



Course Code	CHMT8000A
Course Name	MSc (ENG) Dissertation
Assignment Details	Master's Thesis for Chemical Engineering
Assignment Due Date	28 February 2020

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The phytoremediation potential of water hyacinth

Master of Science in Engineering

by

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September, 2020

ABSTRACT

Water hyacinth was brought to South Africa many decades ago as an ornamental flower. After it entered the water system, water hyacinth became an invasive weed resulting from its rapid growth rate and lack of natural enemies. This rapid growth stems from a high nutrient uptake rate which can be employed to remediate eutrophic water. This study consisted of two parts and investigated the potential for water hyacinth to be used as a tool for the phytoremediation of eutrophic dam water in summer and winter. In summer, individual hyacinth plants were grown in either Hoagland's solution or water from Hartbeespoort Dam, with another set of vessels containing Hartbeespoort Dam water without plants. In winter, only water from Hartbeespoort Dam was used with vessels either containing 10, 4 or no plants. The results show that in summer water hyacinth can remove phosphate and nitrates at a rate of 0.378mg/L and 0.365mg/L per day respectively, while in winter those rates drop to 0.056mg/L and 0.070mg/L per day respectively. It has also been demonstrated that an algae bloom can be expected if the water hyacinth is removed from or allowed to die in eutrophic water where algae spores are present. This study suggests that water hyacinth can be employed in an in-situ steady state system to remediate eutrophic water bodies and reduce the occurrence of algae blooms.

DECLARATION

I, Jessica Elizabeth Auchterlonie, student number 803863, declare that the thesis entitled “The phytoremediation potential of water hyacinth” is my own work except as indicated in the references and acknowledgements. It is submitted in fulfilment of the requirements for the degree of *Master of Science* in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in this or any other university.

A handwritten signature in black ink, appearing to read 'Jessica Auchterlonie', with a horizontal line drawn through the middle of the signature.

Jessica Elizabeth Auchterlonie

Signed at AUCKLAND

On the 26th day of September 2020

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LIST OF TERMS AND ABBREVIATIONS

BOD biological oxygen demand	20, 21
C Control experiment	viii, 29, 44–46
COD chemical oxygen demand	21
DO dissolved oxygen	14
FP Four Plants experiment	vii, viii, 29, 42, 44–46, 53
H Hoagland solution experiment	26, 28, 35–37, 39
HABs harmful algal blooms	6, 7
HB Hartbeespoort Dam water experiment	26, 28, 35–37, 39, 40, 53
HBW Hartbeespoort Dam water without hyacinth experiment	vii, ix, 26, 28, 35, 36, 38–40
TP Ten Plants experiment	vii, viii, 29, 42, 44–46, 53
TSS total suspended solids	20, 21

NOMENCLATURE

c	Concentration ($\text{mg}\cdot\text{L}^{-1}$)
c_0	Initial concentration ($\text{mg}\cdot\text{L}^{-1}$)
r	rate ($\text{mg}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$)
t	time (day)

CHAPTER 1

Introduction and problem statement

1.1 Background

Water is a critical resource across the globe. It is necessary for daily life for drinking, in food production and agriculture, raw material extraction and other sectors. Approximately 70% of global water usage is by the agricultural sector, and this percentage is projected to increase as the global population increases; without a subsequent increase in the efficiency of agricultural production [1]. The average rainfall across the globe is approximately 1000mm annually [2], however, rainfall is not evenly distributed. It is estimated that 785 million people lack access to a potable drinking water source within a 30 minute round trip, 144 million of whom rely on collecting untreated surface water [3].

South Africa is classified as a water-scarce country as it receives approximately half the rainfall of the annual global average and at least 80% of the available water in the country is withdrawn annually for agricultural, domestic, and industrial use [4, 5]. More than 500 dams have been built across the country to store this valuable resource. However, a study conducted over 10 years, from 2002 to 2012, revealed that 62% of South Africa's 50 largest dams were hypertrophic [6]. The eutrophic conditions are mostly as a result of anthropogenic activities such as agricultural runoff and sewage infrastructure breakdowns [7]. Another main source of pollution in South African dams is acid mine drainage (AMD) [8].

Water pollution threatens the already limited water resources in South Africa. A large contributing factor to water pollution is urbanisation. As urbanisation increases, there is a greater amount of physical disturbance to the land as houses, roads and industries are built. The construction of these entities requires multiple chemicals which can cause chemical pollution of the land and water. Rapid urbanisation and expansion put pressure on sewage systems, and the failure of infrastructure can result in sewage entering the water system through groundwater. The demand for natural resources increases as a population grows and industries will expand to keep up with this demand. Energy production, mining and agriculture are three such industries. As the energy industry increases and expands there is a greater demand for coal, putting pressure on the mines, and increased air pollution which

mixes with rainwater and can result in acid rain. As mines expand and increase production there is greater incidence of acid mine drainage in the water system. Increased demand for agriculture has a twofold impact on the water system. Agriculture is a water-intensive industry, as such increased production will increase the water usage by the agricultural sector [4]. Increased agricultural production will require an increase in the use of fertilizers, which are washed into rivers with surface runoff. The fertilizers bring excess nutrients into the water system leading to eutrophic conditions in rivers and dams [8].

Eutrophication substantially affects water quality in South Africa. These eutrophic conditions can, and have, resulted in many deleterious effects on the environment surrounding the dams and the people who rely on the water. The increased nutrient concentrations provide nourishment for algae in the water and increased algae growth rates can result in algae blooms. Certain species of algae, such as cyanobacteria (blue-green algae), produce toxins that are harmful to humans and animals. Some of these toxins may pass through the water purification process and find their way into drinking water, or be ingested by fish and other animals where they may become part of human meals. Those who rely on the rivers for water for domestic use are at the greatest risk [9, 10].

Water Hyacinth (*Eichhornia Crassipes (Mart.) Solms.*) is an aquatic plant native to the Amazon Basin. It was spread from South America to many parts of the world, including South Africa and Australia, as an ornamental flower [11]. Although the water hyacinth is a tropical plant, it has thrived in the new environments, partly due to the lack of natural enemies in these areas [7]. In South Africa, the hyacinth has become a problematic invasive species. It has a high growth rate which is exacerbated by the eutrophic conditions in many South African rivers and dams. A water hyacinth mat's edge can expand by up to 60m/month, in favourable conditions and its seeds remain viable for up to 20 years, on the bottom of the infested water body [12, 13]. The resulting mats can become thick enough to stop the sunlight from penetrating the water, which reduces the photosynthesis of the other aquatic plants below the surface and often results in their death. While this is problematic, a greater problem arises when the water hyacinth dies, as the bacteria responsible for its decomposition use the dissolved oxygen in the water. The depletion of oxygen can cause hypoxic conditions, which can become anoxic/anaerobic in extreme cases. This can result in the death of fish and other aquatic life in the water body [7, 14]. The death and decay of the hyacinth plants can also lead to the re-release of stored nutrients and other contaminants.

South Africa has experienced water hyacinth blooms in the past; however, once the blooms were under control in the early 1990's the government decreased research into eutrophication as it was no longer deemed a priority, and funding was consequently reduced [15]. While large water hyacinth blooms have many detrimental effects on the surrounding environment, there

are some advantages to its growth. The hyacinth plant is capable of absorbing many types of impurities in the water during its growth stage. They can take up large quantities of nutrients, a portion of which are stored and the rest are used for growth. The total nitrogen removal is proportional to the initial nitrogen concentration [16]. Water hyacinth can absorb heavy metals and phenols at low concentrations without detrimental effects to the plant [17–19]. The plants have also been found to reduce biological oxygen demand (BOD) and total suspended solids (TSS). The presence of water hyacinth can reduce the algae growth in a water body through attachment, settling, respiration and growth suppression [20].

Many methods of controlling the water hyacinth have been tried over the years, with varying success in different environments. The three main methods for hyacinth control are mechanical, chemical and biological control [21]. Mechanical control is labour intensive and involves removal by hand or using machines. This can result in fragmentation of the plant, when small pieces of roots and stems are left behind that are able to regrow, which can result in reinfestation [22, 23]. Chemical or herbicide control is less labour intensive, however, it requires specific equipment and knowledge to implement. Applying chemicals to the plants in-situ can negatively impact the water quality, and directly impact animals and non-target plants [24]. Biological control involves releasing natural enemies of the plant which attack the plants, reducing the growth rates and potentially causing plant death. This type of control has had greater success in tropical climates similar to that of the Amazon than in more arid environments such as South Africa, however, it can take many years before the effects are noticed, even in tropical areas [24]. Integrated control is a fourth method of control that combines two or more of the methods, usually chemical and biological control. In South Africa, integrated control is the method with the best historical success rate [24].

1.2 Scope

The intent of this study was to determine the phytoremediation potential of water hyacinth in eutrophic water on a small scale in summer and winter. This study used Hartbeespoort Dam as a case study. In order to accomplish this, two separate experiments were conducted: Experiment one was conducted in summer and Experiment two was conducted in winter. Experiment one consisted of six replicates of Hartbeespoort Dam water with no plants, six replicates of Hartbeespoort Dam water with one water hyacinth each and six replicates of Hoagland's solution with one hyacinth plant each. Experiment two consisted of six replicates of Hartbeespoort Dam water with no plants, six replicates of Hartbeespoort Dam water with four plants each, and six replicates of Hartbeespoort Dam water with ten plants each. The plant growth, phosphate and nitrate concentrations over time and algae growth were measured to determine the ability of water hyacinth to remediate eutrophic dam water

1.3 Outline

This thesis consists of seven chapters. Chapter two presents a review of the literature behind this research project. Chapter three explains the methods and materials used to conduct experiments. Chapter four is an article based on some results which has been submitted for peer review publication to the South African Journal of Chemical Engineering. As the article is presented as submitted, the reader's indulgence is requested as there will be some repetition from other sections. Chapter five contains the results and discussions of further experiments. Chapter six is an overall discussion chapter summarising the data discussed in chapters four and five. Finally, chapter seven concludes the research.

CHAPTER 2

Background

2.1 Introduction

Water hyacinth is believed to have been introduced to many countries, including South Africa, from South America as an ornamental flower [11]. These plants flourished. They spread quickly in the countries they were introduced to as they had no natural enemies and they produce large numbers of seeds that can remain viable for up to 20 years. Water hyacinth can also double in size within a week under the correct conditions [12]. Many factors influence the growth rate of the water hyacinth, including the nitrate and phosphate concentrations in the water, the pH and the season.

There have been many attempts to remove and control this invasive plant, with varying success [25]. In South Africa specifically, control attempts have been hindered by the extreme eutrophication in many of the country's dams, since, in these conditions, the plants grow at an increased rate. The diversity of climatic regions within South Africa has also made it difficult to produce a standard control procedure from a successful experiment [26]. If complete removal is not achieved and maintained the water hyacinth will regrow, from even the smallest cutting. Another issue arising from the control of water hyacinth is that if the plants remain in the water, after chemical or biological control, they will decompose and create anoxic conditions which negatively affects the other aquatic life. [21, 27]

The failure to adequately control water hyacinth has led researchers to try to find ways to utilise the plant. The hyacinth can absorb and store large concentrations of nutrients which has made it ideal to use as compost and fertiliser in lower-income countries. Among other uses such as animal fodder and papermaking, the next most prominent uses for water hyacinth are as a biofuel and for water cleaning [21, 28].

While there are many disadvantages to the growth of water hyacinth, the remediation potential of the plant is well known. The plants have been shown to remove up to 95% biological oxygen demand and up to 70% of the nitrate concentration [29]. Their presence has also been linked to a reduced number of algae blooms and cleaner drinking water [30].

2.2 Water in South Africa

South Africa is classified as a water-scarce country [31]. In the period between 1991 and 2015, South Africa received an average of 490mm of precipitation per year [4] while the global average is approximately 1000mm per year [2]. Approximately 60% of the water in South Africa is used for agriculture, while only 11.5% is used domestically [8]. South Africa is a semi-arid country and only 8% of the land area collects the runoff which produces 50% of the water volume in the river systems [32].

Rand Water defines water pollution as water which is not safe or healthy for people to drink or bathe in, and it has the greatest effect on people who rely on water straight from dams or rivers. According to Rand Water, there are several main causes of water pollution in South Africa. These causes include urbanisation, deforestation, industries, mining, agriculture and energy generation [8]. As a result of scarcity, many dams have been built and the pollution causes these dams to become eutrophic. Another threat to South African water systems is invasive plants, which often use more water than the native species which have adapted to live in reduced water conditions. These invasive species have been responsible for up to 4% of the consumption of available water [32].

2.3 Eutrophication as a global issue

Eutrophication is a growing problem worldwide. It occurs when there is an abundance of nutrients in a still water body [33]. Depending on the context, eutrophication can refer to the process by which the water becomes enriched only or both the process of enrichment and the subsequent problems it causes [34]. Many studies have been done on the causes and effects of eutrophication.

2.3.1 Algae bloom

In 2003, a roundtable discussion was held to come to a consensus of the relationship between eutrophication and harmful algal blooms (HABs). The result of this discussion was seven unanimous consensus statements relating eutrophication and algal blooms. A paper was published in 2008 summarising the consensus statements and providing evidence for each one [35].

A summary of these statements can be found below [35]:

1. The increase in nutrient concentration, and hence decrease in water quality, aids the growth of many types of algal blooms;
2. “The composition – not just the total quantity – of the nutrient pool impacts HABs”;

3. Algal blooms which create high yields of biomass need an external source of nutrients to be sustained;
4. Algal growth can be perpetuated by both continuous and periodic exposure to high nutrient levels;
5. Advancements in technology have improved the detection and prediction of algal blooms and their associated toxins;
6. Studies need to be performed to improve the understanding of how these algal blooms respond to nutrients; and
7. “Management of nutrient inputs to the watershed can lead to significant reduction in HABs.” [35]

2.3.2 Water hyacinth as an exacerbating factor of eutrophication

Alongside the problem of algal blooms in eutrophic water is the problem of water hyacinth growth. This plant has a rapid growth rate and a small increase in the available nutrients can lead to a large increase in plant growth.

Many studies have been performed on the growth of water hyacinth over the decade, with a large number of these occurring in the 1970’s and 1980’s. Funding for these studies, particularly in South Africa, decreased during the 1990’s and 2000’s. Many studies have been conducted on this topic [16, 20, 36–39] and the most relevant will be reviewed.

2.3.2.1 Growth of water hyacinth

A study called “The Phenology and Growth of Water Hyacinth (*Eichhornia Crassipes* (Mart.) Solms) in a Eutrophic North-Central Florida Lake” was performed in 1981 [36]. This study was conducted over a year to monitor the plant growth as the seasons changed. The growth rate was monitored by measuring several plant parameters, such as the maximum leaf length, number of leaves per plant, leaf area, plant density, biomass distribution and root/shoot ratio.

Center and Spencer [36] found that water hyacinth has three growth phases that occur depending on the time of year. The first phase occurs in winter when the plant can suffer from frost damage. During this phase, the distribution of plant weight changed. The proportion of plant weight that was accounted for by the leaves dropped from between 70-75% to between 30-40%. This is largely as a result of frost damage done to the new leaves. In early spring it was recorded that the plants were increasing branching and ramet (daughter plant) production and increasing the number of stems. This was determined to be the second phase. In the third phase, the leaf size increased and the number of leaves per rosette became relatively constant. This occurred in late spring. Along with the three main phases of growth mentioned above, the

article also goes into more detail with regards to the seasonal changes in plant size and growth. It was found that plant density is at its highest in April (early to mid-spring). The density decreases slightly over the change from spring to summer thereafter remaining relatively constant. Autumn brought a slight increase in plant density which was stopped by the frost and in winter it decreased as a result of freezing temperatures. There is a direct correlation between the temperature of the water and the nutrient uptake by water hyacinth [40].

The leaves are a prominent indicator of growth and general health of the plant. During the winter months the leaves become brown and decrease in slightly in size, there are also usually few leaves at this time of the year. In spring the average leaf length increases, with the greatest rate of increase occurring in early spring and a decrease in leaf density occurring in late spring. Relatively constant leaf size was observed in summer, provided that the plants were mature. In autumn, a slight increase in both average leaf length and area was observed followed by a rapid decrease in both parameters. It is interesting to note that the number of leaves per plant remains relatively constant, between 6 and 8 leaves, from late winter to early autumn.

Center and Spencer [36] presented the most prominent factors affecting the growth of water hyacinth. The ambient nutrients and water temperatures were the first set of factors discussed. They found through their sources that $0.1 \text{ mg PO}_4^{3-} / \text{L}$ is the limiting concentration for water hyacinth to grow. By using a ratio of nitrate:phosphate uptake rate of 5-10:1, they found that the limiting concentration for nitrates is $0.5\text{-}1 \text{ mg NO}_3^- / \text{L}$. In order for water hyacinth growth to be sustained, the ambient air temperature must be between $12\text{-}38^\circ\text{C}$ and, for optimum growth, it should be between $22\text{-}35^\circ\text{C}$. For their experiment, the ambient temperature was within the optimum range from mid-spring to mid-autumn, and was almost always within the temperature to sustain growth.

The second factor that was discussed was the competitive interactions between the plants. They found a relationship between population density and plant size. The plants tended to be bigger when the population density was low, and vice versa when the population density was high, the plants tended to be smaller. They surmised that the lower population densities resulted from the smaller plants not being able to compete with the larger plants for sunlight, resulting in the death of the smaller plants and, hence, fewer plants in the area.

Another factor discussed is the effect of external environmental factors such as solar radiation. This factor is closely related to the effects of temperature and it was difficult to separate the effects from temperature and those from solar radiation. However, they considered a combined effect of both and found that the size of the plants correlated with this factor. Plotting the leaf area versus solar radiation gave a correlation of 0.95 and there were very few deviations from

the value of leaf area per unit solar radiation.

In a related study, Kim *et al.* [20] observed that the growth of water hyacinth can suppress the growth of algae in eutrophic water. This occurs as the plants block the sunlight from reaching the algae. The roots of the water hyacinth also acted as a filter to remove algae particles in a process similar to adsorption.

2.3.2.2 Water hyacinth growth rates

Water hyacinth was shown to have a rapid growth rate, especially when there are abundant nutrients available. Reddy and DeBusk [37] conducted an experiment to determine the growth characteristics of three types of aquatic plants, including water hyacinth. Their experiment found that water hyacinth had three distinct growth phases. Its growth had an initial lag phase prior to exponential growth. This was followed by a linear growth phase after which the growth rate became stationary. The maximum growth rates were incurred in the linear growth phase.

In comparison with other plants, water hyacinth had the highest biomass yield of 106 t (dry weight)/ha·year, where the water lettuce and pennywort had growth rates of 72 t and 41 t (dry weight)/ha·year. They found that the plant density during the linear growth phase (operational plant density) was between 500-2000 g dry wt/m², 200-700 g dry wt/m² and 250-650 g dry wt/m² for water hyacinth, water lettuce and pennywort respectively.

Seasonal changes had a significant effect on the growth rate of the aquatic plants, but not on their operational density. Reddy and DeBusk [37] found an inverse relationship between the specific growth rate and the plant density: at low densities the growth rate was high and at high densities the growth rate was low.

2.3.2.3 Nitrogen and water Hyacinth Growth

In their article titled “Influence of Nitrogen Supply Rates on Growth and Nutrient Storage by Water Hyacinth (*Eichhornia crassipes*) Plants” [38], Reddy *et al.* went into greater detail about how water hyacinth responds to changes in nitrogen (N) concentrations, which the previous article mentioned. This article aimed to determine the relationship between nitrogen supply and water hyacinth growth, the effect of nitrogen concentration and plant mass on the ability of the plant to store the nutrients and the relationship between the nitrogen concentration and the storage of phosphorus and potassium.

The experiment was conducted over 83 days. 1000L concrete vaults were filled with 900L of nutrient solution each with a different concentration of nitrogen: 0.5, 2.5, 5.5, 10.5, 25.5 and

50.5 mg N l⁻¹. The experiment was conducted in duplicate. 3 mg/L of phosphorous (P) and 25 mg/L potassium (K) were added and the remaining micronutrients were added in concentrations equivalent to modified Hoagland's solution. To ensure there was always sufficient nutrients for the hyacinth, the solution was replaced every week.

The plants were placed in identical mesh baskets, with a plant density of 4.5 kg/m². Each week the baskets were removed, allowed to drain and weighed. Two plants were removed from each vault every week and were dried at 70°C for 3 days, after which their dry weight was recorded. The plant samples were digested using nitric-perchloric acid and the N and P content was determined using an autoanalyzer and the potassium content was determined using an atomic absorption spectrophotometer. Water samples were taken before the nutrient solutions were replaced and analysed for ammonia, nitrates and ortho-phosphates.

Reddy *et al.*[38] observed an increase in plant density from 13-16 kg/m² in the first month to 36-60 kg/m² in the third month. While the plant density increased, the growth rates decreased from 23-40 g/m²·day to 6.3-26.9 g/m²·day. This led them to conclude that when growing at their highest densities, the plant growth and ramet production was at its lowest. They determined that the biomass increased with increasing nitrogen concentrations up to 5.5 mg/L, and the higher concentrations did not produce a higher biomass yield. The authors found through literature that other experiments showed optimal growth rates occur at a plant density of 20 kg/m².

It was found that the nitrogen storage in the plant tissue did not follow the same pattern as the biomass yield. While the biomass yield remained constant after a point, the nitrogen storage continued to increase as the initial nitrogen concentration was increased. They also found a relationship between the storage of nitrogen and the plant density. The authors concluded that these results showed that water hyacinth had a large capacity to store nutrients even though these nutrients may not have affected the growth of the plant. This conclusion was also made by Fox *et al.* [16]. They observed that the storage of nutrients was ephemeral and the leaves that die will decompose and release the stored nutrients back into the water.

In the nitrogen-limited vaults, more nitrogen was recovered than was added through the nutrient solutions. They attributed this to nitrogen in the rainfall and variances in the nitrate concentration of the well water used to create the nutrient solutions, as well as experimental error. They also speculated that the results could be due to increased biological fixation caused by detrital accumulation in the roots under nitrogen-limited conditions.

It was found that the maximum plant growth and ramet production occurred when there was a nitrogen content in the plant tissue of 14 mg N/g, and similarly to the biomass, an increase in

nitrogen content did not significantly increase the productivity after this point. It was also found that plant densities above 42 kg/m² had the least significant effect on biomass production.

In the nitrogen-limited vaults it was found that nitrogen storage was affected by the plant density, while in the high nitrogen vaults, the nitrogen storage was not affected by the plant density. When the other nutrient storage was examined, it was found that both P and K were proportional to the biomass increase. An increase in nitrogen concentration only increased the phosphorous storage up to an N concentration of 5.5 mg N/L and only increased the potassium storage up to an N concentration of 2.5 mg/L, after which both remained fairly constant. The phosphorous storage was also influenced by plant density.

2.3.2.4 Phosphorous and water hyacinth

Reddy *et al.* conducted a similar experiment in 1990 on the influence of phosphorous on plant growth and nutrient storage [39]. This experiment was set up in the same manner as the nitrogen experiment. The difference between the two experiments was that the phosphorous concentration was the manipulated variable in this study. The starting phosphorous concentrations were 0.06, 0.26, 0.56, 1.06, 2.56 and 10.06 mg P/L and the nitrogen and potassium concentrations were kept constant in each vault at 20 and 25 mg/L respectively. The micro-nutrients were also added in concentrations equivalent to modified Hoaglands solution. These solutions were also replaced weekly.

The following parameters/methods were the same for this experiment as for the nitrogen experiment:

- Vault capacity
- Initial plant density
- Weighing methods (wet and dry)
- Water sampling regime
- Water changing schedule
- Nutrient storage determination

Many of the results found in this experiment were similar to those observed in the nitrogen experiment and, as such, only a summary of these are provided.

- Plant growth increased with increasing P until a concentration of 1.06 mg P/L; anything greater did not increase the growth;
- Productivity was highest at the beginning of the experiment and decreased as plant density increased;
- Phosphorous storage increased with P concentrations and plant biomass, however, after the first 2 months the phosphorous storage decreased despite an increase in plant mass;

- Maximum productivity was reached within the first month, after which an increase in stored P did not increase productivity;
- The stored P in the vaults with low P concentrations decreased in the first week as the plant biomass increased and there was competition for the limited nutrients;
- Stored phosphorous reached steady-state, which indicated nutrient cycling was sufficient to sustain the stored P;
- In the high concentration vaults the P stored was proportional to plant growth until plant density had increased significantly;
- Once plant density had increased significantly, less P was available per unit biomass which resulted in a decrease in stored P;
- Increased availability of P led to increased storage of N and K; the maximum uptake of N and K took place at P concentrations of 256 mg P/L and 1.06 mg P/L respectively;
- The phosphorous uptake was proportional to the N availability, which Reddy *et al.* took to mean the use of P in water hyacinth depended on the ratio of P:N as well as the P concentration; and
- Hyacinth can store large amounts of P.

2.4 Hyacinth control and removal

2.4.1 Physical control

Physical control can be broken down into two subsections: control by hand-tools and control by mechanical equipment. Both methods work using the same principle of physically pulling the hyacinth out of the water. The hand-tool method requires pitchforks while the mechanical method requires specialised equipment. The benefit of physical control is that less plant matter is left in the water to decay, as it is the decomposition process which removes oxygen from the water resulting in the death of other aquatic life and no chemical herbicides or biological agents are introduced into the water. [21]

The disadvantage of physical control is that it is only suitable for small areas and small pieces of plant or seeds could be left behind in the water, which could result in a regrowth of hyacinth. Using hand-tools is also labour intensive and the mechanical method, while it still requires some labourers, is expensive due to the machinery required. The labour requirement, however, could be seen as an advantage in South Africa as it creates jobs. [27]

Although the removal of water hyacinth is desirable, its extraction can lead to an overall decrease in the quality of water in the system. A short-term study in Mexico involved testing the water monthly from a year before the hyacinth was removed and continuing the testing for

a year after the removal [41]. A longer-term study also had the water testing monthly over the 8 year period [30].

Both studies found that removing the water hyacinth leads to overall poorer quality of water. The amount of nutrients in the water increase as the hyacinth was no longer there to remove it, Mangas-Ramírez and Elías-Gutiérrez found that the nitrate and nitrite concentrations increased by approximately 320% and 450% respectively and the ammonia concentration in the water increased from 3mg/L to 60mg/L [41]. The phosphates in both studies were also found to increase.

It was observed in both studies that the increased nutrient concentrations lead to algal blooms. Bicudo *et al.* [30] observed that there were periodic algal blooms before the removal and after the removal, they occurred permanently [30]. Many of the species in the blooms were found to be toxic. The pH was found to increase, largely as a result of the algal blooms [41]. The presence of the algae, as well as the increased nutrient concentrations, lead to decreased water visibility as measured by Secchi disk transparency.

Bicudo *et al.* recorded the dissolved oxygen in the water at the surface and at the depth of 2.5m. They found that dissolved oxygen at the lower level was significantly lower than the surface concentrations, with anoxic periods occurring frequently at the lower level once the hyacinth was removed. This was determined to be the result of the remaining plant mass sinking and decomposing. With their depth studies, they also found that the removal of the hyacinth lead to an increase in the temperature difference between the surface and the bottom of the lake [30].

2.4.2 Chemical/herbicide control

Chemical control of water hyacinth involves spraying the plants with a herbicide. This method requires skill to apply the herbicide on the correct plants. Chemical control is a more expensive option as the herbicides are relatively expensive and are often applied from the air [21].

The two main advantages of chemical control are that the herbicide kills the plants within a short period (usually within 6 weeks) and the herbicide kills the entire plant so there is no chance of the hyacinth growing back from small pieces. This method is also not labour intensive. The disadvantages are that it can cause greater water quality problems as the hyacinth is left in the water to decay, causing anoxic conditions. The herbicides can also drift on to local species, causing unintended damage, and contaminating the water [21].

A study was conducted in India to observe the effect that two different types of herbicide, 2,4-D and Paraquat, at two different concentrations have on the water quality and fish populations. This study was started in 2006 and repeated in 2007 and 2008. The two concentrations that were used were the recommended concentration and double the recommended concentration (it was not specified what these values were). The experiment was set up with 10 430L concrete tanks, 5 tanks had water hyacinth and the other 5 did not. In each set of 5, there was a control with no herbicide, and the other 4 had the two concentrations of both herbicides respectively [42]. It was found that the concentration of both herbicides decreased over time and neither left a residue, however, 2,4-D took 30 days to for this to happen while Paraquat only took 20 days. In terms of dissolved oxygen (DO) content, it was found that both herbicides caused a decrease in DO, although this effect was more pronounced in the tanks with water hyacinth. This was found to be due to the decomposition of the plants. They also found that Paraquat caused a greater decrease in DO than 2,4-D hence they concluded that 2,4-D would be the better option for treating the hyacinth [42]. It was also found that 2,4-D only caused fish mortality in the third year (2008) in the tank with the higher concentration. Further study revealed that this herbicide accumulates in the fish over time. In the tanks containing Paraquat, it was found to cause fish mortality in the first year (2006) in all 4 tanks. The authors concluded that it was the lower dissolved oxygen content which had a greater effect on fish mortality than the herbicides [42].

2.4.3 Biological control

One of the reasons water hyacinth has become such a problem in South Africa is that it is an invasive species. This means that it has no natural enemies in this country, such as pathogens, insects or browsers. Biological control is the process by which these natural enemies are brought to the invading hyacinth as a means to reduce its growth. Several studies have found that biological control works best in tropical areas and larger water bodies [25, 43]. The advantages of biological control are that it is relatively inexpensive and low maintenance as well as being the most environmentally friendly alternative, however, this process can take many years, up to 10 in South Africa, to produce a result [21].

Many biocontrol programs have been attempted in South Africa with little effect [25, 44]. In their paper, Biological Control Initiatives against Water Hyacinth in South Africa: Constraining Factors, Success and New Courses of Action [43], Hill and Olckers investigated the factors that influenced the success of biocontrol in South African dams. They discussed how the wide variety of different climatic zones in South Africa impacted the effectiveness of the biocontrol agents.

The effectiveness of the biocontrol agents is most often compromised by cold weather and, more specifically, frost. The cold temperatures can cause both the plant and insects to become dormant and, when the weather becomes warmer, the plants respond faster than the insects. The insects are then not able to inflict enough damage to the plants to make a significant difference before the weather turns cold again [43].

A second factor to be found influencing the effectiveness of biocontrol is the highly eutrophic state of many South African dams and lakes. The high concentrations of nutrients in the water allows for high growth rates of water hyacinth. Hill and Olckers found that in most cases these high growth rates negated the damage caused by the insects, however, they say there is the potential for the increased nutrient load to lead to an increase in the insect population as more food will be available for the insects. There can also be interference with the biocontrol agents from herbicides, as this has historically been the main method for dealing with hyacinth in South Africa and the majority of the herbicides are toxic to the biocontrol agents [25, 43].

There have been some successful biocontrol experiments in South Africa, the most notable of which, according to Hill and Olckers, occurred on New Year's Dam in the Eastern Cape province of South Africa. Weevils were released onto the hyacinth which covered approximately 80% of the dam and, within 4 years, the surface coverage was less than 10%. The water hyacinth started to grow again and within another 4 years was covering 80% of the dam again, however, the existing weevils (no new insects were released) reduced this back to 10% within the next 2 years [43].

2.4.4 Integrated control

The integrated control method involves combining two or more of the methods mentioned above. The most commonly used combination is chemical and biological control. Mechanical control is occasionally added if there are dense mats of hyacinth that prevent boats from passing through. The only limits to this control method are the relative cost-effectiveness of the different control methods and the balance of potential benefits and risks [21].

An example of this occurred in 1995 in Kwa-Zulu Natal. There had been episodic unmanaged chemical control applied to the Nseleni/Mposha River system for approximately 20 years before the implementation of the integrated control system. Occasional heavy flooding picked up the hyacinth and dragged it away leading to a decline in the already haphazard control system, according to R.W. Jones [26].

The Integrated Water Hyacinth Control Committee was formed in Kwa-Zulu Natal, South Africa, in 1995 to organise the control of water hyacinth. They began by mapping the affected

area and splitting it into manageable sections with groups assigned to each. The first 4 sections were designated for total control of the hyacinth using any methods available and the other 4 were scheduled for containment by biocontrol only. The integrated control of sections 1-4 involved installing permanent booms under the water surface to trap the water hyacinth by its roots which aided the implementation of chemical control by having all the plants in one area. The effect of the herbicide (glyphosate) and biocontrol (weevils) was monitored [26].

The following year the management plan was updated to include a fifth section in the total control program and another weevil species was released. By the end of 1996, the total control program included seven sections. The management plan was followed and updated as necessary in the following years. At the time this article was written, in 2000, 22 km of the river system had been cleared using this method, and only occasional follow-ups were required to ensure there was no new growth [26]. As indicated, water hyacinth can be controlled, however, the correct method of control is often difficult to implement and not infallible.

2.5 Water hyacinth uses

Water hyacinth is a problem worldwide and, as a result of its rapid growth rate, there are large supplies of this plant readily available. As it is difficult to remove, many people have found alternative solutions to the water hyacinth problem that make the hyacinth a resource.

2.5.1 Fertiliser

The most widespread use of water hyacinth is for fertiliser because the plant stores nutrients from the water. It is used in different ways in different contexts. It can be used as soon as it comes out of the water as “green manure” where it is either ploughed into the soil or laid on top. The hyacinth can also be left to dry for a few days and then mixed with ash and animal manure after which it is left in piles where microbial decomposition breaks down many compounds making it well suited as a compost. This process works well in warmer climates, like South Africa [27, 45].

2.5.2 Charcoal briquettes

Another use of water hyacinth as a fuel is to create charcoal briquettes from the plant, which presents an alternative to solid fossil fuels. There are two main methods which have been proposed for the production of water hyacinth briquettes. The method was a concept developed in Kenya [27]. It involved drying the hyacinth and burning it in a kiln. The resulting ash was to be mixed with a binder and pressed into briquettes.

The second method involved mixing empty fruit bunch (EFB) fibres with water hyacinth (WH) in 5 different ratios. The water hyacinth was dried and then blended to 850 μm . The water

hyacinth was then mixed with the EFB in ratios of 100:0, 90:10, 75:25, 50:50 and 25:75 (WH:EBF). Each mixture was added to a binding agent and then put into a cylindrical mould. Experiments were conducted to determine the moisture content, volatile matter content, ash content and fixed carbon percentage of each ratio briquette. These results were also compared to the values of current coal briquettes[46].

It was found that the ratio 25:75 was the most efficient overall, as it has the lowest moisture, ash and fixed carbon content (high moisture content can lead to incomplete burning). This particular briquette had a calorific value of 17.17 MJ/kg. Whilst this value is lower than that of coal (32–42 MJ/kg) it is acceptable as a biofuel. The briquette of this composition also gave the lowest gas emissions. In this article, the authors concluded that this biomass briquette has a high potential for use as a biofuel [46].

2.5.3 Water treatment

Water hyacinth grows best in waters with high nutrient concentrations and, as discussed in Section 2.3.2, can store nutrients even when not using them for growth. This makes the plant suitable for cleaning water. The water hyacinth can be used for both domestic effluent and industrial effluent treatment. More detail on this use can be found in Section 2.7.

2.5.4 Biofuel feed stock

Biofuel is becoming a much more valuable resource as the prices of natural gas and petrol continue to rise. There are several different types of biofuel that fall under two main categories: first-generation and second-generation. The first-generation biofuel feedstocks are all crops such as sugar, starch and vegetable oils. These biofuels are the most widely used but they are undesirable as they put pressure on food sources. The feedstocks for second-generation biofuels do not compete with agricultural resources and are usually waste or by-products from other processes. At this point, second-generation biofuels are still being developed [47].

Water hyacinth has been investigated for use as a second-generation biofuel feedstock as its rapid growth ensures there is a sustainable supply. More detail of types of water hyacinth biofuel can be found in Section 2.6.

2.5.5 Other uses

There are many other uses for water hyacinth such as making paper and using the fibres to make rope and baskets. It has also been used as an animal feedstock and fish feed [27, 48].

2.6 Biofuel production

This Section presents more detail about the methods of production and the outcomes of biofuel experiments. In their paper, Water Hyacinth as a Potential Biofuel Crop, Bhattacharya and Kumar [28] gave several reasons why water hyacinth is a better option for the production of biofuel than terrestrial plants. Firstly, it is potentially less energy-intensive to produce. The plant has a lower lignin content than terrestrial plants (10% as opposed to 15-30%), this compound surrounds the cellulose and protects it from chemical attack. These lignins need to be degraded before the cellulose fermentation can begin. These processes are energy-intensive and expensive, as they are relatively new, therefore the water hyacinth, which has a lower content, will result in lower costs.

Water hyacinth, and other aquatic plants, have a waxy coating on their stems and leaves which are “modified fatty acid deposition[s]”. According to reference [28], these depositions help to increase the biofuel yields. The final reason given in this paper for using water hyacinth instead of crops is the growing shortage of food, as explored in Section 2.5.4.

2.6.1 Bioethanol

Ethanol is typically produced from feedstocks such as corn and sugarcane, as they contain high levels of fermentable carbohydrates and do not need to be substantially pre-treated [49]. As water hyacinth does not contain high levels of carbohydrates, its cellulose needs to be broken down to be able to ferment the hyacinth.

One method of doing this is using sulfuric acid saccharification (hydrolysis of polysaccharides). Masami *et al.* [50] investigated the optimum sulfuric acid concentrations and heating time required to break down the complex carbohydrates into simple sugars. Their method consisted of blending a crop of water hyacinth in distilled water and allowing it to dry overnight and then removing large particles with a sieve. An amount of the water hyacinth powder was added to sulfuric acid and heated in an autoclave at 121°C. The solution was filtered and neutralised and then fermented. The control for this experiment was Japanese cedar, which was subject to the same hydrolysis procedure.

They found that, after hydrolysis, water hyacinth produced a greater concentration of sugar than the cedar. They attributed this to the hydrolysis conditions not being optimal for the Japanese cedar. The sugars produced were then fermented using 13 different strains of yeast to find the optimum strain to ferment water hyacinth. This strain was found to be *Candida intermedia*, and it produced 1.77 ± 0.4 g/L of ethanol.

A similar experiment was performed in 2007 in Thailand [51], in which the xylose and glucose concentrations were measured and a comparison was made between the result achieved using 1% sulfuric acid and 10% sulfuric acid. It followed similar procedures such as blending the hyacinth and drying it, however, the size of the hyacinth particles was approximately 3 times larger and they were dried at a higher temperature. By increasing the sulfuric acid content from 1% to 10%, the authors found that there was a six to ten-fold increase in the amount of xylose present after saccharification, which increased the yield of ethanol. They stated that the rate of degradation depended on the acid concentration and the temperature, and that the neutralisation process at high temperatures generally resulted in greater ability to ferment. Their final result yielded a concentration of 1.01 g/L of ethanol using 10% sulfuric acid.

2.6.2 Biogas

Biogas is one of the most versatile options as a biofuel as it can be made from a wide variety of feedstocks without much pre-treatment. [49]. Currently, the main sources of biogas are from animal manure, crops and municipal waste. Water hyacinth is a good potential feedstock for biogas production, as it has a favourable carbon to nitrogen ratio, however, there are several obstacles that need to be overcome such as the presence of lignins and high water content [48].

In 1993, a study was carried out to determine the difference in biogas production using three different feed materials: urban garbage, fresh water hyacinth and dried water hyacinth. This study was conducted over 278 days in anaerobic digestors. Small amounts of each material were fed into their own 2L fermenter at an average rate of 1g/L·day [52]. The feed materials all had low bulk densities, meaning a large volume of material was required for a relatively small return. Chanakya *et al.* [52] documented the change in the material characteristics and found that the bulk densities of all the materials at the end of the experiment had decreased, leading them to suggest that the waste material can be easily disposed of once spent [52].

All three feed materials reached a maximum gas production of 1 L/day for 1 g total solids/day of feed, after which the production decreased. The garbage and dry water hyacinth reached their maxima after 150 days while the wet hyacinth reached its maximum after 200 days. This led the authors to the conclusion that the maximum solids retention time (SRT) for the garbage and dry hyacinth is 150 days while the maximum SRT for the wet hyacinth was 200 days, with a total solids (TS) feed rate between 125 and 160 g TS/L [52].

In their study, “Production of biogas from water hyacinth (*Eichhornia crassipes* (Mart) (Solms)) in a two-stage bioreactor”, Kivaisi and Mtila [53] investigated the effect of a two stage reactor on the production of biogas from water hyacinth (previously one stage reactors had been used). Their study also compared the production rates of pure water hyacinth and a

mixture of water hyacinth and cow dung in a ratio of 7:3. The only pre-treatment applied to the water hyacinth was allowing it to dry for 5 days and then grinding it to 2mm. They first studied the difference in the degradation of the water hyacinth and the mixture under standard rumen reactor conditions and found that the mixture degraded better under those conditions than the plan hyacinth. Following this the experimented on the mixture to find the optimum reactor conditions. It was found that the optimum conditions were as follows:

- Loading Rate of Volatile Solids: 15.4 g VS/l·day
- Solid Retention Time: 60 hours
- Dilution Rate: 0.5/hour
- Under these conditions, a small reactor is sufficient for biogas production.

2.7 Phytoremediation using water hyacinth

Phytoremediation was first documented more than 300 years ago, however, it was only in the late 20th century that it was scientifically examined [54]. Phytoremediation is a natural process whereby contaminants are removed from an environment by plants. These plants need to be able to take up a variety of pollutants, grow quickly in polluted environments and be easily controlled. As mentioned previously, water hyacinth can take up nutrients and heavy metals with minimal adverse effect on the plant, it has a rapid growth rate and it is resilient [48]. It has also been suggested that plants used for remediation should have a beneficial use after they have been harvested, such as the uses for water hyacinth detailed in Section 2.3.2.1. Water hyacinth has been used in both drinking water purification and sewage treatment facilities [48].

2.7.1 Domestic and industrial effluent remediation

A study was conducted by NASA in the 1970's investigating the use of water hyacinth for domestic and chemical wastewater treatment by monitoring the growth rate of the hyacinth and began developing methods of using the biomass that was produced [29].

At the time of this study, domestic sewage was retained in sewage lagoons for 30-60 days to allow organic material to biodegrade. They measured the effectiveness in terms of the change in biological oxygen demand (BOD) and total suspended solids (TSS) before and after the retention period. The study was started by introducing water hyacinth to the main lagoon at the National Space Technology Laboratories. The water hyacinth reduced the average BOD and TSS by 95%, the nitrogen content was reduced from 12 to 3.4 mg/L and the phosphorous was reduced from 3.7 to 1.6 mg/L. The presence of the water hyacinth also ensured that there were no algal blooms and drastically reduced the odour coming from the lagoons. This was after a retention time of 51 days [29]. A second study was conducted, after the first was successful, with a retention time of 7 days. This lagoon showed an annual decrease of 70% in

BOD and 71% in TSS. The nutrient change was not mentioned for this study. Some problems encountered included the winter damage done to the plants if they were not protected and the presence of spider mites. They also found that, while odour might not be a problem for these lagoons, it could become a problem in lagoons with higher organic loading rate. They suggested keeping the plant population thin so that wind could still mix the surface water and photosynthesis could still occur or to supply mechanical aeration.

Wolverton and McDonald [29] found that water hyacinth easily absorbs heavy metals when employed for chemical waste treatment. In one experiment, the plants were found to have absorbed 594 ppm copper, 297 ppm lead, 113 ppm silver, 164 ppm cadmium, and 286 ppm chromium with no adverse effects to the plant (the initial and final concentrations of these elements in the chemical waste stream were not presented). Wolverton and McKown [17] found that water hyacinth is capable of removing 36.4 g Phenols/kg plant over three days. Smith and Shore [18] found that 258 mg mirex/ kg plant could be removed within a week. Xiaomei *et al.* [19] reported a cadmium absorption of 2040 mg Cd/kg hyacinth over 8 days and a zinc absorption of 9600 mg Zn/kg hyacinth.

The National Space Technology Laboratories applied Wolverton and McDonald's [29] small-scale research to their laboratory effluent. To determine the effectiveness of the water hyacinth, they measured the BOD₅ and the chemical oxygen demand (COD). They found that the water hyacinth reduced the BOD and COD in the 95 m³/day effluent stream by 97% and 88%, respectively. At the time this article was written the water hyacinth system had been in place for 3 years. The plants were removed periodically before they could decay, to prevent the absorbed heavy metals re-entering the water system.

Jianbo *et al.* [55] investigated the use of water hyacinth in a constructed wetland to purify water on a duck egg farm and the effect that feeding the water hyacinth to the ducks has on the quality of the duck eggs. A 64% decrease of COD, 22% decrease of total nitrogen and 23% decrease of total phosphorous were recorded, with an increase of 250% of the transparency of the water. It was also found that feeding the water hyacinth to the ducks led to a 10% increase in egg-laying ratio as well as a 2% increase in egg weight.

2.7.2 Organic solid remediation

A review was conducted in Mauritius by Sooknah [56] to assess the mechanisms of water hyacinth remediation. In this article, Sooknah emphasises the water hyacinth's ability to remove organic matter. Sooknah described the water hyacinth remediation lagoons as "horizontal trickling filters" as the roots of the hyacinth trap the suspended solids. After a large enough amount of these particles have accumulated, they gradually settle to the bottom

of the pond or dam. In some dams, these particles are metabolised by the micro-organisms. The water hyacinth were also found to remove dissolved organic matter. There are several mechanisms by which this occurred, including bio-oxidation by the bacteria on the hyacinth roots, hyacinth absorption and storage, and the accumulation of the organic matter onto particles. Bio-oxidation is the most prevalent mechanism and the bacteria use the organic matter for energy and to produce new cells. This mechanism requires the use of an electron acceptor which is usually oxygen, however, in the absence of oxygen, the bacteria can make use of nitrates and sulfates.

In a water body without hyacinth, it is estimated that the oxygen transfer rate from the atmosphere into the water is $0.5\text{--}1.5\text{ g/m}^3\cdot\text{d}$ [57]. It will be lower in a water body with hyacinth as there is less water surface exposed. Snookah *et al.* [58] suggest that when hyacinths are present the majority of the oxygen in these lagoon comes from the plant roots, at a rate between $2.4\text{ to }10\text{ g/m}^3\cdot\text{d}$. As types of bacteria in the water utilise oxygen to function Problems can be experienced when the bacteria on the plant roots consume greater volumes of oxygen than the roots can transfer. At this point, the water may become anaerobic.

2.7.3 Nitrogen remediation

Fox *et al.* [16] conducted two studies within the same year during different seasons to monitor the change in nitrogen concentration in the water. Each study was conducted for 28 days, Study A was in winter and Study B was in summer. They measured the air temperature, solar radiation and water temperature daily, pH and electrical conductivity weekly and total Kjeldahl nitrogen (TKN), nitrate/nitrite and ammonia concentration at the beginning and end of the experiment. The experiment used 64 ponds and each was filled with 189 L of water from a well and kept at that level with deionised water, and a commercial fertiliser was used to approximate Hoagland's Solution. They created solutions of 0, 40, 80, 100, 150, 200 mg N/L for both studies and added an extra concentration of 300 mg N/L to Study B. The tanks in Study B were required to be topped up with significantly more de-ionised water than Study A as there was increased evaporation, due to the higher temperatures, and the plants grew faster in the summer leading to greater water uptake and evapotranspiration.

Although the plants in Study B grew faster, both studies showed the same trend of faster growth in the tanks with higher nitrogen concentrations. Neither of the studies resulted in a complete depletion of nutrients, however, a greater amount of nitrogen was removed from the water with higher initial nitrogen concentrations. They found that in Study A 59-75% of the nitrogen was removed, however, in Study B only 13-48% of the nitrogen was removed. Another study conducted by Tripathi and Upadhyay [59] showed that 79% of total nitrogen could be removed from a dairy farm wastewater stream when combined with duckweed.

2.8 Algae coverage

The growth of algae in both fresh and marine water bodies can have harmful effects on the environment and people surrounding it. It is important to monitor the initialisation and growth of the algae to minimise its health effects on humans and animals. Many different methods have been developed over the decades for algae growth monitoring. A large proportion of the older methods of algal quantification are disruptive to nature and require the destruction of algae biomass [60].

2.8.1 Health impact of algal blooms

The predominant species of algae in Hartbeespoort Dam is *Microcystis aeruginosa* (Blue-green algae or cyanobacterium) [61]. While this is a single-celled organism it can form dense blooms and mats when grown in warm, eutrophic water and exposed to sufficient sunlight. When the cells die, they rupture and release cyanotoxins into the water. Skin contact with these toxins can lead to rashes and blistering, while ingestion can cause nausea, vomiting, diarrhoea, headaches and fever. Ingestion of increased levels of the toxins can lead to liver damage and has led to the death of animals. While no human deaths have been attributed directly to the toxins, they can build up in shellfish and, when eaten, can cause Paralytic Shellfish Poisoning which has resulted in human fatalities [9, 10].

2.8.2 Invasive algae monitoring

Many of the older methods for quantifying algal colonies are fairly invasive, requiring sample extraction for analysis which often involves the addition of chemicals to rupture the cells. Butterwick *et al.* [62] separated these methods into three different groups: direct counting of cells (A), measurement of a density-dependant physical property (B) and those involving filtering the sample before analysis (C). Group A methods are microscope counts and electronic counters, group B methods are nephelometry, in vivo attenuation and in vivo fluorescence of chlorophyll-a and group C methods are reducing capacity (carbon-equivalent), extracted chlorophyll-a by spectrophotometry and extracted chlorophyll-a by fluorometry [62]. Sample extraction may not be ideal for studies monitoring the biomass change over time [60].

2.8.3 Non-invasive algae monitoring

Alum *et al.* [60] sought to determine a non-disruptive method of quantifying algal growth on concrete surfaces with differing concentrations of additives. Image analysis was compared to standard chlorophyll analysis methods (APHA 1995, Method No. 10200 H. Chlorophyll) to determine the accuracy of image processing, under both laboratory and field conditions. Samples of the different concrete types were cast and sample coupons of 25x25mm were cut. These coupons were placed into an algal growth reactor and incubated for six months. The

samples were analysed periodically using both methods. The image processing method involved subdividing each surface of the concrete cube into a grid sequence of block 6x6mm using an etched glass slide. Photographs of each side of each cube were taken using a high-tech camera at a controlled resolution. The images were processed on Adobe Photoshop to create high-resolution images, after which the green colour of the biomass was isolated. Following this, the images were processed to grayscale and then converted to a binary (pure black and white) images, where the black pixels represented the algae biomass and the white pixels represented the background. The number of black (algae) pixels were counted in Photoshop and divided by the total number of pixels to obtain an area coverage percentage. After image analysis, samples of algae biomass were harvested and mixed with acetone to break the cell walls and extract the chlorophyll. Four successive readings were taken of each sample at four different wavelengths and were adjusted for turbidity. These were used to calculate chlorophyll a, b and c, and determine the algal biomass. This experiment observed a consistent correlation between the aerial density from the image processing and chlorophyll a, b and c. Samples incubated for longer periods tended to have a lower correlation between the image processed area and chlorophyll, this was presumed to be as a result of secondary algal growth. [60].

On a larger scale, satellite imagery has been used to quantify floating macro-algae on large water bodies. The methods used for this are the normalised difference vegetation index (NDVI) and the floating algae index (FAI). External factors such as turbid waters and radiance from atmospheric aerosols can lead to irregular apparent reflectance resulting in inaccurate coverage estimates. Rodrigo *et al.* [63] aimed to develop a method that reduced these inaccuracies presented by the apparent reflectance. They developed the scaled algae index (SAI) to reduce the irregularities by scanning an ocean pixel and normalising the image to that pixel, while still retaining a sufficient contrast between algae pixels and ocean pixels to estimate the algal coverage [63].

2.9 Conclusion

From the research reviewed here, it can be seen that relatively few studies have been performed on this topic in recent years, with the vast majority of papers coming from the early 1980's. This is particularly true for studies in the South African context. As many South African dams are currently facing high amounts of nutrient enrichment and subsequent eutrophication, up to date studies need to be performed to find solutions to the hyacinth infestation in this country, as each incident of water hyacinth growth is unique to that environment.

On the research previously done on the water remediation potential, most of the experiments have been performed in large tanks, in the magnitude of 100 L, containing dozens of hyacinth plants. Many of the studies on these plants have stated that increased plant densities and overcrowding decrease the growth rate due to limited space. This effect is considered in the research presented in this study.

This study will involve monitoring the growth and remediation potential of water hyacinth plants. The plants shall be grown in water from Hartbeespoort Dam as well as Hoagland's solution (a widely used growth medium, containing all the micro-nutrients necessary to sustain the plants, which will aid analysis and comparison between studies). Since removal attempts in South Africa have, thus far, not succeeded an *in-situ* solution may be viable. To further demonstrate the remediation potential of the hyacinth plants, there will be a control with only Hartbeespoort dam water and no plant. In one study a plant-free control was used to show the effect of chemical control on the water, however, it did not compare the general quality of the water with the plant to the control. The water in this control will be compared to the water hyacinth containing water in terms of colour, algae growth and nutrient concentration.

CHAPTER 3

Materials and methods

3.1 Experiment 1 - The phytoremediation potential of water hyacinth: a case study from Hartbeespoort Dam, South Africa

3.1.1 Experimental set-up

On-site tests were performed at Hartbeespoort dam to determine the concentration of phosphates and nitrates in the dam, using a Hach DR/850 Colorimeter (Hach, Loveland, CO, USA) according to the manufacturer's instructions. For this experiment, water hyacinth was grown in the balcony greenhouse in the Oppenheimer Life Sciences Building, University of the Witwatersrand, Johannesburg, South Africa. Eighteen hyacinth plants of equal size and mass were taken from cultures grown in outdoor ponds at the university.

Water hyacinth plants were grown individually in five-litre vessels using either, water from Hartbeespoort dam (Hartbeespoort Dam water experiment (HB)), or tap water with Hoagland's solution added to it (Hoagland solution experiment (H)). The change in water quality was compared to Hartbeespoort dam water without water hyacinth plants (HBW). Each experiment was conducted with six replicates. Water from Hartbeespoort Dam was collected from the shore in 25L plastic jerry cans and stored just above 0°C until it was needed.

The Hoagland solution was prepared by first creating stock solutions of various macro- and micronutrients [28]. Six containers were filled with three litres of distilled water each and a specified volume of each nutrient solution was then added to the water to create the Hoagland solution, the composition of which is presented in Table 3.1. Three litres of Hartbeespoort water (HB) was added to each of the remaining 12 vessels and plants were placed in the six containers with Hoagland solution (H) and into six of the containers with Hartbeespoort Dam water.

The water in the H and HB containers was replaced every five days ("refill periods"). The remediation comparison containers were left for the whole duration of the experiment and were not refilled.

Compounds		Concentration of stock solution [mM]	Volume Stock solution per litre final solution [ml]
Macronutrients	KNO ₃	1000	6
	Ca(NO ₃) ₂ ·4H ₂ O	1000	4
	KH ₂ PO ₄	1000	2
	MgSO ₄ ·7H ₂ O	1000	1
Micronutrients	KCl	15	2
	H ₃ BO ₃	12.5	2
	MnSO ₄ ·H ₂ O	1	2
	ZnSO ₄ ·7H ₂ O	1	2
	CuSO ₄ ·5H ₂ O	0.25	2
	MoO ₃	0.25	2
Fe Na EDTA		64	1

Table 3.1 Composition for Hoagland's stock solution and dilution volumes for use

3.1.2 Plant measurements

Plant measurements were taken once a week for five weeks, maximising experimental time within the time constraints. The position of leaf two was marked at day zero and recorded at the end of the experiment. The number of leaves and ramets of each plant were counted [29]. Each week the stem height, leaf height and leaf width of this shoot were measured. A standard curve was developed to calculate leaf area from leaf width (Equation 3.1). Plants were weighed weekly by removing them from their water which was allowed to drain back into the container for a minute. The wet weight of each plant was then measured to the nearest half gram.

$$\text{area} = 0.0055 * (\text{width})^2 + 0.0158 * (\text{width}) + 0.540 ; \quad r^2 = 99 \quad (3.1)$$

3.1.3 Water analysis

Water samples were taken at least three times during each refill period; the first when the water was replaced, the second on day three and the final sample was taken before the water was discarded. Photographs were taken of the Hartbeespoort dam vessels without water hyacinth when the water samples were taken. Six replicates were required to gather adequate plant data, and two were used for water samples. The water samples were taken from the second and third container of each water type. When the plants had been removed, the water was left to settle for approximately five minutes. 10mL aliquots were then drawn from both replicates of all three experiments for analysis. Nutrients (phosphorous and nitrogen) were measured using a Merck Pharo 300 Spectrophotometer, using Merck phosphate cell tests (1.00474.000) and nitrate tests (1.14773.000), according to the manufacturer's specifications.

The change in concentration over time for each replicate in all three refill periods was plotted and exponential trendlines were fitted. From these trendlines, the average rate of nutrient removal was determined according to Equation 3.2 for each water type, where c is the current concentration, c_0 is the initial concentration, r is the nutrient removal rate and t is time. Simplified first-order kinetics were assumed, as the concentrations measured were low enough to be in the first-order range of Monod kinetics. In the Monod equation, it was assumed that the substrate concentration was much smaller than the half-saturation constant, and that the biomass concentration remained constant. This is a known and understood limitation of the final equation.

$$c = c_0 e^{-rt} \quad (3.2)$$

After the plant growth experiment was concluded the water in the remediation control containers was left to stand for a further 10 days, and the colour change of the water was observed and photographed to monitor any further algae growth or death.

3.1.4 Statistical analysis

The average petiole length, leaf area, number of ramets and number of leaves of the two experiments were compared using a one-way repeated measures ANOVA. A Student's t -test was used to determine the significance of the nutrient removal rates. Separate t -tests were performed to compare the average phosphate removal rate between H and HB, the average nitrate removal rate between H and HB, average phosphate removal rate between HB and HBW and the average nitrate removal rate between HB and HBW. A critical P-value of <0.05 was used for all statistical analyses to define significance.

3.2 Experiment 2 - The release of nutrients by decaying water hyacinth and subsequent algal growth

3.2.1 Experimental set-up

On-site tests were performed at Hartbeespoort Dam to determine the approximate phosphate, nitrate, nitrite and ammonium concentrations. The *in-situ* nitrite and ammonium concentrations were close to and below detection limit respectively, hence only the phosphate and nitrate concentrations were measured during the experiment. Water was collected from the Hartbeespoort Dam and transported to the University of the Witwatersrand, Johannesburg, South Africa. Oval water vessels were set up on the roof of the Richard Ward building, at the University, which were filled with water from Hartbeespoort Dam.

The experiment consisted of three different treatments the C, in which there were no hyacinth plants, the FP in which four hyacinth plants were grown and the TP, in which ten hyacinth plants were grown. Each treatment consisted of six replicates and contained 46L of Hartbeespoort Dam water at the beginning of the experiment. The water level was marked on the side of the water vessels at the beginning of the experiment to account for evaporation and hence close the mass balance.

3.2.2 Plant measurements

84 individual plants were taken from the hyacinth pools on the roof of The Oppenheimer life sciences building at the University and put into water vessels corresponding to each treatment (no plants in any C vessels, 4 plants in each FP vessel and 10 plants in each TP vessel). It was ensured that each plant had 5 leaves and no ramets. The plant measurements were taken once a week for the duration of the experiment. The inner-most leaf was marked at the beginning of the experiment to aid measurements. The petiole length and leaf width were measured and a standard curve was used to calculate leaf area from leaf width (Equation 3.1). The number of leaves and ramets were also counted.

3.2.3 Water analysis

Water samples were taken three times a week for the first 4 weeks. After the plants became dormant, water samples were taken once a week together with the plant measurements. The plant measurements were continued after the plants became dormant to monitor if any new growth started. Six replicates were required to gather adequate plant data, and three were used for water samples. The plants were removed from all buckets and the water was mixed and allowed to settle until the plant matter had sunk from the top layer of water to allow for a clean sample, after which 20ml of water was drawn from the three of the vessels. Nutrients (phosphorous and nitrogen) were measured using a Merck Pharo 300 Spectrophotometer, using Merck phosphate cell tests (1.09713.0001) and nitrate tests (1.14848.0002), according to the manufacturer's specifications. Calibration curves of absorption vs. nutrient concentration were created for both phosphates and nitrates.

To allow water analysis to continue as long as possible, the vessels were not topped up with distilled water. An equation (Equation 3.3) was developed to determine the volume of the vessels from the water height (where the height is in cm).

$$\text{volume} = \int_0^h \pi(0.0183h^2 + 8.24h + 740) dh \quad (3.3)$$

Using the nutrient concentrations and the volumes of water in each water vessel, the mass of the nutrients was calculated, and an equivalent concentration in 46L (the original volume) was calculated.

3.2.4 Algae analysis

Photographs of vessels from which water samples were drawn were taken from directly above the vessel (demonstrated in Figure 3.1) after the plants had been removed and the water had been mixed. These photographs were cropped to the edge of the water in the buckets. A script was written in MATLAB to change the RGB images to greyscale, where pixel luminance ranges from all-black (0) to all-white (255), and then to change the greyscale images to binary images using a luminance threshold of 105 (dimensionless). All pixels with luminance greater than 105 were changed to pure black and all pixels with a luminance less than 105 were changed to all white. In greyscale, the algae pixels had a luminance greater than the threshold of 105, hence the resultant image displayed the algae as black pixels and everything else as white pixels. The script counted and stored the number of black pixels (i.e. algae pixels in each image). To determine a percentage of algae coverage, another set of binary images were produced with a threshold value 5, which returned an image with the entire water surface as black pixels. These black pixels were also counted and stored, after which a ratio of algae to water surface pixels (algae coverage percentage) was calculated, tabulated and written to excel. The image analysis procedure is demonstrated in Figure 3.1.

Equation 3.4 was developed to determine the surface area of the water as the water level dropped. This was multiplied by algae coverage percentage to determine the area of algae coverage for each water vessel over time.

$$\text{surface area} = \pi(0.0183h^2 + 8.24h + 740) \quad (3.4)$$

3.2.5 Statistical analysis

The phosphate and nitrate concentrations, as well as the algae coverage area of all three experiments, were compared using a one-way repeated measures ANOVA. A critical P-value of <0.05 was used for all statistical analyses to define significance.

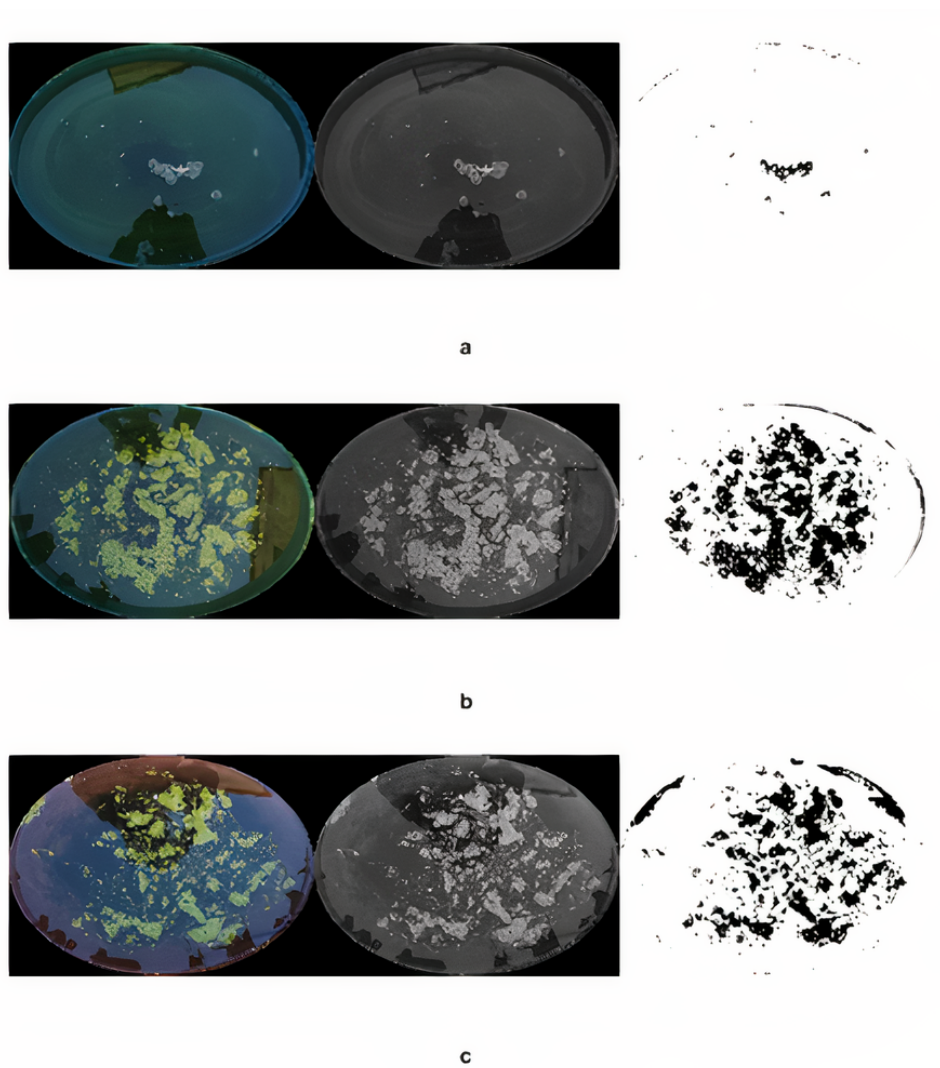


Fig. 3.1 Image analysis procedure for floating algae quantification; from left to right, the RGB image cropped to the water surface, the greyscale image and binary image of (a) the control, (b) the vessel with four hyacinth plants and (c) the vessel with ten hyacinth plants on the 22nd of July 2019

CHAPTER 4

The phytoremediation potential of water hyacinth: a case study from Hartbeespoort Dam, South Africa

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4.1 ABSTRACT

Eutrophication has been a prevalent problem in South African water bodies for many decades, resulting in algal blooms and growth of invasive alien aquatic weeds such as water hyacinth. It was brought to heel in the 1990's, but in recent years this problem has been exacerbated by increasing incidences of untreated sewage entering the river systems, along with other anthropogenic nutrient sources. This study investigated the potential for water hyacinth to be used as a phytoremediation tool to reduce the eutrophic state of Hartbeespoort Dam, and to determine if the water quality, aside from the nutrient concentrations, affects the rate of removal of nutrients by water hyacinth. Water hyacinth plants were grown in either water from the Hartbeespoort Dam or in Hoagland Solution to test the effect of growth medium 'purity' on hyacinth growth. Another set of vessels containing Hartbeespoort Dam water only, without a hyacinth plant, was used to observe the remediation potential of the plant and its effectiveness at suppressing algal growth. The results show that one plant can absorb up to 7,38 mg of Phosphates and 27 mg of Nitrates within 11 days. Water hyacinth can reduce the nutrient concentration of 3 L of Hartbeespoort Dam water from 0,56 mg/L phosphates to 0,05 mg/L phosphate and from 4,1 mg/L nitrates to 0,5 mg/L nitrates in 4 days. These results suggest that water hyacinth can be employed as a phytoremediation tool to mitigate eutrophication in open water bodies and may have the potential to reduce Microcystis blooms.

Keywords: *Eichhornia crassipes*, eutrophication, nitrates, nutrients, phosphates

4.2 LIST OF ABBREVIATIONS

BOD	Biological oxygen demand
TSS	Total suspended solids
H	Hoagland solution experiment
HB	Hartbeespoort Dam water experiment
HBW	Hartbeespoort Dam water without hyacinth experiment

4.3 NOMENCLATURE

c	Concentration ($\text{mg}\cdot\text{L}^{-1}$)
c_0	Initial concentration ($\text{mg}\cdot\text{L}^{-1}$)
r	rate ($\text{mg}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$)
t	time (day)

4.4 INTRODUCTION

Eichhornia crassipes (Mart.) Solms (water hyacinth) is an aquatic plant native to the Amazon Basin that was spread from South America to other parts of the world as an ornamental flower [11]. Its invaded distribution is largely as a result of its rapid growth rate and durability. A water hyacinth mat's edge can expand by up to 60m/month, in favourable conditions and its seeds remain viable for up to 20 years, on the bottom of the infested water body [12, 13]. Aside from the lack of natural enemies in the introduced range, the main factor contributing to excessive water hyacinth growth is anthropogenic eutrophication [7, 14]. In the face of this, many beneficial ways of using water hyacinth have been developed, adding an incentive to harvest the water hyacinth. One of the oldest uses of hyacinth is as a fertiliser as it has a stored nutrients [45, 64]. Other uses include as a biofuel feedstock [50, 51], for charcoal briquettes [11] and for water treatment [29]. Water hyacinth can cause problems in water bodies as the high growth rates result in large mats which the sun cannot penetrate. This often results in the death of other aquatic plants as they are not able to photosynthesise. While this is a significant problem, a greater problem occurs when the hyacinth dies. The bacteria responsible for decomposition of the water hyacinth uses the dissolved oxygen in the water. This results in hypoxic water conditions, which can become anoxic in extreme cases. Fish and other aquatic life can be negatively affected by the lack of oxygen [7, 14].

The nutrient levels in many South African dams have increased to eutrophic levels, resulting in large hyacinth blooms [65]. However research into eutrophication decreased in the 1990's as it was no longer deemed a priority by the government, and funding was consequently reduced [15]. There are, however, some advantages to the growth of water hyacinth in eutrophic water. The plant consumes large amounts of nutrients, a portion of which are stored while the rest are used for growth. Wolverton and McDonald (1979) found that water hyacinth can remove nutrients (nitrogen, phosphorous and potassium) from water while lowering the biological oxygen demand (BOD) and total suspended solids

(TSS) [29]. Wolverton [29], Smith [18] and Xiaomei [19] found that hyacinth is also capable of removing heavy metals, phenols and mirex with limited effects on the plant at low concentrations. This could be an asset when employing the water hyacinth as a remediation tool in dams such as the Hartbeespoort Dam, which have been affected by acid mine drainage and contain heavy metals [66]. Fox et al. [16] found that the removal of total nitrogen by water hyacinth is proportional to the initial nitrogen concentration. Water hyacinth also plays a role in reducing the algae present in a water body. This can occur through attachment, settling, respiration and growth suppression [20]. The algae present in Hartbeespoort Dam primarily consists of *Microcystis aeruginosa* forma *aeruginosa*, which produces cyanotoxins that increase the risk factor for primary liver cancer and kidney damage [67]. The most common method of exposure to these toxins from a polluted water source is through ingestion, whether by drinking the water or eating fish that have come from the water.

Three main methods for control of water hyacinth are mechanical, chemical and biological. Each has varying success in different environments [21]. Mechanical control involves removal by hand or machines, however, it often results in small pieces of roots and stems being left behind which are able to regrow [22]. Herbicides are sprayed on the plants in situ for chemical control requiring equipment and know-how [68]. Herbicides can negatively affect water quality and non-target animals and plants. Biological control involves releasing the plant's natural enemies which attack the plants reducing growth rates and potentially causing mortality [24]. This method has the greatest success in tropical climates; however, it can take many years for the effect to be noticed. Integrated control incorporates biocontrol with herbicide application and has the potential to be a useful management tool for the weed [26]. Most attempts at controlling the water hyacinth have been unsuccessful as a result of the rapid growth rate and the costs involved in removing the hyacinth. In South Africa, the lowest cost for hyacinth removal is R277/ha for integrated control [69]. Biological control has been successful at New Year's Dam in the Eastern Cape during the 1990's. The hyacinth coverage was reduced from 80% to 20% between 1990 and 1995 with the introduction of *Neochetina eichhorniae*. In 1996 several other species were introduced to the dam but did not establish and by 1998 the hyacinth coverage had increased to 80%. It was speculated that this was due to the poor condition of the plants that remained on the dam and incorrect timing of the release. Without further release, the original species *Neochetina eichhorniae* multiplied and were able to reduce the hyacinth coverage to approximately 5% and maintained that coverage from 2002 until 2012. It was determined in 2016 by Hill et al. that the benefit of biocontrol on New Year's Dam exceeded the cost of the control as a result of the water saved by reducing evapotranspiration. They determined that the net benefits of reducing the hyacinth coverage came to between R282 458 and R3 671 954 [70]. There has been some success on Hartbeespoort Dam controlling the hyacinth using chemical control. Between 1977 and 2001 the hyacinth coverage was reduced from approximately 60% to 0,2-5%, at the cost of R4.2 million [69].

The main aim of this study was to determine the potential of water hyacinth to phytoremediate Hartbeespoort Dam water. In order to simulate what could happen if the hyacinth was not present in the dam an experiment was set up that did not include water hyacinth plants in water from Hartbeespoort Dam. Additionally, it was to be determined if water hyacinth grows better in HB water compared to

plants grown in impurity free water made up with similar concentrations of macro- and micronutrients (Hoagland solution) to ensure that the concentrations of heavy metals in Hartbeespoort Dam were below the limit that would affect the plants, as these concentrations were not measured.

4.5 METHODS

4.5.1 Experimental set-up

This experiment was conducted in spring. On site tests were performed at Hartbeespoort dam to determine the concentration of nutrients in the dam, using a Hach DR/850 Colorimeter (Hach, Loveland, CO, USA) according to the manufacturer's instructions. Water hyacinth was grown in the balcony greenhouse in the Oppenheimer Life Sciences Building, University of the Witwatersrand, Johannesburg, South Africa. Eighteen hyacinth plants of equal size and mass were taken from cultures grown in outdoor ponds at the university.

Water hyacinth plants were grown individually in five litre vessels using either, water from Hartbeespoort dam (HB), or tap water with Hoagland's solution added to it (H). The change in water quality was compared to Hartbeespoort dam water without water hyacinth plants (HBW) over the course of the experiment. Treatments had six replicates. Water from Hartbeespoort Dam was collected from the shore in 25L plastic jerry cans and stored just above 0 °C until it was needed.

The Hoagland solution was prepared by first creating stock solutions of various macro- and micronutrients according to Taiz and Zeiger (1991) [71]. Six containers were filled with three litres of distilled water each and a specified volume of each nutrient solution was then added to the water to create the Hoagland solution. Three litres of Hartbeespoort water was added to each of the remaining 12 vessels and plants were placed in the six containers with Hoagland solution and into six of the containers with Hartbeespoort Dam water.

The water in the H and HB containers was replaced every five days ("refill periods"). The remediation comparison containers were left for the whole duration of the experiment and were not refilled.

4.5.2 Plant measurements

Plant measurements were taken once a week for five-weeks. Chlorophyll content of the second leaf was measured using a CCM-300 Chlorophyll Content Meter (Opti-Sciences, NH, USA). The number of leaves and ramets of each plant were counted [72]. The position of leaf two was marked at day zero and recorded at the end of the experiment. Each week the stem height, leaf height and leaf width of this shoot were measured. A standard curve was developed to calculate leaf area from leaf width (Equation 4.1). Plants were weighed weekly by removing them from their water which was allowed to drain back into the container for a minute. The wet weight of each plant was then measured to the nearest half gram.

$$\text{area} = 0.0055 * (\text{width})^2 + 0.0158 * (\text{width}) + 0.540 ; \quad r^2 = 99 \quad (4.1)$$

4.5.3 Water analysis

Water samples were taken at least three times during each refill period; the first when the water was replaced, the second on the third day and the final sample was taken before the water was discarded. Photographs were taken of the remediation control containers when the water samples were taken. While six replicates were required to gather adequate plant data, only two were used for water samples. The water samples were taken from the second and third container of each water type. When the plants had been removed, from the water it was left to settle for approximately five minutes. 10mL of water was then drawn from all three water for analysis. Nutrients (phosphorous and nitrogen) were measured using a Merck Pharo 300 Spectrophotometer, using Merck phosphate cell tests (1.00474.000) and nitrate tests (1.14773.000), according to the manufacturer's specifications.

The change in concentration over time for each replicate in all three refill periods was plotted and trendlines fitted. From these trendlines, the average rate of nutrient removal was determined using Equation 4.2 for each water type. As this system has low nutrient concentrations, first order Monod equations were assumed.

$$c = c_0 e^{-rt} \quad (4.2)$$

After the plant growth experiment was concluded the water in the remediation control containers was left to stand for a further 10 days, and the colour change of the water was observed and photographed.

4.5.4 Statistical analysis

The average petiole length, leaf area, number of ramets and number of leaves of the two experiments were compared using a one-way repeated measures ANOVA. A Student's *t*-test was used to determine the significance of the nutrient removal rates. Separate *t*-tests were performed to compare the average phosphate removal rate between H and HB, the average nitrate removal rate between H and HB, average phosphate removal rate between HB and HBW and the average nitrate removal rate between HB and HBW. A critical P value of <0.05 was used for all statistical analyses.

4.6 RESULTS

4.6.1 Plant growth

The data obtained from the plants was normalised to determine the change in growth over time. None of the plant parameters that were measured over time were significantly different between the two treatments. (Fig. 1a – d). The leaf area was determined using the leaf width and Equation 1. The

number of leaves reached a plateau once the plant had between 7 and 9 leaves, because of outer leaves dying while new leaves are formed in the centre. It can be seen plants grown in the Hartbeespoort Dam water produced more ramets than those grown the Hoagland's solution. A possible reason for this trend is that there are other compounds or organisms in the Hartbeespoort Dam water that increase the growth rate, however this difference is not significant.

All growth parameters measured; petiole length, leaf area, number of ramets and leaves were not different between the HB and H treatments over the duration of the experiment.

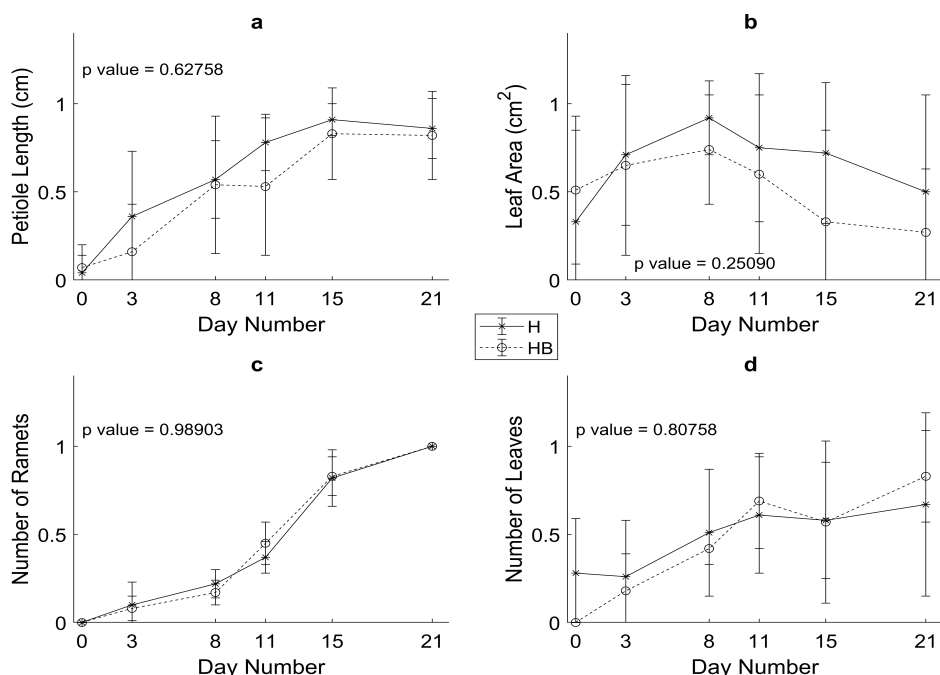


Fig. 4.1 Normalised (a) Petiole length , (b) leaf area, (c) the number of ramets and (d) the number of leaves for water hyacinth plants grown in Hoagland solution (H) water and Hartbeespoort dam (HB) water and over the course of 21 days. n = six plants per mean ($\pm$ SE). Data were tested using a repeated measure ANOVA

4.6.2 Water remediation

Table 4.1 presents a summary of the initial and final concentrations during the three refill periods. The nutrient removal rates were calculated in three intervals which were then used to determine an average removal rate in each vessel.

Figure 2 displays the average nutrient removal rate observed in each water type and compares the relevant experiments to one another (compares H and HB for the effect of water type on removal rate and compares HB and H for the effect of water hyacinth on removal rate). There was no significant difference in the nitrate removal rate between the H solution and HB water, however the difference in the removal rate of phosphate was significantly greater in the HB containers. The nutrient removal rate of both phosphates and nitrates were significantly greater in the HB containers than the removal rates in

the HBW.

	Refil No.	H			HB			HBW		
		Initial (mg/L)	Final (mg/L)	rate (mg/L /day)	Initial (mg/L)	Final (mg/L)	rate (mg/L /day)	Initial (mg/L)	Final (mg/L)	rate (mg/L /day)
Phosphate	1	2.1	1.39	-0.141	0.5	0.04	-0.828	0.65	1.07	0.157
	2	0.73	0.15	-0.322	0.57	0.05	-0.484	0.57	0.26	-0.155
	3	0.87	0.31	-0.335	0.65	0.04	-0.9	0.26	0.08	-0.391
Nitrate	1	3.3	0.5	-0.621	3.75	0.5	-0.714	3.05	4.45	0.1133
	2	1.25	0.5	-0.193	2.55	0.5	-0.343	3.5	1.05	-0.239
	3	1.7	0.5	-0.415	4.15	0.5	-0.664	1.05	0.5	-0.212

Table 4.1 Summary of average initial and final phosphate (PO_4^{3-}) and nitrate (NO_2^{-3}) concentrations and the average rate of nutrient depletion by water hyacinth in Hoagland Solution (H) and in Hartbeespoort Dam water (HB), and in Hartbeespoort Dam water without water hyacinth (HBW) for each refill period.

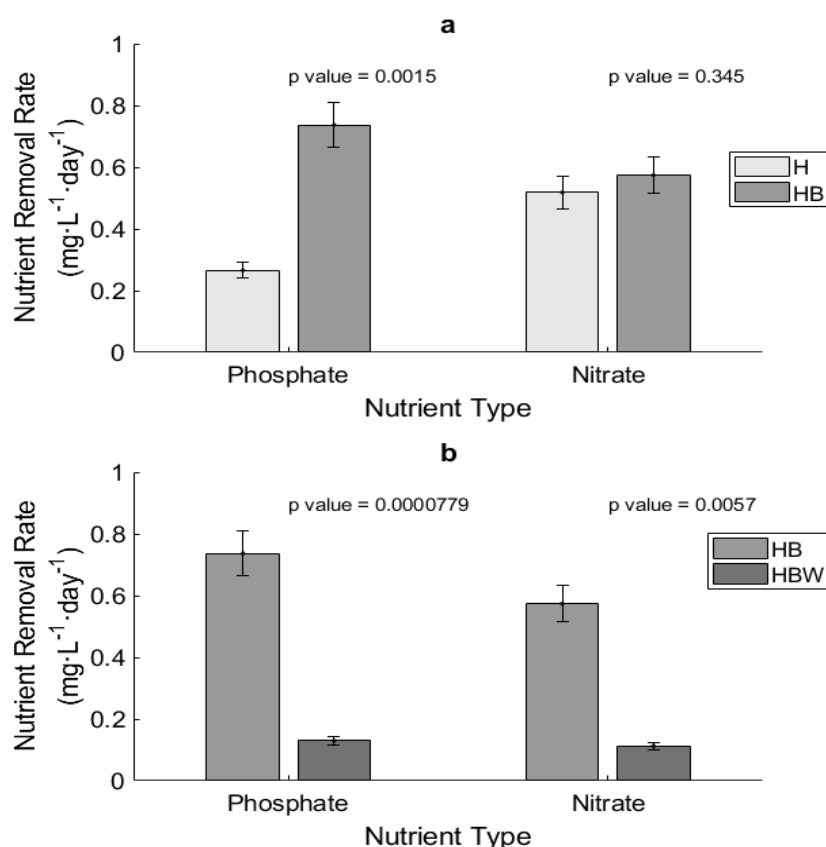


Fig. 4.2 Average rate of depletion of phosphates (a) and nitrates (b) caused by water hyacinth in Hoagland Solution (H) and in Hartbeespoort Dam water (HB) and without water hyacinth in Hartbeespoort Dam water (HBW)

The growth of the algae in the remediation comparison vessels was not quantified; a visual assessment is shown in Figure 3. On the first day the water was fresh and clear with no algae growth. By the third day the water had begun to discolour, and the beginnings of an algae mass could be seen in each of the vessels. By the seventh day the water had turned green and small bubbles could be seen on the surface of the water, which continued to day 11.

By the 16th day of the algae experiment, the water was turning yellow, and scum was seen floating on the surface of the water. By the 21st day, the end of the algae experiment, the water had become more yellow with a greater amount of scum on the surface than was observed on the 16th day. Although the refill periods of the H and HB vessels make a direct comparison of algae growth with HBW difficult, it was noted that there was almost no algae growth in either of the experiments containing water hyacinth. The limited algae growth that was seen occurred in the bottom corners of the vessel and was attached to the side of the vessel, not growing in the centre. There was no water discolouration in the experiments.

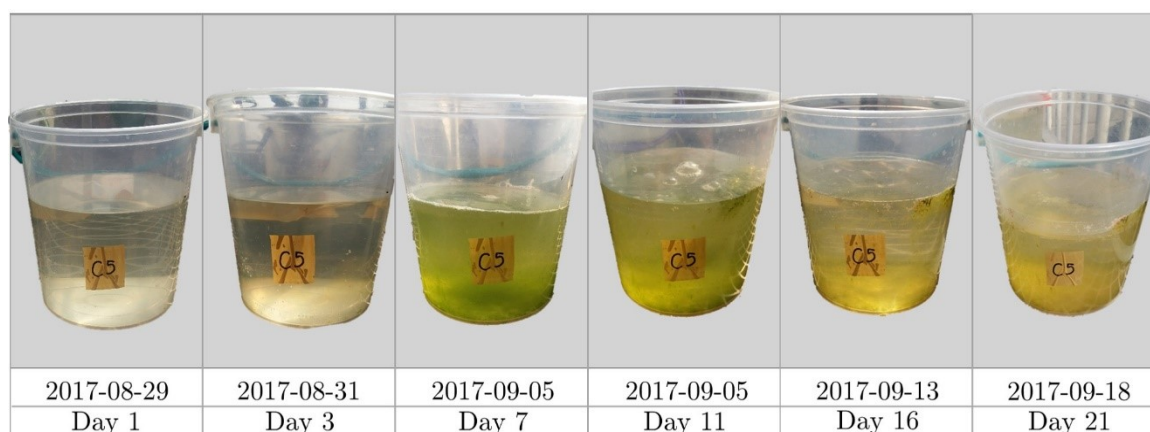


Fig. 4.3 Progression of algal growth in three litres of Hartbeespoort Dam water in five litre containers over 21 days

This shows that there is potential for water hyacinth to be employed in part of the process to treat water, however, further testing will be required to determine what other pollutants may still be present, such as organic and inorganic chemicals, coliforms and cyanotoxins which pose health risks.

4.7 DISCUSSION

4.7.1 Plant growth

The aim of the plant growth experiment was to determine if the type of water and possible presence of other contaminants and microorganisms had an impact on the growth of the water hyacinth. The ability of the water hyacinth to grow in different qualities of water would allow it to be used in various settings to remediate water without adverse effects from the water. From the results depicted in Figure 1 it has been determined that the quality of water in Hartbeespoort Dam does not affect the water hyacinth growth rate, which implies that it can be used in eutrophic dam water to remove excess nutrients. The results of this analysis show that water hyacinth can grow equally well in nutrient rich dam water, which

potentially has added contaminants, such as heavy metals, acid mine drainage and various diseases, (Hartbeespoort water) as it can nutrient rich ‘ideal’ water prepared in a laboratory (Hoagland solution).

4.7.2 Water remediation

The aims of the water remediation experiment were to determine if the quality of water influenced the nutrient removal rate achieved by the water hyacinth and to observe phytoremediation potential of the water hyacinth with reference to phosphates and nitrates, by comparing the nutrient depletion rates when water hyacinth is present and when it is not. The remediation control attempted to simulate what could occur if the water hyacinth were removed from the dam without addressing the nutrient concentrations currently in the dam. There are some limitations to this comparison as in the dam itself there would be continuous inflows of sewerage and surface runoff, bringing new nutrients into the dam. The results in Figure 2a show that the water quality has a significant effect on the removal rate of phosphates as the phosphate removal rate in the Hoagland solution is significantly lower than that observed in the Hartbeespoort Dam water. This suggests the presence of another factor, not explored in this study, in the Hartbeespoort Dam water which aids the uptake of phosphates. Phosphates are the more challenging nutrient to remove from eutrophic water bodies, and their concentration in water bodies is the limiting factor for algal growth. As phosphates were removed from the HB water at a higher rate, this indicates that water hyacinth may be a useful tool to remediate the Hartbeespoort Dam. Subsequent studies will need to be conducted to test this potential further and to determine the reason for the increased phosphate removal rate. Figure 2b shows that both the phosphate and nitrate removal rates are significantly different between the vessels containing water hyacinth (HB) and those without (HBW). This combined with the algal growth observed in the HBW (shown in Figure 3) and lack of algal growth observed in the HB vessels provides a basic insight into what could occur if the water hyacinth is removed. It is possible to expect an algal bloom should the hyacinth be removed without the water from the inlet rivers being treated. This also suggests that the water hyacinth is playing a roll in remediating the dam and reducing the potential for algal blooms.

As a result of the negative impacts of the water hyacinth – such as reducing the dissolved oxygen, blocking sunlight from penetrating the water when it is in thick mats and the decomposition leading to the re-release of nutrients in to the water body – if the water hyacinth were to be employer to remediate water bodies, a system would need to be developed which allows the hyacinth adequate time to grow and remove maximum nutrients from the water, and then be harvested before there is a change for the negative impacts to come into effect. An equilibrium would need to be established between the hyacinth growth rates and the harvesting of the plant, as well as the development and implementation of a harvesting process which is less energy intensive.

4.8 CONCLUSION

Three parameters were investigated to determine the phytoremediation potential of water hyacinth on the Hartbeespoort Dam: the effect that water quality has on the growth of the plant, the effect that the water quality has on the nutrient removal rates of the plant, and the difference in nutrient depletion

when the hyacinth is and is not present in a water body. This study has shown that the water quality does not affect the growth rate of water hyacinth nor its ability to remove nitrates, however it does influence its ability to remove phosphates, possibly due to the presence of a symbiont, or another factor, in the Hartbeespoort Dam water. It would, therefore, be possible to use the hyacinth as a remediation tool in many different environments without a detrimental effect on plant health, however the presence of other factors, or lack thereof, in the water may lead to varied results in the extraction of nutrients, specifically phosphates. The marked difference in the nutrient depletion rates in Hartbeespoort Dam water containing water hyacinth and that without the plant indicates that the plants already play a part in the remediation of the dam water, and could be controlled to allow effective nutrient removal in the dam while reducing the detrimental effects that the hyacinth have on a water body when they form large mats or die and decompose.

The very rapid removal of nutrients is a limitation of the data presented and larger scale experiments will need to be conducted as part of the next phase of research. Notwithstanding this limitation, the experiment has shown that, within a laboratory context, the concentration of nitrates and phosphates in three litres eutrophic water can be reduced to undetectable levels within four days. This data suggests the possibility to use the hyacinth as a remediation tool and the need for more specific and intensive research to determine if an engineering solution for the *in-situ* bioremediation of the Hartbeespoort Dam can be developed.

CHAPTER 5

The removal of phosphates and nitrates by water hyacinth over winter months

5.1 Results

The results obtained during this experiment are displayed in the following sections. The data was recorded and a preliminary analysis was conducted in Microsoft Excel. Following that the data was processed into Figures 5.6 - 5.3 using MATLAB.

5.1.1 Plant growth

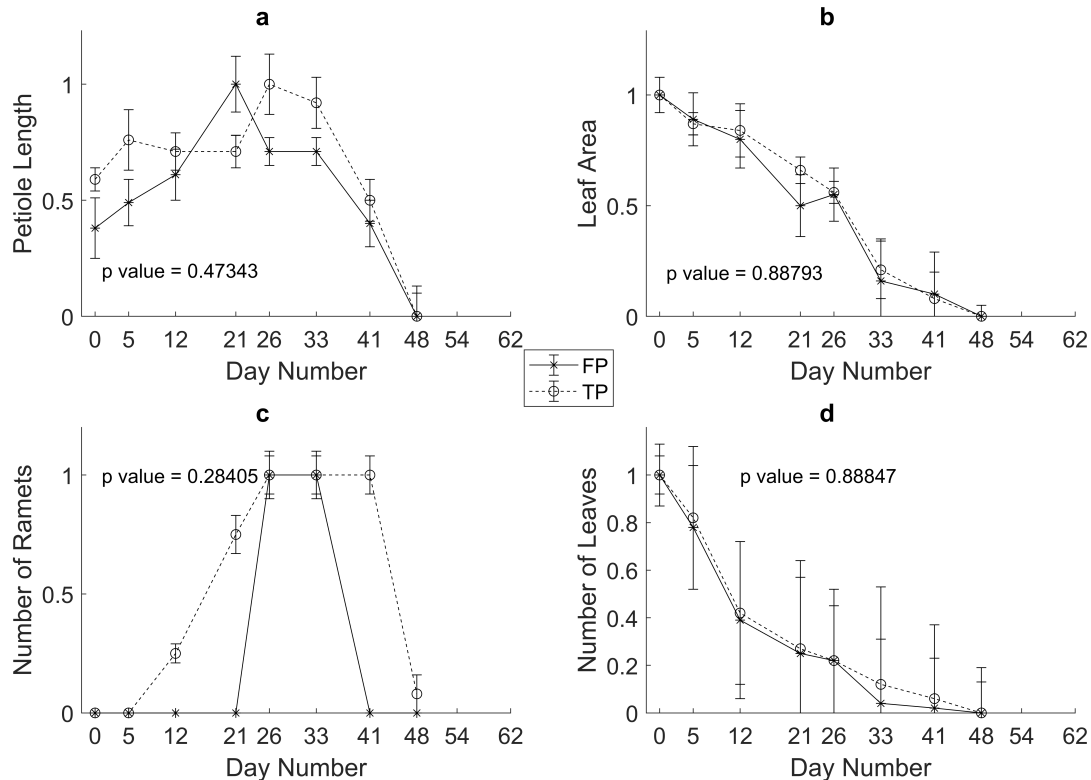


Fig. 5.1 Normalised average (a) Petiole length, (b) leaf area, (c) the number of ramets and (d) the number of leaves for water hyacinth plants grown in Hartbeespoort dam water, under the conditions of four plants per bucket (FP) and ten plants per bucket (TP) and over 48 days. n = six plants per mean ($\pm$ SE). Data were tested using a repeated measure ANOVA

In Figure 5.1 the growth and death of the water hyacinth plants can be seen. Figures 5.1a and c demonstrate a growth followed by plant death, which Figures 5.1b and d show a decline from the start of the experiment. Figure 5.2 was created by extracting and annotating the ramet growth graph from Figure 5.1. The lag, growth, stationary and death phases of the water hyacinth are indicated. Ramet growth and death is the best indicator of the water hyacinth growth states.

Before data analysis was started, the leaf area was calculated using the leaf width and Equation 4.1. The data was then averaged and normalised using Equation 5.1 to better compare the growth over time. A threshold p-value of 0.05 was used to determine significance. Using this threshold value none of the parameters had a significant difference between treatments.

$$x_{normalised} = \frac{x - x_{min}}{x_{max} - x_{min}} \quad (5.1)$$

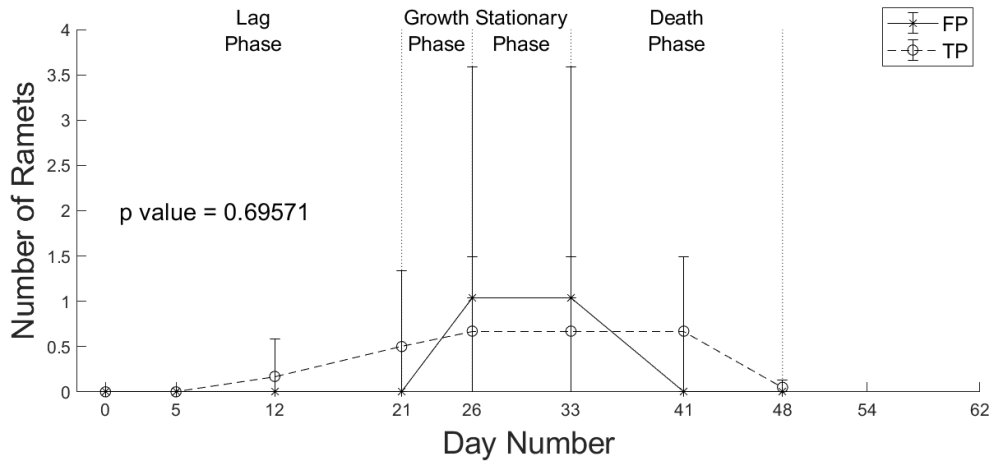


Fig. 5.2 Normalised ramet percentage change over throughout a 62-day experiment showing the growth phases of the ramets

5.1.1.1 Hartbeespoort Dam water hyacinth coverage

A similar method to that used in Section 3.2.4 to determine algae coverage was used to determine the coverage of the floating water hyacinth on Hartbeespoort Dam on the 22nd of July. This was done to provide a sense of context at the beginning of the second experiment. The Google Earth image was close-cropped around the shore of the dam, as some of the terrestrial vegetation was similar in colour to the water hyacinth which would impact the result of the image analysis. The image analysis procedure is presented in Figure 5.3. The result of this analysis gave a percentage coverage of water hyacinth on Hartbeespoort Dam of 27.5%.

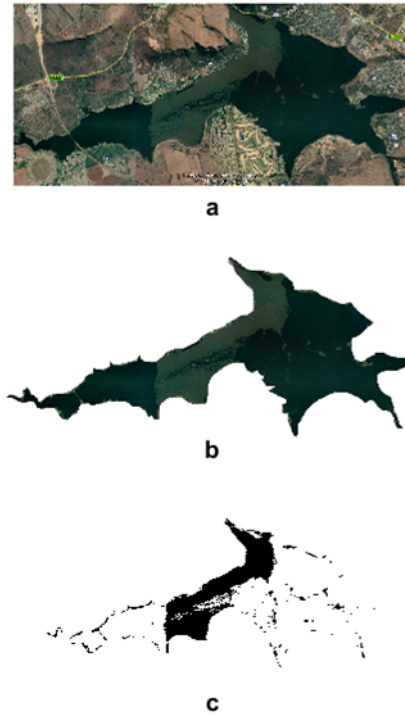


Fig. 5.3 Image Processing for Water hyacinth coverage on Hartbeespoort Dam on the 22nd of July 2019, where (a) is the original RGB image, (b) is the image cropped to the shore and (c) is the binary image.

5.1.2 Water quality

Figure 5.4 displays the change in phosphate and nitrate concentrations over 62 days in the winter months of June to August. Figure 5.4a displays a general decreasing trend from start to end. It took 48 days for the concentration to reach steady state. In Figure 5.4b it can be seen that there is initially an increase in nitrate concentration, after day 21 the concentration decreases until it was beyond detection limit at day 62. In Figure 5.4b, the data points on day 23 were removed as they were determined to be outliers. This was determined as the rest of the nitrate concentrations fit a similar trend except for this point and the phosphate concentration would be replicating the change if they were the correct points. The outliers can most likely be attributed to experimental error on the day. There was not a significant difference in either the nitrate or phosphate concentrations across all three treatments.

Figure 5.5 displays the average removal rates of phosphates and nitrates in all three treatments. The average phosphate removal rates in treatments C, FP and TP are 0.059 mg/L/day, 0.063mg/L/day and 0.045 mg/L/day respectively. The average nitrate removal rates in treatments C, FP and TP are 0.071 mg/L/day, 0.077 mg/L/day and 0.061 mg/L/day respectively. There was no significant difference in the removal of either phosphates or nitrates between any of the treatments.

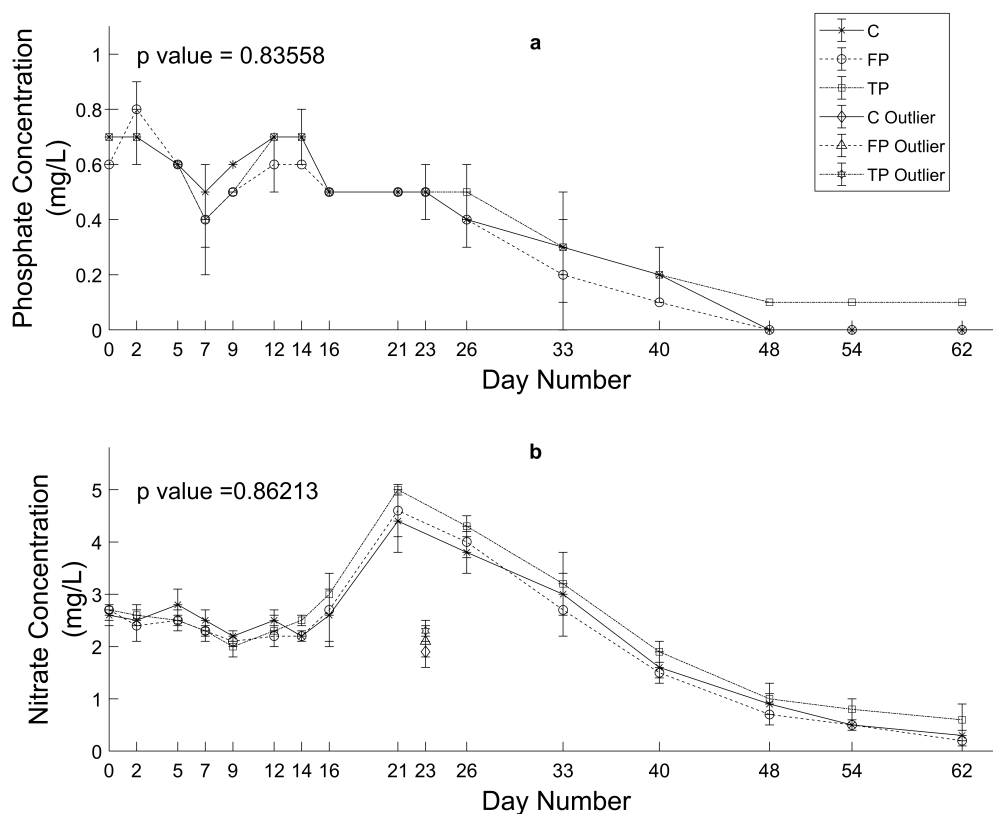


Fig. 5.4 Average change in phosphate (a) and nitrate (b) concentrations in Hartbeespoort Dam water with no water hyacinth plants (C), four water hyacinth plants (FP) and ten water hyacinth plants (TP). Data were tested using a repeated measure ANOVA

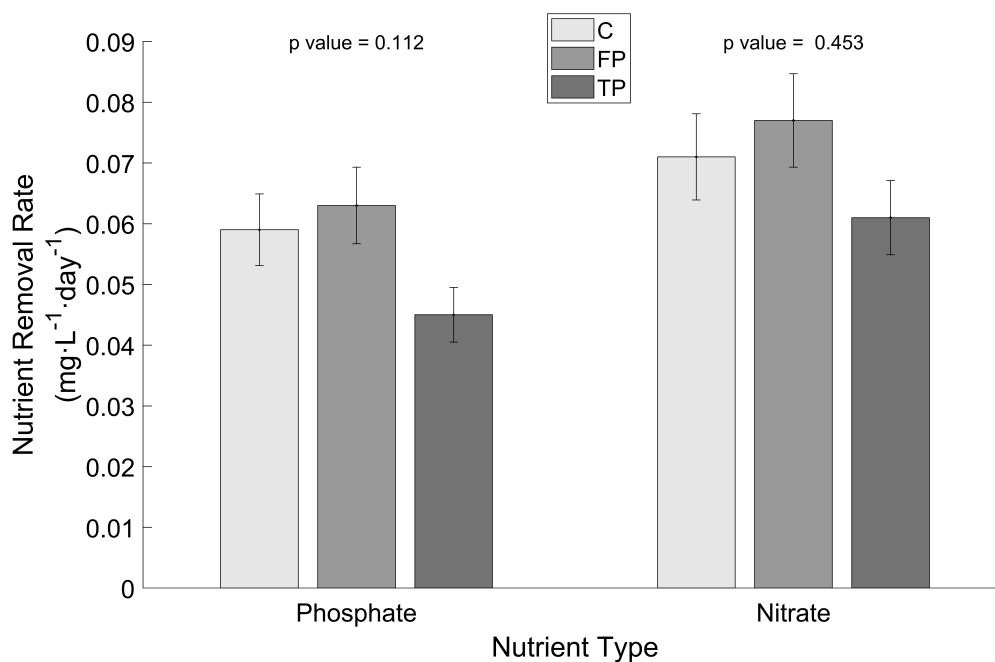


Fig. 5.5 Average phosphate and nitrate removal rates for a control (C), four water hyacinth plants (FP) and ten water hyacinth plants (TP). Data were tested using a repeated measure ANOVA and a p-value of 0.05 for significance

5.1.3 Algal growth

The percentage of algae coverage obtained from the image analysis was used, along with the known surface area of the water, to determine the area of algae coverage. Figure 5.6 displays the average change in algae coverage in each treatment over the 62 days of the experiment. For the first 23 days, there is no change in the average algae coverage. The algae coverage in the FP and TP treatments increase at a similar rate after the 23rd day. The algae coverage in control also begins to increase after the 23rd day, at a slower rate than observed in the other two treatments. In all three treatments, the algae coverage reaches a maximum on the 48th day, after which it started to decrease. There was no significant difference in algae growth between the treatments. The percentage of algae coverage was plotted on a log axis to produce Figure 5.7. In this Figure, the growth phases are marked. The lag phase occurs between days 0 and 23, followed by the exponential growth phase between day 23 and day 33, the stationary phase occurs between days 33 and 54, which is followed by the death phase.

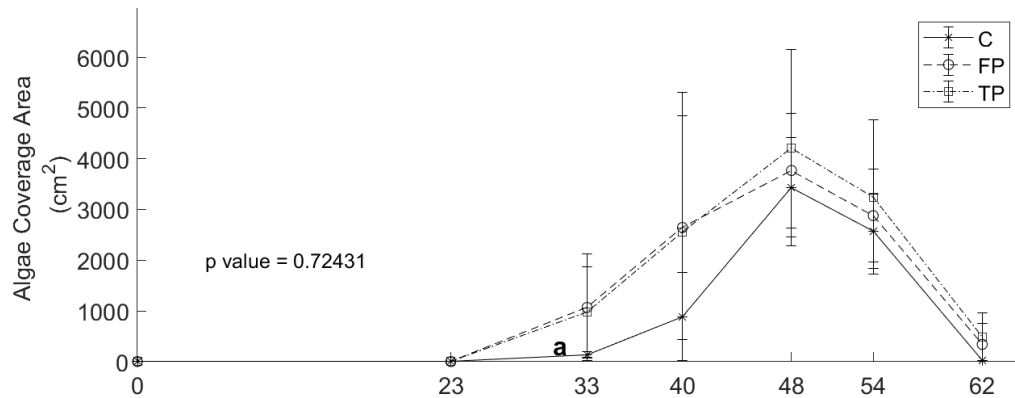


Fig. 5.6 Average change in Algae coverage in Hartbeespoort Dam water with no water hyacinth plants (C), four water hyacinth plants (FP) and ten water hyacinth plants (TP). Data were tested using a repeated measure ANOVA

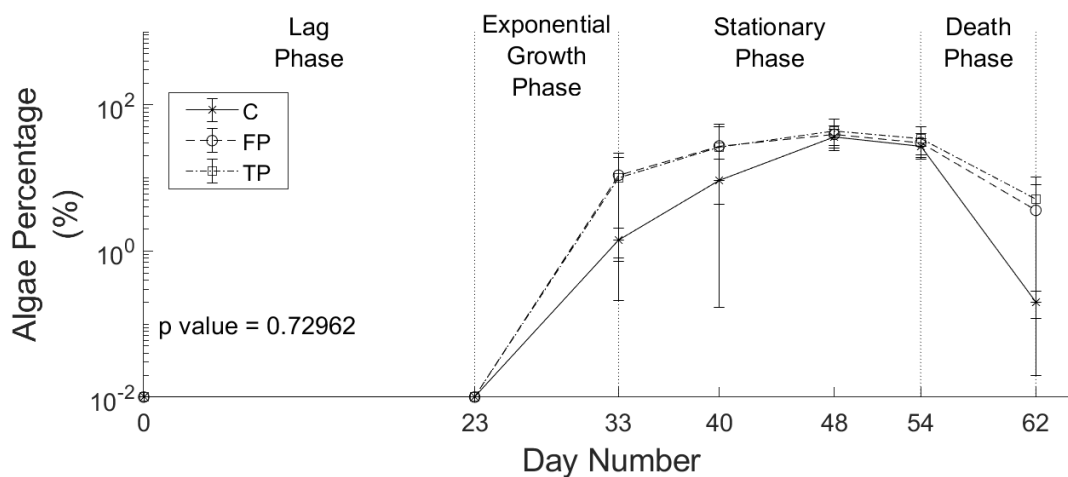


Fig. 5.7 Growth curve for Algae in a batch system with Hartbeespoort Dam water with no water hyacinth plants (C), four water hyacinth plants (FP) and ten water hyacinth plants (TP). Data were tested using a repeated measure ANOVA

5.2 Discussion

5.2.1 Plant growth

The plant experiment aimed to monitor the growth and death of the hyacinth plants in 55L of Hartbeespoort Dam water during winter. Two of the parameters in Figure 5.1, the petiole length and the number of ramets, show that the plants grow initially up until approximately day 26, after which they begin to decrease showing plant death. The two remaining parameters, the leaf area and number of leaves, decrease from the beginning of the experiment. Since these two parameters are both based on the leaf growth, it can be surmised that the growth of the leaves is more susceptible to cold conditions than the growth of the petioles and ramets. This summation is corroborated by Center and Spencer in their article “The phenology and growth of water hyacinth (*Eichhornia Crassipes* (Mart.) Solms) in a eutrophic north-central Florida lake” [36].

The growth phases of the ramets in Figure 5.2 are used as a representation of the water hyacinth growth as it shows the most pronounced growth, stationary and death phases. Growth and death phases are also seen in Figure 5.1a and support the pattern of growth and death observed in Figure 5.2. The ultimate goal of this channel of research is to be able to create a process whereby water hyacinth is allowed to grow in a eutrophic system but removed at a certain point to create steady-state nutrient removal. From Figure 5.2 it has been determined that to create this system, the best time to remove the water hyacinth would be after approximately 26 days during winter to avoid an algae bloom while maximising the nutrient removal as far as possible. Further testing will be required to determine the optimal growth duration during summer. This duration has been chosen as it is at the end of the water hyacinth growth phase, just as the growth is becoming stationary which means the plants do not have chance to die and decay, releasing nutrients that they took up. It is also at the beginning of the accelerated growth phase of the algae. By replacing the plants at this stage of algae growth, it should result in a reduced algae bloom as the average plant size will not decrease to a great extent hence the algae will not receive an increased amount of sunlight which will reduce photosynthesis. Water hyacinth replacement at the beginning of the stationary growth phase will also reduce the re-release nutrients from the plants, which the algae would have used for growth. While this day is not at the point of maximum nitrate removal it is close enough to that point to provide sufficient nutrient removal for this proposed steady-state.

5.2.2 Water quality

The water quality experiment aimed to determine what happens to the concentration of phosphates and nitrates when water hyacinth plants die in situ while algae growth begins. The phosphate and nitrate concentrations were monitored throughout the experiment to determine the effect of the growth and death of the water hyacinth as algae growth begins on the concentrations of the measured nutrients. The initial increase in nitrates in Figure 5.4b could be as a result of the nitrification process, however, these mechanisms were not tested and further studies would have to be conducted to confirm this hypothesis. Following on from the nitrification theory, phosphates are conserved since they cannot be obtained from the atmosphere. As the phosphate concentrations do not increase at the beginning of the experiment it

has been assumed that the increase in nitrates is not a result of the release of nutrients by the water hyacinth. The decrease in phosphate concentration from the start to the end of the experiment, and the decrease of the nitrate concentration from the 21st day until the end of the experiment, can be attributed to the growth of the water hyacinth plants and the subsequent growth of algae.

Once the water hyacinth begins to die and decay the nutrients are released back into the water [21], the fact that this was not seen in the experiment has been attributed to the increased growth of algae depicted in Figure 5.6. When the water hyacinth plants grow, they take up nutrients preferentially over the algae [30], it follows from this that when they began to die, the algae could more effectively compete for nutrients and as the plants began to shrivel this allowed more sunlight to reach the algae for photosynthesis. The growth of the algae after the death of the water hyacinth is what lead to the continued decrease in nutrient concentrations. It is surmised that when the algae died it sank to the bottom of the vessels without rupturing, as ruptured cells would have caused an increase in nutrient concentrations.

5.2.3 Algal growth

The algae experiment aimed to create a method for monitoring algae growth visually to produce a microbial growth curve for the algae as well as to monitor the increased growth of algae once the water hyacinth began to die. The method developed for monitoring algae growth had some advantages and disadvantages. The main advantage of this method was its convenience. The photographs were taken with an easily accessible cellphone camera, the photograph cropping was done using GIMP which is a freely available program and the images were processed using MATLAB which is accessible at many universities for students. The main disadvantage was the quality of the equipment used. The quality of cellphone cameras is highly variable and GIMP may not save the highest quality pictures. This method is sufficient for the purposes of this study and is convenient for studies where specialist equipment is not available, however, it may need to be refined for studies that require higher accuracy.

The algal growth (Figure 5.7) reaches its maximum approximately 12 days after the nitrates (Figure 5.4) and 7 days after the water hyacinth growth (Figure 5.2 reaches its maximum. This possibly occurred because the water hyacinth was removing nutrients preferentially over the algae at the beginning of the experiment but as its growth approach the stationary phase the growth slowed down, allowing the algae to start taking up nutrients [30]. It is suspected that at this point the water hyacinth had also begun releasing nutrients as a result of the leaves the dying and decaying, which the algae were able to take up for its growth, however, these mechanisms were not tested in this experiment and further investigation will be required to confirm this. It has been surmised that a sequence of events is required for algal growth and it is suspected that limiting one of the factors could prevent growth. If the water hyacinth is removed as it gets to its maximum removal state (at approximately day 21-23), this could limit the algae growth as well as maximise the nutrients removed from the system. These mechanisms will need further in depth-testing to confirm this theory. As the nutrient concentration did not increase as algae

coverage declined, it has been assumed that the algae cells did not rupture and release nutrients back into the water, rather that they sunk to the bottom of the vessels.

CHAPTER 6

Discussion

The main objective for this experiment consisted of three parts: to determine if water hyacinth can be used *in-situ* as a tool for phytoremediation of eutrophic dam water, to determine what could occur if water hyacinth is removed from a eutrophic water system and to determine what could occur if the water hyacinth was left to die in a water system. A significantly higher phosphate and nitrate removal rate was observed, on a small-scale, when water hyacinth was present in the water than that observed when water hyacinth was not present. The higher nutrient removal rate demonstrates that water hyacinth can be used as a phytoremediation tool. The water hyacinth was able to remove nutrients in both Hartbeespoort Dam water and Hoagland's solution, demonstrating the potential for the plant to be used in different types of water. In Hartbeespoort Dam water not containing water hyacinth, (imitating what could occur if the plant is removed from a water system) an algae bloom was observed, as well as a significantly lower rate of nutrient removal. When the water hyacinth is no longer present in eutrophic water containing algae spores, the spores can grow at a greater rate resulting in a bloom. If water hyacinth is left to die in a water system and the plants begin to decompose, they re-release nutrients back into the water. This, combined with a reduced nutrient removal rate, can result in an algae bloom as the plants are no longer removing nutrients preferentially over the algae and there are more nutrients available for the algae to consume to reproduce.

The research conducted for this thesis consisted of two experiments: a small-scale experiment conducted in summer and a larger-scale experiment conducted in winter. The experiments focused on Hartbeespoort dam, North West Province, South Africa as a case study. This was done in the first experiment by determining to what extent water hyacinth can be used for remediation in three litres of water with one plant, the impact of the type of water on phosphate and nitrate removal and the effect that water hyacinth has on the removal of phosphates and nitrates from Hartbeespoort dam water. In the second experiment, this was done by monitoring the growth and death of water hyacinth in winter in 75 litres of water and differing number of plants, determining the rates of phosphate and nitrate removal by plant growth, and by monitoring and mapping the growth of algae after the hyacinth death.

It was hypothesised that the water hyacinth plants would be an effective tool for phytoremediation as a result of their high nutrient uptake rates. The type of water (Hoagland's solution and Hartbeespoort Dam water) was not thought to have an impact on the growth of the plant or its ability to remove nutrients as water hyacinth has been shown to take up heavy metals and other such contaminants with little to no detrimental effect on the plant [17–19]. The presence of water hyacinth is thought to result

in a higher rate of phosphate and nitrate removal than the rate of removal in the vessels without water hyacinth. It was also hypothesised that the vessels without water hyacinth will experience an algae bloom.

For the second experiment, it was hypothesised that the water hyacinth would experience growth at the beginning of the experiment but would die towards the end of the experiment as a result of exposure to cold and possible frost [29]. The rates of phosphate and nitrate removal should be lower in the second experiment than the first as the water hyacinth growth rate is lower in winter than in summer and it is thought that the plants will die in the winter. As a result of plant death, it is thought that there will be an algae bloom as the water hyacinth will no longer inhibit algal growth [30].

6.1 Water hyacinth plants

Experiment One demonstrated that the type of water does not have a significant effect on the growth of the plants as they grew equally well in Hoagland's solution and water from Hartbeespoort Dam as seen in Figure 4.1. Experiment Two only used water from Hartbeespoort dam as a growth medium for the water hyacinth since another comparison was not needed to determine if water hyacinth grew better in Hartbeespoort Dam water or in a standard solution.

Experiment One and two showed the difference in water hyacinth growth in summer and winter, respectively. To more effectively compare the growth of the hyacinth plants in the two experiments, a parameter of growth needed to be chosen which is an appropriate analogue for the growth of the plant as a whole. According to literature, the water hyacinth leaf population shifts from making up approximately 70% of the plant weight to only making up approximately 35% of the plant weight in winter [36]. Experiment Two demonstrates that the number of leaves and their area decreased from the beginning of the experiment in winter demonstrating that the leaves are more susceptible to cold and frost than the petioles and ramets hence it was decided that the leaf parameters were not adequate to use as an analogue for the growth of the plant. The change in petiole length and the number of ramets in Experiment Two both show an increase at the beginning of the experiment and a decrease at the end (Figure 5.1). The change in the number of ramets has a more defined growth curve, shown in Figure 5.2, and hence was chosen as the parameter to adequately depict the water hyacinth growth. In Experiment One, none of the parameters reached a stationary growth phase and hence did not influence the choice of ramets as the analogue for plant growth. In Figure 4.1, it could be seen that the number of ramets increased steadily for the duration of Experiment One, it is inferred from this that the plants were growing at a relatively constant rate for the whole experiment.

6.2 Phosphates and nitrates

These experiments have shown that the phosphate and nitrate removal rates of water hyacinth in summer are an order of magnitude greater than in winter with the average removal rate of the summer

experiment being 0.378mg/L/day for the phosphates and 0.365mg/L/day for the nitrates, and the average removal rate from the winter experiment being 0.056mg/L/day for phosphates and 0.070mg/L/day for nitrates respectively. In winter the plants decreased in size as a result of a lower growth rate, and hence the nutrient uptake by the plants was decreased [36]. It had previously been found that the temperature of the water directly affects the nutrient absorption by water hyacinth [40].

In Experiment One, it was observed that the nitrate removal rates between Hoagland's solution and Hartbeespoort Dam water were not significantly different, however, the removal rates of phosphates were significantly different. The removal of phosphates from Hartbeespoort dam water was significantly higher than those in the Hoagland's solution, which suggest the presence of a compound or organism in the Hartbeespoort water which aids the uptake of phosphates, however, this mechanism was not tested in this set of experiments. Phosphate is the limiting factor for nutrient uptake as the plants cannot retrieve phosphates from the atmosphere, the presence of an organism or compound which increases the uptake of phosphates would decrease the time that the plants take to remediate a system. There was a significant difference in the removal of phosphates and nitrates in Experiment One between Hartbeespoort Dam water with and without water hyacinth plants. This supports the hypothesis that water hyacinth can be used to remediate eutrophic water as the removal rates were significantly higher in the vessels with water hyacinth than without. The nutrient concentrations in the Hartbeespoort dam water with the plants were reduced to undetectable levels after three days while it took approximately nine days for the nitrates and eleven days for the phosphates to become undetectable in the vessels without water hyacinth. Experiment Two did not show a significant difference in the removal of phosphates and nitrates which is assumed to be as a result of the already low rates of removal in winter. From the plant growth in Experiment Two, it has been assumed that the plant growth in Experiment One should reach a similar stationary growth phase followed by a death phase after a longer period. It is suggested that the stationary growth phase is the best period at which to remove the water hyacinth plants from a steady-state system to obtain maximum nutrient removal without the re-release of nutrients when the plants die. In order to reduce the impact of the lag phase at the beginning of the plant growth, it is suggested that the plants be entered into the system in batches at staggered time intervals since the first batch would be nearing the growth phase as the second batch were entered into the system and so forth.

6.3 Algae

The aim of the experiment set without water hyacinth in Experiment One and two was to simulate what could occur in a eutrophic water body if water hyacinth was removed from that system. For the vessels with water from Hartbeespoort Dam in Experiment One, an algae bloom was observed in the vessels without water hyacinth while no algae bloom was observed in those with hyacinth. As the same water was used in both experiment sets, it can be surmised that the water hyacinth prevented the growth of algae and, hence, if the water hyacinth is removed from a eutrophic water body where algae spores are present, an algae bloom could ensue. In Experiment Two, both the control and the vessels with water hyacinth experienced an algae bloom. As a result of this, the data from Experiment Two cannot be used to corroborate the findings from Experiment One that the removal of water hyacinth leads to an algae

bloom. However, by comparing the results from Experiment One and Experiment Two from the experiment sets with Hartbeespoort Dam water and water hyacinth plants (HB from Experiment One and FP and TP from Experiment Two), it can be seen that the plant death *in-situ* which occurs in winter can lead to an algae bloom. This is as a result of the re-release of nutrients by the decaying water hyacinth plants and the shrivelling of the plants as they die, allowing more sunlight to reach the algae spore for photosynthesis. To prevent the algae bloom in winter, it is suggested that the plants be removed and replaced more frequently as the temperatures get colder.

CHAPTER 7

Conclusion

7.1 Limitations and recommendations

The results reported in this study should be considered in light of some limitations. This research focused on the Hartbeespoort Dam and was conducted on a small scale over a short period. The nutrient extraction rates observed in this study may be valid only at Hartbeespoort Dam as water bodies in different climates may have differing concentrations of nutrients and other unknown contaminants which could change the rate of nutrient removal. Two separate experiments were conducted for summer and winter, the results obtained from these separate experiments may differ if the experiment is run continuously through summer and winter. The results of the experiment may be affected by the scale of the experiment. Result bias may also have occurred in the algae image processing experiment as a result of the quality of the camera and image editing software used; a lower quality camera could have a lower pixel density which could affect the accuracy of the pixel count and simpler editing software could have resulted in too much or too little of the image being cropped. It is recommended that this study be repeated on a larger scale over a longer time frame, with water from various water bodies and in different climates. Higher quality camera and editing software are also recommended. To enhance this study it is also recommended that the Hartbeespoort Dam water be tested for the presence of agents increasing the rate of phosphate removal and that the mechanism of nutrient uptake by water hyacinth be compared to that of the algae present in the dam. The presence of hyacinth is also a limitation – it is not possible to recommend the introduction of an invasive alien species into a water body to induce remediation; rather, if hyacinth is there, there exists the potential to exploit it for beneficial remediation.

7.2 Conclusion

Notwithstanding these limitations, this study has shown that water hyacinth can be used as a phytoremediation tool on a small-scale. This was determined through two experiments, one conducted in summer and the other in winter. The parameters measured for these experiments were: the effect that the type of water has on the growth rate of water hyacinth, the effect the type of water has on the removal of phosphates and nitrates, the difference in nutrient depletion when the hyacinth is and is not present in a eutrophic water body, the effect that plant death and algae growth has on the phosphate and nitrate concentrations, the difference in nutrient removal rate in summer and winter and the difference in algae growth in summer and winter. The type of water (Hartbeespoort Dam water or Hoagland's solution) was not found to impact the growth of the water hyacinth plants or the removal of nitrates,

however, the rate of phosphate removal was significantly higher in the Hartbeespoort Dam water, possibly due to the presence of a symbiont, or another factor, in the dam water. In summer the rate of removal of phosphates and nitrates was significantly higher in the Hartbeespoort Dam vessels with water hyacinth present than those without the plant. This begins to show the suitability of water hyacinth as a phytoremediation tool, as the concentration of phosphates and nitrates were reduced to undetectable levels within four days when water hyacinth was present, while it took eleven days for the concentration to be reduced in the vessels without water hyacinth. An algae bloom was experienced only in the vessels without water hyacinth, which showcases the plants' ability to suppress algae growth. In winter an algae bloom was experienced in all vessels which has been attributed to the water hyacinth plants not being able to suppress the growth of the algae as a result of low plant growth rates and plant death. It has been concluded that the death of the water hyacinth led to a re-release of phosphates and nitrates which provided nutrients for the algae to grow. The growth of the algae in all vessels in winter demonstrates what could happen in the dam as the plants begin to die.

These results suggest the possibility of developing a steady-state engineering solution using a problematic invasive weed to help solve the problem of eutrophication in South African dams. They also show that the water hyacinth may already play a part in the remediation of Hartbeespoort Dam, however, the death of the plants *in-situ* could be re-contributing to the trophic state of the dam and providing a platform for increased algae growth in winter. The water hyacinth should be removed from the system when they reach a stationary growth phase to circumvent the re-release of nutrients. A steady-state would need to be maintained by adding new water hyacinth to the system, preferably when partially through the lag phase of growth, to maximise the nutrient removal. This study demonstrates the potential for water hyacinth to be used in an *in-situ* steady-state system for the removal of nutrients from eutrophic dam water.

REFERENCES

- [1] Tariq Khokhar. Globally, 70% of freshwater is used for agriculture, 2017.
- [2] Michael Pidwirny. Chapter 8: Introduction to the hydrosphere, 2014.
- [3] World Health Organisation. Drinking-water, 2019.
- [4] The World Bank Group. Climate change knowledge portal, 2019.
- [5] Paul Reig, Andrew Maddocks, and Francis Gassert. World's 36 most water-stressed countries, 2013.
- [6] Mark William Matthews. Eutrophication and cyanobacterial blooms in south african inland waters: 10years of meris observations. *Remote Sensing of Environment*, 155:161–177, 2014.
- [7] Doster E. Zitomer R. A. and Wilson A. E Chislock, M. F. Eutrophication: Causes, consequences, and controls in aquatic ecosystems. *Nature Education Knowledge*, 4(4):8, 2013.
- [8] Water Wise; Rand Water. Water situation in south africa, 2019.
- [9] Governemnt of South Australia. Blue green algae, July 2019.
- [10] T. Le. Microcystis: Toxic blue-green algae, February 2009. For the Office of Environmental Health Hazard Assessment.
- [11] Shahabaldin Rezania, MohanadossPonraj, Mohd Fadhil Md Din, Ahmad Rahman Songip, Fadzlin Md Sairan, and Shreeshivadasan Chelliapan. The diverse applications of water hyacinth with main focus on sustainable energy and production for new era:an overview. *RenewableandSustainableEnergyReviews*, 2015.
- [12] Tsutomu Imaoka and Seiji Teranishi. Rates of nutrient uptake and growth of the water hyacinth. *Water Research*, 1988.
- [13] Anushree Malik. Environmental challenge vis a vis opportunity: The case of water hyacinth. *Environment International*, 33(1):122–138, 2007.
- [14] Guy Rogers. War on river weed hots up. *The Herald*, 2018.
- [15] Lani van Vuuren. Microscope refocused on sa water quality threat : eutrophication. *WaterWheel*, 7:14–17, 2008.

- [16] L. J. Fox, P. C. Struik, B. L. Appleton, and J. H. Rule. Nitrogen phytoremediation by water hyacinth (*Eichhornia crassipes* (Mart.) Solms). *Water, Air, and Soil Pollution*, 194:199–207, 2008.
- [17] M M McKnown B C Wolverton. Phytoremediation potential of aquatic macrophyte, azolla. *Aquatic Botany* 2, 191 (1976)., 2:191, 1976.
- [18] VanZandt F L Shore R W Smith. Water hyacinths for removal of mirex from water. *Journal of the Mississippi Academy of Sciences Supplement* 22, 20 (1977)., 1977.
- [19] Lu Xiaomei, Maleeya Kruatrachue, Prayad Pokethitiyookb, and Kunaporn Homyokb. Removal of cadmium and zinc by water hyacinth, *Eichhornia crassipes*. *ScienceAsia*, 30:93–103, 2004.
- [20] Youngchul Kim and Wan-Joong Kim. Roles of water hyacinths and their roots for reducing algal concentration in the effluent from waste stabilization ponds. *Water Research*, 34(13):3285–3294, 2000.
- [21] eThekweni Municipality Environmental Planning Climate Protection Department. *Water Hyacinth Control: Insight into Best Practice, Removal Methods, Training and Equipment*. 2013.
- [22] Wm. T. Penfound and T. T. Earle. The biology of the water hyacinth. *Ecological Monographs*, 18(4):447–472, 1948.
- [23] Martin P. Hill and Julie Coetzee. The biological control of aquatic weeds in south africa: Current status and future challenges. *Bothalia - African Biodiversity Conservation*, 47:1 – 12, 00 2017.
- [24] Biological and integrated control of water hyacinth, *Eichhornia crassipes*. *Second Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth*, page 152, 2000.
- [25] J. A. Coetzee and M. P. Hill. Biological control of water hyacinth – the south african experience. *EPPO Bulletin*, 38:458–463, 2008.
- [26] R.W. Jones. Integrated control of water hyacinth on the nseleni/mposa rivers and lake nsezi, kwa zulu-natal, south africa. *Biological and Integrated Control of Water Hyacinth, Eichhornia crassipes*, 2001.
- [27] Intermediate Technology Development Group. Water hyacinth control and possible uses. techreport, United Diversity Library, 2009.
- [28] Anjanabha Bhattacharya and Pawan Kumar. Water hyacinth as a potential biofuel crop. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 9, 2011.
- [29] Billy C Wolverton and Rebecca C McDonald. The water hyacinth: From prolific pest to potential provider. *Ambio*, 8(1):2–9, 1979.
- [30] Denise De C. Bicudo, Barbara M. Fonseca, Luis M. Bini, Luciane O. Crossetti, Carlos E. De M. Bicudo, and Tatiane Araújo Jo-Jesus. Undesirable side-effects of water hyacinth control in a shallow tropical reservoir. *Freshwater Biology*, 52:1120–1133, 2007.

- [31] Simon Damkjaer and Richard Taylor. The measurement of water scarcity: Defining a meaningful indicator. *Ambio*, 46(5):513–531, 2017.
- [32] Christine Colvin, Dean Muruven, David Lindley, Helen Gordon, and Klaudia Schachtschneider. Water: Facts and futures, rethinking south africa’s water future. techreport, World Wildlife Fund, 2016.
- [33] Britt-Marie Haahti, Eva Hedenström, Sebastian Linke, Cecilia Lundberg, Gunilla Reisner, and Markus Wanamo. Case-study report: Eutrophication. techreport, Environmental Risk Governance of the Baltic Sea, 2010.
- [34] National-Eutrophication-Monitoring-Programme-Design. Eutrophication, 2003.
- [35] J. Heisler, P.M. Glibert, J.M. Burkholder, D.M. Anderson, W. Cochlan, W.C. Dennison, Q. Dortch, C.J. Gobler, C.A. Heil, E. Humphries, A. Lewitus and R. Magnien, H.G. Marshallm, K. Sellner, D.A. Stockwell, D.K. Stoecker, and M. Suddleson. Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae*, 2008.
- [36] Ted D. Center and Neal R. Spencer. The phenology and growth of water hyacinth (*eichhornia crassipes* (mart.) solms) in a eutrophic north-central florida lake. *Aquatic Botany*, 10:1–32, 1981.
- [37] K. R. Reddy and W. F. DeBusk. Growth characteristics of aquatic macrophytes cultured in nutrient-enriched water: I. water hyacinth, water lettuce, and pennywort. *Economic Botany*, 38(2):229–239, 1984.
- [38] K.R. Reddy, M. Agami, and J.C. Tucker. Influence of nitrogen supply rates on growth and nutrient storage by water hyacinth (*eichhornia crassipes*) plants. *Aquatic Botany*, 36:33–43, 1989.
- [39] K.R. Reddy, M. Agami, and J.C. Tucker. Influence of phosphorus on growth and nutrient storage by water hyacinth (*eichhornia crassipes* (mart.) solms) plants. *Aquatic Botany*, 37:355–365, 1990.
- [40] M. M. Gpetrucio and F. A. Esteves. Uptake rates of nitrogen and phosphorus in the water by *eichhornia crassipes* and *salvinia auriculata*. *Revista Brasileira de Biologia*, 60:229–236, 2000.
- [41] Ernesto Mangas-Ramírez and Manuel Elías-Gutiérrez. Effect of mechanical removal of water hyacinth (*eichhornia crassipes*) on the water quality and biological communities in a mexican reservoir. *Aquatic Ecosystem Health and Management*, 7:161–168, 2004.
- [42] P. Janaki, S. Meena, and C. Chinnusamy. Persistence of 2,4-d and paraquat in aquatic system and its effect on dissolved oxygen and fish mortality. *IJTA*, 28(3-4):497–502, 2010.
- [43] M.P. Hill and T. Olckers. Biological control initiatives against water hyacinth in south africa: Constraining factors, success and new courses of action. *Biological and Integrated Control of Water Hyacinth, Eichhornia crassipes*, 2001.
- [44] Bronwen May and Julie Coetzee. Comparisons of the thermal physiology of water hyacinth biological control agents: predicting establishment and distribution pre- and post-release. *Entomologia Experimentalis et Applicata*, 147:241–250, 2013.

- [45] R. Teygeler. Water hyacinth paper. contributions to a sustainable future. *Paper and Water*, 2000.
- [46] Shahabaldin Rezania, Mohd Fadhil Md Din, Siti Fatimah Kamaruddin, Shazwin Mat Taib, Lakhveer Singh, Ee Ling Yong, and Farrah Aini Dahalan. Evaluation of water hyacinth (*eichhornia crassipes*) as a potential raw material source for briquette production. *Energy*, 2015.
- [47] Types of biofuel, 2010.
- [48] Harshit Sharma and M.S. Chauhan. Water hyacinth: Control and utilization. *International Journal of Civil Engineering and Technology*, 8:765–772, 2017.
- [49] Dr. Ann C. Wilkie. Feedstocks for biogas production, 2017.
- [50] GO. Ogawa Masami, Ishida Yukinari Usui, and Naoto Urano. Ethanol production from the water hyacinth *eichhornia crassipes* by yeast isolated from various hydrospheres. *African Journal of Microbiology Research*, 2:110–113, 2008.
- [51] Chartchalerm Isarankura-Na-Ayudhya, Tanawut Tantimongcolwat, Thikamporn Kongpanpee, Ponpitak Prabkate, and Virapong Prachayasittikul. Appropriate technology for the bioconversion of water hyacinth (*eichhornia crassipes*) to liquid ethanol: Future prospects for community strengthening and sustainable development. *EXCLI Journal*, 2007.
- [52] H.N. Chanakya, Sushama Borgaonkar, G. Meena, and K.S. Jagadish. Solid-phase biogas production with garbage or water hyacinth. *Bioresource Technology*, 46:227–231, 1993.
- [53] A.K. Kivaisi and M. Mtila. Production of biogas from water hyacinth (*eichhornia crassipes*) (mart) (solms) in a two-stage bioreactor. *World Journal of Microbiology and Biotechnology*, 14:125–131, 1998.
- [54] M. M. Lasat. Phytoextraction of metals from contaminated soil: A review of plant/soil/metal interaction and assessment of pertinent agronomic issues. *Journal of Hazardous Substance Research*, 2:1–25, 1999.
- [55] LU Jianbo, FU Zhihui, and YIN Zhaozheng. Performance of a water hyacinth (*eichhornia crassipes*) system in the treatment of wastewater from a duck farm and the effects of using water hyacinth as duck feed. *Journal of Environmental Sciences*, 20:513–519, 2008.
- [56] R. Sooknah. A review of the mechanisms of pollutant removal in water hyacinth systems. *Science and Technology - Research Journal*, 6, 2000.
- [57] Karl Imhoff, W.J. Muller, and D.K.B. Thistlethwayte. Disposal of sewage and other water-borne wastes. *New Biological Books*, 1971.
- [58] K. K. Moorhead and K. R. Reddy. Oxygen transport through selected aquatic macrophytes. *Journal of Environment Quality*, 17(1):138–142, 1988.
- [59] B. D. Tripathi and Alka R. Upadhyay. Dairy effluent polishing by aquatic macrophytes. *Water, Air, and Soil Pollution*, 2003.

- [60] A. Alum, B. Mobasher, A. Rashid, and M. Abbaszadegan. Image analyses-based nondisruptive method to quantify algal growth on concrete surfaces. *Journal of Environmental Engineering*, 135(3):185–190, 2009.
- [61] Jacobus Petrus Brink. *The cultivation and harvesting of micro-algal biomass from the Hartbeespoort Dam for the production of biodiesel*. PhD thesis, North-West University, 2011.
- [62] C. Butterwick, S.I. Heaney, and J.F. Talling. A comparison of eight methods for estimating the biomass and growth of planktonic algae. *British Phycological Journal*, 17(1):69–79, 1982.
- [63] Rodrigo A. Garcia, Peter Fearn, John K. Keesing, and Dongyan Liu. Quantification of floating macroalgae blooms using the scaled algae index. *Journal of Geophysical Research: Oceans*, 118(1):26–42, 2013.
- [64] Intermediate Technology Development Group. Water hyacinth control and possible uses, 2009.
- [65] Hartbeespoort Dam Rehabilitation Steering Committee. History. 2019, 2017.
- [66] Aqeelah Davis. Hydrogeological characteristics of hartbeespoort dam. *School of Geosciences, Masters of Science in Hydrogeology*:74, 2017.
- [67] Damjana Drobac, Nada Tokodi, Jelica Simeunovic, Vladimir Baltic, Dina Stanic, and Zorica Svircev. Human exposure to cyanotoxins and their effects on health. *Arhiv Za Higijenu Rada i Toksikologiju*, 64(2):119–130, 2013.
- [68] European and Mediterranean Plant Protection Organization. Eichhornia crassipes. *National regulatory control systems*, 39:460–464, 2009.
- [69] E. van Wyk and B. W. van Wilgen. The cost of water hyacinth control in south africa: a case study of three options. *African Journal of Aquatic Science*, 27(2):141–149, 2002.
- [70] G.C.G. Fraser, M.P. Hill, and J. A. Martin. Economic evaluation of water loss saving due to the biological control of water hyacinth at new year’s dam, eastern cape province, south africa. *African Journal of Aquatic Science*, 41(2):227–234, 2016.
- [71] L. Taiz and E. Zeiger. *Mineral nutrition.*, pages 100 – 119. 1991.
- [72] Angela Bownes, Martin P. Hill, and Marcus J. Byrne. Assessing density–damage relationships between water hyacinth and its grasshopper herbivore. *Entomologia Experimentalis et Applicata*, 137(3):246–254, 2010.

Appendices

Appendix A

Experiment 1 - Nutrient concentrations

Refill No.	Phosphate Concentration (mg/L)									
	Date	Day	H2	H3	HP 2	HP 3	HB 2	HB 3	C2	C3
1	2017/08/28	0	2.34	1.68	2.15	2.05	0.43	0.56	0.64	0.65
	2017/08/29	1	1.55	0.79	1.96	1.31	0.14	0.14	0.66	0.67
	2017/08/30	2	1.2	0.56	1.79	0.97	0.12	0.08	0.72	0.68
	2017/08/31	3	2.18	0.67	1.88	0.9	0.02	0.05	1.07	1.07
2	2017/08/31	3	0.56	0.53	0.76	0.7	0.51	0.62	0.59	0.55
	2017/09/03	6	0.55	0.32	0.53	0.05	0.06	0.21	0.34	0.55
	2017/09/05	8	0.06	0.1	0.21	0.08	0.06	0.04	0.34	0.17
3	2017/09/05	8	0.64	0.64	0.8	0.93	0.66	0.63	0.34	0.17
	2017/09/06	9	0.29	0.27	0.58	0.53	0.08	0.1	0.21	0.16
	2017/09/08	11	0.1	0.13	0.38	0.24	0.04	0.03	0.1	0.06

Refill No.	Nitrate Concentration (mg/L)									
	Date	Day	H2	H3	HP 2	HP 3	HB 2	HB 3	C2	C3
1	2017/08/28	0	2	2.4	2.3	4.3	4.1	3.4	3.2	2.9
	2017/08/29	1	1.4	1.5	1.9	1.9	1.2	1.8	3.8	3.9
	2017/08/30	2	1.1	0.6	1.2	1	0.5	0.5	3.6	4.1
	2017/08/31	3	0.5	0.5	0.5	0.5	0.5	0.5	4.4	4.5
2	2017/08/31	3	0.9	0.6	1	1.5	2.1	3	3.8	3.2
	2017/09/03	6	0.5	0.5	0.5	0.5	0.5	0.5	1.3	2.3
	2017/09/05	8	0.5	0.5	0.5	0.5	0.5	0.5	1.1	1
3	2017/09/05	8	1.8	1.8	1.7	1.7	4.3	4	1.1	1
	2017/09/06	9	1.2	1.1	1.4	1.1	1.2	1.1	0.5	0.5
	2017/09/08	11	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Appendix B

Experiment 1 - Water hyacinth measurements

Stem Height Marked Leaf (cm)													
Date	Day Number	H						HB					
		1	2	3	4	5	6	1	2	3	4	5	6
28-Aug	1	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.0	0.0	0.0
31-Aug	4	0.3	0.0	0.6	1.0	0.4	0.0	0.0	0.1	0.0	0.7	0.2	0.0
05-Sep	9	0.8	0.5	0.9	0.5	0.4	0.5	1.0	0.0	0.3	0.7	0.9	0.3
08-Sep	12	0.6	1.0	1.0	0.6	0.8	0.7	1.0	0.2	0.4	0.8	0.7	0.0
12-Sep	16	0.9	1.0	0.8	1.0	0.9	1.0	0.8	0.3	1.0	0.8	1.0	1.0
18-Sep	22	1.0	0.9	1.0	0.6	1.0	0.7	0.9	1.0	0.8	1.0	0.9	0.3

Leaf height Marked Leaf (cm)													
Date	Day Number	HP						HB					
		1	2	3	4	5	6	1	2	3	4	5	6
28-Aug	1	1.0	0.0	1.0	0.5	0.0	0.8	0.0	0.0	0.7	0.7	1.0	1.0
31-Aug	4	0.5	0.0	1.0	0.0	0.4	0.0	0.0	0.2	0.7	0.3	0.8	0.5
05-Sep	9	0.0	0.7	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5
08-Sep	12	0.3	0.5	1.0	1.0	0.8	0.8	0.0	0.6	0.6	1.0	0.0	0.5
12-Sep	16	1.0	1.0	0.0	0.0	0.8	1.0	0.5	0.6	0.1	0.0	0.5	0.5
18-Sep	22	0.0	0.5	0.0	1.0	1.0	0.8	1.0	0.8	0.0	0.0	1.0	0.0

Leaf Width Marked Leaf (cm)													
Date	Day Number	HP						HB					
		1	2	3	4	5	6	1	2	3	4	5	6
28-Aug	1	0.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	1.0	0.9	0.5	0.7
31-Aug	4	0.0	1.0	1.0	0.8	0.5	1.0	0.0	0.0	1.0	0.9	1.0	1.0
05-Sep	9	1.0	1.0	1.0	1.0	0.5	1.0	1.0	1.0	0.3	1.0	0.5	0.7
08-Sep	12	0.0	1.0	1.0	1.0	0.5	1.0	0.0	1.0	0.3	1.0	1.0	0.3
12-Sep	16	1.0	0.9	0.0	1.0	0.5	1.0	1.0	1.0	0.0	0.0	0.0	0.0
18-Sep	22	0.0	0.0	0.0	1.0	1.0	1.0	0.0	1.0	0.3	0.1	0.0	0.3

*0 indicates dried leaf

Leaf Area Marked Leaf (cm ²)													
Date	Day Number	HP						HB					
		1	2	3	4	5	6	1	2	3	4	5	6
28-Aug	1	0.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	1.0	0.9	0.5	0.7
31-Aug	4	0.0	1.0	1.0	0.7	0.5	1.0	0.0	0.0	1.0	0.9	1.0	1.0
05-Sep	9	1.0	1.0	1.0	1.0	0.5	1.0	1.0	1.0	0.3	1.0	0.5	0.7
08-Sep	12	0.0	1.0	1.0	1.0	0.5	1.0	0.0	1.0	0.3	1.0	1.0	0.3
12-Sep	16	1.0	0.8	0.0	1.0	0.5	1.0	1.0	1.0	0.0	0.0	0.0	0.0
18-Sep	22	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.9	0.2	0.1	0.0	0.3

0 indicates dried leaf

Number of Ramets													
Date	Day Number	HP						HB					
		1	2	3	4	5	6	1	2	3	4	5	6
28-Aug	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31-Aug	4	0.0	0.0	0.2	0.0	0.3	0.1	0.1	0.1	0.0	0.2	0.1	0.0
05-Sep	9	0.1	0.1	0.3	0.3	0.3	0.2	0.1	0.1	0.3	0.2	0.1	0.1
08-Sep	12	0.3	0.3	0.4	0.4	0.5	0.4	0.4	0.3	0.5	0.6	0.4	0.4
12-Sep	16	0.5	0.9	0.9	0.9	0.9	0.9	0.9	0.7	0.7	0.8	0.9	1.0
18-Sep	22	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Number of Leaves													
Date	Day Number	HP						HB					
		1	2	3	4	5	6	1	2	3	4	5	6
28-Aug	1	0.7	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
31-Aug	4	0.0	0.5	0.8	0.3	0.0	0.0	0.0	0.5	0.3	0.0	0.0	0.3
05-Sep	9	0.3	1.0	0.8	0.7	0.3	0.0	0.3	0.5	0.3	0.5	0.3	0.5
08-Sep	12	0.3	1.0	1.0	0.7	0.3	0.3	0.7	1.0	0.7	1.0	0.3	0.5
12-Sep	16	0.7	0.5	0.0	1.0	0.7	0.7	1.0	0.0	1.0	0.0	0.7	0.8
18-Sep	22	1.0	0.0	0.0	1.0	1.0	1.0	1.0	0.5	1.0	0.5	1.0	1.0

Appendix C

Experiment 2 - Nutrient concentrations

Phosphate Data										
Date	Day No.	Concentration (mg/L)								
		C1	C3	C5	FP1	FP3	FP5	TP1	TP3	TP5
2019/06/19	0	0.77	0.6	0.6	0.64	0.62	0.66	0.62	0.59	0.85
2019/06/21	2	0.75	0.65	0.81	0.84	0.72	0.79	0.63	0.61	0.78
2019/06/24	5	0.68	0.71	0.67	0.67	0.67	0.65	0.64	0.66	0.63
2019/06/26	7	0.4	0.41	0.67	0.31	0.36	0.61	0.31	0.35	0.62
2019/06/28	9	0.6	0.65	0.65	0.58	0.63	0.63	0.56	0.62	0.61
2019/07/01	12	0.65	0.81	0.91	0.64	0.74	0.77	0.75	0.79	0.83
2019/07/03	14	0.84	0.9	0.86	0.75	0.78	0.77	0.74	0.77	0.83
2019/07/05	16	0.59	0.7	0.71	0.64	0.74	0.69	0.65	0.72	0.74
2019/07/10	21	0.64	0.74	0.71	0.65	0.74	0.74	0.67	0.75	0.74
2019/07/12	23	0.6	0.85	0.77	0.65	0.77	0.78	0.68	0.79	0.75
2019/07/15	26	0.58	0.81	0.76	0.62	0.83	0.79	0.66	0.81	0.91
2019/07/22	33	0.19	0.83	0.72	0.03	0.63	0.79	0.25	0.72	0.9
2019/07/29	40	0.25	0.64	0.47	0.21	0.45	0.46	0.36	0.39	0.86
2019/08/06	48	0.11	0.27	0.2	0.11	0.25	0.18	0.29	0.26	0.36
2019/08/12	54	0.32	0.37	0.045	0.2	0.28	0.29	0.27	0.38	0.44
2019/08/20	62	0.26	0.34	0.42	0.36	0.32	0.22	0.24	0.51	0.48

Absorbance										
Date	Day No.	C1	C3	C5	FP1	FP3	FP5	TP1	TP3	TP5
2019/06/19	0	0.377	0.294	0.295	0.315	0.306	0.325	0.304	0.289	0.417
2019/06/21	2	0.368	0.317	0.396	0.409	0.354	0.386	0.309	0.298	0.382
2019/06/24	5	0.331	0.346	0.327	0.328	0.328	0.319	0.312	0.325	0.309
2019/06/26	7	0.196	0.204	0.328	0.153	0.179	0.299	0.157	0.174	0.306
2019/06/28	9	0.292	0.319	0.318	0.284	0.308	0.311	0.274	0.304	0.3
2019/07/01	12	0.317	0.396	0.445	0.314	0.359	0.377	0.368	0.387	0.408
2019/07/03	14	0.411	0.437	0.419	0.465	0.382	0.374	0.362	0.375	0.406
2019/07/05	16	0.289	0.345	0.349	0.313	0.36	0.335	0.319	0.35	0.361
2019/07/10	21	0.314	0.361	0.345	0.319	0.36	0.359	0.328	0.368	0.363
2019/07/12	23	0.295	0.415	0.374	0.317	0.378	0.379	0.332	0.386	0.368
2019/07/15	26	0.283	0.395	0.372	0.306	0.405	0.386	0.324	0.298	0.399
2019/07/22	33	0.095	0.404	0.351	0.18	0.31	0.384	0.125	0.354	0.44
2019/07/29	40	0.127	0.314	0.23	0.107	0.22	0.226	0.177	0.191	0.42
2019/08/06	48	0.058	0.137	0.104	0.059	0.126	0.091	0.0146	0.133	0.18
2019/08/12	54	0.162	0.181	0.221	0.101	0.237	0.199	0.206	0.42	0.285
2019/08/20	62	0.132	0.168	0.209	0.176	0.157	0.112	0.123	0.252	0.237

Tub Volume (L)										
Date	Day No.	C1	C3	C5	FP1	FP3	FP5	TP1	TP3	TP5
2019/06/19	0	46	46	46	46	46	46	46	46	46
2019/06/21	2	46	45	45	45	44	45	45	45	45
2019/06/24	5	46	42	42	43	42	42	43	42	43
2019/06/26	7	46	41	41	41	41	41	41	41	42
2019/06/28	9	46	39	39	40	39	40	40	39	40
2019/07/01	12	46	37	37	38	37	37	38	37	39
2019/07/03	14	46	35	32	38	34	34	38	36	41
2019/07/05	16	35	34	34	35	34	35	36	34	36
2019/07/10	21	31	30	30	32	30	31	32	31	33
2019/07/12	23	32	28	27	30	27	28	30	30	34
2019/07/15	26	28	27	27	28	26	27	29	27	30
2019/07/22	33	26	21	21	23	21	22	25	22	27
2019/07/29	40	21	17	16	19	14	17	21	17	22
2019/08/06	48	12	10	10	13	9	12	15	11	16
2019/08/12	54	8	6	6	9	4	7	11	6	13
2019/08/20	62	5	2	2	5	1	2	7	2	10

Date	Day No.	Mass (mg)								
		C1	C3	C5	FP1	FP3	FP5	TP1	TP3	TP5
2019/06/19	0	35.42	27.60	27.60	29.44	28.52	30.36	28.52	27.14	39.10
2019/06/21	2	34.50	28.94	36.06	37.50	32.00	35.21	28.16	27.16	34.92
2019/06/24	5	31.28	30.03	28.33	28.55	28.22	27.58	27.36	27.93	27.04
2019/06/26	7	18.40	16.74	27.34	12.79	14.61	25.01	12.85	14.30	25.85
2019/06/28	9	27.60	25.57	25.56	23.14	24.58	24.93	22.49	24.42	24.68
2019/07/01	12	29.90	30.07	33.76	24.23	27.15	28.82	28.66	29.37	32.05
2019/07/03	14	38.64	31.50	27.52	28.50	26.52	26.18	28.12	27.72	34.03
2019/07/05	16	20.54	23.92	24.23	22.50	24.86	23.85	23.15	24.65	26.76
2019/07/10	21	20.05	22.55	21.60	20.65	21.99	22.93	21.69	22.92	24.48
2019/07/12	23	19.46	24.19	20.90	19.35	20.90	22.20	20.25	23.52	25.33
2019/07/15	26	16.14	21.68	20.30	17.59	21.45	21.66	19.22	21.78	27.31
2019/07/22	33	4.91	17.18	14.90	0.70	13.04	17.36	6.14	15.82	24.43
2019/07/29	40	5.17	10.82	7.36	3.99	6.48	7.78	7.45	6.59	18.90
2019/08/06	48	1.37	2.83	2.08	1.48	2.20	2.10	4.31	2.78	5.93
2019/08/12	54	2.65	2.24	0.27	1.88	1.16	2.15	2.96	2.39	5.63
2019/08/20	62	1.22	0.79	0.98	1.69	0.37	0.51	1.70	1.19	4.56

Date	Day No.	Equivalent Concentration in 46L (mg/L)								
		C1	C3	C5	FP1	FP3	FP5	TP1	TP3	TP5
2019/06/19	0	0.77	0.60	0.60	0.64	0.62	0.66	0.62	0.59	0.85
2019/06/21	2	0.75	0.63	0.78	0.82	0.70	0.77	0.61	0.59	0.76
2019/06/24	5	0.68	0.65	0.62	0.62	0.61	0.60	0.59	0.61	0.59
2019/06/26	7	0.40	0.36	0.59	0.28	0.32	0.54	0.28	0.31	0.56
2019/06/28	9	0.60	0.56	0.56	0.50	0.53	0.54	0.49	0.53	0.54
2019/07/01	12	0.65	0.65	0.73	0.53	0.59	0.63	0.62	0.64	0.70
2019/07/03	14	0.84	0.68	0.60	0.62	0.58	0.57	0.61	0.60	0.74
2019/07/05	16	0.45	0.52	0.53	0.49	0.54	0.52	0.50	0.54	0.58
2019/07/10	21	0.44	0.49	0.47	0.45	0.48	0.50	0.47	0.50	0.53
2019/07/12	23	0.42	0.53	0.45	0.42	0.45	0.48	0.44	0.51	0.55
2019/07/15	26	0.35	0.47	0.44	0.38	0.47	0.47	0.42	0.47	0.59
2019/07/22	33	0.11	0.37	0.32	0.02	0.28	0.38	0.13	0.34	0.53
2019/07/29	40	0.11	0.24	0.16	0.09	0.14	0.17	0.16	0.14	0.41
2019/08/06	48	0.03	0.06	0.05	0.03	0.05	0.05	0.09	0.06	0.13
2019/08/12	54	0.06	0.05	0.01	0.04	0.03	0.05	0.06	0.05	0.12
2019/08/20	62	0.03	0.02	0.02	0.04	0.01	0.01	0.04	0.03	0.10

Nitrate Data										
Date	Day No.	Concentration (mg/L)								
		C1	C3	C5	FP1	FP3	FP5	TP1	TP3	TP5
2019/06/19	0	2.5	2.5	2.7	2.7	2.8	2.7	2.6	3.0	2.5
2019/06/21	2	2.5	2.5	2.6	2.4	2.3	2.8	2.5	2.8	2.8
2019/06/24	5	2.7	3.4	2.7	2.8	2.6	2.9	2.6	2.8	2.8
2019/06/26	7	2.4	3.0	2.7	2.5	2.7	2.6	2.6	2.8	2.3
2019/06/28	9	2.3	2.4	2.6	2.3	2.5	2.6	2.1	2.5	2.4
2019/07/01	12	2.6	2.8	3.2	2.7	2.6	3.0	2.4	2.9	3.0
2019/07/03	14	2.2	3.0	3.1	2.7	2.9	2.9	2.9	3.1	2.9
2019/07/05	16	2.7	3.7	3.9	2.9	3.2	4.7	3.3	4.0	4.3
2019/07/10	21	5.4	7.3	7.0	5.8	7.4	7.2	7.0	7.5	7.0
2019/07/12	23	2.3	3.6	3.3	3.0	3.4	3.9	3.3	3.4	3.4
2019/07/15	26	5.5	6.9	6.9	6.0	6.9	7.3	6.5	7.2	7.0
2019/07/22	33	4.4	7.4	6.8	4.2	6.3	6.3	5.1	6.7	6.5
2019/07/29	40	4.1	3.7	4.2	3.8	4.1	4.0	3.9	5.7	4.0
2019/08/06	48	3.7	4.2	2.9	2.9	2.9	2.8	3.2	2.8	3.8
2019/08/12	54	3.1	3.9	3.7	2.8	3.7	3.4	2.9	4.5	3.6
2019/08/20	62	4.1	3.7	4.2	3.8	4.1	4.0	3.9	5.7	4.0

Date	Day No.	Absorbance								
		C1	C3	C5	FP1	FP3	FP5	TP1	TP3	TP5
2019/06/19	0	0.348	0.343	0.393	0.377	0.413	0.383	0.368	0.451	0.360
2019/06/21	2	0.351	0.350	0.347	0.335	0.321	0.363	0.353	0.383	0.363
2019/06/24	5	0.382	0.448	0.371	0.379	0.367	0.391	0.361	0.379	0.390
2019/06/26	7	0.325	0.382	0.372	0.326	0.380	0.345	0.334	0.388	0.313
2019/06/28	9	0.314	0.320	0.367	0.315	0.332	0.364	0.304	0.345	0.342
2019/07/01	12	0.372	0.400	0.442	0.388	0.373	0.434	0.353	0.408	0.419
2019/07/03	14	0.322	0.365	0.383	0.371	0.357	0.360	0.398	0.381	0.359
2019/07/05	16	0.432	0.496	0.515	0.448	0.484	0.607	0.452	0.524	0.555
2019/07/10	21	0.677	0.893	0.854	0.727	0.903	0.882	0.859	0.909	0.860
2019/07/12	23	0.303	0.447	0.411	0.386	0.416	0.459	0.409	0.423	0.418
2019/07/15	26	0.693	0.842	0.843	0.745	0.840	0.886	0.806	0.877	0.859
2019/07/22	33	0.576	0.900	0.833	0.547	0.777	0.784	0.646	0.821	0.799
2019/07/29	40	0.540	0.494	0.554	0.510	0.542	0.529	0.521	0.713	0.532
2019/08/06	48	0.499	0.546	0.409	0.409	0.406	0.398	0.445	0.397	0.502
2019/08/12	54	0.428	0.517	0.498	0.399	0.499	0.463	0.406	0.582	0.483
2019/08/20	62	0.540	0.494	0.554	0.510	0.542	0.529	0.521	0.713	0.532

Date	Day No.	Tub Volume (L)								
		C1	C3	C5	FP1	FP3	FP5	TP1	TP3	TP5
2019/06/19	0	46	46	46	46	46	46	46	46	46
2019/06/21	2	46	45	45	45	44	45	45	45	45
2019/06/24	5	46	42	42	43	42	42	43	42	43
2019/06/26	7	46	41	41	41	41	41	41	41	42
2019/06/28	9	46	39	39	40	39	40	40	39	40
2019/07/01	12	46	37	37	38	37	37	38	37	39
2019/07/03	14	46	35	32	38	34	34	38	36	41
2019/07/05	16	35	34	34	35	34	35	36	34	36
2019/07/10	21	31	30	30	32	30	31	32	31	33
2019/07/12	23	32	28	27	30	27	28	30	30	34
2019/07/15	26	28	27	27	28	26	27	29	27	30
2019/07/22	33	26	21	21	23	21	22	25	22	27
2019/07/29	40	21	17	16	19	14	17	21	17	22
2019/08/06	48	12	10	10	13	9	12	15	11	16
2019/08/12	54	8	6	6	9	4	7	11	6	13
2019/08/20	62	5	2	2	5	1	2	7	2	10

Date	Day No.	Mass (mg)								
		C1	C3	C5	FP1	FP3	FP5	TP1	TP3	TP5
2019/06/19	0	115.0	115.0	124.2	124.2	128.8	124.2	119.6	138.0	115.0
2019/06/21	2	115.0	111.3	115.7	107.1	102.2	124.8	111.8	124.7	125.4
2019/06/24	5	124.2	143.8	114.2	119.3	109.5	123.0	111.2	118.5	120.2
2019/06/26	7	110.4	122.5	110.2	103.1	109.5	106.6	107.8	114.4	95.9
2019/06/28	9	105.8	94.4	102.2	91.8	97.6	102.9	84.3	98.5	97.1
2019/07/01	12	119.6	103.9	118.7	102.2	95.4	112.3	91.7	107.8	115.9
2019/07/03	14	101.2	105.0	99.2	102.6	98.6	98.6	110.2	111.6	118.9
2019/07/05	16	94.0	126.8	132.6	101.9	107.5	163.4	117.8	135.8	153.7
2019/07/10	21	168.1	223.7	212.5	185.0	220.9	224.4	227.6	228.9	232.9
2019/07/12	23	74.6	102.4	89.6	89.3	92.3	111.0	98.3	101.2	114.8
2019/07/15	26	153.4	184.1	183.9	169.9	177.2	199.6	190.7	193.5	211.0
2019/07/22	33	114.8	153.3	140.6	97.1	130.0	139.4	124.8	146.9	176.0
2019/07/29	40	85.1	62.4	66.4	72.9	59.5	67.8	81.5	96.3	88.8
2019/08/06	48	46.6	43.7	30.2	39.2	25.4	32.9	48.2	30.0	62.0
2019/08/12	54	25.5	23.6	22.1	26.5	15.4	25.2	31.6	28.3	45.9
2019/08/20	62	19.3	8.6	9.9	18.0	4.8	9.4	27.9	13.3	38.4

Date	Day No.	Equivalent Concentration in 46L (mg/L)								
		C1	C3	C5	FP1	FP3	FP5	TP1	TP3	TP5
2019/06/19	0	2.50	2.50	2.70	2.70	2.80	2.70	2.60	3.00	2.50
2019/06/21	2	2.50	2.42	2.52	2.33	2.22	2.71	2.43	2.71	2.73
2019/06/24	5	2.70	3.13	2.48	2.59	2.38	2.67	2.42	2.58	2.61
2019/06/26	7	2.40	2.66	2.40	2.24	2.38	2.32	2.34	2.49	2.08
2019/06/28	9	2.30	2.05	2.22	1.99	2.12	2.24	1.83	2.14	2.11
2019/07/01	12	2.60	2.26	2.58	2.22	2.07	2.44	1.99	2.34	2.52
2019/07/03	14	2.20	2.28	2.16	2.23	2.14	2.14	2.40	2.43	2.58
2019/07/05	16	2.04	2.76	2.88	2.22	2.34	3.55	2.56	2.95	3.34
2019/07/10	21	3.65	4.86	4.62	4.02	4.80	4.88	4.95	4.98	5.06
2019/07/12	23	1.62	2.23	1.95	1.94	2.01	2.41	2.14	2.20	2.50
2019/07/15	26	3.34	4.00	4.00	3.69	3.85	4.34	4.15	4.21	4.59
2019/07/22	33	2.50	3.33	3.06	2.11	2.83	3.03	2.71	3.19	3.83
2019/07/29	40	1.85	1.36	1.44	1.59	1.29	1.47	1.77	2.09	1.93
2019/08/06	48	1.01	0.95	0.66	0.85	0.55	0.72	1.05	0.65	1.35
2019/08/12	54	0.56	0.51	0.48	0.58	0.34	0.55	0.69	0.62	1.00
2019/08/20	62	0.42	0.19	0.22	0.39	0.10	0.20	0.61	0.29	0.83

Appendix D

Experiment 2 - Water hyacinth measurements

[illegible][illegible][illegible]

Date	Day Number	Leaf Width												
		Plant Number	Bucket	1	2	3	4	5	6	7	8	9	10	Average
2019/07/10	21		FP1	3.1	3.9	2.8	3.1							3.2
			FP2	3.1	2.7	3.6	3.4							3.2
			FP3	3.2	3.2	0	2.5							2.2
			FP4	4.5	3	2.5	3.2							3.3
			FP5	0	3.2	3.4	3.6							2.6
			FP6	3.1	2.7	0	2.9							2.2
			Average											2.8
			TP1	2.8	2.9	4.4	3.9	3.1	3	2.9	2.4	0	3.1	2.9
			TP2	2.5	3.3	3.2	3.5	0	2.9	4	2.4	3	3.9	2.9
			TP3	4	3.4	2.8	3.4	3.3	3.2	3	3.6	0	2.9	3.0
			TP4	3	2.8	3.8	0	3.4	2.3	3.1	3.7	2.8	2.7	2.8
			TP5	3.9	3.4	2.5	3.5	3	0	3.6	3.3	3.9	0	2.7
			TP6	0	2.6	3.3	2.9	3.8	0	3.2	2.9	3.4	0	2.2
			Average											2.7

Date	Day Number	Leaf Width												
		Plant Number	Bucket	1	2	3	4	5	6	7	8	9	10	Average
2019/07/15	26	FP1		2	2.7	3.3	2.9							2.7
		FP2		2.9	2.8	0	3.3							2.3
		FP3		3.2	3.1	0	2.5							2.2
		FP4		4.5	3	2.5	2.9							3.2
		FP5		0	0	3.4	3.6							1.8
		FP6		3.2	2.5	0	2.7							2.1
		Average												2.4
		TP1		2.9	3	4.4	3.9	2.8	3	3	2.5	0	3.2	2.9
		TP2		3.2	2.5	3	3.1	0	3	3.9	0	2.9	3.9	2.6
		TP3		3.5	3	2.9	3.3	0	3.4	0	0	0	0	1.6
		TP4		3.2	0	3.5	0	4	2.8	2.9	3.6	2.7	2.9	2.6
		TP5		3.8	3.4	0	3.1	3	2.9	3.6	0	2.7	0	2.3
		TP6		0	3	3.4	3.7	3.4	0	3.3	3.1	3.4	0	2.3
		Average												2.4

Date	Day Number	Leaf Width												
2019/07/22	33	Plant Number	Bucket	1	2	3	4	5	6	7	8	9	10	Average
		FP1		0	0	0	0							0.0
		FP2		0	0	3.2	0							0.8
		FP3		0	0	0	0							0.0
		FP4	4.3	0	2.3	0								1.7
		FP5		0	0	2.7	3.4							1.5
		FP6		0	0	0	0							0.0
		Average												0.7
		TP1		0	0	0	0	0	3.1	0	0	0	0	0.3
		TP2	3.2	0	0	2.7	0	2.6	0	0	2.9	1.9		1.3
		TP3		0	0	2.9	1.5	0	0	3.2	0	0	0	0.8
		TP4		0	2.8	0	0	3.4	2.7	0	0	0	0	0.9
		TP5	3	3.3	0	3.1	2.9	3.3	3.6	0	0	0		1.9
		TP6		0	0	3.2	0	0	0	3.1	0	3.6	0	1.0
		Average												1.0

Date	Day Number	Leaf Width												
		Plant Number	Bucket	1	2	3	4	5	6	7	8	9	10	Average
2019/07/30	41	FP1		0	0	0	0							0.0
		FP2		0	0	0	0							0.0
		FP3		0	0	0	0							0.0
		FP4		3.2	0	0	0							0.8
		FP5		0	0	3	3.4							1.6
		FP6		0	0	0	0							0.0
		Average												0.4
		TP1		0	0	0	0	0	0	0	0	0	0	0.0
		TP2		3.2	0	0	0	0	3.2	0	0	2.6	3.9	1.3
		TP3		0	0	0	0	0	0	0	0	0	0	0.0
		TP4		0	0	0	0	3.3	0	0	0	0	0	0.3
		TP5		2.9	2.7	0	0	0	0	3.4	0	0	0	0.9
		TP6		0	2.7	0	0	0	0	0	0	0	0	0.3
		Average												0.5

Date	Day Number	Leaf Width												
		Plant Number	Bucket	1	2	3	4	5	6	7	8	9	10	Average
2019/08/06	48	FP1		0	0	0	0							0.0
		FP2		0	0	0	0							0.0
		FP3		0	0	0	0							0.0
		FP4		0	0	0	0							0.0
		FP5		0	0	0	0							0.0
		FP6		0	0	0	0							0.0
		Average												0.0
		TP1		0	0	0	0	0	0	0	0	0	0	0.0
		TP2		0	0	0	0	0	0	0	0	0	3.8	0.4
		TP3		0	0	0	0	0	0	0	0	0	0	0.0
		TP4		0	0	0	0	0	0	0	0	0	0	0.0
		TP5		0	0	0	0	0	0	3.5	0	0	0	0.4
		TP6		0	0	0	0	0	0	0	0	0	0	0.0
		Average												0.1

Date	Day Number	Leaf Area (Calculated from Leaf Width)												
		Bucket												
2019/06/19	0	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	7.83	10.9	8.65	11.4								9.694375
		FP2	7.05	9.97	9.97	4.98								7.99235
		FP3	8.65	6.68	7.83	9.97								8.28315
		FP4	12.4	7.05	4.98	9.52								8.4839
		FP5	11.4	8.65	7.83	8.65								9.1315
		FP6	8.65	8.65	9.52	9.97								9.200075
		Average	Average											8.79755833
		TP1	5.96	8.24	10.4	9.08	8.65	5.96	9.52	4.98	5.96	9.52	7.83141	
		TP2	7.05	9.97	6.68	9.08	10.4	5.62	9.52	9.52	5.96	6.68	8.05209	
		TP3	10.4	10.9	7.43	8.24	10.4	8.65	10.4	7.05	10.4	14	9.79822	
		TP4	9.97	5.96	10.4	8.24	7.83	6.68	9.52	9.97	7.83	9.52	8.59561	
		TP5	9.52	7.43	9.08	9.08	6.68	8.24	7.83	8.65	9.52	9.08	8.51208	
		TP6	10.9	9.08	6.68	9.97	13.4	12.4	5.96	9.97	7.83	14	10.01911	
		Average	Average											8.80142

Date	Day Number	Leaf Area (Calculated from Leaf Width)												
		Bucket												
2019/06/24	5	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	7.05	9.52	5.96	10.4								8.242075
		FP2	7.05	5.96	8.65	7.05								7.179575
		FP3	5.29	8.65	6.31	4.67								6.23275
		FP4	13.4	6.68	4.67	14								9.686825
		FP5	7.83	7.83	7.83	3.55								6.759875
		FP6	7.83	8.65	9.52	9.08								8.771775
		Average												7.81214583
		TP1	5.29	7.43	9.97	9.08	7.43	5.96	5.29	4.09	6.68	9.52	7.07601	
		TP2	7.05	9.97	6.68	5.62	9.52	5.62	9.52	5.96	5.96	9.52	7.5437	
		TP3	5.96	9.52	6.68	7.83	7.83	7.83	9.08	6.31	9.52	6.68	7.72478	
		TP4	9.52	5.62	9.52	7.43	7.83	6.31	8.24	7.43	6.31	6.68	7.4909	
		TP5	9.08	7.43	4.09	7.05	6.31	7.83	7.83	4.98	9.52	7.05	7.11767	
		TP6	9.52	8.65	6.31	8.65	14	12.9	5.96	9.52	6.68	9.52	9.17014	
		Average												7.6872

Date	Day Number	Leaf Area (Calculated from Leaf Width)												
		Bucket												
2019/07/01	12	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	5.96	6.31	7.05	8.24								6.89135
		FP2	7.83	5.62	7.83	8.24								7.379975
		FP3	6.68	7.83	4.37	4.98								5.963775
		FP4	12.4	6.31	4.67	7.83								7.800375
		FP5	7.43	8.24	7.43	4.37								6.869525
		FP6	8.24	6.68	6.31	7.05								7.06975
		Average												6.99579167
		TP1	5.29	9.97	6.68	14.5	8.24	5.96	5.96	4.67	7.05	8.24		7.6581
		TP2	5.96	10.4	7.05	7.83	9.52	5.96	9.52	5.29	3.06	10.4		7.50658
		TP3	4.98	8.24	6.68	7.05	8.65	8.65	9.08	5.62	4.37	9.97		7.32962
		TP4	7.05	6.68	5.62	3.55	7.05	6.31	7.83	8.24	5.62	5.96		6.3919
		TP5	9.08	6.68	4.67	7.83	5.96	7.83	8.24	5.29	9.08	4.67		6.93322
		TP6	5.29	9.08	6.68	7.83	11.4	13.4	6.68	9.52	7.05	9.97		8.69241
		Average												7.41863833

Date	Day Number	Leaf Area (Calculated from Leaf Width)												
		Bucket												
2019/07/10	21	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	6.31	9.52	5.29	6.31								6.861275
		FP2	6.31	4.98	8.24	7.43								6.74045
		FP3	6.68	6.68	0.54	4.37								4.566525
		FP4	12.4	5.96	4.37	6.68								7.35025
		FP5	0.54	6.68	7.43	8.24								5.722
		FP6	6.31	4.98	0.54	5.62								4.363375
		Average												5.93397917
		TP1	5.29	5.62	11.9	9.52	6.31	5.96	5.62	4.09	0.54	6.31	6.11645	
		TP2	4.37	7.05	6.68	7.83	0.54	5.62	9.97	4.09	5.96	9.52	6.16361	
		TP3	9.97	7.43	5.29	7.43	7.05	6.68	5.96	8.24	0.54	5.62	6.42258	
		TP4	5.96	5.29	9.08	0.54	7.43	3.81	6.31	8.65	5.29	4.98	5.73648	
		TP5	9.52	7.43	4.37	7.83	5.96	0.54	8.24	7.05	9.52	0.54	6.10093	
		TP6	0.54	4.67	7.05	5.62	9.08	0.54	6.68	5.62	7.43	0.54	4.77783	
		Average												5.88631333

Date	Day Number	Leaf Area (Calculated from Leaf Width)												
		Bucket												
2019/07/15	26	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	3.06	4.98	7.05	5.62								5.176275
		FP2	5.62	5.29	0	7.05								4.49195
		FP3	6.68	6.31	0	4.37								4.34105
		FP4	12.4	5.96	4.37	5.62								7.086775
		FP5	0	0	7.43	8.24								3.9178
		FP6	6.68	4.37	0	4.98								4.00625
		Average												4.83668333
		TP1	5.62	5.96	11.9	9.52	5.29	5.96	5.96	4.37	0	6.68	6.12615	
		TP2	6.68	4.37	5.96	6.31	0	5.96	9.52	0	5.62	9.52	5.39573	
		TP3	7.83	5.96	5.62	7.05	0	7.43	0	0	0	0	3.39023	
		TP4	6.68	0	7.83	0	9.97	5.29	5.62	8.24	4.98	5.62	5.42316	
		TP5	9.08	7.43	0	6.31	5.96	5.62	8.24	0	4.98	0	4.76307	
		TP6	0	5.96	7.43	8.65	7.43	0	7.05	6.31	7.43	0	5.02871	
		Average												5.021175

Date	Day Number	Leaf Area (Calculated from Leaf Width)												
		Bucket												
2019/07/22	33	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	0	0	0	0								0
		FP2	0	0	6.68	0								1.6693
		FP3	0	0	0	0								0
		FP4	11.4	0	3.81	0								3.80025
		FP5	0	0	4.98	7.43								3.102625
		FP6	0	0	0	0								0
		Average												1.42869583
		TP1	0	0	0	0	0	6.31	0	0	0	0	0	0.63149
		TP2	6.68	0	0	4.98	0	4.67	0	0	5.62	2.83		2.47699
		TP3	0	0	5.62	2.01	0	0	6.68	0	0	0	0	1.43146
		TP4	0	5.29	0	0	7.43	4.98	0	0	0	0	0	1.77045
		TP5	5.96	7.05	0	6.31	5.62	7.05	8.24	0	0	0	0	4.02392
		TP6	0	0	6.68	0	0	0	6.31	0	8.24	0	0	2.12285
		Average												2.07619333

Date	Day Number	Leaf Area (Calculated from Leaf Width)												
		Bucket												
2019/07/30	41	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	0	0	0	0								0
		FP2	0	0	0	0								0
		FP3	0	0	0	0								0
		FP4	6.68	0	0	0	0							1.6693
		FP5	0	0	5.96	7.43								3.3496
		FP6	0	0	0	0								0
		Average												0.83648333
		TP1	0	0	0	0	0	0	0	0	0	0	0	0
		TP2	6.68	0	0	0	0	0	6.68	0	0	4.67	9.52	2.75441
		TP3	0	0	0	0	0	0	0	0	0	0	0	0
		TP4	0	0	0	0	7.05	0	0	0	0	0	0	0.70505
		TP5	5.62	4.98	0	0	0	0	7.43	0	0	0	0	1.80338
		TP6	0	2.7	0	0	0	0	0	0	0	0	0	0.27
		Average												0.92214

Date	Day Number	Leaf Area (Calculated from Leaf Width)												
		Bucket												
2019/08/06	48	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	0	0	0	0								0
		FP2	0	0	0	0								0
		FP3	0	0	0	0								0
		FP4	0	0	0	0								0
		FP5	0	0	0	0								0
		FP6	0	0	0	0								0
		Average												0
		TP1	0	0	0	0	0	0	0	0	0	0	0	0
		TP2	0	0	0	0	0	0	0	0	0	0	9.08	0.9082
		TP3	0	0	0	0	0	0	0	0	0	0	0	0
		TP4	0	0	0	0	0	0	0	0	0	0	0	0
		TP5	0	0	0	0	0	0	0	7.83	0	0	0	0.78301
		TP6	0	0	0	0	0	0	0	0	0	0	0	0
		Average												0.28186833

Date	Day Number	Petiole Length												
		Bucket												
2019/06/19	0	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	3.2	2.6	3	2.8								2.9
		FP2	1.4	0.4	0.9	1.6								1.075
		FP3	1.3	0.5	4	1.2								1.75
		FP4	2.1	1.2	0.9	1.4								1.4
		FP5	3.6	0.6	1.3	1.3								1.7
		FP6	1.1	2.5	2.5	2.7								2.2
		Average												1.8375
		TP1	2.2	1.5	4	3.7	1.5	2.3	1.7	1.2	0.6	1.2	1.99	
		TP2	2	1.1	2.4	2.4	2.3	3.7	1.7	1.6	1.8	1.9	2.09	
		TP3	2.5	1.5	3.2	0.8	3.4	1	1.2	1.2	3	3.4	2.12	
		TP4	3	0.8	2.4	2.6	0.8	1.6	1.2	2.9	1.2	1.4	1.79	
		TP5	2	1.1	3.6	1.2	1.1	2	1.4	3	3.1	2.9	2.14	
		TP6	2.4	1.4	1.5	2.3	3.3	2.4	1.3	3.3	0.9	2.4	2.12	
		Average												2.041667

Date	Day Number	Petiole Length												
		Bucket												
2019/06/24	5	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	2.8	2.8	3.2	2.4								2.8
		FP2	1.5	0.9	1.9	1								1.325
		FP3	1.8	1.3	4.4	0.8								2.075
		FP4	2	1.1	0.8	1.2								1.275
		FP5	3	0.8	2.5	2.2								2.125
		FP6	1.3	3.2	3.9	2.6								2.75
		Average												2.058333
		TP1	1.2	1.5	3.9	2.8	1.6	2.7	2	1.5	1.1	1.2	1.95	
		TP2	2	1.4	2.8	2.8	1.3	3.6	1.6	1.7	2	3.6	2.28	
		TP3	2.5	1.8	3	1.3	2.5	1.2	1.3	1.3	3.5	3.3	2.17	
		TP4	2.8	1.1	3.2	3.9	1.2	1.3	1.5	2.6	1.2	1.8	2.06	
		TP5	1.9	1.4	3.7	1.2	1.2	2.8	2.3	2.8	4	3.8	2.51	
		TP6	2.6	2.8	1.8	3.1	3.2	2.8	1.6	3	0.8	2.8	2.45	
		Average												2.236667

Date	Day Number	Petiole Length												
		Bucket												
2019/07/01	12	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	3.3	2.4	3	2.7								2.85
		FP2	1.5	1.3	1.8	1.6								1.55
		FP3	3.2	1.3	4	1.1								2.4
		FP4	2.1	1.2	1	1.1								1.35
		FP5	2.6	1.2	1.7	2.8								2.075
		FP6	1.2	2.8	2.4	2.6								2.25
		Average												2.079167
		TP1	1.5	3.9	1.5	3	1.6	2.6	2	1.5	0.9	1.2	1.97	
		TP2	2.2	1.3	2.9	2.4	1.6	3	1.3	1.7	2	2.1	2.05	
		TP3	2.7	1.7	2.8	3.8	2.8	1.5	1.5	1.4	3	4	2.52	
		TP4	2.8	0.5	2.8	2.4	1.1	1.5	2	2	1.8	2	1.89	
		TP5	1.5	1.2	4	1.2	0.9	2.2	1.5	4	3	2.6	2.21	
		TP6	2.2	1.4	1.6	1.9	2.8	2.7	3.3	3	1.2	3.8	2.39	
		Average												2.171667

Date	Day Number	Petiole Length												
		Bucket												
2019/07/10	21	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	3.5	4.1	2.7	2.7								3.25
		FP2	1.5	0.8	3.8	1.9								2
		FP3	3.2	1.1	4.3	1.2								2.45
		FP4	2.4	1.2	0.8	1.4								1.45
		FP5	3.2	2.3	1.9	3.1								2.625
		FP6	1.4	3	2.8	2.8								2.5
		Average												2.379167
		TP1	1.6	1.2	2.4	2.7	1.6	2.4	2	1.5	2.6	1.8		1.98
		TP2	1.9	1.7	2.4	2.7	1.3	3	1.3	1.6	1.7	1.8		1.94
		TP3	3	1.6	2.9	1	3.6	1.1	2.8	1.3	3.8	3.8		2.49
		TP4	3	1	2.8	2.5	1.1	1.3	2.1	2.7	1.3	1.7		1.95
		TP5	2.1	0.8	4	1.2	1.2	2.7	1.5	2.9	3.2	2.8		2.24
		TP6	2.7	1.5	1.3	2.6	3.3	2.5	3	3.2	1.3	3.3		2.47
		Average												2.178333

Date	Day Number	Petiole Length												
		Bucket												
2019/07/15	26	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	3.4	2.9	3.1	2.7								3.025
		FP2	1.7	0.8	2.3	1.2								1.5
		FP3	3.4	1.2	4.4	1.2								2.55
		FP4	2.3	1.3	1	1.4								1.5
		FP5	3.4	2.6	1	1.4								2.1
		FP6	1.2	3.3	3.4	1.8								2.425
		Average												2.183333
		TP1	1.8	1.7	3.1	3.1	1.7	2.8	2	1.8	3	1.5		2.25
		TP2	2.3	1.7	2.4	2.7	2.5	3	3.8	1.8	2	1.9		2.41
		TP3	3.9	1.3	3.1	3.1	3.7	1.2	1.8	1.4	3.7	3.6		2.68
		TP4	2.9	2.8	3	3.2	3.3	1.7	1.8	2.7	1.5	2.3		2.52
		TP5	1.8	1.3	4.7	1.2	1	2.2	1.6	3.1	4	2.9		2.38
		TP6	3	2.6	1.5	2.7	2.4	2.7	3.5	3.3	3.5	3.4		2.86
		Average												2.516667

Date	Day Number	Petiole Length												
		Bucket												
2019/07/22	33	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	3	2.8	3.4	3.1								3.075
		FP2	2	0.9	1.2	4.1								2.05
		FP3	3.5	1.1	0	1.1								1.425
		FP4	2.2	1.4	1.1	1.7								1.6
		FP5	3.4	2.6	2	1.3								2.325
		FP6	1.4	3.7	2.9	2.6								2.65
		Average												2.1875
		TP1	1.5	1.7	3.3	2.8	1.8	2.9	2.4	1.7	3.2	1.4		2.27
		TP2	2.3	1.5	2.3	2.6	2.1	3.3	1.5	2	1.9	3		2.25
		TP3	3.1	0	3.5	3.1	3.9	1.4	2	1.6	3.8	4.1		2.65
		TP4	3.3	1.1	3.2	2.8	1.4	1.8	2	3.2	1.7	2.1		2.26
		TP5	2.2	1.2	4.4	1.4	1.2	2.3	1.5	3.7	3.3	2.7		2.39
		TP6	3.2	1.7	1.7	2.8	3.5	2.7	3.5	3.4	1.2	3.7		2.74
		Average												2.426667

Date	Day Number	Petiole Length											
		Bucket											
2019/07/30	41	Plant Number	1	2	3	4	5	6	7	8	9	10	Average
		FP1	3.5	2.5	3.3	2.9							3.05
		FP2	2	1.2	2.6	1.5							1.825
		FP3	3.5	1.2	0	0							1.175
		FP4	2.4	1.4	1	1							1.45
		FP5	0	3.7	1.9	1.2							1.7
		FP6	1.4	3.5	0	2.7							1.9
		Average											1.85
		TP1	1.9	2	3.4	2.8	2.7	0	2.6	1.8	3.4	1.4	2.2
		TP2	2.7	2.9	2.4	2.8	2	1.2	1.5	2.3	2.3	2	2.21
		TP3	2.9	2	3.9	0	3.6	0	1.9	1.8	0	0	1.61
		TP4	3.2	1.2	3.2	3.9	0	3.6	1.9	1.8	0	0	1.88
		TP5	2	1.3	0	1.4	1	2.4	1.6	3.4	3.2	0	1.63
		TP6	0	1.5	1.8	2.6	3.6	0	3.8	3.3	3.7	0	2.03
		Average											1.926667

Date	Day Number	Petiole Length											
		Bucket											
2019/08/06	48	Plant Number	1	2	3	4	5	6	7	8	9	10	Average
		FP1	0	0	3.5	3.3							1.7
		FP2	0	0	2.1	1.4							0.875
		FP3	3.7	1.8	0	0							1.375
		FP4	2.3	1.5	0	1.6							1.35
		FP5	0	2.6	2	1.2							1.45
		FP6	1.4	2.9	0	2.9							1.8
		Average											1.425
		TP1	0	1.6	0	0	1.9	0	2.4	0	1.2	1.7	0.88
		TP2	2.6	1.9	0	2.9	2	1.2	1.6	2.4	1.9	1.9	1.84
		TP3	0	2.3	0	0	3.9	0	2	2.8	0	0	1.1
		TP4	0	1.2	0	3.4	3.5	1.7	1.7	0	2	2.3	1.58
		TP5	2.1	1.3	0	1.4	0	2.5	1.6	3	3.1	0	1.5
		TP6	0	1.7	1.4	3.8	0	0	3.6	0	1.2	0	1.17
		Average											1.345

Date	Day Number	Number of leaves											
		Bucket											
		Plant Number	1	2	3	4	5	6	7	8	9	10	Average
2019/06/19	0	FP1	5	5	5	5							5
		FP2	5	5	5	4							4.75
		FP3	5	5	5	5							5
		FP4	5	5	5	5	5						5
		FP5	5	5	4	5							4.75
		FP6	5	5	5	5							5
		Average											4.91667
		TP1	5	5	5	5	4	4	5	5	5	5	4.8
		TP2	5	5	5	5	4	5	5	5	5	5	4.9
		TP3	5	5	5	5	5	5	5	5	5	5	5
		TP4	4	5	5	5	5	5	5	5	5	5	4.9
		TP5	5	5	5	5	5	5	5	5	5	5	5
		TP6	5	5	5	5	5	5	5	5	5	5	5
		Average											4.93333

Date	Day Number	Number of leaves											
		Bucket											
2019/06/24	5	Plant Number	1	2	3	4	5	6	7	8	9	10	Average
		FP1	5	4	4	4							4.25
		FP2	4	4	3	5							4
		FP3	4	5	3	5							4.25
		FP4	5	4	3	4							4
		FP5	4	4	3	3							3.5
		FP6	4	4	4	4							4
		Average											4
		TP1	3	4	4	3	4	4	5	5	4	4	4
		TP2	5	5	4	4	4	4	5	4	4	5	4.4
		TP3	3	4	4	5	3	4	3	3	6	6	4.1
		TP4	5	5	4	6	5	3	4	4	4	4	4.4
		TP5	5	5	3	5	3	4	4	3	4	4	4
		TP6	3	3	3	3	6	4	4	5	4	6	4.1
		Average											4.16667

Date	Day Number	Number of leaves											
		Bucket											
2019/07/01	12	Plant Number	1	2	3	4	5	6	7	8	9	10	Average
		FP1	2	2	3	2							2.25
		FP2	1	3	2	2							2
		FP3	3	2	1	3							2.25
		FP4	4	2	3	2							2.75
		FP5	2	4	2	2							2.5
		FP6	2	3	2	2							2.25
		Average											2.33333
		TP1	1	2	3	2	1	2	3	2	3	2	2.1
		TP2	2	3	4	2	2	5	4	2	3	4	3.1
		TP3	1	2	4	2	2	3	2	2	2	1	2.1
		TP4	2	2	4	3	3	2	3	2	2	1	2.4
		TP5	3	5	2	3	3	2	4	2	2	3	2.9
		TP6	2	1	3	2	3	2	3	3	4	2	2.5
		Average											2.51667

Date	Day Number	Number of leaves												
		Bucket												
2019/07/10	21	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	2	1	1	3								1.75
		FP2	1	1	2	1								1.25
		FP3	2	1	0	1								1
		FP4	5	2	2	1								2.5
		FP5	1	1	1	3								1.5
		FP6	2	2	1	2								1.75
		Average												1.625
		TP1	0	3	2	2	1	2	1	1	0	1		1.3
		TP2	3	3	4	1	0	5	4	1	3	3		2.7
		TP3	2	1	3	2	2	2	1	1	1	1		1.6
		TP4	2	1	3	1	3	2	1	2	2	1		1.8
		TP5	2	3	1	2	2	0	4	2	2	1		1.9
		TP6	1	1	1	1	1	4	1	3	2	4	1	1.9
		Average												1.86667

Date	Day Number	Number of leaves											
		Bucket											
2019/07/15	26	Plant Number	1	2	3	4	5	6	7	8	9	10	Average
		FP1	3	3	1	3							2.5
		FP2	1	1	0	1							0.75
		FP3	1	2	1	1							1.25
		FP4	3	1	2	2							2
		FP5	1	1	1	3							1.5
		FP6	1	2	1	2							1.5
		Average											1.58333
		TP1	1	2	2	2	2	2	2	1	1	1	1.6
		TP2	2	3	2	2	0	4	3	0	2	3	2.1
		TP3	2	1	3	2	1	2	1	0	1	1	1.4
		TP4	1	2	1	2	2	2	1	2	2	1	1.6
		TP5	2	4	0	2	1	1	4	1	2	1	1.8
		TP6	1	1	1	1	3	1	1	2	2	1	1.4
		Average											1.65

Date	Day Number	Number of leaves												
		Bucket												
2019/07/22	33	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	1	1	1	1								1
		FP2	1	0	2	0								0.75
		FP3	0	0	1	0								0.25
		FP4	3	0	1	0								1
		FP5	0	1	2	2								1.25
		FP6	0	1	1	1								0.75
		Average												0.83333
		TP1	0	0	1	1	1	2	1	0	0	0		0.6
		TP2	2	3	1	2	1	2	2	1	2	3		1.9
		TP3	1	1	2	1	1	1	2	1	1	1		1.2
		TP4	1	1	1	1	2	1	1	1	1	1		1.1
		TP5	2	2	1	1	1	1	4	1	1	1		1.5
		TP6	1	1	1	1	2	1	1	1	1	1		1.1
		Average												1.23333

Date	Day Number	Number of leaves												
		Bucket												
2019/07/30	41	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	1	1	1	1								1
		FP2	1	0	1	1								0.75
		FP3	0	1	0	0								0.25
		FP4	2	0	0	0								0.5
		FP5	0	1	2	1								1
		FP6	1	1	1	1								1
		Average												0.75
		TP1	1	1	1	1	1	1	1	1	1	1	1	1
		TP2	2	2	1	1	1	1	2	1	3	3		1.7
		TP3	1	1	1	0	1	0	1	1	0	1		0.7
		TP4	1	0	1	1	2	0	1	1	1	1		0.9
		TP5	2	2	0	1	0	1	3	1	1	1		1.2
		TP6	1	1	1	1	1	0	0	1	0	0		0.6
		Average												1.01667

Date	Day Number	Number of leaves												
		Bucket												
2019/08/06	48	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	1	1	1	1								1
		FP2	1	0	1	1								0.75
		FP3	0	0	0	0								0
		FP4	1	0	0	0	1							0.5
		FP5	0	1	1	1								0.75
		FP6	1	1	1	1								1
		Average												0.66667
		TP1	1	1	1	0	1	1	1	0	0	1		0.7
		TP2	1	1	0	1	1	1	1	1	2	2		1.1
		TP3	1	1	1	0	1	0	1	1	0	1		0.7
		TP4	1	0	1	1	0	1	1	1	1	1		0.8
		TP5	1	0	0	1	0	1	3	1	1	1		0.9
		TP6	0	1	1	1	0	0	0	0	0	0		0.3
		Average												0.75

Date	Day Number	Number of Ramets											
		Bucket											
2019/06/19	0	Plant Number	1	2	3	4	5	6	7	8	9	10	Average
		FP1	0	0	0	0							0
		FP2	0	0	0	0							0
		FP3	0	0	0	0							0
		FP4	0	0	0	0							0
		FP5	0	0	0	0							0
		FP6	0	0	0	0							0
		Average											0
		TP1	0	0	0	0	0	0	0	0	0	0	0
		TP2	0	0	0	0	0	0	0	0	0	0	0
		TP3	0	0	0	0	0	0	0	0	0	0	0
		TP4	0	0	0	0	0	0	0	0	0	0	0
		TP5	0	0	0	0	0	0	0	0	0	0	0
		TP6	0	0	0	0	0	0	0	0	0	0	0
		Average											

Date	Day Number	Number of Ramets												
		Bucket												
2019/06/24	5	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	0	0	0	0								0
		FP2	0	0	0	0								0
		FP3	0	0	0	0								0
		FP4	0	0	0	0	0							0
		FP5	0	0	0	0								0
		FP6	0	0	0	0								0
		Average												0
		TP1	0	0	0	0	0	0	0	0	0	0	0	0
		TP2	0	0	0	0	0	0	0	0	0	0	0	0
		TP3	0	0	0	0	0	0	0	0	0	0	0	0
		TP4	0	0	0	0	0	0	0	0	0	0	0	0
		TP5	0	0	0	0	0	0	0	0	0	0	0	0
		TP6	0	0	0	0	0	0	0	0	0	0	0	0
		Average												

Date	Day Number	Number of Ramets												
		Bucket												
2019/07/01	12	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	0	0	0	0								0
		FP2	0	0	0	0								0
		FP3	0	0	0	0								0
		FP4	0	0	0	0	0							0
		FP5	0	0	0	0	0							0
		FP6	0	0	0	0	0							0
		Average												0
		TP1	0	0	0	0	0	0	0	0	0	0	1	0.1
		TP2	0	0	0	0	0	0	0	0	0	0	0	0
		TP3	0	0	0	0	0	0	0	0	0	0	0	0
		TP4	0	0	0	0	0	0	0	0	0	0	0	0
		TP5	0	0	0	0	0	0	0	0	0	0	0	0
		TP6	0	0	0	0	0	0	0	0	0	0	0	0
		Average												0.016667

Date	Day Number	Number of Ramets												
		Bucket												
2019/07/10	21	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	0	0	0	0								0
		FP2	0	0	0	0								0
		FP3	0	0	0	0								0
		FP4	0	0	0	0	0							0
		FP5	0	0	0	0	0							0
		FP6	0	0	0	0	0							0
		Average												0
		TP1	0	0	0	0	0	0	0	1	0	0	1	0.2
		TP2	0	0	0	0	0	1	0	0	0	0	0	0.1
		TP3	0	0	0	0	0	0	0	0	0	0	0	0
		TP4	0	0	0	0	0	0	0	0	0	0	0	0
		TP5	0	0	0	0	0	0	0	0	0	0	0	0
		TP6	0	0	0	0	0	0	0	0	0	0	0	0
		Average												0.05

Date	Day Number	Number of Ramets											
		Bucket											
2019/07/15	26	Plant Number	1	2	3	4	5	6	7	8	9	10	Average
		FP1	0	0	0	0							0
		FP2	0	0	0	0							0
		FP3	0	0	0	0							0
		FP4	0	0	0	0	0						0
		FP5	0	0	0	0	0						0
		FP6	1	0	0	0	0						0.25
		Average											0.041667
		TP1	0	0	0	0	0	0	1	0	0	1	0.2
		TP2	0	0	0	0	1	0	0	0	0	0	0.1
		TP3	0	0	0	0	0	0	0	0	0	0	0
		TP4	0	0	1	0	0	0	0	0	0	0	0.1
		TP5	0	0	0	0	0	0	0	0	0	0	0
		TP6	0	0	0	0	0	0	0	0	0	0	0
		Average											0.066667

Date	Day Number	Number of Ramets												
		Bucket												
2019/07/22	33	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	0	0	0	0								0
		FP2	0	0	0	0								0
		FP3	0	0	0	0								0
		FP4	0	0	0	0	0							0
		FP5	0	0	0	0	0							0
		FP6	1	0	0	0	0							0.25
		Average												0.041667
		TP1	0	0	0	0	0	0	0	1	0	0	1	0.2
		TP2	0	0	0	0	0	1	0	0	0	0	0	0.1
		TP3	0	0	0	0	0	0	0	0	0	0	0	0
		TP4	0	0	1	0	0	0	0	0	0	0	0	0.1
		TP5	0	0	0	0	0	0	0	0	0	0	0	0
		TP6	0	0	0	0	0	0	0	0	0	0	0	0
		Average												0.066667

Date	Day Number	Number of Ramets												
		Bucket												
2019/07/30	41	Plant Number	1	2	3	4	5	6	7	8	9	10	Average	
		FP1	0	0	0	0								0
		FP2	0	0	0	0								0
		FP3	0	0	0	0								0
		FP4	0	0	0	0	0							0
		FP5	0	0	0	0	0							0
		FP6	0	0	0	0	0							0
		Average												0
		TP1	0	0	0	0	0	0	0	1	0	0	1	0.2
		TP2	0	0	0	0	0	1	0	0	0	0	0	0.1
		TP3	0	0	0	0	0	0	0	0	0	0	0	0
		TP4	0	0	1	0	0	0	0	0	0	0	0	0.1
		TP5	0	0	0	0	0	0	0	0	0	0	0	0
		TP6	0	0	0	0	0	0	0	0	0	0	0	0
		Average												0.066667

Date	Day Number	Number of Ramets											
		Bucket	1	2	3	4	5	6	7	8	9	10	Average
2019/08/06	48	Plant Number											
		FP1	0	0	0	0							0
		FP2	0	0	0	0							0
		FP3	0	0	0	0							0
		FP4	0	0	0	0	0						0
		FP5	0	0	0	0							0
		FP6	0	0	0	0							0
		Average											0
		TP1	0	0	0	0	0	0	1	0	0	1	0.2
		TP2	0	0	0	0	1	0	0	0	0	0	0.1
		TP3	0	0	0	0	0	0	0	0	0	0	0
		TP4	0	0	0	0	0	0	0	0	0	0	0
		TP5	0	0	0	0	0	0	0	0	0	0	0
		TP6	0	0	0	0	0	0	0	0	0	0	0
		Average											0.05

Appendix E

Other documents



Exit Form for Undergraduate and Postgraduate Students

Student name/s and Student number/s

Jessica Auchterlonie
803863

Please complete where appropriate:

Was laboratory work undertaken (Y/N) Y

Laboratory room number/s used RW 204

The Laboratory Manager confirms the following: (Y/N)

Return of all keys NA

Return of all library books NA

Return of notebook or computer NA

Return of workshop materials (Tools etc.) Y

Return of all PPE Y

The work station in lab is cleaned Y

All equipment is returned in good order Y

All samples and waste are disposed correctly Y

All incidents are reported NA

Comments (if required):

Supervisor Name: Craig Sheridan

Supervisor Signature:

Date: ...22nd November 2019.....

Lab Supervisor / Manager Name: Janet Smith

Lab Manager / Supervisor Signature:

Date: 25/11/19

South African Journal of Chemical Engineering

The phytoremediation potential of water hyacinth: a case study from Hartbeespoort Dam, South Africa --Manuscript Draft--

Manuscript Number:	
Article Type:	Technical note
Keywords:	Eichhornia crassipes; eutrophication; nitrates; nutrients; phosphates
Corresponding Author:	Craig Sheridan University of the Witwatersrand Johannesburg, South Africa
First Author:	Jessica Auchterlonie
Order of Authors:	Jessica Auchterlonie
	Christin-Leigh Eden
	Marcus Byrne
	Nicolaas Venter
	Craig Sheridan
Abstract:	Eutrophication has been a prevalent problem in South African water bodies for many decades, resulting in algal blooms and growth of invasive alien aquatic weeds such as water hyacinth. It was brought to heel in the 1990's, but in recent years this problem has been exacerbated by increasing incidences of untreated sewage entering the river systems, along with other anthropogenic nutrient sources. This study investigated the potential for water hyacinth to be used as a phytoremediation tool to reduce the eutrophic state of Hartbeespoort Dam, and to determine if the water quality, aside from the nutrient concentrations, affects the rate of removal of nutrients by water hyacinth. Water hyacinth plants were grown in either water from the Hartbeespoort Dam or in Hoagland Solution to test the effect of growth medium 'purity' on hyacinth growth. Another set of vessels containing Hartbeespoort Dam water only, without a hyacinth plant, was used to observe the remediation potential of the plant and its effectiveness at suppressing algal growth. The results show that one plant can absorb up to 7,38 mg of Phosphates and 27 mg of Nitrates within 11 days. Water hyacinth can reduce the nutrient concentration of 3 L of Hartbeespoort Dam water from 0,56 mg/L phosphates to 0,05 mg/L phosphate and from 4,1 mg/L nitrates to 0,5 mg/L nitrates in 4 days. These results suggest that water hyacinth can be employed as a phytoremediation tool to mitigate eutrophication in open water bodies and may have the potential to reduce Microcystis blooms.
Suggested Reviewers:	Evans Chirwa, PrEng (SA), PE (MD-USA), MSc (UKY), PhD (UKY), MSA Professor and incumbent of the Sedibeng Water Chair in Water Utilisation Enginee, University of Pretoria evans.chirwa@up.ac.za Expert on SA water.
	Ndeke Musee, BSc (Eng) Associate Professor, University of Pretoria ndeke.musee@up.ac.za Expert in South African water
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	Chris Buckley, BScEng, MScEng Head of the Polution Research Group, University of Kwazulu-Natal buckley@ukzn.ac.za Expert on South African Water

Opposed Reviewers:	
--------------------	--

SUBMISSION OF THESIS/DISSERATIO/RESEARCH REPORT FOR EXAMINATION

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The phytoremediation potential of water hyacinth

10. Did your research involve animal experimentation or human participants as defined in A.4 of the Senate Standing Orders on Higher degrees?

If **YES**, please certify that clearance was obtained from the relevant Ethics committee

Clearance Number: _____

	NO
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11. I understand that I may not graduate unless my University fees have been paid in full and I have no Library holds.

12. Name of Supervisor(s)

Craig Sheridan School: Chemical and Metallurgical Engineering

Nikolaas Venter School: Animal, Plant and Environmental Sciences

Signature of Candidate: 

Date: 31/01/2020

