.6957	30.8479	63.67	0.015
Residual	2	0.9690	0.4845		
Total	5	63.3480			

Since there are many micro and macro-nutrients two of each nutrient were added as well as one ratio.

## Appendix 2: Statistical output for analysis of variance (ANOVA) for incubation statistics

### Analysis of variance

Variate: NH4\_mg\_Kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Amendment	3	12422.72	4140.91	230.54	<.001
Day	5	280312.99	56062.60	3121.19	<.001
Amendment.Day	15	36422.20	2428.15	135.18	<.001
Residual	46	826.25	17.96		
Total	71	329993.36			

Variate: NO3\_mg\_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Amendment	3	30.153	10.051	3.19	0.032
Day	5	2274.544	454.909	144.40	<.001
Amendment.Day	15	536.725	35.782	11.36	<.001
Residual	46	144.913	3.150		
Total	71	2986.838			

Variate: P\_mg\_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Amendment	3	51.928	17.309	9.47	<.001
Day	5	324.879	64.976	35.53	<.001
Amendment.Day	15	246.076	16.405	8.97	<.001
Residual	46	84.117	1.829		
Total	71	707.499			

Variate: pH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates.*Units* stratum					
Amendment		3	5.966949	1.988983	486.85 <.001
Day		5	6.033824	1.206765	295.38 <.001
Amendment.Day		15	0.544026	0.036268	8.88 <.001
Residual		46	0.187931	0.004085	
Total		71	12.733732		

### Appendix 3: Statistical output for analysis of variance (ANOVA) for leaching analysis

Analysis of variance

Variate: NH4\_mg\_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates stratum					
		2	3.6028	1.8014	2.58
Replicates.*Units* stratum					
DW_type		2	21.9871	10.9935	15.72 <.001
Hours		3	266.8235	88.9412	127.20 <.001
DW_type.Hours		6	287.4740	47.9123	68.52 <.001
Residual		22	15.3826	0.6992	
Total		35	595.2700		

Variate: NO3\_mg\_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates stratum					
		2	24.46	12.23	0.44
Replicates.*Units* stratum					
DW_type		2	1333.94	666.97	24.18 <.001
Hours		3	664.68	221.56	8.03 <.001
DW_type.Hours		6	1293.79	215.63	7.82 <.001
Residual		22	606.84	27.58	
Total		35	3923.72		

Variate: P\_mg\_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates stratum					
		2	15074.79	7537.39	19.87 <.001
Replicates.*Units* stratum					
DW_type		3	6503.60	2167.87	32.71 <.001
Hours		3	3952.17	1317.39	19.87 <.001
DW_type.Hours		9	2169.97	241.11	3.64 0.004
Residual		30	1988.54	66.28	
Total		47	15074.79		

**Appendix 4:** Statistical output for analysis of variance (ANOVA) for pot trial statistics and pictures

Variate: C\_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
DW_type		5	20.160		4.032	1.92	0.131
Rates		1	2.283		2.283	1.09	0.308
DW_type.Rates		5	16.210		3.242	1.55	0.217
Residual		22	46.119		2.096		
Total		35	85.765				

Variate: N\_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
DW_type		5	3.797569		0.759514	92.68	<.001
Rates		1	0.063470		0.063470	7.74	0.011
DW_type.Rates		5	0.142555		0.028511	3.48	0.018
Residual		22	0.180299		0.008195		
Total		35	4.214935				

Variate: P\_mg\_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
DW_type		5	0.64370		0.12874	1.64	0.192
Rates		1	0.01312		0.01312	0.17	0.687
DW_type.Rates		5	0.11825		0.02365	0.30	0.907
Residual		22	1.73063		0.07866		
Total		35	2.50744				

Variate: Uptake\_N

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
DW_type		5	4.183E-03		8.365E-04	215.58	<.001
Rates		1	2.595E-04		2.595E-04	66.88	<.001
DW_type.Rates		5	3.406E-04		6.811E-05	17.55	<.001
Residual		22	8.537E-05		3.880E-06		
Total		35	4.886E-03				

Variate: Uptake\_P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
DW_type		5	0.00056447		0.00011289	10.72	<.001
Rates		1	0.00002399		0.00002399	2.28	0.145
DW_type.Rates		5	0.00004763		0.00000953	0.90	0.496

Residual	22	0.00023167	0.00001053
Total	35	0.00087217	

### Residual soil analysis

Variate: N\_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2	0.000634	0.000317	0.22	
Replicates.*Units* stratum					
DW_type	3	11.399929	3.799976	2586.75	<.001
Incubation	1	0.582910	0.582910	396.80	<.001
DW_type.Incubation	3	0.120894	0.040298	27.43	<.001
Residual	14	0.020566	0.001469		
Total	23	12.124932			

Variate: C\_%

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2	0.14487	0.07244	3.77	
Replicates.*Units* stratum					
DW_type	3	10.16287	3.38762	176.10	<.001
Incubation	1	0.14717	0.14717	7.65	0.015
DW_type.Incubation	3	1.10909	0.36970	19.22	<.001
Residual	14	0.26932	0.01924		
Total	23	11.83332			

Variate: P\_mg\_kg

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2	0.2103	0.1051	0.49	
Replicates.*Units* stratum					
DW_type	3	138.1937	46.0646	214.13	<.001
Incubation	1	16.0295	16.0295	74.51	<.001
DW_type.Incubation	3	31.9922	10.6641	49.57	<.001
Residual	14	3.0117	0.2151		
Total	23	189.4375			

Variate: pH\_KCl

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2	0.49743	0.24871	4.03	
Replicates.*Units* stratum					
DW_type	3	0.74005	0.24668	3.99	0.030
Incubation	1	0.95242	0.95242	15.41	0.002
DW_type.Incubation	3	0.50206	0.16735	2.71	0.085
Residual	14	0.86508	0.06179		
Total	23	3.55704			

Variate: EC\_dS\_cm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2	0.000258	0.000129	0.04	
Replicates.*Units* stratum					
DW_type	3	0.491017	0.163672	55.16	<.001
Incubation	1	0.920417	0.920417	310.19	<.001
DW_type.Incubation	3	0.042150	0.014050	4.74	0.018
Residual	14	0.041542	0.002967		
Total	23	1.495383			

**Appendix 5:** Statistical output for analysis of variance (ANOVA) for field statistics and pictures

Variate: FW

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	3098006.	1032669.	1.60	0.265
Residual	8	5171736.	646467.		
Total	11	8269742.			

Variate: DM

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	3	3601.82	1200.61	13.72	0.002
Residual	8	700.02	87.50		
Total	11	4301.84			



### Analysis of variance

Variate: %C

Source of variation	d.f.	s.s.	m.s.	v.r.	F	pr.
Treatments		3	13.921	4.640	4.27	0.062
Residual		6	6.515	1.086		
Total	11	22.436				

Variate: %N

Source of variation	d.f.	s.s.	m.s.	v.r.	F	pr.
Treatments		3	2.48399	0.82800	53.10	<.001
Residual		6	0.09357	0.01559		
Total	11	2.67834				

Variate: PH

Source of variation	d.f.	s.s.	m.s.	v.r.	F	pr.
Treatments		3	0.32207	0.10736	6.05	0.030
Residual		6	0.10653	0.01776		
Total	11	0.46720				



Variate: EC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
Treatments		3	0.7436917		0.2478972	339.33	<.001
Residual		6	0.0043833		0.0007306		
Total		11	0.8080917				

Variate: C\_N

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
Treatments		3	25.4353		8.4784	15.48	0.003
Residual		6	3.2869		0.5478		
Total		11	29.0911				

Variate: P

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.		
Treatments		3	66.179		22.060	11.85	0.006
Residual		6	11.174		1.862		
Total		11	78.487				