THE POTENTIAL USE OF WETLAND PLANT SPECIES WITHIN A RENOSTERVELD SETTING FOR THE PHYTOREMEDIATION OF GLYPHOSATE AND FERTILISER

by
Dylan Michael Jacklin

Thesis presented in fulfilment of the requirements for the degree of Master of Science in the Faculty of Science at Stellenbosch University

Supervisor: Mr J de Waal
Co-supervisor: Dr IC Brink

December 2018

Department of Geography and Environmental studies

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third-party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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DEDICATION

The author wishes to dedicate this thesis to his mother.

ABSTRACT

In South Africa, fertiliser and herbicide pollutants resulting from various agricultural practices lead to a degradation of surface freshwater and groundwater quality. Nitrogen and phosphorous, and glyphosate derived from agricultural fertiliser and herbicide applications, respectively, significantly contribute to watercourse toxicity. Adjacent to many of the surface freshwater systems are some of the South Africa's most productive agricultural fields, which convert the surrounding natural ecosystems in favour of the crops produced. As a result, the degradation of natural vegetation and deterioration of freshwater quality is observed. The critically endangered status of some Renosterveld vegetation types is the product of agricultural expansion, nutrient loading through fertilisation and the spraying of herbicides.

The characteristics of phytoremediation provide an attractive alternative for the pollutant biofiltration of freshwater aquatic ecosystems. A buffer of Renosterveld vegetation along river corridors may be a solution for agricultural pollutant remediation prior to entering the watercourses. As a result of its successful uptake and metabolism capabilities of fertilisers and herbicides, inexpensiveness, aesthetic advantages and long-term use, it has become a remediation technology of choice in developing countries. The utilisation of wetland plants occurring within Renosterveld vegetation for pollutant extraction from agricultural practices will increase river corridor biodiversity, creating indigenous refuges, and facilitating habitat connectivity.

Considering this, the study aims to delineate the potential use of wetland plant species indigenous to Renosterveld for the effective removal of agricultural pollutants. The evaluation of plant species' pollutant removal efficiency in comparison to unvegetated soil will substantiate its use in vegetative buffer strips. The potential use of indigenous species as an alternative to invasive alien plant (IAP) species, currently considered successful phytoremediators, will aid in conserving the Renosterveld ecoregion.

An experimental phytoremediation system was designed and constructed under laboratory conditions to investigate the pollutant removal potential of indigenous vegetation. Five pollutant parameters, namely ammonia, nitrate, soluble reactive phosphorous and two glyphosate concentrations (0.7 and 225 mg/L), were selected to reflect environmental stresses on 14 indigenous wetland species. The high but non-lethal glyphosate dosage strength was selected by means of a dual species dilution series experiment, where two plant species were subjected to ten different glyphosate concentrations. The dosage strength was selected at a concentration where plants did not display signs of mortality.

Effluent analyses indicated the exceptional removal efficiencies of the indigenous wetland species across both fertiliser and herbicide pollutants, with the two most beneficial species identified as the species selected for this test aquic *Phragmites australis* and *Cyperus textilis*. The unvegetated soil control further exhibited efficient pollutant removal. However, indigenous vegetation consistently displayed greater pollutant removal than the unvegetated soil control. When compared to the IAP and Palmiet (*Prionium serratum)* multi-plant

community assemblage, the indigenous species indicated similar pollutant removal efficiencies, justifying the use of indigenous plant species over the alien invasive equivalent.

Phytoremediation presented significant potential for the utilisation of non-invasive wetland plant species in agricultural pollutant remediation, ameliorating freshwater aquatic ecosystems, and aiding the conservation of the already fragmented landscape.

OPSOMMING

In Suid-Afrika lei kunsmis- en onkruiddoder-besoedelingstowwe wat aanwesig is tydens verskeie landbou aktiwiteite tot die afname van die oppervlak varswater asook ondergrondse water. Stikstof, fosfaat en glifosaat afkomstig vanuit landboukunsmis en onkruiddoders dra aansienlik by tot die toksisiteit van waterlope. Sommige van Suid-Afrika se mees produktiewe landbouindustriee is aangresend tot oppervlak-varswatersisteme, en vereis die omskakeling van die omliggende natuurlike ekosisteme om die groei van aangeplante gewasse te bevorder. Die agteruitgang van plantegroei en natuurlike habitatte asook die varswater-kwaliteit word dus waargeneem. Renosterveld-plantegroei is sodoende op ‘n kritiese bedreigde vlak weens verskeie bevrugtingvoedingstowwe, asook die bespuiting van onkruiddoders. Die eienskappe van fytoremediëring bied 'n aantreklike alternatief vir die biofiltrasie van besoedeling met betrekking tot varswater-akwatiese ekosisteme. 'n Buffer van Renosterveld-plantegroei kan 'n effektiewe oplossing bied vir die herstelling van landboubesoedelende middels voordat dit waterlope binnedring. Die suksesvolle opname en metabolieringsvermoëns met betrekking tot kunsmis en onkruiddoders, tesame met die koste-effektiwiteit, estetiese voordele en langtermyn toepaslikheid, het dit alreeds die vooraanstaande remediëringstegnologie in verkeie ontwikkelende lande gemaak. Dit sal ook inheemse toevlugsoorde skep wat habitatverbindings fasiliteer. In die lig hiervan is die studie daarop gemik om die potensiële gebruik van vleiland-plantspesies te definieer vir die effektiewe verwydering van Landbou-besoedelstowwe. Die evaluering van inheemse vleiland plantspesies se verwyderingsdoeltreffendheid in vergelyking met onbegroeide grond sal die gebruik daarvan in vegetatiewe bufferstroke staaf. Die potensiële gebruik van inheemse-spesies as 'n alternatief vir indringerplantspesies, wat tans as suksesvolle fytoremediatore beskou word, sal help om die area te bewaar. 'n Eksperimentele fytoremediëringsisteem is ontwerp en gebou onder laboratoriumtoestande om die verontreinigingspotensiaal van inheemse plantegroei te ondersoek. Vyf besoedelende parameters, naamlik ammoniak, nitraat, oplosbare reaktiewe fosfor en twee glifosaat konsentrasies (0,7 en 225 mg / L), is geidentifiseer en gekies om omgewingstressors op 14 inheemse vleiland-spesies te weerspieël. Die hoë, maar nie-dodelike glifosaat-dosis sterkte is gekies deur middel van 'n eksperiment wat ‘n dubbele spesies verdunning reeks bevat, waar twee plantspesies aan tien verskillende glifosaat konsentrasies blootgestel was. Die dosis sterkte is die konsentrasie waar plante nie tekens van mortaliteit vertoon het nie.

Verskeie uitvloeisel-ontledings dui op die uitsonderlike doeltreffendheid van die verwydering van inheemse vleiland-plantspesies oor beide kunsmis- en onkruiddoder-besoedelingsstowwe met die twee mees voordelige spesies geïdentifiseer as *Cyperus textilis* en *Phragmites australis,* onderskeidelik. Die grondbeheer het verder doeltreffende kontaminante verwyder. Vleiland-plantegroei het egter meer kontaminante as die onbedekte grondbeheer verwyder. In vergelyking met die indringerspesies insluitend Palmiet, het inheemse-plantegroei soortgelyke besoedelingsverwydering-doeltreffendheid aangedui, wat die gebruik van inheemse

plantspesies oor die uitheemse indringende plus Palmiet ekwivalent regverdig. Fytoremediasie het aansienlike potensiaal getoon vir die gebruik van die nie-indringende vleiland-spesies in die voorkoming van landbou-besoedelstowwe asook die verbetering van varswater-akwatiese ekosisteme, wat in geheel tot die bewaring van die reeds gefragmenteerde landskap sou bydra.

THE RUFFORD FOUNDATION

The Rufford Foundation is a UK registered charity that funds nature conservation projects across the developing world.

The author gratefully acknowledges the Rufford Foundation for their generous financial assistance to this research project [23296-1], granting stability for the duration of the project.

ACKNOWLEDGEMENTS

I would like to thank the following people and organisations for their assistance and contribution to the success of this study:

- My supervisor Mr J de Waal (Geography and Environmental Studies, Stellenbosch University) for his guidance and constant support for the duration of the project.
- My co-supervisor Dr IC Brink (Water and Environmental Engineering, Stellenbosch University) for her unwavering interest in and support of the project, the use of her Water Quality Laboratory, sharing her expertise in water quality and granting me the opportunity to construct the phytoremediation system.
- The Rufford Foundation (United Kingdom) for their financial support, granting me the opportunity to complete the project.
- The Overberg Renosterveld Conservation Trust (Napier, Western Cape) for sharing their expertise, encouragement and the use of the Haarwegskloof Research Unit. The author wishes to thank Dr Odette Curtis (Director, ORCT) and Mr Jannie Groenewald (Reserve and Centre Manager, ORCT) for assistance during specimen collection and friendship for the duration of the study.
- Dr Kirsty Carden (Research Coordinator, UCT) and Dr Kevin Winter (Chair Water Task Team, UCT) for exploring the use of ecological infrastructure in protecting water, advising during the study.
- Prof Martin Kidd (Centre for Statistical Consultation, Stellenbosch University) for his assistance with statistical analysis.
- Mr Stefan Milandri (Senior Professional Officer, City of Cape Town) for his continuous support and guidance throughout the study.
- Mr Johann van Biljon (Director, Intaba Environmental Services) for his guidance regarding plant choice and capabilities.
- Mr Johann Nieuwoudt (Laboratory Technician, Stellenbosch University) for his interest, assistance and support during the construction of the phytoremediation system.
- Mr Michael Jacklin for his support, encouragement and espousal of the project.
- Ms Lari Opperman for her assistance and inspiration throughout.

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NOMENCLATURE

Abbreviations

AMD	Acid mine drainage
AMPA	Aminomethylphosphonic acid
BLSA	BirdLife South Africa
CAPE	Cape Action Plan for the Environment
CBO	Community-based Organisation
CFR	Cape Floristic Region
CLRP	Cape Lowlands Renosterveld Project
CSIR	Council for Industrial and Scientific Research
CSP	Conservation Stewardship Programme
DAFF	Department of Agriculture, Forestry and Fisheries
DEA	Department of Environmental Affairs
DWS	Department of Water and Sanitation
EEC	Expected Environmental Concentration
FFI	Fauna & Flora International
GEF	Global Environment Facility
IPA	Isopropylamine
IPPS	Individual Plant Per Silo
IUCN	The International Union for Conservation of Nature
MPPS	Multiple Plants Per Silo
NGO	Non-governmental Organisation
NWA	National Water Act, Act 36 of 1998
NWRS	National Water Resource Strategy
ORCT	Overberg Renosterveld Conservation Trust
PMO	Phosphorous Management Objective
PNC	Plan for Nature Conservation
POEA	Polyoxyethylene tallow amine
SAICE	South African Institution of Civil Engineers
SAWQG	South African Water Quality Guidelines
SuDS	Sustainable Urban Drainage Systems
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation
WWF-SA	World Wildlife Fund-South Africa

CHAPTER 1 : INTRODUCTION

As a result of increased agricultural developments in the Western Cape, the immense degradation of critically endangered natural Renosterveld vegetation and the deterioration of freshwater quality have been observed (Von Hase et al. 2003; Curtis 2013). The need to mitigate environmental degeneration necessitates proactive biodiversity conservation strategies, with the aim of ameliorating the freshwater aquatic ecosystems. Through the identification of indigenous plant species capable of the phytoremediation of agricultural contaminants, strategies may be devised for the inclusion of naturally occurring plants into vegetated buffer strips adjacent to waterways. The purpose of the vegetative buffers would be to connect the remaining Renosterveld fragments, combat biodiversity loss and subsequently improve the quality of the water transported back to the freshwater systems.

South Africa's freshwater quality and resource availability is declining due to increased pollution by mining, afforestation, power generation, urbanisation, industry and, most importantly, agricultural practices (Constantine, Musingafi & Tom 2014). It has been widely accepted that a principal cause of water quality deterioration globally is nutrient enrichment (King et al. 2012). The role of excessive amounts of the nutrients phosphorous (P) and nitrogen (N) contribute significantly to surface water eutrophication and water-quality degradation (Lee, Rast and Jones 1978; Schoumans et al. 2014; Xi 2018). Non-point source pollution of terrestrial surface and groundwaters by pesticides within intensive agricultural regions include: non-irrigated, irrigated field and pasturage (Barcelo 1997; Budd et al. 2009). These fertiliser and pesticide pollutants originating from various agricultural operations lead to the deterioration of surface freshwater and groundwater (Zalidis et al. 2002; Arumi, Oyarzún & Sandoval 2005; Lam, Schmalz & Fohrer 2010).

Herbicides are introduced in the environment with the intention of applying effects on specific target organisms (Pilon-Smits 2005; Chèvre et al. 2006). Unfortunately, the toxic action is not only exerted on the area where it is applied, but through overhead spray, leaching and soil erosion transport, significant amounts of the herbicide and its degradation products reach the freshwater aquatic systems, most acutely where agricultural pastures border surface waters (Beach & Carlson 1993; Barcelo 1997; Kanwar, Colvin & Karlen 1997; Dabrowski 2001; Chèvre et al. 2006; Pérez, Vera & Miranda 2011; Mensah, Palmer & Muller 2013; ACBIO 2015; Tran et al. 2017).

The aquatic freshwater organisms are generally exposed to a combination of these pesticides, resulting in a collection of single compounds below the no-effect concentration, causing the accumulation mixture to exhibit degradatory effects (Faust et al. 1994; Gilliom et al. 1999; Backhaus et al. 2000; Chèvre et al. 2006). Gilliom et al. (1999) reveals that pesticide mixtures below individual acceptable concentrations for human health, may however present toxic effects on freshwater aquatic organisms.

The nutrient level of the 20 largest freshwater river catchments within South Africa, based on dissolved- inorganic nitrogen ($NO_3^- + NO_2^-$) and -phosphorous (PO_4^{3-}), indicate that the nutrient levels within most watercourses exceed the recommended water quality guidelines for plant life (De Villiers & Thiart 2007).

Generally, a similar trend exists in all but six of South Africa's largest river catchments, with dissolved-phosphorous levels surpassing the recommended quantity for the protection of aquatic animal life, demonstrating an alarming upward trend in dissolved PO_4^{3-} levels in 60% of the rivers (Von Hase et al. 2003; De Villiers & Thiart 2007; Curtis 2013). This nutrient proliferation poses an urgent and expensive environmental conservation threat to water quality and biodiversity. The negative environmental effects of eutrophication include a reduction in biodiversity and impaired performance of freshwater aquatic ecosystems, as well as a deterioration in surface water quality (Schoumans et al. 2014).

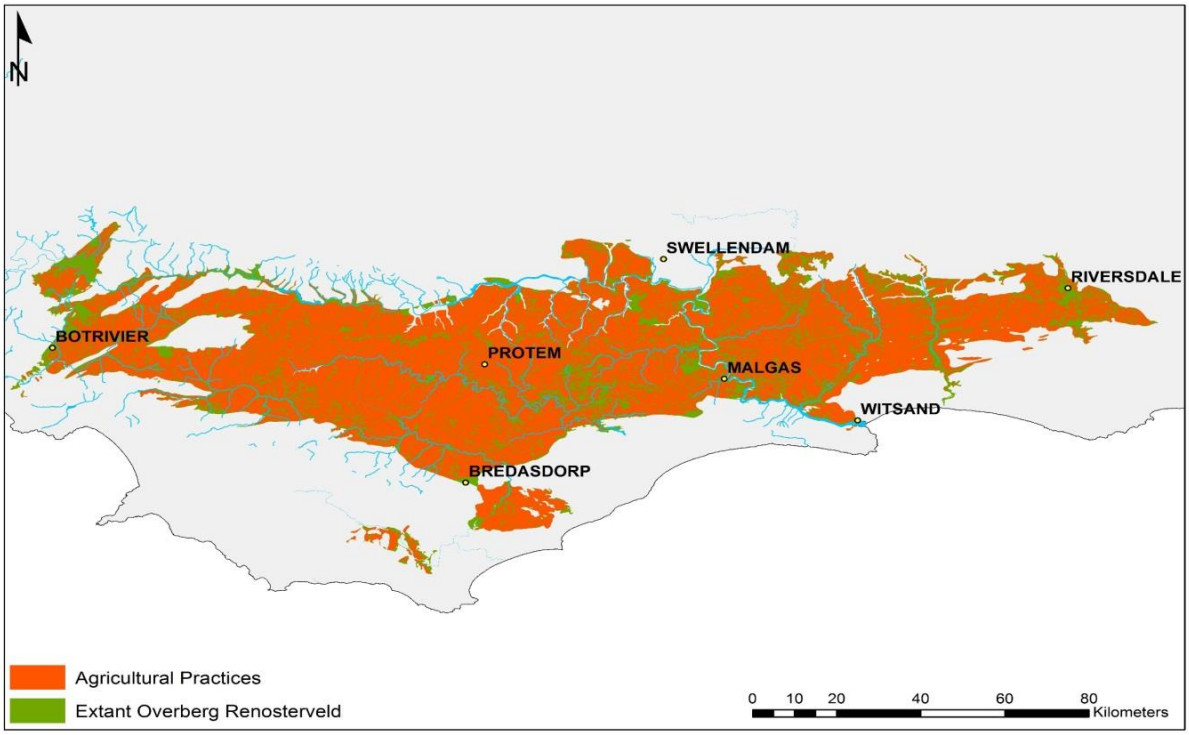

Figure 1.1: Agricultural dominance in the Overberg (data source: SANBI 2003).

Adjacent to many of the freshwater surfaces are some of South Africa's most productive agricultural industries, that aim to simplify the surrounding ecosystems in favour of the crops and in some cases, livestock produced (Giliomee 2006). The Renosterveld lowlands vegetation, situated in the Cape Floristic Region (CFR), are the most transformed and under-conserved areas within the CFR (Kemper, Cowling & Richardson 1999; Rouget, Richardson & Cowling 2003). The severity of Renosterveld transformation illustrated in Figure 1.1 above, indicates that more than 90% of the natural vegetation has already been ploughed for agricultural development (McDowell & Moll 1992; Kemper, Cowling & Richardson 1999; Curtis 2013). Classified as critically endangered, the Renosterveld

ecoregion is extremely vulnerable to extinction (Curtis 2013). The use of indigenous Renosterveld vegetation as a functioning pollutant buffer between the planted agricultural pastures and the river systems will aid the conservation effort by establishing river corridors, connecting the remaining fragments (Curtis 2013).

The practice of phytoremediation utilizes vegetation for the *in situ* treatment of polluted soils, sediments and water, applicable to areas that contain either organic, nutrient or metal pollutants (Schnoor et al. 1995; Hughes et al. 1997; Dietz & Schnoor 2001). The practice is appropriate for any scenario where the pollutants are sequestered, degraded, immobilised or metabolised, by plant roots (Anderson, Guthrie & Walton 1993; Schnoor et al. 1995; Terry & Banuelos 2000; Dietz & Schnoor 2001).

Due to the high cost attributed to water treatment, developing countries generally do not possess the financial capacity and expertise to perform advanced water treatment systems (Mara 2004; Henze 2008). However, plant cultivation and harvesting technologies are relatively inexpensive processes compared to advanced wastewater treatment systems, thus phytoremediation may provide an attractive alternative for the biological treatment of freshwater aquatic ecosystems (Sekabiera et al. 2011). A buffer of Renosterveld vegetation may accordingly be a relevant solution when agricultural pollutant remediation is required.

1.1 Problem Statement

The world is rapidly squandering its biological wealth, the result of various human activities, such as pollution, habitat destruction and invasion by alien plants and animals. These activities escalate and threaten the continued existence of many species. Due to the exceptional level of Renosterveld transformation, the remaining habitat only occurs as fragments in transformed agricultural landscapes (Von Hase et al. 2003; Milton 2007). Changes in fire regimes (total exclusion or use of fire in the wet season), invasion by alien plants, livestock overgrazing on fragments, and nutrient enrichment appear to be diminishing the value of Renosterveld for both conservation and grazing scenarios (Milton 2007; Rebelo et al. 2015).

In aquatic systems both N and P nutrients have been widely recognised as algal biomass and aquatic plant growth limiting nutrients (Lee, Rast & Jones 1978). The nutrients become readily available through the fertilisation of the adjacent agricultural farmlands, where an excess in agricultural nutrients is the main source of N and P pollution to freshwater aquatic systems (Lam, Schmalz & Fohrer 2010; Bayram et al. 2013; Strokal & Kroeze 2013; Schoumans et al. 2014; Hashemi et al. 2016). The degradation of Renosterveld has diminished many of the vegetative buffer zones along water corridors, which act as a natural layer of protection between the watercourse and the adjacent farmland (Giliomee 2006).

Eutrophication as a result of pollution has become a major problem for South Africa, with approximately 62% of the largest waterbodies listed as hypertrophic, these catchments

display a more nutrient-rich environment than eutrophic water bodies (Matthews & Bernard 2015). Eutrophication has the potential to deoxygenate a waterbody, causing toxic blue-green algae blooms that decimate the heterogeneity of a natural system (Foehrenbach 1972).

Ecosystem processes and agricultural practices ensure that herbicides ultimately make their way into aquatic ecosystems (Mensah, Palmer & Muller 2013). In the case of glyphosate, the active ingredient in many herbicides, the pollutant has been known persist in the surface- and ground-water of the Western Cape since the 1990s (London et al. 2005). For the control of both aquatic and terrestrial weeds, glyphosate-based herbicide use is common in South Africa. This is as a result of the lack of South African Water Quality Guidelines accentuating its effects on non-target organisms (Mensah, Palmer & Muller 2013). Distressingly, its use for the control of aquatic alien plant species has been encouraged by national departments and commercial farmers (London et al. 2005; Maharaj 2005; Dalvie et al. 2011).

The Council for Industrial and Scientific Research (CSIR) indicated that elevated concentrations of pesticides from agricultural runoff have been detected in a variety of the country's major river systems (Oelofse & Strydom 2010).

With the Western Cape province currently experiencing the worst water shortage in 113 years, in particular the west coast and central Karoo that have already been declared agricultural drought disaster areas, the remaining natural vegetation fragments are subjected to added pressure (Botai et al. 2017). Warranted by a combination of environmental stresses, the responsible and sustainable use and reuse of freshwater becomes critically important. In this current predicament, the protection of a shared water resource must be declared across all spheres.

The nutrient removal and nutrient recovery capabilities of plant species occurring within the critically endangered Renosterveld vegetation type, makes the vegetation particularly attractive for effluent polishing. An integrated phytoremediation system can serve as a buffer between the agricultural farmlands and the receiving waterbody, reducing excess nutrient loads. Furthermore, the buffer zones' potential to act as a barrier against contaminated water runoff in conservation corridors requires further investigation.

1.2 Thesis Statement

"Wetland plant species that naturally occur within Renosterveld vegetation and among other areas, have the phytoremediatory potential to reduce herbicide and fertiliser nutrient loads."

1.3 Research Aim

Investigate the phytoremediation capabilities of plant species found within the critically endangered Renosterveld vegetation type but are not limited to the vegetation type, allowing

comparison with accepted invasive plant species, currently utilised in sustainable urban drainage systems and constructed wetlands.

1.4 Research Objectives

Technical Objectives:

- Design and construct a laboratory experiment capable of evaluating the nutrient uptake of the following:
    - The purification potential of 14 individual indigenous wetland species
    - The combination purification potential of four indigenous wetland species plant communities
    - The combination purification potential of four Sustainable Urban Drainage System (SuDS) species plant communities
- Identify 14 indigenous wetland plant species physiologically capable of nutrient uptake and capture through roots within the rhizosphere.
- Evaluate previous research into SuDS to determine the most effective plant species regarding nutrient uptake potential.
- Determine optimal herbicide and fertiliser products, ensuring favourable water solubility, corresponding with products generally used in the particular agricultural practices.
- Initiate two herbicide and one fertiliser treatment over a 40-day period.

Research Objectives:

- Comparing the remediation efficacy of a Renosterveld community VS unvegetated soil.
- Identifying individual indigenous wetland species' pollutant removal.
- Analysing the effect of glyphosate dosage strength on herbicide remediation.
- Quantify the influence of selected water quality parameters.
- Compare remediation efficacy of indigenous wetland vegetation VS invasive alien plant (IAP) and Palmiet vegetation assemblage.
- Rank species within the Renosterveld community effective across pollutants.
- Analysing the root growth length on cumulative pollutant removal.
- Evaluating the temporal effect on cumulative pollutant removal for the duration of the study (March 3rd - May 18th).

1.5 Methodology

The phytoremediatory capabilities of different plant species are analysed and ultimately compared by means of the integration of a water-quality analysis system, capable of demonstrating plant efficacy in nutrient removal. A phytoremediation system has been designed and constructed for this specific purpose, allowing the comparison of chemical

parameters between effluent and influent water solutions. Fourteen indigenous wetland plant species that occur within Renosterveld among other areas and four plant species currently used in SuDS and constructed wetlands are selected for this study. Plant inclusion was based on a variety of environmental and feasibility factors, further discussed in Chapter 3, Section 3.3. Site specific sediment was collected from the Overberg to use as growth medium for the experiment, with soil analyses conducted. Thereafter, plants were collected and transplanted from the field into the experimental system.

Ammonium chloride (NH_4Cl), potassium nitrate (KNO_3) and di-potassium hydrogen phosphate (K_2HPO_4) were the analytical representative fertiliser substances for the provision of ammonia (NH_3), nitrate (NO_3^-) and ortho-phosphate (PO_4^{3-}) respectively within the experiment. *Springbok 360 SL™*, selected according to its widespread use in the farming community, was the source of glyphosate ($C_3H_8NO_5P$) evaluated at two different concentrations in the phytoremediation system.

The water quality parameters selected for analysis were selected to comply with the requirements for a successful water-quality assessment (South Africa 1999). The parameters are indicators of water quality, used as a method to assess ecosystem health (Scherman, Muller & Palmer 2003). The selected parameters are further discussed in Chapter 2, Section 2.5 and Chapter 3, Section 3.6.4. They are as follows: pH, dissolved oxygen (DO) and electrical conductivity (EC) as indicators in the general characterisation of water quality (Golterman 1969; South Africa 1996d; Clesceri, Greenberg & Eaton 1998; Ngwenya 2006).

Nutrient enrichment analysis was achieved by examining N-NO_3^-, N-NH_3 and P-PO_4^{3-}. The forms of N and P are of greatest interest in water quality analyses as they are the major nutrient contributors to water pollution, with eutrophication as a result (South Africa 1996a; Clesceri, Greenberg & Eaton 1998; Pegram & Görgens 2001; Dallas & Day 2004). Glyphosate analyses are undertaken due to the general use of the herbicide in agricultural practices, more specifically in Canola cultivation (Curtis 2017, Pers com; De Kock 2017, Pers com; Nolte 2017, Pers com). The contamination of water from runoff and spray drift is a major concern of the herbicide in question (Clesceri, Greenberg & Eaton 1998).

The major delineations of the study are that Renosterveld vegetation was represented by 14 indigenous plant species that occur within Renosterveld but are widespread and not specifically associated with Renosterveld, while the alien invasive plants were limited to four plant species. The time frame for experimentation lasted four months, with four rounds of sampling and one baseline sampling round executed within this time.

The assumptions made during the innovation of the biofiltration system and major limitations of the study are discussed in Section 3.9 and 5.3 respectively.

CHAPTER 2 : LITERATURE REVIEW

This chapter outlines previous research and provides an understanding of the principal concepts for the study. The components identified as relevant to this research have been sub-categorised within this chapter and are reviewed individually. All aspects need to be extensively explored to allow for an appropriate research design and a coherent understanding and interpretation of the findings. The fundamental areas of research are as follows:

- Renosterveld habitat, transformation and conservation
- Freshwater pollution from agriculture
- Water-quality and the consequence of degradation
- Phytoremediation
- Parameters indicative of water quality

2.1 Renosterveld habitat, transformation and conservation

This section of the literature review describes the regional conditions and the importance of the Renosterveld vegetation type, as well as its conservation-efforts, -history and -challenges.

2.1.1 The Regional Context/Background to the Cape Floristic Region

The decay of the world's natural environments is a universal dilemma, as their suitability for cultivation have been heavily exploited since the emergence of large-scale agriculture (Curtis 2013).

At the Southern tip of Africa, the Cape Floristic Region (CFR) is recognised as one of the richest habitats in the world, regarding its floristic heterogeneity and endemism (Goldblatt & Manning 2000; Von Hase et al. 2003). Due to the extensive flora of the CFR, it is regarded the smallest, yet richest plant kingdom of the world's six floral kingdoms (Curtis 2013; D'Alton et al. 2015). In terms of botanical diversity, the CFR is home to one of the richest regions, and the highest known concentration of threatened and rare species worldwide (Cowling & Hilton-Taylor 1994). Despite inhabiting only a small fragment (4%) of the area, the Cape is responsible for nearly 44% of the flora in southern Africa (Von Hase et al. 2003).

With such an exceptionally high plant species diversity and endemism: covering an area of 87 892 km^2, 9000 plant species are found of which approximately 70% are endemic (Goldblatt & Manning 2000; Von Hase et al. 2003; Giliomee 2006). The CFR has been recorded as one of only 25 globally acknowledged biodiversity hotspots (Myers et al. 2000; Von Hase et al. 2003). For its tiny size, the biome most likely has the richest flora worldwide (Russell 1984; McDowell & Moll 1992).

Due to human-induced pollution and habitat destruction increase, the world is rapidly losing its biological wealth (Giliomee 2006). As a result of habitat transformation, the CFR's

unique biodiversity is under enormous pressure, associated with agriculture and urban developments, unfeasible harvesting techniques, as well as unsuitable land-use planning resulting in the spread of alien invasive species (Von Hase et al. 2003). More problematic, the Western Cape's most productive and fertile farmlands are located within the CFR (Giliomee 2006).

Extensive agriculture reshapes the natural landscape into a highly fragmented state, which inflicts a variety of adverse effects on the coherence of these systems (McDowell & Moll 1992; Giliomee 2006; Curtis 2013). One such fragmented system, occurring in a very diverse landscape, is Renosterveld.

2.1.2 Introduction to Renosterveld

Renosterveld is a vegetation type, illustrated in Figure 2.1 below, occurring within the Fynbos biome of the southern and south-western Cape Province (McDowell & Moll 1992). In the lowlands of the CFR, in response to lower rainfall and a switch to more fertile, clay- and shale-based soils, the vegetation evolves into Renosterveld (Curtis 2013). Renosterveld is a dominant vegetation type, with moderate rainfall of 350 to 650 mm/annum (McDowell & Moll 1992; Von Hase et al. 2003; Krug 2004; Curtis 2013). When rainfall levels exceed an average of 800mm/annum, the Fynbos elements start to dominate Renosterveld vegetation (Von Hase et al. 2003).

Both Fynbos and Renosterveld vegetation types are characterised by very high species diversity (Cowling & Holmes 1992; Kemper et al. 1999; Curtis 2013). When differentiating between the two, Renosterveld is identified with the three pivotal fynbos indicators (proteas, ericas and restios) being absent whilst being dominated by Asteraceous shrubs and perennial grasses (McDowell & Moll 1992; Von Hase et al. 2003; Curtis 2013). The vegetation type is considered the richest bulb habitat in the world and is renowned for its incredible spring flower displays (D'Alton et al. 2015). Iridaceae (iris family), Liliaceae (lily family) and Oxalidaceae (oxalis family) are well represented in Renosterveld vegetation, whereas Poaceae (grasses) form an essential component when grazing is not too intense (McDowell 1988; McDowell & Moll 1992). Iridaceae are some of the endemic and threatened plant species occurring in Renosterveld with 330 species known to exist, 160 species listed as threatened and nearly 250 species endemic to the ecoregion (Von Hase et al. 2003).

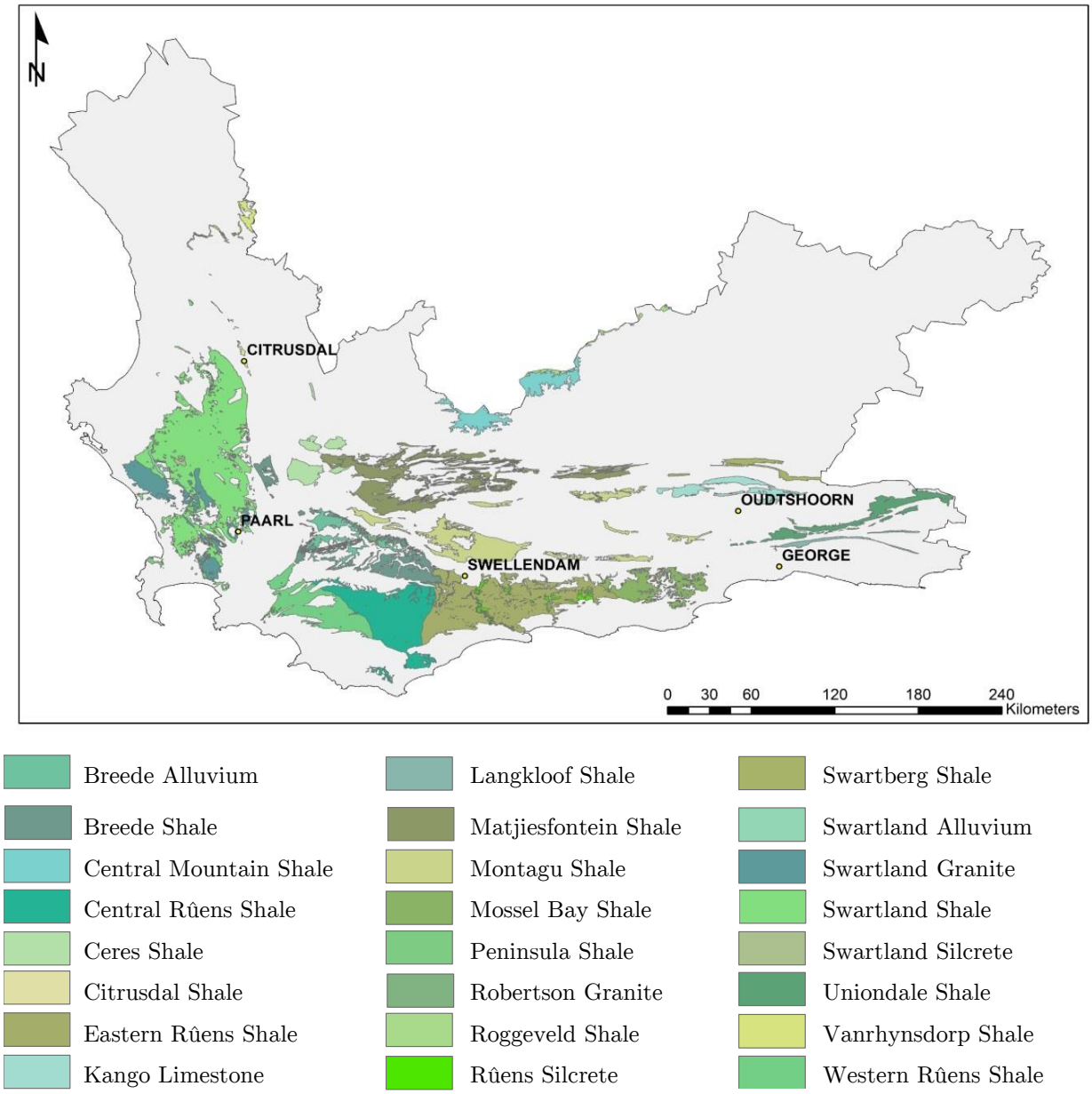

Figure 2.1: Renosterveld vegetation types and their natural distribution (data source: SANBI 2003).

The name 'Renosterveld' stems from the Afrikaans words 'Renoster' and 'veld' meaning rhino and vegetation respectively. Whilst the precise origin of the name is uncertain, it is traditionally believed that the vegetation was named after the Black Rhinoceros which used to inhabit the area (Krug 2004; Curtis 2013). McDowell & Moll (1992) argue a different origin for the term 'Renosterveld', and believes it was derived from the characteristic dominant grey-green Renosterbos (*Elytropappus rhinocerotis).* Although naturally occurring in Renosterveld, the presence of Renosterbos is an indication of an altered and degraded landscape. Veld dominated by Renosterbos is an indication of overgrazing, a lack of fire regimes (old veld) or both (D'Alton et al. 2015).

Four Renosterveld types are recognised in the Overberg, Figure 2.2 (Rutherford & Mucina 2006):

- Western Rûens Shale Renosterveld
- Central Rûens Shale Renosterveld
- Eastern Rûens Shale Renosterveld
- Rûens Silcrete Renosterveld

A consequence of the rich soil fertility associated with a natural Renosterveld distribution, the area is encroached by an ever-expanding agricultural presence (Von Hase et al. 2003; Rutherford & Mucina 2006). As a result, all the Renosterveld vegetation types in the Overberg are listed as critically endangered (Curtis 2013).

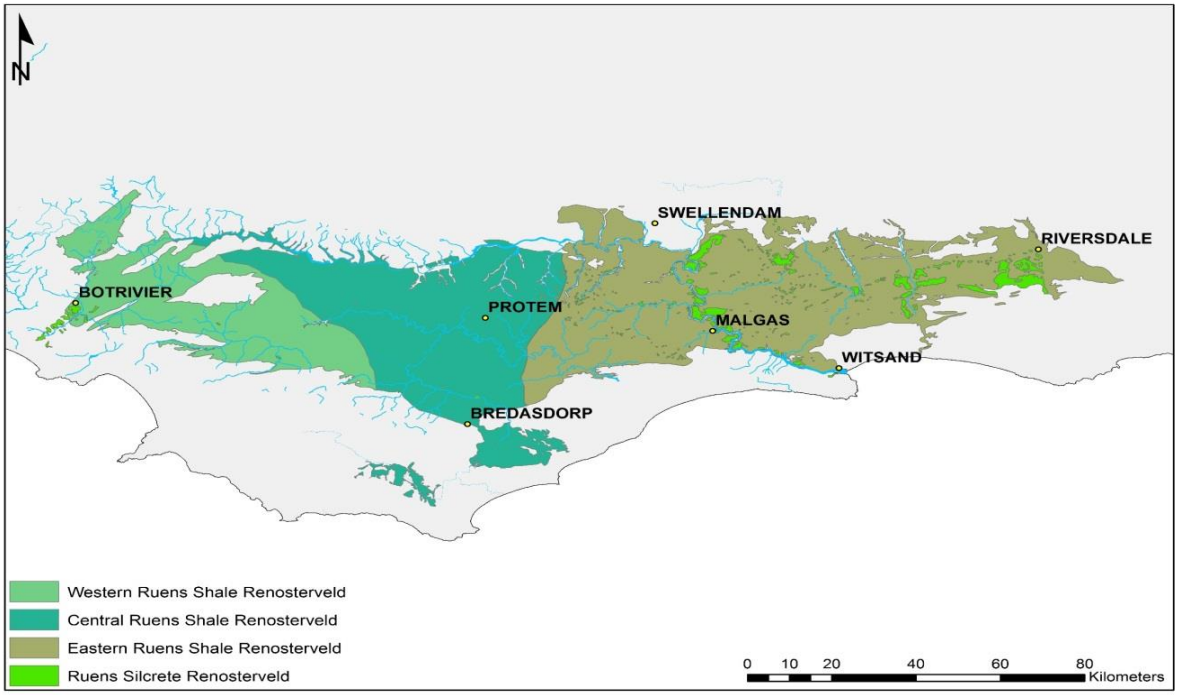

Figure 2.2: The distribution of Overberg Renosterveld (data source: SANBI 2003).

The first significant human influence on the Renosterveld landscape occurred when the European settlers arrived, with the Knoekhoen (indigenous people to the area, 2000 years ago) adopting a herding lifestyle (Krug 2004). The European settlers hunted big herds of game into extinction, changing the face of the vegetation, as a result of the absence of grazing animals (Krug 2004; Curtis 2013).

Renosterveld vegetation dominates the extant natural vegetation of the Overberg region in the Western Cape, with the coastal region comprised of coastal, lowland and limestone-based fynbos types (Curtis 2013). Farming practices in the Overberg consist of grain, livestock, vineyards and flower crops (Curtis 2013). The northern extent of the Overberg is composed of predominantly mountain fynbos vegetation types (Curtis 2013). Between the mountains and the coast the soil becomes more fertile and rich in clay derived from the shale, and this is where Renosterveld habitats naturally occur (Curtis 2013).

Renosterveld naturally occurs on richer substrate than fynbos, making it more prone to clearance for agriculture and vulnerable to the detrimental effects of fragmentation (Stindt & Joubert 1979; Cowling, Pierce & Moll 1986; Jarman 1986; McDowell & Moll 1992; Curtis 2013). As a consequence of the rich soil fertility, Renosterveld has been extensively converted to croplands, with less than 5% remaining (D'Alton et al. 2015).

The early farmers regarded the Renosterbos as an indicator for good fertile farmland, moderate rainfall and flat topography, conditions ideal for crop farming (Krug 2004). Over time, the Renosterveld associated with low-lying areas were forced to make way for wheat, grain and cereal (Canola) fields, vineyards, olive groves and orchards (Krug 2004).

Figure 2.3: Renosterveld vegetation replaced by Canola crops.

Agricultural activities have fundamentally impacted the Cape Lowland. As a result the region has now become famous for its striking green wintertime wheat- and grain-fields and yellow rolling hills of Canola pastures illustrated in Figure 2.3 above (Von Hase et al. 2003; Curtis 2013).

2.1.3 Renosterveld pressures and transformation

Due to Renosterveld's rich soil fertility, it was considered more valuable as a ploughed land for growing grain and cereal crops and artificial pasture (Lucerne) than left as natural pasture (Curtis 2013). Commercial agricultural practices transformed the land until all that was left were the areas that were too steep, too rocky or too wet to plough (Kemper et al. 1999).

2.1.3.1 Current state of Renosterveld

Evident from the current fragmented landscape, irreversible change or destruction of natural ecosystems are generally caused through the practice of agronomy (Harris & Hawkes 1978;

McDowell & Moll 1992). Table 2.1 illustrates the extent of the different land-uses in the Overberg area, with agriculture dominant. It is important to note that the majority of the remaining natural vegetation consists of fynbos vegetation and may not be regarded as pristine Renosterveld.

Table 2.1: The extent of the different land-uses in the Overberg area (Von Hase et al. 2003).

Land-use	(%)
Urban areas	0.40
Agricultural landscapes	85.83
Alien plants	1.15
Natural vegetation	12.62
Total area (ha)	552 426.8

The natural Renosterveld lowland habitat has undergone exceptional transformation, promoting a highly fragmented state with only 18 000 remnants of natural vegetation, of which most are smaller than half a hectare, scattered throughout the landscape (Von Hase et al. 2003; Curtis 2013). Table 2.2 below, depicts the remaining fragments and their associated sizes. As illustrated, the majority of the fragments (greater than 50% of the population) are smaller than half a hectare with approximately 1 000 fragments larger than 10 ha.

Table 2.2: The distribution of the remaining Renosterveld fragments in different size classes (Von Hase et al. 2003).

Fragment size (ha)	Overberg (no. of fragments)	Swartland (no. of fragments)	Elgin (no. of fragments)
0 – 0.5	9 719	453	693
0.5 – 1	1 997	68	44
1 – 5	2 860	266	34
5 – 10	627	115	7
10 – 100	701	219	9
100 – 500	84	39	4
500 – 1 000	11	8	0
> 1 000	4	7	0
Total (no. of fragments)	16 003	1 175	791

Newton & Knight (2004) suggest that the Western Cape-based intensification of domestic livestock breeding that occurred 200 years ago, severely transformed the extent and species structure of Renosterveld vegetation. The distribution change is illustrated in Figure 2.4 below:

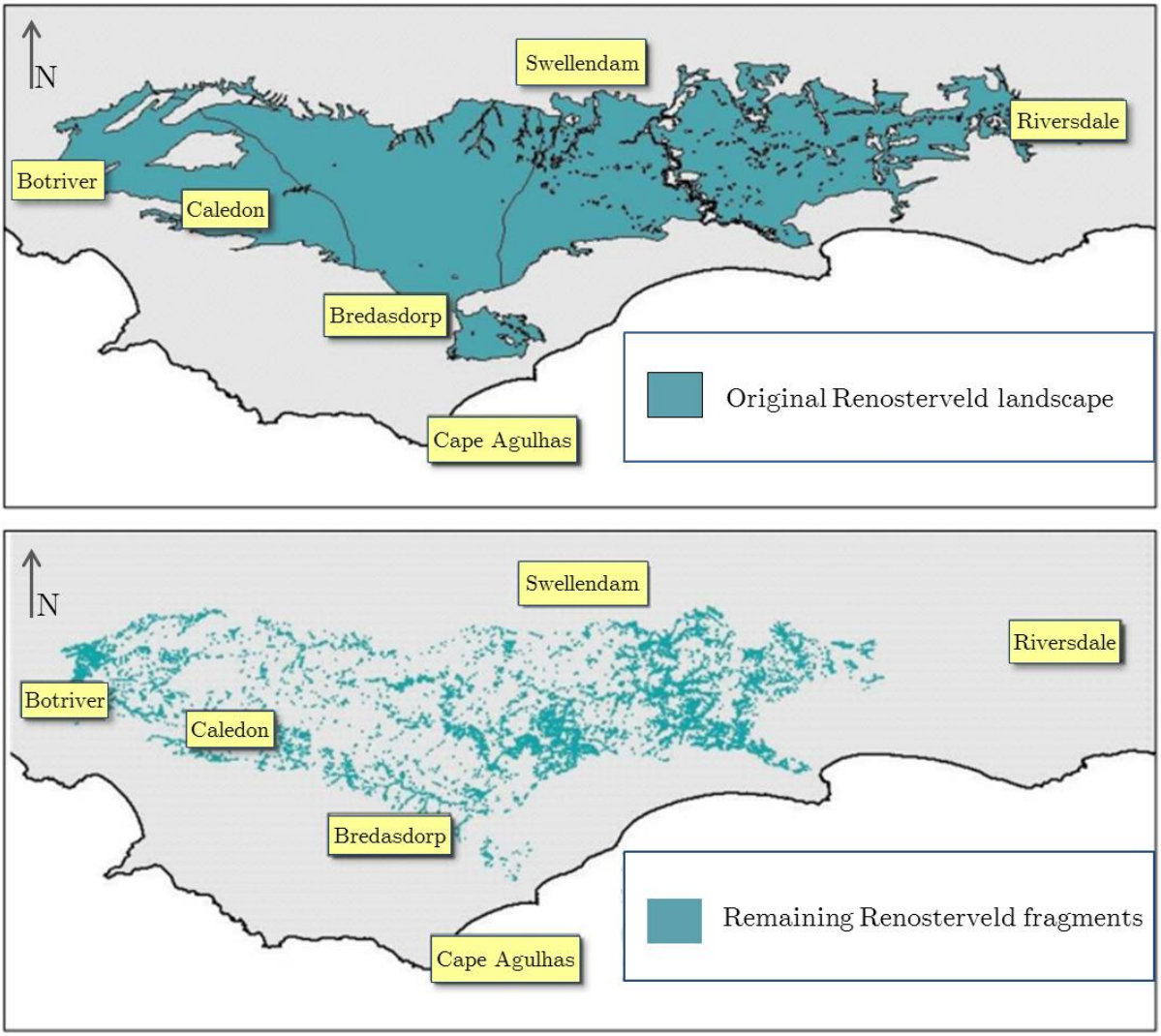

Figure 2.4a (above) and 2.4b (below): Illustration of the amount Renosterveld has been lost over 300 years. Figure 2.4a represents the original Renosterveld in the Overberg, where Figure 2.4b denotes the extant distribution in 2013 (adapted from Curtis 2013).

Within the CFR, the Renosterveld lowlands are the most transformed and under-conserved areas (Rouget, Richardson & Cowling 2003). Lowland Renosterveld was recognised as a top conservation priority within the CFR in 1999, by the Cape Action Plan for the Environment (CAPE) (Cowling et al. 1999; Cowling & Pressey 2003). The vegetation type was further deemed 100% irreplaceable on the basis of extremely high levels of natural habitat loss (greater than 90% of the habitat has been lost). Thus targets aimed at achieving long-term persistence of the natural ecosystem were established (Von Hase et al. 2003; Krug 2004).

2.1.3.2 The cause of Renosterveld fragmentation

The most significant pressures on natural Renosterveld are agricultural expansion, nutrient loading though fertilisation, spraying of herbicides and the spread of alien invasive plants (Krug 2004). The crops that are effectively replacing the natural veld are predominantly grain, wheat, barley, oats and rye, while livestock farming in the Overberg is focused on

sheep and cattle (Kemper, Cowling & Richardson 1999; Von Hase et al. 2003). Curtis (2013) adds incorrect grazing, inappropriate fire regimes promoting degradation and virgin-cropland transformation as current day management-related risks.

Agricultural ploughing, as illustrated in Figure 2.5 below, has severely transformed more than 90% of Renosterveld for grain, cereal and artificial pastures (McDowell & Moll 1992; Kemper, Cowling & Richardson 1999; Giliomee 2006; Curtis 2013).

Figure 2.5: Renosterveld degradation by agricultural practices, natural vegetation is ploughed and replaced with crops.

Although the widespread indiscriminate spraying of herbicides and pesticides are regarded as a less obvious negative effect, the increased leaching of fertiliser from neighbouring pastures are directly associated with freshwater nutrient loading and spread of alien perennial grasses, particularly in the watercourses (e.g. *Hyparrhenia spp.*) of the Overberg (Von Hase et al. 2003). Due to agricultural influence, many natural habitats located on steep slopes become seeded with exotic species, which are then further fortified with the treatment of fertilisers (Tainton 1976; McDowell & Moll 1992).

The increased frequency of sporadic winter drought results in severe financial difficulties for many farms in the region (Curtis 2013; Botai et al. 2017). Lumsden, Schulze & Hewitson (2009), noted that farmers are anxious to maintain a hold on their farms and livelihoods, with added stress due to a weakening economic climate and climate variability. The added stress may be the root of poor decision-making, where virgin land is developed or the ill-timed and frequent burning of vegetation results in irreversible damage to biodiversity and functioning of Renosterveld ecosystems (Curtis 2013). Although the importance of conserving Renosterveld has been accepted for many years, very little progress has been made to meet the conservation targets (Cowling, Pierce & Moll 1986; Jarman 1986; Rebelo 1995). These targets and their accompanying projects will be discussed in the following section.

2.1.4 Historical Conservation Plans

The Cape Action for People and the Environment (CAPE) was initiated in 1998 supported with an initial contribution from the Global Environment Facility (GEF), with its initial phase originating in 2001 (Von Hase et al. 2003). CAPE comprised stakeholders from various sectors including provincial and national government, non-governmental organisations (NGO's), the private sector, parastatals and community-based organisations (CBO's) (Von Hase et al. 2003).

There are however conservation initiatives in the Cape Lowlands that predate CAPE, originally initiated due to concern regarding the biodiversity and continuing habitat loss of Renosterveld and sand-plain fynbos (Parker 1982; Cowling, Pierce & Moll 1986; McDowell & Moll 1992; Rebelo 1995; Kemper, Cowling & Richardson 1999; Kemper et al. 2000). Jarman (1986) identified a number of lowland habitat sites for conservation, these sites promulgated the Plan for Nature Conservation (PNC) (Burgers, Nel & Pool 1987; Von Hase et al. 2003). The PNC plan was not structured around a methodical approach, where important sites were highlighted on the basis of what was known instead of what was not known about the Renosterveld regions (Von Hase et al. 2003). This was deemed inadequate and CAPE was, therefore, justified to undertake a conservation assessment of a more strategic and comprehensive fine-scale nature of the extant Renosterveld areas in the lowlands (Von Hase et al. 2003).

The Cape Lowlands Renosterveld Project (CLRP), initiated by the Botanical Society of South Africa, emphasised a shift from a predominantly reactive stance to more proactive conservation initiative (Von Hase et al. 2003). The CLRP received financial contribution through the Table Mountain Fund regulated by the World Wildlife Fund-South Africa (WWF-SA) and was the first to address fine-scale conservation planning in a priority area of the CFR (Cole et al. 2000). The plan focused on the highly transformed and fragmented Renosterveld lowlands, containing thousands of natural remnants.

CAPE's implementation programme, the Conservation Stewardship Programme (CSP), coincided with the CLRP conservation plan in providing an opportunity for working together with additional conservation initiatives. Most of the remaining Renosterveld fragments are located on privately owned land, therefore the CSP developed off-reserve mechanisms which include incentives encouraging conservation commitments by private landowners to achieve set conservation goals (Pence, Botha & Turpie 2003; Winter 2003). Curtis (2013) explains that only a small number of farms adopted the Stewardship Agreements or Conservation Easements. Strategically, every remaining Renosterveld segment cannot be protected, thus the CLRP rather focuses on areas that maintain a reasonable chance of surviving (Von Hase et al. 2003). Ecological processes encourage the survival of the system's biodiversity elements, necessitating significant attention in the projects' planning and implementation phases (Von Hase et al. 2003).

The importance of the CLRP hinges on the fact that the project fits into the broader context of conservation in the CFR, in that many conservation initiatives are relevant to its success, for instance CAPE and CSP (Von Hase et al. 2003). For successful conservation, all ecosystems and their species must be conserved. To secure long-term persistence of these ecosystems the ecological and evolutionary processes needed to maintain them were accepted as conservation targets (Krug 2004).

Capacity constraints and the need for conservation action within the landscape resulted in various organisations developing models for conservation on private land, such as CapeNature, WWF-SA, Overberg Renosterveld Conservation Trust (ORCT), BirdLife South Africa (BLSA), Fauna & Flora International (FFI) and SANParks (D'Alton et al. 2015; Curtis 2018a). Two primary models have been developed in working towards sustainable living landscapes (D'Alton et al. 2015):

2.1.4.1 Biodiversity Easement Programme

This programme is operated by CapeNature to guarantee the perseverance of biodiversity in a changing climate, with the goal that privately-owned areas consisting of high biodiversity receive a secure conservation status and are linked to a network of other conservation areas in the corresponding landscape (D'Alton et al. 2015). The landowners must achieve tangible benefits for initiating conservation actions, and may choose from a variety of options within the Stewardship Programme (D'Alton et al. 2015):

- Contract Nature Reserve - Sites of critical biodiversity importance, containing threatened ecosystems with exceptional biodiversity assemblages.
- Protected Environment - Groups of landowners with various land-use activities across the landscape.
- Biodiversity Agreement - Conservation-worthy land, protected for a minimum of ten years.

2.1.4.2 Conservation Easement Programme

The Conservation Easement Programme is a new concept in South Africa, piloted by the ORCT in the Overberg. The easement programme is applicable to both present and future landowners, by its inclusion into the title deeds of the property. The programme requires approval from local authorities and the Department of Agriculture at both local and national level. Similar to the Stewardship Programme it can be done with an approved NGO (D'Alton et al. 2015; Curtis 2018a). At the time of this writing (2018), the conservation easements signed in South Africa have all focused on protecting Renosterveld, managed by the ORCT. The easements offer landowners an easy opportunity to conserve their land, demarcating areas for conservation and agriculture (Curtis 2018a). The ORCT further provides management support to the existing easements and generate detailed management plans, with regular inputs from the landowners, for future endeavours (Curtis 2018b).

Curtis (2013), reiterates that the management of lowland fragments at the farm and landscape level is vital for their continued functioning as sustainable ecological systems.

A comprehensive study carried out by Hashemi et al. (2016) reviewed 130 papers published between 1995 and 2014, based on work in catchment or watershed levels, excluding for instance groundwater studies. This study concluded that stakeholder inclusion is particularly relevant for farm and catchment management to better foster policies and incentives.

2.1.5 River corridors for Renosterveld conservation

Successful biodiversity river corridors in the CFR are identified as: a last line of biodiversity under threat as a result of pollution; a resourceful strategy for the development of integrative and inclusive biodiversity and human wellbeing; and a stimulus for cultural and economic development (West, Cairns & Schultz 2016).

Heterogeneous plant assemblages are structurally and functionally supported by riverine ecosystems which link the major habitats of the Cape Lowlands by promoting diversification and dispersal in plants (Von Hase et al. 2003). These river corridors establish refuges during times of drought and major climatic events (Von Hase et al. 2003).

Von Hase et al. (2003), identifies two types of river corridors:

- The river systems that traverse two or more biogeographic areas within the CFR - identified by CAPE as landscape river corridors; and
- Perennial river systems that have continuous flow in the stream bed for the duration of the year, during years of normal rainfall - identified by CLRP as fine-scale river corridors.

The former is described by Rouget et al. (2003), as broad-scale, with functioning processes for instance the migration and exchange between coastal and inland biotas. The latter encapsulates processes that act at a finer scale.

Finer scale processes include the dispersal of seeds along river stretches, movement of pollinators, water run-off and nutrient distribution (Von Hase et al. 2003). Although rivers within the lowlands have been extensively transformed, maximum species variety are often located on the remaining vegetation, as these areas are unsuitable for ploughing (Kemper, Cowling & Richardson 1999; Kemper et al. 2000). All river systems with less than 20% transformation through either urban development, agriculture and the alien plant invasion are considered extant (Kemper, Cowling & Richardson 1999).

Table 2.3: Degree of habitat transformation on the Overberg river systems (Von Hase et al. 2003).

River system	Total area (ha)	Extant (%)	Transformed (%)	Urban development (%)
CAPE river corridors	23 976	10.4	86.9	2.7
CLRP fine-scale corridors	38 329	27.3	71.4	1.2

The establishment of biodiverse corridors along roads, fences and rivers serve as refuges for predatory insects which contribute to the biological control of aphids (Dennis & Fry 1992; Giliomee 2006). Field boundaries aid plot to plot connectivity of surface runoff and subsurface flows - the vegetated boundaries act as a filter for surface runoff with root systems of plants extracting water and chemicals from shallow ground systems, affecting subsurface flows (Schoumans et al. 2014).

2.1.5.1 Management of three types of areas

The management of the natural corridors is essential in connecting the fragmented landscape, linking the extant islands together. The importance has been identified with measures put in place as a guideline for the correct use and management of corridors (D'Alton et al. 2015):

- Zero ploughing through or within 10m of a watercourse, where a buffer of 20 - 30m should be fostered on either side of the watercourse.

The Renosterveld fragments are not isolated islands, their acknowledgment as part of a vast interconnected network of fragments is imperative for constructive management (D'Alton et al. 2015):

- The lack of effort to connect fragments to one another will result in the loss of many more species, as a result of a loss of processes.
- Losses of processes include, but are not limited to, pollination, seed dispersal and predator-prey interactions contributing to a loss in biodiversity.
- Small islands of Renosterveld act as 'stepping stones' for insect and animal movement, irrespective of the size and species richness.

The management of agriculture production land encapsulating Renosterveld fragments, is as important for landscape conservation as the direct management of the remaining fragments (D'Alton et al. 2015):

- Herbicide drift or fertiliser runoff into watercourses and natural veld is detrimental to the veld.
- A buffer of undisturbed veld around watercourses is paramount, as this will aid in protecting the natural habitats and reduce the impact of chemical runoff, often leading to eutrophication and salinisation.

The optimal management and feasibility of buffer zones adjacent to water corridors and production lands are of interest in this study.

2.1.6 Significance of Habitat Connectivity

Habitat connectivity is essential in maintaining natural ecosystems, whereas transformation triggered by habitat fragmentation carries detrimental effects to heterogeneity (Von Hase et al. 2003). Smith & Hellmann (2002) reveal that, among many conservation biologists, habitat fragmentation is globally accepted as a concern with regards to biodiversity loss. The

fragmentation process always occurs via similar consequences, yet subsequent outcomes vary drastically (Haila 2002; Von Hase et al. 2003). This variation makes it difficult to identify specific suggestions for land-use planning and management protocols (Villard 2002).

Fragmented areas directly contribute to the sustainability of the landscape (Kemper, Cowling & Richardson 1999). Habitat connectivity explores the processes of migration and exchange of biota, pollination, and predator-prey relationships between these areas (Von Hase et al. 2003). With the current forced separation, atypical high levels of extinction can be expected due to changes in the climate, as a result of species' insufficient mobility to 'migrate' across the transformed landscape (Newton & Knight 2010).

In spite of the difficulty for application, convincing evidence recognising the significance of habitat connectivity in biodiversity has emerged (Von Hase et al. 2003). An extensive study executed in Australia investigated the performance, dispersal, reproduction and survival of birds in a fragmented landscape with less than 7% of natural habitat remained (Brooker, Brooker & Cale 1999; Brooker & Brooker 2001; Smith & Hellmann 2002). The study found that the degree of connectivity was instrumental for the survival of the target species (Von Hase et al. 2003). In competently-connected habitat regions, compared to poorly connected neighbourhood patches, the opportunity for successful dispersal, territory establishment and survival was exceptional (Brooker & Brooker 2001; Brooker & Brooker 2002). Connections have been identified between healthy ecosystems and farming landscapes, a shift in maintaining living landscapes (D'Alton et al. 2015).

The agricultural sectors have subsequently realised that loss of biodiversity, soil quality, soil health, as well as functioning watercourses and rivers hold negative effects for production landscapes and so the need to promote an alternative modus operandi to the one they had been accustomed to was required (D'Alton et al. 2015).

Global benefits to agriculture in habitat connectivity are vast and may include (Giliomee 2006):

- The transfer of desirable traits from 'wild' plants to crop plants.
- New crops for medicines, oils and fibres derived from 'wild' plants.
- The natural areas create habitats for natural enemies, which may support the biological control of pests and diseases.
- Ecological processes, such as soil formation, nutrient cycling, erosion control, water storage and pollination.

Growing evidence indicates that by increasing agro-biodiversity on farm scale pest populations are stabilised and the reliance for insecticides is reduced (Gollin & Smale 1998; Altieri 1999). Increased agro-biodiversity is achieved by planting a mixture of crops, adding buffer strips promoting natural vegetation and planting windbreaks consisting of indigenous plant species (Giliomee 2006). The tools for habitat management by both conservation and

agriculture started to overlap significantly, resulting in today's interdisciplinary environmental laws, which are remarkably similar across both spheres (D'Alton et al. 2015).

2.1.7 Renosterveld conservation

Cowling & Bond (1991), bluntly raised the question: how many reserves are needed and do we require sporadic large areas, or are several small fragments as effective? What you want to preserve is just as relevant as what is threatening its preservation (McDowell & Moll 1992). The study of natural ecosystems is an example of the former, whereas farmers' attitudes toward this issue is an example of the latter (McDowell 1988; McDowell & Sparks 1989).

The International Union for Conservation of Nature (IUCN) recommends that, globally, 10% of each habitat type must be protected by effective conservation. For Renosterveld to meet this threshold it is necessary to reclaim and restore all transformed areas that display conservation potential, as well as the degraded areas resulting in fragmentation (Krug 2004).

McDowell & Moll (1992) explained that the conservation status of publicly owned Renosterveld was abysmally low in 1992 (Edwards 1974; Jarman 1986). Kemper, Cowling & Richardson (1999) continued that, in 1996 the proportion of lowland fynbos and Renosterveld which was formally conserved was at a distressing 5% and 1.6% respectively (Low & Rebelo 1998).

Work executed on Renosterveld, reviewing the importance of habitat connectivity, indicated connectivity within Renosterveld in addition to the relationship between fynbos and Renosterveld as critical to its survival (Von Hase et al. 2003). Both categories maintain different processes, suggesting Renosterveld connectivity to play a vital role for: plant and animal dispersal, pollination and genetic exchange (Von Hase et al. 2003). In contrast to managing a homogenous system, the management of fragmented fynbos is complicated but critical to Renosterveld's survival, as increased levels of endemism and diversity are coupled with increased risk of mortality, thus requiring area-specific management scenarios (Curtis 2013).

Flow pathways of nutrients within agricultural catchments are very diverse, where scenarios to target load reductions need to be adapted to local geographic and climatic conditions. For scenarios to be successful, it is of paramount importance that human modifications of land use, as well as land- and water-management be taken into consideration (Hashemi et al. 2016). The implementation of river corridors are presently accepted as effective management strategies for successful load reduction.

Within Renosterveld, exceptionally high plant diversity can be found in small fragments (Kemper, Cowling & Richardson 1999; Curtis, Stirton & Muasya 2013). In view of the fact that nearly all the remaining vegetation fragments are required to fulfil a modest reservation target, the conservation of the extant 'islands' is fundamental to any successful

implementation plan (Kemper, Cowling & Richardson 1999). Although the small fragments are highly disturbed by grazing, trampling, crop spraying and frequent fires, they maintain similar community structure compared to large fragments that seemingly portray the pre-agricultural vegetation (Kemper, Cowling & Richardson 1999). Kemper, Cowling & Richardson (1999), applies this notion to justify all remnants of Renosterveld, irrespective of size, must be reviewed as conservation-worthy. Protecting these reserve islands in isolation from the rest of the landscape, would eventually lead to the collapse of the whole ecosystem as a result of the loss of processes such as pollination, seed dispersal and predator-prey interactions (D'Alton et al. 2015).

Curtis (2013), suggests that several Renosterveld reserves are paramount for its long-term conservation and its associated ecological processes within, due to the high species turnover across habitat and landscape gradients. The sustainability of Overberg Renosterveld is influenced by the establishment of interconnected reserves which include the entire range of management regimes and micro-habitats, in order to integrate diversity throughout (Curtis 2013). Notwithstanding the development of reserves, this alone will not be sufficient for the continuing persistence of Renosterveld as a functioning ecological entity. As such, landowner buy-in through tangible incentives is essential (Curtis 2013).

The negative effects of fragmented habitats can generally be negated by correct management practices, with different species responding differently to a variety of management interventions, determining an appropriate management technique for these habitats is pivotal to reduce the effects of fragmentation (Curtis 2013).

There are a variety of methods that farmers and conservationists can employ with the aim of increasing biodiversity and combating habitat loss, all contributing towards sustainable management. Some of the most effective methods for sustainability are intercropping, establishing buffer strips along natural corridors, retaining riparian zones and regions with high natural vegetation, reducing pesticides and the provision of financial incentives to landowners for biodiversity conservation (Giliomee 2006).

2.2 Freshwater pollution from agriculture

Freshwater is highly susceptible to pollution and contamination, constituting a shared common-pool natural resource for which everyone must be held responsible (Constantine, Musingafi & Tom 2014). Fertilisers and pesticides derived from various agricultural practices, lead to the degradation of both surface and groundwater (Barcelo 1997; Donoso, Cancino & Magri 1999; Zalidis et al. 2002; Lam, Schmalz & Fohrer 2010; Smith & Siciliano 2015). The geographic distribution of fertiliser and pesticide pollutions generally follow patterns of agricultural use, occurring as seasonal pulses following periods of increased application (Gilliom et al. 1999).

Agriculture is responsible for large-scale water quality degradation as a consequence of nutrient loading, nitrogen (N) and phosphorous (P), to surface and groundwater (Norse 2005; Schachtschneider, Muasya & Somerset 2010; Bouraoui & Grizzetti 2014; Hashemi et al. 2016). Erosion from tilling processes further causes the loss of P in mountainous areas by detaching the soil particles during overland flow (Schoumans et al. 2014). When the topography is flatter, surface water pollution is a consequence of soil leaching and artificial drainage (Grant et al. 1996; Ulén & Mattsson 2003; Chapman et al. 2005; Heathwaite, Quinn & Hewett 2005; Nelson, Parsons & Mikkelsen 2005). Organic particles leached from the lands are transported with N > P, due to the C:N:P ratio (100:10:1) of soil organic matter, thus the concentration of soluble inorganic P heavily relies on the amount of adsorbed P (Stevenson & Cole 1999).

Agricultural soil erosion contributes significantly to the loss of nutrients via the transportation of soil particles (Schoumans et al. 2014). The subsequent loss of pesticides and NO_3^- through surface runoff and erosion decreases the quality of ground- and surface water, this can however be mitigated with the implementation of appropriate crop management practices and vegetative remediation mechanisms (Kanwar, Colvin & Karlen 1997).

2.2.1 Fertilisers

Diffuse water pollution from agricultural fertiliser applications carries immense cost to society, taking into account the environmental and ecosystem damage, lost aquaculture and fisheries income and the increased treatment costs for drinking water (Norse et al. 2001; Norse 2005; Smith & Siciliano 2015). A more unperceived effect of excessive fertiliser use is its contribution to greenhouse gas emissions (Liu et al. 2011).

2.2.1.1 Ecosystem significance of fertilisers

The application of fertilisers as nutrient diffuse-source pollutant inputs, has the ability to enhance crop growth and improve soil nutrient richness (Lam, Schmalz & Fohrer 2010). However, excessive nutrient input will result in the impairment of water quality (Lam, Schmalz & Fohrer 2010; Schoumans et al. 2014). An increase in N concentration, evident post fertiliser crop application, is generally found within adjacent water systems (Van Es, Sogbedji & Schindelbeck 2006). Increased P concentrations occur in waters that receive leaching or runoff deposition from cultivated land that undergo fertilisation (McColl & Hughes 1981).

2.2.1.2 Sources of fertiliser pollution

The nutrient losses from agriculture are predominantly leached into water systems via surplus runoff as nutrient transport flowing over and under farmland. Non-point sources of pollution, Table 2.4 below, further act as significant contributors of N and P to aquatic ecosystems (Dallas & Day 2004). The P loss through soil leaching is determined by the

extent of soil-phosphate saturation, with the amount of soluble organic nutrients dependent on: soil organic matter content, physical and chemical attributes of organic material, soil adsorption affinity and the pH of the soil (Van der Zee 1990; McDowell & Koopmans 2006).

Table 2.4: Major sources of N and P (adapted from Dallas & Day 2004).

Source of nutrients		
Climatic	Soil and rock weathering	
	Erosion	
	Rainfall	
	Runoff variability	
Catchment characteristics	Geology	
	Land form	
Anthropogenic	Point source	Sewage discharge
		Industrial effluent
		Intensive animal undertaking
		Detergents
	Diffuse source/Non-point	Agricultural terrestrial runoff
		Soil mantle disturbance
		Fertiliser application
		Manure
		Urban runoff
	Atmospheric deposition	Gases released from agriculture
		Fossil fuel burning

Fertiliser applications produce steep nutrient concentrations in the water runoff, soil solution and tile drains, subsequently generating increased N and P concentrations (Lee, Rast & Jones 1978; Preedy et al. 2001; Van Es, Schindelbeck & Jokela 2004; Schelde et al. 2006; Smith et al. 2007; Allen & Mallarino 2008; Hahn et al. 2012; Schoumans et al. 2014).

2.2.1.2.1 Nitrogen

Extreme nutrient availability, in this case N, is a consequence of intensive agriculture where the loss of N and P from the soil is a regular occurrence (Breeuwsma & Silva 1992; Sharpley et al. 1994; Van Es, Schindelbeck & Jokela 2004; Van Es, Sogbedji & Schindlebeck 2006).

The N cycle depicted by Figure 2.6 below, differs from P recycling as N may enter and leave the cycle as gaseous N (N_2) through nitrogen-fixation and bacterial and chemical denitrification in oxygen-poor conditions (Golterman et al. 1980; Dallas & Day 2004). These processes become active in sediments and heavily polluted waters (South Africa 1996d).

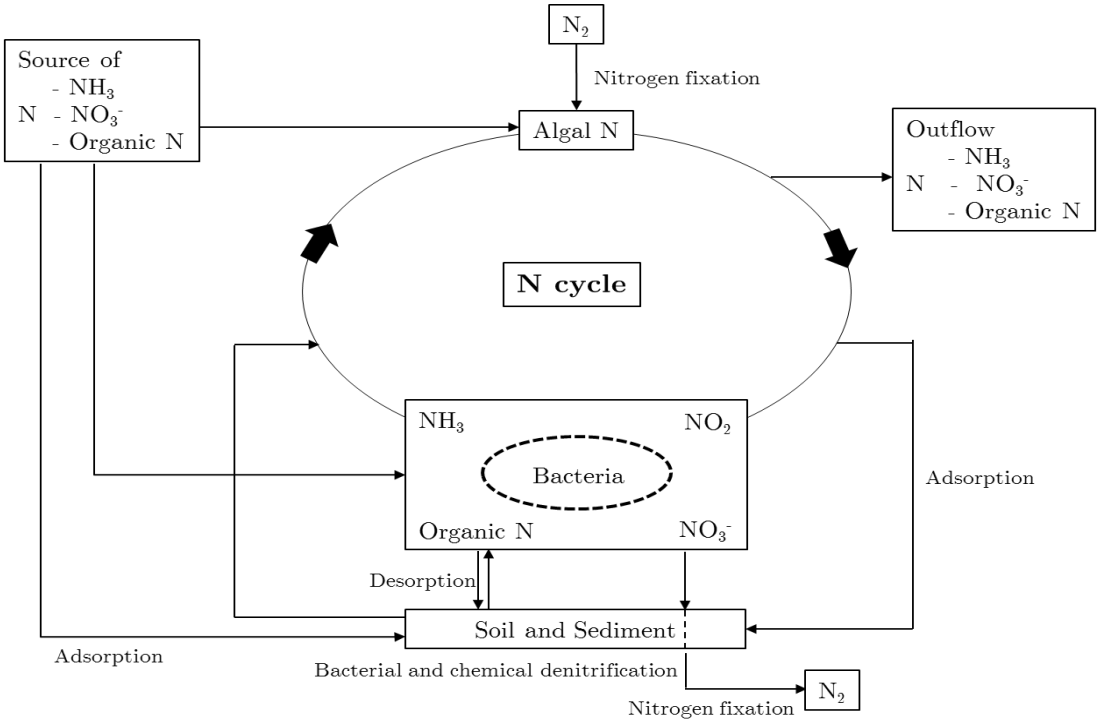

Figure 2.6: Schematic representation of the N cycle (adapted from Dallas & Day 2004).

For the majority of applied fertilisers, the average N:P ratio is between 2 and 4, although the N:P uptake by major crops varies between 4.5 and 9, resulting in the excessive application of fertiliser for farming practices, leading to the accumulation of P in the soil (Eghball & Power 1999; Maguire, Brake & Plumstead 2006).

2.2.1.2.2 Phosphorous

Surface waters receive P through surface flows, rather than groundwater, due to phosphates binding to most soils and sediment (Correll 1998). The PO_4^{3-} ion is readily adsorbed by particles in the sediment and water column, due to its strong surface-active characteristics (Webster, Ford & Hancock 2001).

During periods of low-flow the sediment and soil act as a sink for P that enters the stream at high concentrations, in anoxic (high-flow) conditions the adsorbed P is then released from the sediment (Dallas & Day 2004). The P cycle is depicted by a flow diagram, Figure 2.7, indicating its origin, processes involved and outflow.

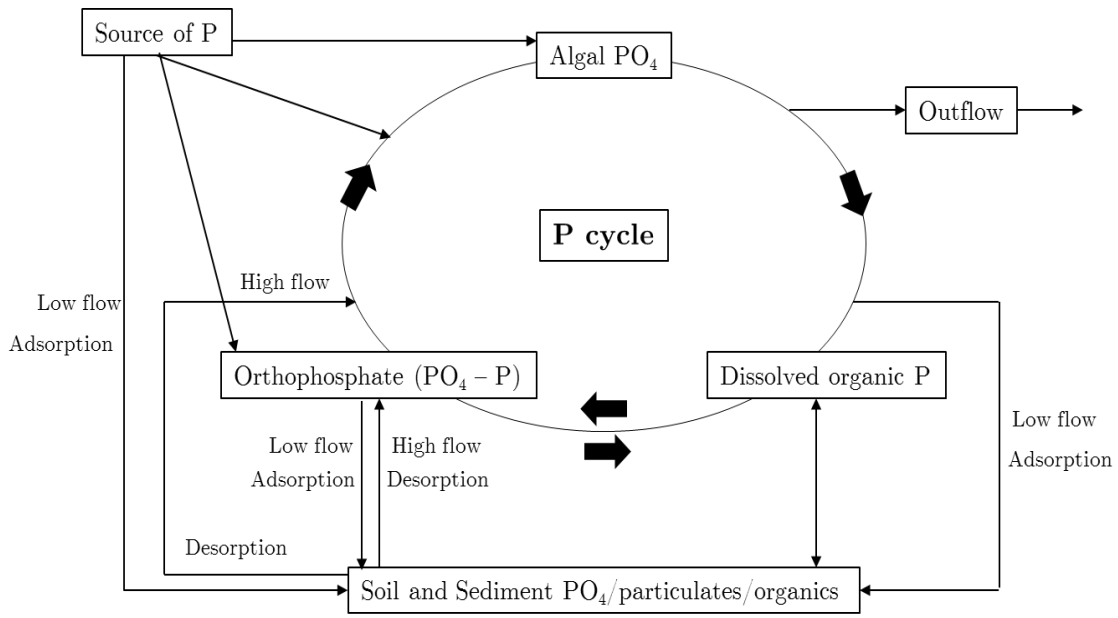

Figure 2.7: Schematic representation of the P cycle (adapted from Dallas & Day 2004).

The soil P status of the ploughed layer represents the amount of soluble inorganic P in surface runoff water, where P enrichment is present in fine eroded material (Allen et al. 2006; Schiettecatte 2006). Ortho-phosphates, known as soluble reactive phosphorous (SRP), entering the surface water as runoff from agricultural fertiliser applications is common (Clesceri, Greenberg & Eaton 1998).

2.2.1.3 Improving fertiliser application practices

It is important to improve the fertiliser application practices in response to a degrading environment. The nutrient status of the soil, coupled with the crop requirements must indicate the application rate and composition (N:P:K) of the fertiliser, however, many farming communities still falsely believe that considerable/over application of fertiliser will positively influence the crop yield, without affecting the ecosystem (Schoumans et al. 2014).

Although crop dependent, the recommended amount of P necessary can be reduced by up to 75% when it is band applied 'bandplaas', the practice of placing the fertiliser on the target site, rather than spreading it (Van Dijk & Van Geel 2012). The crop yield improves due to an increase in nutrient availability, with minimal nutrient loss as a result of uptake efficiency (Schoumans et al. 2014).

Alternative strategies that result in more efficient nutrient uptake by plants, with less nutrient runoff are: split nutrient fertiliser applications (i.e. dividing total nitrogen application into two or more treatments); improved timing of application; and the use of controlled release fertiliser (Lopez-Bellido, Lopez-Bellido & Lopez-Bellido 2006; Burton et al. 2008). By avoiding the application of fertiliser before prolonged and heavy rainfall events and injecting or ploughing the fertiliser directly after application, the risk of nutrient leaching is decreased substantially (Dallas & Day 2004; Schoumans et al. 2014). Soil and crop management are established nutrient-loss mitigation techniques, with a variety of

options available, namely direct drilling (zero ploughing), shallow cultivation and organic matter induced ploughing (Schoumans et al. 2014). Different cropping systems, specifically no-tillage, are used for its potential to improve soil, by decreasing soil erosion and thereby also particulate nutrient losses (Holland 2004). The main soil tillage mitigation options are (Lundekvam & Skoien 1998):

- No till/direct drilling: plant residue covers ≥30% of the soil
- Shallow cultivation: ≤10cm soil tillage, without an inversion process
- Ploughing: Inversion process at 20cm depth
- Contour ploughing

2.2.1.4 Mitigation options of water polluted with particulate and dissolved nutrients

Restrictive land use practices, as a result of the need to control diffuse pollution, has the potential to increase the efficiency of pollutant removal in certain affected areas (Bouraoui & Grizzetti 2014). Agricultural pollution occurs in natural areas that interact with a hydrographical network, as some areas are nutrient sources and others sinks, the catchment's buffer capacity must be considered (Forman & Godron 1981; Schoumans et al. 2014). Options for reducing nutrient losses at catchment scale are (Schoumans et al. 2014):

- Water and nutrient storage and trapping within buffer strips along watercourses
- Water and nutrient uptake by vegetation and biota
- Biogeochemical transformation
- Dilution

Schoumans et al. (2014) review the three main surface water management techniques for the improvement of the ecological quality of water systems, Table 2.5 below, are; river maintenance and river restoration, lake re-establishment and wetland restoration and construction.

Table 2.5: Mitigation strategies for the management of aquatic ecosystems (adapted from Schoumans et al. 2014).

Strategy	Aim	Measure
Maintain and restore rivers	Increase capacity to retain nutrients	Limit cutting and removal of vegetation and lessen the removal of gravel that impedes flow Re-meander, restore flood plains and reconnect inundation areas
Rehabilitate and restore lakes	Reduce lake water P concentration	Control P inlet and extend residence time Add solutions to bind P from sediments
Restore and construct wetlands	Retain wetland nutrient loss from upstream fields	Creating wetlands in agricultural areas with substantial P losses

Surface water management plans as seen above, are frequently administered in River Basin Management as they are inexpensive and efficient for the removal of N and P by increasing the nutrient retention and improving stream ecology (Schoumans et al. 2014).

Management strategies for the mitigation of nutrient losses at a catchment scale, can be grouped into three main categories, Table 2.6 below; field water management, land use changes and landscape management (Schoumans et al. 2014).

Table 2.6: Mitigation strategies for the management of nutrient loss at catchment scale (adapted from Schoumans et al. 2014).

Strategy	Aim	Measure
Water Management	Blocking or reducing overland flow will alter runoff	Creating ponding systems
		Construction of grassed waterways
		Sediment box creations
		Improving surface irrigation
	Avoid below surface losses via leaching	Removing trenches and ditches
		Install drains
		Allow drainage water for meadow irrigation
Land use management	Improves sinks and sources by adapting agricultural use patterns	Alternate arable land and grassland
		Design crops with high nutrient uptake to bottom lands
	Protect vulnerable areas through nature development	Set aside agricultural land
Landscape management	Reduce losses from farmlands	Decrease volume of dirty water produced
	Reduce losses from livestock	Hinder surface water contact: fences and bridges
	Reduce surface runoff and soil erosion	Re-site paths: trails and roads
	Nutrient interception from runoff, erosion and subsurface losses of water	Vegetated buffer strips

From Table 2.6, there are a variety of water system management options available for the mitigation of nutrient enrichment. Landscape management strategies focus on the management of polluted water, with the primary aim of decreasing the volume of dirty water produced, and the secondary aim of preventing the contaminated water from reaching surface water, by utilizing available plant and geomorphological disturbances. Finally, interfaces along permanent streams and ditches are used to control the direct inputs to surface water (Schoumans et al. 2014).

2.2.2 Pesticides

A rapid increase in pesticide use is expected to occur in developing countries, in contrast to already developed countries (Barcelo 1997). The occurrences of pesticide contamination and its associated degradation products in water depends on several physico-chemical,

agricultural and environmental factors (Barcelo 1997). The majority of non-point source pollution of ground- and surface-waters by pesticides originates from agriculture, which include non-irrigated, irrigated/speciality crop production, pastureland and rangeland (Barcelo 1997).

2.2.2.1 Ecosystem significance of pesticides

Pesticides, specifically herbicides, introduced into the environment are done so with the intention of applying effects on one or more specific target organisms, this sets them aside from other introduced organic compounds (Pilon-Smits 2005; Chèvre et al. 2006).

Unfortunately the toxic action is not only exerted onto the area where it is applied, but through overhead spray, leaching potential, soil erosion and transport, significant amounts of chemicals and their degradation products reach aquatic systems (Beach & Carlson 1993; Barcelo 1997; Kanwar, Colvin & Karlen 1997; Chèvre et al. 2006; Mensah, Palmer & Muller 2013; Tran et al. 2017). Non-point source pesticide pollution enters streams and rivers via leaching, spray drift and runoff, most acute where agricultural pastures border surface waters, as seen in Figure 2.8 below (Barcelo 1997; Dabrowski 2001; Pérez, Vera & Miranda 2011; ACBIO 2015). Aquatic freshwater organisms in an ecosystem are generally exposed to a mixture of pesticides, as single contaminant occurrences are unusual (Gilliom et al. 1999; Chèvre et al. 2006). Conveying that even if individual single compounds within a blend of pesticides are below the no-effect concentration (nontoxic), the accumulation mixture works together exhibiting a degradatory effect (Faust et al. 1994; Backhaus et al. 2000). Gilliom et al. (1999) reveal that pesticide mixtures, even below individual acceptable concentrations, may have toxic effects on aquatic organisms.

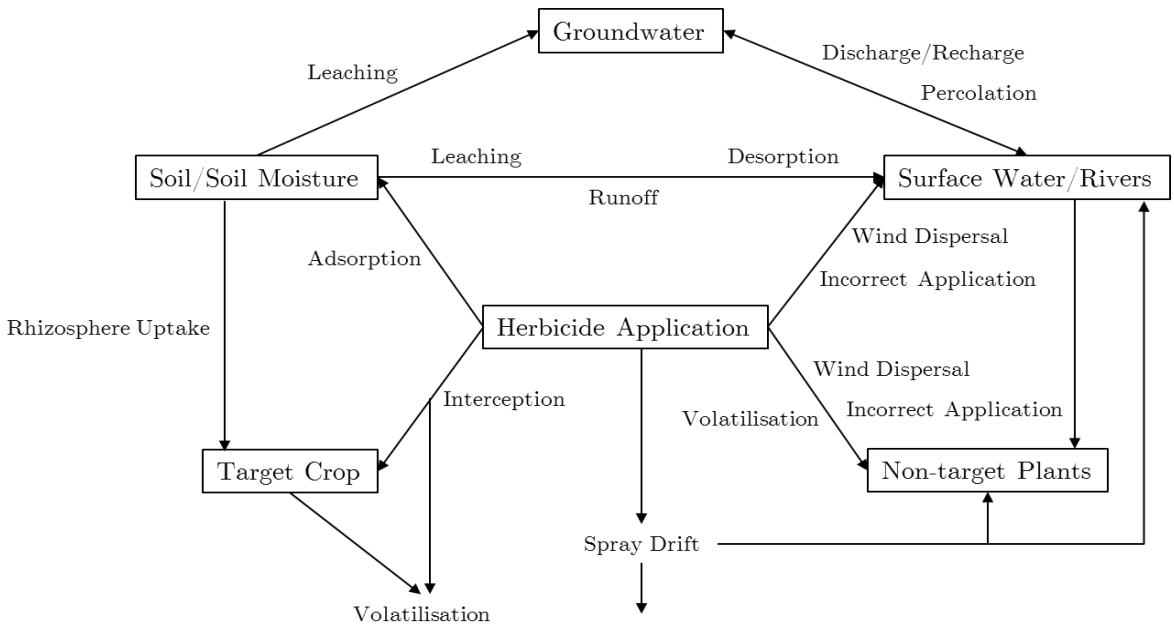

Figure 2.8: Herbicide to crop pathways, with multiple modes of transport to the target organism (adapted from Dallas & Day 2004).

The mobility of a pesticide influences its bioavailability and transfer to the environment, depending on the adsorption and desorption of soil particles and transportation in water (Moorman, Jayachandran & Reungsang 2001). Processes that directly control the transport of herbicide from soil to water are factors that influence the persistence of pesticides in media with degradation and adsorption most prominent (Linn et al. 1993). The environmental threats as a result of herbicide exposure include: damage to non-target vegetation, residue in soil and water, toxicity to non-target organisms and threats to human health (London et al. 2005; Radosevich, Holt & Ghersa 2007). Herbicides have the capacity to reduce species diversity, change community structure, alter nutrient cycling and reduce the resilience of ecosystems, all exhibiting detrimental effects to the aquatic environments' water quality and ecosystem functioning systems (Pérez, Vera & Miranda 2011).

2.2.2.2 Glyphosate

Glyphosate, with chemical formulation $C_3H_8NO_5P$ is a systematic broad-spectrum herbicide used in agriculture for controlling both annual and perennial weeds (Lipok, Studnik & Gruyaert 2010; Dosnon-Olette et al. 2011; Gomes et al. 2016a). Glyphosate comfortably dominates the global herbicide market, accounting for more than 25% of pesticide production, thus regarded as the world's biggest selling agro-chemical (Barcelo 1997; Coupe et al. 2012; ACBIO 2015; Rolando et al. 2017; Tran et al. 2017). Due to the increased cultivation of genetically modified glyphosate-resistant crops, there is a significant increase in herbicide use (Kolpin et al. 2006).

The glyphosate-based herbicides are composed of the isopropylamine (IPA) salt of glyphosate as the active ingredient and is water soluble due to this salt (Arysta 2011). The herbicide is deemed to be chemically stable in water and will not undergo photochemical degradation, the combination of this stability and its mobility in soil is indicative of its potential for surface and groundwater contamination (Lipok, Studnik & Gruyaert 2010). Glyphosate is adjudged to display little degradation, resulting in low toxicity. However, evidence suggest otherwise, through rapid negative responses of marine communities when exposed to glyphosate (Pesce et al. 2009; Vera et al. 2010).

The active ingredient of the herbicide is considered to exhibit phytotoxic traits, as a result of its non-selectivity the non-target phytotoxicity becomes a problem (Clesceri, Greenberg & Eaton 1998; Lipok, Studnik & Gruyaert 2010). However, a number of studies have indicated the importance of other components within herbicide formulations: the presence of IPA and polyoxyethylene tallow amine (POEA), the surfactants, focusing on their adverse impacts on aquatic biota (Krogh et al. 2003). Although the herbicide is designed to eradicate plants only, several countries have recorded numerous negative effects on human health and non-target animals as a result of glyphosate in aquatic ecosystems (Giesy, Dobson & Solomon 2000; Tsui & Chu 2003; Xie, Liu & Xu 2010; Tzaskos et al. 2012; Battaglin et al. 2014; ACBIO 2015; Carneiro et al. 2015; Tran et al. 2017).

2.2.2.2.1 Water solubility of chemicals

Water solubility is the concentration of a chemical dissolved in water, at the stage where the water is both in contact with the pure chemical and at equilibrium (Barcelo 1997). The water solubility is an indication of a chemical's, in this case the pesticide, tendency to be removed from soil by runoff- and irrigation-water, allowing it to reach surface water (Barcelo 1997). Glyphosate, has been proclaimed to be an insoluble chemical when dissolved in water, with a strong affinity to be deposited onto surface soil, hindering transport to surface water systems (Barcelo 1997). However, this statement cannot be used in predicting leaching through soil, a consequence of its regular detection in surface waters, discrediting the solitary use of water solubility as an indicator of glyphosate mobility (Barcelo 1997; Glusczak et al. 2007; Dosnon-Olette et al. 2011; Pérez, Vera & Miranda 2011).

2.2.2.2.2 Water-octanol partition coefficient

Reported as a logarithm, log K_{ow} or log P_{ow}, the parameter is the ratio of the equilibrium concentrations of the two-phase system, consisting of water and n-octanol (Barcelo 1997). Generally, substances with a log K_{ow} >3.0 indicate accumulation in biological membranes. Barcelo (1997) reports that the glyphosate substance is a potential leacher within a soil-water medium, adding to its mobility.

2.2.2.2.3 Glyphosate half-life

Pesticides do not possess one single half-life, resulting in approximations for the exponential decay function with measurements strongly dependent on environmental conditions (Barcelo 1997). The half-life of glyphosate in natural waters was found to be less than 14 days, with shorter half-lives in harder waters measured at 11 days (Pérez, Vera & Miranda 2007). Similarly Maqueda et al. (2017), found the half-life of glyphosate to be between 6.3 and 11 days. In contrast, Struger et al. (2008) reported a significantly longer half-life of glyphosate in water and soil as 70 and 60 days respectively. Gomes et al. (2016a), indicated the half-life under laboratory conditions to range between 30 to 40 days, but in the field can vary from 2 to 197 days.

Table 2.7: Glyphosate half-life from literature.

Medium	Literature Half-Life (days)				
	(Pérez, Vera & Miranda 2011)	(Pérez et al. 2007)	(Maqueda et al. 2017)	(Struger et al. 2008)	(Gomes et al. 2016a)
Natural water	14	6.3 - 11	70		2 - 197
Hard water	11				
Soil			60		
Laboratory conditions					30 - 40

Half-lives reported in literature do not reflect local climatological conditions, as most studies have reported half-lives for Europe and the US, they are not applicable elsewhere (Barcelo 1997).

To combat half-life uncertainty, this study measures the degradation of the influent water supply during each round of sampling. This will indicate whether any concentration discrepancies are present among standardised influent glyphosate solutions.

2.2.2.2.4 Cations antagonistic to glyphosate activity

Glyphosate activity diminishes in the presence of cations found in hard water (Hall, Hart & Jones 2000). Shea & Tupy (1984) reported a reduction in glyphosate phytotoxicity in the presence of high levels of the calcium cation (Ca^{2+}). Cationic mineral nutrients such as iron (Fe^{2+}), aluminium (Al^{3+}), calcium, zinc (Zn^{2+}), sodium (Na^{+}) and magnesium (Mg^{2+}) all reduce herbicidal activity (Sprankle, Meggitt & Penner 1975; Stahlman & Phillips 1979; Duke et al. 1983; Subramaniam & Hoggard 1988). The reduction in phytotoxicity seems to be caused by some cations, although other factors may also be responsible (Stahlman & Phillips 1979).

The reduction of glyphosate phytotoxicity within this study is not of critical importance as the cation concentrations within the standardised influent solutions, were comfortably below the reported 400 mg/L required to impede herbicide activity.

2.2.2.3 Glyphosate in aquatic environments

Glyphosate-based herbicides are regularly detected in surface water long after application, a cause of concern (Glusczak et al. 2007; Dosnon-Olette et al. 2011; Pérez, Vera & Miranda 2011). Groundwater contamination may further act as a source of pesticides for surface water, adding to the potential exposure risk to aquatic freshwater ecosystems (Gilliom et al. 1999).

Glyphosate absorption by soil is determined by the physical and chemical characteristics of the soil, with desorption from the soil as high as 80% of the absorbed herbicide, elevating off-target movement of glyphosate into water ecosystems over large distances (up to 15km from application) to undesired places (Comes, Bruns & Kelley 1976; Piccolo, Celano & Pietramellara 1992; Cerdeira & Duke 2006). The IPA salt of glyphosate then contributes to the toxicity of freshwater invertebrates and fishes (Giesy, Dobson & Solomon 2000; ACBIO 2015). Because of this mobility, the herbicide has been found to induce morphological changes in some vertebrates (Relyea 2012). The microbial biodegradation of glyphosate occurs predominantly in soil and to a lesser extent in aquatic sediment and water, with the major intermediate end product of metabolism (metabolite) being aminomethylphosphonic acid (AMPA) (Lipok, Studnik & Gruyaert 2010; Pérez, Vera & Miranda 2011). Although generally considered to be moderately toxic to aquatic ecosystems (between 1 and 100 mg/L), some plants are reported to be more sensitive to glyphosate concentrations (between

0.1 and 1 mg/L) than others (Pérez, Vera & Miranda 2011). Numerous studies suggest glyphosate to cause detrimental impacts on aquatic freshwater ecosystems, Table 2.8 below (Pérez, Vera & Miranda 2011; ACBIO 2015; Gomes et al. 2016b):

Table 2.8: The glyphosate impact.

Impact	Reference
Inhibiting microbial growth at lower concentrations than claimed	(Clair et al. 2012)
Glyphosate's ability to rapidly 'bind' to soil particles, minimising risk of leaching, vary depending on chemical properties	(Helander, Saloniemi & Saikkonen 2012)
Glyphosate impair water intake by plants and interfere with nutrient uptake	(Cakmak et al. 2009; Zobiole et al. 2010)
Glyphosate weed control linked with increased plant disease in crops	(Johal & Huber 2009)
Glyphosate may inhibit nitrogen fixation or simulation in soybean crops	(Zablotowicz & Reddy 2007; Bellaloui et al. 2009)
Glyphosate toxicity to earthworms	(Antoniou, Robinson & Fagan 2012)

Although herbicides are designed to exterminate undesired terrestrial plants, the most threatened group of non-target organisms are aquatic plants and algae (Pérez, Vera & Miranda 2011). These aquatic plants play a significant role in the functioning of aquatic ecosystems; stabilising sediment and sedimentation rates, flow velocity, nutrient uptake and recirculation (Scheffer et al. 1993). Microalgae additionally provides the basis of food webs in aquatic environments, a fundamental role in the functioning of aquatic ecosystems (Wetzel 2001). Aquatic plants are more sensitive to this pollution than microalgae (Pérez, Vera & Miranda 2011). Within a freshwater ecosystem, when a concentration of 8 mg/L of the active ingredient is applied algae is hindered, however, cyanobacterial growth is favoured (Lipok, Studnik & Gruyaert 2010; Vera et al. 2010). Glyphosate application at similar concentrations causes a significant increase in planktonic assemblages, as a result of glyphosine presence (Grossbard & Atkinson 1985; Pérez et al. 2007). Cyanobacteria reveal a remarkable tolerance to glyphosate. Lipok, Studnik & Gruyaert (2010), observed that cyanobacterial growth is effectively inhibited when the concentration of glyphosate nears 7 mg/L.

The impact of non-point source pesticide pollution can be successfully minimised through the implementation of effective buffer zones between water bodies and crops, although, as of yet no provision has been made for buffer zones in South African regulations (Cole et al. 1997; Dabrowski 2001; Dallas & Day 2004; ACBIO 2015).

2.2.2.3.1 Application

The method of application is crucial to determine the potential contamination of aquatic ecosystems (South Africa 1996b). Aerial herbicide applications pose significantly greater

threats to aquatic ecosystems than selective ground application (Dallas & Day 2004). Much of the herbicide culminates in surface water as a result of spray drift with some of the active ingredient washed by surface runoff and nutrient leaching post terrestrial application, the amount entering aquatic systems can then be significant (Dallas & Day 2004).

Alarmingly when pesticides undergo ground and sprayed application, only 20% and 60% reach their target organisms respectively, with glyphosate-based herbicides inhibiting the nutrient uptake of plants in non-target organisms (ACBIO 2015). When applied near aquatic environments, glyphosate can enter surface and subsurface waters through leaching and runoff from terrestrial application (Abrantes et al. 2009; Dosnon-Olette et al. 2011).

To quantify the amount of pesticides that enter surface waters via runoff and leaching, a number of factors need to first be taken into account. These include the interval between pesticide applications, rainfall events, slope and soil type, as well as the quantity of applied pesticides and the expanse and properties of buffer strips (Barcelo 1997; Cole et al. 1997; Dabrowski 2001).

2.2.2.3.2 Expected environmental glyphosate concentrations

The expected environmental concentrations (EEC) of glyphosate in aquatic environments, indicate the maximum number of applications per growing season at the maximum rate of application, calculated from the application methods of the product label (Duffus, Nordberg & Templeton 2007). With a variety of different glyphosate-based products on the market, each recommended for certain target weeds, a broad spectrum of EECs are applied:

Table 2.9: Expected Environmental Concentrations of Glyphosate in aquatic environments from literature.

Expected Environmental Concentrations	Literature
1.87 mg/L	(Chen, Hathaway & Folt 2004)
2.6 mg/L	(Relyea 2005; Pérez, Vera & Miranda 2011)
3.73 mg/L	(Perkins, Boermans & Stephenson 2000)
10.13 mg/L	(Mann & Bidwell 1999)

From Table 2.9 above, it is clear that the expected concentration of glyphosate in aquatic environments differs substantially. Standing waters and irrigation canals create conditions where the EEC concentrations are quickly achieved. At any of the above mentioned EECs the effect of glyphosate with its formulations are hazardous to the freshwater aquatic environment, where significant environmental degradation has been reported at concentrations lower than 2.6 mg/L (Pérez, Vera & Miranda 2011).

2.2.2.4 International pesticide regulations

Internationally, a significant variation of the maximum permissible levels of pesticides in water is extensive due to the concerns regarding its adverse health and environmental

impacts, the World Health Organisation (WHO) and the US Environmental Protection Agency (USEPA) leading the way (London et al. 2005). International regulations for acceptable glyphosate levels vary between countries, although these are generally set at very low concentrations (Sayre 1988). The Canadian water quality guidelines for glyphosate, 0.8 mg/L, is used as a benchmark for the protection of aquatic ecosystems (Struger et al. 2008; Tran et al. 2017). For drinking water in Canada, the maximum acceptable concentrations of all pesticides is 0.1 mg/L, compared to the European Economic Community guideline of 0.005 mg/L (Sayre 1988). These rigorous requirements are mirrored by the European Commission for herbicides in general, with a permissible value of 0.0001 mg/L (You, Kaljurand & Koropchak 2003).

According to the requirement of glyphosate concentration set out by USEPA, the threshold level on water systems is 0.7 mg/L, similar to the Canadian regulations (You, Kaljurand & Koropchak 2003). The WHO has indicated a recommended concentration for glyphosate for drinking water as 0.9 mg/L (WHO 2005; Tran et al. 2017).

2.2.2.5 South African pesticide regulations

The complete disregard of pesticide pollution in South African water sources is reflected in the current legislation, regarded as being inadequate by regulatory standards (London et al. 2005; ACBIO 2015). The South African Water Quality Guidelines (SAWQG) does not make provision for the evaluation of glyphosate-based herbicides effect on water resources (Mensah, Palmer & Muller 2013). The SAWQGs further lack information on the use of indigenous plant species, as biofilters for pollutant extraction, for the effective protection of its aquatic biota against the glyphosate biocide (Mensah, Palmer & Muller 2013).

The Department of Agriculture Forestry and Fisheries (DAFF) acknowledges that the Fertilisers, Farm Feeds, Agricultural Remedies and Stock Remedies Act (36 of 1947) is outdated and needs revision. The revision will consider the cumulative effects of pesticides and mandating buffer zones to protect aquatic freshwater systems from pesticide applications (ACBIO 2015). Mensah, Palmer & Muller (2013), proposed parameters (for short-term and long-term exposure) to water quality guidelines for South Africa, for the protection of aquatic ecosystems from glyphosate-based herbicides. They focused on *RoundUp®*, a product from *Monsanto Ltd®*, the world's most popular herbicide and a specialist glyphosate-based non-selective weed killer (Mensah, Palmer & Muller 2013). Short-term guidelines are used for the protection of organisms from mortality events such as inappropriate application (spraying during unfavourable conditions or severe wind) and extreme worst case scenarios, as a result of not following product label instructions (Mensah, Palmer & Muller 2013). These guidelines are used as a limited mitigation technique to minimise the impact on aquatic biota. Long-term guidelines are applied for the indefinite protection of all aquatic species and their different life stages during chronic exposure. Chronic exposure to a pesticide may be the result of moderate desorption from sediment or

soil, entry by water runoff or repeated application (Mensah, Palmer & Muller 2013). The proposed water quality guidelines, differentiating between short-term and long-term, have been recommended by only a handful of countries for the protection of aquatic organisms. Illustrated in Table 2.10 below:

Table 2.10: The water quality guidelines for Glyphosate and RoundUp® (CCME 2012; Mensah, Palmer and Muller 2013).

Country	Glyphosate (mg/L)		*RoundUp®* (mg/L)	
	Short-term	Long-term	Short-term	Long-term
Australia & New Zealand	1.2			
Canada	27	0.8		0.065
South Africa			0.250	0.002

From above, Mensah, Palmer & Muller (2013), proposes short-term and long-term water quality guidelines for *RoundUp®* as 0.250 mg/L and 0.002 mg/L respectively, the only clearly differentiated short-term water quality guideline ever reported for this specific glyphosate-based herbicide. Australia and New Zealand, alongside Canada, are among the few countries that have established water quality guidelines for monitoring glyphosate. However, the Australasian countries do not differentiate between short-term and long-term exposure, applied to monitor both acute and chronic situations (Mensah, Palmer & Muller 2013).

Differentiated exposure guidelines are useful for the protection of South Africa's aquatic life against glyphosate-based herbicides. The water quality guidelines, if used as part of an integrated water resources management plan, may be useful in protecting South African aquatic ecosystems from glyphosate-based pollution (Mensah, Palmer & Muller 2013).

2.2.2.6 Glyphosate from a South African perspective

Based on import data between 2006 and 2011, glyphosate import increased by 177% an increase of 12 to 20 million litres (ACBIO 2015).

First registered in 1975 in the South African agricultural sector, glyphosate-based herbicides are among the leading products used for the control of weeds and alien invasive species (Mensah, Palmer & Muller 2013; ACBIO 2015). Ecosystem processes and agricultural practices ensure that these herbicides ultimately make their way into aquatic ecosystems (Mensah, Palmer & Muller 2013).

Since the 1990s, glyphosate has been known to persist in surface- and ground-water of the Western Cape (London et al. 2005). Distressingly, its use for the control of aquatic alien plant species has been encouraged by national departments and commercial farmers alike (London et al. 2005; Maharaj 2005; Dalvie et al. 2011). For the control of both aquatic and terrestrial weeds, glyphosate-based herbicide use is common in South Africa, as a result of

the absence of SAWQGs indicating its effects on non-target organisms (Mensah, Palmer & Muller 2013).

According to the CSIR, elevated concentrations of pesticides from agricultural runoff have been detected in a variety of the country's major river systems e.g. Breede, Berg and Orange Rivers (Oelofse & Strydom 2010). Despite these findings, the impact of pesticides on South African surface waters is ultimately not considered as a part of water resource management (Archer & Van Wyk 2015).

2.3 Water-quality and the consequence of degradation

This section outlines the repercussions of water quality degradation, highlighting the environmental hazards that emerge as a result thereof and revises previous research focusing on freshwater aquatic ecosystem contamination. Applicable and reliable information from water-quality monitoring is crucial to improve knowledge on the water quality of various river systems, establishing effective and efficient water-quality management structures.

2.3.1 River systems

A rapidly growing population is the main contributor to the widespread pollution of entire river systems. The damage has become so extensive that very few rivers persist in their natural condition (Ngoye & Machiwa 2004; Ngwenya 2006). Davies (1998), characterises rivers by both physical and chemical conditions that are progressively and continuously modified downstream from origin to the coast. Whilst there is natural spatial variation regarding the constituents and attributes of water, within and between rivers, the majority of surface water bodies exhibit natural intra and inter annual variation (King et al. 2003).

Rivers are the outcome of surface runoff and base flow which also transports pollutants from natural and anthropogenic sources with which they interact along their course (Davies & Day 1998; Ferrier & Edwards 2002). Pollutants are contained within solution or particulate matter, often reflecting the catchment activity (Tong & Chen 2002; King et al. 2003; Dallas & Day 2004).

From a South African perspective, there are six main areas of concern with regard to water quality pollution (Kidd 2011):

- Eutrophication
- Salinisation
- Pollution
- Proliferation of alien species
- Faulty waste-water systems due to management failure
- Acid mine drainage (AMD)

An added problem in South African environmental protection is the pollution of an aquatic system by other substances such as toxins and organic waste (Kidd 2011). Alien plants and

animals, of whom many benefit from eutrophic conditions, have a negative impact on indigenous species (Kidd 2011). Many of the activities within the water system, anthropogenic or environmental, contribute to the enrichment of nutrients and cyanobacteria, causing eutrophication, posing a significant threat to freshwater surface bodies (Matthews & Bernard 2015).

2.3.2 Eutrophication

Eutrophication is the fertilisation process of natural waters (Lee, Rast & Jones 1978). Increased biological growth due to an increase in nutrient availability, is regarded as a global threat to water quality resources and biodiversity (Foehrenbach 1972; Revenga & Kura 2003; De Villiers & Thiart 2007; Smith & Schindler 2009; Corcoran 2010; Harding 2015). Eutrophication is the consequence of a variety of contributing factors; animal and human waste, fertilisers and stormwater run-off (Wiseman & Sowman 1992; Kidd 2011). The WHO observes the concept of eutrophication to describe the qualitative conditions of an aquatic environment that has been disrupted (Bergman et al. 2013; Harding 2015). Globally, surface water is increasingly affected by eutrophication which controls the ubiquitous increase in cyanobacterial blooms (Khan et al. 2014; Carvalho et al. 2017). A general consensus worldwide is that water-quality issues will progressively and significantly constrain economic development (Harding 2015).

2.3.2.1 The cause and source of eutrophication-related water quality problems

In the mid-20th century, the contribution of surplus nutrients N and P in the eutrophication of surface water was recognised (Redfield 1958; Vollenweider 1986; Schoumans et al. 2014). The significance of PO_4^{3-} as a promoter of excess fertilisation problems in fresh- and marine water was subject to considerable controversy (Lee, Rast & Jones 1978). Water plants and algae growth is the result of nutrient enrichment in a water body, affecting the health of the ecosystem (Kidd 2011).

The major nutrients from anthropogenic disturbances of concern with respect to eutrophication in natural water bodies are N, P and carbon (C) (Oswald 1978; De Villiers & Thiart 2007). The non-point source of aquatic environmental pollution, dispersing inorganic N and P, are agricultural activities (use of manure and nitrogenous fertilisers and the cultivation of N-fixing crops), with a global intensification of nutrients dominated by agricultural sources (Vitousek et al. 1997; Carpenter et al. 1998; Howarth et al. 2000; De Villiers & Thiart 2007; Estrada-Vasquez 2018) The waste generated from agricultural practices is delivered into and transported by the river systems, which are often reflected by a mixture of water quality problems that include eutrophication, increased salinity and acidification (Ngwenya 2006). Within a water body the internal P loading (recycling from sediments) is minimal compared to the external P loads (input from land and the atmosphere), indicating that the solution to controlling excess fertilisation in waters is by controlling the available P from external sources (Lee, Rast & Jones 1978).

Carbon dioxide (CO_2) is abundant in the atmosphere and can readily enter waterways through numerous processes, thus by reducing the N and P concentrations readily available eutrophication is restrained (Van der Merwe 2016). The available C in the form of CO_2 and carbonate species rarely limits the total algal biomass bloom in a waterbody, thus C is not deemed the limiting factor with P-enrichment causing noxious cyanobacterial blooms (Lee, Rast & Jones 1978; Chorus & Bartram 1999; Harding 2015).

In aquatic systems both N and P have been widely recognised as elements that are algal biomass and aquatic plant growth limiting nutrients (Lee, Rast & Jones 1978). The nutrients become readily available through the fertilisation of the adjacent agricultural farmlands, where an excess in agricultural nutrients cause N and P pollution (Buck, Niyogi & Townsend 2004; Lam, Schmalz & Fohrer 2010; Bayram et al. 2013; Strokal & Kroeze 2013; Schoumans et al. 2014; Hashemi et al. 2016). These practices contribute 60% of Total N (TN) and 50% of Total P (TP) to surface waters (Poggi-Varaldo & Estrada-Vasquez 2018).

2.3.2.2 P as the eutrophication limiting nutrient

Schoumans et al. (2014) confirm that the measurement of relative concentrations of TN and TP coupled with bioassays is an accurate method for establishing which of these nutrients limit the growth of algae in aquatic systems (Redfield 1958; Atkinson & Smith 1983; Smith 1983; Hecky, Campbell & Hendzel 1993). Notably, in the majority of freshwater systems P is the limiting nutrient, present in the lowest amount in relation to phytoplankton requirements (Lee 1973; Lee, Rast & Jones 1978; Herath 1997; Carpenter 2008; Schoumans et al. 2014). However, regarding aquatic marine systems, N is identified as the growth-limiting nutrient, particularly in summer (Ärtebjerg & Carstensen 2001; Anderson, Glibert & Burkholder 2002; Schoumans et al. 2014). Lee, Rast & Jones (1978), further report aquatic plant and algae growth in freshwater systems as P limited, with N as a nutritive element ranked second. Caution must accompany the interpretation of the limiting nutrient of an aquatic system, as factors such as light and physical conditions play a role (Correll 1998; Reynolds & Davies 2001; Schoumans et al. 2014).

Algal growth can start at N and P concentrations as low as 0.3 mg N/L and 0.01 mg P/L respectively (Oswald 1978; Van der Merwe 2016). Although the exact concentration for establishment varies, a P threshold greater than 0.01 mg P/L in South Africa is regarded as indicative of causing eutrophication growth in water bodies (Khalid 2014).

2.3.2.3 Water quality monitoring parameters

The recommended water quality criterion varies slightly between literature sources and the SAWQGs for aquatic ecosystems. Table 2.11 below indicates the recommended nutrient concentrations from different departments, limnologists and researchers.

Table 2.11: Recommended levels of nutrients in aquatic systems, identified from literature (Oswald 1978; Grobler & Siberbauer 1985; South Africa 1996d; Van Ginkel et al. 2001; De Villiers & Thiart 2007; Harding 2015).

Nutrient Parameter	Literature adaptations							
	SAWQG for aquatic ecosystems (South Africa 1996d)	(De Villiers 2007)			(Harding 2015)	(Van Ginkel 2001)	(Grobler & Siberbauer 1985)	(Oswald 1978)
	Preventing eutrophication	Pristine	Aquatic animals	Preventing eutrophication				
[1]NO_3^- (End product of the oxidation of organic N and NH_3)	Maximum permissible: 40 mg [2]N/L (Israel 2015).		2 – 3.6 mg N/L					
NO_2^- (Inorganic intermediate)			0.08 – 0.35 mg N/L					
NO_3^- + NO_2^- (Dissolved inorganic N)		<0.04 mg N/L						
NH_3	Target range: 0.007 mg N/L		0.05 – 0.35 mg N/L Short term					
	Chronic effect value: 0.015 mg N/L		0.01 – 0.02 mg N/L Long term					
	Acute effect value: 0.1 mg N/L							
TN (Total inorganic N) (NH_3 + NH_4^+ + NO_2^- + NO_3^-)	<0.5 mg N/L			<0.4 mg N/L				<0.3 mg N/L
PO_4^{3-} (SRP)	<0.005 mg P/L	<0.005 mg P/L	0.02 – 100 mg P/L	<0.02 mg P/L			<1 mg P/L	
TP (Total phosphorous)				<0.03 mg P/L	<0.06 mg P/L	<0.13 mg P/L		<0.1 mg P/L

[1] Areas marked in grey are of interest in this study. These parameters are tested during experimental analysis.
[2] mg N/L = mg Nitrogen/Litre & mg P/L = mg Phosphorous/Litre.

From Table 2.11 above, near pristine conditions are prevalent when dissolved inorganic N (NO_3^- + NO_2^-) is <0.04 mg N/L and dissolved inorganic ortho-phosphate/soluble reactive phosphorous (SRP) reported as PO_4^{3-} is <0.005 mg P/L (De Villiers & Thiart 2007).

For the protection of aquatic animals, the criteria for inorganic N in the form of NO_3^- and NO_2^- are 2 – 3.6 mg N/L and 0.08 – 0.35 mg N/L respectively, and 0.02 – 0.1 mg P/L for SRP (De Villiers & Thiart 2007). The most toxic form of inorganic N to aquatic animals is un-ionized NH_3 with a recommended water quality criteria of 0.05 – 0.35 mg N/L and 0.01 – 0.02 mg N/L for short-term and long-term exposure respectively (De Villiers & Thiart 2007). Available data on South African river catchments suggest that, Ammonium (NH_4^+) concentrations are negligible and thus not of concern with regard to water quality criteria (De Villiers & Thiart 2007).

In preventing eutrophication, recommended levels of inorganic N and P are lower than registered for aquatic animals. Distressingly, there is no established criteria regarding the lower limit of P required for freshwater plant growth, with levels >0.03 mg TP/L as conducive for eutrophication (De Villiers & Thiart 2007). Dissolved values of N and P favouring eutrophication are accepted at >0.4 mg TN/L and >0.03 mg TP/L (De Villiers & Thiart 2007). NO_3^- + NO_2^- accounts for most of the TN, whereas the dissolved SRP is merely a fraction of TP, thus a moderate threshold value of 0.02 mg PO_4^{3-} -P/L combined with the recommended 0.4 mg NO_3^- + NO_2^- -N/L is applied for eutrophication control in freshwater systems (De Villiers & Thiart 2007).

2.3.2.4 Eutrophication from a South African perspective

Nutrient enrichment and subsequent toxin-producing cyanobacteria (blue-green algae) blooms present a considerable threat to the quality of surface water bodies in South Africa (Matthews & Bernard 2015).

Eutrophication has become a significant problem in South African rivers. 2009/2010 witnessed an increase in water crisis reports with numerous rivers, reservoirs and coastal lakes in South Africa no longer having the resilience to naturalize nutrients or sequestrate toxicants (Harding 2015). In spite of this, preventing eutrophication is a low priority in South Africa (Oelofse & Strydom 2010; Matthews & Bernard 2015). Hitherto, water quality issues have been centred around salinisation with a shift in concern from eutrophication, fertilisers and pesticides in South African rivers and reservoirs (Adebayo et al. 2014; Harding 2015). As early as 1979, Cillie, Coombs & Odendaal (1979) noted, by reducing the pollution of South Africa's limited water resource coupled with the multiple cycle reuse of water and ultimately the incorporation of desalination, a future crisis can be averted.

For South African reservoirs, the Phosphorus Management Objective (PMO) is set at 0.13 mg TP/L, this value is more than twice the level at which cyanobacterial blooms become problematic (Van Ginkel et al. 2001; Rossouw, Harding & Fatoki 2008; Harding 2015). Matthews & Bernard (2015) determined cyanobacterial blooms and cyanobacterial surface

scum for South Africa's 50 largest surface water bodies. The study confirmed that 62% is highly nutrient enriched or hypertrophic, while 54% had evidence of already undergoing cyanobacterial blooms, presenting a health hazard. Conradie & Barnard (2012) suggest that if the current trend persists, an increase of toxin-producing cyanobacteria will be observed. Confirming this statement, available data indicates frequent and widespread seasonal occurrence of cyanobacterial blooms in South Africa (Downing 2004; Harding 2015).

For sustainable economic growth in South Africa, De Villiers & Thiart (2007) argues that the fertiliser applications need to be considerably increased. Presently the cumulative river fluxes of N and P are 2.5×10^6 kg N/year and 0.3×10^6 kg P/year (soluble P only), equivalent to 25% of annual soil nutrient loss (De Villiers & Thiart 2007). The cost of nutrient removal by rivers is significant, in terms of equivalent fertiliser cost, contribution of agricultural output and the associated cost of the degradation of ecosystem services in terms of water quality and biodiversity (Bationo, Lompo & Koala 1998; Nandwa & Bekunda 1998).

2.3.2.5 Eutrophication in the Breede River

Nationally, nutrient accumulation in freshwater river systems has shifted its trend from rapidly increasing to slightly increasing, as a result of fertiliser application governance. De Villiers & Thiart (2007) recorded an increase in dissolved inorganic N levels and a significant upward trend in dissolved SRP for the intensively cultivated Breede River system, consistent with an increase in agricultural activity and use of fertilisers for the area, contrasting the national trend. The Breede River produces seasonal concentration nutrient profiles that peak during high river runoff conditions, observing an increase in $NO_3^- + NO_2^-$ and PO_4^{3-}. The profiles are consistent with agricultural fertiliser applications (De Villiers & Thiart 2007). Agricultural activity further exhibits a diffuse source of $NO_3^- + NO_2^-$ in river systems through the burning of biomass in the dry season, with elevated $NO_3^- + NO_2^-$ levels manifested in low-flow conditions, whereas the background $NO_3^- + NO_2^-$ is raised as a result of runoff from fertilisation in high-flow conditions (De Villiers & Thiart 2007).

The National Water Resources Strategy (NWRS) communicated that all aquatic ecosystems cannot be managed to an equivalent standard of protection. Thus tolerating a level of degradation, to sustain socio-economic development is necessary (South Africa 2014). However many believe that this level has long been exceeded necessitating increased attention (Von Hase et al. 2003; De Villiers & Thiart 2007; Harding 2015).

In South Africa, water does not receive the attention that it requires, evident, *inter alia*, through wastage, pollution and degradation, hindering sustainability (Harding 2015). The resource quality has been diminished by mineralisation (salinisation), eutrophication and AMD, in addition to pollution from urban runoff linked with increased migration of rural dwellers to the cities (Grobler & Toerien 1986; Nyenje et al. 2010; Harding 2015). The quality of South African water resources, Table 2.12 below, illustrates the percentage of the resource under threat and the proportion which has already become critical.

Table 2.12: The conservation status of South African water resources (Harding 2015).

SA water resources	Threatened (%)	Critically endangered (%)
Rivers	60	25
Wetlands	65	48
Reservoirs	42	no data

Concerning from the evidence above, the NWRS did not address the issue of eutrophication in reservoirs in their reports (2009 – 2013), a recent planning model for a catchment long plagued by eutrophication from the Department of Water and Sanitation, only addresses salinity under the topic of water quality (Harding 2015).

The Department of Environmental Affairs (DEA) acknowledges that, although substantial research has been carried out on eutrophication in South Africa, collective understanding of the problem remains limited- even though it is clear that eutrophication in water sources results in the reduction in quality of life for South Africans (South Africa 2012).

The South African Institution of Civil Engineers (SAICE) reported within their 2011 'Infrastructure Report Card', that the salinisation and eutrophication of rivers and reservoirs continues, subsequently increasing the cost of water treatment and damaging the environment (Wall 2011).

The recommended TP value for South African reservoirs (<0.13 mg TP/L, Table 2.11) merely allows oligotrophic and mesotrophic reservoirs to degrade to hypertrophy. Even more concerning is the fact that the Minister of Water Affairs referred to the value as a tool in South Africa's armoury to combat eutrophication (Harding 2015). South Africa, with 76% of impounded water affected by eutrophication, is at this stage ill-equipped in managing the problem (Harding 2015).

2.3.2.6 Consequences of Eutrophication

The detrimental environmental effects associated with eutrophication are the decreased function and biodiversity of aquatic ecosystems and diminishing surface water quality (Scheffer 1998; Smith, Tilman & Nekola 1999). Algal blooms that are naturally associated with eutrophication produce toxic algal substances that decimate fish, cause disease in animals and is detrimental to human health (Carpenter, Pritchard & Whaley 1969; Main et al. 1977; Jaworski 1981; Falconer 1989; Kotak, Prepas & Hrudey 1994; Lawrence, Martin & Cooke 1994; De Villiers & Thiart 2007; Schoumans et al. 2014). Therefore, as the quality of South African river systems continues to deteriorate at an unprecedented rate, the health of aquatic ecosystems, agriculture and water users are compromised (Ngwenya 2006). Nutrient enrichment changes the competitive balance between plant species, with the outcome being the decay of aquatic plant communities which produce food, shelter and breeding habitats for an assortment of animal species (De Villiers & Thiart 2007). For many decades,

mortality of animals from cyanotoxins has been prevalent in South Africa (Harding & Paxton 2001; Harding 2015).

Nutrient increase in aquatic ecosystems may also influence the toxicity of pesticides and herbicides, adding complexity to the present understanding of invertebrate community responses (Alexander et al. 2013; Harding 2015). In South Africa's freshwater ecosystems these values are unknown and the effect of nutrient enrichment will have unknown impacts (De Villiers & Thiart 2007). For the control of eutrophication, the nutrient loads must be reduced (Schoumans et al. 2014). To this end, rigorous management of P is the primary global objective in the reduction of noxious algal blooms in surface waters, although P management alone will not solve the eutrophication problem, as the examination of N availability is also key (Paerl 2014; Paerl et al. 2014; Xu et al. 2014). Although P control as the limiting nutrient is important, it is only one factor in an elaborate combination of climatic, watershed and in-lake factors (Harris 1994; Harding 2015).

For South Africa to ensure sustained economic growth and development, water which is accepted as a critical resource in the country needs to be sustainably managed (Matthews & Bernard 2015). The deteriorating water quality in South African river systems present a significant challenge, perceived as a major hindrance in the country's capability to provide sufficient water of suitable quality to meet its current and future needs (South Africa 2004a; Otieno & Ochieng 2004; Ngwenya 2006).

Phytoremediation is a potential solution to the ongoing freshwater aquatic nutrient pollution problem in South Africa. The use of indigenous vegetation, increasing biodiversity, along river corridors located between agricultural practices and water systems has the ability to extract harmful pollutants from the soil and water. Successful pollutant extraction results in the sustained management of the critical resource, ensuring the provision of sufficient water of suitable quality to meet the needs of the future.

2.3.3 Salinisation

Salinisation may occur either as the result of natural processes in parent material or irrigation runoff from agricultural lands, evident in 90% of South Africa's total land surface (Owojori et al. 2009).

Salinity influences the soil water availability for plant uptake, increasing salinity reduces soil water availability (Ayers & Westcot 1976; Chhabra 2017). Salinity also affects agriculture, with excessive quantities of soluble salts in the root zone, crops display escalated difficulty in extracting water from the saline soil solution (Ayers & Westcot 1976). Increased salinity negatively influences the animal and plant life of an aquatic ecosystem and may lead to ecosystem degradation (Scherman, Muller & Palmer 2003; Kotzee 2010).

South African rivers are in general naturally saline (South Africa 2003). Human activities are responsible for an increase in salinity, resulting in water unfit for irrigation and treatments costs too expensive (South Africa 2003).

2.3.3.1 Regulating essential ecosystem processes

Periods of higher salinity are natural phenomena in semi-arid areas, attributed to high evaporation conditions and inflow variability. Water is pumped from catchments to regulate ecosystem processes, providing dilution and flushing stored salts from the dry summer season (Jolly, McEwan & Holland 2008; Kotzee 2010). Salinity impacts the growth and survival of microorganisms, plants, soil and aquatic organisms (Lippi et al. 2000; Ramoliya, Patel & Pandey 2004; Kadukova & Kalogerakis 2007; Owojori et al. 2009).

For plants to extract water from a saline solution they must overcome both the soil water potential and the osmotic potential, indicating how hard plant roots must work to draw water from the rhizosphere (Ayers & Westcot 1976).

2.3.3.2 Salinity within the Breede River

The saline levels, since the 1960s, in the Breede River have increased significantly over the summer periods (Kirchner et al. 1997). The Breede River and its catchment is characterised by agricultural land-use, with water quality issues originating from agricultural salinisation (Scherman, Muller & Palmer 2003). To supply the demand for agricultural irrigation, water is pumped from rivers such as the Breede where the salinity is extensive and ultimately affects crop production (Biesenbach & Inc 1989; Moolman & De Clercq 1993; Owojori et al. 2009; Bothma 2016, Pers com).

Farmers within the lower catchment of the Breede River Valley rely heavily on irrigation to supply their crops. However, they often experience issues with highly-saline water (Kirchner et al. 1997; Bothma 2016, Pers com; Swart 2018, Pers com). The high salinity within the Breede River is believed to be caused by the high volume of irrigation return flows, the geology of the area and a variety of agricultural application practices (Bester 2011). During the summer dry season, the river becomes more saline due to an increase in irrigation from agriculture, resulting in higher return flows (Kirchner et al. 1997; Scherman et al. 2003).

The major saline contributor remains the irrigation return flows emanating from excessive leaching from the adjacent agricultural practices, resulting in elevated sodium chloride (NaCl) levels (Kirchner et al. 1997; Scherman, Muller & Palmer 2003). Salt and saline sediment contribution is aggravated through mobilization when additional irrigation lands are prepared (Kirchner et al. 1997).

2.3.3.3 Managing and improving water saline content within the Breede River

By law the Department of Water and Sanitation (DWS) must provide water of an acceptable quality to farmers (Bester 2011; Bothma 2016, Pers com). For the Breede catchment, 'freshening water' is released from the Brandvlei dam in an attempt to dilute the

highly saline water accumulated from agricultural runoff, 25% of all releases are as a result of dilution purposes (Kirchner et al. 1997). In 1999, due to over irrigation practices for the season increasing the saline level, 25 million m^3 of water was released from the Brandvlei dam (Bruwer 2012).

For the management of effective irrigation, the Breede River Valley systems are of a very high standard, however farmers have little knowledge of theoretical irrigation scheduling and often rely on previous experience, resulting in over irrigation and higher return flows which raise the salinity of the river (Bester 2011).

2.3.4 Targeting nutrient enrichment in freshwater ecosystems

The enrichment of nutrients, N and P, is the main contributor to cyanobacterial blooms. Mitigation options have been proposed to manage the nutrient content within the freshwater systems. For a sustainable solution, mitigation techniques of proactive approach are advised.

2.3.4.1 Wetlands and Sustainable Urban Drainage Systems (SuDS)

Wetlands have an exceptional ability to remove nutrients associated with agricultural runoff. Both constructed and natural wetlands provide an excellent solution from a water management perspective (Hammer 1992; Reed, Crites & Middlebrooks 1995; Comin et al. 1997; Kovacic et al. 2000; Fink & Mitsch 2004; De Villiers & Thiart 2007). The majority of wetland retention rates of nutrients are far greater than the agricultural runoff rates. Fink & Mitsch (2004), express that the standard NO_3^- and TP nutrient retention rates within wetlands ranging between 3000 - 285000 kg N km^{-2}/year and 100 – 71 000 kg P km^{-2}/year respectively. Constructed wetlands can be an effective tool in reducing agricultural fertiliser and glyphosate loading, specifically surface runoff and leaching of N and P, to surface freshwater and aquatic ecosystems and for attaining safe drinking water standards (Kovacic et al. 2000).

Thus, the diminishing level of dissolved N and P in freshwater ecosystems and the prevention of groundwater pollution depend significantly on wetlands to retain nutrients (De Villiers & Thiart 2007). Small riparian wetlands are in many instances the interface between intensive agricultural cultivated hill slopes and plateaus, and water bodies. The wetland's efficiency in the reduction of NO_3^- pollution has been extensively studied in natural and artificial wetlands (Fisher & Acreman 2004; Machefert & Dise 2004; Kadlec 2009).

It is important to note that wetland destruction results in the exponential nutrient enrichment of downstream freshwater systems, resulting in enhanced eutrophication, deteriorating water quality and accelerated biodiversity loss (De Villiers & Thiart 2007). Many countries are replacing conventional engineering approaches with alternatives that aim to manage the quantity and quality of water runoff, and recycle the return flow to a pre-contaminant state (Bratieres et al. 2008; Hatt, Fletcher & Deletic 2009). Two techniques are applied for ideal water management practice, Water Sensitive Urban Design (WSUD) and

Sustainable Urban Drainage Systems (SuDS) (Bratieres et al. 2008; Milandri et al. 2012). SuDS utilize a treatment train of elements to achieve three objectives- the reduction of runoff volumes, improving runoff quality, and improving site amenity and biodiversity (Milandri et al. 2012). SuDS are currently gaining acceptance and are being used to ameliorate and extract a range of pollutants and treatment elements (Bratieres et al. 2008; Milandri et al. 2012).

2.3.4.2 Vegetative buffers in water corridors

The management of vegetation along buffer strips is a significant factor for the removal of stored nutrients, with the goal of increasing buffer lifespan and preventing the loss of nutrients through leaching. Enhancing plant ability for the extraction of mobilised nutrients offers two benefits; removing pore water nutrients that would otherwise leach out and provide removal pathways through vegetative harvesting (Lee et al. 2000; Schoumans et al. 2014). In the occurrence of polluted water from tile drains, the particulate and dissolved nutrients are filtered by meadows or riparian zones (Tanner, Nguyen & Sukias 2005; Stutter, Langan & Lumsdon 2009; Stutter, Chardon & Kronvang 2012).

The presence of glyphosate in the environment creates hazardous effects on non-target organisms, thus techniques capable of reducing glyphosate leached from agricultural soils must be developed (Tesfamariam et al. 2009). The use of riparian buffer strips constitute a solution, by limiting the transport of agricultural contaminants into adjacent waterways when desorbed and mobile glyphosate becomes available for root uptake and degradation (Beltrano et al. 2013; Gomes et al. 2016b). The pollutant is retained in the tissues of the vegetation, resulting in a reduction of glyphosate bioavailability in runoff to aquatic ecosystems (Gomes et al. 2016b). Situated between agricultural lands and aquatic ecosystems, plants within riparian buffer strips are exposed to both glyphosate and nutrient runoff, similar to glyphosate's competition for space in soil adsorption it vies for access to plant membrane carriers (Denis & Delrot 1993; Morin et al. 1997; Gomes et al. 2016b).

Riparian buffer strips are shown to reduce the risk of surface water contamination via surface runoff and nutrient leaching (Lowrance, Leonard & Sheridan 1985). Two types of riparian buffers can be distinguished in terms of their hydrological conditions (Schoumans et al. 2014):

- Unsaturated, vegetated buffer strips
- Saturated, riparian wetlands or wet meadows

The majority of P transportation to watercourses are bound to particles, with the principal physical process occurring within buffer strips being sedimentation (Hoffmann et al. 2009; Schoumans et al. 2014). Hoffmann et al. (2009), reviewed the efficiencies of riparian buffers in the retention of TP and reported that it varied between 41% and 92%, with effectiveness depending on many factors, for instance the nature of contributing sources, slope, soil type, vegetation, flow and hydrological soil conditions (Schoumans et al. 2014).

Bouraoui et al. (2014) reiterated the importance of using vegetative buffer strips for the uptake of nutrients. In contrast to the mitigation options by Schoumans et al. (2014), which focused heavily on the reduction of P losses from the agricultural sector to improve surface water quality, his work concentrates on the reduction of N. Reviewing modelled mitigation options to reduce diffuse N water pollution from agriculture in Europe, 70% specifically identifies either buffer strips or wetlands as proficient options (Lam, Schmalz & Fohrer 2010; Bouraoui & Grizzetti 2014). Riparian zone buffer strips reduce the risk of surface water contamination via surface runoff. The zones are three-dimensional assemblages of vegetation and organisms adjacent to flowing water, establishing an ecotone between the terrestrial and aquatic ecosystems (Lowrance, Leonard & Sheridan 1985; Dallas & Day 2004).

In another study, Poggi-Varaldo & Estrada-Vasquez (2018), found that soil-plant systems were effective in buffering against the accumulation of inorganic N, which included the volatilization and denitrification of N in soil. The denitrification process removes NO_3^- through the provision of reactive carbon for bacteria by plants, with PO_4^{3-} primarily removed through plant and algae cell uptake (Terry & Banuelos 2000).

2.3.4.3 Erosion

Significant P and N losses occur through the transportation of eroded particles from agricultural soils. Thus soil erosion from arable and grassland environments must be reduced. Soil and crop management implementing tillage strategies, soil conditioner applications, crop rotation and crop management directly impact soil erosion. Therefore it is possible to reduce nutrient losses by adapting management strategies in erosion-prone areas (Schoumans et al. 2014). Many of these techniques are currently (2018) implemented by the Overberg agricultural community (Bothma 2016, Pers com; Lynch 2016, Pers com; Groenewald 2017, Pers com; Swart 2018, Pers com).

Poggi-Varaldo & Estrada-Vasquez (2018), affirms that soil erosion is still one of the major causes of damage to land and pollution of surface waters. By reducing the loss of soil particles and nutrients through erosion from agricultural fields, the nutrient loading into catchments are mitigated.

2.3.4.4 Relevant legislation for the protection of water bodies

It is crucial to implement environmental legislation in the protection of aquatic freshwater ecosystems, this will initiate a variety of widespread beneficial consequences for the environment (De Villiers & Thiart 2007; Poggi-Varaldo & Estrada-Vasquez 2018).

The National Water Act, 36 of 1998 (NWA) ensures the nation's water resources are protected, used, developed, conserved, managed and controlled in ways that take into account a variety of social, economic and environmental role players (Kidd 2011). According to the DWS in Kidd (2011), the major sources of surface water pollution are from agricultural drainage and wash-off (irrigation return flows, fertilisers, pesticides and runoff

from feedlots). The NWA aims to achieve effective sustainable management of the country's water resources, to ensure sufficient access to the population and meet the needs of the environment (Kidd 2011).

The National Water Resource Strategy 2004 (NWRS) of the NWA revolves around three main objectives: achieving equitable access to water, achieving sustainable use of water and achieving efficient and effective water use for social and economic benefit (South Africa 2014).

The National Environmental Management Act, 107 of 1998 (NEMA) makes provision for water pollution, stating that any person causing significant pollution or degradation to the environment must take reasonable measures to prevent such pollution from occurring, continuing or recurring (South Africa 1998a).

The National Environmental Management: Biodiversity Act, 10 of 2004 (NEM:BA) is based on the 'White Paper on the Conservation and Sustainable Use of South Africa's Biological Diversity', its objective is to provide for the management and conservation of South Africa's biodiversity within NEMA's framework (South Africa 1997; South Africa 2004b; Kidd 2011).

The National Environmental Management: Protected Areas Act, 57 of 2003 (NEM:PAA), has a similar legislative development process as NEM:BA and aims to consolidate and vindicate all the protected areas legislation in South Africa (Kidd 2011). The Act includes prominent international environmental issues such as endangered species, conservancies and biosphere reserves (South Africa 2003).

Pesticides and fertilisers in South African legislation are controlled by the Fertilisers, Farm, Feeds, Agricultural Remedies and Stock Remedies Act, 36 of 1947, this act is controlled by the Department of Agriculture (South Africa 1996a).

International law treaties are important in the practical protection of freshwater aquatic systems, some of the agreements that are important for this study are as follows;

- The African Convention on the Conservation of Nature and Natural Resources 1968 (replaced by a newer convention in 2002), this convention makes special provision for endangered species and deals with issues relating to conservation, education and research (Kidd 2011).
- Convention on Wetlands of International Importance 1971 (RAMSAR Convention), the convention recognises the ecological and other values of wetlands, restraining the degradation of wetlands and promoting its wise use (RAMSAR 1994).
- Convention on Biological Diversity 1992, the convention is aimed at guaranteeing the conservation of biological diversity and the sustainable use of the biotic components within (UN 1992).
- Convention on Persistent Organic Pollutants (Stockholm Convention) 2001, the POPS convention aims to eliminate or restrict the production and use of Persistent Organic Pollutants. Organic compounds that are resistant to environmental

degradation through chemical, biological and photolytic processes, due to their potential adverse impacts on human health and the environment (UNEP 2009).

2.4 Phytoremediation

Phytoremediation is the *in situ* use of plants to remedy contaminated soils, sediments and groundwater of organic and inorganic pollutants (Schnoor et al. 1995; Cunningham et al. 1997; Campbell 1999; Dietz & Schnoor 2001; Pilon-Smits 2005; Dosnon-Olette et al. 2011). The clean-up (destruction, inactivation or immobilisation to a harmless form) of pollutants is primarily mediated by photosynthetic plants (Terry & Banuelos 2000). The use of plants in the removal of toxic metals, pesticides, chemicals and nutrients as methods of soil and water remediation is cost-effective and environmentally sound (Raskin et al. 1994; Terry & Banuelos 2000; Dietz & Schnoor 2001; Dosnon-Olette et al. 2011).

The sorption and uptake processes are regulated by the physicochemical properties of the compounds, with relatively hydrophobic chemicals (logarithm octanol-water coefficients = 0.5 - 3.0) the most likely to be bioavailable to rooted vascular plants (Schnoor et al. 1995; Dietz & Schnoor 2001). The organic chemicals that pass through the plant membranes are translocated to stem and leaf tissue, where they are converted to amino acids, and further compartmentalized in the tissues of the plant as bound residue (Dietz & Schnoor 2001).

Various phytoremediation applications exist, suitable for different classes of extracted pollutants, Table 2.13 below. These mechanisms for phytoremediation make use of different plant properties, and generally implement different species of plants for each (Cunningham et al. 1997; Pilon-Smits 2005).

Table 2.13: Different phytoremediation technologies (adapted from Dietz & Schnoor 2001; Pilon-Smits 2005).

Technologies	Classes of pollutants
Constructed wetlands	NO_3^-, PO_4^{3-} and herbicides
Rhizofiltration	Radionuclides
Phytostabilization	Prevent leaching and runoff of pollutants, herbicides
Phytoextraction	Metals, nutrients and herbicides
Phytostimulation	Hydrophobic organics/Petroleum hydrocarbons (log K_{ow} >3.5)
Phytodegradation	Herbicides, nutrients (NO_3^- and PO_4^{3-})
Phytovolatization	Volatile organic compounds

Phytotransformation and rhizosphere bioremediation as depicted by Terry & Banuelos (2000), considers similar phytoremediatory mechanisms for nutrient and herbicide extraction as the above constructed wetlands, phytostabilisation, phytoextraction and phytodegradation. These mechanisms are used interchangeably.

Phytostabilisation involves the reduction of the mobility of pollutants in soil (Pilon-Smits

2005). Phytoextraction is regarded as a sub-process of phytoremediation in which plants remove dangerous elements or compounds from a soil-water solution, which may be toxic to organisms even at low concentrations (Dietz & Schnoor 2001). Phytodegradation is the process by which substances extracted by a plant from the soil and water are broken down (Terry & Banuelos 1999).

2.4.1 The phytoremediation process

The first step in the phytoremediation of organic chemicals is sorption to roots, where the chemicals are taken up, translocated, metabolized or volatized by plants (Dietz & Schnoor 2001; Pilon-Smits 2005). The combination of plants and their rhizosphere organisms phytoremediate in different ways, with different phytoremediatory mechanisms, Figure 2.9 below, suitable for different pollutants. Chemical contaminants in soil and water that are intercepted by roots, bind to the root structure and cell walls, hemicellulose within the cell wall and bind hydrophobic organic chemicals effectively (Dietz & Schnoor 2001; Pilon-Smits 2005).

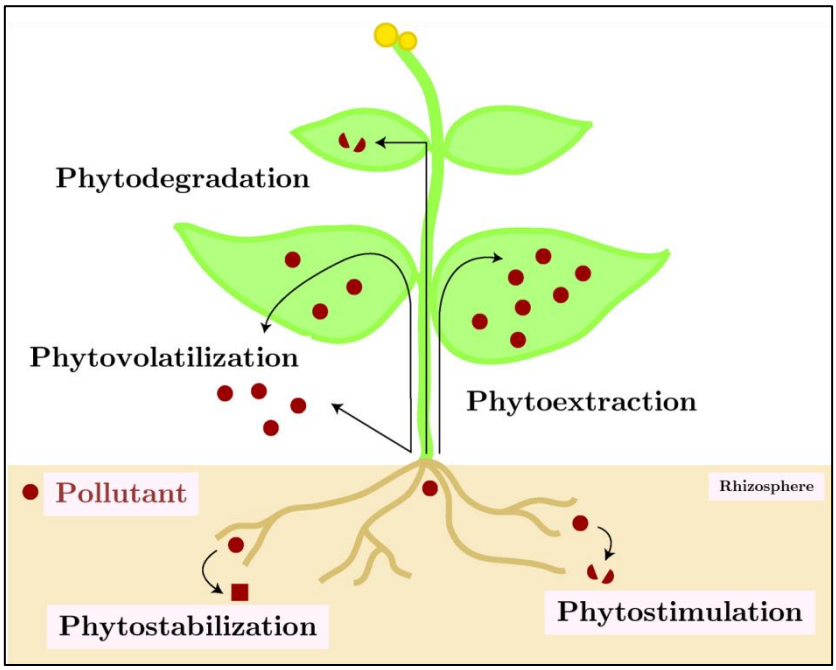

Figure 2.9: Plants facilitating phytoremediatory mechanisms for the biodegradation of pollutants (adapted from Pilon-Smits 2005).

Hydrophobicity relates to the octanol-water partition coefficient (log K_{ow}) of the organic substances. The larger the hydrophobicity of the substance, the greater its tendency to partition out of its aqueous phase and onto the roots (Cunningham et al. 1997; Dietz & Schnoor 2001). Burken & Schnoor (1998), reveal a similar relationship for organic contaminants in hydroponically grown plants, where organic chemicals with log K_{ow} >3.0 are highly sorbed by roots.

The partitioning of hydrophobic chemicals in organic phases is not the only mechanism in the binding of chemicals to roots, where the specific sorption at chemical sites, volatilization and enzymatic transformation by membrane-bound proteins are mechanisms of potential importance (Dietz & Schnoor 2001; Pilon-Smits 2005). The rapid transformation of contaminants by the root surface, due to extracellular- or membrane-bound enzymes, causes binding to roots through irreversible chemical transformations (Dietz & Schnoor 2001).

Vascular rooted plants must extract water and nutrients to grow, these are transported into cells through membrane channels or membrane-bound proteins that bind to the nutrients and transport them into the cell (Dietz & Schnoor 2001). The direct uptake of organics by rooted vascular plants from contaminated sites is an efficient removal mechanism (Schnoor et al. 1995; Dietz & Schnoor 2001; Pilon-Smits 2005).

Hydrophobic pollutants, log K_{ow} >3.0, are chemicals too strongly bound to the surface of roots and soils, they get stuck in the membranes of the plants and will not undergo transport through the plant (Cunningham et al. 1997; Dietz & Schnoor 2001; Pilon-Smits 2005). Water soluble chemicals, log K_{ow} <0.5, are not sufficiently sorbed by roots and have transport difficulty through the plant, evident by Table 2.14 below.

Table 2.14: Potential uptake of organics by plants (adapted from Dietz and Schnoor 2001; Pilon-Smits 2005).

Octanol-water partitioning coefficient (log K_{ow})	Hydrophobicity	Transport through plant
<0.5	Hydrophilic chemicals	Not sufficiently sorbed, no active transport through plant
0.5 – 3.0	Moderately hydrophobic organic chemicals	Efficient removal mechanism
>3.0	Hydrophobic chemicals	Bound too strong, cannot be translocated easily within plant

The removal rate of pollutants is directly related to an increase in pollutant loading (Terry & Banuelos 2000). When saturation is well beyond the enzyme uptake and cellular transport mechanisms, scenarios of mass removal is produced (Terry & Banuelos 2000).

2.4.2 Phytoremediation of fertilisers

Non-point sources of pollution, including diffuse pollution from agriculture, contribute nutrients to surface waters as a result of nutrient leaching or surplus runoff over or under farmland (Chapman et al. 2005; Schoumans et al. 2014).

NO_3^- and PO_4^{3-} are the most common of all pollutants, existing in elevated concentrations from agricultural runoff derived from intensive agricultural fertiliser applications (Dwivedi et al. 2007). Reilly, Horne & Miller (2000), proved NO_3^- removal effectiveness and reliability of constructed wetlands for the protection of human health and reduction of eutrophication.

PO_4^{3-} fertilisation of soil is a common agricultural practice, assuring plant development and growth, its high water solubility results in the easy transfer, through runoff, to aquatic systems (Gomes et al. 2016b).

Schnoor et al. (1995), found potential in the application of phytoremediation for the bioremediation of agricultural non-point source pollution of pesticides and fertilisers. Similarly, Campbell & Ogden (1999), confirmed the utilisation of plant species in the prevention of herbicides and fertilisers from contaminated surface and groundwater samples. Notably, vegetation planted along stream banks adjacent to planted pastures can reduce NO_3^- levels in leachate from fertilised fields through soluble inorganic-N and NH_4^+-N uptake and their conversion into protein and N-gas (Campbell 1999).

2.4.3 Biofilter design

The quality of water discharge from vegetative biofilters exceeds the water quality of unvegetated media, with these differences particularly applicable in the remediation of N and P (Bratieres et al. 2008; Read et al. 2010). Previous research demonstrates biofilters to portray significant ability in the reduction of N (15 – 65%) and P (70 – 94%) concentrations, N removal varies considerably due to the leaching of NO_3^- from these systems (Bratieres et al. 2008; Milandri et al. 2012). Milandri et al. (2012), accentuate the need for a biofiltration system to include diverse plant species to target specific pollutants.

Bratieres et al. (2008), found that biofiltration systems removed at least 80% of P, irrespective of the design. This emphasizes the role of the soil in removing certain pollutants. The choice of plant species is essential for targeting specific pollutants, as some species display affinity, through nutrient uptake, to certain pollutants (Read et al. 2010; Milandri et al. 2012).

2.4.4 Phytoremediation of pesticides/glyphosate

Pesticides are the most common groundwater pollutants, with NO_3^- second, in intensive agricultural practices (Dwivedi et al. 2007). The potential of Phytoremediation to treat pesticides from polluted agricultural runoff is an effective technique for water security (Schnoor et al. 1995; Pilon-Smits 2005).

2.4.4.1 Potential use

The efficiency of removal rates for pesticides have been extensively researched pertaining to experimental wetlands (Alvord & Kadlec 1995; Terry & Banuelos 2000). *Typha* is found capable of decreasing the half-life of some pesticides from 90 days to 5 days- similar to published literature on terrestrial soils, with pesticides becoming immobilized rather than degraded (Terry & Banuelos 2000). Cunningham et al. (1997), communicate that the translocation to aboveground plant tissues is most efficient for pesticides with intermediate polarity, i.e. log $K_{ow} = 1.8$.

Dosnon-Olette et al. (2011) further indicate that glyphosate affects the growth rate of some plant species at 0.08 mg/L. The effectiveness of phytoremediation technologies, for glyphosate removal, indicated a removal yield of 8% in soil medium and 11% in the presence of plants (Dosnon-Olette et al. 2011). Glyphosate has a low octanol-water partition coefficient (log K_{ow} = -3.5 to -2.8), indicating a hydrophilic relationship, thus difficulty for glyphosate to pass through the membranes and infiltrate plants. However, once in the aquatic environment it rapidly dissipates and may bind to the plant roots (Chamberlain, Evans & Bromilow 1996; Schuette 1998; Pilon-Smits 2005; Dosnon-Olette et al. 2011; Coupe et al. 2012). Root binding creates opportunity for pesticide degradation within the rhizosphere.

Vegetation, established in buffers strips, planted adjacent to pastures along a watercourse can slow the migration of volatile organic chemicals and transform some pesticides into CO_2 (Campbell 1999). Due to glyphosate's hazardous effects on the environment, specifically non-target organisms, techniques to reduce glyphosate leaching from agricultural soils must be developed (Tesfamariam et al. 2009). Riparian buffer strips offer an alternative in limiting agricultural pollutants (enhancing microbial degradation) into adjacent waterways (Beltrano et al. 2013).

2.4.4.2 Impact of PO_4^{3-} on glyphosate uptake

The P content of the soil is a key factor in controlling glyphosate availability, with inorganic PO_4^{3-} and glyphosate's methylphosphonic group competing for adsorbing sites, as a result, glyphosate's availability in a soil solution is determined by the soil's capacity to adsorb PO_4^{3-} (Bott et al. 2011; Gomes et al. 2015; Gomes et al. 2016b). As glyphosate desorbs it becomes mobile and available for root uptake (Beltrano et al. 2013; Gomes et al. 2016b). After herbicide extraction, the herbicide is retained in plant tissue, resulting in the reduction of glyphosate bioavailability for runoff into aquatic systems (Gomes et al. 2016b). The riparian buffer strips form a barrier against agricultural pollution, limiting its runoff. A consequence of its ability to extract compounds through roots, playing a critical role in buffer strips efficacy (Gomes et al. 2015).

In particular plant species PO_4^{3-} is responsible for glyphosate uptake and translocation, consequently resulting in increased glyphosate phytoremediation with the bioavailability of PO_4^{3-} (McWhorter, Jordan & Wills 1980; Denis & Delrot 1993; Bott et al. 2011; Gomes et al. 2016b). Glyphosate effects plant's physiological processes such as photosynthesis, with PO_4^{3-} aiding the plant's tolerance to some of the herbicide's deleterious effects (Bott et al. 2011; Gomes et al. 2016b). Gomes et al. (2016b), noticed increased P nutrition in glyphosate-treated plants and increased glyphosate uptake by plants that were subjected to PO_4^{3-} fertilisation, thus an inter-molecule relationships exists that is beneficial to both species, a synergistic effect reflecting a mutualistic interaction.

2.4.4.3 Phytotoxicity of glyphosate re-mobilised by PO_4^{3} fertilisation

In the presence of PO_4^{3-}-fertilisation, the application promotes significant plant damage (Bott et al. 2011). Plant degradation is associated with shikimate (metabolic route used by bacteria, fungi, algae, parasites and plants for biosynthesis) accumulation in the root tissue, Aminomethylphosphonic acid (AMPA - the primary degradation product of glyphosate) toxicity causing a decline in germination and lack of growth-stimulation in the presence of glyphosate (Bott et al. 2011). The re-mobilisation of glyphosate as a result of PO_4^{3-}-fertilisation represents additional transfer pathways for glyphosate to non-target plants.

2.4.5 Advantages of phytoremediation

Phytoremediation is popular due to its cost-effectiveness, aesthetic advantages and long-term applicability (Schnoor et al. 1995; Cunningham et al. 1997; Dietz & Schnoor 2001). The successful uptake and metabolism capabilities of organic xenobiotic chemicals, fertilisers, pesticides and explosive compounds by plants has already been established (Anderson, Guthrie & Walton 1993; Schnoor et al. 1995; Hughes et al. 1997; Newman et al. 1997; Burken & Schnoor 1998; Raskin & Ensley 2000; Terry & Banuelos 2000; Dietz & Schnoor 2001).

The costs associated with environmental remediation is staggering, with US $25 – $50 billion/annum spent worldwide (Glass 1999; Pilon-Smits 2005). Because phytoremediation has biological processes and is essentially solar-driven, it is on average tenfold cheaper than soil excavation, soil washing/burning and pump-and-treat-stations, or engineered-based remediation methods (Glass 1999). Compared with traditional best management practices for pollutant control, an apparent advantage of phytoremediation is cost. The traditional remediation methods rely on electricity, pumping, oxygen additions and construction of large concrete/steel vessels where phytoremediation uses the abundant solar energy and requires no elaborate containment systems (Terry & Banuelos 2000). The *in situ* treatment of pollution contributes to phytoremediation's cost-effectiveness and may reduce exposure of polluted substrate to humans and the environment (Pilon-Smits 2005). In developing countries, as a result of this cost-efficiency and implementation ease, phytoremediation may become a technology of choice for remediation projects (Pilon-Smits 2005).

2.4.6 Limitations of phytoremediation

With the introduction of phytoremediation, there may be potential introduction of contaminants or metabolites into the food chain of aquatic organisms, coupled with long clean-up times required to achieve regulatory levels and the toxicity exposure at waste sites when vegetation is established and maintained (Dietz & Schnoor 2001; Dosnon-Olette et al. 2011). During the establishment of vegetation for phytoremediation at polluted sites, toxicity could be an issue. Palliating strategies include adding nutrients and soil amendments to ameliorate this issue and promote vegetative establishment (Dietz & Schnoor 2001; Pilon-

Smits 2005). With plant establishment and contaminant concentration decrease, the vegetation undergoes vigorous growth and remediation can occur (Dietz & Schnoor 2001; Pilon-Smits 2005). Soil properties, toxicity level and climate at the site of pollution must be suitable for plant growth, with phytoremediation also limited by root depth growth as plants need to be able to reach the pollutant, this can be circumvented by the pumping-up of polluted water for plant irrigation (Cunningham et al. 1997; Pilon-Smits 2005).

The clean-up of soil via the accumulation of nutrients by phytoremediation may take years, limiting applicability, the process is also limited by the bioavailability of nutrients, however this can be resolved by adding soil amendments (Pilon-Smits 2005). For plants to be suitable in the phytoremediation of contaminated water, they should maintain a good health status, in turn maximising the efficiency of the purification process (Baker 2000; Sulmon et al. 2007; Dosnon-Olette et al. 2011).

Engineered plants for biofiltration have already been used in agricultural practices for the remediation of *RoundUp®*, active ingredient glyphosate (Dietz & Schnoor 2001).

2.5 Water quality parameters

The NWA (Act 36 of 1998) defines an ecological reserve as the quantity and quality of water necessary to protect aquatic ecosystems, ensuring ecological sustainability throughout the use of the resource (South Africa 1998b). The recommended water quality parameters are indicators of water quality, indicative of the ecological state of an aquatic system (Scherman, Muller & Palmer 2003).

The DWS have recommended water quality variables within their *RDM Manual* (Resource Direct Measures) for the protection of South Africa's water resources. The manual specifies the minimum requirements to successfully undertake a water quality assessment (South Africa 1999). System variables (total dissolved solids, electrical conductivity and pH), nutrients (nitrates, ammonia and phosphates) and toxic substances (herbicides) are used to determine the water quality of a system (South Africa 1999). The apparatuses and methods used in analysing the parameters are further discussed in Chapter 3.

2.5.1 pH

In water, pH is accepted as an indicator for acidification and used in the general characterisation of water quality (Golterman 1969; Clesceri, Greenberg & Eaton 1998; Ngwenya 2006). pH is defined as a measure of hydrogen ion activity and an indicator of hydrogen ion concentration present in water (South Africa 1996a). With an increase in the hydrogen ion concentration ($[H^+]$), the solution becomes more acidic (a pH decrease), however, with $[H^+]$ decrease the solution is more alkaline resulting in pH increase (South Africa 1996b; Clesceri, Greenberg & Eaton 1998). The aforementioned changes in pH affect the water chemistry within an aquatic system (Dallas & Day 2004). Only an extreme pH will have direct consequences on water (South Africa 1996b).

Many chemical species in water are determined by the pH of the medium in which they are found, thus both the toxicity and solubility of metals and non-metallic ions in water are affected by pH (South Africa 1996b; Dallas & Day 2004). When the pH is altered, pH <7, some metals become increasingly toxic, in contrast, at a pH >8 non-metallic ions become toxins (Ngwenya 2006). NH_4^+ which is not toxic for example, can be transformed into highly toxic unionized NH_3 (South Africa 1996; Clesceri, Greenberg & Eaton 1998; Dallas & Day 2004). Consequently, extreme pH influences the suitability of water for ecological, agricultural, industrial and domestic use (Pegram & Görgens 2001).

South African rivers are relatively well buffered, with pH ranges between 6 and 8. However, in the drainage of catchments containing certain types of vegetation (e.g. fynbos) the pH may drop as low as 3.9, due to the presence of organic acids (South Africa 1996a). These acidic conditions exist in parts of the south-western and southern Cape (South Africa 1996a). South African rivers are infrequently very alkaline, however, elevated pH values can be triggered by biological activity present in eutrophic systems (South Africa 1996a; Dallas & Day 2004). When alkaline conditions are the result of intense photosynthetic activity of aquatic plants, the effect of pH is diminished, this scenario is generally accompanied by high levels of dissolved oxygen (South Africa 1996a). Adsorptive properties of large molecules and particulate matter in water depend on surface charges. pH can alter the ability of nutrients like PO_4^{3-} to adsorb to the materials, of significance when low pH leads to the release of toxic metals from sediment (South Africa 1996a).

In freshwater systems, for every 20°C temperature increase, the pH decreases by 0.1 units, changes in temperature are thus insignificant in the measure of pH in aquatic systems (South Africa 1996a). The value of determining pH as a measure of water quality for this study is negligible due to its non-toxicity to plant and animal life at a controlled temperate environment.

2.5.2 Total dissolved solids/Electrical conductivity (TDS/EC)

TDS concentration is a measure of the quantity of all compounds that are dissolved in water (South Africa 1996b). With the majority of substances in water containing an electric charge, the TDSalt concentration/conductivity is an estimate of the concentration of TDS in the water (South Africa 1996c; Clesceri, Greenberg & Eaton 1998). The conversion factor of EC to TDS ranges from 5.5 to 7.5, where the average factor for most water is 6.5, with the exact factor depending on the ionic composition of the water (South Africa 1996c; Ngwenya 2006). Equation 2.1 is used as a general approximation for TDS concentrations in South African inland waters (South Africa 1996c):

$$TDS\ (mg/L) = EC\ (mS/m\ at\ 25\ °C) \times 6.5 \qquad \text{Equation 2.1}$$

Where

TDS	=	total dissolved solids (mg/L)
EC	=	electrical conductivity (mS/m)

EC measures the ability of water to conduct an electrical current, as a result of ion presence exhibiting an electric current. The majority of organic compounds do not dissolve in water, and consequently don't affect the EC (South Africa 1996c). Measuring the total amount of dissolved material in water, EC is generally used as a characterisation of water quality (Dallas & Day 2004; Ngwenya 2006). The toxicological conditions define the concentration-response relationship between a driving water quality parameter and ecosystem health (Hart, Maher & Lawrence 1999; Scherman, Muller & Palmer 2003).

TDS and EC are representatives for salinity, a system variable that regulates essential ecosystem processes (Palmer & Scherman 2000). The accumulation of salts downstream is due to both natural and anthropogenic processes, such as industrial effluent and surface water runoff. In South African rivers it is notable that this is mainly due to leaching from agricultural sites (Dallas & Day 2004).

2.5.3 Dissolved Oxygen (DO)

Adequate DO concentrations are critical for the survival and functioning of aquatic systems (Dallas & Day 2004). DO may be measured as mg/L or as the percentage of the saturation concentration, with the concentration relating to the absolute amount of oxygen that an organism requires, measured as the instantaneous concentration at 06h00 (South Africa 1996c; Dallas & Day 2004). The instantaneous concentration measured at 06h00 relates to the point in time when the biological activity in water is at its lowest reaction phase.

Gaseous oxygen (O_2) from the atmosphere dissolves in water and is also generated by aquatic plants and phytoplankton (South Africa 1996c). Generally DO decreases in aquatic ecosystems inflict adverse effects on the aquatic organisms, which depend on oxygen for their efficient functioning (Dallas & Day 2004). The increase in DO is termed super-saturation and indicates eutrophication within a water body (South Africa 1996b; Dallas & Day 2004). A variety of factors, Table 2.15, may cause the increase/decrease of DO in an aquatic system. DO concentrations depend significantly on the rate of photosynthesis, respiration and advective transport (Dallas & Day 2004). These are influenced by solar exposure, pollution, sedimentation and precipitation.

Table 2.15: Factors causing an increase/decrease in DO (Dallas & Day 2004).

DO increase	DO decrease
Atmospheric re-aeration	Increasing temperature and salinity
Increased atmospheric pressure	Respiration of aquatic organisms
Decreasing temperature and salinity	Decomposition of organic material
Photosynthesis by plants	Chemical breakdown of pollutants

In natural conditions, the concentration of DO fluctuates diurnally, due to the relative rates of photosynthesis and respiration of aquatic biota. Thus DO is lowest near dawn and

increases during the day, peaking in the afternoon and decreases during the night (South Africa 1996c; Dallas & Day 2004). Within aquatic habitats such as wetlands with a large amount of organic matter, low concentrations of DO are natural phenomena (Cooper 1993). When toxic pollutants such as NH_3 are present, the aquatic systems undergo more stress, with substances becoming increasingly toxic as DO concentrations are reduced (South Africa 1996c; Clesceri, Greenberg & Eaton 1998; Dallas & Day 2004).

It has been reported that the acute toxicity of several common toxicants increases twofold as the DO concentration is halved (Dallas & Day 2004).

2.5.4 Nutrient enrichment

Various plant nutrients are required for normal growth and reproduction, with N and P most commonly implicated with eutrophication through nutrient enrichment in aquatic systems (Dallas & Day 2004). The majority of nutrients are not toxic (with NH_3 as an exception) but may significantly impact the structure and functioning of biotic communities in high concentrations (South Africa 1996c).

N and P are generally considered to be indicators of water pollution (Pegram & Görgens 2001). N and P, though naturally occurring nutrients, control the degree of eutrophication and excessive growth of aquatic plants in South Africa (Davies & Day 1998; Pegram & Görgens 2001; Dallas & Day 2004). The major nutrients that contribute to eutrophication are P as PO_4^{3-} and N as NO_3^-, NO_2^- and NH_4^+ ions (Dallas & Day 2004). The N and P nutrient uptake by plants is in the inorganic form, with microbes responsible for organic to inorganic conversion (South Africa 1996a; Dallas & Day 2004).

2.5.4.1 Nitrogen

The forms of N of greatest interest in water are $NO_3^- > NO_2^- > NH_3 >$ organic N (Clesceri, Greenberg & Eaton 1998). Occurring abundantly in nature, N is a major component of all living organisms, with a significant contribution towards essential photosynthesis processes (Dallas & Day 2004). Inorganic N is present in many forms in both natural and polluted waters. Common water quality tests include NH_3 and NO_3^- forms (South Africa 1996a). From a South African context, inorganic N concentrations in unpolluted aerobic surface waters are generally below 0.5 mg/L and may increase to 10 mg/L in highly enriched waters (South Africa 1996c).

2.5.4.1.1 Nitrate

Nitrates are the end product of the aerobic stabilization of N, transported to freshwater systems via the effluent runoff of agricultural fertilisers (South Africa 1996a; Dallas & Day 2004). NO_2^- is the inorganic intermediate in the conversion of NH_3 to NO_3^- (South Africa 1996c). Natural surface waters are seldom rich in NO_3^-, despite the many sources, due to the photosynthetic action that continually converts them to organic N in plant cells. Although

due to the leaching of organic and inorganic fertilisers, NO_3^- concentrations >150 mg N/L can be present (South Africa 1996c; Dallas & Day 2004).

In natural waters NO_3^- is found in concentrations between 1 and 10 mg/L, with higher concentrations indicating the effects of N-containing fertilisers, since the NO_3^- ion is only poorly absorbed in soil and easily reaches the freshwater aquatic ecosystems (Rump 2002).

NO_3^- may become toxic in high concentrations, resulting in a threshold recommendation of 10 mg/L NO_3^- imposed on drinking water for the prevention of methemoglobinemia in infants (Clesceri, Greenberg & Eaton 1998; Dallas & Day 2004).

Compared to NO_2^-, NO_3^- is the more stable form and is significantly more abundant in aquatic environments (South Africa 1996d). The rapid conversion of NO_2^- to NO_3^-, and vice versa, is controlled by bacterial processes (South Africa 1996d). In aerobic conditions, nitrifying bacteria converts NO_2^- to NO_3^-. In anaerobic conditions however, NO_3^- is reduced to NO_2^- by denitrifying bacteria, which is the most important process of NO_3^- is loss from freshwater aquatic systems (South Africa 1996d).

2.5.4.1.2 Ammonia

NH_3 and NH_4^+ are reduced forms of inorganic N, with proportions controlled by water pH and temperature, the inorganic N is of concern due to its stimulatory effect on plant and algae growth (South Africa 1996d). NH_3 is toxic to aquatic organisms, this toxicity is directly related to the concentration of the un-ionized form of the NH_4^+ ion, although contributing to eutrophication it consists of little or no toxicity (Williams, Green & Pascoe 1986; South Africa 1996d; Dallas & Day 2004). This toxicity is increased as the DO concentrations are reduced, CO_2 is increased and with a salinity increase (Dallas & Day 2004).

NH_3 is generally found in waters in concentrations below 0.1 mg/L (Dallas & Day 2004). NH_3 gas is extremely water soluble, with the toxicity related to the amount of ammonium hydroxide (NH_4OH) in a solution. At low to medium pH, NH_4^+ dominates but as pH increases NH_3 is formed (Schubaur-Berigan et al. 1995; South Africa 1996d; Dallas & Day 2004). The un-ionized NH_3, inhibits cellular metabolism of many animals, by decreasing the oxygen permeability of the cell membrane, causing respiratory issues (Clesceri, Greenberg & Eaton 1998; Dallas & Day 2004).

2.5.4.2 Phosphorous

In natural conditions P is almost entirely presented in phosphate form, by the three major groups of phosphates that include ortho-phosphates, condensed phosphates and organically bound phosphates (Clesceri, Greenberg & Eaton 1998). Phosphorous most commonly occurs in the dissolved form as the inorganic PO_4^{3-} ion, where SRP that is immediately available is seldom (<0.01 mg/L) found in polluted water, as a result of plant utilisation (Clesceri, Greenberg & Eaton 1998; Dallas & Day 2004). Phosphorous that is soluble and readily

available is classified as soluble reactive phosphorous, consisting predominantly of ortho-phosphates (Clesceri, Greenberg & Eaton 1998; Van der Merwe 2016). Ortho-phosphates applied to agricultural land as fertilisers are carried into surface waters via stormwater runoff (Clesceri, Greenberg & Eaton 1998). The added P may increase diatom biomass, with growth of cyanobacteria (a prominent symptom of eutrophication) resulting in increased photosynthesis, where a system may shift from heterotrophic to autotrophic according to bacterial activity (Peterson et al. 1985).

2.5.5 Glyphosate

The contamination of water from runoff and spray drift is a major concern of glyphosate-based herbicides (Clesceri, Greenberg & Eaton 1998). Pesticides are transformed by biotic and abiotic processes post application, with photolysis and hydrolysis the two main abiotic processes in aquatic systems (Barcelo 1997). During photolysis, the rate of degradation is affected by light energy and intensity, and the duration of sunshine (Barcelo 1997).

Organophosphate herbicides are more biodegradable and less subject to biomagnification than DDT for instance, its toxic action is by the inhibition of acetylcholinesterase, an enzyme transmitting nerve impulse to all animals (Dallas & Day 2004). The effect of environmental parameters on the degradation rate of glyphosate is poorly understood, with light emerging as the principal component influencing the rate of pesticide degradation (Barcelo 1997).

2.6 Conclusion

This chapter produces a background on the state of Renosterveld vegetation as well as the factors threatening its existence and the options available to mitigate its degradation. The chapter initially described the Renosterveld habitat, highlighting the ecoregion's transformation to a critically endangered state and provided its historic conservation efforts. Conservation methods that have been proven effective elsewhere were acknowledged, and their possibility for integration into critically endangered Renosterveld conservation was discussed. Second, the driving forces behind freshwater aquatic ecosystem pollution were evaluated, with agricultural practices identified as the leading contributor. Third, the chapter assessed the current state of South Africa's freshwater systems, unveiling widespread pollution of entire river systems, predominantly as a result of; eutrophication, pollution, the proliferation of alien invasive species and faulty wastewater systems. The mitigation techniques currently employed to combat the nutrient enrichment of freshwater ecosystems were discussed. The processes, feasibility, advantages and limitations of phytoremediation as a mechanism for fertiliser and herbicide soil-, sediment- and water-pollution remediation were evaluated. Current research on the effectiveness of phytoremediation across environmental pollutants was given. Finally, the water quality parameters of interest in this study are discussed, as well as their potential contribution to freshwater aquatic degradation. All pollutants negatively influence biodiversity conservation and water quality amelioration.

CHAPTER 3 : METHODOLOGY

This chapter discusses the design, construction, development and execution of the thesis. All aspects contained within this section are critical for the successful examination and comparison of the purification efficacies in a variety of plants. The location in the Renosterveld ecoregion that promulgated this and other similar research projects is identified, where the need for conservation has been emphasized in Chapter 2. Precise planning and implementation of the study parameters combined with experimental research was vital to accomplish the objectives of this project. The foundational areas of planning, designing, construction and execution of an effective system capable of analysing phytoremediatory capabilities were as follows:

- Study area in need of conservation and the site of the laboratory experiment
- Experimental design, construction and set-up of the phytoremediation system
- Indigenous and alien plant species included in the study
- Selection of Fertiliser and Herbicide
- Experimental procedure and data analysis
- Limitations and assumptions

3.1 Study area

The location of the natural Renosterveld ecoregion that encouraged the need for this study and the laboratory experimental site are included within this section. The field location identified the vegetation available for use in the phytoremediation system, the soil growth medium incorporated and the specific fertiliser and herbicide applied with their corresponding dosage strengths. Time spent in the field promulgated discussion with conservation ecologists whom further encouraged the choice of plant species.

The laboratory site represents the location of the phytoremediation experimental system in the Waterlab in the department of Civil Engineering at Stellenbosch University.

3.1.1 Field location

The field location was chosen as a result of discussions with the Overberg Renosterveld Conservation Trust (ORCT) research centre. The centre was then used as a base during soil excavation, water sampling and plant extraction, with numerous conservation ecologists and botanists at hand.

The Breede River, Figure 3.1 below, originates in the mountains of the Ceres basin, in the Western Cape Province of South Africa. The river predominantly travels in a west to east direction with its outlet located at Witsand (GPS co-ordinates: 34°23'53.51"S, 20°50'27.87"E) (Curtis 2013).

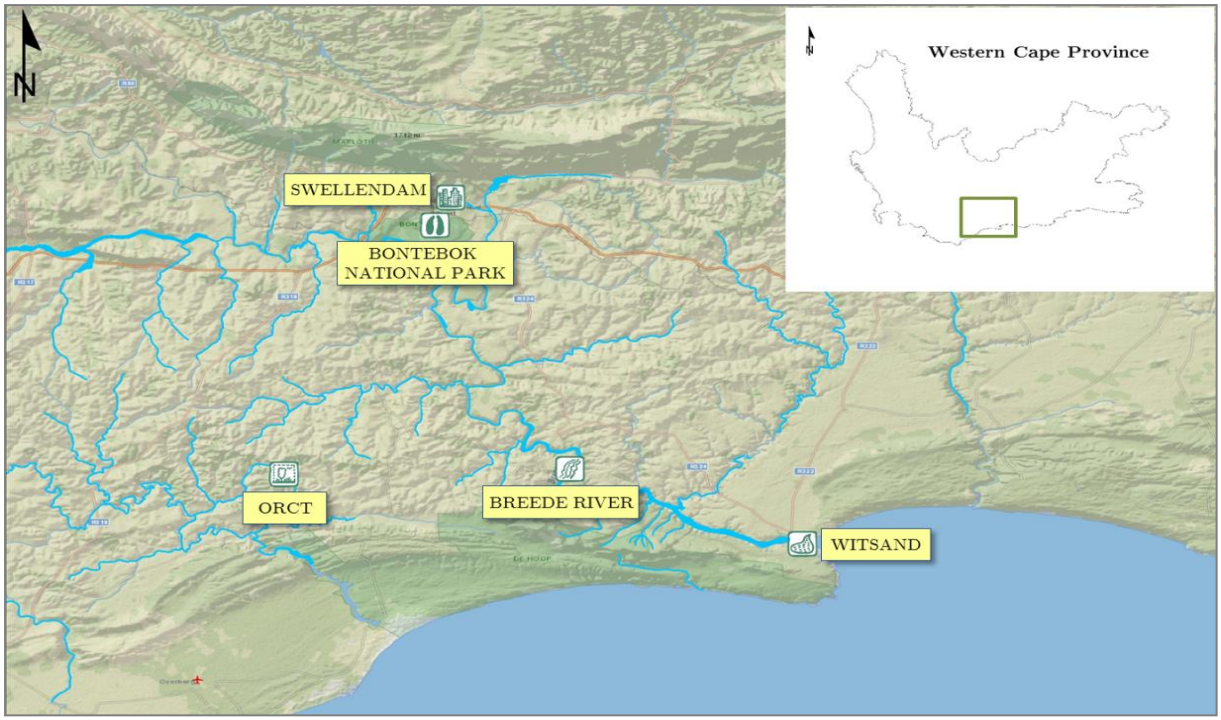

Figure 3.1: The Breede River Valley in the Overberg (data source: SANBI 2003).

Rainfall is bimodal (spring and autumn peaks) in the Overberg (Von Hase et al. 2003). Although there are four Overberg Renosterveld types, for this study the focus is on Rûens Silcrete Renosterveld, occurring in a long thin strip along the Breede River, illustrated in Figure 3.2 below (Curtis 2013).

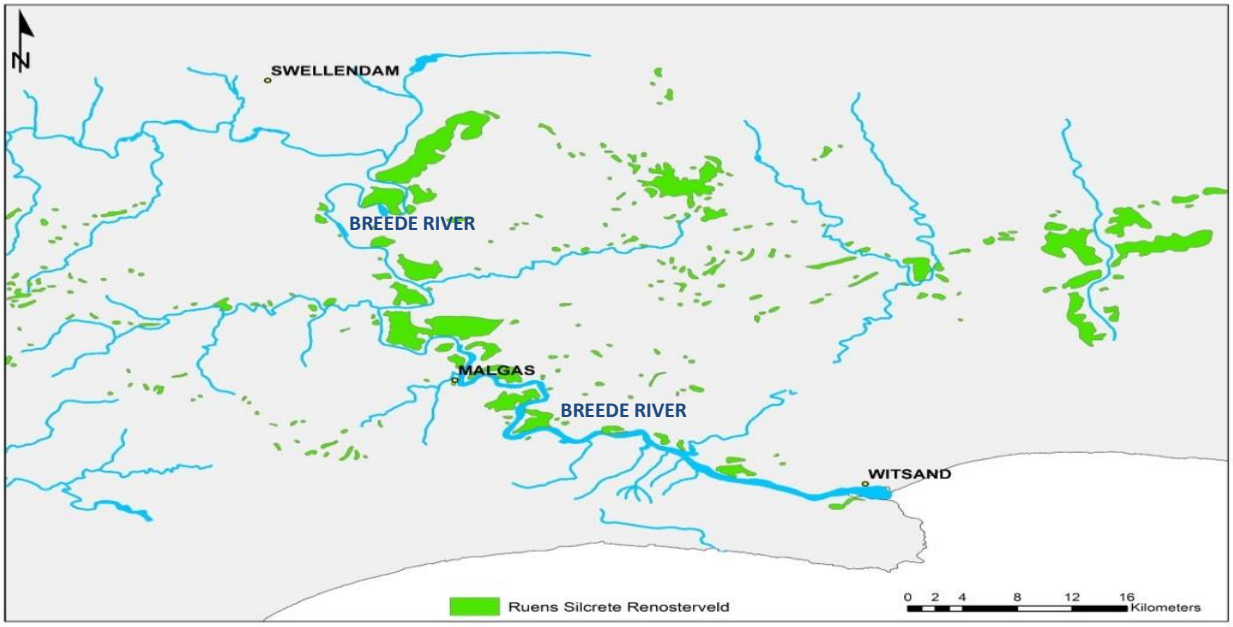

Figure 3.2: Natural distribution of Rûens Silcrete Renosterveld along the Breede River (data source: SANBI 2003).

The Breede River was selected for its course through one of South Africa's most prominent agricultural areas, encompassing grazing meadows, cereal and grain fields and wine and fruit farms (Cowling, Pierce & Moll 1986). Agricultural practices along the Breede River have decimated the naturally occurring Rûens Silcrete Renosterveld (Von Hase et al. 2003; Rutherford & Mucina 2006; Curtis 2018b), evident from Figure 3.3 below.

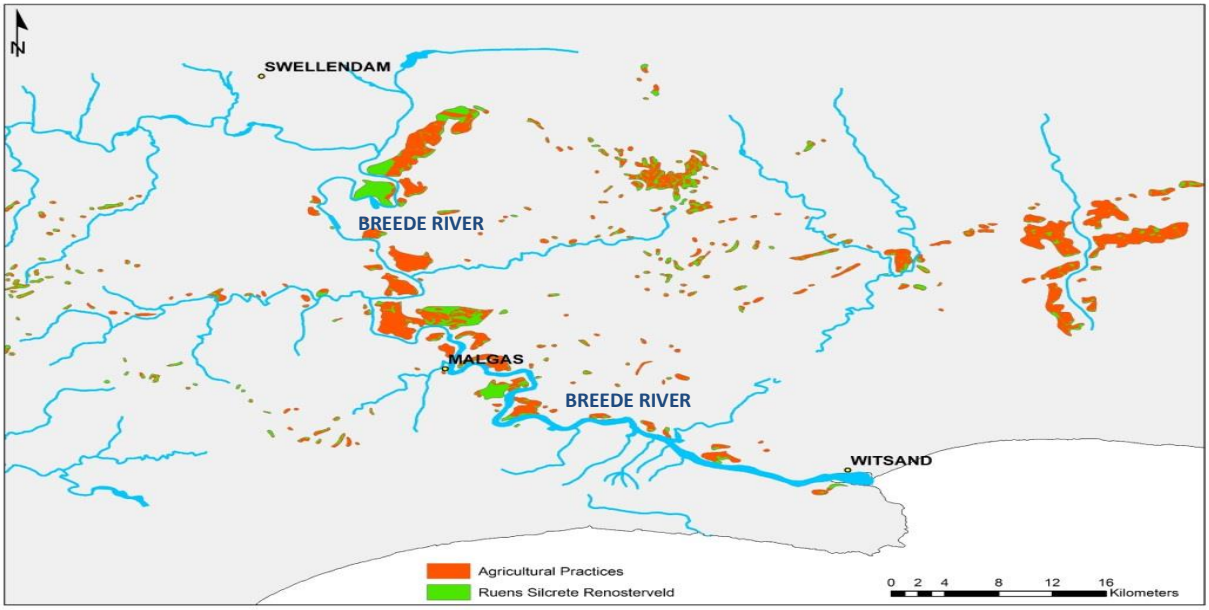

Figure 3.3: Agricultural dominance on Rûens Silcrete Renosterveld (data source: SANBI 2003).

The production of Canola (*Brassica napus*) is increasing in popularity as a seed crop in the Western Cape, used in the production of cooking oil and margarine in South Africa (Coetzee 2017). The application of agricultural fertilisers and pesticides to these fields pollute the adjacent freshwater systems, via surface leaching and runoff, threatening the health of the aquatic ecosystems and water-quality (Von Hase et al. 2003; Bester 2011; Schoumans et al. 2014). The degrading water quality further disturbs the farms along the river, forcing farmers to treat the water prior to irrigating their crops (Bothma 2016, Pers com). This treatment substantially increases the cost of farming. Additionally, irreversible conversion of the natural Renosterveld vegetation type to croplands occurs at an alarming rate, with organic and inorganic pollutants adding more pressure on the natural veld.

3.1.2 Laboratory site

The laboratory experiment (April 2017 – May 2018) was conducted in the Water Laboratory at the Department of Civil Engineering at Stellenbosch University. The designed system prevented precipitation events from altering the quantity or quality of water used in the experiment, whilst maintaining ambient solar radiation and temperature. The purpose of the laboratory experiment was to investigate the water remediation potential by wetland plants, of agricultural polluted water, that are found within critically endangered Renosterveld vegetation types and are widespread throughout Southern Africa and to allow comparison

between indigenous species tested and invasive alien species currently being utilised in South African sustainable urban drainage systems (SuDS).

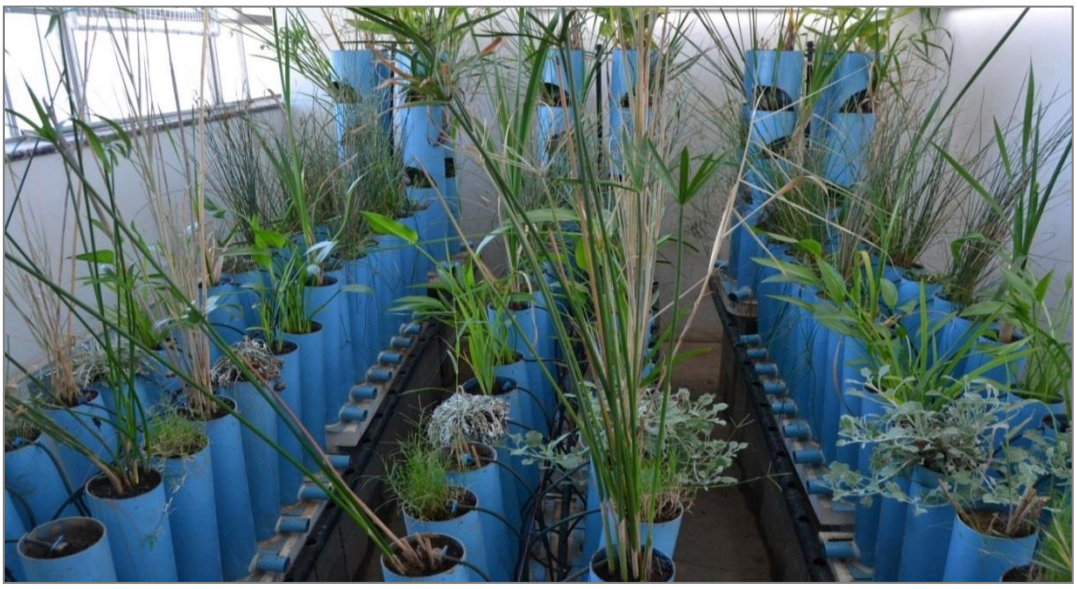

Figure 3.4: Constructed laboratory phytoremediation system.

Effluent samples were collected and analysed in the Water Quality Lab at Stellenbosch University. The lab is equipped to measure all nutrient- and water quality-parameters. The equipment utilised for effluent analyses are illustrated in Figure 3.5 below.

Figure 3.5: Equipment for nutrient and water quality parameter analysis.

The *HQ440d Benchtop Multi- Parameter Meter*™ was used for the analyses of pH, EC and DO. The *DR3900 Benchtop Spectrophotometer*™ and *TNTplus*™ test kits were used in the analyses of NH_3, NO_3^- and PO_4^{3-}. The DO probe was calibrated by filling a volumetric flask with di-ionized H_2O to a height of 6.4mm and shaken, the probe was inserted and left to calibrate for 30minutes. The EC probe was calibrated by rinsing the probe with di-ionized H_2O and submersing the tip of the probe in a Hydrochloric acid solution until calibration was secured. The pH probe was calibrated by rinsing the probe with di-ionized H_2O, after which the probe was inserted into known pH solutions of 4.00, 7.00 and 10.00 for successful calibration.

3.2 Experimental design

The system was designed to test and compare the individual purification capabilities of 14 indigenous, mainly wetland plant species and a soil control. The aim was to establish the potential use of the species in buffer strips, SuDS treatment trains, wastewater phytoremediation and constructed wetlands. Species inclusion was considered on merit, regarding potential efficacy in the removal of contaminants, and ease of use i.e. growth rate, invasiveness, hardiness and availability, information deduced from previous research (Bratieres et al. 2008; Read et al. 2008; Read et al. 2010; Milandri et al. 2012). The phytoremediatory capabilities were further compared to plant species that have been investigated locally and internationally regarding their accumulative purification properties. Although effective, the majority of these plants pose alien invasive threats. As a result, the experimental system included a plant community analysis, by comparing the purification capabilities of four plant species found within Renosterveld habitats and three invasive alien plant (IAP) species and Palmiet (selected due to its rapid growth properties and growth characteristics as well as use in constructed wetlands). Bearing in mind that all plants differ in nutrient extraction and affinity, a combination of species was identified to be used as a collective, with maximised pollutant removal as the goal.

3.2.1 Design and construction of the phytoremediation system

(As illustrated in Appendix A, Table A.1 and A.2)

In order to assess and compare the nutrient extraction capabilities of the different plant species, a system capable of incorporating multiple plants over a variety of species was needed. The system was required to integrate five influent parameters across three treatment pathways, capable of guaranteeing uniform standardised influent irrigation throughout. The design and construction of the phytoremediation system is given below.

3.2.1.1 Individual plant per silo design (IPPS)

A total of 90 silos were constructed from Ø110mm x 500mm polyvinyl chloride (PVC) piping, each with a Ø40mm x 180mm threaded slit drainage pipe that protruded from the sealed base of each silo, enabling effluent collection into sampling containers directly below. The base of each silo was sealed with a 150mm x 150mm square PVC sheet, perpendicular to the length of the silo.

A mould was designed, adding support to the effluent drainage pipe, by tailoring a cube of isoboard into a shape that securely fit underneath the outlet pipe. The mould assisted effluent removal by steering the filtered effluent water solution into the drainage pipe to allow for discharge. Isoboard was used instead of polystyrene, due to polystyrene's water absorption and expansion properties. Expansion of the mould would add unwanted pressure on the sealant of the silo. Isoboard is also unreactive to the pollutants in the study (PPC 2018). A diagram depicting the design of the silos is given by Figure 3.6 below.

The silos allowed for the analyses of 14 different individual species and one unvegetated soil control medium, receiving three different treatments; fertiliser, herbicide and a municipal tapwater control. The municipal control treatment is later converted to a weaker/nontoxic (compared to the first herbicide treatment) herbicide dosage. For each treatment, all species were represented by two plants, adhering to a duplicate study design.

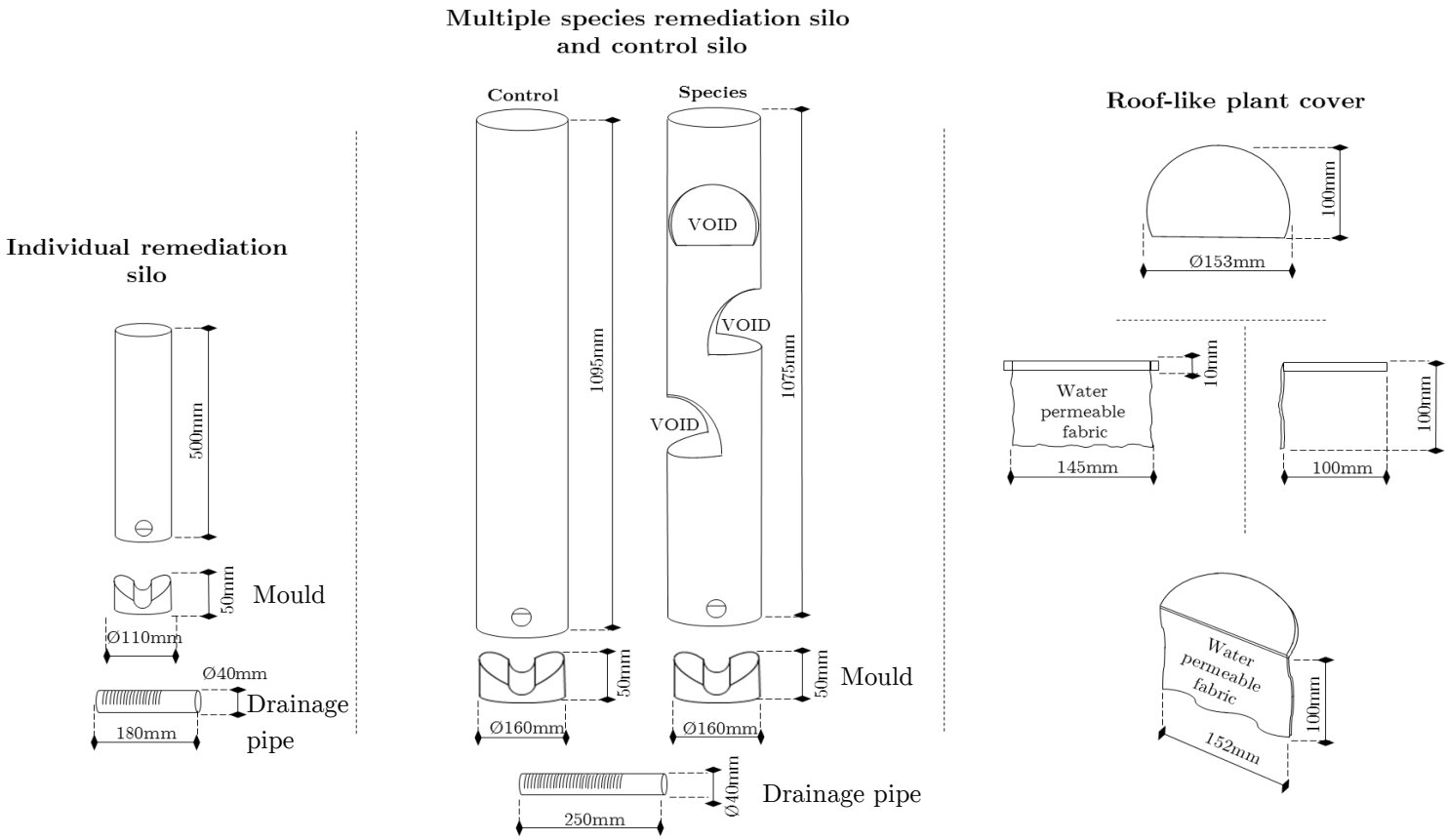

Figure 3.6: Silo design for both the individual and multiple species treatments, and a roof-like plant cover design for the multiple specie silos.

3.2.1.2 Multiple plants per silo design (MPPS)

For the analyses of combined species remediation, a total of 15 silos were constructed from Ø160mm x 1075mm PVC piping, each with a Ø40mm x 250mm threaded slit drainage pipe protruding from the sealed base of each silo, enabling effluent collection into sampling containers directly below. As with the design for individual species remediation silos, a mould was also created to support effluent drainage. Four silos were used per treatment with four different species per silo. The indigenous plant species selected are; *Phragmites australis, Cyperus textilis, Typha capensis* and *Cynodon dactylon* whereas the IAP species are; *Canna indica, Arundo donax* and *Pennisetum clandestinum.* The fourth species added to this IAP assemblage is *Prionium serratum.* For each treatment investigating phytoremediation capabilities, two silos of indigenous species and two silos of alien invasive species are used.

The alien invasive species were selected with regard to their frequent use in SuDS (Bratieres et al. 2008; Read et al. 2008; Milandri et al. 2012). The paired silos each consisting of four plant species adhered to the duplicate experimental design. Voids were tailored along the length of the silos to enable plant establishment at different intervals of the silo, at each void a plant species was introduced (the quantity per species depended on the surface cover of each species, quantity of grass > quantity of sedges). Equivalent growing space/volume of soil media was created approximate to the individual plants per silo design. This volume growth medium equivalence ensured that the effect of soil degradation and soil adsorption was constant between the different silo sizes, as illustrated by Figure 3.8. It is important to acknowledge the effect of soil in pollutant remediation, thus controls were designed to establish exactly what this contribution is. For each growing compartment (void) a roof-like structure was designed and applied overhead, Figure 3.6 above, with the role of ensuring the stability of the soil medium, preventing cascading of collapsed media onto and potentially harming the plants. A water-permeable fabric was attached to the roof-like structure allowing the free movement of water and pollutants throughout the silo, however acting as a barrier for soil threatening to collapse onto the plants.

Similar to the individual plant per silo experiment, a soil control for each treatment was included in the multiple plants per silo study, Figure 3.7, establishing the role of soil degradation and adsorption in pollutant remediation.

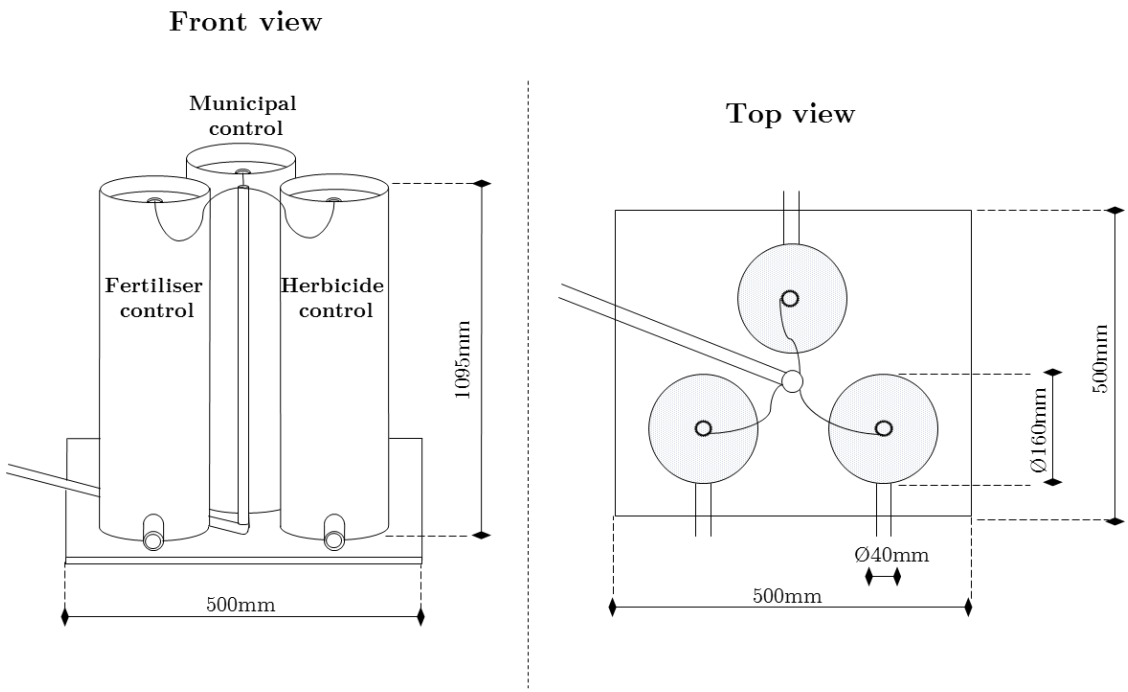

Figure 3.7: Soil control for multiple species/community analysis.

3.2.1.3 Representative soil growth volume calculations

The subsequent calculations and steps are illustrated in Figure 3.8 at the end of Section 3.2.1.5, inserted to grant clarity of the procedure followed. The dimensions of the individual

plant per silo study (IPPSs) were taken as a representative of the desired volume for the study. The dimensions of the silos *h* x *w(Ø)* are as follows;

Where

h = height (cm) = 50cm (chosen).

w(Ø) = radius (cm) = 5.2cm (inner radius of Ø110mm silo).

Considering only a portion of the silo is used as growth medium and the rest as filtration, the effective growth height per soil medium is 30cm. The following equation is used:

$$V = \pi r^2 h \qquad \text{Equation 3.1}$$

Where

V = volume (cm^3)

π = pi

r = radius (cm)

h = height (cm)

Growth height is 30cm, thus the soil growth volume is:

$$V = \pi r^2 h$$
$$V = \pi(5.2)^2(30)$$
$$V = 2548.46\text{cm}^3$$

The soil growth volume for all plants per silo is standardised at 2548.46cm^3. This value is used throughout and is equivalent to the multiple plants per silo study (MPPSs) and the soil control. The MPPSs soil control volume must integrate the soil growth volume of the IPPSs and adapt it to represent a culmination of four plants. Due to the voids created to allow for efficient plant growth, the volume is adjusted; this is to accommodate the area lost by the void. The resulting calculated adaptations are as follows:

The silo volume at void:

$$V = \pi r^2 h$$
$$V = \pi(7.65)^2(10)$$
$$V = 1838.54\text{cm}^3$$

Where

r = radius of silo (cm) = 7.65cm (inner radius of Ø160mm silo).

h = height of void (cm) = 10cm (chosen for freedom of plant growth).

The volume of void:

$$\frac{2}{3}V = 1838.54\text{cm}^3$$
$$V = 1225.69\text{cm}^3$$

Where

V = the volume represented by two-thirds of the area if the void expanded through the entire width of the silo (cm^3). This value takes into account the shape of the void and the area that it intrudes. The value, 1225.69cm^3, delineates the area that is not available to the plant roots for growth.

Volume beyond the void, separated from plant by a water permeable fabric:

$$V = 1838.54 - 1225.69$$
$$V = 612.85\text{cm}^3$$

Where

V = volume adjacent to the void area separated by the water permeable fabric, this volume is accessible for plant growth (cm^3).

Additional volume required to be representative of the initial 2548.46cm^3 set out by IPPSs:

$$x + 612.85\text{cm}^3 = 2548.46\text{cm}^3$$
$$x = 1935.61\text{cm}^3$$

Where

x = the volume required to be representative of the desired volume constant for plant growth (cm^3).

Height of required volume above void:

$$V = \pi r^2 h$$
$$1935.61 = \pi(7.65)^2 h$$
$$h = 10.53\text{cm}$$
$$h \approx 11\text{cm}$$

Where

V = volume required to meet the representative growth medium (cm^3).

r = radius of the silo (cm).

h = height of the area of the void to represent 1935.61cm^3 (cm).

The total growing area per plant:

$$h = h(void) + h(hav)$$
$$h = 10\text{cm} + \pm 11\text{cm}$$
$$h \approx 20\text{cm}$$

Where

h = cumulative height of void area and area above void (cm).

$h(void)$ = height of void area (cm).

$h(hav)$ = height of area above void (cm).

In estimating a representative soil volume for the MPPSs control, the cumulative height required of four plant species is taken:

$$h \approx 20\text{cm x } 4$$
$$h \approx 80\text{cm}$$

The height of the MPPSs is used to estimate the height of its soil control. As a result of pollutant removal effected predominantly by the distance that pollutants are transported through a medium rather than volume.

3.2.1.4 Drainage layers

Paired drainage layers were added below the soil medium comprising of coarse sand and gravel, the layers covered the drainage pipe. The aim of the drainage layers was to prevent sedimentation within the slits of the drainage pipe, preventing clogging of the effluent runoff. The thickness of the drainage layers are comparable with similar studies (Bratieres et al. 2008; Read et al. 2008; Milandri et al. 2012). The layer depths are illustrated by Figure 3.8 and represented in Table 3.1 below:

Table 3.1: Drainage layer thickness.

Material	Size (mm)	Layer thickness (mm)	
		Individual plant per silo	Multiple plants per silo
Sand	0.6 - 2	50	100
Gravel	7 - 18	100	120

3.2.1.5 Soil used as growth medium

(As illustrated in Appendix B, Table B.1)

The soil used as the growth medium for this study was excavated from the Overberg region in the Western Cape (GPS co-ordinates: 34°20'15.82"S, 20°20'33.85"E), the soil was selected to reflect the natural conditions for indigenous plant root growth and pollutant adsorption. The plants are naturally accustomed to the soil enabling plant familiarity to the soil further alleviates stresses during plant extraction and transplantation, establishing ideal growing conditions. All visible organic matter was removed from the soil, prior to drying, and weighed in preparation of a sieve analysis. Soil was collected by hand with 0.229m^3 and 0.152m^3 required for 90 Ø110mm- and 15 Ø160mm- growth silos respectively. Both dry and wet sieve analyses were conducted for the classification of the soil medium, executed at the Geotechnical and Transport Engineering laboratory at Stellenbosch University. A soil sample was taken from the collected media, consisting of 23g, 1320g and 3967g for clay, silt and sand respectively. The sieve analysis classified the soil, by implementing the USDA classification system, as Sandy Loam. This texture allowed the diffusion of water through the medium, establishing potential for remediation analysis.

3.2.1.6 Root length measurement

Each individual species' root length growth was examined after conclusion of pollutant extraction analyses. Plants were removed from their respective growth silos, where after soil and organic matter was cleaned from the roots structure. The root length values measured during the initial transplantation process was subtracted from the total root length, taken from the start of the stem/taproot to the tip of the longest root. An association between the root length growth value and the pollutant removal efficiencies was evaluated to indicate interrelationships. The longer and denser the plant roots, the greater remediation the plant displays, promoting the importance of plant physiology in phytoremediation technologies, with rhizosphere processes crucial in pollutant extraction (Read et al. 2008; Read et al 2010).

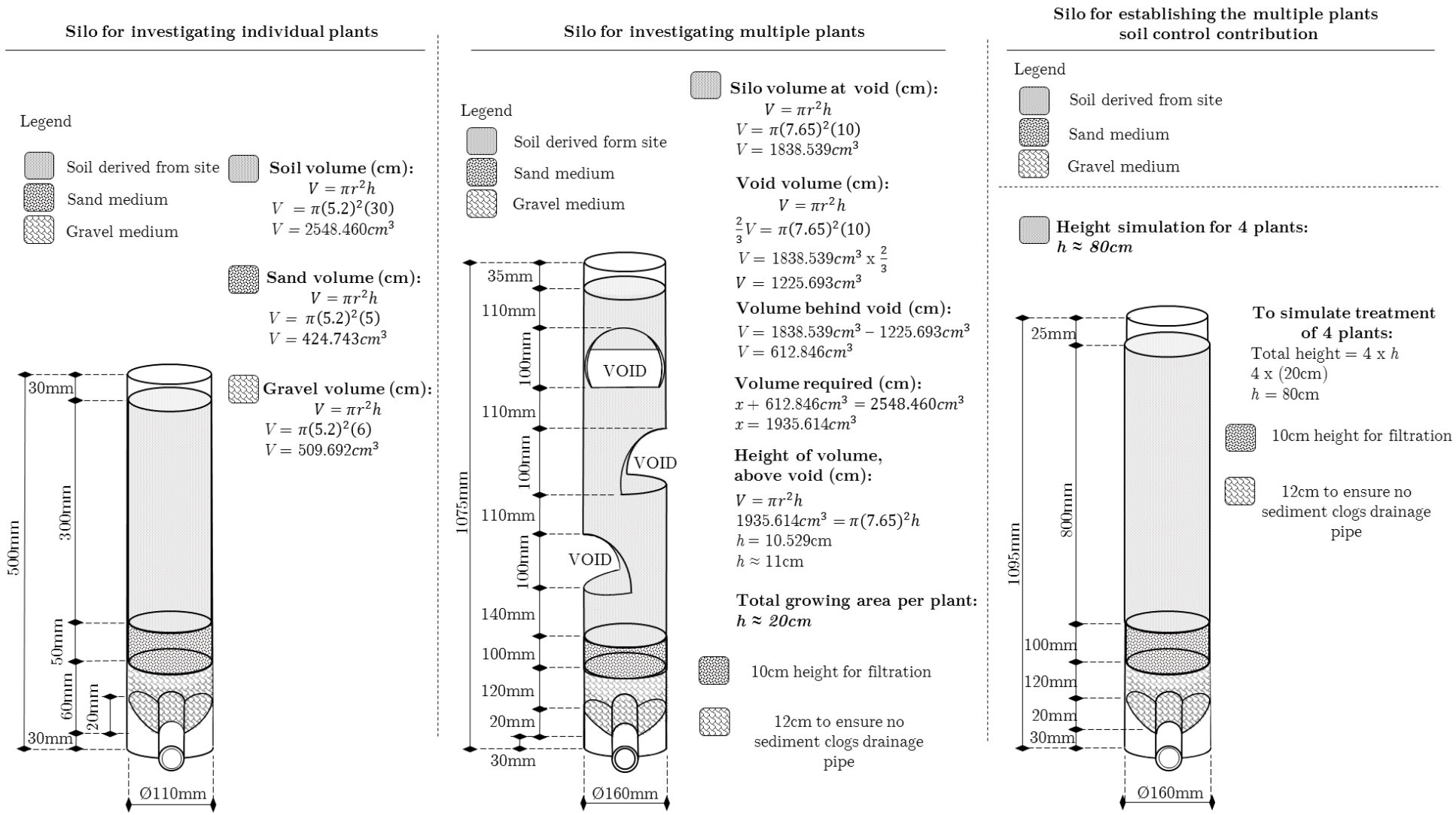

Figure 3.8: Calculation of silo dimensions for soil medium required.

3.2.1.7 Irrigation

An automated irrigation system was installed to ensure a consistent irrigation regime, frequency set for irrigation every 72 hours. The system was fitted with three submersible pumps, one for each of the three treatments, within their respective water storage containers. The capacities of the containers were 45 L with each fitted with an external clear pipe marked to indicate the volume of the solution within.

The municipal control container was fitted to a municipal tap to refill the water volume as the submersible pump transported solution to the system. The capacity within the container was controlled by a domestic toilet flow inlet control valve connected to a float ball, Figure 3.9a below, ensuring a constant water level.

Each submersible pump transferred the water from their respective storage containers using 15mm irrigation pipes attached to 35 treatment silos via drippers, each pipe was fitted with an *Emjay®* filter, figure 3.9b below, to extract any material that may impede the flow.

Figure 3.9a (left) and 3.9b (right): Figure 3.9a illustrates the flow inlet control and submersible pump. Figure 3.9b depicts the Emjay® filters incorporated in the irrigation line.

Added storage tanks were constructed for the herbicide and fertiliser containers, Figure 3.10a below. Two 70 L storage tanks were included in the study to increase the mixed herbicide and fertiliser solution capacities. The tanks were placed above the experimental setup on scaffolding to allow transport of fluid to the 45 L containers below, containing the submersible pumps, by gravitational flow. The containers were fitted with an external clear pipe marked at 3 L intervals, indicating the level of the solution within. The solution was transported to the submersible pump containers by 15mm irrigation lines, controlled by internal valves, shutting when there is a need to inhibit the flow. The capacity of the submersible pump containers were controlled by attaching the irrigation inflow, from the storage tanks above, to a domestic toilet flow inlet control valve. The valve was connected to a float ball. This ensured a consistent volume of solution for irrigation into the study silos.

Figure 3.10a (left) and 3.10b (right): Figure 3.10a added influent storage for fertiliser and herbicide. Figure 3.10b illustrates the 15mm pipes connecting the added storage to the influent containers.

Different drippers were used for the different silo sizes, 870 mL/h and 2070 mL/h for the smaller IPPS and the larger MPPS respectively. The treatments consisted of municipal tap water (as the control), a fertiliser solution and a herbicide solution. These were mixed prior to each treatment cycle. Each container housed an additional submersible pump, to prevent stagnation and ensure complete dispersion of nutrients and chemicals within.

After transplantation, the plants received municipal tap water from September 2017 – March 2018, this period allowed the plants to acclimatize to their new growing conditions. This process was mitigated by establishing practically similar environmental growing conditions, with the soil excavated from the field site. The duration of the acclimatisation period granted the species sufficient time to develop into stronger more mature plants, spreading their roots throughout the silos and, if any adverse impacts resulting from plant transplantation and translocation were experienced, they recuperated.

3.2.1.8 Lighting

An indoor laboratory phytoremediation system is prone to have irregular natural light for uniform plant growth, thus artificial lighting was provided to produce a uniform distribution. The lighting was distributed by fluorescent tubes via *Osram® Biolux™* lamps due to their wavelength distribution comparable with sunlight (Osram 2018). Eight 58W Biolux tubes were mounted throughout the system, placed at specific locations to ensure uniform light distribution.

The fluorescent lights were controlled by a mechanical timer, switching the lights on and off according to a programmable schedule. The timer was programmed to display light between 05h30 and 20h00, to reflect natural growing conditions.

3.3 Plants for phytoremediation

Plant species vary with regard to their pollutant removal abilities, with the most effective plant species characterised by long roots, deep root depth, and heavy root mass (Read et al. 2010). The introduction of certain plant species for phytoextraction may however pose a set of alien invasive problems, hence it is necessary to alternatively investigate the removal efficiencies of plant species indigenous to contaminated areas (Schachtschneider, Muasya & Somerset 2010; Leguizamo, Gómez & Sarmiento 2017). Different phytotechnologies utilise different plant properties and typically implement different plant species for each scenario. Properties that have been accepted as advantageous to phytoremediation are: fast growing, high biomass, competitive and high tolerance to pollution (Pilon-Smits 2005).

The pollutant removal efficiency of indigenous plant species and alien invasive plant species were investigated and compared. With the establishment of an indigenous plant community displaying non-invasive properties, capable of remediating pollutants either matching or superior to the more invasive plant species, the community contributes to conservation by increasing the natural biodiversity and heterogeneity of an ecoregion. The implementation of these species as phytoextractors, rather than their invasive counterparts, will benefit the biodiversity conservation initiatives. The properties will assist in determining which plants to incorporate into the system. The process of species consideration for this study was according to each attribute's contribution as listed from most important to least important:

- Previous literature identifying plants used as phytoremediators.
- Personal communication (Carden 2016; Lynch 2016; Cowen 2017; Curtis 2017; February 2017; Groenewald 2017; Jacobs 2017; Milandri 2017; Poulsen 2017; Van Biljon 2017).
- Plant properties.
- Invasiveness/Indigenous species.
- Tolerance to pollution.
- Availability.

3.3.1 Indigenous wetland species from Renosterveld

The laboratory experiment made use of 14 indigenous wetland plant species, based on plant properties (root length and structure), conservation status, visual proliferation, availability, invasive properties, and potential to tolerate increased moisture and conditions of drought. The experimental layout of the different plant species is illustrated in Figure 3.11 at the end of Section 3.3.3. The selected plants, Table 3.2 below, consist of wetland and dryland species, creating opportunity to establish a community along a river bank gradient. This dryland to wetland variability increases the hardiness of the community during seasonal fluctuations, where intermittent conditions of drought and saturation exist. The species selected represent genera that are capable of rapidly maturing during the experimental timeframe, providing an accurate representation of each species' capacity for nutrient removal.

Table 3.2: Indigenous species.

Species	Common name	[3]South African Distribution
[4]*Cynodon dactylon*	Scutch Grass	EC, FS, GP, KZN, LP, MP, NW, NC and WC
Cyperus textilis	Mat Sedge	EC, KZN and WC
Phragmites australis	Fluitjiesriet	EC, FS, GP, KZN, LP, MP, NW, NC and WC
Typha capensis	Bulrush	EC, FS, GP, KZN, LP, MP, NW, NC and WC
Juncus effusus	Common Rush	EC, FS, KZN and WC
Carpobrotus edulis	Sour Fig	EC, FS, GP, KZN, LP, MP, NW and WC
Arctotis acaulis	Renoster Marigold	EC, NC and WC
Zantedeschia aethiopica	Arum-Lily	EC, KZN, MP, NC and WC
Aristea capitata	Blue Sceptre	WC
Juncus lomatophyllus	Leafy Juncus	EC, GP, KZN, LP, MP and WC
Bolboschoenus maritimus	Alkali Bulrush	EC, GP, NC and WC
Isolepsis prolifera	Vleigras	EC, KZN and WC
Juncus kraussii	Dune Slack Rush	EC, FS, KZN and WC
Eleocharis limosa	Schrad	EC, FS, GP, KZN, LP, MP, NW, NC and WC

All selected plant species are wetland plants found I wetlands that run through many vegetation types in Africa, they are indigenous to South Africa and can be readily found in Renosterveld ecoregions and distributed throughout the country.

3.3.1.1 Cynodon dactylon

Cynodon dactylon is globally considered as an effective option for the *in situ* decontamination of pollutants (Leguizamo, Gómez & Sarmiento 2017). The species has further displayed resistance to glyphosate (Cerdeira & Duke 2006). For the phytoremediation of crude oil-contaminated soil and fertiliser, the treatment incorporating *Cynodon dactylon* presented the greatest degradation compared to treatments without (White et al. 2006). The grass species was also found to be efficient in the phytoremediation of heavy metals from stream ecosystems, mine tailings and contaminated soils, where the mean total heavy metal concentrations were found in the order; roots > leaves > stems (Shu et al. 2002; Soleimani et al. 2009; Sekabiera et al. 2011; Leguizamo, Gómez & Sarmiento 2017). Although *Cynodon dactylon* has continuously been proven to be effective in soil and water pollutant removal, there are risks associated with the species inclusion. Proposed South African legislation further seeks to convert *Cynodon dactylon* to a category II species, meaning that it can only be propagated, owned, transported or planted with a permit (ISSA 2018). The grass may become invasive as a result of its ability to infiltrate non-vegetated areas (Fourie 2010). This attribute also contributes to its efficacy in phytoremediation systems.

[3] EC = Eastern Cape, FS = Free State, GP = Gauteng, KZN = KwaZulu-Natal, LP = Limpopo, MP = Mpumalanga, NW = North West, NC = Northern Cape and WC = Western Cape.

[4] Plant species in grey shading are selected for the multiple plant per silo analyses, with pollutant removal efficacies compared with three IAP species and Palmiet.

3.3.1.2 Phragmites australis

The establishment of *Phragmites australis* beds has been identified as an effective mechanism for the removal of metals and NO_3^- from landfill leachate, groundwater and mine tailings in constructed wetlands (Peverly & Wang 1995; Terry & Banuelos 2000; Lin et al. 2002; Shu et al. 2002). Milandri et al. (2012), validated the use of *Phragmites australis* in SuDS biofiltration treatment trains, as a result of its PO_4^{3-} and NH_3 percent removal. An additional study presented the reduction in pesticides of two constructed wetlands, from non-point source agricultural runoff, to values regarded as non-toxic for aquatic life two, the wetlands were covered with vegetation including *Phragmites australis* (Blankenberg, Braskerud & Haarstad 2006). The plant species is an effective phytostabilizer during revegetation of waterlogged mine tailings and low nutrient environments (Deng, Ye & Wong 2004). Nakamura & Shimatani (1997), indicate that due to the large size of the reed community (in terms of covering %), displays large potential for water purification and evaporation. Some concerns regarding the reed is its relatively quick encroachment in streams and wetlands, when exposed to high nutrient loads (South Africa 1996a; Schachtschneider, Muasya & Somerset 2010; Milandri et al. 2012).

3.3.1.3 Typha capensis

Typha capensis has been observed to accumulate heavy metals in constructed wetlands (Deng, Ye & Wong 2004). According to Kotzee (2010), its wide distribution may be attributed to the plant's ability to survive during extreme weather conditions, out-competing other species. The plant favours intensive agricultural pollution, influencing the nutrient load of wetlands (Khalid 2014). Similar to *Phragmites,* in high nutrient load conditions the plant encroaches streams and wetlands at a relatively quick rate (Milandri et al. 2012).

3.3.1.4 Juncus effusus

Juncus effusus exhibits effective phytoremediation of heavy metal concentrations (Deng, Ye & Wong 2004). Aggressive growth is observed in heavily saturated soils, but can withstand periods of drought (Kotzee 2010). Leguizamo, Gómez & Sarmiento (2017), reported the species to portray non-invasive behaviour when planted for the phytoremediation of heavy metals in constructed wetlands. In contrast to this observation, some researchers still believe *Juncus effusus* pose an alien invasive threat when introduced into areas where it does not naturally occur, detrimental to fish when a large aquatic area is covered (South Africa 1996a; Schachtschneider, Muasya & Somerset 2010).

3.3.1.5 Carpobrotus edulis

Carpobrotus edulis has been found to be effective in the removal of nutrients in stormwater biofiltration systems, removing a high proportion of pollutants (Milandri et al. 2012).

3.3.1.6 Zantedeschia aethiopica

Zantedeschia aethiopica is a naturally occurring plant found throughout the Western Cape. Consistently accompanied by *Typha capensis,* the plant has been proven to efficiently

remove nutrients from stormwater pollution (Kotzee 2010; Milandri et al. 2012; Khalid 2014).

3.3.1.7 Juncus kraussii

Juncus kraussii produces new culms throughout the year, requiring supplemental nutrients for consumption (Congdon & McComb 1980). The plant species has the ability to extract a variety of nutrients. The plants usually form dense colonies in wetlands (Kotzee 2010).

The selected indigenous plants for this study that weren't motivated through literature have not previously been studied in terms of their phytoremediatory capabilities. These species are included on account of their plant properties, personal communication with conservationists, whether they are indigenous to the contaminated area and availability. Although some concerns arose regarding the use of certain species when exposed to high levels of nutrients, these traits may subsequently improve the species; phytoremediatory efficacy. All 14 species could potentially be used for the phytoremediation of agricultural pollutants, thus included within this preliminary experiment.

3.3.2 Alien invasive and Palmiet assemblage

Three IAP species and Palmiet were selected with regard to their current use in constructed wetlands, wastewater treatment and SuDS biofiltration treatment trains. These species, Table 3.3, have proven excellent remediators of polluted water, and thus are commonly used internationally (Schachtschneider, Muasya & Somerset 2010; Milandri et al. 2012).

Table 3.3: Invasive alien plant species and Palmiet.

Species	Common name	Invasive status (NEM:BA)
Pennisetum clandestinum	Kikuyu	Category 1b
Arundo donax	Giant reed	Category 1b
Canna indica	Canna	Category 1b
[5]*Prionium serratum*	Palmiet	Non-invasive. Included on account of aggressive growing properties (Groenewald 2017, Pers com; Curtis 2017, Pers com).

3.3.2.1 Pennisetum clandestinum

Pennisetum clandestinum is a popular plant for the phytoremediation of landfill leachate, mining wastes and heavy metal contaminated water and soils (Bech et al. 2002; Söğüt et al. 2005; Erdogan et al. 2008; Mukhopadhyay & Maiti 2010; Okem, Kulkarni & Van Staden 2015). Pant et al. (2004), also observed the successful use of the grass in the phytoremediation of N and P from manure and fertiliser pollutants. A variety of different studies further observed its applicability in the degradation of pesticides (Singh et al. 2004; Wang et al. 2012; Ibrahim et al. 2013; Qu et al. 2017). In a study examining the performance

[5] *Prionium serratum* is not classified as an alien invasive species in South Africa, however included in this study as one, due to the immense size of the plant, its proliferation properties and common use in constructed wetlands (Groenewald 2017, Pers com).

of plant species in removing nutrients from stormwater in biofiltration systems, *Pennisetum* was found to be the most successful with 90% removal of PO_4^{3-}, NH_3 and NO_3^- (Milandri et al. 2012). *Pennisetum* can however pose a major threat to terrestrial and aquatic ecosystems. The grass is an aggressive species, proliferating in areas where natural competition is absent or if the grass is not sufficiently controlled (O'Farrell, Donaldson & Hoffman 2010; NZBS 2012).

3.3.2.2 Arundo donax

Arundo donax has been proven to display great tolerance to heavy metals from contaminated soil and water, with potential phytoextraction of pollutants (Deng, Ye & Wong 2004; Han & Hu 2005; Mirza et al. 2010; Kausar et al. 2012; Bonanno 2013; Sabeen et al. 2013; Barbosa et al. 2015). Schröder (2007), investigated the potential use of *Arundo donax* for the phytoremediation of pesticides and found the plant's metabolism to promote outstanding candidacy. The species is characterised as an alien invasive species and prioritised in numerous plant control projects to manage the ecology of wetlands (Khalid 2014). Its introduction may pose a variety of problems to an ecosystem (Schachtschneider, Muasya & Somerset 2010).

3.3.2.3 Canna indica

Canna indica has predominantly been used in the phytoremediation of heavy metal contaminated industrial sludge. Due to its low maintenance cost the plant is suitable for the extraction of most metals (Cheng et al. 2002; Bose et al. 2008; Subhashini & Swamy 2014). The plant's exposure to pesticides demonstrates an effective mechanism in soil and water pollutant degradation (Cheng et al. 2007; Xiao, Cheng & Wu 2010). Yavari, Malakahmad & Sapari (2015), presented the plant as a viable replacement for the treatment of oil spills, as current clean-up and recovery techniques are challenging and expensive. The plant has further been proven as an effective mechanism in the phytoremediation of metals from fertiliser application (Chou, Yeh & Lin 2006). Due to its hardiness, the plant is regarded as portraying invasive properties.

3.3.2.4 Prionium serratum

NOTE: In this study, P*rionium serratum* is listed with the IAP assemblage on account of its aggressive growing properties and concerns from conservationists (Groenewald 2017, Pers com), and not with regards to its 'alien status' or interactions within an ecosystem. Although included in the IAP assemblage, it is endemic to South Africa and of this author's opinion that *Prionium serratum* does certainly not present invasive properties to the freshwater systems of the Western Cape. In fact Palmiet wetlands are ecosystems that greatly reduce the erosive damage done by floodwater and the removal of these plants may result in streams becoming choked by sediment and banks eroded by unchecked floodwater. Thus, the ecosystem services of Palmiet may be invaluable in the sustainability of aquatic systems. The author acknowledges the fact that more research has to be conducted to establish the exact role of the plant species.

Prionium serratum is a super-dominant ecosystem engineer, although completely salt and shade intolerant (Sieben 2012; Rebelo et al. 2014). Recovery of the plant in degraded freshwater systems has been identified as a major role player in the recovery of ecological networks (Samways & Pryke 2016). In a study conducted by Rebelo (2018), Palmiet wetlands appeared to act as a sink for water cations, anions, dissolved silicon and nutrients. The plants portray the ability to tolerate inorganic and biological stressors (to an extent) and may be used in freshwater ecosystems in the presence of these stressors (Lacoul & Freedman 2006). Palmiet plants and their wetlands' water purification capabilities are affiliated with aquatic ecosystems illustrating pristine water quality (Rebelo et al. 2013). *Prionium serratum* restoration may offer crucial ecosystem services; including water purification and flood attenuation (Blignaut & Aronson 2008; Rebelo et al. 2014). The benefits of Palmiet wetlands include; the slowing force of floods, purification of water, habitat provision for biodiversity and sediment retention (Rebelo 2018).

3.3.3 Plant removal and collection

Five of the 18 plant species[6] were removed near the Overberg Renosterveld Conservation Trust research unit (Haarwegskloof), located on Rûens Silcrete Renosterveld, GPS coordinates 34°20'18.7"S, 20°19'34.1"E, in the Overberg. Four species[7] were removed from the Buffeljagsrivier area, GPS coordinates 34°04'17.7"S, 20°32'33.2"E, near the town of Swellendam in the Overberg. Four more species[8] were removed from the outskirts of Stellenbosch, GPS coordinates 33°52'53.3"S, 18°49'59.8"E, in different canals and catchments. Three plant species[9] were removed from the Berg River, GPS coordinates 33°52'58.9"S, 19°02'32.9", near the town of Franschoek. The remaining two species[10] were sourced from New Plant Nurseries situated in the Southern Cape. All plant species were carefully transported to the laboratory location, in the Waterlab of the Civil Engineering Department at Stellenbosch University and planted in September 2017 receiving tap water irrigation for six months - allowing time to mature and adjust to growing conditions. Thereafter, the plants received standardised contaminated water treatments. During the transplantation process, special care was taken to remove all visible foreign organic matter and soil, limiting external factors contributing to the phytoremediation process, ensuring equal conditions throughout the system (Jacobs 2017, Pers com). The soil collected from the relevant field site was inserted and circulated in a 50 L pan mixer at the Civil Engineering department at Stellenbosch University. The pan mixer ensured completely mixed soil conditions before transfer into the growth silos prior to transplantation. If trimming of the stem was necessary, it was done in a manner that wouldn't alter plant development.

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[6] *Pennisetum, Cyperus, Phragmites, Juncus effusus and Juncus kraussii*
[7] *Cynodon, Typha, Bolboschoenus and Eleocharis*
[8] *Arundo, Canna, Carpobrotus and Zantedeschia*
[9] *Prionium, Juncus lomatophyllus and Isolepsis*
[10] *Arctotis and Aristea*

Indigenous species

MPPS

Species name:	Common name:	Species name:	Common name:
Cynodon dactylon	Scutch Grass	*Zantedeschia aethiopica*	Arum-Lily
Cyperus textilis	Mat Sedge	*Aristea capitata*	Blue Sceptre
Phragmites australis	Fluitjiesriet	*Juncus lomatophyllus*	Leafy Juncus
Typha capensis	Bulrush	*Bolboschoenus maritimus*	Alkali Bulrush
Juncus effusus	Common Rush	*Isolepsis prolifera*	Vleigras
Carpobrotus edulis	Sour Fig	*Juncus kraussii*	Dune Slack Rush
Arctotis acaulis	Renoster Marigold	*Eleocharis limosa*	Schrad

Invasive alien plants (IAP)/SuDS and endemic Palmiet

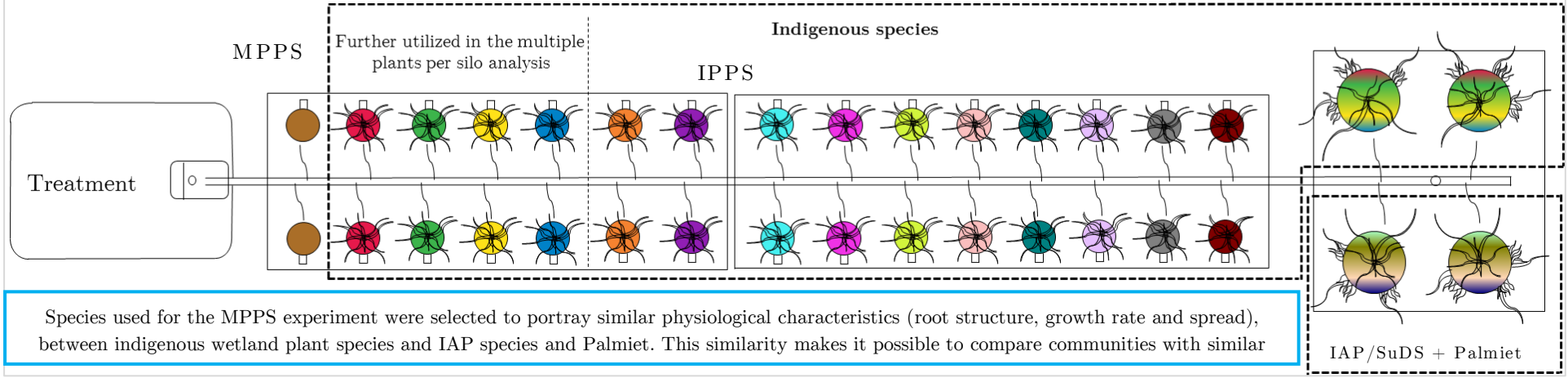
MPPS

Species name:	Common name:
Pennisetum clandestinum	Kikuyu
**Prionium serratum*	Palmiet
Arundo donax	Giant Reed
Canna indica	Canna
Soil control	

The multiple plant treatment encapsulates 4 plant species in a larger silo. The plants undergo the same treatment to establish whether the relationship between species alter their remediation potential.

*NOTE: Although *Prionium serratum* is included with the IAP assemblage, the plant is endemic to South Africa, included with the assemblage on accord of its aggressive growing properties and concern from conservationists (Groenewald, Pers com).

IPPS = Individual Plant Per Silo
MPPS = Multiple Plants Per Silo



Species used for the MPPS experiment were selected to portray similar physiological characteristics (root structure, growth rate and spread), between indigenous wetland plant species and IAP species and Palmiet. This similarity makes it possible to compare communities with similar

Figure 3.11: Species distribution within the laboratory phytoremediation system.

3.4 Fertiliser selection[11]

Initially the selected fertilisers were products regularly applied in the Overberg for the fertilisation of Canola, designed to resemble real world environmental conditions. The proposed products were subsequently excluded on the ground of inaccurate labelling, as exact known influent concentrations are vital to experimental feasibility and chemical reagents available were limited to experimental analyses. Analytical grade compounds with known concentrations were rather included as representatives of the DAFF recommended fertiliser applications (South Africa 2016).

3.4.1 Optimal fertiliser requirements for Canola production

Canola is a relatively new crop in South Africa. In 1992, only 400 tonnes canola seed was produced, compared with 1 690 375 tonnes in 2015 (South Africa 2016). Currently South Africa imports more than half of its oil every year, with the production of Canola lower than the demand. Canola is mainly grown in the Western Cape Province as a winter crop.

Canola is best suited for clay-loam soils and cannot tolerate waterlogged soils. The crop should be planted in April to early June to achieve highest yields, with significant yield reduction if planting is delayed post 15th June.

3.4.1.1 Application of N recommendation for the study area

The key element for the improvement of Canola yield is N, about 55 kg N is removed by the crop per ha to produce one ton of seed (South Africa 2016). High N fertilisation rates stimulate larger leaves, increased transpiration and moisture use. The Department of Agriculture, Forestry and Fisheries (DAFF) have compiled recommendations for Canola, Table 3.4 below, relating to the area of production.

Table 3.4: Nitrogen recommendation for Canola in the Southern Cape (adapted from South Africa 2016).

Rainfall (mm/annum)	Yield potential (1000kg/ha)	N application (kg/ha)
< 450	1.0	10 – 35
[12]450 - 500	1.5	40 – 45
> 525	2.0	50 - 55

The Overberg, more specifically Eastern Rûens Shale Renosterveld, represents the focus area of this study, which receives between 400 - 500 mm rainfall per annum (Curtis 2013). Therefore the N application per season for this study is regarded as 40 kg/ha.

[11] The values displayed in Section 3.4 and 3.5 are limited to three decimal places for comprehension ease. For calculation accuracy, the original values (not rounded up) are used. This causes some decimal discrepancies with the values displayed.

[12] The selected Nitrogen concentration applied.

3.4.1.2 Application of P recommendation for the study area

The P content of the topsoil is determined by the Citric acid ($C_6H_8O_7$) concentration (South Africa 2016). The optimal P content for the production of Canola should be 20 mg/kg, as shown in Table 3.5 below (South Africa 2016). The soil citric acid content of the experiment was analysed by Bemlab in Somerset West, indicating a recommended application of 15 kg/ha P to achieve optimal P content.

Table 3.5: Phosphorous recommendation for Canola (adapted from South Africa 2016).

Soil citric acid content (mg/kg)	Recommendation (kg/ha)
10	30
20	24
30	18
[13]40	15
50+	0

3.4.2 Calculating initial N and P for application

The recommended guidelines reported in Section 3.4.1, represent required applications of 40 kg/ha N and 15 kg/ha P. The amount required must take into account the soil area of the phytoremediation system, as follows;

$$A = \pi r^2 \text{ x } Silos_{total} \qquad \text{Equation 3.2}$$

Where

A	=	Total area of silos surfaces (m^3)
r	=	Radius of silo (m) = 0.055m
$Silos_{total}$	=	Total silos per study = 30 silos/5 silos

Soil area (m^2) of 30 silos at Ø110mm:

$$Ø110mm\ Area = \pi(0.055)^2 \text{ x } 30$$
$$= 0.285\text{m}^2$$

Soil area (m^2) of 5 silos at Ø160mm:

$$Ø160mm\ Area = \pi(0.08)^2 \text{ x } 5$$
$$= 0.101\text{m}^2$$

Volume solution (H_2O + Fertiliser/Herbicide) irrigated to each silo:

Ø110mm silo at 870 ml/h = 0.653L over 45minutes

$$30\ Silos = 0.6525L \text{ x } 30$$
$$30\ Silos = 19.575\ \text{L}$$

[13] The selected Phosphorous concentration applied.

Ø160mm silo at 2070 ml/h = 1.553L over 45minutes

$$5\ Silos = 1.5525L \text{ x } 5$$

$$5\ Silos = 7.763 \text{ L}$$

Total volume irrigated every three days:

$$19.575 + 7.763 = 27.338 \text{ L/3days}$$

3.4.2.1 Inflow N applied at 40kg/ha

Length of Canola growing season is taken as 365 days, with irrigation scheduled every three days.

A third of the growing season:

$$365days \div 3 = 121.667$$

$$Mass\ \text{N}/m^2\ per\ planting\ season = \frac{40kg/1ha}{121.67}$$

$$= 0.033 \text{ g/m}^2$$

$$\text{Multiply by } 100 = 3.288 \text{ g/m}^2$$

The mass N/m^2 is multiplied by 100 for practicality. Equipment available for this study did not meet the requirements to accurately weigh 0.033 g/m^2. A hundred-fold increase in mass allows the measurement for N. This conversion is further applied in calculating P for consistency between the ratios required of the nutrients.

Mass required every three days for individual silo sizes:

$$Mass \text{ N } for\ Ø110mm\ silos = 3.288 \text{ x } 0.285(\text{Soil area of 30 silos})$$

$$= 0.937 \text{ g/3days}$$

$$Mass \text{ N } for\ Ø160mm\ silos = 3.288 \text{ x } 0.101(\text{Soil area of 5 silos})$$

$$= 0.331 \text{ g/3days}$$

The most popular fertiliser applied to Canola is *CanolaFeed™* a product from *Nulandis®* (Raubenheimer 2018, Pers com). The *CanolaFeed™* product is a fertiliser mixture designed to increase the yield of Canola crops, through the provision of trace element nutrition (Nulandis 2018). The N content in *CanolaFeed™* as an active ingredient is 91g N/kg (Nulandis 2018).

Total mass N required from the fertiliser:

Mass of fertiliser to provide required N from 91g N/kg fertiliser for Ø110mm silos:

$$\frac{0.937g}{xkg} = \frac{91g}{1kg}$$

$$x = 0.01\text{kg Fertiliser required}$$

Mass of fertiliser to provide required N from 91g N/kg fertiliser for Ø160mm silos:

$$\frac{0.331g}{xkg} = \frac{91g}{1kg}$$

$$x = 0.004\text{kg Fertiliser required}$$

Total mass of fertiliser to provide required N for irrigation:

$$\textit{Mass } \text{N } \textit{Ø110mm silos} + \textit{Mass } \text{N } \textit{Ø160mm silos} = \textit{Total mass } \text{N } \textit{required}$$
$$0.01kg + 0.004kg = 0.014\text{kg}$$

Mass of fertiliser required for 0.014kg N:

$$CanolaFeed^{TM} = \frac{91g}{1kg} = \frac{xg}{0.014kg}$$
$$x = 1.268\text{g } CanolaFeed^{TM}$$

N concentration applied to the system:

$$\text{N } conc. = \frac{1267.792mg}{27.338L}$$
$$= 46.376 \text{ mg/L}$$

3.4.2.2 Inflow P applied at 15kg/ha

The duration of the Canola growing season is taken as 365 days, with irrigation scheduled every three days. After a soil citric acid analysis conducted, according to the DAFF guidelines for Canola, a 15kg/ha application is recommended.

A third of the growing season:

$$365days \div 3 = 121.667$$

$$\textit{Mass } \text{P}/m^2 \textit{ per planting season} = \frac{15kg/1ha}{121.667}$$
$$= 0.012 \text{ g/m}^2$$
$$\text{Multiply by } 100 = 1.233 \text{ g/m}^2$$

As with the case in N, the required mass P/m^2 is extremely small, this causes difficulty during weighing of the required mass. Any mistake at such a small mass would result in exponential inaccuracies in the ratio of N and P. Thus, the 0.012 g/m^2 is multiplied by 100 for practicality.

Mass required every three days for individual silo sizes:

$$\textit{Mass } \text{P } \textit{for 30, Ø110mm silos} = 1.233 \text{ x } 0.285(\text{Soil area of 30 silos})$$
$$= 0.351 \text{ g/3days}$$

$$\textit{Mass } \text{P } \textit{for 5, Ø160mm silos} = 1.233 \text{ x } 0.101(\text{Soil area of 5 silos})$$
$$= 0.124 \text{ g/3days}$$

P is more often than not applied separately, to decrease climatic stresses (Komen 2018, Pers com; Swart 2018, Pers com). There are a number of different techniques to apply the

fertiliser, with "bandplaas" being most effective (Swanepoel 2018, Pers com; Swart 2018, Pers com). Bandplaas places the fertiliser at the stem of the plant to increase nutrient contact with plant roots, rather strenuous the fertiliser directly influences the crop (Barnard 2018, Pers com).

Farmers and fertiliser representatives alike, differ with regard to best practices for P application (Nolte 2017, Pers com). Barnard (Pers com, 2018), advises the use of a fertiliser with 90g P/kg content. Although an exact 90g P/kg fertiliser could not be sourced, a substitute was identified as *Garden Phosphate™* a product of *ProtekSA®* registered by *Arysta Lifescience®*. The fertiliser contains only P as an active ingredient as 83g/kg, a similar concentration as advised.

Total mass P required from the fertiliser:

Mass of fertiliser required to provide required P from 83g P/kg fertiliser for 30, Ø110mm silos:

$$\frac{0.351g}{xkg} = \frac{83g}{1kg}$$

$$x = 0.004kg \text{ Fertiliser required}$$

Mass of fertiliser required to provide required P from 83g P/kg fertiliser for 5, Ø160mm silos:

$$\frac{0.124g}{xkg} = \frac{83g}{1kg}$$

$$x = 0.001kg \text{ Fertiliser required}$$

Total mass of fertiliser to provide required P for irrigation:

$$Mass \text{ P } Ø110mm\ silos + Mass \text{ P } Ø160mm\ silos = Total\ mass \text{ P } required$$

$$0.004kg + 0.001\ kg = 0.006kg$$

Mass of fertiliser required for 0.005kg P:

$$\frac{83g}{1kg} = \frac{xg}{0.006kg}$$

$$x = 0.475\text{g } Garden\ Phosphate^{TM}$$

P concentration applied to the system:

$$\text{P } conc. = \frac{475.422mg}{27.338L}$$

$$= 17.391 \text{ mg/L}$$

3.4.3 Shortfall of commercial products

Prior to experimentation, samples of the two fertilisers were analysed to confirm the concentrations given by their labels. After analyses, the results of both products returned concentrations above the measurable limits of the laboratory reagents for this study, due to funding constraints the sampling of more samples was not feasible. It was established that

the products contained much higher concentrations of N and P than initially conveyed. For this study the commercial products were not used as knowledge of the exact concentrations were vital to compare effluent from influent.

It was decided to exclude the two commercially available products, as discrepancies regarding the initial concentrations were not acceptable. The fertiliser products were replaced with laboratory grade chemicals, of which there was zero uncertainty regarding the makeup.

3.4.4 Calculation of Laboratory/Analytical compounds

The N and P concentrations recommended by DAFF, i.e. 46.376 mg/L N and 17.391 mg/L P, were applied. Three chemical grade compounds were used to recreate the aforementioned fertilisers. The exact concentrations as stipulated in 3.4.2.1 and 3.4.2.2 were used. In commercial fertilisers NH_4 and NO_3^- is generally the source of N with PO_4^{3-} the source of P. Although analytical grade chemicals are used, the elemental N:P ratio is consistent, a reflection of the N:P ratio of agricultural fertilisers. Table 3.6 illustrates the nutrients and their corresponding compounds:

Table 3.6: Concentration of analytical compounds, derived from the DAFF recommendations.

Nutrient	DAFF recommendation		Product
	kg/ha	mg/L	
N	40	46.376	NH_4^+ - N NO_3^- - N
P	15	17.391	PO_4^{3-} - P

N and P are represented by analytical NH_4Cl + KNO_3 and K_2HPO_4 respectively. The concentration of the analytical grade compound is calculated from the initial 46.376 mg/L and 17.391 mg/L of N and P respectively. N is sourced from NH_4^+ and NO_3^-, as is the case in fertilisers worldwide, with P sourced from PO_4^{3-}. The combination of NH_4^+ and NO_3^- produces the elemental N concentration, evident from Table 3.7 below.

Table 3.7: Synthetic fertiliser composition.

DAFF recommendation			Representative analytical substances		
Nutrient	Product	Conc. (mg/L)	Representative substance	Formula	Representative substance conc. (mg/L)
N	NH_4^+ - N	37.096	Ammonium chloride	NH_4Cl	141.659
	NO_3^- - N	9.274	Potassium nitrate	KNO_3	66.939
P	PO_4^{3-} - P	17.39	Di-Potassium-H-phosphate	K_2HPO_4	97.788

The concentration of the analytical grade compounds represent the initial concentrations calculated from the DAFF recommendations for Canola production. Although the ratios of N:P between elemental and compound formulations may differ, due to other chemicals also contributing to the compound's molar mass i.e. Chloride in NH_4Cl, the elemental N:P ratios are represented. Therefore, the influent concentrations, Table 3.7 above, for this study are 141.659 mg/L, 66.939 mg/L and 97.788 mg/L for NH_4Cl, KNO_3 and K_2HPO_4 respectively.

3.5 Herbicide selection

A glyphosate-based herbicide was selected for this study on a basis of relevance, consistently used by the agricultural sector. The most popular pesticide globally is *RoundUp®* from *Monsanto Ltd*, active ingredient glyphosate (Pieterse 2017, Pers com). The Canola farming community of the Overberg applies *Springbok 360 SL™*, a product of *Arysta LifeScience®*, before the planting of crops post rain (Swart 2018, Pers com; Groenewald 2017, Pers com; Bothma 2016, Pers com; De Kock 2017 Pers com).

Due to its similarities with *RoundUp®*, the product has become the most widely used pesticide in the area (Nolte 2017, Pers com). The popularity of the herbicide supports its inclusion in this study.

3.5.1 Determining initial glyphosate concentration

Two dosage strengths were selected to analyse the phytoremediation capabilities of selected plant species. The dosage concentrations were chosen to represent a nontoxic contamination and worst case scenario contamination, at 0.7 mg/L and 225 mg/L respectively.

3.5.1.1 Acute nontoxic contamination to aquatic ecosystems

Glyphosate threshold concentration for the protection of aquatic ecosystems, supplied by the Canadian water quality guidelines and the EPA, is 0.8 mg/L and 0.7 mg/L respectively (You, Kaljurand & Koropchak 2003; Struger et al. 2008; Tran et al. 2017). These values are the maximum concentrations for glyphosate at which aquatic ecosystem degradation does not occur.

For the acute nontoxic contamination experiment, a glyphosate concentration of 0.7 mg/L was irrigated onto the plants without municipal tap water dilution between treatments. The diluted strength of the solution confirmed zero to negligible damage to the plant, representing optimal root contaminant uptake.

3.5.1.2 Worst case scenario acute contamination

Both the information booklet supplied by *Arysta LifeScience™* for the proper use of *Springbok 360 SL™* and the current management practices of farmers in the area, were used to establish the concentration of the herbicide used in the field (Arysta 2011; Bothma 2016, Pers com; Swart 2018, Pers com).

The effect of different glyphosate solutions on indigenous plant species in general were given through personal communication with Environmental Practitioners, Table 3.8 below (Muir 2018, Pers com). These values are an adaptation of the recommended dosage strengths.

Table 3.8: Glyphosate effect on plants (Muir 2018, Pers com).

Glyphosate	
Dosage (% of product conc.)	Effect on plant
2	Specimen dies 2 days post treatment
0.5	Specimen dies 25 days post treatment
0.25	Specimen wilts but is sub-lethal
<0.25	Zero effect on specimen

The treatment concentrations are significantly higher than the recommended dosage strengths supplied by the product booklet and previous research. These discrepancies prompted an analysis of dosage strengths on indigenous plant species, with the goal of identifying a plausible worst-case scenario initial influent concentration for the phytoremediation experiment.

It was revealed that farmers' herbicide applications at extreme concentrations are a common practice, either through application error or lack of product dosage understanding (De Kock 2017, Pers com; Nolte 2017, Pers com). This results in the transport of severe herbicide pollutants into aquatic ecosystems (Bothma 2016, Pers com; De Kock 2017, Pers com; Swart 2018, Pers com). The following experiment was conducted to reveal possible human-error worst case influent concentrations.

3.5.2 Plant response to a range of dosage strengths

(As illustrated in Appendix C, Table C.1 and C.2)

Ten glyphosate concentrations were selected and applied to two indigenous plant species over a four week period. *Typha capensis* and *Cynodon dactylon* were selected to evaluate plant degradation from varying concentrations over time, Figure 3.12 below.

The two species were selected due to their physiological characteristics differing significantly. The lack of similarity regarding their physiology and appearance create broad toxin responses across plant species. This will aid in establishing plant responses between the wetland plant species that naturally occur within Renosterveld vegetation.

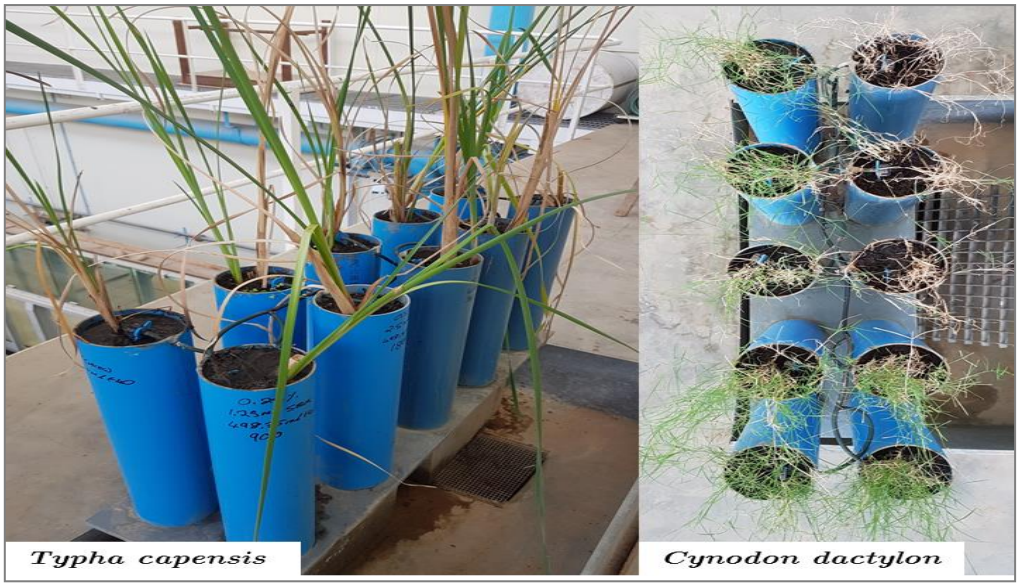


Figure 3.12a (left) and 3.12b (right): Figure 3.12a *Typha capensis* and Figure 3.12b *Cynodon dactylon* exposed to ten different glyphosate dosages.

The concentration range, as illustrated in Table 3.9 below, follows a dilution series method. The dosage concentrations were obtained by personal communication regarding human-error worst case scenario treatments. The initial corresponding dosages were transferred to each plant dissolved in 500 mL municipal water, the plants were irrigated at a rate of 653 mL/3days.

Table 3.9: Dilution series method after 30 days.

Springbok 360 SL™ (360 g/L Glyphosate)				
Mortality	Solution – Springbok™ %	Glyphosate Concentration mg/L (ppm)	H_2O Volume mL	Springbok™ Volume mL
YES	100	360 000	0	500
	5	18 000	475	25
	2	7 200	490	10
	1	3 600	495	5
	0.5	1 800	497.5	2.5
NO	0.25	900	498.75	1.25
	0.125	450	499.375	0.625
	[14]0.0625	225	499.6875	0.3125
	0.00625	22.5	499.96875	0.03125
	0.003125	11.25	499.984375	0.015625
	0.0015625	5.625	499.9921875	0.0078125

[14] The selected toxic glyphosate concentration to be applied.

The concentrations are selectively strong, reflecting extreme situations in the environment, at conditions where plant mortality rate is high, although not high enough as to wilt off and die immediately. From the dilution series experiment, plants exhibited survival at 900 mg/L and lower, with no immediate sign of degradation during the short scope of the analysis. However, with increased exposure this author expects that the plants would display signs of mortality and finally death.

For the worst-case scenario experiment, a constant glyphosate solution was irrigated on the plants with no municipal water dilution over an extended period. For this reason, a weaker concentration was selected for irrigation during this process, so as to avoid increased mortality with time. The selected dosage for the study, as illustrated in Table 3.9 above, and as a result of personal communication with environmental practitioners, pesticide agents and Canola farmers regarding human error applications, was 225 mg/L (De Kock 2017, Pers com; Groenewald 2017, Pers com; Muir 2018, Pers com; Swart 2018, Pers com).

3.6 Experimental procedure

The method of applying the standardised fertiliser and herbicide influent solutions (as discussed in the previous sections), sampling processes and analyses of parameters and methods are discussed in the following section:

3.6.1 Observation of extreme salinity

(As illustrated in Appendix D, Table D.1)

With the initial irrigation of municipal water, the system was observed to be strongly saline, due to the leaching of salts from the soil material. Considering the water used for irrigation was recycled, as to ensure minimal nutrient loss during this process, the salts within the soil leached into the water. The salts accumulated in the water containers, attaining a level which hinders plant access to soil water (Sheldon et al. 2004).

The desirable range for most established plants is 0.75 – 1.25 mS/cm, with the upper range capable of reducing some sensitive plants (Parida & Das 2005). An EC analysis was executed on the system's water, producing an EC measurement of 33.7 mS/cm, an extremely strong saline level, resulting in the plant wilting and certain death if allowed to persist. The system was therefore flushed every day over a period of 11 days to reach a suitable salinity levels, increasing the osmotic potential of the soil solution (Sheldon et al. 2004).

The salt source was identified as the soil collected in the Overberg. Natural processes guarantee that an acceptable saline range is established. The soil content is highly saline, however, the freshwater systems/rivers "flush" the salts, effectively diluting the level of salinity. The rivers are seen as continuous diluters of the salts. As rivers lose flow, the soil dries and the concentration of salt in the soil solution increases - decreasing the solution's osmotic potential (Sheldon et al. 2004). This experiment recycled the inflow prior to contamination, thus recycling the leached salts onto the plants.

3.6.2 Contamination treatment

After the initial six-month irrigation with municipal water, the pollutant treatments commenced. The irrigation regime, every three days, was based on the saturation and permeability of the growth silos. A dosage of 0.653 L/3days and 1.553 L/3days, for the IPPS and MPPS respectively, was regarded as the optimum volume and rate for irrigation.

The plants received treatment over a continuous 50-day period with the treatment dosages, Table 3.10 below, irrigated 20 days prior to the first round of sampling. The 20-day period allowed sufficient time to transport the excess uncontaminated municipal water that may linger within the silos, out of the system, ensuring negligible dilution of the pollutants.

Every ten days the influent solutions were drained and replaced with a fresh mixture of pollutants, this hindered the effect of pollutant degradation in the storage tanks.

Table 3.10: Influent treatment concentrations.

Pollutant	Parameter	Concentration (mg/L)
Fertiliser	Ammonia-N	37.096
	Nitrate-N	9.274
	Orthophosphate-P	17.39
Herbicide	Glyphosate	225
		0.7

Table 3.10, illustrates the standardised influent treatment concentrations of the pollutants. By draining and remixing the influent solutions, the dosage strengths remained constant.

3.6.3 Sampling process

Samples were collected on five occasions during the study. The first round of sampling was initiated on the 3rd of March 2018, examining the baseline nutrient concentrations; this determined the nutrient concentrations within the effluent prior to treatment. The baseline determination allowed for precise comparison between influent and effluent water. The second round of sampling occurred 20 days post initial treatment. Thereafter sampling was undertaken every ten days. The percentage removal by all specimens was compared as influent concentrations were premixed to standardised levels and baseline concentrations were known.

Treatment effluent water was collected by collection containers directly below the drainage pipes of each silo. Water samples were also collected from each influent storage container and each silo effluent collection container per species. Two plants per species received treatment, establishing experimental duplication and reducing outlier influence.

The effluent solutions were collected in 90 mL specimen containers, with twin plant species effluent solutions combined post effluent collection. The sampling process was undertaken at

06h00 as for measuring the instantaneous concentration, this is the moment where DO is at its lowest concentration in a 24 hour period (South Africa 1996d). The instantaneous measurement represents the moment where biological activity is reduced, delivering representative water quality conditions. The influent, baseline and effluent concentration data are all presented in Appendix E, Table E.1.

3.6.4 Analysis

In order to evaluate the efficacy of the experiment's pollutant removal, various water quality parameters were measured within the experimental time frame. These include pH, dissolved oxygen (DO), electrical conductivity (EC), ammonia (N-NH_3), nitrate (N-NO_3^-), orthophosphate/soluble reactive phosphorus (P-PO_4^{3-}/SRP) and glyphosate ($C_3H_8NO_5P$). The mg/L NH_3-N, mg/L NO_3-N and mg/L PO_4-P is measured to ascertain the N and P content within the effluent solution. The following section gives a brief summary of methods and equipment used for parameter analysis.

3.6.4.1 pH, DO and EC

The pH, DO and EC were calculated, utilizing the *Hach®* manufactured *HQ440d Benchtop Multi-Parameter Meter™.* The instrument is a handheld water-quality tool, incorporating parameter specific probes to instantaneously measure specific water quality parameters. The *IntelliCAL™ PHC281* probe calculated the pH, whereas the *IntelliCAL™ LDO101* probe measured the DO concentrations. Further, the *IntelliCAL™ CDC401* probe calculated the *in situ* EC concentrations. Calibration of the probes was done with the use of the *Hach®* buffer and standard solutions prepared at known concentrations for pH, DO and EC, as mentioned in Section 3.1.2.

3.6.4.2 Ammonia

The N-NH_3 concentrations were calculated colorimetrically utilizing the *Hach® DR3900 Benchtop Spectrophotometer™,* applying the *TNTplus™832* test kit. The test kit has a range of 2 to 47 mg/L NH_3 -N, with the 10205 Salicylate method as the method applied.

The effluent sample was filtered with a 0.45 µm syringe filter before analysis. The *TNTplus™832* test vial contains a *DosiCap™* zip cap, a double ended cap with the test reagents enclosed in the sealed end of the cap. With the use of a pipet 0.2 mL of filtered effluent was added to the test vial, the cap was then inverted and fastened onto the vial, allowing the reagents side to react with the effluent sample. The vial was further shaken 2-3 times as to dissolve the reagent in the cap. The solution reacted for 15minutes, after which it was colorimetrically examined by the spectrophotometer. The NH_3 concentration results were displayed in mg/L NH_3-N.

3.6.4.3 Nitrate

The N-NO_3^- concentrations were calculated colorimetrically utilizing the *Hach® DR3900 Benchtop Spectrophotometer™,* applying the *TNTplus™835* test kit. The test kit has a range of 0.23 to 13.50 mg/L NO_3^--N, with the 10206 Dimethylphenol method as the method applied.

The effluent sample was filtered with a 0.45 µm syringe filter before analysis. The effluent samples were diluted before analysis after initial tests exhibited some concentrations above the 13.5 mg/L NO_3^--N upper limit, this ensures test accuracy. With the use of a pipet 1.0 mL of filtered effluent was added to the test vial, followed by 0.2 mL of *Solution A* (provided by the *TNTplus™835* test kit). Thereafter the cap of the vial was tightened and the vial was inverted until completely mixed. The solution reacted for 15minutes, after which it was colorimetrically examined by the spectrophotometer. The NO_3^- concentration results were displayed in mg/L NO_3^--N.

3.6.4.4 Soluble reactive phosphorous (SRP)

The SRP/PO_4^{3-}-P concentrations were calculated colorimetrically utilizing the *Hach® DR3900 Benchtop Spectrophotometer™,* applying the *TNTplus™845* test kit. The test kit has a range of 2 to 20 mg/L PO_4^{3-}-P, with the 10210 Ascorbic Acid method as the method applied.

The effluent sample was filtered with a 0.45 µm syringe filter before analysis. With the use of a pipet 0.4 mL of filtered effluent was added to the test vial, followed by 0.5 mL of *Solution B* (provided by the *TNTplus™845* test kit). The initial cap was exchanged for a grey *DosiCap™* C, also provided by the test kit. The vial was inverted 2-3 times allowing mixture with the solution within. The solution was given 10minutes to react, after which it was colorimetrically examined by the spectrophotometer. The SRP results were displayed in mg/L PO_4^{3-}-P.

3.6.4.5 Glyphosate

The method for glyphosate effluent sample collection is identical to the collection procedure for the fertiliser nutrients. The samples were analysed by the Central Analytical Facility: LCMS division at Stellenbosch University.

The waters acuity ultra-performance liquid chromatography (UPLC) was coupled to a Xevo Triple Quadrupole Tandem Mass Spectrometer (MS/MS) (Waters, Milford, MA, USA) and used for high-resolution UPLC-MS/MS analysis (Waters 2018). Glyphosate was further separated by multiple reaction monitoring (MRM) using electrospray ionisation in a positive mode.

The operating parameters used are illustrated in Table 3.11 below:

Table 3.11: Operating parameters for Xevo.

Parameter	Value
Capillary voltage	3.5 V
Cone voltage	15 V
Collision energy	20 eV (electron Volt)
Source temperature	140 °C
Desolvation temperature	400 °C
Desolvation gas	800 L/h
Cone gas	50 L/h

The separation of the solvent component from the particle was achieved with a Hypercarb (2.1 x 100mm, 5u particle size, Thermo) column at 45 °C coupled with a flow rate of 0.4 mL/min. An injection volume of 1 µl was used and the mobile phase consisted of water acidified with 1% acetic acid (A), at gradients illustrated in Table 3.12 below, and Methanol acidified with 1% acetic acid (B).

Table 3.12: Gradient used for Glyphosate analysis.

Percentage Acetic acid (A) (%)	Time (min)
98	0 - 0.5
98 - 94	0.5 - 4
94 - 50	4 - 4.1
50 - 10	4.1 - 6
10 - 98	6 - 6.2
98	6.2 - 10

The glyphosate concentrations are displayed as mg/L - glyphosate. UPLC is an accurate and precise confirmation method as it uses two columns, with either glyphosate or AMPA available for analysis.

3.7 Data Analysis

The data generated from the experimental phytoremediation system is examined and analysed, identifying potential wetland plant species for effective agricultural pollutant treatment and their implementation in vegetative buffer strips. The use of indigenous wetland plant species that naturally occurs within Renosterveld is to encourage the biodiversity of the critically endangered vegetation type, with the goal of effectuating these findings into the already fragmented landscape.

The implementation of non-invasive plant species support vegetative biodiversity, whilst ameliorating the water quality of aquatic freshwater systems.

The following phytoremediatory aspects are examined in Chapter 4:

- Comparing the remediation efficacy of a Renosterveld community VS unvegetated soil.
- Identifying individual indigenous wetland species' pollutant removal.
- Analysing the effect of glyphosate dosage strength on herbicide remediation.
- Quantify the influence of selected water quality parameters.
- Compare remediation efficacy of indigenous wetland assemblage VS invasive alien plant (IAP) and Palmiet assemblage.
- Rank species within the Renosterveld community effective across pollutants.
- Analysing the root growth length on cumulative pollutant removal.
- Evaluating the temporal effect on cumulative pollutant removal for the duration of the study (March 3rd - May 18th).

The information gathered for these analyses was obtained from the experimental phytoremediation system at Stellenbosch University.

For the evaluation of removal efficiency, the baseline concentration values needed to be known. These values are the initial nutrient content within the growth silos prior to each round of sampling. The baseline concentration of every growth silo was analysed before contaminants were added to the system. The measured baseline concentrations were deducted from the measured effluent concentrations to allow for the calculation of percentage removal for each sampling round. The following equation was used;

$$\frac{Influent\ conc. - (Effluent\ conc. - Baseline\ conc.)}{Influent\ conc.} \times \frac{100}{1} \qquad \text{Equation 3.3}$$

Where

Influent conc.	=	Influent concentration (mg/L)
Effluent conc.	=	Effluent concentration (mg/L)
Baseline conc.	=	Baseline concentration (mg/L)

The Kruskal-Wallis H-test, non-parametric ANOVA, was used for the evaluation of plant species occurring in Renosterveld as a community VS unvegetated soil, thereafter a Student's t-test was used for the evaluation of the individual wetland species' pollutant removal values and the multiple indigenous wetland plant species VS multiple IAP species and Palmiet. The ranking of the plant species occurring in the Renosterveld community with regard to species pollutant remediation across all pollutants required a one-way ANOVA, with a normal Kruskal-Wallis H-test performed.

Statistical analyses were executed in *Python*™ by means of the data analytical library.

3.8 Limitations of the study

The laboratory experiment did not take site-specific conditions such as hydrology and climate into account. Even if the laboratory results determine vegetated biofilters (indigenous or alien species) to be exceptionally effective at treating contaminated water, the results need to be confirmed under field conditions.

The alteration of soil media and the limited growth area may influence the natural phytoremediatory abilities of the laboratory plants. This may affect the characteristics of plants in the natural domain. The plants have however displayed exceptional growth in the experimental setup, an indication that alterations of their natural morphological growth properties were negligible.

One of the major limitations of the study was time and funding constraints, for this reason the data collected was limited. The parameters analysed, frequency and quantity of sampling opportunities were limited to four rounds, of which five parameters were examined. Due to cost and logistical constraints this approach was necessary to ascertain a paired-study design by comparing treatments through time against a control and against other species. However, although it is possible to evaluate the relationship between plant species with regard to pollutant remediation and to determine the temporal effect on remediation, sample replication was not possible. The lack of replicates (at least three samples per species per time) is a major limitation to the study, which results in less confidence relating to significance of the p-values in determining differences between plants through time. The limited data points collected constrained the statistical accuracy of analyses and without replication one cannot minimize confounding factors like the health and vigour of the individual plants, however, with recognition of this limitation, the six objectives are addressed and discussed in Chapter 4.

3.9 Phytoremediation system assumptions

Various assumptions were made during system design and construction. The assumptions made during the development of the phytoremediation system, and their corresponding summaries:

- Completely mixed conditions existed within the fertiliser and herbicide treatment tanks. Observations during the laboratory experiment suggest that this assumption was justifiable. The smaller submersible pumps added to each container ensured effective mixing of soluble compounds. The choice of water soluble liquid herbicide and analytical grade chemicals guaranteed the complete dispersal of contaminants in the solution.
- There were no significant variations between influent treatments. A mixing regime was established to provide fresh influent at standardised concentrations every ten days. The influent concentrations were also analysed before each round of

sampling, to determine the natural degradation (half-life) of the solutions. The remixing process warranted uniform influent concentrations throughout the phytoremediation laboratory experiment. The assumption was therefore justifiable.

- Ammonia volatilisation was negligible. The application method suggests that this assumption was justifiable. NH_3 volatilisation is a chemical process occurring at the soil surface when NH_4^+-containing fertilisers are converted to NH_3 gas at high pH (Schwenke & DPI 2014). N losses are minimal when the fertiliser is combined, but may be high when the fertiliser is surface applied (Bacon & Freney 1989; Turner et al. 2010). The fertiliser method of application in the laboratory experiment incorporated the N into the system, rather than surface application. This theoretically limited the NH_4^+ conversion to NH_3 gas.
- Nitrate denitrification was negligible. NO_3^- denitrification is a microbially facilitated process for the reduction of NO_3^-. Gaseous N_2 occurring within the soil profile is produced where there is sufficient NO_3^- in anoxic conditions, such as in slowly draining soils, this may be high in waterlogged soils (Schwenke & DPI 2014). For successful denitrification an oxygen concentration (dissolved and freely available) near depletion, is required (Seitzinger et al. 2006). The conditions within the phytoremediation system do not support NO_3^- denitrification reactions, however some may be present in microsites but not likely to be significant.
- Nitrate leaching was accounted for. NO_3^- leaching occurs with water drainage through the profile, with minimal large-scale loss of NO_3^- below the root zone in the rhizosphere (Schwenke & DPI 2014). By analysing the effluent runoff and comparing the results with the known standardised influent and uncontaminated baseline concentrations, the NO_3^- leached was recorded. The solubility and mobility of NO_3^- creates opportunity for rhizosphere uptake. The assumption was therefore justifiable.
- Evaporation losses were negligible. Due to the small surface area of the silos, limited moisture was lost to the atmosphere, compared to the water content remaining within the silos. All storage tanks and mixing containers were sealed, allowing zero moisture to be evaporated. The water capacities, determined by analysing the exterior clear tubing that indicates the volume within the containers and tanks, illustrated the negligible loss of moisture. This assumption was therefore justifiable.
- Uniform influent flow rate over all irrigation drippers. The irrigation drippers incorporated within the phytoremediation system are standardised products, with consistent flow at 2 L/h and 8 L/h for the individual plants per silo and multiple plants per silo experiments respectively. The drippers are connected to submersible pumps with uniform power output, ensuring interchangeable flow rates.

The exact flow of each dripper was further measured to ensure uniform irrigation volume. The assumption is correct.

- Uniform soil media within growth silos. Soil collected from the relevant field site was inserted and circulated in a 50 L pan mixer at the Civil Engineering department at Stellenbosch University. The pan mixer ensured completely mixed soil conditions before transferring into the growth silos prior to transplanting. The assumption was therefore justifiable.
- All visible organic matter in the form of foreign roots, bulbs and detritivores were removed from soil medium. Prior to mixing, all the visible organic matter was manually removed either by hand or with a 5mm sieve. The removal of foreign organic matter was important in guaranteeing limited contributions from unknown objects during phytoremediation. The efforts towards organic matter removal confirm the assumption to be justifiable.
- Light distribution was uniform throughout the biofiltration experiment. *Osram® Biolux™* lamps with wavelength distribution comparable to sunlight were provided for standardizing lighting conditions (Osram 2018). Eight 58W Biolux tubes were mounted throughout the system, placed at specific locations to ensure uniform light distribution. The consistent growth rates between plants with different locations in the laboratory experiment, warranted uniform light distribution, thus indicating a justifiable assumption.
- Glyphosate degradation was accounted for. As reported by literature, half-lives reported do not reflect local climatological conditions (Barcelo 1997). Uncertainty regarding herbicide degradation was countered by analysing the influent water supply at every round of sampling, this indicated any herbicide discrepancies among glyphosate solutions. The assumption was therefore justifiable.

CHAPTER 4 : EXPERIMENTAL RESULTS AND DISCUSSION

The data generated from the experimental phytoremediation system is analysed and discussed within this chapter. The results obtained were used to identify potential plant species indigenous to Renosterveld and other wetland areas for the treatment of polluted water and their possible implementation in vegetative buffer strips, treatment facilities and biofiltration trains. This chapter is subdivided to allow for analysis of results and discussion of the research objectives contained within this study. The following phytoremediatory objectives are discussed:

- Compare the remediation efficacy of a Renosterveld community VS unvegetated soil.
- Identifying individual indigenous wetland species' pollutant removal.
- Analysing the effect of glyphosate dosage strength on herbicide remediation.
- Quantify the influence of selected water quality parameters.
- Compare remediation efficacy of indigenous wetland assemblage VS invasive alien plant (IAP) and Palmiet assemblage.
- Rank species within the Renosterveld community effective across pollutants.
- Analysing the root growth length on cumulative pollutant removal.
- Evaluating the temporal effect on cumulative pollutant removal for the duration of the study (March 3rd - May 18th).

The results discussed within this chapter were obtained from experimental tests conducted in the laboratory experiment at Stellenbosch University.

Four statistical hypothesis tests were used (advised by the Centre for Statistical Consultation at Stellenbosch University), to determine if two sets of data are significantly different from each other. The Kruskal-Wallis H-test, evaluated indigenous plants species VS unvegetated soill. The Student's t-test was used for the evaluation of individual wetland species' pollutant remediation and multiple indigenous wetland plant species VS IAP species and Palmiet. Regression analysis was used to assess the influence of selected water quality parameters with the temporal effect on pollutant remediation evaluated by means of trend lines. The ranking of the plant species occurring in the Renosterveld community with regard to species pollutant remediation across all pollutants required a one-way ANOVA, thereafter a normal Kruskal-Wallis H-test was performed.

Statistical analyses were executed in *Python*™ by means of the data analytical library.

4.1 Experimental results

Eight objectives were executed on the laboratory phytoremediation system, as mentioned above. The removal efficiency of the phytoremediation system was evaluated by examining the influent and effluent concentrations of N-NH_3, N-NO_3^- and P-SRP, and the 0.7 mg/L and 225 mg/L glyphosate concentrations for agricultural fertilisers and herbicides respectively.

4.1.1 Influent concentrations

The influent dosages were assigned and premixed as the concentrations given in Section 3.4 and 3.5 (Table 3.10), representing the standardised influent concentrations for both fertiliser and herbicide contaminants.

4.1.2 Effluent concentrations

(Illustrated in Appendix E, Table E.1)

The concentrations that were periodically measured for the 0.45 µm filtered effluent samples were collected with sample containers directly below the drainage pipes, immediately followed by sample analysis.

4.1.3 Baseline concentrations

For the evaluation of removal efficiencies (the difference between the influent and effluent concentrations), the baseline concentration values are needed. The baseline values indicated the initial nutrient content within the growth silos prior to the addition of pollutants. Without this information, one cannot deduce the removal efficiencies of the system. The initial baseline concentration of every growth silo was measured before contaminants were added to the system.

The baseline concentrations were deducted from the measured effluent concentrations, allowing the calculation of percentage removal for each species per sampling round. The following equation was used:

$$\frac{Influent\ conc. - (Effluent\ conc. - Baseline\ conc.)}{Influent\ conc.} \times \frac{100}{1} \qquad \text{Equation 4.1}$$

Where

Influent conc.	=	Influent concentration (mg/L)
Effluent conc.	=	Effluent concentration (mg/L)
Baseline conc.	=	Baseline concentration (mg/L)

4.1.3.1 Baseline fertiliser content

In evaluating percentage removal, indicating removal efficiency for the first sampling round (post contamination), the initial baseline concentrations were used, as illustrated by the data representing the first analysis in Appendix E, Table E.1. The percentage removal considered the concentration of the nutrients within the growth silos prior to the addition of the standardised influent concentrations. Thereafter (second, third and fourth rounds of sampling), the baseline concentration values were not taken as the initial concentrations (prior to influent treatment), but rather assumed to be the effluent concentrations measured from the previous sampling round. Each analysed data point served as the new baseline

concentration for the subsequent sampling round. For example, the baseline concentration of sample 1 during the second round of effluent sampling in evaluating removal efficiency, was assumed to incorporate the effluent concentrations analysed during the first round of sampling for sample 1. These steps were included to achieve the most updated known concentration of the nutrients within the growth silos, allowing removal efficiency comparison between different rounds of sampling. This method calculated a running mean removal.

4.1.3.2 Baseline herbicide content

The initial measurement of herbicide concentrations within the growth silos were deemed unnecessary, as the soil was collected from an area where zero to minimal pesticide application took place.

4.2 Remediation efficacy of a Renosterveld community VS unvegetated soil

The objective of this experiment was to observe and determine the usefulness of vegetation in agricultural pollutant remediation. The experiment included all 14 indigenous plants as well as the unvegetated soil control. The pollutant parameters of importance included all fertiliser nutrients (NH_3, NO_3^- and PO_4^{3-}) and the 225 mg/L glyphosate herbicide concentration.

4.2.1 Analysis of vegetative pollutant removal

Vegetative pollutant removal in comparison with unvegetated soil was achieved by either examining the effluent pollutant concentrations post drainage, establishing percentage removal of pollutants (with baseline concentrations known) or analysing the differences among pollutant concentration group running means for vegetation and soil only.

The third technique computes the Kruskal-Wallis H-test for independent samples. The Kruskal-Wallis H-test is a non-parametric version of ANOVA, applicable on two or more independent samples of different sizes. The phytoremediation system's removal efficiency compares the average pollutant removal of indigenous wetland plant species as a Renosterveld community with the pollutant removal of the unvegetated soil control.

4.2.1.1 Pollutant removal efficiency by evaluating effluent concentration

The average concentration of pollutants from the 14 plant species found in Renosterveld vegetation types and across wetlands, as a collective Renosterveld community, compared to soil is illustrated in Figure 4.1 to Figure 4.4. The effluent pollutant concentrations are depicted on the vertical axis as a function of time, indicating days of sampling. Baseline nutrient and herbicide concentrations were taken into consideration to allow for comparison between influent and effluent, avoiding deflated results.

The assumption that nutrients are absent within the growth silos prior to each sampling round produces deflated results. Calculating the removal efficiencies considers both the nutrient uptake and extraction by vegetation and soil of the standardised influent concentrations, with the already occurring nutrient content within the silos.

Figure 4.1 compares the effluent ammonia, NH_3-N concentrations of the 14 plants found within Renosterveld vegetation with soil for the duration of the experiment. The standardised influent NH_3 concentration, as depicted below was 37.096 mgN/L.

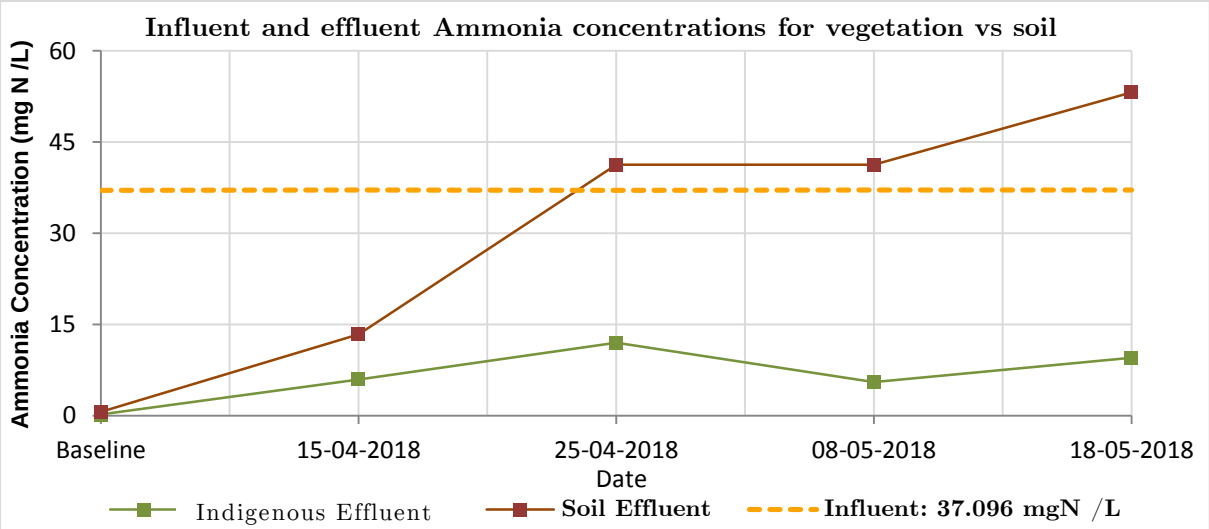


Figure 4.1: Ammonia influent and effluent for vegetation and unvegetated soil.

The vegetative effluent NH_3-N concentration trend differs significantly to that of unvegetated soil. The effluent concentration of vegetation was consistently below 15 mgN/L, as well as comfortably below the initial NH_3 dosage concentration of 37.096 mgN/L. The rapid accumulation of NH_3-N in the absence of vegetation is evident by the soil effluent trend, a concentration increase with time. In the absence of vegetation, the effluent rapidly exceeded the initial NH_3 dosage. The effluent trends indicate that the selected Renosterveld community was better equipped to restrain nutrient accumulation in comparison to the unvegetated soil.

Figure 4.2 compares the effluent concentrations of the indigenous vegetation with unvegetated soil for nitrate, NO_3^--N over the duration of the experiment. The standardised influent NO_3^- concentration, as depicted below was 9.274 mgN/L.

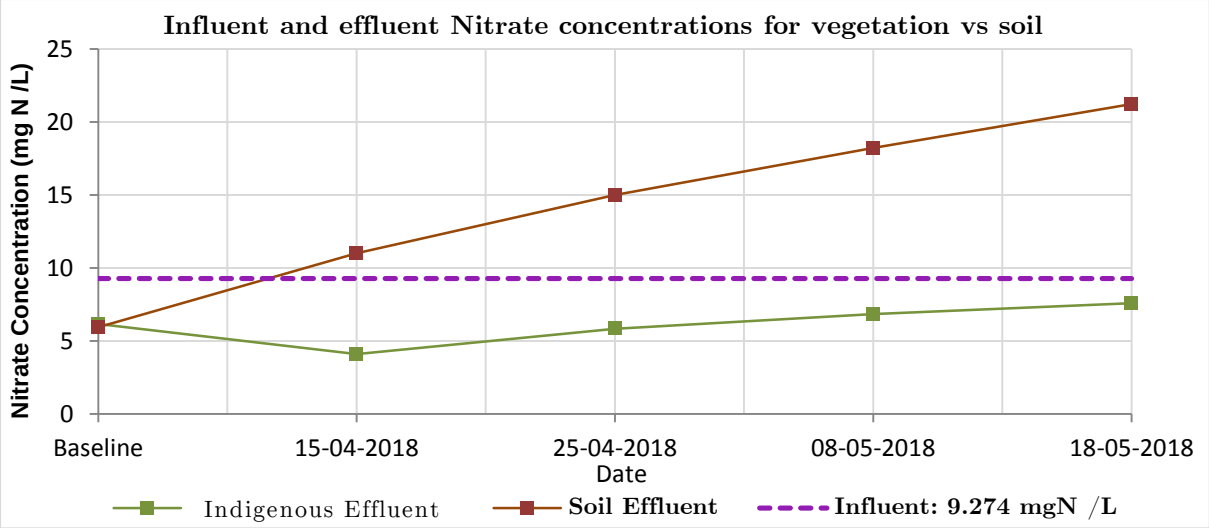


Figure 4.2: Nitrate influent and effluent for vegetation and unvegetated soil, with evident nitrate accumulation in unvegetated soil.

The vegetated removal efficiency of NO_3^--N was significantly better than the unvegetated soil, evident by comparing the effluent concentrations in Figure 4.2 above. The vegetated effluent trend differs to that of unvegetated soil, with the vegetation effluent value below the initial NO_3^- dosage concentration of 9.274 mgN/L for the duration of the study, although at a less remarkable proportion than for NH_3. Nutrient accumulation was more pronounced in the unvegetated soil, as a result of vegetation's ability to extract nutrients from a soil-water solution. The NO_3^--N effluent concentration in unvegetated soil rapidly accumulated, whereas it was restrained by the indigenous plants. This indicates that the vegetation displayed greater removal efficiency of NO_3^- than soil only.

Figure 4.3 compares the effluent soluble reactive phosphorous (SRP), PO_4^{3-}-P concentrations of the selected vegetation with unvegetated soil for the duration of the experiment. The standardised influent PO_4^{3-} concentration, as depicted below was 17.39 mgP/L.

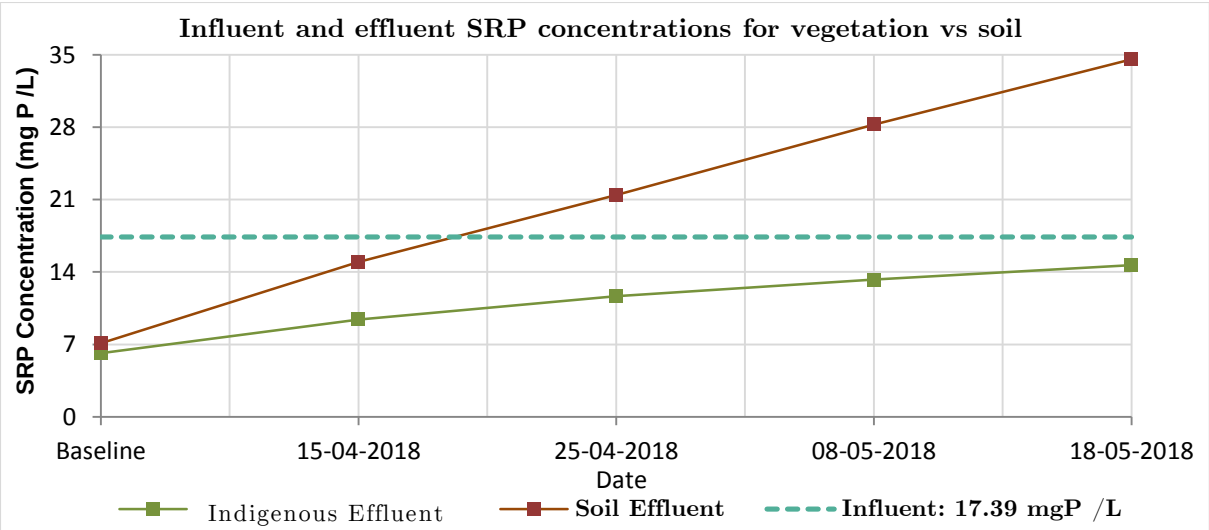


Figure 4.3: SRP influent and effluent for vegetation and soil control.

The SRP effluent concentrations seen in Figure 4.3, indicate the indigenous species' removal efficiency as being more pronounced than that of unvegetated soil. The SRP effluent rapidly accumulated in the soil control in the absence of vegetation, whereas nutrient accumulation in the effluent was on average restrained in the presence of vegetation. The findings indicate the wetland plant species as displaying a stronger SRP removal efficiency than soil only.

Figure 4.4 compares the effluent herbicide concentration of the indigenous vegetation with soil control, for the duration of the experiment, with glyphosate at a standardised influent concentration of 225 mg/L.

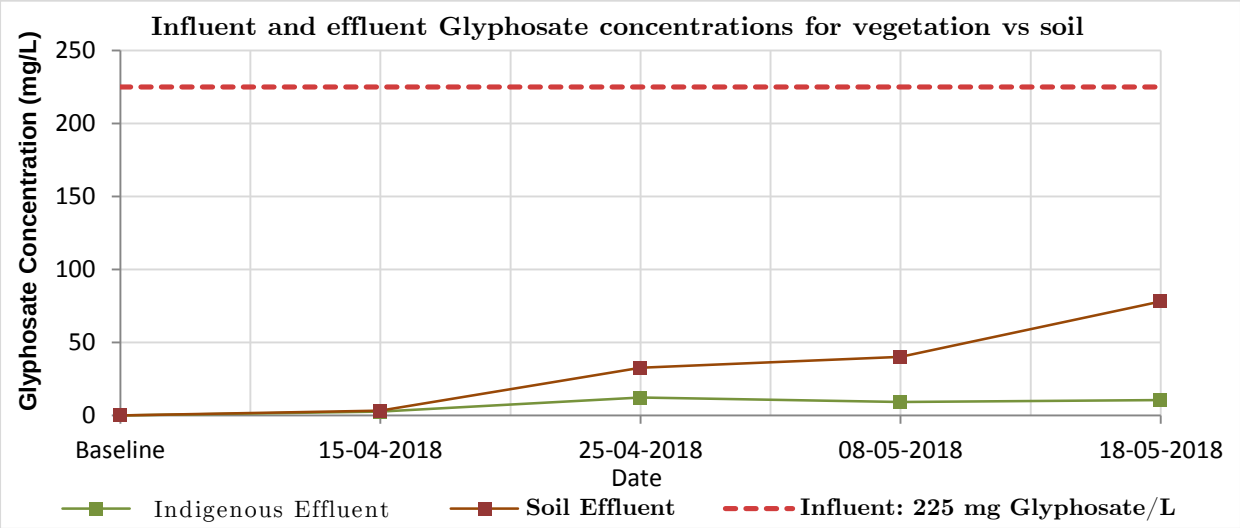


Figure 4.4: Glyphosate influent and effluent for vegetation and unvegetated soil.

The effluent glyphosate concentration trend in Figure 4.4, indicates that glyphosate experienced significant degradation, immobilization and removal from its initial 225 mg/L influent concentration. Both the vegetation and the unvegetated soil control media displayed exceptional effluent concentration decrease. It is apparent, however, that the vegetation effluent was consistently measured below the effluent concentration of the unvegetated soil. These trends, although significantly reduced, display indigenous vegetation as capturing, removing or degrading the herbicide more effectively than the soil control.

4.2.1.2 Removal efficiency by evaluating percentage removal

An alternative method in evaluating the pollutant removal efficiency of the wetland plant species that are found in Renosterveld compared to soil is by examining the percentage of the pollutant removed, instead of the effluent concentrations. The percentage removal of nutrients and herbicide pollutants are illustrated in Table 4.1 and 4.2 and Figure 4.5 and Figure 4.6. The percentage pollutant removal is depicted on the vertical axis as a function of time, indicating days of sampling. Baseline nutrient and herbicide concentrations (as discussed in Section 4.1.3) were taken into consideration to allow influent and effluent comparison.

Figure 4.5 compares the average percentage removal of all nutrients (NH_3-N, NO_3^--N and PO_4^{3-}-P) for indigenous vegetation and the unvegetated soil control for the duration of the experiment.

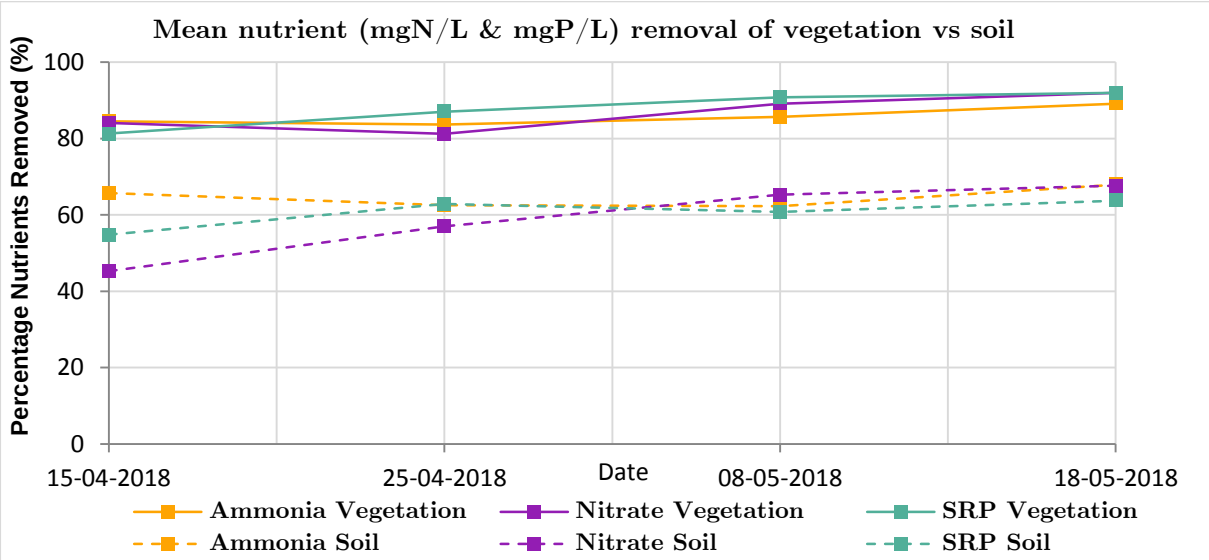


Figure 4.5: Mean nutrient percentage removal for vegetation and unvegetated soil.

Plant species within the Renosterveld community reduced the effluent concentration of the nutrients. The percentage removal averaged 85.75%, 86.62% and 87.78% for NH_3-N, NO_3^--N and PO_4^{3-}-P (SRP) respectively. The percentage nutrient removal of the plant community was consistently greater than that of the unvegetated soil control, evident in Table 4.1 below.

Table 4.1: Average percentage nutrient removal.

Nutrient	Mean percentage removal (%)	
	Indigenous vegetation	Soil control
Ammonia (NH_3-N)	86	65
Nitrate (NO_3^--N)	87	59
Soluble Reactive Phosphorous (PO_4^{3-}-P)	88	61

The average percentage nutrient removal in Figure 4.5 and Table 4.1, indicate the indigenous vegetation on average to be more effective in the removal of nutrient pollutants than soil. There was no obvious difference in NH_3, NO_3^- and PO_4^{3-} remediation within vegetation or the soil control, whereas, considerable percentage nutrient removal variation existed between vegetated and unvegetated media. This is as a result of factors discussed in Section 4.3.2.1.

From Figure 4.5 above, it is evident that the removal efficiencies improved with time for the duration of the study. The unvegetated soil NO_3^- remediation improved with time, this may be due to bacterial establishment in the soil. Generally, bacterial establishment in

unvegetated soil does not seem to considerably improve the remediation of nutrients. However, vegetation displays the greatest remediation of nutrients, with rhizosphere root bacteria as possible contributors.

Figure 4.6 compares the average percentage removal of herbicides for vegetated and unvegetated soil for the duration of the experiment.

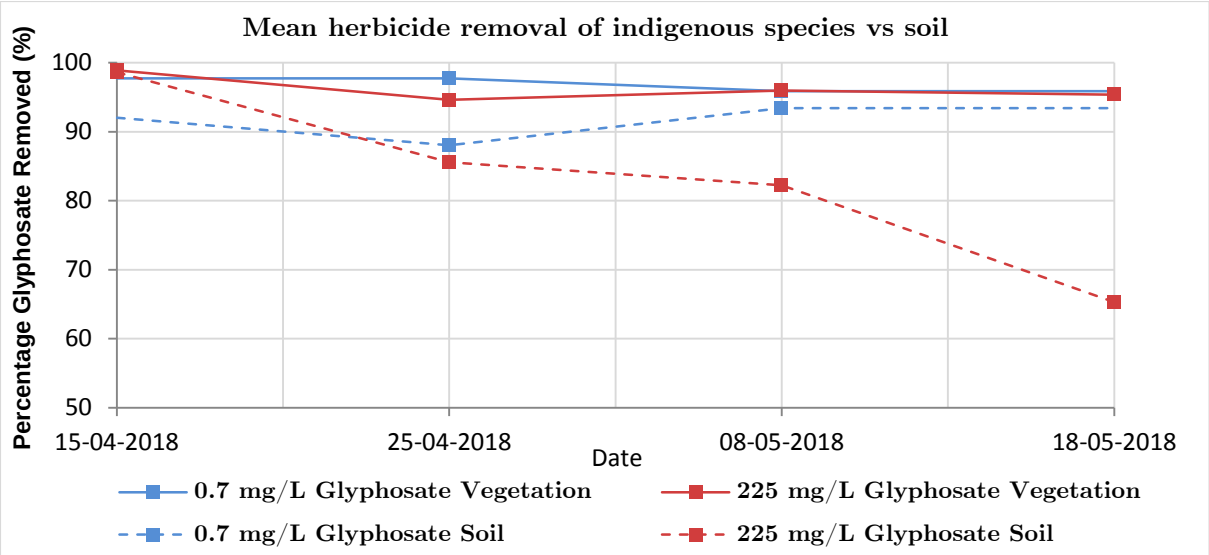


Figure 4.6: Mean herbicide percentage removal for vegetation and unvegetated soil.

Indigenous vegetation removed a greater percentage of 0.7 mg/L glyphosate and 225 mg/L glyphosate, compared to soil only. Although percentage removal of the unvegetated soil is very high (Figure 4.6 and Table 4.2), it is evident that vegetation more effectively remediated pollutants at both glyphosate concentrations. From Figure 4.6, the percentage removal of the soil control dropped with time. This indicates that in the absence of vegetation, the herbicide accumulated, resulting in increased leaching and transportation of glyphosate. Environmentally, this may result in increased agricultural pollution of adjacent freshwater aquatic systems.

Table 4.2: Average percentage herbicide removal.

Herbicide	Mean percentage removal (%)	
	Indigenous vegetation	Soil control
0.7 mg/L Glyphosate	96	92
225 mg/L Glyphosate	97	83

From Table 4.2 above, the vegetation and the unvegetated soil control showed exceptional percentage herbicide removal values (percentage >80%), as a result of factors discussed in Section 4.3.2.2.

4.2.1.3 Kruskal-Wallis H-test

The Analysis of Variance (ANOVA) test has important assumptions that need to be satisfied in order for the associated p-value to be valid.

1. The samples are independent.
2. Each sample is from a normally distributed population.
3. The population standard deviations of the groups are all equal.

If all of the above assumptions are satisfied, the property is known as homoscedasticity.

In the case that these assumptions are not definitely met (t-test not satisfied) for a given set of data, a Kruskal-Wallis H-test is possible, however with some loss of power. The Kruskal-Wallis method was used, due to the data somewhat satisfying a normal distribution (Appendix F, Figure F.1). Analysis of variance between the vegetated and the unvegetated soil control, regarding percentage pollutant removal produced the findings in Table 4.3 below. A significance level (α) of 0.05 has been selected to assess whether the differences between means are statistically significant.

Table 4.3: Kruskal-Wallis H-test for vegetation VS unvegetated soil.

Statistical significance of Renosterveld removal VS soil				
	NH_3	NO_3^-	SRP	225 mg/L Glyphosate
P-value	0.00152	0.00304	0.00227	0.01775

n = 4, df = 3.

P-value ≤ α: Differences between means are statistically significant. From the results above, there is statistical significance, p-value ≤0.05, across the pollutant parameters analysed. Therefore, the removal of pollutants by vegetation is significantly more effective than the unvegetated soil control.

4.3 Individual indigenous wetland species' pollutant removal.

(Further illustrated in Appendix F, Figure F.2 at a 95% Confidence Interval)

The objective of this test was to identify the pollutant removal performance of individual indigenous species. Individual plant species displayed unique remediation capabilities, with affinity to different pollutants. Mechanisms within phytoremediation make use of different plant properties, and generally implement different species of plants for each (Read et al. 2008; Read et al. 2010). The percentage removal of individual plant species found within Renosterveld across the fertiliser and herbicide pollutants is illustrated in the following section.

The comparison of two groups require a t-test (Student's t-test), with the difference in removal percentages between the unvegetated soil control and individual wetland plant species compared. The p-values generated indicated to what extent the individual indigenous plant species' pollutant removal values differed in relation to the unvegetated soil control. The results generated by the Student's t-test are illustrated by Table 4.6, Section 4.3.2

4.3.1 Individual wetland species percentage pollutant removal

All 14 indigenous plant species that naturally occur within Renosterveld were contaminated, with individual percentage pollutant removal capabilities evaluated and compared with the remaining plant species and the unvegetated soil control, illustrated in Table 4.4 and 4.5 and Figure 4.7 and 4.8.

4.3.1.1 Plant species nutrient removal

The 14 indigenous plant species all reduced the nutrient concentrations of the effluent, with removal averaging 85.75%, 86.62% and 87.78% for NH_3, NO_3^- and SRP respectively.

The percentage nutrient removal values of all wetland plant species displayed exceptional removal efficiencies (percentage removal >80%), except two; *Carpobrotus edulis* and *Arctotis acaulis*, both displaying mean percentage nutrient removal values of 68%, Figure 4.7 below.

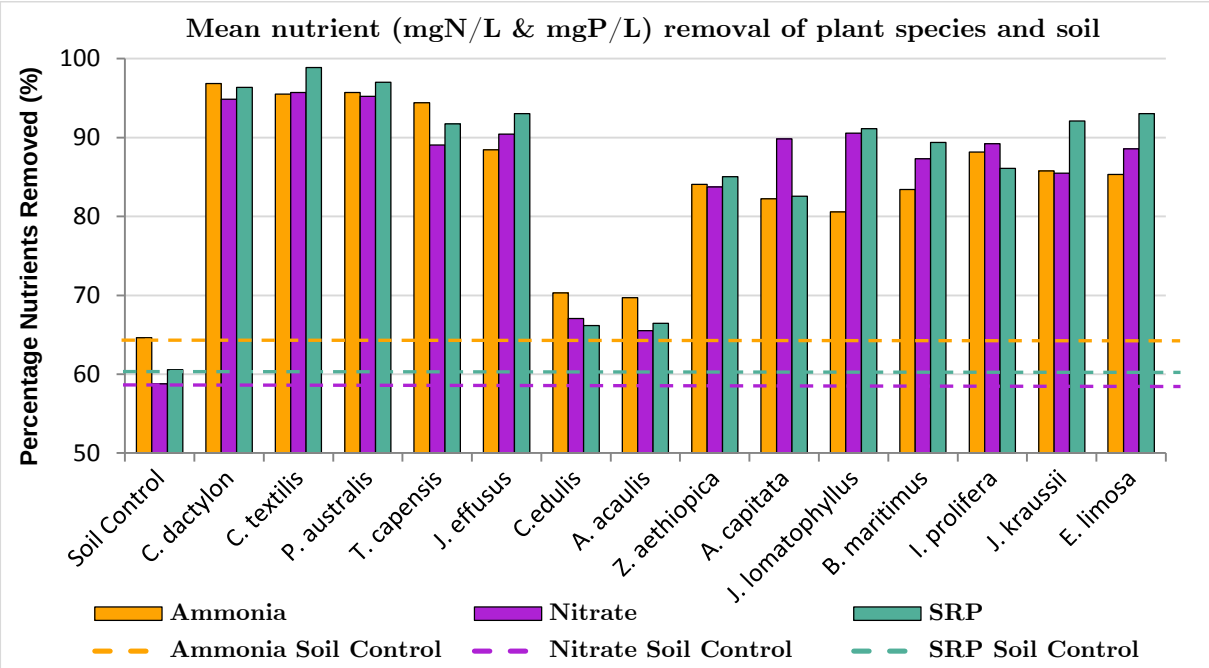


Figure 4.7: Average percentage nutrient removal of wetland plant species and soil control.

Figure 4.7 above illustrates which plants of the Renosterveld community display greater removal efficiencies than the unvegetated soil control, with regard to the nutrient parameters.

4.3.1.1.1 Ammonia (mgN-NH_3/L)

All indigenous plant species were effective in removing NH_3 with effluent concentrations being reduced by between 70 - 97% (average NH_3 removal 86%), while the unvegetated soil control removed an average of 65% (Table 4.4 and Figure 4.7). The three most effective species: *Cynodon dactylon*, *Phragmites australis* and *Cyperus textilis*, were on average 31% more effective than the soil control. Similar percentage NH_3 removal ranges were found by

comparable local and international SuDS and biofiltration studies, focusing on different plant species and pollutant sources (Bratieres et al. 2008; Milandri et al. 2012).

4.3.1.1.2 Nitrate (mgN-NO_3^-/L)

Previous research utilizing different plant species, focusing on stormwater treatment systems, found between 15 - 65% TN removal as a result of <20% NO_3^- retention (Read et al. 2008; Milandri et al. 2012). As with NH_3, all the indigenous plant species were effective in removing NO_3^- from the influent solution. The removal efficiency ranged between 65 - 96% (average NO_3^- removal 87%), while the unvegetated soil control removed on average 59% (Table 4.4 and Figure 4.7).

The three most effective species: *Cyperus textilis*, *Phragmites australis* and *Cynodon dactylon*, were on average 36% more effective than the soil control.

In contrast to the findings of previous research, this study showed far greater NO_3^- removal. This may be due to the specific plant species under study, micro-organisms, the establishment of bacterial assemblages in the rhizosphere and the drip irrigation applied as opposed to rapid high volume irrigation (Bratieres et al. 2008; Read et al. 2008). The irrigation system fitted in this study percolated the influent through the silos at a slower rate, allowing more time for the uptake of water and dissolved nutrients (Milandri et al. 2012).

4.3.1.1.3 Soluble Reactive Phosphorous (SRP or mgP-PO_4^{3-}/L)

Following the trend, all the indigenous plant species displayed a strong removal efficiency for SRP, with the percentage SRP removed ranging from 66 - 99% (average SRP removal 88%), while the unvegetated soil control removed on average 61% (Table 4.4 and Figure 4.7).

The three most effective species: *Cyperus textilis*, *Phragmites australis* and *Cynodon dactylon*, on average displayed a removal efficiency of 36% greater than the soil control. The SRP removal values are similar to the findings from comparable studies focusing on mine-leachate and stormwater (Bratieres et al. 2008; Read et al. 2008; Milandri et al. 2012).

Plant species occurring within Renosterveld ranked according to their mean nutrient removal efficiencies is given in Table 4.4 below.

Table 4.4: Mean percentage nutrients removed by individual wetland plant species.

	Percentage nutrient removal		
Rank	NH_3	NO_3^-	PO_4^{3-}
1	*Cynodon dactylon* (97%)	*Cyperus textilis* (96%)	*Cyperus textilis* (99%)
2	*Phragmites australis* (96%)	*Phragmites australis* (95%)	*Phragmites australis* (97%)
3	*Cyperus textilis* (96%)	*Cynodon dactylon* (95%)	*Cynodon dactylon* (96%)
4	*Typha capensis* (94%)	*Juncus lomatophyllus* (91%)	*Juncus effusus* (93%)
5	*Juncus effusus* (88%)	*Juncus effusus* (90%)	*Eleocharis limosa* (93%)
6	*Isolepsis prolifera* (88%)	*Aristea capitata* (90%)	*Juncus kraussii* (92%)
7	*Juncus kraussii* (86%)	*Isolepsis prolifera* (89%)	*Typha capensis* (92%)
8	*Eleocharis limosa* (85%)	*Typha capensis* (89%)	*Juncus lomatophyllus* (91%)
9	*Zantedeschia aethiopica* (84%)	*Eleocharis limosa* (89%)	*Bolboschoenus maritimus* (89%)
10	*Bolboschoenus maritimus* (83%)	*Bolboschoenus maritimus* (87%)	*Isolepsis prolifera* (86%)
11	*Aristea capitata* (82%)	*Juncus kraussii* (85%)	*Zantedeschia aethiopica* (85%)
12	*Juncus lomatophyllus* (81%)	*Zantedeschia aethiopica* (84%)	*Aristea capitata* (83%)
13	*Carpobrotus edulis* (70%)	*Carpobrotus edulis* (67%)	*Arctotis acaulis* (66%)
14	*Arctotis acaulis* (70%)	*Arctotis acaulis* (65%)	*Carpobrotus edulis* (66%)
15	Soil control (65%)	Soil control (59%)	Soil control (61%)

Table 4.4, indicates that the indigenous community is more effective than unvegetated soil for the remediation of agricultural nutrients.

4.3.1.2 Plant species herbicide removal

The 14 indigenous plant species effectively remediated herbicide pollution for two glyphosate concentrations (0.7 mg/L and 225 mg/L). The average removal efficiency for both herbicide concentrations are displayed in Figure 4.8 below.

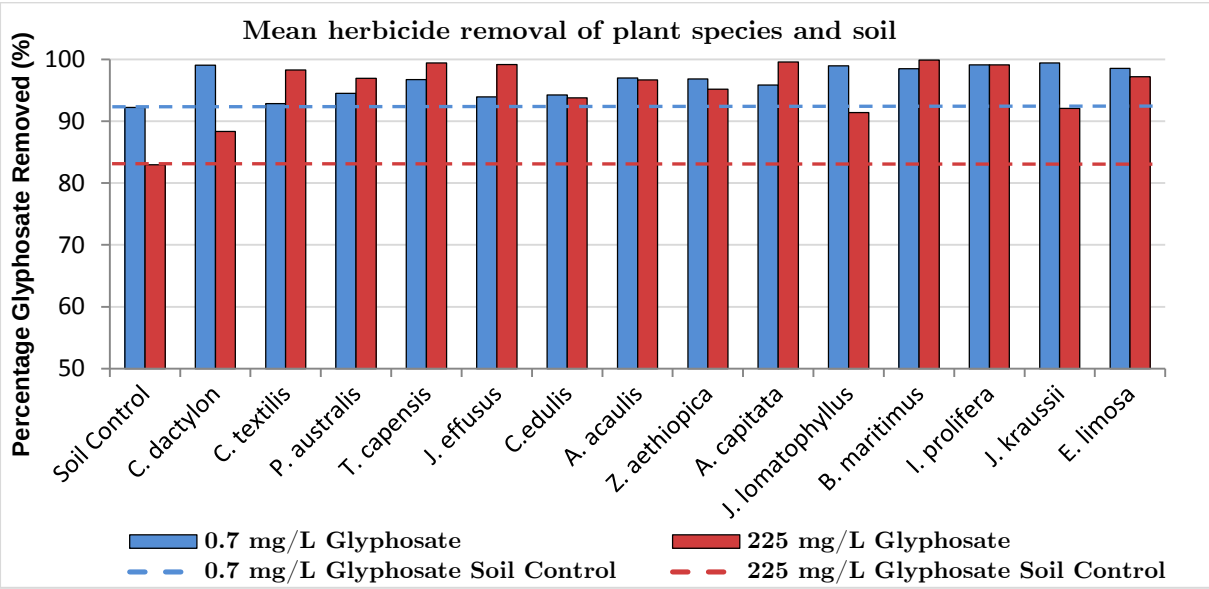


Figure 4.8: Average percentage herbicide removal of wetland plant species and soil control.

4.3.1.2.1 Glyphosate (0.7 mg/L)

The percentage removal of vegetation for the 0.7 mg/L glyphosate influent was reduced by between 92.84 - 99.39% (average glyphosate removal 96.81%), while the unvegetated soil control removed on average 92.21% (Table 4.5 and Figure 4.8). The three most effective species: *Juncus kraussii*, *Isolepsis prolifera* and *Cynodon dactylon*, were on average 6.97% more effective than the soil control.

4.3.1.2.2 Glyphosate (225 mg/L)

Similar to the removal efficiency of the less concentrated glyphosate solution, the percentage removal of vegetation for the 225 mg/L glyphosate influent ranged between 88.34 - 99.86% (average glyphosate removal 96.21%), with the soil control on average removing 82.93% (Table 4.5 and Figure 4.8). The three most effective species: *Bolboschoenus maritimus*, *Aristea capitata* and *Typha capensis*, were on average 16.67% more effective than the soil control.

Across fertiliser and herbicide pollutants, *Cynodon dactylon* was deemed to be an outstanding performer with regard to pollutants removed. The three most effective species for the removal of fertiliser pollutants are consistent throughout the different nutrient parameters, whereas, the three most effective species for the removal of herbicide pollutants vary drastically.

Table 4.5: Mean percentage herbicide removed by wetland plant species.

	Percentage Glyphosate Removal	
Rank	0.7 mg/L	225 mg/L
1	*Juncus kraussii* (99.39%)	*Bolboschoenus maritimus* (99.86%)
2	*Isolepsis prolifera* (99.09%)	*Aristea capitata* (99.56%)
3	*Cynodon dactylon* (99.06%)	*Typha capensis* (99.39%)
4	*Juncus lomatophyllus* (98.96%)	*Juncus effusus* (99.17%)
5	*Eleocharis limosa* (98.52%)	*Isolepsis prolifera* (99.11%)
6	*Bolboschoenus maritimus* (98.48%)	*Cyperus textilis* (98.26%)
7	*Arctotis acaulis* (97.00%)	*Eleocharis limosa* (97.17%)
8	*Zantedeschia aethiopica* (96.81%)	*Phragmites australis* (96.92%)
9	*Typha capensis* (96.70%)	*Arctotis acaulis* (96.69%)
10	*Aristea capitata* (95.83%)	*Zantedeschia aethiopica* (95.18%)
11	*Phragmites australis* (94.51%)	*Carpobrotus edulis* (93.76%)
12	*Carpobrotus edulis* (94.26%)	*Juncus kraussii* (92.08%)
13	*Juncus effusus* (93.93%)	*Juncus lomatophyllus* (91.38%)
14	*Cyperus textilis* (92.84%)	*Cynodon dactylon* (88.34%)
15	Soil control (92.21%)	Soil control (82.93%)

Both the vegetation and the unvegetated soil control displayed exceptional glyphosate remediation. It is noted however that all 14 indigenous plant species displayed greater

removal efficiencies than the unvegetated soil control, over both concentrations. The majority of the glyphosate is reduced by soil media, with vegetative processes adding to pollutant extraction. An indication that vegetation does indeed have a role to play is shown by the additional pollutant removal in all silos in the presence of vegetation. This reiterates the importance of the soil media as a role player in the removal of glyphosate.

4.3.2 Student's t-test for individual wetland plant species removal

The need to compare the average percentage pollutant removal between individual plant species and the unvegetated soil control, generalized a t-test for all nutrient pollutants (NH_3, NO_3^- and SRP) and the 225 mg/L glyphosate herbicide. The 0.7 mg/L glyphosate herbicide is excluded from the t-test due to limited data points available for evaluation. The results of the t-test of means for two independent sample scores are illustrated in Table 4.6 below.

The findings of the Student t-test display similar results to the percentage pollutant removal analyses in Section 4.3.1. The p-values indicate significant differences between the majority of the indigenous species and the unvegetated soil control. Species with significant differences are illustrated by p-values ≤0.05.

Table 4.6: t-test evaluation for percentage removal between individual plant species and unvegetated soil control[15].

Percentage removal differences with soil			
Species	P-value	Species	P-value
NH_3		NO_3^-	
Cynodon dactylon	0.000000232	*Cyperus textilis*	0.000179
Cyperus textilis	0.000000422	*Cynodon dactylon*	0.000216
Phragmites australis	0.000000530	*Phragmites australis*	0.00025
Typha capensis	0.000000935	*Juncus lomatophyllus*	0.000915
Isolepsis prolifera	0.0000203	*Isolepsis prolifera*	0.00103
Zantedeschia aethiopica	0.0000266	*Eleocharis limosa*	0.00115
Juncus effusus	0.0000951	*Typha capensis*	0.00118
Juncus kraussii	0.000304	*Juncus effusus*	0.00133
Aristea capitata	0.000461	*Zantedeschia aethiopica*	0.0014
Bolboschoenus maritimus	0.00242	*Aristea capitata*	0.00287
Juncus lomatophyllus	0.00317	*Juncus kraussii*	0.0066
Eleocharis limosa	0.00613	*Bolboschoenus maritimus*	0.022
[16]*Arctotis acaulis*	0.0627	*Carpobrotus edulis*	0.151
Carpobrotus edulis	0.0687	*Arctotis acaulis*	0.234

[15] Sample size (n) = 4, all species consisting of duplicates within the experimental system.

[16] Grey shaded species displayed removal values that were not significantly different in comparison with the unvegetated soil control.

SRP		225 mg/L Glyphosate	
Cyperus textilis	0.000000886	*Bolboschoenus maritimus*	0.0242
Cynodon dactylon	0.00000129	*Aristea capitata*	0.0258
Phragmites australis	0.00000153	*Typha capensis*	0.0266
Juncus lomatophyllus	0.0000183	*Juncus effusus*	0.0284
Typha capensis	0.0000421	*Isolepsis prolifera*	0.0284
Eleocharis limosa	0.0000495	*Cyperus textilis*	0.0346
Juncus effusus	0.0000831	*Eleocharis limosa*	0.043
Bolboschoenus maritimus	0.000346	*Phragmites australis*	0.046
Juncus kraussii	0.00127	*Arctotis acaulis*	0.048
Zantedeschia aethiopica	0.00129	*Zantedeschia aethiopica*	0.0656
Isolepsis prolifera	0.00196	*Carpobrotus edulis*	0.0892
Aristea capitata	0.0304	*Juncus kraussii*	0.13
Carpobrotus edulis	0.038	*Juncus lomatophyllus*	0.145
Arctotis acaulis	0.142	*Cynodon dactylon*	0.254

n = 4, df = 3.

From Table 4.6, the difference in removal efficiency between vegetation and unvegetated soil is more prevalent for the removal of fertiliser nutrients, than herbicides.

From the t-test, displaying p-values, the similarity in fertiliser nutrient removal between wetland plants and unvegetated soil is illustrated by significantly low p-values. In contrast, the indigenous plant species did not display significant variation throughout the Renosterveld community for glyphosate removal. Similar glyphosate removal efficiencies are displayed, with larger p-values, between the plant species and the unvegetated soil control.

4.3.2.1 Factors influencing nutrient remediation

The remediation of nutrients can be theoretically attributed to a variety of processes involved; phytotransformation, rhizosphere bioremediation, phytostabilisation, phytoextraction, phytovolatilization, phytostimulation and phytodegradation (Terry & Banuelos 2000; Dietz & Schnoor 2001; Pilon-Smits 2005).

4.3.2.2 Factors influencing glyphosate remediation

The exceptional reduction in glyphosate concentration within the effluent solution can be theoretically attributed to a variety of processes involved; glyphosate adsorption to soil, the microbial degradation of glyphosate occurring predominantly in soil, phytostabilisation, rhizosphere bioremediation, root binding, herbicide immobilization and a reduction in herbicide half-life as a result of plant interaction (Comes, Bruns & Kelley 1976; Piccolo, Celano & Pietramellara 1992; Alvord & Kadlec 1995; Chamberlain, Evans & Bromilow 1996; Schuette 1998; Terry & Banuelos 2000; Dietz & Schnoor 2001; Pilon-Smits 2005; Cerdeira &

Duke 2006; Lipok, Studnik & Gruyaert 2010; Pérez, Vera & Miranda 2011; Dosnon-Olette et al. 2011; Coupe et al. 2012; Beltrano et al. 2013; Gomes et al. 2016a).

An important deduction, the most effective plant species for the remediation of glyphosate is not consistent with the most effective species for nutrient remediation. Comparing the most effective remediators for the different herbicide dosage concentrations, variation among plant species is further supported. However, there is little variation between plant species when exposed to the same pollutant parameter.

4.4 Effect of glyphosate dosage strength on remediation

The objective was to observe different herbicide dosage strengths on the phytoremediation capabilities of indigenous vegetation, within the experimental phytoremediation system. All indigenous plants and the soil control were subject to two notably different glyphosate concentrations. The first concentration of 0.7 mg/L was selected to represent the nontoxic threshold at which aquatic ecosystems do not undergo degradation (You, Kaljurand & Koropchak 2003).

In contrast, the second 225 mg/L concentration was selected to present an extreme worst case scenario human-error dosage at which plant mortality is high (De Kock 2017, Pers com; Muir 2018, Pers com; Swart 2018, Pers com). The removal efficiency, degradation, phytostabilisation, bioremediation, root binding and immobilization influence of the plants at the different herbicide concentrations are compared, allowing evaluation regarding their phytoremediation capabilities with regard to different stresses.

4.4.1 Analysis of herbicide dosage effect

Evaluating two remarkably different concentrations is achieved by analysing the effluent concentrations change over the duration of the experiment. This method may be used, as the baseline concentration values indicating pollutant content within the treatment silos prior to influent irrigation, is taken as 0 mg/L glyphosate.

Figure 4.9 compares the effluent concentration of 0.7 mg/L glyphosate for vegetation and the unvegetated soil control. The effluent concentrations are depicted on the primary vertical axis whilst the influent concentration is depicted on the secondary vertical axis, both as a function of time.

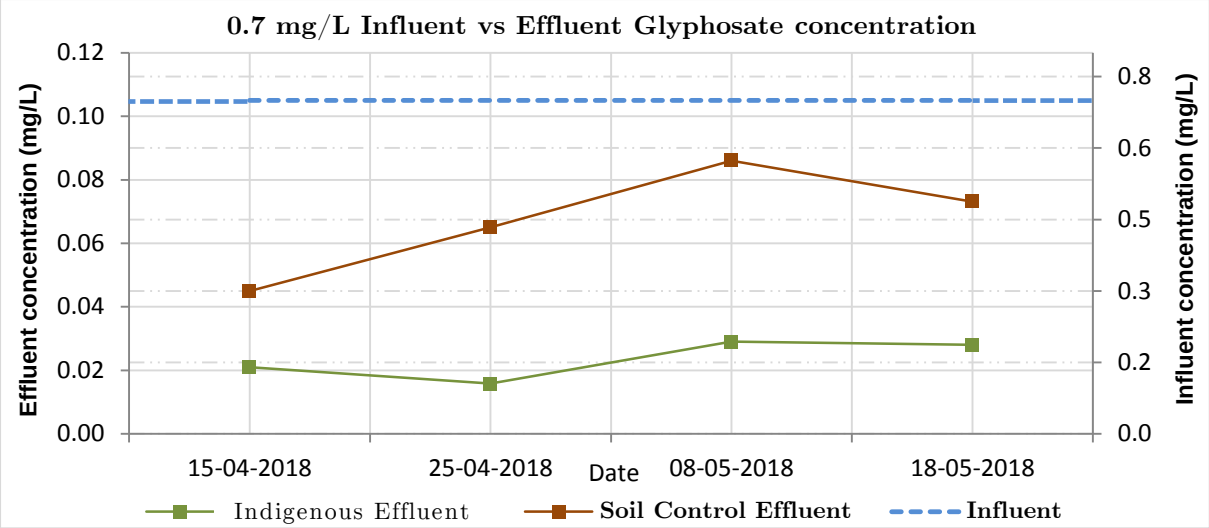


Figure 4.9: The 0.7 mg/L glyphosate influent and effluent removal of vegetation and soil control.

From Figure 4.9 above, the vegetation samples consistently removed glyphosate for the duration of the experiment, whereas remediation by unvegetated soil was less effective. The plant species treatment is seen to be more effective for the removal of 0.7 mg/L glyphosate, derived by the constant lower effluent concentrations produced by plant remediation.

Figure 4.10 compares the effluent concentrations of 225 mg/L glyphosate between vegetation and the unvegetated soil control. The effluent concentrations are depicted on the primary vertical axis whilst the influent concentrations are depicted on the secondary vertical axis, both as a function of time.

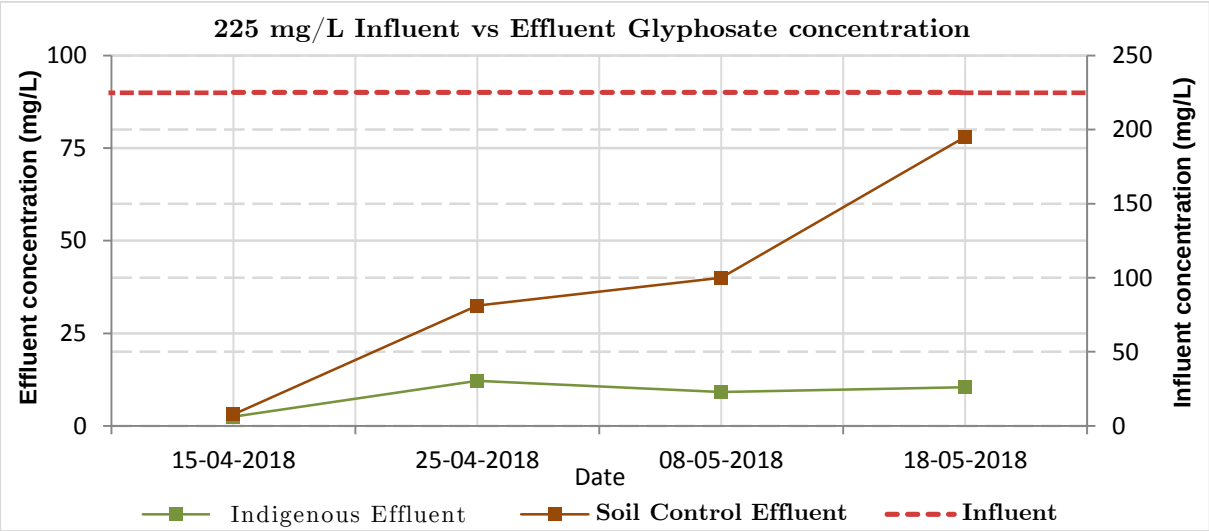


Figure 4.10: The 225 mg/L Glyphosate influent and effluent removal of vegetation and soil control.

Vegetation consistently removed glyphosate throughout the experiment, Figure 4.10 above, similar to the findings of the 0.7 mg/L glyphosate analysis. The soil control presented less remediation than the vegetated treatment. Although the vegetation exhibited little change, a considerable increase was noted in the soil control trend. The accumulation of 225 mg/L glyphosate in unvegetated soil throughout the experiment indicated that it did not

successfully remediate the herbicide solution. The indigenous vegetation remediated the 225 mg/L glyphosate more effectively than unvegetated soil.

The findings of the 225 mg/L glyphosate effluent removal between a community of wetland plant species and the soil control are dissimilar to the effluent removal of 0.7 mg/L glyphosate. The herbicide dosage strengths do influence the removal efficiency of vegetation. With time the vegetation will wilt and die, returning pollutants to the soil (Bratieres et al. 2008; Read et al. 2008; Milandri et al. 2012). The unvegetated soil control rapidly loses the ability to hinder pollutant accumulation at extreme glyphosate dosage concentrations, making it less effective than its vegetated counterparts.

4.5 Influence of selected water quality parameters

(Further illustrated in Appendix G, Figure G.1)

Water quality parameters are known to affect phytoremediation (South Africa 1996a; South Africa 1996b; Clesceri, Greenberg & Eaton 1998; Dallas & Day 2004). Therefore, the aim of this test was to analyse their influence on the remediation of pollutants within this study. The pH, EC and DO of all plant species within the phytoremediation experiment, regardless of influent treatment, was measured. The relationship between the selected water quality parameters and pollutant removal was analysed to gain an understanding of their role in freshwater aquatic ecosystem. The effect of the water quality parameters on glyphosate degradation was also evaluated.

4.5.1 Analysis of water quality parameter influence

Establishing the influence of the selected water quality parameters (pH, EC and DO) on the removal efficiency of vegetation and the unvegetated soil, was achieved by means of a regression analysis on the parameters in question. The percentage removal across all pollutant parameters did not display interdependence with the water quality parameters, which may be as a result of the limited data points.

The coefficient of determination of the water quality parameters and the percentage removal by vegetation and unvegetated soil indicated, at best, a 0.45 correlation, for NH_3-N and DO. Due to limited data points measured, as a result of time and cost constraints, statistical analyses depicting strong correlation are not easily achieved. Therefore, this result does not disprove possible correlations, which may have been achieved had more data been available. These limitations contribute to the need for further research, where the relationship between effluent pollutant removal, water quality parameters and vegetation type needs to be further explored.

The P content of the soil is a key factor in controlling glyphosate availability, with PO_4^{3-} /SRP and the methylphosphonic group of glyphosate competing for root and soil adsorbtion sites, therefore, glyphosate's bioavailability in a soil-water solution is regulated by the soil's

capacity to adsorb PO_4^{3-} (Bott et al. 2011; Gomes et al. 2015; Gomes et al. 2016a; Gomes et al. 2016b). As glyphosate desorbs it becomes mobile and available for root uptake (Beltrano et al. 2013). In some plant species, PO_4^{3-} fertilisation considerably increases glyphosate's uptake by plant roots and leaf translocation, resulting in glyphosate phytoremediation increase with PO_4^{3-} bioavailability (Gomes et al. 2015; McWhorter et al. 1980; Denis & Delrot 1993; Bott et al. 2011). Regression analysis predicted a slight positive correlation of the dependent SRP variable from the 0.7 mg/L- and 225 mg/L-glyphosate independent variables, as 0.0029 and 0.0829 respectively. The R^2-values produced, indicated that SRP did not significantly explain the variability dependence of the glyphosate data around its mean.

4.6 Multiple indigenous wetland plant species VS multiple invasive alien plant (IAP) and Palmiet species

This objective evaluated and compared the removal efficiencies of indigenous wetland plant species, which naturally occur within Renosterveld amongst other vegetation areas, displaying rapid transpiration and growth and IAP species and Palmiet currently used in SuDS, constructed wetlands and biofiltration treatment trains. The silos equipped with multiple plants were used for this experiment. Plants of similar physiology selected from literature were used for community comparison (Read et al. 2010). The removal efficiencies of the plant species within the indigenous wetland community and IAP and Palmiet community were compared.

The indigenous wetland plants selected for this test included: *Phragmites australis*, *Cyperus textilis*, *Typha capensis* and *Cynodon dactylon*. The IAP species were: *Canna indica*, *Arundo donax* and *Pennisetum clandestinum,* with *Prionium serratum* included in the assemblage. All pollutants and selected water quality parameters were analysed. The aim was to examine whether potential exists to integrate the less invasive indigenous plant species instead of the IAP and Palmiet species currently used in local and international constructed wetlands, SuDS and biofiltration systems (Bratieres et al. 2008; Read et al. 2008; Schachtschneider, Muasya & Somerset 2010; Milandri et al. 2012).

4.6.1 Analysis of wetland plants VS IAP and Palmiet percentage pollutant removal

The selected communities of wetland plant species VS IAP and Palmiet species display a normal distribution (illustrated in Appendix F, Figure F.4). This allowed a Student's t-test application, to determine the degree that the communities differ with regard to their pollutant extraction capacities.

Figure 4.11 to Figure 4.16 illustrate the remediation of pollutants for the indigenous community, IAP community and Palmiet and their soil control. The percentage pollutant removed is depicted on the vertical axis as a function of time on the x-axis.

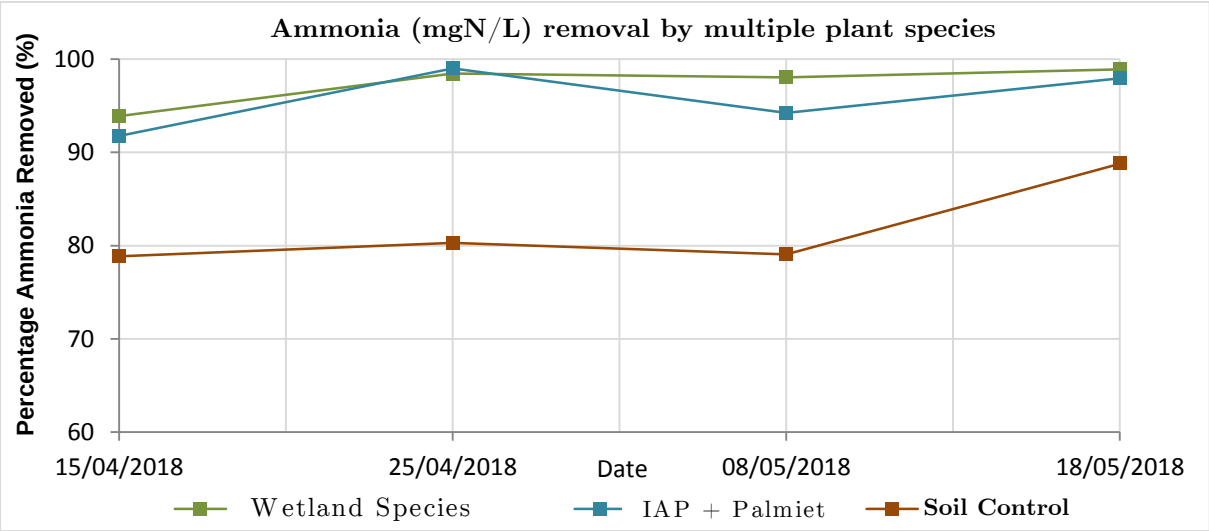


Figure 4.11: Percentage Ammonia removed between plant communities.

Figure 4.11 depicts the percentage NH_3-N removed. Both the selected wetland plant species that naturally occur within Renosterveld vegetation and IAP and Palmiet vegetation display greater removal than the unvegetated soil control. The vegetated media are very similar with regards to removal capabilities, with both plant communities displaying excellent remediation. There is no conclusive evidence to select one community above the other, for the removal of NH_3.

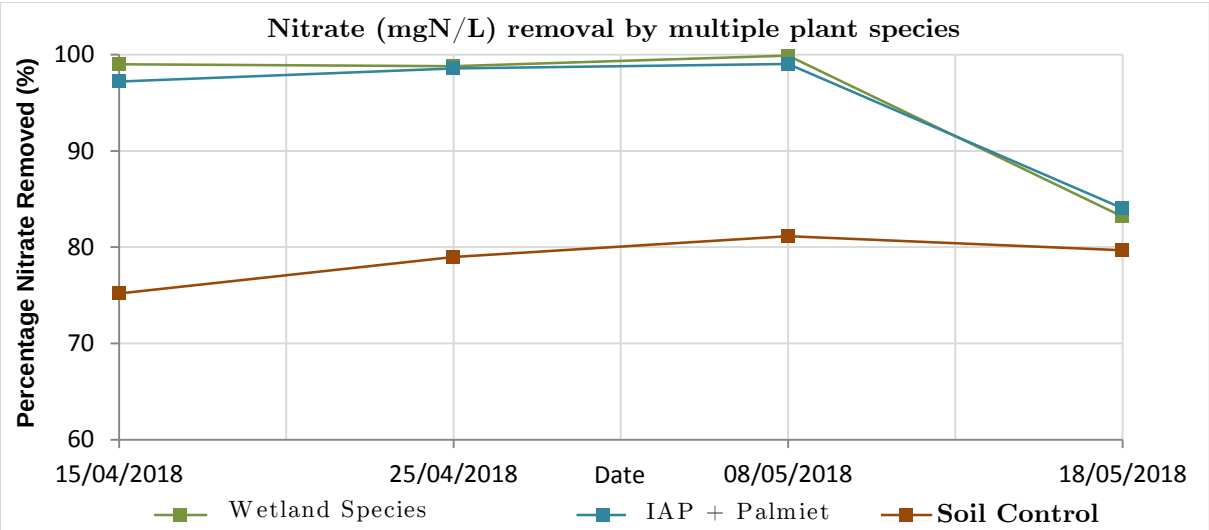


Figure 4.12: Percentage Nitrate removed between plant communities.

Figure 4.12 illustrates the percentage NO_3^--N removed. The chosen indigenous wetland plant assemblage and IAP and Palmiet assemblage more effectively remediated NO_3^- than the

unvegetated soil control. The vegetation presented very similar trends with regard to pollutant removal, at the start and midway of the experiment efficient removal is apparent, however, the communities became less effective with time. There is no conclusive evidence in selecting one community above the other, for the removal of NO_3^-.

From Figure 4.12 it is evident that the NO_3^- concentration rapidly increased at the final sampling date, this may be the result of a variety of factors. Nitrifying bacteria may theoretically be responsible for the increase in NO_3^-, due to microorganisms converting NH_3 to NO_3^-, however this conversion would display a slight decrease in NH_3 at the date in question, thus we reject this hypothesis (Van der Merwe 2016).

The nitrification process encompasses a secondary conversion by which bacteria (*Nitrobacter, Nitrospina* and *Nitrococcus*) converts NO_2^- to NO_3^- (Van der Merwe 2016). In agriculture, irrigation with dilute solutions of NH_3 results in an increase in soil NO_3^- through the action of nitrifying bacteria (South Africa 1996b; Clesceri, Greenberg & Eaton 1998). The paired NO_3^- concentration increase for indigenous wetland species and IAP species with Palmiet is presumed to be as a result of the nitrification conversion of NO_2^- to NO_3^-. The biological oxidation of NH_3 to NO_2^- followed by the oxidation of the NO_2^- to NO_3^- may be a possible source of NO_2^-. An evaluation to test this hypothesis requires the analyses of microorganism establishment and NO_2^- formulation.

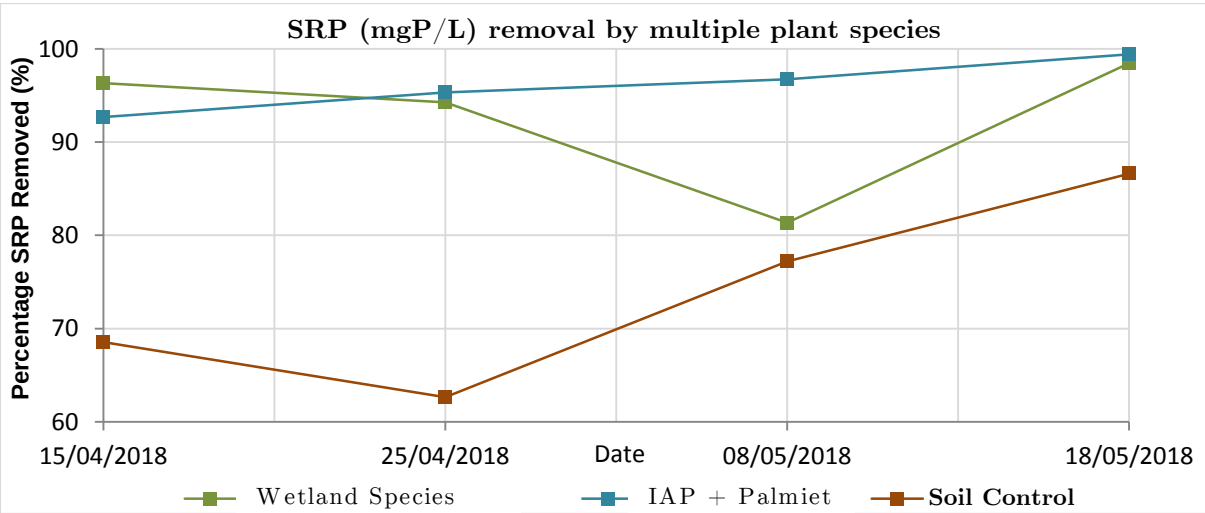


Figure 4.13: Percentage SRP removed between plant communities.

Figure 4.13 illustrates the percentage removal of SRP. Both indigenous wetland- and IAP with Palmiet- vegetation presented stronger removal efficiency than the unvegetated soil control. However, the IAP and Palmiet vegetation trend demonstrates that this community is more consistent with regards to the removal of SRP. Indigenous species do, however, display similar removal efficiency to the IAP and Palmiet species with time. There is no conclusive evidence to select one community above the other, for the removal of SRP.

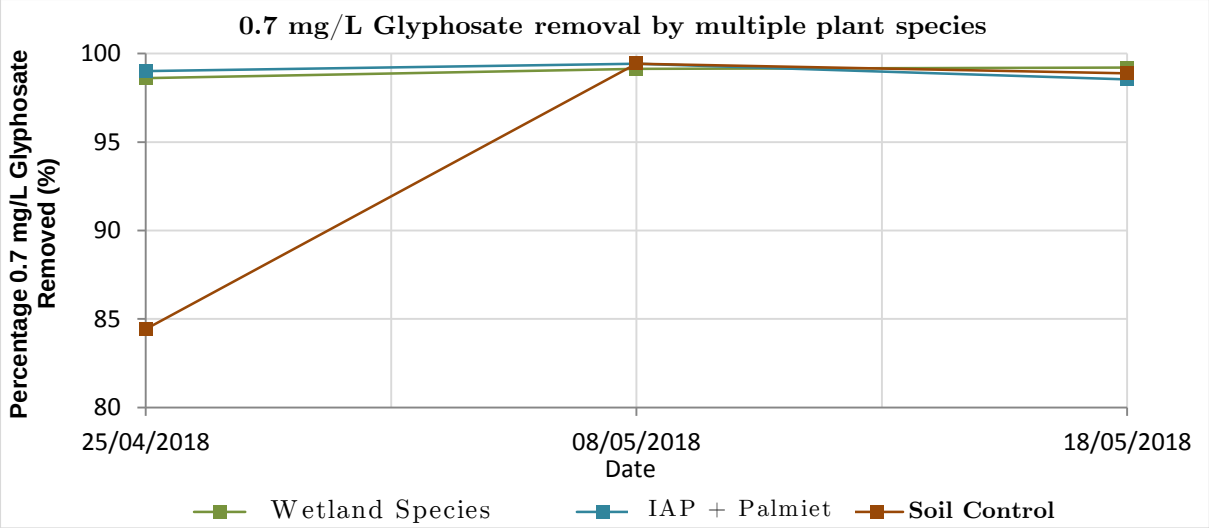


Figure 4.14: Percentage 0.7 mg/L glyphosate removed between plant communities.

Figure 4.14 illustrates the percentage removal of 0.7 mg/L glyphosate. Although both indigenous wetland vegetation and IAP with Palmiet vegetation indicated more effective glyphosate removal than the unvegetated soil control for the first data point, a conclusion cannot be made. Due to insufficient data points collected, as a result of time and funding constraints, with regard to this pollutant parameter during the less invasive indigenous VS IAP and Palmiet experiment.

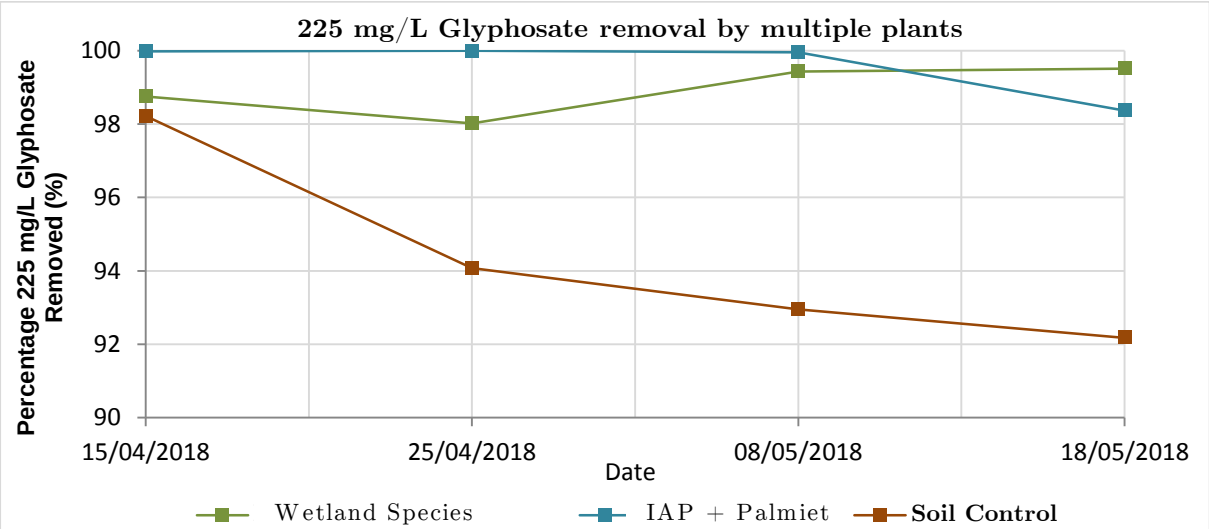


Figure 4.15: Percentage 225 mg/L glyphosate removed between plant communities.

Figure 4.15 illustrates the percentage removal of 225 mg/L. Both the indigenous wetland vegetation and IAP with Palmiet vegetation presented greater removal of 225 mg/L glyphosate than the unvegetated soil control. In contrast to the consistent trend upheld by the two vegetative media, the soil control depicts a negative trend, as a result of a reduction in influent pollutant extraction or degradation. There is no conclusive evidence in selecting one community above the other, for the removal of 225 mg/L glyphosate.

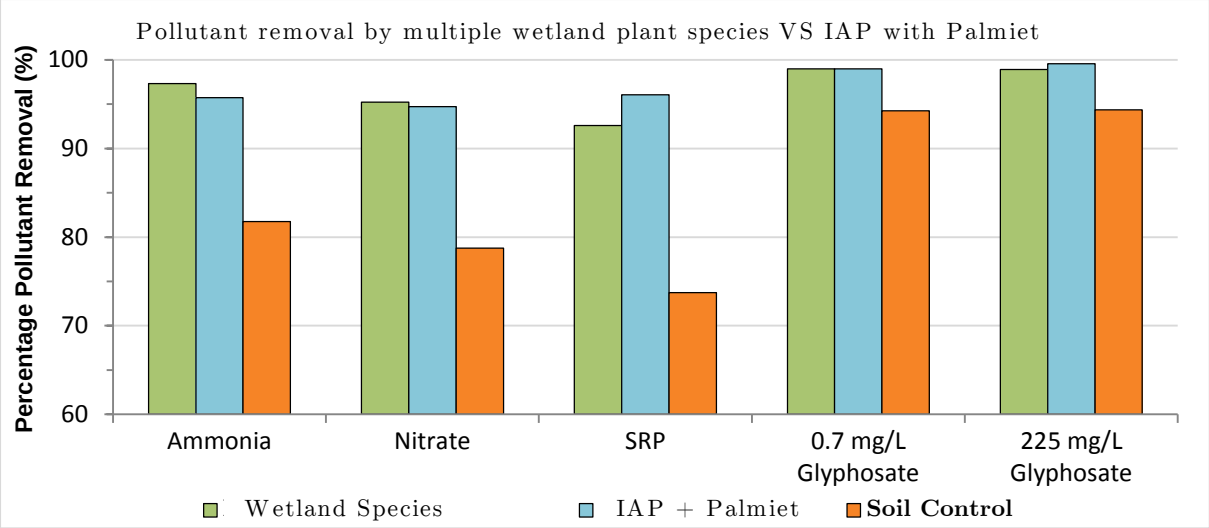


Figure 4.16: The percentage pollutant removal of wetland plant species, IAP species with Palmiet and soil control.

Figure 4.16 above, represents the difference in the mean percentage pollutants removed between the indigenous vegetation, the IAP and Palmiet vegetation and the unvegetated soil control. We can infer that there is no evidence that suggest one community to be more effective in removing pollutants than the other. It is however evident that both the indigenous and IAP with Palmiet assemblages are more effective for the removal of pollutants than the unvegetated soil control. This finding is further supported by a t-test, evaluating the relationship between the three media.

The Student's t-test determined if the removal efficiencies of the two independent media are significantly different from each other. The findings are illustrated in Table 4.7 below.

Table 4.7: t-test evaluation for percentage removal between media.

Percentage removal differences between media				
	Pollutant			
Comparison	NH_3	NO_3^-	SRP	225 mg/L Glyphosate
Indigenous VS soil	0.000523	0.00394	0.0136	0.00829
IAP with Palmiet VS soil	0.00147	0.00282	0.0031	0.00495
Indigenous VS IAP with Palmiet	[17]0.233	0.463	0.216	0.135

n = 4, df = 3.

From Table 4.7 above, the pollutants removed by both indigenous and IAP with Palmiet vegetation is statistically significantly different (P-value ≤0.05), in relation to the removal of the unvegetated soil, indicating both vegetation types as particularly more effective across all pollutants than soil alone. The p-values, generated by comparing indigenous and IAP with

[17] P-values >0.05 indicate that the two independent media are not significantly different, thus displaying similar pollutant extraction capacities.

Palmiet vegetation are >0.05 across all pollutants, the two vegetation types are very similar with regard to pollutant extraction or degradation. The t-test findings, indicating large p-values between indigenous and IAP with Palmiet vegetation, conclude that one vegetative community is not significantly more effective in removing pollutants than the other, thus wetland species that naturally occur within Renosterveld may be used instead of their more invasive counterparts.

The percentage removal similarities between the selected indigenous plant species and the IAP species with Palmiet, and their dissimilarity to the unvegetated soil control at a 95% Confidence Interval are further illustrated in Appendix F, Figure F.5.

4.7 Ranked community composition effective for all pollutants

The objective of this test was to evaluate and determine the potential of indigenous plant species best suited in phytoremediation systems. Individual species remediation aids the establishment of biodiverse plant assemblages. The effective removal over all pollutant parameters is taken into consideration, with a rank order established. Pollutant removal was compared to the unvegetated soil control to establish sediment contribution in the remediation of pollutants. The pollutant parameters comprised all fertiliser nutrients (NH_3, NO_3^- and PO_4^{3-}) as well as the 225 mg/L glyphosate-based herbicide concentration.

4.7.1 Analysis of cumulative pollutant remediation

Cumulative pollutant removal examines the mean percentage pollutant removal of individual species across the pollutant parameters in its entirety. The phytoremediatory capabilities of the individual plant species are used to identify a community of plants capable of targeting a selection of pollutants in agricultural runoff. The Kruskal-Wallis H-test for ranks is a one-way ANOVA, comprising of a non-parametric method to compare two independent sample scores. The test analysed the mean percentage removal of the individual species across the pollutants with time, indicating the cumulative pollutant species remediation. The p-values obtained when comparing the remediation values of individual species with the unvegetated soil, indicate to what extent the different individual plant species differ with regard to their cumulative pollutant removal. P-values ≤0.05 rejects the hypothesis that unvegetated soil and indigenous species display similar pollutant remediation. The plant species are ranked according to individual pollutant remediation, indicating significance of difference and not magnitude of difference.

From Table 4.8 below, it is evident that the three most effective species; *Phragmites australis*, *Cyperus textilis* and *Cynodon dactylon*, display significantly more effective cumulative pollutant removal compared to the unvegetated soil control. An essential finding of the current study is that the entire indigenous wetland plant community was individually more effective in remediating or degrading pollutants than the unvegetated soil control.

Table 4.8: Rank order of wetland species cumulative pollutant removal efficiencies, with regard to the soil control.

Rank	Wetland species	P-value
1	*Phragmites australis*	0.00000000113
2	*Cyperus textilis*	0.00000000217
3	*Cynodon dactylon*	0.0000000105
4	*Juncus lomatophyllus*	0.000000148
5	*Typha capensis*	0.000000584
6	*Zantedeschia aethiopica*	0.00000193
7	*Juncus effusus*	0.0000088
8	*Juncus kraussii*	0.0000116
9	*Isolepsis prolifera*	0.0000337
10	*Eleocharis limosa*	0.0000642
11	*Aristea capitata*	0.000115
12	*Bolboschoenus maritimus*	0.000742
13	*Carpobrotus edulis*	0.00751
14	*Arctotis acaulis*	0.0474

n = 4, df = 3.

Table 4.8 illustrates that the pollutant removal values of all indigenous plant species are statistically significantly different compared to the unvegetated soil control. With p-values consistently <0.05 for all plant species, we can conclude that indigenous plant species are significantly more effective than unvegetated soil, in the remediation of nutrients and herbicides.

Indigenous vegetation presented specific species that were more effective in removing particular pollutants and not others. For example, *Cynodon dactylon* removed 96% of the nutrients (NH_3, NO_3^- and PO_4^{3-}), but was least effective for the removal of 225 mg/L glyphosate with an average removal of 88.34%. A similar finding with *Cyperus textilis*, which on average removed 97% of all the nutrients, but was the least effective in the removal of 0.7 mg/L glyphosate, displaying an average removal of 92.84% (Table 4.4 and 4.5).

4.8 Root growth effect in pollutant removal

This analysis indicates the effect of plant root growth in pollutant extraction efficacies. The effective removal over all the fertiliser and herbicide pollutants were taken into consideration.

4.8.1 Analysis of Root growth effect in pollutant removal

Evaluating the effect of plant growth was achieved by analysing the cumulative pollutant removal values, acknowledging root length growth. The species root lengths were examined by removing the plants from their growth silos after experimental conclusion. The root length measured during the transplantation process was subtracted from the total root length to determine the experimental root growth value. Table 4.9 lists the 14 selected

wetland plant species in cumulative pollutant removal rank order displaying significance but not magnitude with their measured corresponding root length values.

Table 4.9: Root growth of ranked wetland plant species for the duration of the study.

Species root growth	
Wetland species	Root growth (cm)
Phragmites australis	35
Cyperus textilis	31
Cynodon dactylon	28
Juncus lomatophyllus	28
Typha capensis	17
Zantedeschia aethiopica	26
Juncus effusus	24
Juncus kraussii	24
Isolepsis prolifera	22
Eleocharis limosa	19
Aristea capitata	15
Bolboschoenus maritimus	16
Carpobrotus edulis	6
Arctotis acaulis	5

From Table 4.9 above, the root length of plant species potentially accounted for the phytoremediatory capabilities of pollutants. It is evident that plants with longer roots were more effective in extracting pollutants from the soil. The longer and denser the plant roots, the greater removal efficiencies they displayed. This promotes the importance of plant physiology in selecting phytoremediation technologies, with the rhizosphere the most important area in pollutant extraction (Terry & Banuelos 1999; Dietz & Schnoor 2001; Read et al. 2008; Read et al. 2010).

4.9 Temporal effect on cumulative pollutant removal

(Further illustrated in Appendix F, Figure F.3)

The objective of this test was to evaluate the effect of time on the removal efficiency of the selected wetland plant species and the unvegetated soil control. With time, in the presence of different pollutants and pollutant concentrations, the metabolic processes and physiology of plants are altered (Terry & Banuelos 1999; Arias-Estévez et al. 2008; Read et al. 2010). The removal efficiencies of all pollutants were incorporated to determine efficiency for the duration of the study.

4.9.1 Analysis of the temporal effect on cumulative pollutant removal

In determining the effect of time on pollutant remediation, we presented the potential threshold at which plant species lose their phytoremediatory capabilities after being exposed

to pollutants. The cumulative pollutant removal efficiency values of individual wetland plant species and the unvegetated soil control are evaluated at each round of sampling to establish remediation change with time. Figure 4.17 illustrates the temporal effect of the three most effective species: *Phragmites australis*, *Cyperus textilis* and *Cynodon dactylon*, with their corresponding trend lines.

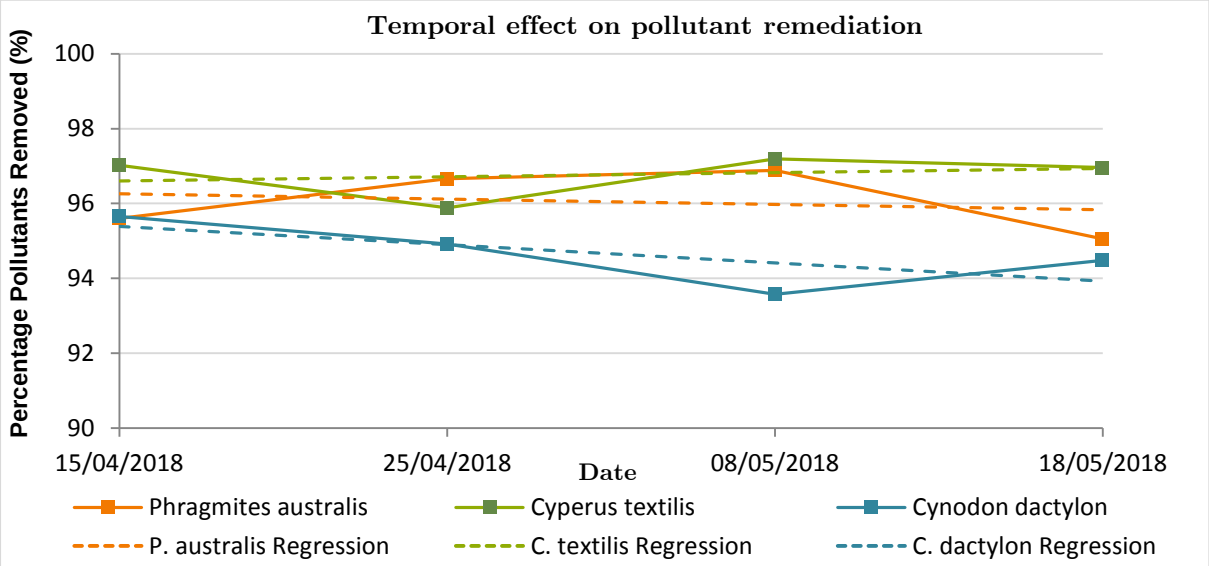


Figure 4.17: Temporal effect on the remediation of the three most effective species.

From Figure 4.17, the removal efficiency of the three most effective plant species are relatively stable with time. The plant species do not seem to lose efficacy with the undeviating addition of different pollutants, for the duration of the study. The steady trend suggests that the wetland plant species indigenous to Renosterveld, at least for the time period under study, are potentially very strong candidates for agricultural pollutant remediation. The plant species successfully withstood the deleterious effects of the pollutant parameters under study, whilst effectively extracting these pollutants from the soil-water solution over two months. This finding is reflected by the remaining wetland plant species, indicating resistance to damage for the duration of the study, at the same time mitigating the pollutants that they encounter. The temporal effect on specific removal efficiencies of the remaining Renosterveld community are represented in Appendix G, Figure G.1.

4.10 Application of findings

The results revealed that plants differ with regard to pollutant remediation, emphasizing the need to include a variety of plants in the design of a phytoremediation system to ensure effective remediation of agricultural pollutants. The inclusion of plants differing in pollutant extraction and physiology will not only target a broader range of agricultural pollutants but also support biodiversity (Terry & Banuelos 2000; Milandri et al. 2012; Curtis 2017). The addition plants that display less effective pollutant removal plays an important role, their presence in the field will hinder flow rates, encouraging infiltration into the soil and improve

water quality. The establishment of biodiverse indigenous Renosterveld buffers in corridors along rivers serve as refuges for predatory insects, contributing to the biological control of insects (Dennis & Fry 1992; Giliomee 2006).

Plant heterogeneity for the remediation of pollutants is consistent with the findings of previous studies, indicating that plant species vary in their pollutant-removal performances, proving that the design of an effective remediation system must incorporate a range of species (Bratieres et al. 2008; Read et al. 2008; Milandri et al. 2012). In line with similar research, the current study found unvegetated soil to aid the remediation of all pollutant parameters, especially regarding the removal of herbicides (Read et al. 2010; Milandri et al. 2012). Soil pollutant accumulation in phytoremediation systems have long-term maintenance implications, for instance pollutant leaching into groundwater systems. The maintenance and mitigation of pollutant accumulation both require measures such as replacing the soil and harvesting the vegetation for disposal, or use as biofuel (Zhu et al. 2009). When a plant incorporated into a phytoremediation system reaches mortality and starts to decompose, the plant material will return the non-translocated, non-metabolized and non-volatized extracted pollutants into the soil. By harvesting vegetation used in phytoremediation systems that has become prone to mortality for biofuel, may solve this key ecological infrastructure limitation.

Phytoremediation reduces the impact of pollutants on receiving freshwater aquatic ecosystems in the short-term, these systems must be appropriately designed to include the monitoring of pollutant accumulation and the need to replace contaminated soil.

CHAPTER 5 : CONCLUSION

The study determined wetland plant species present in Renosterveld ecosystems to be capable of phytoremediating agricultural pollutants, thus mitigating its degradatory effects on aquatic freshwater ecosystems and improving the quality of water. The thesis statement; "Plant species within Renosterveld vegetation have the phytoremediatory potential to reduce herbicide and fertiliser nutrient loads", is supported by the results obtained from the research. The research objectives of this study were to evaluate, and:

- Compare the remediation efficacy of a Renosterveld community VS unvegetated soil.
- Identify individual indigenous wetland species' pollutant removal.
- Analyse the effect of glyphosate dosage strength on herbicide remediation.
- Quantify the influence of selected water quality parameters.
- Compare remediation efficacy of indigenous wetland vegetation VS invasive alien plant (IAP) and Palmiet vegetation assemblage.
- Rank species within the Renosterveld community effective across pollutants.
- Analyse the root growth length on cumulative pollutant removal.
- Evaluate the temporal effect on cumulative pollutant removal for the duration of the study (March 3rd - May 18th).

5.1 Summary of findings

The designed system can be used to investigate the phytoremediatory capabilities of plant species, whilst acknowledging the contribution of soil in pollutant remediation. The proposed phytoremediation system allows accurate examination of the removal efficiencies of multiple pollutant parameters across communities of several plant species.

Pollutant remediation between wetland plant species and unvegetated soil indicated that, all indigenous plant species display greater pollutant removal than unvegetated soil. However, soil media contributed significantly towards the pollutant removal efficiencies of vegetation and in many cases remediated the pollutants to an extent. The presence of vegetation consistently exceeded the removal efficiencies of soil media alone.

All wetland plants were effective in removing the fertiliser nutrients (NH_3, NO_3^- and PO_4^{3-}). The species displaying the greatest percentage fertiliser removal were *Cynodon dactylon*, *Phragmites australis* and *Cyperus textilis*. Remediation of the glyphosate-based herbicide pollutants was exceptional, with soil media contributing significantly, depicting *Juncus kraussii*, *Isolepsis prolifera* and *Cynodon dactylon* as the most prominent species for the removal of 0.7 mg/L glyphosate and *Bolboschoenus maritimus*, *Aristea capitata* and *Typha capensis* as the most effective species in the removal of 225 mg/L glyphosate.

Wetland vegetation consistently removed greater percentages of both glyphosate concentrations compared to the unvegetated soil control throughout the experiment, with glyphosate accumulation successfully hindered in the presence of vegetation. At an extreme dosage, reflecting a human-error/worst case scenario concentration, the unvegetated soil

control unsuccessfully restrained glyphosate accumulation, resulting in the rapid build-up of glyphosate in the soil.

Selected water quality parameters (pH, EC and DO) were found not to significantly increase/decrease phytoremediation of species in this study, with similar findings in the case of the soil control.

The four combined indigenous plant species (*Phragmites australis*, *Cyperus textilis*, *Typha capensis* and *Cynodon dactylon*) used in the community analysis were found to exhibit equivalent removal efficiencies across all pollutants when compared to the three IAP species (*Canna indica*, *Arundo donax and Pennisetum clandestinum*) and *Prionium serratum*, with both communities displaying exceptional phytoremediation. Both communities additionally display greater remediation than the unvegetated soil control. At locations subjected to high agricultural and urban pollution, resulting in the degradation of indigenous vegetation, there is a need to treat pollutants (Schachtschneider, Muasya & Somerset 2010). Phytoremediation is a popular technology of choice, due to its cost-effectiveness, aesthetic advantages and long-term applicability. Remediation analysis of indigenous wetland plant species VS invasive alien plant species and Palmiet indicated that invasive plants can be substituted with the less invasive indigenous plant species without losing remediating efficacy, in this case Renosterveld. This in turn contributes to the conservation of endangered vegetation, by increasing the natural biodiversity of an ecoregion.

The cumulative pollutant removal (nutrient and herbicide concentrations) identified plant species within a community, in this case wetland plants found in Renosterveld and other areas, which exhibit significant remediation of contaminated soil-water across all pollutants. The mean cumulative percentage pollutant removal of individual species found within Renosterveld ranged from 78.3 - 96.24% (average cumulative pollutant removal 90.63%), with the soil control removing on average 71.82%. A Kruskal-Wallis H-test indicated that the three most effective indigenous species with regard to their individual p-values when compared to the unvegetated soil control are: *Phragmites australis, Cyperus textilis* and *Cynodon dactylon*. These removal efficiencies are comparable to the findings from similar local and international studies that focused on the biofiltration of stormwater and mine-leachate using wetland plants (Bratieres et al. 2008; Read et al. 2008; Milandri et al. 2012).

5.2 Wetland plant species use for phytoremediation in Renosterveld

Various factors influence the capacity of vegetative buffers in removing agricultural pollutants from surface water-runoff and groundwater, in turn influencing nutrient and herbicide availability for extraction. The location, topography, adjacent agricultural practices, plant choice (biodiversity), pollutants and orientation of extant Renosterveld fragments all contribute to the successful implementation of conservation river corridors

capable of phytoremediation. For instance, not only must plants survive the local climatic conditions and varying agricultural pollutant products, but pollutant retention varies between plant species. The phytoremediatory role of plant species occurring in conservation corridors will provide a holistic approach to conserving the fragmented landscape.

The Renosterveld community utilised within this study displayed exceptional removal efficiencies across all pollutants, making them attractive options for inclusion in vegetative buffer strips along river corridors. The implementation of both dryland and wetland plant species in this study, allows for the design of biodiverse vegetative buffer strips, with plant species selected capable of thriving at various distances from aquatic freshwater systems and along the slope of a riverbank. Indigenous species which exhibit poor remediation capabilities contribute to buffer strips by potentially hindering flow rates, and stabilising sedimentation. Reducing the runoff flow and transport of sediments, pollutant infiltration into the soil is encouraged, with the rhizosphere the most active location for pollutant extraction.

Vegetative buffers implemented in river corridors adjacent to cultivated land, extract N and P from agricultural fertiliser runoff prior to pollutant deposition into watercourses. Improving the water quality restrains cyanobacterial bloom establishment, the result of rapid N and P accumulation in freshwater systems, with eutrophication and salinisation processes regulated.

Incorporating indigenous wetland plant species, both excellent and poor phytoremediators of agricultural pollutants, create a mutualistic relationship. The vegetation hinders surface and subsurface flow rates, establishing ideal conditions for nutrient sorption to roots, where N and P are extracted, translocated, metabolised or volatised by plants. The pollutants in the soil and water bind to the roots and cell walls, hemicellulose within the cell and are transported to different parts of the plants. For herbicide pollutants, mechanisms for remediation are: glyphosate adsorption to soil, microbial degradation of glyphosate occurring predominantly in soil, phytostabilisation, rhizosphere bioremediation, root binding, herbicide immobilisation and a reduction in herbicide half-life as a result of plant interaction.

Habitat connectivity is essential in maintaining natural ecosystems, whereas transformation triggered by habitat fragmentation exhibits detrimental effects to heterogeneity. The fragmented areas directly contribute to the sustainability of the landscape. Indigenous Renosterveld plant species contribute to the biodiversity of the fragmented landscape, aiding conservation of this critically endangered vegetation type. The establishment of conservation corridors as a result of their purification potential link the islands of Renosterveld fragments.

5.3 Limitations

Although the potential use wetland plant species within Renosterveld for the phytoremediation of agricultural pollutants is demonstrated, several factors limited the investigation. The limitations include and are further discussed below:

- The limited growth space of the specimen silos, with increased acclimatisation as a consequence.
- Only one standardised concentration was selected for fertiliser analysis.
- Soil media was moderately altered from the natural composition to allow for water permeability, saturation and percolation.
- Experimentation and sampling were limited by time and funding constraints.

The limited growth space available per silo may have influenced the condition of the plants, as plant roots in some instances required tailoring to allow for transplantation. The process however, ensured that the plant roots were dispersed across the extent of the silos. As a result, pollutants were subjected to contact with roots. The dispersal of plant roots within the silos prevented the percolation of pollutants through the silos without exposure to roots, granting plants the opportunity for pollutant extraction or stabilization.

The fertiliser pollutant removal efficiency parameters, consisting of NH_3, NO_3^- and PO_4^{3-} were analysed at only one standardised concentration for each of the nutrients; 37.096 mgN/L, 9.274 mgN/L and 17.39 mgP/L respectively.

To allow for the percolation of irrigated influent, the soil media was moderately altered with the addition of a handful of pebbles to each silo. The pebbles were evenly distributed throughout the system, by rotating the soil and pebble composition within a pan mixer, aiding water percolation through the growth silos.

The parameters and plant species tested within this experiment were limited by time and funding constraints. The selection of indigenous plant species, pollutants (*Springbok*™ and *Canola Feed*™) and the standardised pollutant concentration parameters were allocated for this study to reflect real-world conditions.

CHAPTER 6 : RECOMMENDATIONS

From the knowledge attained in this study, the potential use of non-invasive (to an extent invasive regarding *Cynodon dactylon*) wetland species naturally found within critically endangered Renosterveld vegetation for the phytoremediation of agricultural pollutants was deemed significant and requires further investigation. The recommendations are as follows:

6.1 Future research

- Investigate the removal efficiency of plant species subjected to a variety of fertiliser influent concentrations.
- A wider range of species selected with regard to plant physiology traits, for collective Renosterveld community removal efficiency analysis.
- The analyses of additional contaminants such as heavy metals, pathogens and suspended solids should be included.
- A larger quantity of and more frequent sampling for the evaluation of pollutant removal must be performed.

6.2 Practical application of Renosterveld for phytoremediation

- The potential use of vegetation in wastewater treatment facilities should be explored.
- The possibility of extracting long-established plants utilised as phytoremediators reaching mortality status (prior to death and depositing pollutants back into the soil) for use as biofuel.
- Investigate the effectiveness of phytoremediation in a Renosterveld field setting.
- Explore the ecosystem response of aquatic-, as well as terrestrial-plant and animal species as a result of the integration of the species under study.
- A cost analysis is recommended to determine the feasibility of active river corridor restoration, implementing efficient phytoremediators, at specific locations.

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PERSONAL COMMUNICATIONS

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Bothma B 2016. Farm Manager, Molenkop. Buffeljagsrivier. Interview on 15 August regarding the Canola farming community and irrigation from the Breede River.

Carden K 2016. Research Coordinator, Future Water and University of Cape Town. Cape Town. Interview regarding Sustainable Urban Drainage Systems and nutrient pollution.

Cowen O 2017. PhD Student, University of Cape Town. Haarwegskloof. Interview on 15 November regarding potential plant species for phytoremediation.

Curtis O 2017. Director, Overberg Renosterveld Conservation Trust. Haarwegskloof. Interview on 15 July regarding Renosterveld vegetation and potential indigenous species for phytoremediation.

De Kock E 2017. Sales Agent, ProtekSA. Cape Town. Interview on 8 March regarding Glyphosate-based herbicides and *Springbok*™ specifically.

February R 2017. Implementation Manager, WWF Water Balance Programme. Stellenbosch. Email correspondence on 13 November regarding potential use of indigenous plant species for phytoremediation and *Prionium serratum* collection.

Groenewald J 2017. Reserve and Centre Manager, Overberg Renosterveld Conservation Trust. Haarwegskloof. Interview on 15 July regarding Renosterveld vegetation and potential indigenous species for phytoremediation.

Jacobs S 2017. Senior Lecturer, Department of Conservation Ecology and Entomology, Stellenbosch University. Stellenbosch. Interview on 21 July regarding phytoremediation as a whole and potential species to be included.

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Milandri S 2017. Environmental Practitioner, Department of Environmental Affairs. Cape Town. Interview on 14 March regarding phytoremediation systems and potential plant species to be included.

Muir D 2018. Specialist Programme Manager, Department of Environmental Affairs. Stellenbosch. Telephonic interview on 2 February regarding Glyphosate dosage strengths and its effect on freshwater aquatic ecosystems.

Nolte S 2017. Business Manager, Arysta LifeScience. Stellenbosch. Interview on 1 July regarding fertiliser and herbicide products applicable to the study, specifically *Springbok™*.

Pieterse P 2017. Senior Lecturer, Department of Agronomy, Stellenbosch University. Stellenbosch. Interview on 9 May regarding herbicides in general.

Poulsen Z 2017. PhD Student, University of Cape Town. Haarwegskloof. Interview on 15 July regarding Renosterveld vegetation and the effect of agriculture.

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APPENDICES

Appendix A: Design of the phytoremediation system

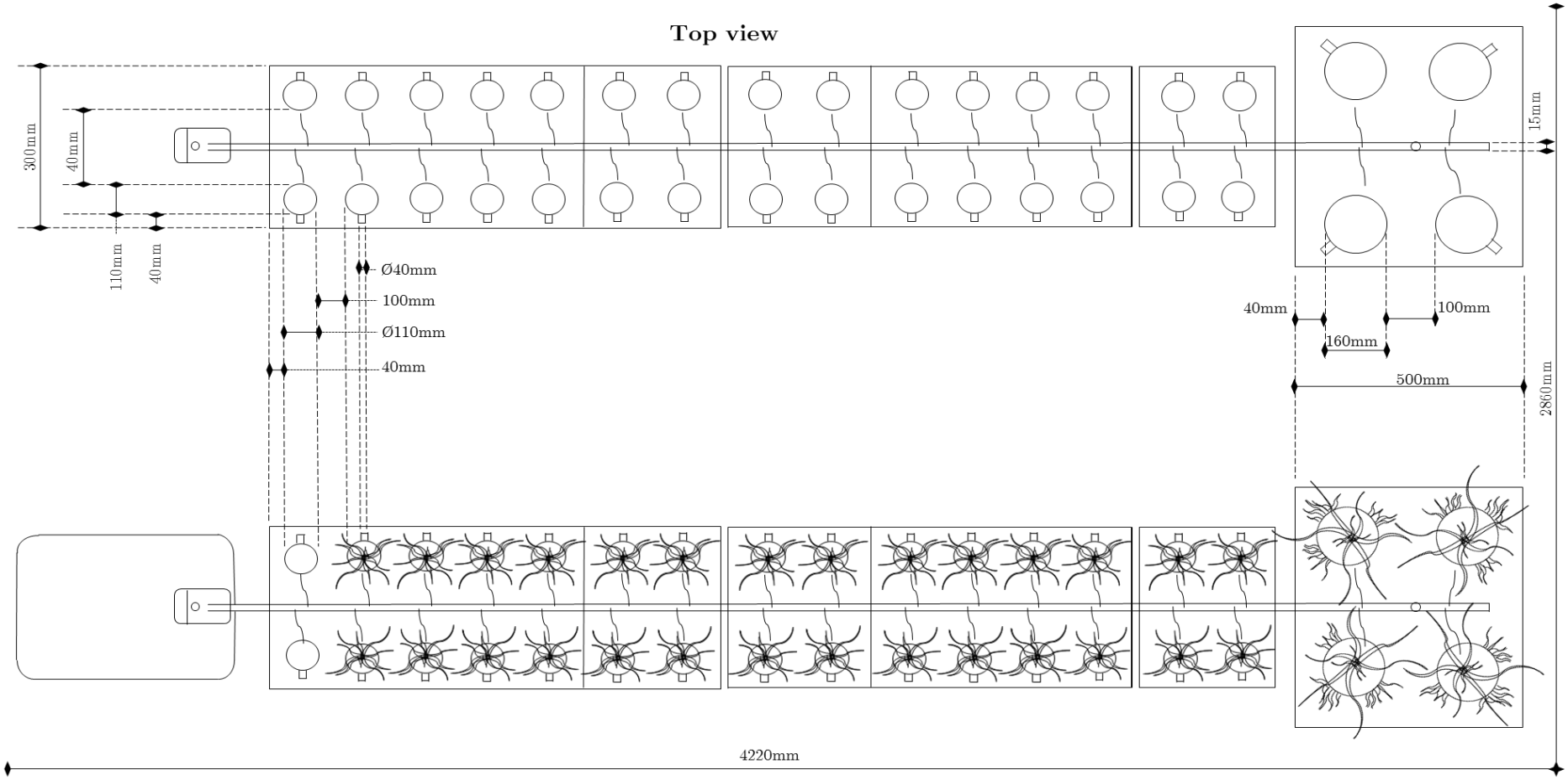


Figure A.1: Top view of the laboratory phytoremediation system.

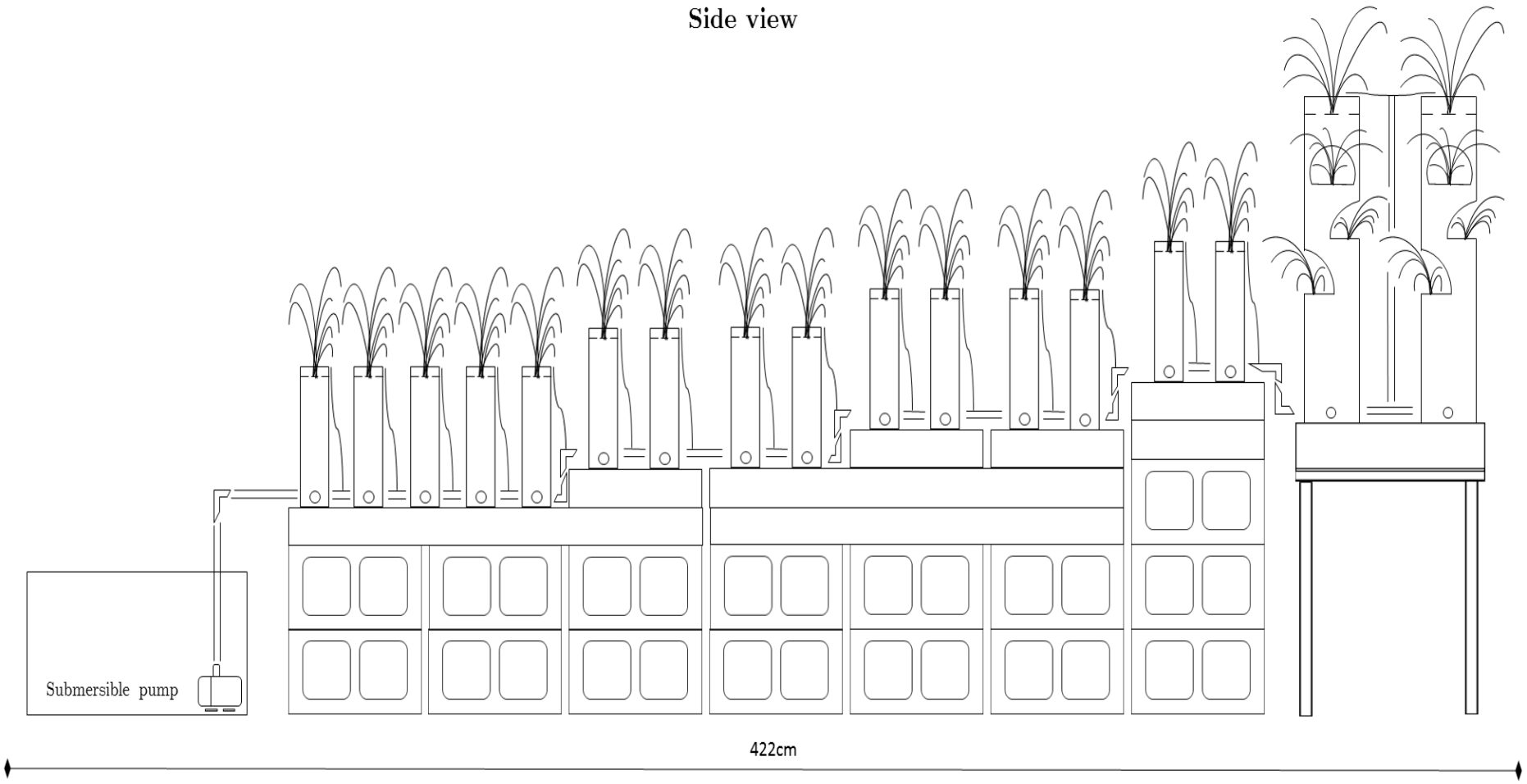


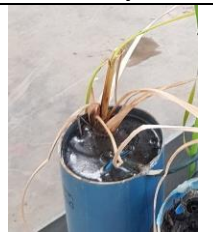
Figure A.2: Side view of the laboratory phytoremediation system.

Appendix B: Sieve Analysis

Table B.1: Wet and dry sieve analysis.

Sieve Analysis		
Sieve Size	Mass of Sieve (g)	Mass of Soil (g)
Wet sieve analysis		
< 0.02µm	1100	23
Dry sieve analysis		
<75µm	1180	1320
75µm	1076	788
150µm	2426	621
300µm	2832	658
600µm	2146	231
1.18mm	1348	156
2.36mm	1120	94
4.75mm	1294	76
6.70mm	1550	23
9.5mm	1480	0

Appendix C: Plant Glyphosate degradation with time.

Concentration	*Typha capensis* Time					
	0 Days	5 Days	10 Days	15 Days	20 Days	25 Days
5%						
2%						
1%						

0.5%						
0.25%						
0.125%						
0.0625%						

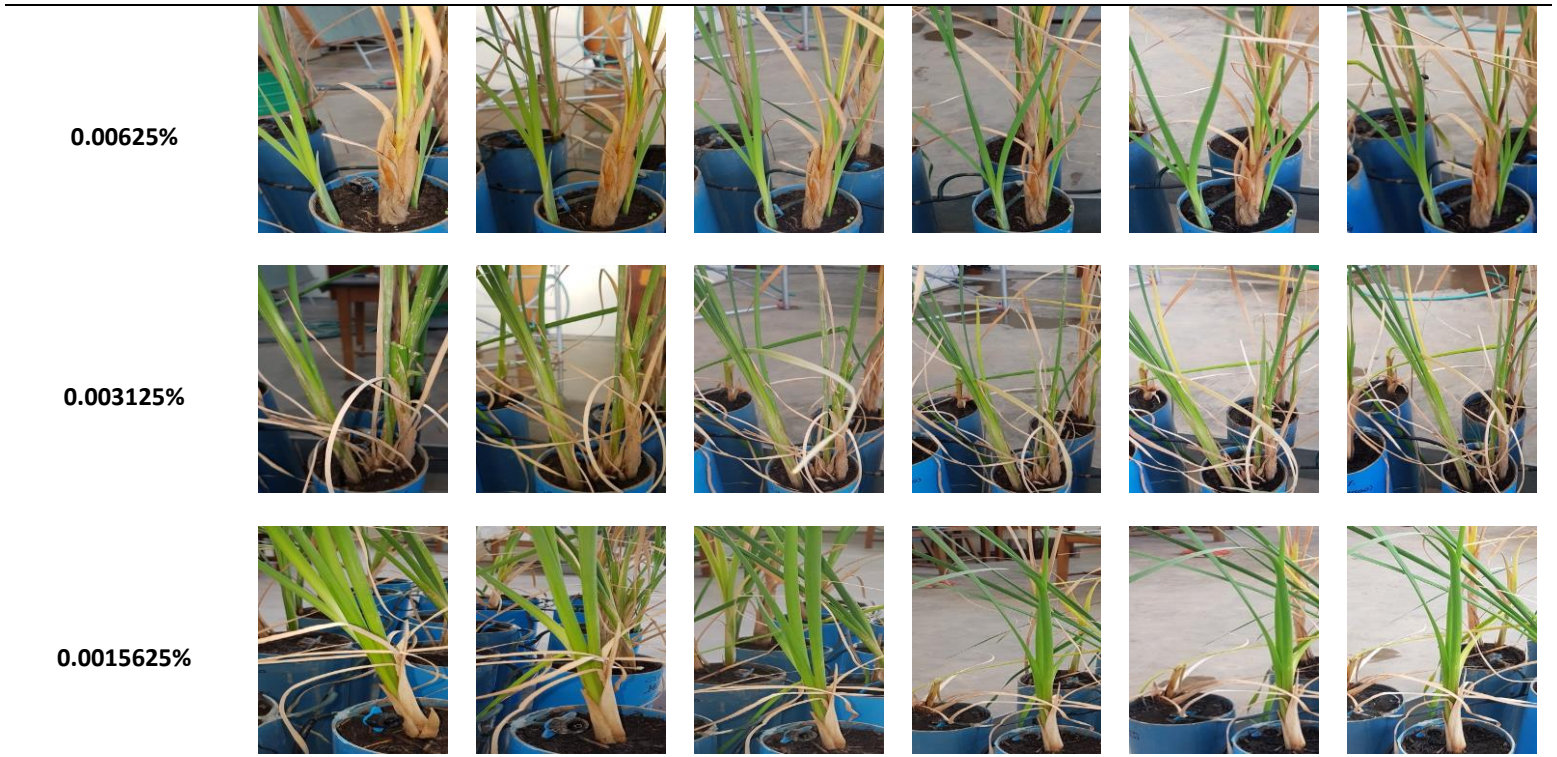
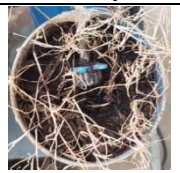
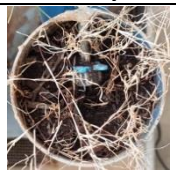
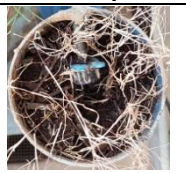


Figure C.1: Typha capensis response to a range of Glyphosate dosage strengths.

Concentration	*Cynodon dactylon* Time						
	0 Days	5 Days	10 Days	15 Days	20 Days	25 Days	30 Days
5%							
2%							
1%							
0.5%							
0.25%							

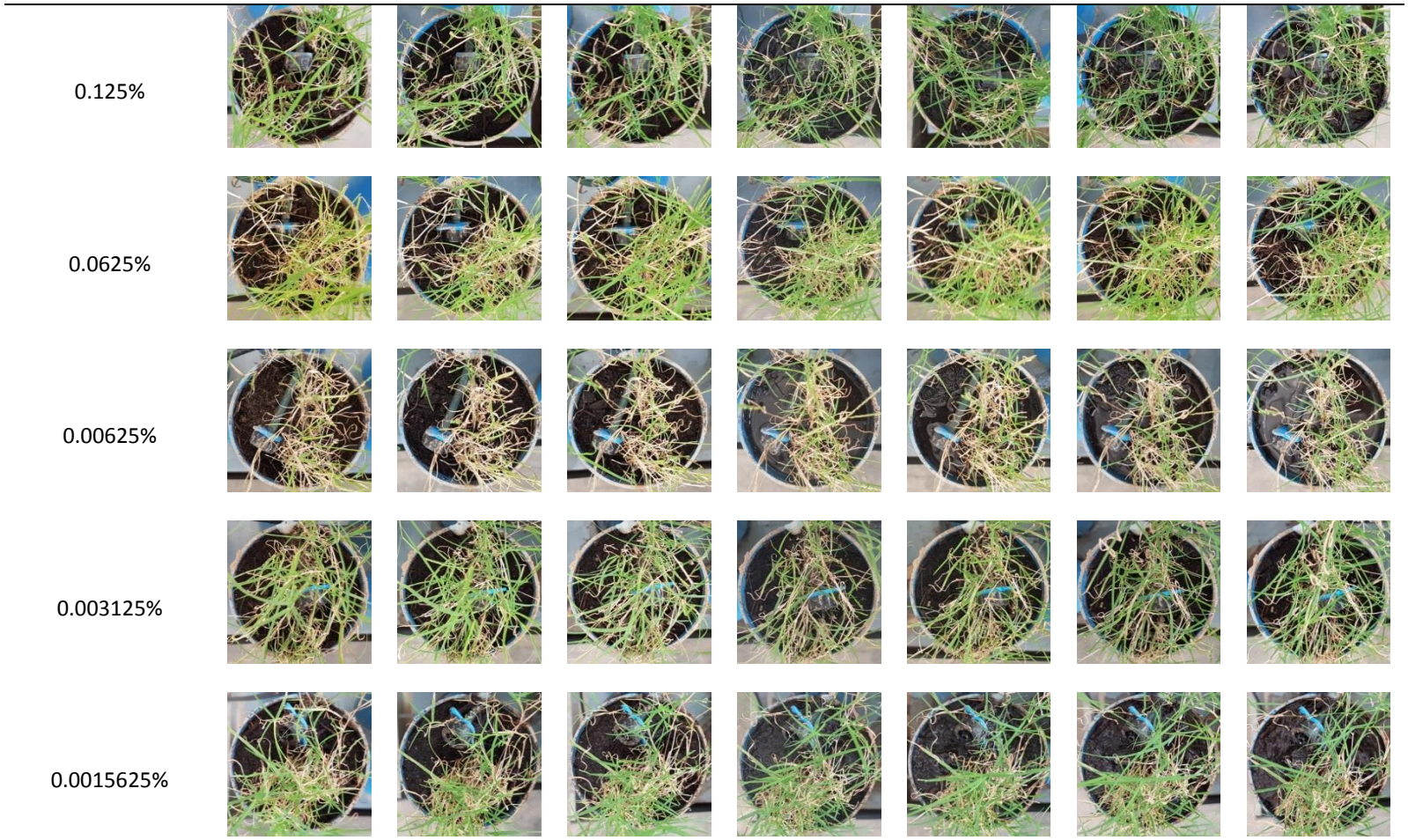


Figure C.2: Cynodon dactylon response to a range of Glyphosate dosage strengths.

Appendix D: Salinity accumulation

Table D.1: Observation of extreme salinity.

Accumulation of Salt in System			
Large Container	**pH**	**Dissolved Oxygen (mg/L)**	**Electroconductivity (µS/cm)**
06/11/2017	8.01	9.56	17050
08/11/2017	8.86	9.16	13490
09/11/2017	7.8	8.57	11430
10/11/2017	8.12	9.19	10140
13/11/2017	8.16	8.81	6860
14/11/2017	8.3	9.5	6400
15/11/2017	8.29	9.19	5980
16/11/2017	8.7	9.59	5750
17/11/2017	8.39	9.31	5370
Small Container	**pH**	**Dissolved Oxygen (mg/L)**	**Electroconductivity (µS/cm)**
08/11/2017	7.98	9.28	8680
09/11/2017	8.21	9.21	7080
10/11/2017	8.55	9.24	4810
13/11/2017	8.06	8.75	1748
14/11/2017	8.07	9.42	1591
15/11/2017	8.25	9.1	1518
16/11/2017	8.37	9.31	1231
17/11/2017	8.5	8.63	1334
Water Tank	**pH**	**Dissolved Oxygen (mg/L)**	**Electroconductivity (µS/cm)**
31/10/2017	7.62	8.64	33700
01/11/2017	7.35	9	1361
02/11/2017	7.39	8.86	1352
08/11/2017	7.41	8.93	155.8
Tap Water	**pH**	**Dissolved Oxygen (mg/L)**	**Electroconductivity (µS/cm)**
31/10/2017	7.13	8.53	47

Appendix E: Data capturing

Table E.1: Influent, baseline and effluent concentration data.

Datum	Sample	NH3 mg/L	NO3 mg/L	SRP mg/L	pH	EC µS/cm	DO mg/L	
03/04/2018	IPPS Soil	0.634	5.94	7.12	9.26	3170	9.64	Baseline Fertiliser and Water Quality Parameter Values measured prior to the addition of pollutants
	C. dactylon	0.523	3.33	11.8	8.36	315	9.55	
	C. textilis	0.192	3	9.78	8.84	983	9.82	
	P. australis	0.391	3.79	7.76	9.01	1283	10.36	
	T. capensis	0.113	1.51	2.76	8.66	421	10.29	
	J. effusus	0.108	3.28	2.85	8.99	1012	10.03	
	C. edulis	0.264	3.92	11.8	8.91	856	10.03	
	A. acaulis	0.048	1.4	2.23	8.89	534	9.84	
	Z. aethiopica	0.141	2.82	3.48	8.17	341	9.08	
	A. capitata	0.295	2.59	1.46	8.33	299	9.2	
	J. lomatophyllus	0.077	1.25	10.4	8.37	566	9.98	
	B. maritimus	0.092	3.16	10.2	8.73	952	9.77	
	I. prolifera	0.106	1.89	4.29	8.79	712	11.08	
	J. kraussii	0.159	2.2	4.05	9	225	10.76	
	E. limosa	0.16	2.61	3.33	8.66	641	10.83	
	SuDS	0.118	6.62	7.95	8.51	1567	10.29	
	Multiple R.veld	0.38	5.02	7.72	8.39	556	7.75	
	MPPS Soil	0.105	2.23	9.3	8.76	697	10.19	

Datum	Sample	NH3 mg/L	NO3 mg/L	SRP mg/L	pH	EC µS/cm	DO mg/L	Glypho 225 mg/L	Glypho 0.7 mg/L
	IPPS Soil	13.35	11.02	14.98	8.62	706	11.05	3.0989	
	C. dactylon	1.89	3.96	12.56	8.81	534	10.77	5.7002	

15/04/2018	C. textilis	1.585	3.51	9.8	8.92	447	10.44	10.0357	
	P. australis	1.656	4.58	8.737	8.93	865	11.09	0.1245	
	T. capensis	2.56	3.12	5.12	8.62	1147	10.34	1.2872	
	J. effusus	6.578	4.111	3.212	8.87	759	10.11	0.3858	
	C. edulis	11.523	8.323	18.33	8.72	591	10.08	1.475	
	A. acaulis	12.259	6.23	10.012	8.18	541	9.87	1.509	
	Z. aethiopica	6.98	4.325	8.36	8.41	631	10.07	1.2213	
	A. capitata	5.66	3.123	9.351	8.54	516	9.9	0.7506	
	J. lomatophyllus	11.21	2.83	11.231	8.42	444	10.12	3.6138	
	B. maritimus	9.88	3.351	13.258	8.53	851	9.86	0.6088	
	I. prolifera	4.12	3.29	9.231	8.7	697	10.9	5.4426	
	J. kraussii	3.81	2.24	8.555	8.54	636	10.24	2.0912	
	E. limosa	3.46	4.41	3.96	8.58	660	10.85	0.4153	
	SuDS	3.18	6.878	9.222	8.43	617	10.16	0.04	
	Multiple R.veld	2.65	5.111	8.357	8.65	710	10.05	2.8029	
	MPPS Soil	7.95	4.53	14.77	8.89	621	10.61	4.01	
Datum	Sample	NH3 mg/L	NO3 mg/L	SRP mg/L	pH	EC µS/cm	DO mg/L	Glypho 225 mg/L	Glypho 0.7 mg/L
25/04/2018	IPPS Soil	27.253	15.01	21.43	8.65	700	11.1	32.4825	0.0839
	C. dactylon	2.754	4.38	13.321	8.78	539	10.72	30.508	0.0043
	C. textilis	3.777	3.782	10.3	8.95	438	10.55	5.522	0.0448
	P. australis	2.592	5.22	9.13	8.96	870	11.15	4.961	0.0197
	T. capensis	4.87	4.325	6.233	8.6	992	10.33	2.0613	0.0054
	J. effusus	10.125	6.012	6.11	8.89	754	10.18	7.0057	0.0108
	C. edulis	23.067	11.36	24.252	8.8	595	10.01	18.4195	0.0731
	A. acaulis	22.058	9.52	15.98	8.2	540	9.96	15.2748	0.0067
	Z. aethiopica	13.36	5.987	10.651	8.45	622	10.04	12.8907	0.0026

	A. capitata	14.562	5.55	10.5	8.51	501	10.01	1.987	0.0285
	J. lomatophyllus	17.083	3.27	13.231	8.45	446	10.02	29.4985	0.005
	B. maritimus	16.587	7.31	16.241	8.56	858	9.8	0.0715	0.0058
	I. prolifera	10.212	4.82	11.6	8.72	701	10.94	0.2105	0.0049
	J. kraussii	11.79	4.71	9.011	8.55	636	10.08	29.6025	0.0031
	E. limosa	15.237	5.51	6.667	8.54	660	10.73	12.202	0.0067
	SuDS	3.555	7.01	10.033	8.44	602	10.22	0.01	0.0069
	Multiple R.veld	3.23	5.22	9.356	8.63	713	10.12	4.4585	0.0098
	MPPS Soil	15.256	6.48	21.265	8.92	627	10.54	13.32	0.109
Datum	Sample	NH3 mg/L	NO3 mg/L	SRP mg/L	pH	EC µS/cm	DO mg/L	Glypho 225 mg/L	Glypho 0.7 mg/L
08/05/2018	IPPS Soil	41.253	18.23	28.253	8.59	703	11.6	39.9873	
	C. dactylon	1.998	5.01	13.985	8.85	538	10.69	25.0147	
	C. textilis	2.11	4.29	10.4	8.89	451	10.38	0.0592	
	P. australis	2.55	5.3	9.5	8.9	864	11.12	8.1974	
	T. capensis	1.13	4.51	8.212	8.56	1087	10.38	2.0671	
	J. effusus	5.25	6.15	6.61	8.89	754	10.15	0.0339	
	C. edulis	13.6	14.067	30.23	8.69	563	10.07	18.5835	
	A. acaulis	13.444	12.541	21.69	8.15	538	9.78	5.9581	
	Z. aethiopica	4.656	7.54	12.6	8.51	635	10.09	13.0343	
	A. capitata	7.658	6.14	12.21	8.49	511	9.91	0.378	
	J. lomatophyllus	6.84	4.424	14.33	8.47	438	10.15	22.2806	
	B. maritimus	5.832	7.71	17.35	8.49	846	9.86	0.072	
	I. prolifera	4.569	5.2	12.254	8.71	701	10.91	1.2656	
	J. kraussii	3.535	6.625	9.1	8.55	641	10.28	24.1197	
	E. limosa	3.777	6.366	7.14	8.57	662	10.86	6.4548	
	SuDS	2.258	7.1	10.6	8.46	619	10.18	0.1082	0.004

	Multiple R.veld	1.111	5.23	12.6	8.69	711	10.12	1.2747	0.006
	MPPS Soil	7.87	8.23	25.232	8.91	628	10.56	15.8631	0.004
Datum	Sample	NH3 mg/L	NO3 mg/L	SRP mg/L	pH	EC µS/cm	DO mg/L	Glypho 225 mg/L	Glypho 0.7 mg/L
18/05/2018	IPPS Soil	53.156	21.23	34.56	8.62	706	11.05	78.021	0.0252
	C. dactylon	2.958	5.23	14.32	8.81	534	10.77	43.7039	0.0089
	C. textilis	3.265	4.587	10.56	8.92	447	10.44	0.0726	0.0555
	P. australis	4.55	5.555	9.85	8.93	865	11.09	14.4456	0.0572
	T. capensis	3.62	5.57	8.51	8.62	1147	10.34	0.0336	0.0408
	J. effusus	7.25	6.82	7.69	8.87	759	10.11	0.0576	0.0742
	C. edulis	21.547	16.14	35.35	8.72	591	10.08	17.6873	0.0072
	A. acaulis	23.014	14.2	25.57	8.18	541	9.87	7.0858	0.0353
	Z. aethiopica	10.58	8.854	13.9	8.41	631	10.07	16.235	0.042
	A. capitata	12.357	6.36	13.6	8.54	516	9.9	0.8659	0.0299
	J. lomatophyllus	11.9	4.755	16.58	8.42	444	10.12	22.1776	0.0096
	B. maritimus	8.22	7.87	17.58	8.53	851	9.86	0.4632	0.0155
	I. prolifera	7.533	5.89	13.95	8.7	697	10.9	1.0884	0.0079
	J. kraussii	9.639	7.58	9.55	8.54	636	10.24	15.4354	0.0054
	E. limosa	6.854	6.85	8.18	8.58	660	10.85	6.3916	0.014
	SuDS	3.02	8.58	10.7	8.43	617	10.16	3.6704	0.0102
	Multiple R.veld	1.52	6.789	12.87	8.65	710	10.05	1.0993	0.0056
	MPPS Soil	12.03	10.115	27.56	8.89	621	10.61	17.6033	0.0079

Appendix F: Data distribution.

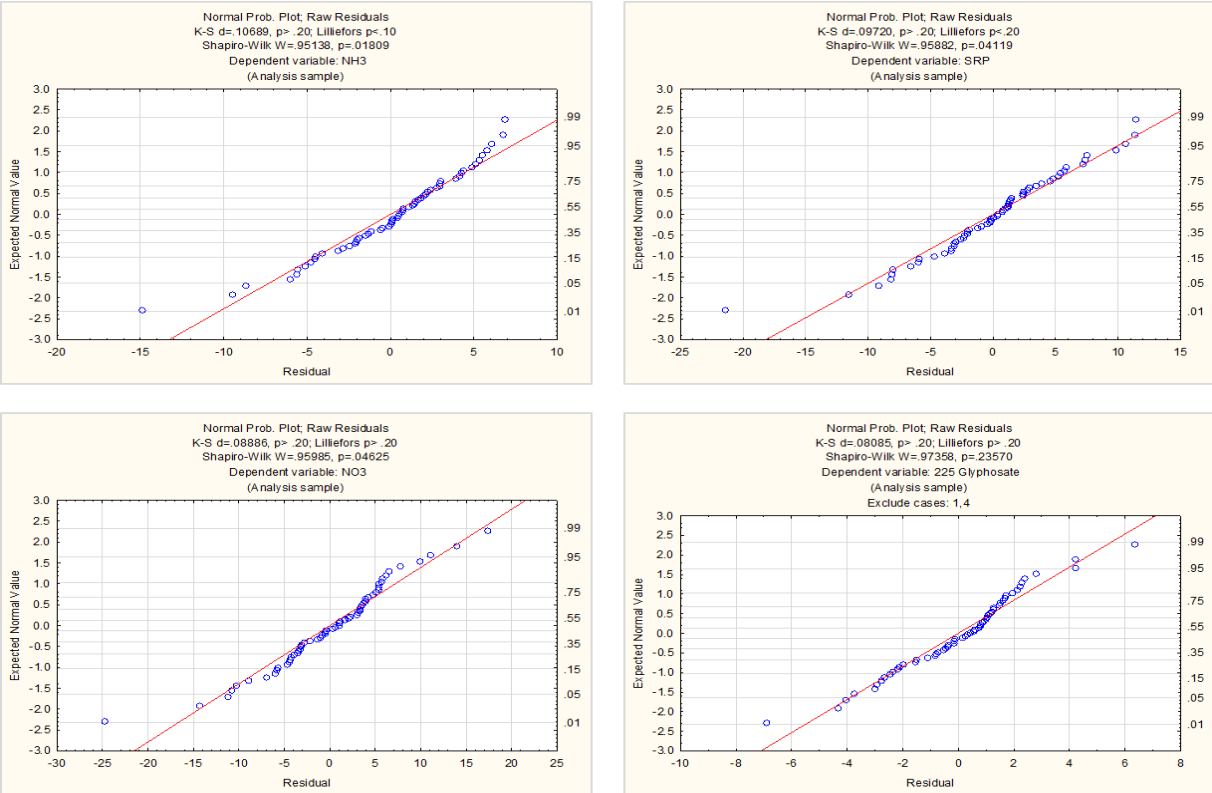


Figure F.1: Data distribution satisfying a normal distribution.

Figure F.2: Main effect analysis of variance with error bars at 95% Confidence Interval to the mean.

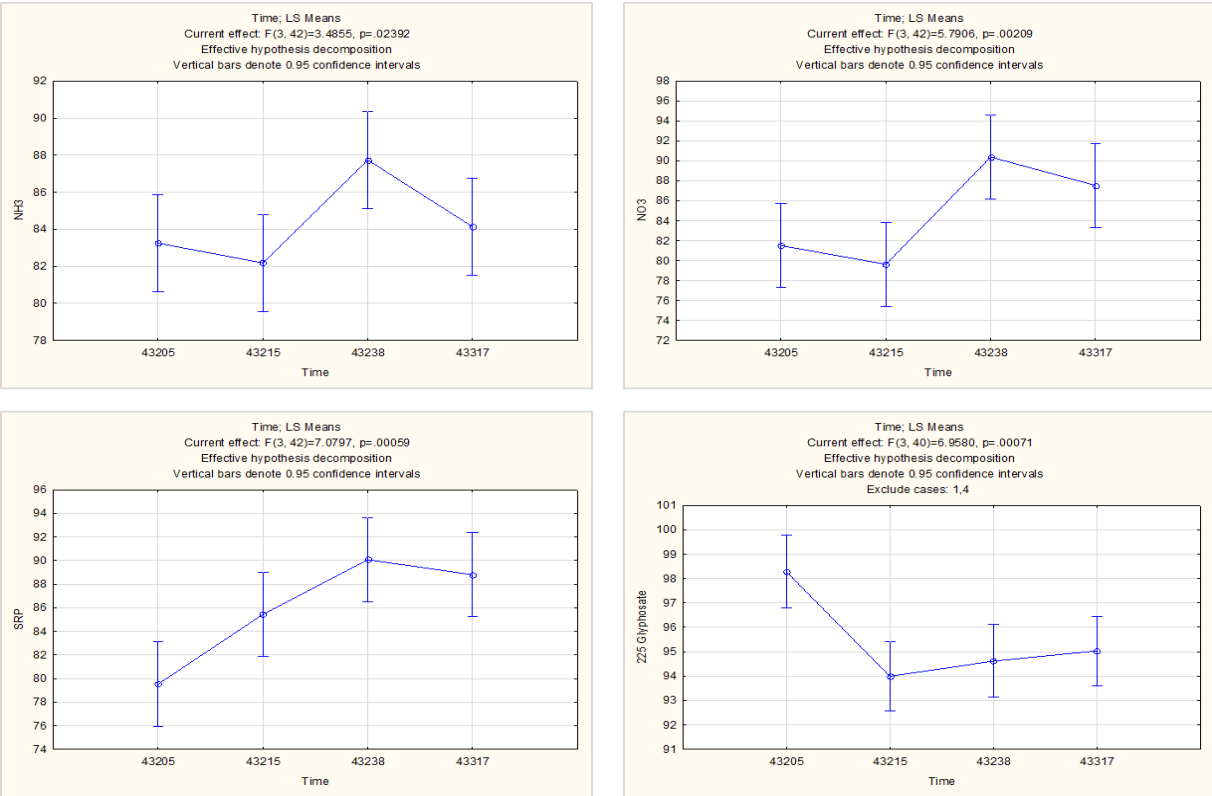


Figure F.3: Temporal effect on remediation efficacy of wetland plant species.

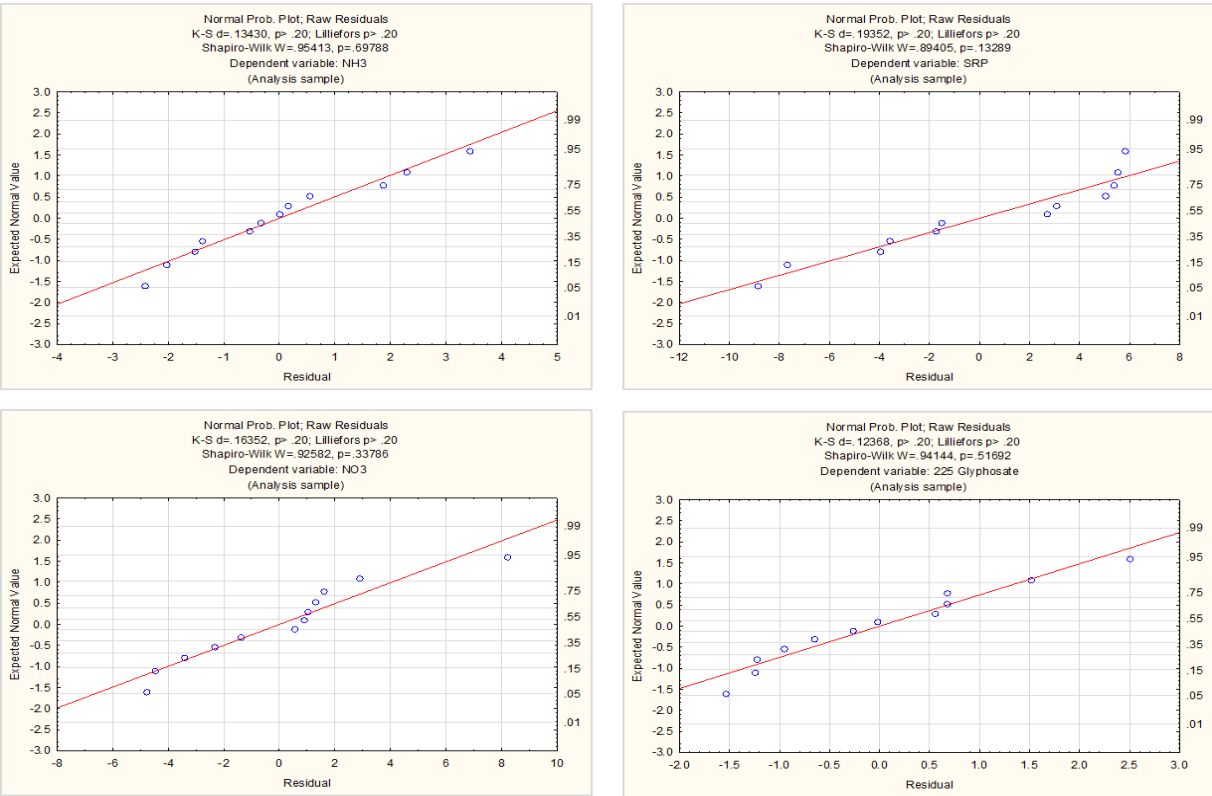


Figure F.4: Data distribution satisfying a normal distribution for Renosterveld, IAP with Palmiet and soil control.

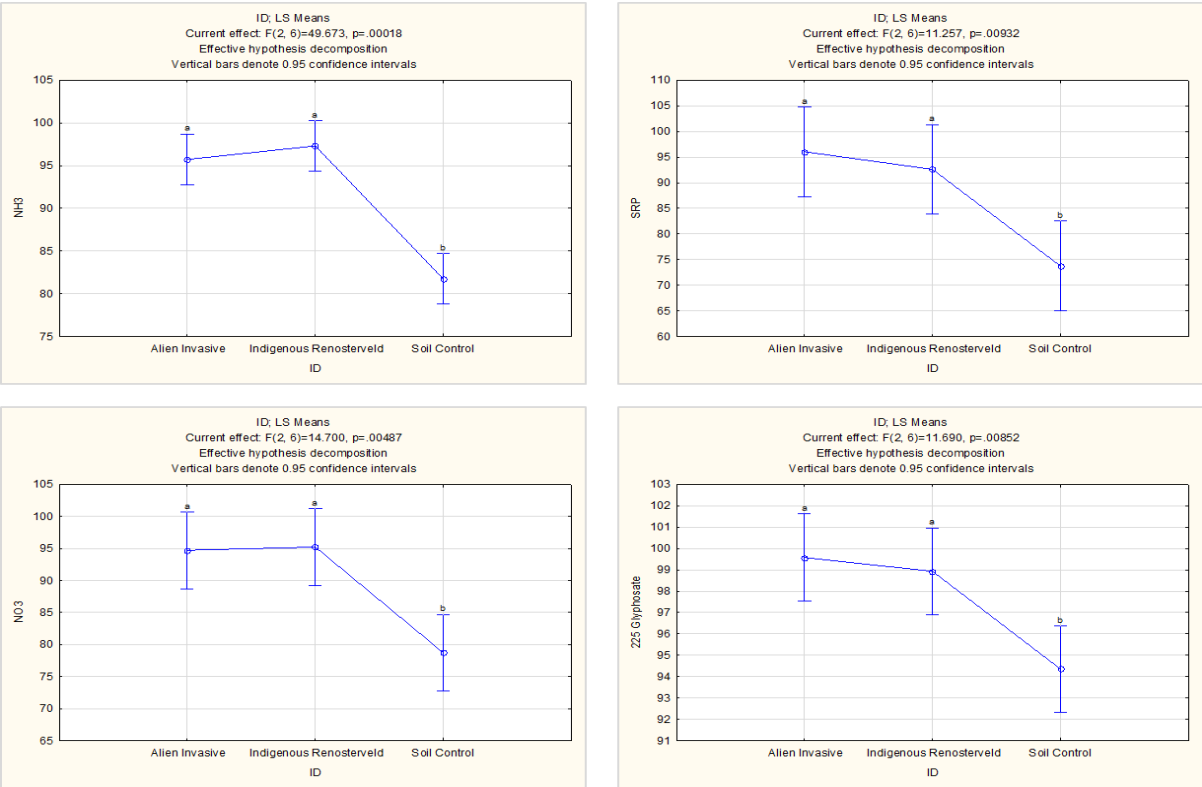
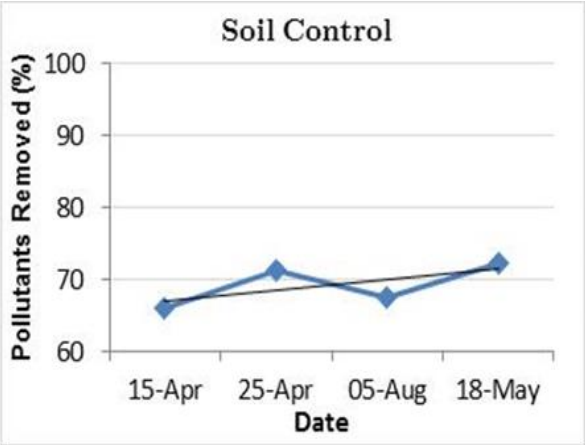
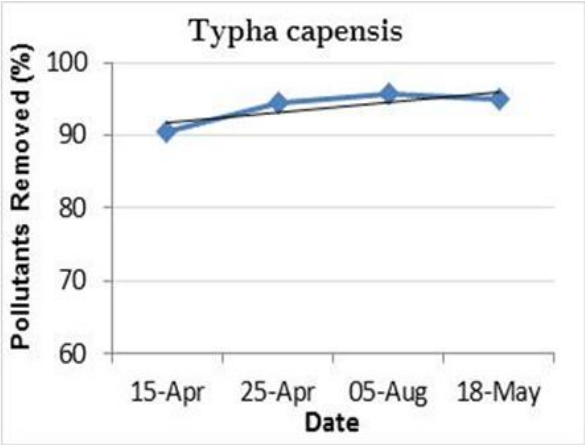
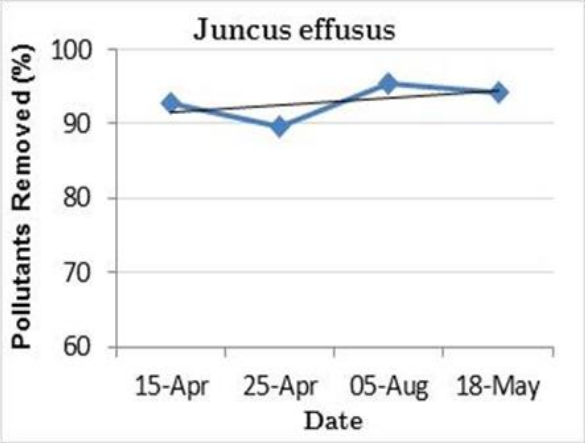


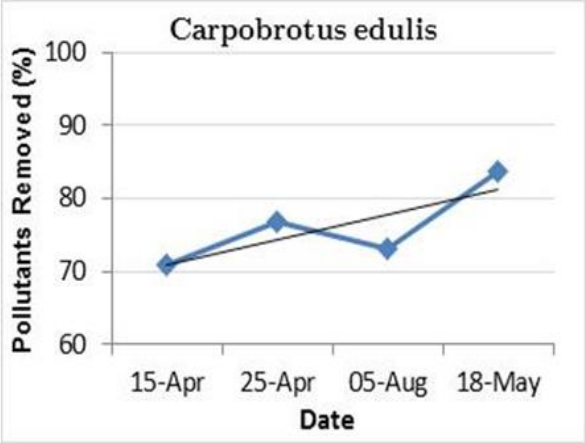
Figure F.5: Main effect analysis of variance with error bars at 95% Confidence Interval to the mean for Renosterveld, IAP with Palmiet and soil control.

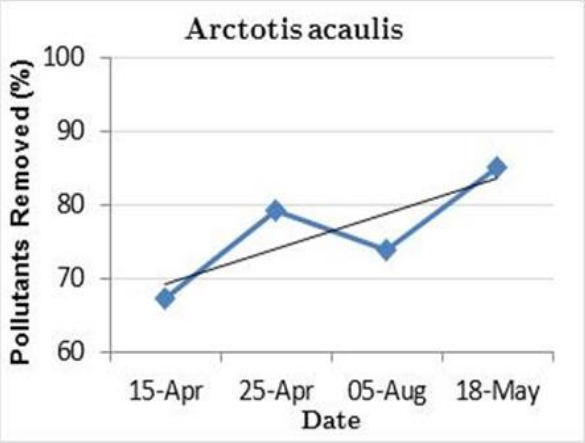
Appendix G: Temporal effect.

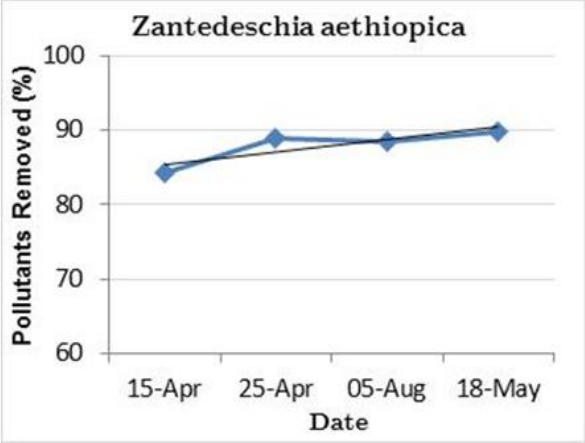












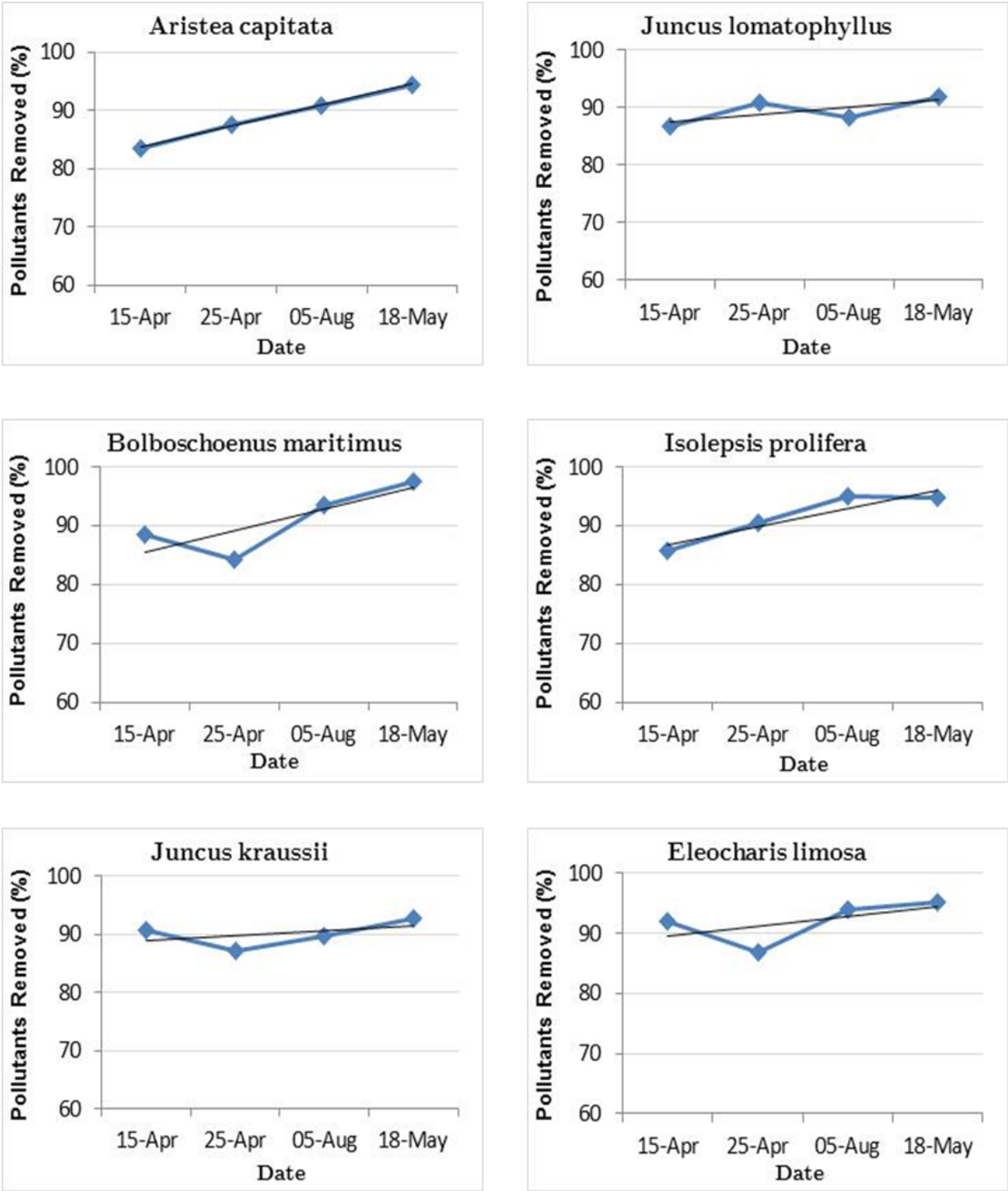


Figure G.1: Temporal effect on cumulative pollutant removal of the remaining indigenous plants.