A remote sensing-based approach for detecting and mapping the accumulation of heavy metal in *Phragmites australis* (Cav.) Trin. ex Steud. and *Arundo donax L.* in wetlands influenced by gold mining

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Abstract

Arundo donax L. and *Phragmites australis* (Cav.) Trin. ex Steud. are two morphologically similar species which play an important role in removal of heavy metals from acid mine drainage (AMD) polluted wetlands. Mapping the distribution of the two species in wetlands and monitoring the level of heavy metal contaminants accumulated in their tissues is very crucial for effective wetland management. Determining the phytoremediating efficiency between the two wetland species would help promoting the native *P. australis* for a wide use across the country by replacing *A. donax*, declared as invasive weed category '1b' according to the National Environmental Management: Biodiversity (NEM:BA) Act, 2004 (Act NO. 10 of 2004) of Alien and Invasive Species Lists, 2016, that places it under strict environmental restrictions prohibiting any propagation or spreading of the species. This study investigated the efficiency of *A. donax* and *P. australis* in the uptake of copper from artificially simulated acid mine drainage by mixing copper ($CuSO_4.5H_2O$) and nitric acid (HNO_3) in tubs in a glasshouse experiment. Both *A. donax* and *P. australis* were able to uptake substantial amounts of copper from the tubs. There were significant differences between the control and treatment of the leaf and root samples of *A. donax* with mean differences of 2.876 ± 0.364 and 25.603 ± 3.119 mg/kg, respectively, and the stems and roots of *P. australis* with mean differences of 6.512 ± 2.01 and 16.10 ± 3.62 mg/kg, respectively. The native *P. australis* had a higher translocation factor (0.4), which makes it more preferable for phytoremediation where harvesting of the above ground biomass is required to remove the accumulated copper for safe disposal, than the alien *A. donax* (0.2). Using ASD hyperspectral data measured from the glasshouse plants, the utility of the Random Forest (RF) and Support Vector Machines (SVM) classification algorithms coupled with the Guided Regularized Random Forest (GRRF) for feature selection, was tested in the discrimination between the two species grown under control and treatment conditions (copper + nitric acid). Results showed that leaf copper concentration is inversely related to mean leaf spectral reflectance. Spectral discrimination was more efficient between treatment plants of the two species (Overall Accuracy = 85.2 % and 81.4 % for RF and SVM, respectively) than between the control plants (Overall Accuracy = 64.2 % and 75 % for RF and SVM, respectively). This was because although the two species are morphologically similar under healthy conditions, and hence similar spectral reflectance, they responded to heavy metal pollution accumulation in their tissues differently, which resulted in more separable spectral data. The RF classification results were more robust than those from the SVM in the discrimination of the two plants under treatment conditions.

The potential of the multispectral Sentinel-2 data to detect and map *A. donax* and *P. australis* effectively was investigated in wetlands around the city of Johannesburg and the results showed that *P. australis* was more abundant and wide spread than *A. donax*. Although there was some spectral confusion in separating the two species, the RF and SVM yielded recommendable and equal accuracies in the mapping process (overall accuracy =91.2 % and kappa coefficient = 0.89 for both RF and SVM, respectively). The study concludes that the Sentinel-2 data coupled with either RF or SVM classification algorithms were effective for monitoring the *A. donax* and *P. australis* species in wetlands.

Preface

The research work described in this thesis was carried out in the School of Geography, Archaeology and Environmental Studies, University of the Witwatersrand, Johannesburg, from July 2016 to April 2021, under the supervision of Professor Elhadi Mohammed I. Adam (School of Geography, Archaeology and Environmental Studies, University of the Witwatersrand, Johannesburg, South Africa) and Professor Solomon W. Newete (Agricultural Research Council-Institute for Soil, Climate and Water (ARC-ISCW) and a visiting Associate Professor at the School of Animal, Plant and Environmental Sciences (APES) at Wits University.

I would like to declare that the research work reported in this thesis has never been submitted in any form for any degree or diploma to any tertiary institution. It, therefore, represents my original work. Where use has been made of the work of other authors or organizations it is duly acknowledged within the text or references chapter.

Loveness Mabhungu ________________________________Date:_22 August 2021

As the candidate's supervisor, I certify the above statement and have approved this thesis for submission.

1. Prof Elhadi Mohammed I. Adam: __________________________ Date: _________

2. Prof Solomon W. Newete: __________________________ Date: ____________

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I, Loveness Mabhungu, declare that:

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Declaration 2 - Publication and manuscripts

Mabhungu, L., Adam, E. and Newete, S.W. (2019). Monitoring of phytoremediating wetland macrophytes using remote sensing: the case of common reed (*Phragmites australis* (cav.) Trin. Ex steud.) and the giant reed (*Arundo donax* L.). A. *Applied Ecology and Environmental Research*, *17*(4), pp.7957-7972.

Mabhungu, L., Newete, S.W. and Adam, E. (*Under review*). The Comparison of Copper Uptake between *Arundo donax* L. and *Phragmites australis* (cav.) Trin. Ex steud.. *Ecology, Environment and Conservation*.

Mabhungu, L., Adam, E. and Newete, S.W. (*Under review*). Spectral discrimination between *Arundo donax* L. and *Phragmites australis* (cav.) Trin. Ex steud. grown in copper contaminated conditions using in situ field spectral measurements. *International Journal of Environment and Pollution*.

Mabhungu, L., Adam, E. and Newete, S.W. (*In preparation*). Mapping *Arundo donax* L. and *Phragmites australis* (cav.) Trin. Ex steud. using Sentinel-2 data in Johannesburg, South Africa.

Signed________________________________

Dedication

This research is dedicated to my late father, Mr Albert Mapfaire and my mother, Mrs Juliet Mapfaire, my brothers: Christopher, Herbert, Elliot and Taona, and my sisters: Irikidzai and Precious, and my husband, Dr I Mabhungu. I thank you for the unwavering inspiration, support and prayers for my success.

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I am grateful to the University of the Witwatersrand for giving me an opportunity to study for a PhD in Environmental Studies. My thanks go to the management at the Hofmeyer greenhouse for giving me an opportunity to conduct my experiments at their facility, to Dr. Jeanne d'Arc Mukarugwiro, Mr. Hadisu Abubakar, and Mr. Marko Freddy Mudede for their assistance in monitoring of plants and data collection at the glasshouse. I thank Mr. Ronald Sithole for assisting during collection of reed rhizomes for the glasshouse experiment and Simbarashe Jombo for assisting in data collection in the field, and Emmah Mandishona for her moral support.

I thank my fellow colleagues at Bindura University Science Education, Faculty of Agriculture and Environmental Science, for the stimulating discussions we had on my research as well as their constructive criticisms. Special thanks go to the management of Bindura University of Science Education for granting me study leave so that I could pursue my studies.

Last but not least, I would like to thank my husband Dr Isaac Mabhungu for the support I received in putting this document together, financial, social, moral and spiritual support during the period of my study.

Above all, I would like to thank GOD for making it possible for me to start and finish the PhD study.

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CHAPTER ONE

1. General Introduction

1.1 Heavy metal pollution in wetland systems in South Africa

Wetlands are aquatic and semiaquatic ecosystems, subjected to a permanent or periodic water logging with low depth (Leguizamo *et al.*, 2017). They can be natural or artificial, with water either flowing or static (Ramsar Convention Secretariat, 2016). Wetlands offer a wide range of functions to human society and the environment, including, *inter alia*, water storage, wastewater treatment, transformation of nutrients, and habitat for wildlife (Ramsar Convention Secretariat, 2016). Pollution of wetland systems from heavy metals is a worldwide problem (Parveen *et al.*, 2016), mainly because heavy metals are nonbiodegradable, persistent in nature, biomagnified throughout the food chain, and serious health hazards to human beings even at low concentrations (Charlesworth *et al.*, 2011; Saxena *et al.*, 2019). Most wetlands in South Africa receive effluent from surrounding processing industries and outflow from mining industries. The major source of heavy metal pollution is acid mine drainage (AMD) mainly due to abandoned gold and coal mining sites (Liphadzi and Kirkham, 2005; Tutu *et al.*, 2008). Acid mine drainage is formed when sulfide minerals are exposed to water and oxygen resulting in the formation of sulfuric acid, metal ions and sulfate (Skousen *et al.*, 2019). The low pH, toxic heavy metals and sulfate content characteristic of AMD lead to grievous impacts on the environment, particularly on soil, water resources and aquatic communities (Kefen *et al.*, 2017). Acid mine drainage pollution of wetlands can eventually end up in streams, rivers or dams, which supply potable water for human beings (Sukumaran, 2013).

The heavy metals such as but not limited to Ni, Zn, Pb, Cu and As have been found to be present in sediments of many wetlands around the world (Schaller *et al.*, 2013). Thus, wetlands are considered as sinks for metals in the environment. Acid mine drainage from gold mining in the Witwatersrand basin of South Africa has resulted in severe damages to both soil ecology and aquatic life in surrounding wetland systems (Odoh *et al.*, 2019). The toxic heavy metals disturb the redox status (balance between oxidants and antioxidants) in plants' systems, which leads to physiological damages due to oxidative stress (Saxena *et al.*, 2019). Hence, heavy metal pollution in wetlands may cause destruction of wetland vegetation or alteration of vegetation composition, which in turn affects wild animals which use the plants as habitats. The toxicity of heavy metals on wetland plants depends on the plant species in question, and its tolerance level to the metal pollutant present in the wetland. Most aquatic plants cannot survive elevated heavy metal concentrations and low pH in wetlands contaminated by AMD pollution. Certain aquatic macrophytes, categorised as either emergent, floating-leaved or submerged macroscopic plant species, can withstand the high metal concentrations and low pH in contaminated wetlands (Malthus, 2017). They can readily absorb and accumulation the soluble and bioavailable contaminants from water without showing any visible symptoms of pollutant toxicity (Nakbanpote et al., 2016). In this regard, wetland emergent macrophytes have proven to be the most tolerant to high concentrations of heavy metals in their tissues (Sheoran and Sheoran, 2006). Examples of such emergent aquatic macrophytes in South Africa are *Cyperus papyrus, Arundo donax* L., *Phragmites australis* (Cav.) Trin. ex Steud., *Typha latifolia*, and *Typha domingensis* (De Villiers et al., 2011; Zingelwa and Wooldridge 2016). Such plants can be used as bio-indicators to evaluate the presence and amount of certain heavy metals in wetlands. They have a very extensive root system and root surface area that enhances contaminant uptake by adsorption of metal cations onto their negatively charged root surfaces (Newete and Byrne, 2016). The eventual harvest of the phytoremediating plant biomass allows the abatement of the wetland contaminates before plants senescence and rot to reverse the sequestered contaminants in the plant tissue back to the wetland. Wastewater treatment using green plant in constructed wetlands is a widely recognised sustainable practice for many municipalities, industries and mining companies across the world (Liu *et al.*, 2007; Vymazal and Březinová, 2015b).

1.2 Heavy metal uptake by aquatic macrophytes

Phytoremediation is a cost-effective and environmentally friendly technique of abating environmental pollution where pollutants are extracted, degraded, contained, or immobilized by green plants (Bello *et al.*, 2018). Aquatic macrophytes have been effectively used for phytoremediation of heavy metal contaminated wetlands (Eid *et al.*, 2020). Characteristic features that make aquatic macrophytes efficient heavy metal accumulators include their strong growth traits even in highly contaminated environments, high biomass, stress tolerance, mostly unpalatable to animals, and ability to hyperaccumulate contaminants in their tissues (Prabakaran *et al.*, 2019). Such plants include *A. donax, P. australis, Phalaris arundinacea, Scirpus* spp. *and Typha* spp. among others (Vymazal and Březinová, 2016; Newete and Byrne, 2016; Galal *et al.*, 2017). Most of these plants have been used for phytoremediation of AMD affected natural and/or constructed wetlands in South Africa (Tutu *et al.*, 2008; De Villiers *et al.*, 2011). Uptake of heavy metals by plants happens from the rhizosphere and involves the following stages: mobilization of heavy metals in wetland medium, subsequent uptake by plant roots, translocation of the accumulated metals from roots to above ground tissues through the xylem vessels, and sequestration of the metals in plant tissues and tolerance (Prabakaran *et al.*, 2019; Saxena *et al.*, 2019). Due to the role they play in up-taking and hence containing heavy metals and other contaminants from sediments and water, wetland plants help in maintaining aquatic ecosystems, and are often considered as biological filters (Galal *et al.*, 2017). Remediation of the heavy metal-polluted wetlands could be achieved by harvesting the metal accumulating plants (Al-Homaidan *et al.*, 2020).

Arundo donax and *P. australis* are morphologically similar reeds widely distributed in wetlands across South Africa (De Villiers *et al.*, 2011; Canavan *et al.*, 2018). They are both C_3, tall, robust and perennial emergent species that survive well in contaminated industrial and mining areas (Bello *et al.*, 2018). They both form dense stands which crowd or shade other wetland vegetation (Everitt *et al.*, 2004; Kawanabe *et al.*, 2012). *Phragmites australis* is the most frequently used and studied aquatic plant species for heavy metal uptake in constructed wetlands (Vymazal and Krőpfelová, 2005; Vymazal and Březinová, 2016). It is among the most common plants of wetlands in South Africa, while *A. donax* is a declared invasive alien weed according to the National Environmental Management: Biodiversity (NEM:BA) Act, 2004 (Act No. 10 of 2004) Alien and Invasive Species Lists, 2016. The heavy metal uptake capacities for *P. australis* and *A. donax* have never been compared under controlled or experimental conditions (Mabhungu *et al.*, 2019). Such information if present, would be necessary in deciding which of the two plant species to use for phytoremediation in constructed wetlands, since the latter is an invader. Thus, there is a need to compare heavy metal uptake capacities between the two species under experimental conditions.

Although all forms of aquatic plants (emergent, submerged and floating aquatic species) are used for phytoremediation of heavy metal contaminants (Newete and Byrne, 2016), emergent aquatic macrophytes like *P. australis* and *A. donax* have proven to be more resilient and effective than others (Sheoran and Sheoran, 2006; Yang *et al.*, 2006). Depending on the plant tissues involved in the removal of pollutants and their specific sites of accumulation in the plants, phytoremediation can occur in different forms, and these are phytostabilisation, phytoextraction, rhizofiltration, phytostabilization, phytodegradation, and rhizodegradation (Pilon-Smits, 2005; Newete and Byrne, 2016). Phytoextration involves translocation of contaminants from the roots to be accumulated in above ground plant tissues, and subsequent biomass harvest for safe disposal (Prabakaran *et al.*, 2019). The efficiency of plants for phytoextraction can be measured using their translocation factor (TF) and /or bioconcentration factor (BCF). TF refers to the ratio of heavy metal concentration in shoots to concentration in roots, while BCF is the ratio of metal concentration in roots to concentration in soil or water (Goel *et al.*, 2009; Antoniadis *et al.*, 2017). A plant with both TF and BCF of above 1 is a potential candidate for phytoextraction (Yoon *et al.*, 2006).

1.3 Evaluating the effeciency of phytoremediating plants using remote sensing

Despite the many services that wetlands provide to the ecosystem, wetlands continue to be degraded and poorly managed (Orimoloye *et al.*, 2019). Effective and accurate mechanisms for assessing and monitoring wetlands and their associated attributes are paramount (Orimoloye *et al.*, 2018). Monitoring of heavy metal uptake by phytoremediating plants is crucial for effective wetland management. It provides information on the ecological risks of contaminated wetlands and helps in determining when to harvest the biomass before the plants die and decompose, releasing the contaminants up taken in plant tissues back into the source (Mabhungu *et al.*, 2019; Lassalle *et al.*, 2021).

The conventional method of determining metal accumulation in plant tissue is through field sampling and laboratory analysis which are exorbitantly expensive, destructive and labour intensive (Liu *et al.*, 2010b; Van Deventer and Cho, 2014). Thus, there is a need for an effective and non-destructive method to monitor heavy metal uptake by various wetland plants. Effective mapping of aquatic species and monitoring their heavy metal uptake can be achieved through remote sensing. In addition to detecting heavy metal-induced stresses in plants, high spectral and spatial resolution satellite imageries are also effective in discriminating between morphologically similar species like *A. donax* and *P. Australis*, and thus facilitate wetland managment. Adam *et al.* (2010) reviewed the use of hyperspectral data to identify wetland vegetation. The spectral response properties of vegetation are determined by the biochemical content (e.g. chlorophyll) and anatomical (e.g. canopy architecture) structure of their leaves or crowns (Curran, 1989). The heavy metal uptake by wetland plants can be detected with remote sensing using the reflectance-sensisitve plant chlorophyll as a proxy to metal-induced plant stress (Newete *et al*., 2014; Liu *et* *al.*, 2020). Different heavy metals have different effects on the spectral reflectance properties of leaves. For example, zinc accumulated in plant leaves causes a blue shift in the red edge position (REP) and a decrease in near infrared reflectance (Sridhar *et al.*, 2007). Accumulated Cd also results in a blue shift of REP and an overall increase of reflectance in the visible region (Sridhar *et al.*, 2007). An increase in leaf copper concentrations in plants results in increased leaf spectral reflectance, cause a blue shift of about 5 to 15 nm, increases the red edge from 4.5534 to 8.9475 nm and decreases the chlorophyll absorption depth (Li *et al.*, 2008). Among the numerous toxic heavy metals present in wetland pollution, certain metals like Cu and Zn are also important micronutrient required for plant growth and health (Petrovic, and Krivokapic, 2020). Copper is an important component of enzymes responsible for cell metabolism, chloroplast formation and it maintains the membrane structure of thylakoids in plants (Adrees *et al.*, 2015). On the other hand, Cu toxicity in plants may result in stunned root and leaf development, reduced plant growth and biomass, poor seed germination and impaired respiration. In this study the potential of using high resolution remote sensing to discriminate and monitor *A. donax* and *P. australis* plants under copper stress from acid mine drainage was investigated. The two species play an important role in phytoremediation of wetland systems (Bello *et al.*, 2018; Bonanno, 2012), and are often planted in artificial wetlands for purposes of wastewater and sludge treatment (Bonanno, 2013; Uggetti *et al.*, 2012). *Phragmites australis* is the most commonly used and studied plant species (Vymazal and Březinová, 2016) for phytoremediation of wetlands, and *A. donax,* which is an alien species in South Africa, is morphologically similar to *P. australis*. Both species are widely distributed in most wetlands in the country.

1.4 Research Aim and Objectives

The main aim of this study is to assess the use of remote sensing to monitor the uptake of copper by *Arundo donax* and *Phragmites australis* plants growing in acid mine drainage polluted wetlands.

The specific objectives of the study are to:

1. measure heavy metal concentrations in sediments and plant tissues of *A. donax* and *P. australis* planted in healthy medium (control), and in Cu + acid treated medium (treatment), under glasshouse conditions.

2. compare the spectral reflectance of *A. donax* and *P. australis* plants growing under both control and treatment glasshouse conditions.
3. evaluate the relationships between plant copper concentration, chlorophyll and leaf spectral reflectance for *A. donax* and *P. australis*.
4. map the spatial distribution of *A. donax* and *P. australis* reeds in wetlands in Johannesburg, South Africa using Sentinel-2 data.

1.5 Scope of the study

This study investigated the potential of remote sensing in monitoring the uptake of copper contaminant by *Phragmites australis* and *Arundo donax* grown in a simulated acid mine drainage polluted wetland. Both plants grow naturally in wetlands, can survive in AMD polluted environments and are commonly used for phytoremediation to treat municipal, industrial and mine wastewater in wetlands. The study focused on copper as contaminant under acidic conditions. The capacities of the two plant species in copper uptake under control and treatment (Cu + acid) conditions were compared in a glasshouse experiment (Chapter 3). The relationship of copper uptake by plants and leaf chlorophyll was assessed. Hyperspectral data acquired with an Analytical Spectral Devices (ASD) was used to assess the spectral properties of *A. donax* and *P. australis* under control and treatment conditions (Chapter 4). The effects of copper uptake on leaf spectral properties were determined. The Random Forest (RF) and Support Vector Machines (SVM) classification algorithms along with the Guided regularized random forest (GRRF) for feature selection were used to discriminate between the two species.

The final section of the study (chapter 5) looked at the potential to discriminate and map *A donax* and *P. australis* using Sentinel-2 imagery. The RF and SVM classification models were employed for this purpose.

1.6 Study area

The first phase (objective 1 to 3) of this study was conduct in a glasshouse experiment set at the University of the Witwatersrand. For objective 4, the study area was wetland areas in Johannesbug South Africa, where *A. donax* and *P. australis* grow. The study area covered parts of Soweto, Roodepoort and Randburg regions in Johannesburg, and is situated between latitudes 26° 09’ S to 26° 17’ S and longitudes 27° 51’ E to 27° 59’ E. Johannesburg has a subtropical highland climate characterized by hot, rainy days and cool evenings in summer and relatively dry sunny days and cold nights in winter. Wetlands in Johannesburg, for example the Klip river wetland areas, receive pollution from industry and gold mining fields (active and abandoned) in the area (McCarthy *et al.*, 2007). Although *P. australis* is the major vegetation cover in wetland areas in Johannesburg, both *A. donax* and *P. australis* grow in wetlands around the study area (McCarthy *et al.*, 2007). Figure 1.1 shows the study area map.

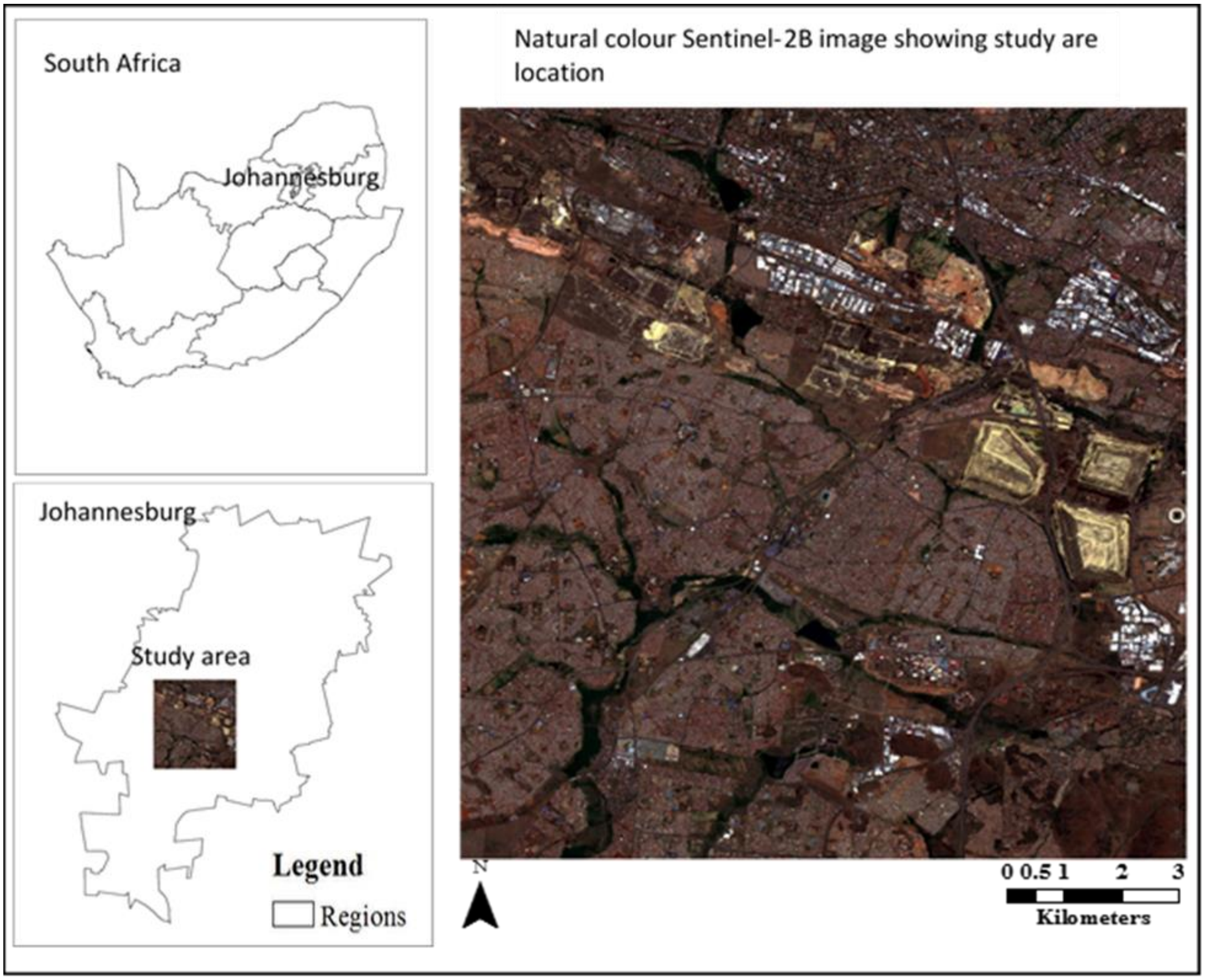

Figure 1.1 Map of South Africa showing the location of the study area in Johannesburg and the natural color Sentinel-2 satellite image of the study area. The study area covers parts of Soweto, Roodepoort and Randburg regions in Johannesburg.

1.7 Thesis outline

To achieve the main objectives of this study, the thesis is organized as a collection of four research papers, one of which is already published, two papers have been submitted to peer reviewed international journals for publication and the last one is in preparation. The papers were prepared as stand-alone articles which can be read separately from the rest of the thesis. Some replications and overlaps occur in the sections "Introduction" and "Materials and methods" in the different chapters. This problem is deemed to be of little significance considering the critical peer review process and the fact that the different chapters are papers that can be read separately without losing the overall context. The thesis consists of six chapters and these are as follows:

Chapter 1 gives a general introduction to the thesis. It describes the research objectives, scope of the study and outlines the thesis outline.

Chapter 2 contains a detailed literature review on the potential of remote sensing for monitoring heavy metal uptake by phytoremediating wetland plants. The efficiency of phytoremediation by emergent aquatic macrophytes in wetlands with specific reference to two morphologically similar reeds, *A. donax* and *P. australis,* in South Africa is reviewed in detail. The research gaps for selection of best performing plants for phytoremediation between *A. donax* and *P. australis,* and the application of hyperspectral remote sensing in monitoring wetland phytoremediation are introduced.

Chapter 3 compares the heavy metal uptake effeciency between *A. donax* and *P. australis* species under experimental conditions, using artificially mixed solutions of copper ($CuSO_4.5H_2O$) and nitric acid (HNO_3) to simulate an acid mine drainage condition. This was conducted in a glasshouse experiment. One-way Analysis of variance (ANOVA) from SPSS was used for comparing heavy metal concentrations, pH, EC and chlorophyll content between the two species grown in a control and treatment conditions, and simple linear regression model was used to assess the relationship between leaf copper concentration and chlorophyll content. Phytoremediation capacities of the two species were assessed using their BCF and TF.

Chapter 4 contains an assessment on the ability to discriminate between *A. donax* and *P. australis* species in control and treatment (copper + acid) conditions, using the ASD spectral measurements from the two plants grown in a glasshouse experiment. The effect of the different treatments to which the two plants were exposed on their spectral reflectance properties was evaluated. The efficiencies of RF and SVM models in discriminating the two plant species using features selected from the GRRF were compared.

Chapter 5 covers an assessment on the effectiveness of Sentinel-2 data in mapping *A. donax* and *P. australis* reeds in wetlands around the city of Johannesburg using the RF and SVM classifiers. The spatial distribution of *A. donax* and *P. australis* and other land cover types in the study area were included in the map produced.

Chapter 6 provides a synthesis of all the first 5 chapters. It summarizes the findings and conclusions from the preceding chapters and outlines some relevant recommendations for future research on applications of remote sensing in monitoring phytoremediating plants like *A. donax* and *P. australis*.

A single reference list is provided at the end of the thesis.

CHAPTER TWO

2. Literature Review

This chapter is based on the paper published as:

Mabhungu, L., Adam, E. and Newete, S.W. (2019). Monitoring of phytoremediating wetland macrophytes using remote sensing: the case of common reed (*Phragmites australis* (cav.) Trin. Ex steud.) and the giant reed (*Arundo donax* L.). A. *Applied Ecology and Environmental Research*, *17*(4), pp.7957-7972.

Abstract

Contaminants from various anthropogenic activities such as agriculture, mining, and recreation negatively affect wetland water quality and vegetation health and composition. Phytoremediation is an effective, sustainable and eco-friendly pollution abatement method using green plants. *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. are two morphologically similar reeds commonly used for phytoremediation in wetlands of South Africa. *Arundo donax* is however, a declared category ’1b’ weed under the National Environmental Management: Biodiversity Act (NEM:BA, Act No. 10 of 2004), requiring its immediate removal. Thus, determining the phytoremediation efficiency of the two reeds could help making the right choice between them for potential use in wetlands. Furthermore, the efficiency of wetlands depends on a robust monitoring system for phytoremediating plants to determine the appropriate time for biomass harvest before they die and release contaminants taken up in plant tissues back into the source through decomposition. Remote sensing can be used for mapping and monitoring of such aquatic macrophytes at species level effectively. Satellites imageries with high spatial and spectral resolutions not only are capable of detecting heavy metal-induced plant stresses, but also can effectively discriminate between morphologically similar species like *A. donax* and *P. australis* and facilitate wetland managment

Keywords: Acid Mine Drainage; Heavy metal uptake, Mapping; Hyperspectral; Species discrimination

2.1 Introduction

Phytoremediation is a relatively low-cost and environmentally friendly method of reducing environmental pollutants to harmless levels using green plants (Emmanuel *et al.*, 2014; Kaewtubtim *et al.*, 2016; Newete and Byrne, 2016). Based on the plant tissues involved in removal of pollutants and their specific sites of accumulation in the plants, phytoremediation is sub-categorized as phytoextraction, rhizofiltration, phytovolotalization, phytostabilization, phytodegradation, and rhizodegradation (Pilon-Smits, 2005; Newete and Byrne, 2016). Phytostabilisation refers to the process of plants stabilising pollutants in the soil to harmless levels; phytoextraction is when pollutants are transported and concentrated in the above ground plant tissues and involves subsequent harvest of aerial plant biomass; rhizofiltration is when plants, or microorganisms associated with the rhizosphere, remove contaminants from water; phytodegradation occurs when plant enzymes break down pollutants inside their tissues and convert them into harmless compounds; phytovolatilization involves extraction of volatile compounds by plants which are released as volatile compounds through the leaves (Raskin, 1997; Wong, 2003). Phytoremediation can occur in both dry and wet environments. Phytoextraction is commonly used for cleaning contaminants from soil, rhizofiltration to clean contaminants from water medium, while phytostabilization, phytovolatilization and phytodegradation can be used to clean either from soil or water mediums (Frick *et al.*, 1999; Wong, 2003; Yang *et al.*, 2005). The degree of pollutant accumulation by plants tends to increase with the wetness of the environment (Aryal *et al.*, 2016). Compared to conventional or engineering methods of cleaning polluted environments, phytoremediation is potentially cheaper, environmentally friendly, sustainable, and has the possibility of bio-recovering heavy metals (Yang *et al.*, 2005).

According to Ramsar Convention Secretariat (2016) wetlands are "areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six metres". Wetlands are essential to both humans and the ecological environment, and once their health and functions are disturbed, the effects are detrimental on all life forms supported by wetlands. Their functions include nutrient removal from water, trapping of sediments, pollutant trapping, control of erosion, stream flow augmentation, provision of habitat for wildlife, and recreational benefits (Bruneau, 2017; Luo *et al.*, 2017). According to Zedler and Kercher (2004), wetlands account for approximately 6 % of the world's land surface. Depending on their positioning, wetlands are subjected to impacts from human activities such as agriculture, mining and urban development. Impacts of mining on wetlands include water pollution from Acid Mine Drainage (AMD) and heavy metals, land degradation, vegetation biodiversity degradation, and destruction of a wide range of habitat for wetland wildlife (Gupta and Nikhil, 2016). From these, the major effect of mining activities on wetlands is AMD pollution, which emanates from seepage of both active and abandoned mining areas near wetlands (Newete and Byrne, 2016). Acid mine drainage is the outflow of acidic water from mining sites, and this is mainly due to the oxidation of iron pyrites (FeS_2) (Akcil and Koldas, 2006; Ochieng *et al.*, 2010). Due to its low pH of usually 2 - 3, AMD dissolves mining ores, and thus contains, high concentrations of metals which include manganese, aluminum, iron, nickel, zinc, cobalt, copper, and cadmium (Bell *et al.*, 2001; Coetzee *et al.*, 2006). Acid mine drainage pollution from wetlands can also end up in streams, rivers and dams, which supply potable water for humans (McCarthy and Humphries, 2013). This makes the water quality unsuitable for human consumption and adds a toll to the costs of water purification. Research has shown that low pH and high concentrations of heavy metals negatively affect wetland species composition (Ochieng *et al.*, 2010). Destruction of wetland vegetation or alteration of vegetation composition will also affect wild animals harboured in such plants. However, some aquatic plant species have adapted mechanisms to survive in such conditions (Deng *et al.*, 2004; Papazoglou *et al.*, 2005; Vymazal and Březinová, 2015b), thus, they can be used as bio-indicators to evaluate the presence and amount of heavy metal contaminants in wetlands. These plants have an extensive root system and root surface area that enhances contaminant uptake by adsorption of metal cations onto the negatively charged root surfaces (Newete *et al.*, 2016; Newete and Byrne, 2016). The subsequent harvest of the phytoremediating plants with a cocktail of organic and inorganic contaminants from polluted wetlands means an improvement of the existing water quality. Thus, many artificial wetlands are constructed for treatment of wastewater using green plants (Liu *et al.*, 2007; Vymazal and Březinová, 2015b). Among the common emergent macropytes used in wetlands are *Phragmites australis* (Cav.) Trin. ex Steud. (Vymazal and Brezinova, 2016), *Arundo donax* L. (Elhawat *et al.*, 2014), *Phalaris arundinacea Scirpus* spp. and *Typha* spp. (Papazoglou *et al.*, 2005; Newete and Byrne, 2016). From these, *P. australis* is the most commonly used and studied plant species (Vymazal and Březinová, 2016). This could be because it is a cosmopolitan plant species which is widely distributed across the world (Adams and Bate, 1999). *Arundo donax*, which is morphologically similar to *P. australis*, is another such species with wide geographical distribution in many polluted wetland systems of South Africa outside its natural habitat (Rouget *et al.*, 2004). *Phragmites australis* also known as common reed, is native to South Africa (Adams and Bate, 1999), while *A. donax*, known as the Spanish or the giant reed, is a declared Category 1b weed in South Africa (Henderson, 2001; Department of Environmental Affairs, 2016). Although the two reeds have proved to be effective as tools of phytoremediation in wetlands, their capacities to uptake heavy metals have not been compared under similar and controlled conditions.

Although the efficiency of metal uptake by phytoremediating plants depends on plant species, size of plant biomass particularly large root surface area in aquatic macrophytes, type and amount of elements targeted for removal (Newete and Byrne, 2016), it is equally important to monitor the level of such pollutants' removal from the polluted environment and accumulation in the plant tissues. This is because it allows subsequent management of the phytoremediating plants by determining the appropriate time for harvest of plant biomass and safe disposal (Carson *et al.*, 2018). The conventional method of determining metal accumulation in plant tissue in the laboratory is often expensive, destructive and labour intensive (Van Deventer and Cho, 2014). Thus, there is a need for effective and non-destructive method to monitor heavy metal uptake by various wetland plants. Remote sensing has the potential to be that method. This is because the spectral reflectance by green vegetation depends, *inter alia*, on the amount of specific biochemical in plant leaves (Gates *et al.*, 1965; Zwiggelaar, 1998). Remote sensing has extensively been used to monitor the effect of individual metal pollutants on plants using their effects on chlorophyll and net photosynthesis (Sridhar *et al.*, 2007).

There is a large number of literature reviews on phytoremediation (Raskin *et al.*, 1997; Pilon-Smits, 2005; Laghlimi *et al.*, 2015; Rizwan *et al.*, 2017) and for wetlands (Matagi *et al.*, 1998; Usharani and Vasudevan, 2016; Newete and Byrne, 2016; Leguizamo *et al.*, 2017). However, only few of them focused on wetland phytoremediating plants. This chapter will therefore, review the efficiency of phytoremediation by emergent aquatic macrophytes in wetlands with specific reference to two morphologically similar reeds, *P. australis* and *A. donax,* in South Africa. It will also investigate the potential of remote sensing for monitoring heavy metal accumulation by the plants effectively.

2.2 Reeds in South Africa

Reed is the general botanical term used for tall, grass-like plants that grow in wet places and occur in reed beds. They are all members of the order Poales, but under different families. Table 2.1 gives a list of some of the commonly known reeds. From the list in Table 2.1, *Cyperus papyrus*, *A. donax*, *Calamagrostis* species, *P. australis* and *Typha* species were identified in wetlands of South Africa (De Villiers *et al.*, 2011). *Cyperus papyrus* is a tall perennial grass-like emergent plant. It is naturally found in KwaZulu-Natal, Mpumalanga and Limpopo provinces. It grows along the edges of rivers, seasonal or permanent pools, or swamps. The reed is unpalatable and has low forage quality, and thus does not support many plantivores (De Villiers *et al.*, 2011). *Calamagrostis* is a perennial grass which inhabits vleis and marshes. It is sparsely distributed in Eastern Cape, Mpumalanga, Gauteng, North West and Northern Cape provinces of South Africa (De Villiers *et al.*, 2011). *Typha* species is widely distributed in South Africa and it grows in wetlands and all aquatic habitats (Masoko *et al.*, 2008; Ilfergane, 2016).

Table 2.1 Some of the common reeds known to grow in wetlands across the world

Family	**Reed's common name and species name**
Poaceae grass	Common reed *(Phragmites australis* (Cav.) Trin. ex Steud.)
	Giant reed (*Arundo donax* L.)
	Burma reed (*Neyraudia reynaudiana*)
	Reed canary-grass (*Phalaris arundinacea*)
	Reed sweet-grass (*Glyceria maxima*)
	Small-reed (*Calamagrostis species*)
Cyperaceae (sedge)	Paper reed or papyrus (*Cyperus papyrus*)
Sparganiaceae	Bur-reed (*Sparganium species*)
Typhaceae	Reed-mace (*Typha species*)
Restionaceae	Cape thatching reed (*Elegia tectorum*)
	Thatching reed (*Thamnochortus insignis*)

Arundo donax and *P. australis* are tall, robust and perennial grass-like plants with hollow stems, and are widely distributed in Western Cape, Eastern Cape, KwaZulu-Natal, Mpumalanga and Gauteng provinces of South Africa (De Villiers *et al.*, 2011). Morphologically the two reeds are very similar. They are both aggressive invaders outside their native ranges (Lambert *et al.*, 2010). The slight differences between them are shown in Table 2.2 as extracted from Lusweti (2011) and CABI (2019). Images for *A. donax* and *P. australis* (Figure 2.1) in Johannesburg, South Africa, extracted from google earth, are shown below.

Table 2.2 Morphological differences between *Phragmites australis* (Cav.) Trin. ex Steud. and *Arundo donax* L.

***Arundo donax* L.**	***Phragmites australis* (Cav.) Trin. ex Steud.**
Very tall grass, 2 – 7 m tall	relatively tall grass, 1.5 – 3m tall
Relatively broad leaves, 10 – 80 mm wide	relatively narrow leaves 10 – 35 mm wide
color: glaucous (yellow-green to a bluish-gray)	color: - grey-green

Arundo donax is native to the Mediterranean Basin and the middle east in Asia, and some parts of Africa and southern Arabian Peninsula, while *P. australis* is a cosmopolitan species with wide distribution spanning the five continents (excluding the polar continents) of the world, and it is also known as native to South Africa (Henderson and Cillier, 2004). *Arundo donax* is a naturalised alien species in South Africa, and was introduced in the late 1700s for soil erosion control (Milton, 2004; Canavan *et al.*, 2017). It invades riparian areas across South Africa and its spread is facilitated by human activities such as building of dams and soil stabilisation (Canavan *et al.*, 2017). Alien invasive plants have negative impacts on ecosystems' biodiveresity. They form dense stands which reduce biodiversity of indigenous communities, affect food-webs, and hence change ecosystem processes (Milton, 2004). *Arundo donax* is highly flammable and can alter fire regimes in invaded areas, thus changing the communities of native plants into a dense mono stand of *A. donax* (Lusweti, 2011). To address the invasive potential and negative impacts of *A. donax* in South Africa, biological control methods are being considered, for example use of *Rhizaspidiotus donacis* (Leonardi) (Hemiptera: Diaspididae) insect, which feeds on rhizhomes, stems and leaves (Pillay, 2016; Canavan *et al.*, 2017).

Figure 2.1 Image of the Giant Spanish reed (*Arundo donax* L.) (A) and the common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) (B). Source: US Department of State Geographer, Google earth, 2018

Both *A. donax* and *P. australis* can adapt well in both dry and damp or wet areas, as well as in heavily polluted areas. As such, they are common in most wetlands. Their dense stands often crowds or shade other wetland vegetation. Their ability to uptake and accumulate nutrients and other pollutants makes them the most suitable macrophytes for phytoremediation in polluted wetlands (Bonanno, 2012; Aminsharei *et al.*, 2017; Bello *et al.*, 2018). Thus, the two plants are widely used for phytoremediation in constructed and natural wetlands.

2.3 Phytoremediation by wetland plants

Wetland vegetation is an important part of the wetland ecosystem, as it plays a major role in environmental sustainability (Adam *et al.*, 2010). Naturally wetlands are sinks for many contaminants, and as such they accumulate materials resulting from both terrestrial and wetland disturbances like sediments, nutrients, salts, heavy metals and other contaminants (Kaplan *et al.*, 2017). According to De Villier *et al.* (2011), 50 % of wetlands in South Africa have already been destroyed due to anthropogenic activities such as agriculture, mining, and urban development. Disturbances from these activities negatively affect the native wetland ecosystems. As a result of this, wetlands are vulnerable to invasion, and presence of particular invasive macrophytes in a wetland may be an indicator of the status of the wetland system (Zedler and Kercher, 2004). Most invasive macrophytes are hyperaccumulators and have the ability to absorb harmful substances and pollutants from wetlands into their plant tissues (Zedler and Kercher, 2004). High concentration of pollutants in plant tissues is an important indicator of water quality status in wetlands and other water systems (Deng *et al.*, 2004; Allende *et al.*, 2011). All forms of aquatic plants (emergent, submerged and floating aquatic species) are used for phytoremediation of polluted wetlands (Newete and Byrne, 2016). Nevertheless, emergent aquatic macrophytes like *P. australis* and *A. donax* are more prevalent in most wetland systems and have proven to be more resilient and effective in removal of heavy metals (Sheoran and Sheoran, 2006; Yang *et al.*, 2006). The degree to which plants accumulate heavy metals is determined by the individual plant uptake capacity and intracellular transportation within the plant (Yang *et al.*, 2005). For example, *P. australis* has the potential to accumulate Cd, Cu, Cr, Ni and Pb up to 0.1 % of plant dry mass and Fe and Zn up to 1 % plant dry mass (Kalra, 1998; Sasmaz *et al.*, 2008). Research conducted by Yang *et al.* (2006) in Guangdon province, China, showed that *Typha latifolia*, *P. australis* and *Cyperus malaccensis* significantly removed 94 % of Cd, 99.04 % of lead (Pb), and 97.30 % of zinc (Zn) from a constructed wetland. Vymazal and Březinová (2016) revealed that *P. australis* planted in constructed wetlands removed 59 % Zn, 55 % Cd and 38 % Cr, from the inflow annual load of the metals into wetlands. Vymazal and Březinová (2016) also concluded that the plant shoots in constructed wetlands can sequester up to 55 % Cr, 49 % Zn and 71 % Cd, of the total heavy metals removed by the *P. australis*. In another study 19 plant species, among which were *P. australis* and *Cyperus* species, were planted in a constructed wetland in China and irrigated with waste water containing concentrations of Zn, Cd and Pb at 5.0, 0.5 and 2.0 mg/L, respectively. The results showed that the plants had more than 90 % removal efficiency for the heavy metals (Liu *et al.*, 2007). Bello *et al.* (2018), in Saudi Arabia, investigated the capacity of *P. australis* in removing Cd, Pb and Ni in hydroponic experiments with 5 mg/L concentration for each heavy metal. The results showed that the capacity of heavy metal removal by *P. australis* was 93 %, 95 % and 84 % for Cd, Pb and Ni, respectively. In another research conducted in Catania, Italy, the roots, stems, and leaves of *A. donax* were tested as potential bio-monitors of trace elements such as Al, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn. The results showed that *A. donax* had the capacity to significantly bioaccumulate the trace elements, with root/sediment Bioaccumulation factors of: Cr (0.08), Cu (0.05), Mn (0.02), Ni (0.1), and Pb (0.03) (Bonanno, 2012). Mojiri *et al.* (2015) investigated the effectiveness of *P. australis* for heavy metal uptake from municipal waste leachate. At the end of their experiment, the amount of Fe, Mn, Cu and Ni removed by *P. australis* were 25.049, 9.623, 6.112, and 0.900 mg/kg, respectively. The translocation factors were 0.34, 0.89, 1.30 and 1.01, respectively. Translocation Factor (TF) is the ratio of metal concentration in shoots to the metal concentration in roots, and measures the ability of a plant to translocate metals from roots to shoots (Mojiri *et al.*, 2015). A plant has potential to be used for phytoextraction when its TF for a particular metal is above 1. Thus, according to Mojiri *et al.* (2015), it is apparent that *P. australis* has the potential for the phytoextraction of Cu and Ni, from wetlands, but not for Fe and Mn.

Aquatic plants are very useful in natural and constructed wetlands in the abatement of water contaminants and improving the water quality for domestic and agricultural purposes. Table 2.3 is a summary of some literature showing the removal of heavy metals from contaminated wetlands by aquatic macrophytes.

A number of researchers worldwide have studied on the efficiencies of *P. australis* and *A. donax* for heavy metal uptake (Papazoglou *et al.*, 2005; Bonanno, 2012; Kumari and Tripathi, 2015b), including in South Africa (Van der Merwe *et al.*, 1990; Zingelwa and Wooldridge, 2009). Kumari and Tripathi (2015b) demonstrated the removal of Cu, Cd, Cr, Ni, Fe, Pb and Zn from urban sewage mixed with industrial effluents using *P. australis* and *T. latifolia,* as a cost effective and promising technology in India. Their results showed that *P. australis* perfomed better than *T. latifolia* for all the metals, and mixing the two plants increased the removal efficiency of the metals from the effluent. Papazoglou *et al.* (2005) investigated *A. donax* irrigated with tap water containing increased concentrations of Cd and Ni in Greece. At the end of the experiment the parameters measured, namely, stem height and diameter, number of nodes, fresh and dry weight

Table 2.3 Removal of heavy metal contaminants from wetlands by aquatic macrophytes

Species	Metals purified	Place	Reference
Typha capensis and *Arundo donax* L.	Zn, Mn, Ni and Fe	Johannesburg, South Africa; Natural wetland	Van der Merwe *et al.*, 1990
Cyperus vaginatus	Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb	South Australia; Constructed wetland	Aryal *et al.*, 2016
Typha domingensis, *Phragmites australis* (Cav.) Trin. ex Steud. and *Arundo donax* L.	Al, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Zn.	Italy; Natural wetland	Bonanno, 2013
Phragmites australis (Cav.) Trin. ex Steud. and *Bolboschoenus maritimus*	Cr, Ni, Cu and Zn	Northeast Italy; Constructed wetland	Bragato *et al.*, 2006
Bolboschoenus Maritimus	Al, Fe, Cu, Zn	Western Cape, South Africa; Natural wetland	Shuping *et al.*, 2011

of leaves, and net photosynthesis, were found not affected, indicating that *A. donax* tolerates high concentrations of Cd and Ni, and thus can be used for phytoremediation. Van der Merwe *et al.* (1990) measured the accumulation of Zn, Mn, Ni and Fe by *A. donax* and *T. capensis* under acidic and alkaline conditions in the Burgspruit catchment area near Germiston in Ekurhuleni (South Africa) and concluded that the metal accumulation capacity of *A. donax* was higher than that of *T. capensis*. Zingelwa and Wooldridge (2009) investigated the mineral element uptake and accumulation by *T. latifolia* and *P. australis* from waste water in constructed wetlands in South Africa and found that *T. latifolia* accumulated more mineral elements (N, P, K, Ca and Al) than *P. australis*. However, only few or no studies compared the capacities of *A. donax* and *P. australis* in heavy metal accumulation under experimental or controlled environment. Bonanno (2013) compared the trace element bioaccumulation capacities of the two plant species under field or natural conditions, and the results showed that *P. australis* is a better heavy metal accumulator than *A. donax*. He measured the heavy metals Al, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn in *Typha domingensis*, *P. australis* and *A. donax* naturally growing along a stream in Italy. The results showed that the bioaccumulation capacities decreased in the order of root>stem>leaf in *T.*

domingensis, and root>leaf>stem in *P. australis* and *A. donax*. Generally the bioaccumulation capacities for *P. australis* and *T. domingensis* were found to be higher than that of *A. donax*. Thus, if the native *P. australis* is more effective in removal of heavy metals than its counterpart *A. donax* in wetlands, it is imperative to promote the former to eventually replace the notorious invader *A. donax* in wetlands of South Africa. However, to ascertain this, research on the subject should focus on comparing the heavy metal uptake capacities betwen the two plant species under controlled/experimental conditions. This is because they are widely distributed in many parts of the country and they both provide similar economic and ecological functions. They are useful as thatching, ornamental and musical-instrument (like flutes) materials and source of energy, and wildlife habitat (Bonanno, 2012; Shuai *et al.*, 2016). However, because of their similar morphology, discrimination between the two species and monitoring their occurrence and their environmental foot prints in the field is often difficult.

2.4 Monitoring heavy metal uptake by aquatic macrophytes

While many aquatic macrophytes have proven to be valuable for phytoremediation, the process requires continuous monitoring to ensure timely harvest of plant biomass and their replacement for continuous and effective removal of water contaminants (Carson *et al.*, 2018). An effective tool of monitoring is required to determine the heavy metal accumulation in the phytoremediating plant tissues and their health status to avoid metal-induced stress and subsequent death of plant tissues before their harvest and removal (Vassilev *et al.*, 2004). Traditional methods of monitoring heavy metal uptake by plants involve sampling plant tissues and sample preparation for laboratory analysis. For example tree-ring analysis, which involves chemical analysis of samples from successive growth rings, can be done to monitor patterns of heavy metal uptake by plants over years (Lepp, 1975). In the same manner samples of plant roots, stems, and leaves can be analysed in the laboratory to determine the heavy metal uptake ability by plants (Vymazal and Březinová, 2016). However, for monitoring large areas, traditional surveys involve lots of plant sample collection and preparation, which may be difficult, expensive, too technical, time consuming and poses safety issues on chemical handling and disposal. Sampling of representative plant tissue could also be constrained due to inaccessiblity. Remote sensing is a modern and alternative technology for monitoring such wetland phytoremediating plants. It is also an effective, non-destructive tool for monitoring heavy metal accumulation by wetland plants (Van Deventer and

Cho, 2014). Remote sensing can be used to acquire spatial and temporal variations of accumulated heavy metal concentrations over large and inaccessible areas, as opposed to the in-situ traditional methods which are limited to small and accessible areas (Liu *et al.*, 2010b).

2.4.1 Monitoring of heavy metal uptake by wetland plants with remote sensing

Remote sensing is the technology for obtaining information about an object without a direct contact to the object under observation. Monitoring of vegetation using remote sensing is possible because vegetation under different conditions and of different characteristics and quality has unique spectral characteristics in the electromagnetic spectrum (Knipling, 1970). The spectral response properties of vegetation are determined by the biochemical content (e.g. chlorophyll) and anatomical (e.g. canopy architecture) structure of their leaves or crowns (Curran, 1989). All vegetation has a spectral response curve, which is unique to each species. High chlorophyll levels result in increased absorption (reduced reflection) in the red region of the electromagnetic spectrum, and broadening of the absorption pit in the red (660–680 nm) (Horler *et al.*, 1983). It also shifts the red edge reflectance slope (680–760 nm) and the point of maximum slope in the red edge known as the chlorophyll red edge position (REP) towards the longer wavelengths, referred to as a red shift (Horler *et al.*, 1983). Accordingly, decreased chlorophyll results in increased reflectance in the red region, and a shift of the red edge slope and REP towards the shorter wavelengths, which is referred to as a blue shift. Increases in biomass result in increase reflection in the near-infrared (NIR) region (Rouse Jr., 1974). Various heavy metal concentrations also have significant effects on the spectral reflectance properties of leaves. According to Sridhar *et al.* (2007), Zn accumulated in plant leaves causes a blue shift in the REP and a decrease in NIR reflectance. Accumulated Cd also results in a blue shift of REP and overall increase of reflectance in the visible region (Sridhar *et al.*, 2007). Lead results in increased reflectance in the NIR region (Clevers *et al.*, 2004). Liu *et al.* (2010b) studied the possibility of using a small scale hand held hyperspectral sensor to estimate heavy metal (Pb, Cu and Zn) concentrations in *P. australis*. The study revealed that heavy metal concentrations affected chlorophyll levels in the plants, which in turn determine the hyperspectral measurements for the plant leaves. It was also found that chlorophyll concentrations for the sampled leaves varied inversely with concentrations of Pb, Cu and Zn, and chlorophyll concentration accounted for about 30 % of the variations in the three heavy metals, respectively. Linear combination of normalized band depths at wavelengths 537 (green), 667 (red) and 747 (near infrared) nm were found to explain 82 % of the variation of chlorophyll concentration (Liu *et al.*, 2010b). Thus, it was concluded that it is possible to use laboratory-based hyperspectral data to estimate concentrations of Pb, Cu and Zn in *P. australis* (Liu *et al.*, 2010b). In another study, Shakya *et al.* (2008) also concluded that high concentrations of heavy metals in plants determine their chlorophyll content, which can be measured by field hyperspectral data or hyperspectral images. Research by Li *et al.* (2008) investigated the biogeochemistry responses of vegetation *Rhus chinensis* Mill in a copper mine area to heavy metal contamination. The results showed that there was a significant correlation between copper concentration in leaves and leaf reflectance. Increased copper concentrations were found to: increase the leaf spectral reflectance by about 5 % to 30 %, cause a blue shift of about 5 to 15 nm, increase the red edge from 4.5534 to 8.9475 nm and decrease the chlorophyll absorption depth. Thus, remote sensing can be used to differentiate between healthy and stressed vegetation and to determine the level of specific heavy metals in plants. Some plants used for phytoremediation in wetlands like *P. australis* and *A. donax* are morphologically similar, and it is also important to be able to discriminate between such species using remote sensing.

2.4.2 Remote sensing to discriminate between wetland plants

Successful management of wetlands requires proper and up to date mapping and discrimination of wetland plants (Hirano *et al.*, 2003; Davranche *et al.*, 2010). While the physical methods of wetland plant survey is time consuming and have accessibility and many other logistical problems, remote sensing can be an effective and alternative tool for such purpose. Discrimination of plant species using remote sensing is possible because different vegetation types reflect differently to electromagnetic radiation. Plant leaves of different species have different biophysical and biochemical characteristics, and these affect their leaves' spectral properties, and make it possible to discriminate between different plant species (Kumar *et al.*, 2001). Extensive research has been done using remote sensing to discriminate between plant species (Adam *et al.,* 2010). For example, Dubula *et al.* (2016) proved the potential of remote sensing in discriminating invasive plant species in the Klipriviersberg nature reserve in Johannesburg, South Africa. Their research concluded that at both individual level and plot level, the near infrared region of the electromagnetic spectrum could be used to discriminate between *Asparagus laricinus* and other vegetation species in the nature reserve. Pu (2009) used spectrometry to identify 11 forest species. They concluded that two classification algorithms, Artificial Neural network (ANN) and Linear Discriminant Analysis (LDA) were effective in discriminating plant species using selected spectral variables that are linked to water content, pigments and other biochemicals.

Even though remote sensing is proven as a practical and cheap method for discriminating plant species, there are some challenges involved when discriminating wetland plant species. It is not easy to detect and discriminate wetland vegetation types using optical remote sensing, because of very high spatial and spectral variability, due to very short ecotones between wetland vegetation units (Adam and Mutanga, 2009; Zomer *et al.*, 2009). Thus, the selection of the appropriate spatial and spectral resolution and the best process to use to extract the spectral information is of paramount importance (Elhadi *et al.*, 2009). According to the review paper by Adam *et al.* (2010), hyperspectral remote sensing, especially field spectrometry, is more appropriate and accurate in discrimination of wetland plant species compared to aerial photography and multispectral remote sensing such as Landsat TM and SPOT. This is because hyperspectral sensors have hundreds of narrow continuous spectral bands ranging between 400 to 2500 nm, which make them capable to provide more details on vegetation types (Zomer *et al.*, 2009). In this regard, a study by Smith and Blackshaw (2003) confirmed that plant species discrimination using hyperspectral data yielded more accurate results than that of multispectral data. The research also indicated that the visible (400-700 nm) and the red edge (700-730 nm) bands were the regions significantly useful for plant species discrimination. Schmidt and Skidmore (2003) also concluded that increased spectral resolution improved accuracy in discrimination of vegetation species of similar structure in a wetland. The purpose of their research was to discriminate 27 wetland plant species, including *P. australis* using field spectrometry. Their results showed that more than 75 % of the possible pairs of plant species showed significant differences based on their spectral reflectance measurements. Furthermore, research by Vaiphasa *et al.* (2005) concluded that field spectral measurements of crown leaves of various mangrove species were efficient in discriminating mangrove species. In their research a field spectrometer, with 2151 bands ranging from 350 to 2500 nm, was used to measure leaf spectral reflectance of 16 mangrove species. Statistical analysis of the spectral measurements indicated that the 16 species were statistically different at most spectral locations. Adam *et al.* (2012) also used ASD spectrometry measurements to discriminate among four wetland vegetation species, *Cyperus papyrus* L., *P. australis*, *Echinochloa pyramidalis* and *Thelypteris interrupta* in Greater St Lucia Wetlands Park in South Africa. Van Deventer and Cho (2014) also proved that field spectroscopy can be a quick and cheap method to assess the health and condition of vegetation affected by acid mine drainage.

2.5 Conclusion

Arundo donax and *P. australis*, among other macrophytes, are very important in phytoremediation of heavy metal contaminated wetland systems. Their efficiencies in heavy metal uptake have never been compared under same and controlled environment. While the two species could be important phytoremediating plants, determining their efficiency could help their management as weeds, particularly considering the fact that *A. donax* is an infamous invader of the water system in South Africa. It will also help determine the effective plant of choice for phytoremediation in constructed wetlands. Remote sensing could be an effective method for monitoring of heavy metal-induced stress in both plant species to facilitate their management before they die and release the contaminants removed from water and accumulated in plant tissue back to the source. Although the two plant species are morphologically similar, remote sensing can effectively discriminate between them. Research on these reeds should focus on comparing the heavy metal uptake capacities between the two under controlled/experimental conditions, and determine the best method to discriminate between them using remote sensing for monitoring their efficiency in heavy metal uptake.

CHAPTER THREE

3. The comparison of copper uptake between *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud.

This chapter is based on:

Mabhungu, L., Newete, S.W. and Adam, E. (*In review*). The comparison of copper uptake between *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud., *Ecology, Environment and Conservation.*

Abstract

The heavy metal uptake efficiency and the relationship between leaf copper concentration and chlorophyll for *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud., were assessed under experimental conditions in tubs replicated 12 times each. Half of the replicates in each species were exposed to a solution of Copper (5 mg/L) and nitric acid (pH 3.5) artificially mixed to simulate an acid mine drainage (AMD), while the remaining half were kept as control plants without any of the treatment. Measurements of pH, electrical conductivity and chlorophyll content were recorded from all the tubs. Samples of soil, water and plant tissues from each tub were analyzed for copper, and the Bio-concentration Factors (BCF) and Translocation factors (TF) of each species were computed. Copper uptake decreased in the order roots>leaves>stems for *A. donax* and root>stems>leaves for *P. australis*. Both species exceeded the benchmark of BCF >1 for plants considered as good for phytoremediation. Nevertheless, *P. australis* had a higher TF (0.4) than *A. donax* (0.2), and thus it is the preferred plant for phytoremediation. Regression analysis showed a significantly positive relationship ($p < 0.05$) between leaf copper concentration and chlorophyll content. However, the low R^2 (0.29) obtained meant that the independent variable (chlorophyll), could explain only a very little percentage of the variation in the dependent variable.

***Keywords**:* acid mine drainage, bio-concentration factor, phytoremediation, translocation factor

3.1 Introduction

Acid Mine Drainage (AMD), from the mining industry, is a major source of pollution affecting surface and ground water bodies in South Africa (Limpitlaw, 1996; McCarthy and Humphries, 2013). Wetlands are a common feature along streams and rivers in mining areas of South Africa, where they are subjected to AMD pollution (Tutu *et al.*, 2008). Artificial wetlands have been constructed in mining areas to allow purification of water before discharge into lakes and other water bodies (Tutu *et al.*, 2008). AMD is characterised by low pH, between 2 and 4, and elevated concentrations of heavy metals including copper, aluminium, manganese, zinc, iron, nickel, cobalt and cadmium (Bell *et al.*, 2001; Seadira, 2014). It occurs when acidic waters are released from mining sites to the environment due to exposure of sulphide minerals (mostly pyrite $FeS_{2(s)}$) to oxidising conditions (Bell *et al.*, 2001; Pat-Espadas *et al.*, 2018). The equation below, extracted from Pat-Espadas (2018), represents the chemical formation of AMD:

$$FeS_{2(s)} + \frac{15}{4}O_2 + \frac{7}{2}H_2O \rightarrow Fe(OH)_{3(s)} + 2SO_4^{2-} + 4H^+ \tag{3.1}$$

Acid mine drainage pollution in wetlands affects, not only the wetlands and their ecology, but seepage of the acidic waters may end up in streams, rivers or dams, which supply potable water for human beings, leading to bioaccumulation and bio-magnification in the food chain (Sukumaran, 2013). For example, an investigation by McCarthy and Humphries (2013) revealed that the cause for sudden deterioration in water quality of the Boesmanspruit Dam, which supplies portable water to Carolina town in Mpumalanga, South Africa, was AMD from coal fields, which had polluted upstream wetlands. The dam's water was highly acidic with elevated concentrations of total dissolved solids, heavy metals and nutrient ions, making it unsuitable for house hold use. On the other hand, Bell *et al.* (2001) found that water quality in the Blesbokspruit dam was below South African standards for portable water, and this was because of AMD pollution from the Witbank coalfield. Conventional methods of heavy metal removal from polluted wetlands involve chemical treatment of AMD and adsorption of metal pollutants by non-living biomass. These methods require huge capital for operation and management (Sukumaran, 2013; Kumari and Tripathi, 2015a). On the other hand, phytoremediation, based on the natural ability of certain plants to extract, sequester, degrade, or render contaminants in the environment harmless, is both cost effective and environmentally friendly (Sukumaran, 2013; Seadira, 2014; Kumari and Tripathi, 2015a). Among wetland plants, emergent plants are able to uptake heavy metals into their tissues several times above normal thresholds. Examples of such plants are *Phragmites australis* (Cav.) Trin. ex Steud., *Arundo donax* L., *Phalaris arundinacea*, *Scirpus spp*. and *Typha spp*. (Papazoglou *et al.*, 2005; Vymazal and Březinová 2016; Newete and Byrne, 2016), and most have been used for phytoremediation of AMD affected natural and/or constructed wetlands in South Africa (Tutu *et al.*, 2008; De Villiers *et al.*, 2011). *Arundo donax* and *P. australis* are two morphologically similar wetland reeds that are commonly used in wetlands of South Africa for heavy metal removal (Van der Merwe *et al.*, 1990; Zingelwa and Wooldridge, 2009). While *P. australis* is a native species in South Africa, *A. donax L.* is a naturalised alien invasive species (Henderson and Cillier, 2004; Milton, 2004; Rouget *et al.*, 2004). The two species play an important role in phytoremediation of wetland systems (Bello *et al.*, 2018; Bonanno, 2012), and are often planted in artificial wetlands for purposes of wastewater and sludge treatment (Bonanno, 2013; Uggetti *et al.*, 2012). They have well-developed root systems, large biomass and significantly high tolerance to toxicity which enable them to remove trace elements from wetlands and sequester them across their various tissues (Bonanno *et al.*, 2018).

The degree to which plants accumulate heavy metals is determined by the individual plant uptake capacity and intracellular transportation within the plant (Yang *et al.*, 2005). The stages involved in the heavy metal accumulation process are mobilisation of the metal ions in the rhizosphere, transport of metals across the plasma membrane of root cells, xylem loading and translocation, and detoxification and sequestration of heavy metals at the whole plant and cellular levels (Khan *et al.*, 2000). Heavy metals absorbed by plants are distributed between below ground biomass (root system) and the above ground biomass (stems and leaves). To a larger extent, the concentration of heavy metals stored in the below ground biomass of wetland plants is higher than in the above ground biomass, and it varies with inflow loads of the metals into the wetland (Phillips *et al.*, 2015). The rate of distribution of the metals from the roots (below ground) to stems and leaves (above ground biomass) vary with plant species, as well as with the metal in question. For above ground biomass, generally, the main storage site for accumulated metals is the leaf cell vacuole (Tong *et al.*, 2004). Thus, with the exception of Zinc, which is mainly used for growth purposes in the stems, the concentrations of accumulated heavy metals are commonly higher in the leaves than in stems (Vymazal and Březinová, 2015a).

Copper is crucial to plant health in many ways. It is an important component of enzymes responsible for cell metabolism, chloroplast formation and it maintains the membrane structure of thylakoids in plants (Adrees *et al.*, 2015). On land surfaces, Cu binds with organic matter in the soil and that significantly reduces its availability for plant uptake. However, in aquatic systems, plants are directly exposed to Cu toxicity (Fernandes and Henriques, 1991).

Tutu *et al.* (2008) characterised AMD pollution in the Witwatersrand Basin, South Africa. The surface waters sampled had pH levels between 2.3 and 4, and electro-conductivity (EC) values exceeding 400 µs/cm. For copper, most samples had concentrations below 5mg/L but the highest recorded in the basin was 11.43 mg/L. The normal range of copper concentrations in plant tissues for plants growing in non-contaminated environments is from 8 to 13ppm (Fernandes and Henriques, 1991). Generally, plant tissue copper concentration above 20ppm is toxic to plant growth, as it negatively affects root elongation, pigment synthesis and photosynthesis, and result in symptoms of senescence (Fernandes and Henriques, 1991; Ali *et al.*, 2002; Newete *et al.*, 2016). However, the toxicity thresholds are highly variable, and may be as high as 575ppm (for example in sedges), or 1,000 ppm, in metallophytes (Fernandes and Henriques, 1991).

The heavy metal uptake capacities for *A. donax* and *P. australis* have never been compared under controlled or experimental conditions (Mabhungu *et al.*, 2019). Comparison under field conditions by Bonanno (2013) showed that *P. australis* had a higher heavy metal uptake capacity than *A. donax* It is important to determine which of the two species is more effective in phytoremediation under same and controlled conditions in order to promote the native *P. australis* for phytoremediation in constructed wetlands, should it be found more effective. Thus, the current study compared the heavy metal uptake efficiency between the two species under experimental conditions, using copper contaminant.

3.2 Materials and Methods

3.2.1 Glasshouse experiment

A glasshouse experiment was setup at the University of the Witwatersrand, Johannesburg, South Africa in January 2018. Rhizomes of giant reed (*A. donax*) and common reed (*P. australis*) were collected from Lake Florida, Johannesburg, South Africa, and planted in rooting beds filled with sand. After 21 days, rhizome cuttings with stem height of about 12 cm bearing one plant each were selected and transplanted into 12 twenty-litre plant tubs each for *A. donax* and *P. australis*. Two rhizomes were planted into each tub. Each tub contained layers of gravel, sand soil and top soil starting from the bottom up. The tubs were irrigated when necessary to maintain the water levels at about 8 cm above the top soil layer. Immersible pumps were setup into each pot to keep the water agitated and circulating, to mimic a wetland environment. Three months after transplanting the plants, half of the tubs from each plant species were treated with copper to make a concentration of 5mg/L while the other half was kept free of Cu as a control. Drops of nitric acid were added to each of the copper treated tubs to attain an average pH of 3.5. This was to mimic copper contamination from acid mine drainage pollution in wetlands. At this stage water electrical conductivity (EC) and pH were measured from each tub. At the end of the experiment, two months after the Cu + acid treatment addition, leaf chlorophyll content was measured from each tub. pH and EC were also measured for the second time. Samples of soil, water and plant tissues (leaves, stems and roots) were prepared from each tub for copper analysis.

3.2.2 Chlorophyll measurement

Leaf chlorophyll content was measured using the non-destructive hand held SPAD-502 (Minolta Camera Co., Osaka, Japan) chlorophyll meter. The device measures leaf absorbance at two wavelengths, 400-500 nm and 600-700 nm, and converts it into digital signals, and then into a SPAD value, which is proportional to extractable chlorophyll concentration in the leaf (Minolta, 1989; Süß *et al.*, 2015). SPAD values range from 0 to 99 and they are positively correlated with destructive chlorophyll measurements with r^2 above 0.9 (Wood *et al.*, 1993; Rodriguez and Miller, 2000). The higher the chlorophyll content, the healthier the plant. Chlorophyll content was measured from four randomly selected leaves from each tub, then averaged to give a mean value.

3.2.3 Copper concentration assessment in plant tissues, soil and water

Plant, water and soil samples were collected from all tubs for copper analysis. Plant samples collected from both control and Cu + acid treatment tubs of both species were separated as leaves, stems and roots, packed in labelled paper bags, and oven dried at 70 °C for 72 hours. Soil samples from each of the tubs were air dried for three days before being packed in labelled plastic bags (500g each). Water samples were collected in 500ml plastic bottles and kept in a refrigerator until transferred to the laboratory for analysis. Finally, all samples (soil, water and plant tissue) were sent to the Agricultural Research Council-Institute for Soil Climate and Water (ARC-ISCW, Pretoria) Laboratory for copper analysis. The inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to analyse for copper.

3.2.4 Assessment of phytoremediation effeciency of Phragmites australis and Arundo donax

The potential of *A. donax* and *P. australis* for phytoremediation was assessed using Bioconcentration factor (BCF) and Translocation factor (TF) (Mishra and Pandey, 2019). Bio-concentration factor measures the capacity of a plant species to accumulate a particular metal from the surrounding environment into its tissues (Ladislas *et al.*, 2012; Ndeda and Manohar, 2014; Mishra and Pandey, 2019). This was calculated for leaves, stems and roots of both plant species, and for both water and soil mediums, as indicated below:

$$\text{Bio-concentration Factor (BCF)} = \frac{\text{Concentration of Cu in plant tissue}}{\text{Concentration of Cu in external environment}} \tag{3.2}$$

Phytoremediation is possible when the BCF for a particular metal in a particular plant tissue is above 1 (Nazir *et al.*, 2011; Mishra and Pandey, 2019). Translocation Factor (TF) describes the ratio of metal concentration in shoots to the metal concentration in roots, and measures the ability of a plant to translocate metals from roots to shoots (Mojiri *et al.*, 2015). It was determined by the equation below:

$$\text{Translocation factor (TF)} = \frac{\text{Concentration of Cu in shoot}}{\text{Concentration of Cu in root}} \tag{3.3}$$

A plant has potential for phytoremediation when its TF is above 1.

3.2.5 Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey post hoc test from SPSS was used to compare means of copper concentration, leaf chlorophyll content, pH and EC for various data sets. The simple linear regression model was used to determine the relationship between leaf copper concentration and chlorophyll content. The significance of the overall regression model (p-value) and the percentage of variance explained by the independent variable (R^2) were used to determine whether the linear regression model is a good fit for the relationship between leaf copper concentration and leaf chlorophyll. All statistical tests were performed at 95 % confidence level. Data was presented as mean ± standard error.

3.3 Results

3.3.1 Electrical conductivity and pH

The pH and EC results showed some significant differences (($F_{(7, 40)} = 582.37$, $p < 0.0005$) and ($F_{(7, 40)} = 38$, $p < 0.0005$), respectively) between the different treatments (Figure 3.1 and 3.2). After the addition of copper + acid to treatment tubs at the start of the experiment (Initial), the mean EC significantly increased ($p < 0.0005$) by 98 % from 587 ± 10 µs/cm in the *A. donax* tubs and by 116 % from 441 ± 22 µs/cm in the *P. australis* tubs. At the end of the experiment (Final), the mean pH in the treatment tubs significantly increased ($p < 0.0005$) to levels comparable with the control tubs, 8.35 ± 0.02 and 8.34 ± 0.16 for *A. donax* and *P. australis* respectively, and the EC significantly decreased ($p < 0.0005$) by 58.4 % from 1162.67 µs/cm for *A. donax* and by 48.0 % from 955.5 µs/cm for *P. australis*. There was no significant differences in the mean pH and EC between the treatment tubs of the two species ($p > 0.05$) measured at the start and end of the experiment.

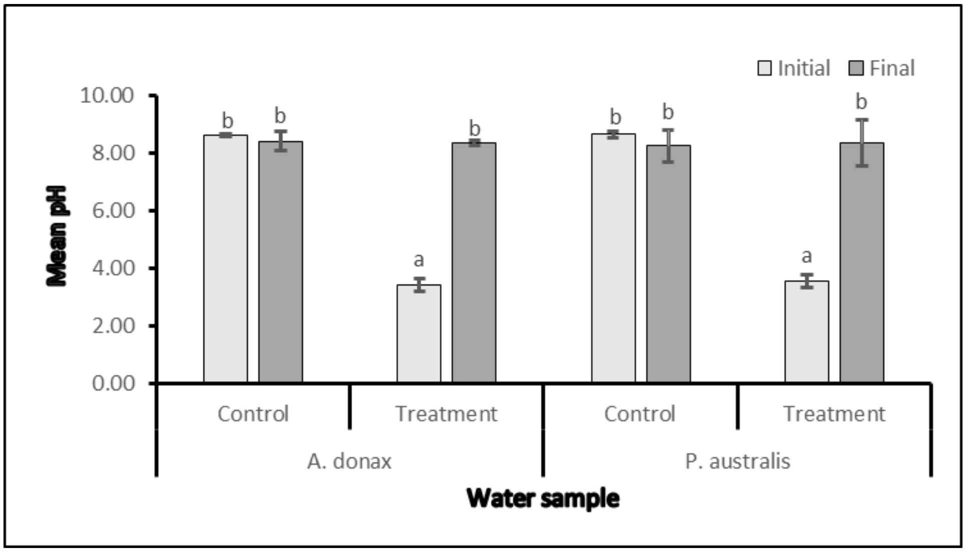

Figure 3.1 Mean water pH for control and treatment tubs of *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud.. All means compared by one-way analysis of variance followed by Tukey post hoc test. Those followed by the same letter(s) are not significantly different ($P > 0.05$). Error bars: ± 2SE

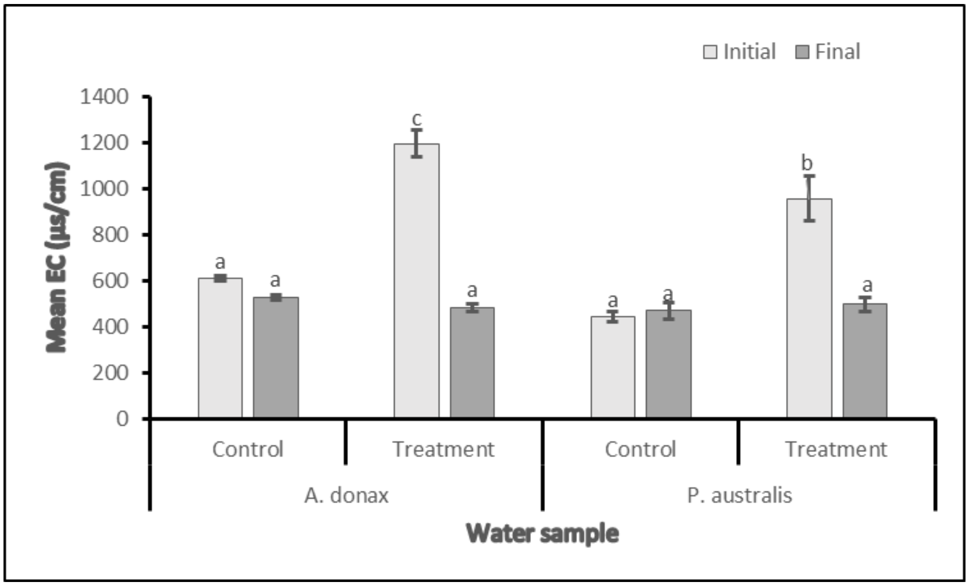

Figure 3.2 Mean water EC for control and treatment tubs of *A. donax L*. and *P. australis*. All means compared by one-way analysis of variance followed by Tukey post hoc test. Those followed by the same letter(s) are not significantly different (P > 0.05). Error bars: ± 2SE

3.3.2 Copper concentrations in soil and water

There were significantly higher levels of Cu in the treatment than in the control tubs ($p < 0.05$) for both soil and water samples and in *A. donax* and *P. australis* (Figure 3.3). The mean concentration of copper was significantly higher (more than 250 times higher) in soils than in water in both species ($p < 0.05$). The mean soil copper concentration in *P. australis* treatment tubs was significantly higher than in *A. donax* tubs with mean difference of 3.49 ± 0.81 ppm ($p = 0.002$). No significant difference was noted on mean water copper concentration between treatment tubs of the two species ($p = 0.732$).

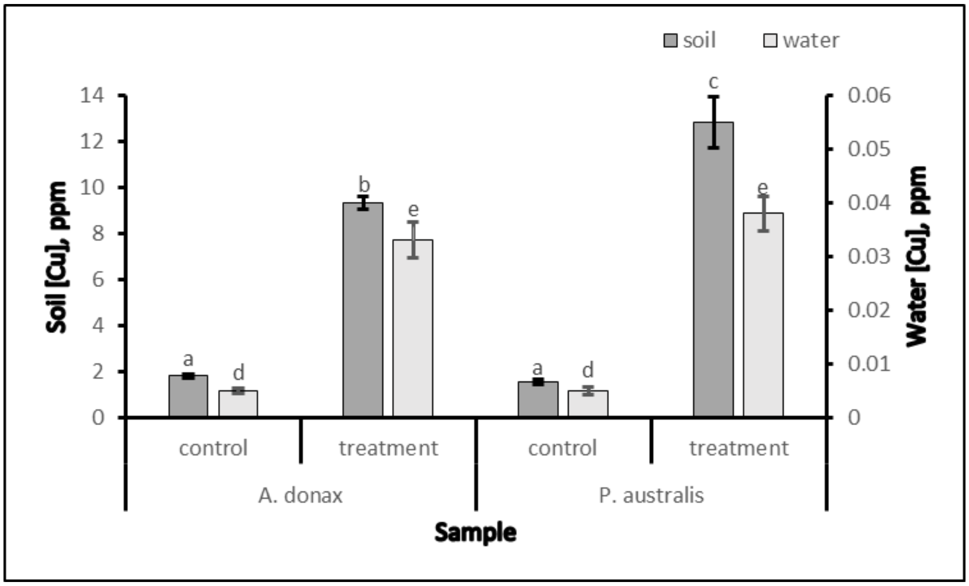

Figure 3.3 Mean soil and water copper concentrations for control and treatment tubs of *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud.. tubs. Means compared by one-way analysis of variance followed by Tukey post hoc test. Those followed by the same letter(s) are not significantly different (P > 0.05). Error bars: ± 2SE

3.3.3 Copper accumulation in plants' tissues

Copper uptake was assessed in the tissues of *A. donax* and *P. australis* grown in control (healthy) and copper + acid treatments. Significant differences were observed between the control and treatment of the leaf and root samples of *A. donax* with mean differences of 2.876 ± 0.364 and 25.603 ± 3.119 mg/kg, respectively, and the stems and roots of *P. australis* with mean differences of 6.512 ± 2.01 and 16.10 ± 3.62 mg/kg, respectively (Figure 3.4). Copper concentrations in the treatment plants decreased in the order roots>leaves>stems for *A. donax* and root>stems>leaves for *P. australis*. Comparison of means of copper concentrations between treatment samples of the two species showed significant differences ($p < 0.05$) in stem and root samples. The mean copper concentration in the stems of *P. australis* (13.20 ± 1.95) was significantly higher than those in the *A. donax* (6.77 ± 0.80) by 95 %, and in roots of *A. donax* (34.95 ± 3.06) significantly higher than of *P. australis* (26.75 ± 1.99) by 30 %.

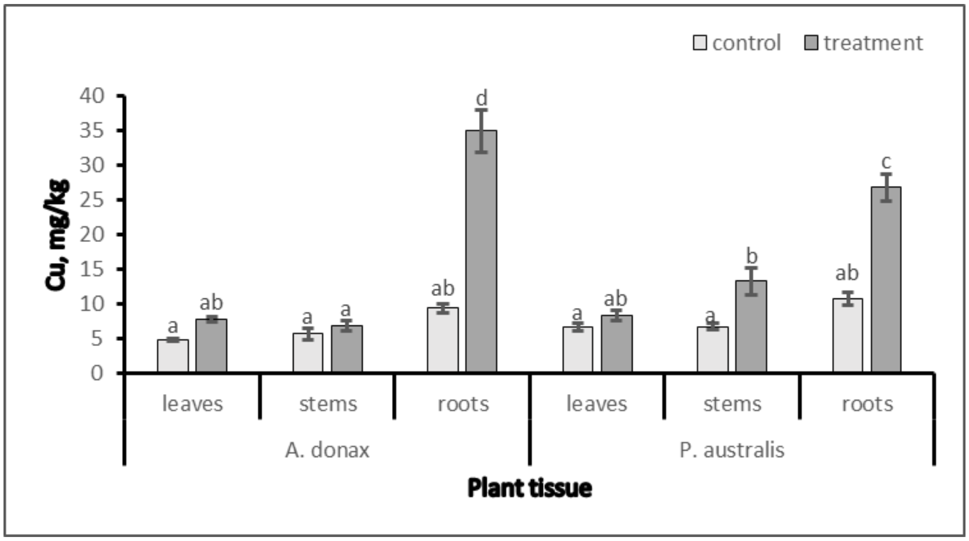

Figure 3.4 Copper accumulation by tissues of *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud.. All means compared by one-way analysis of variance followed by Tukey post hoc test. Those followed by the same letter(s) are not significantly different ($P > 0.05$). Error bars: ± 2SE.

Copper accumulation by treatment plants was more pronounced in roots than in above ground tissues in both plant species, as revealed by their bio-concentration factors (BCFs), deteremined with respect to both soil and water mediums (Table 3.1). Phytoremediation is considered more effective when the BCF for a particular metal in a particular plant tissue is above one. Thus, from this study the potential for phytoremediation of contaminated water was proved through accumulation in all the plant tissues, while for soils phytoremediation is possible through accumulation in roots of both plants and stems of *P. australis* only. The translocation factors for the two plant species were 0.4 and 0.2 for *P. australis* and *A. donax*, respectively.

Table 3.1 Bio-concentration factors for *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. plants grown in treated environment.

	Bio-concentration factor (BCF)					
	Leaves		**Stems**		**Roots**	
Plant species	**Soil**	**Water**	**Soil**	**Water**	**Soil**	**Water**
A. donax	0.82	231.07	0.7	203.04	3.7	935.54
P. australis	0.64	276.64	1.03	441.81	2.1	895.06

3.3.4 Leaf chlorophyll content

The SPAD chlorophyll values ranged from 34.63 to 39.37 for *A. donax* and 33. 87 to 43.77 for *P. australis* (Table 3.2). One-way ANOVA test ($F_{(3,38)} = 11.935$, $p < 0.0005$) and post hoc test revealed significant differences between means of the leaf chlorophyll content between the two species.

Table 3.2 Mean chlorophyll content for *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. leaves. Means compared by one-way analysis of variance followed by Tukey post hoc test. Those followed by the same letter(s) are not significantly different (P > 0.05). Error bars: ± 2SE.

Treatment		**Mean chlorophyll index ± S.E**
Arundo donax	Control	35.70 ± 0.72^{a}
	Treatment	36.14 ± 0.7^{a}
Phramites australis	Control	34.82 ± 1.79^{a}
	Treatment	40.37 ± 0.97^{b}

3.3.5 The relationship between copper accumulation in plant tissues and chlorophyll content

Linear regression from SPSS was used to determine the relationship between copper accumulation in plant leaves and chlorophyll content, and whether chlorophyll content values can be used to estimate leaf copper concentration. The regression model showed there was a significant linear relationship ($F_{(1,21)} = 8.574$, $p = 0.008$) between leaf copper concentration and chlorophyll content. However, the R^2 value (0.29) showed that chlorophyll could explain only a small proportion of the variation in leaf copper concentration (Figure 3.5). The regression equation was as follows:

$$\text{Leaf\_copper\_concentration} = -6.39 + (0.35 \times \text{Chlorophyll\_content}) \qquad (3.4)$$

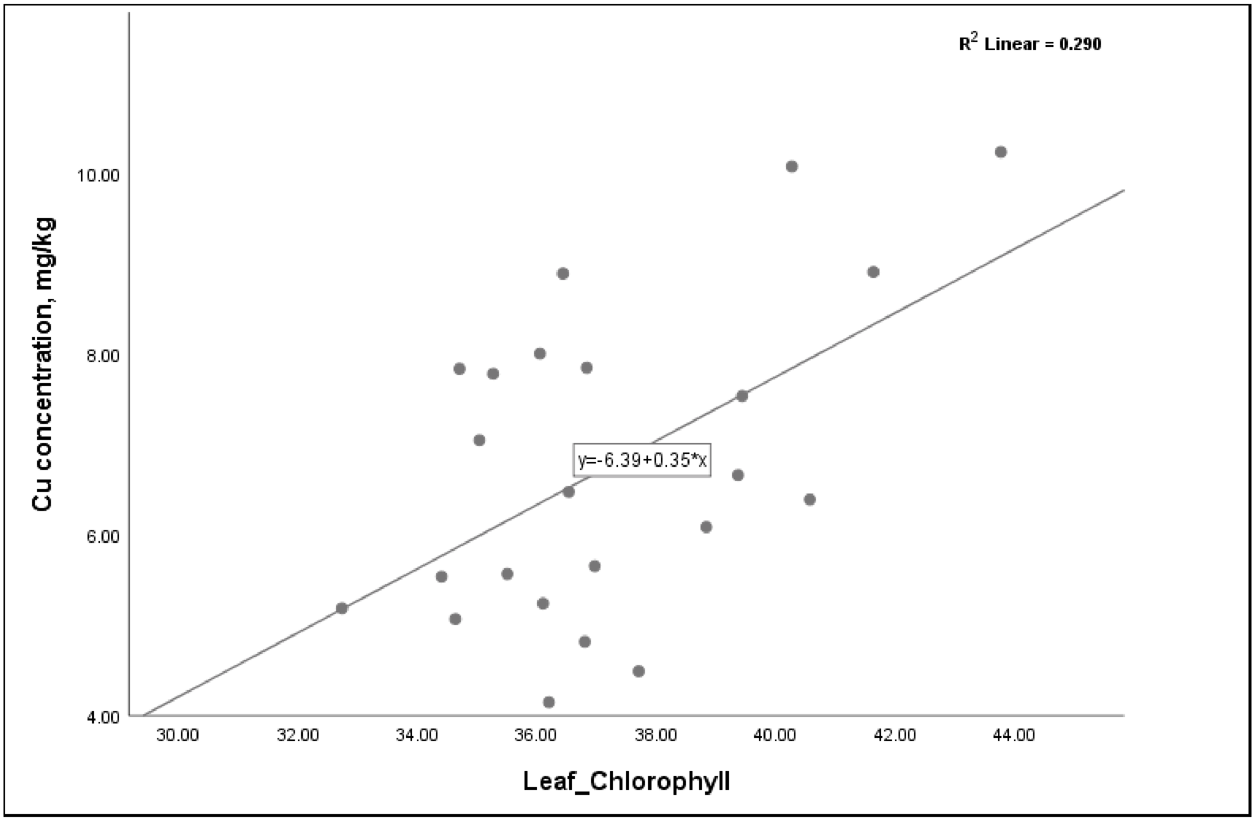

Figure 3.5 Relationship between leaf chlorophyll content and leaf copper concentration produced using the linear regression model.

3.4 Discussion

3.4.1 Copper accumulation by Arundo donax **and** *Phragmites australis*

Both *A. donax* and *P. australis* showed potential to remove substantial amounts of copper from acid mine drainage contaminated wetland environments. Treatment plants for both species accumulated greater copper concetration in their tissues than those planted in the controls (Figure 3.4). Several other studies have also proved the two plant species as candidates for heavy metal uptake in aquatic systems (Papazoglou *et al.*, 2005; Mishra *et al.*, 2008; Kumari and Tripathi, 2015a). The distribution pattern of copper concentrations in plant tissues was roots>leaves>stems for *A. donax* and root>stems>leaves for *P. australis*. The distribution pattern in tissues of *A. donax* is in agreement with results from Bonanno (2013). More copper was stored in the root system of both plant species, than the amount translocated to the above ground tissues (stems and leaves). Thus, the roots play a significant role in immobilizing the heavy metals. Other studies also got simillar results (Deng *et al.*, 2004; Phillips *et al.*, 2015). In Deng *et al.* (2004), concentrations of

Pb, Zn, Cu and Cd in 12 emergent wetland plants were investigated in China, and it was concluded that for all the four metals, concentrations were significantly higher in roots than in the shoots. The retention of more copper in roots compared to above ground tissues is a defence mechanism to restrain heavy metals from reaching the metal toxicity sensitve phtosythetic organs (Ho *et al.*, 2008; Newete and Byrne, 2016).

Bio-concentration factors (BCFs) higher than 1 obtained for roots of both plant species and stems of *P. australis* indicate their efficiency for phytoremediation through accumulation of copper in the respective tissues. Based on the BCFs obtained for soil and water mediums for *A. donax* (0.82 and 231.07; 0.7 and 203.04; 3.7 and 935.54, for leaves, stems and roots, with respect to soil and water mediums respectively) and *P. australis* (0.64 and 276.64; 1.03 and 441.81; 2.10 and 895.06, for leaves, stems and roots, with respect to soil and water mediums respectively) (Table 3.1), neither *A. donax.* nor *P. australis* is a hyperaccumulator for copper. Hyperaccumulators for copper are plants with BCFs above 10 with respect to soil and above 1000 with respect to water, and that can accumulate more than 1,000 mg/kg of Cu (Baker and Brooks, 1989, Baker *et al.*, 1994; Zayed et.al., 1998; Zhu *et al.*, 1999; Hammad, 2011). The *A. donax* and *P. australis*, in this study, had BCFs above 1 and TFs lower than 1, indicating that the plants are tolerant to copper contamination under acidic conditions and are siutable for phytoremediation, but they are not good candidates for phytoextraction. Both plant species have the ability to uptake substantial amounts of copper into their roots, but restrict its transfer to above ground tissues. Phytoremedition in such plants is mainly through phytostabilisation and / or rhizofiltration (Pivetz, 2001; Newete and Bryne, 2016). The results in this study are in agreement with Liu *et al.* (2010a) where *P. australis* had a TF around 0.7 for copper, but contradicts with findings of Mojiri *et al.* (2015), where the TF was above 1 for copper. Although the BCFs and TFs for the native *P. australis* and the alien invader *A. donax* were found to be in the same range, the former had a higher capacity to transfer copper to above ground tissues (TF = 0.4), which can later be harvested to remove the contaminant load from the wetland environment. Since the two species showed no significant difference in Cu removal from the tubs, the native *P. australis* should be the preferred plant for phytoremedaiton under the restrictive regulation of National Environmental Management: Biodiversity Act (NEM:BA) No. 10 of 2004 (Department of Environmental Affairs, 2016). Thus, between the two species, *P. australis* is a better candidate for phytoremediation in wetlands contaminated with copper from AMD in South Africa than *A. donax*.

3.4.2 Chlorophyll concentration and relationship with copper accumulation

Leaf chlorophyll concentration, among other factors, determines the photosynthetic potential and primary productivity of a plant (Li *et al.*, 2018). The higher the chlorophyll content, the healthier the plant is. In this study, mean chlorophyll content was higher in treatment plants than in the control plants. This means the treated plants did not accumulate copper to phytotoxic levels. Copper is a significant micronutrient for plants, and acts as a catalyst in respiration and photosynthesis. It is required for the formation of proteins and enzymes in the mitochondria and chloroplasts where chlorophyll is formed (Adrees *et al.*, 2015). Hence, an increase in accumulated copper in leaves for both plant species between control and treatment resulted in more chlorophyll production. The low pH and decreased EC in treatment tubs did not have negative impacts on chlorophyll content.

Although the low p value from the linear regression model ($p < 0.05$) indicated a statistically significant relationship between leaf copper concentration and SPAD chlorophyll values, the low R-squared value showed that the chlorophyll content can only explain 29 % of the variation in leaf copper concentration. Hence, given the chlorophyll content, it is not possible to predict or estimate accurately the corresponding leaf copper concentrations for the plants using the regression model. The amount of copper accumulated in the treatment leaves (mean = 7.70 mg/kg and 8.7 mg/kg for *A. donax.* and *P. australis*, respectively) in this study did not get to levels that negatively affect chlorophyll production, as opposed to those reported by Shaheen *et al.* (2019a), where copper accumulation in plants showed toxicity symptoms in leaves at concentrations above 75 mg/kg. High copper concentration, above phytotoxicity levels for a particular plant species, leads to decreased chlorophyll content, and may show symptoms like chlorosis, stunted growth, necrosis and leaf discoloration (Esmaeilzadeh *et al.*, 2016). The weak correlation between leaf copper concentration and chlorophyll as depicted by the regression model may be explained by the fact that the accumulated copper was too small to affect or degrade chlorophyll level in the leaves.

3.5 Conclusions

Copper accumulation in plant tissues of *A. donax* and *P. australis* varied in the order of roots>leaves>stem for *A. donax* and root>stems>leaves for *P. australis*. Based on their translocation factors, the *P. australis* which showed a higher translocation factor, is a better candidate for phytoextration than *A. donax* and considering that *P. australis*is native to South Africa, its preference as a tool of phytoremedaiton is safer and sustainable as opposed to the exotic reed, the *A. donax* whose propagation is restricted by environmetal and biodiversity regulations of the country. There was a significant positive relationship between leaf copper concentration and chlorophyll content. The levels of copper accumulated by the plants were too small to cause any degrading effects to leaf chlorophyll content.

CHAPTER FOUR

4. Spectral discrimination between *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. grown in copper contaminated conditions using in situ field spectral measurements

This chapter is based on:

Mabhungu, L., Adam, E. and Newete, S.W., (*Under review*) Spectral discrimination between *Arundo donax L.* and *Phragmites australis* (Cav.) Trin. ex Steud. grown in copper contaminated conditions using in situ field spectral measurements. *International Journal of Environment and Pollution.*

Abstract

The purpose of this chapter was to discriminate between *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. species under healthy (control) and copper + acid (treatment) conditions. The specific objectives were (i) to determine if copper and acid treatment had a significant effect on the spectral reflectance properties of plant leaves, (ii) to discriminate between *A. donax* and *P. australis* species both under control and copper + acid treatment conditions and, (iii) to compare the random forest (RF) and support vector machines (SVM) models in discriminating the two plant species using features selected from the GRRF. *Arundo donax* and *P. australis* were grown under experimental conditions in tubs replicated 26 times each. Half of the tubs for each species were exposed to 5 mg/L of copper and nitric acid (pH 3.5) artificially mixed to simulate acid mine drainage in wetland environments. Leaf spectral reflectance and chlorophyll content were measured from each tub. Plant leaf, water and soil samples from all tubs were analyzed for copper concentration. Results showed that copper uptake influenced the spectral properties of the plants. The RF produced higher level of separability (overall accuracy = 85.2 % and Kappa = 0.7) between *A. donax* and *P. australis* compared to SVM (overall accuracy = 81.4 %, Kappa = 0.63). Overall, there was high efficiency in using the GRRF coupled with either RF or SVM classification for discriminating and mapping the two plants.

Key words: Acid mine drainage; *Arundo donax* ; environmental pollution; guided regularized random forest; Hyperspectral data; *Phragmites australis*; Random Forest; spectral discrimination; support vector machines.

4.1 Introduction

Wetland ecosystems are a vital ecological infrastructure that provide multiple services such as sediment and pollution trapping, stream flow augmentation, removal of nutrients from water, control of erosion, recreational benefits as well as proving habitats for wildlife (Desta *et al.*, 2012; Luo *et al.*, 2017). The capabilities of wetlands in reducing and controlling different contaminants such as heavy metals are well documented (Mabhungu *et al.*, 2019; Newete and Byrne, 2016). According to De Villier *et al.* (2011), human activities including mining, agriculture and development of urban areas have negatively impacted 50 % of wetlands in South Africa. Wetlands are common along most streams and rivers in mining areas of South Africa (Tutu *et al.*, 2008). Impacts of mining on streams in South African include heavy metal and Acid Mine Drainage (AMD) pollution, degradation of land and vegetation biodiversity, and destruction of habitats for wetland wildlife animals (Gupta and Nikhil, 2016). In such highly polluted areas, wetlands play a crucial role in maintaining biodiversity and controlling pollution (Xu *et al.*, 2020). Wetland plants have proved vast potential in removing metals from water and soils through accumulation in aboveground plant tissues ‘‘phytoextraction’’ and/or below ground in plant roots “rhizofiltration” (Tomé *et al.*, 2008; Weis and Weis, 2004). Heavy metal concentration in wetland plants vary considerably between species and wetland systems. This is due to the fact that some wetland plant species, such as *Arundo donax L.* (giant reed) (Song *et al.*, 2014; Yang *et al.*, 2012) and *Phragmites australis* (Cav.) Trin. ex Steud. (common reed) (Shaheen *et al.*, 2019b; Xu *et al.*, 2020), exhibit a high metal accumulation capacity.

Both *P. australis* and *A. donax* are tall, $C_{3,}$ robust and perennial aquatic emergent species that are widely distributed across South African wetlands (Canavan *et al.*, 2018; De Villiers *et al.*, 2011). They also grow in many contaminated areas such as industrial and mining areas (Bello *et al.*, 2018). They both form dense stands which crowd or shade other wetland vegetation (Everitt *et al.*, 2004; Kawanabe *et al.*, 2012). Despite many similarities between the two, *A. donax* is relatively taller, with broader leaves and larger above-ground biomass than *P. australis* (Lusweti, 2011). *Arundo donax* has been cultivated across Asia, southern Europe, North Africa, and the Middle East for thousands of years, while *P. australis* is native to most countries in America, Africa, Europe, Asia and Australia (Lambert *et al.*, 2010; CABI, 2019). *Phragmites australis* is native in South Africa (Canavan *et al.*, 2018), while *A. donax* is an alien species deliberately introduced into the country in the late 1700, for purposes of soil erosion control (Guthrie, 2007).

The two aquatic macrophytes play an important role in phytoremediation of wetland systems (Bello *et al.*, 2018; Bonanno, 2012), and are often planted in artificial wetlands for purposes of wastewater and sludge treatment (Bonanno, 2013; Uggetti *et al.*, 2012). They have well-developed root systems, large biomass and significantly high tolerance to toxicity which enable them to remove trace elements from wetlands and sequester them across their various tissues (Bonanno *et al.*, 2018). Despite their significant roles in phytoremediation of contaminated wetlands, the two species' stands are increasingly expanding in South Africa, which might have negative impacts on ecosystems (Canavan *et al.*, 2017; Canavan *et al.*, 2018).

Monitoring of the two wetland species' performance for phytoremediation in wetlands, requires timely and accurate spatial and temporal information on their distribution as well as abundance. This information is necessary to enhance the understanding of the roles and the functions of *A. donax* and *P. australis* in wetlands ecosystems as well as to control the trends and the patterns in their spread. The traditional method for vegetation species discrimination and distribution mapping needs rigorous field work, involving taxonomical information, collateral and ancillary data analysis, and the visual observation and identification of species quality, and quantity (Austin and Heyligers, 1989; Godínez-Alvarez *et al.*, 2009; Smartt and Grainger, 1974). This method is labourious, expensive and time-consuming and sometimes inappropriate as some areas are not easily accessible (Adam *et al.*, 2010). On the other side, remote sensing is a relatively new alternative for mapping vegetation, which is time- and cost- effective, up-to-date and comparatively accurate (Lee and Yeh, 2009). Remote sensing records the electromagnetic radiation reflected or emitted by the earth's surface, which in turn, is influenced by both the properties of the object and the radiation hitting the object (Aggarwal, 2004). Identification and mapping plant species using remote sensing is attainable because different vegetation species possess different biochemical and biophysical characteristics, which in turn influence the plants' leaves' spectral properties (Kumar *et al.*, 2001). Vegetation spectral response is influenced by leaf pigments in the visible region, leaf internal structure in the near infrared region (700 to 1299 nm), and by water and other bio-chemicals in the mid infrared (1300 to 2500) (Fernandes *et al.*, 2013; Kumar *et al.*, 2002). However, unlike terrestrial plants, wetland plants and their properties are not as easily detectable. This is because the reflectance spectra of wetland vegetation canopies are often very similar and are combined with reflectance spectra of the underlying soil, and hydrologic regime (Adam *et al.*, 2010).

Since *A. donax* and *P. australis* morphologically and ecologically resemble each other (Saltonstall *et al.*, 2010), their reflectance properties overlap, and it becomes difficult to spectrally discriminate between them (Everitt *et al.*, 2004; Fernandes *et al.*, 2013). However, when subjected to environmental stress and/ or climatic or phenological changes, the two plant species may respond or adapt to the new conditions differently. For instance, heavy metals accumulated by plants alter their morphological, physiological and structural properties (Emamverdian *et al.*, 2015). Presence of heavy metals in plant leaves affect their spectral reflectance (Liu *et al.*, 2010b; Shakya *et al.*, 2008). This is because the concentrations of heavy metals determine chlorophyll content levels, which in turn determine spectral reflectance of plant leaves. Liu *et al.* (2010b), indicated that chlorophyll concentrations in *P. australis* leaves varied inversely with concentrations of Zn, Cu and Pb. Liu et al (2010b) also found that approximately 30 % of the variations in leaf Cu, Pb and Zn concentrations were explained by chlorophyll concentration in the leaves. Moreover, Fernandes *et al.* (2013) found that *A. donax* and *P. australis* were not spectrally separable at the vegetative period, but could be spectrally discriminated at senescent period. To detect such small variation between the vegetation spectra, sensors with high spectral resolution are recommended (Adam *et al.*, 2010). The narrow bandwidths (less than 10nm) of hyperspectral remote sensing make it possible to detect local variation in reflectance features that might otherwise be masked within broader bands of multispectral scanners (Terrence *et al.*, 2018). For example, Schmidt and Skidmore (2003) successfully used field spectral reflectance to discriminate between 27 saltmarsh vegetation species including *P. australis* in the Dutch Waddenzee wetland. In another study, ASD spectrometry data were used to discriminate among four wetland plant species, *P australis*, *Cyperus papyrus L.*, *Thelypteris interrupta* and *Echinochloa pyramidalis* in Greater St Lucia Wetlands Park, South Africa (Adam *et al.*, 2012). Fernandes *et al.* (2013) evaluated the possibility of using field spectrometry to spectrally separate *A. donax* from surrounding vegetation, in different phenological periods. Using the Kruskal–Wallis and the Post hoc Dunn tests, followed by classification and regression trees (CART) for dimensionality reduction, it was possible to spectrally separate *A. donax* from all other surrounding vegetation, except for the *P. australis*. Only few wavelengths, mainly the red-edge region, and in some cases the visible and mid infrared regions, were found to separate *A. donax* and *P. australis* in the senescent period.

Although hyperspectral remote sensing allows for comprehensive mapping and vegetation discrimination at species level, it has its own limitations. Plant adaptations to harsh environmental conditions, and/ or phenological changes may result in different spectral reflectance from plants of the same species (Fernandes *et al.*, 2013; Mansour *et al.*, 2012). This may also result in overlap in spectral signature from different plant species (Fernandes *et al.*, 2013) belonging to the same Plant Functional Type (PFT). Plant Functional Type refers to plants growing in a specific geographical area that respond to changes in global climate or environmental conditions in a similar way. Vegetation within one PFT show certain similar morphological, physiological and phenological traits, and may comprise of species cutting across taxonomical classes (Ustin and Gamon, 2010). The physical environment and stress conditions of plants determine their biochemical and structural traits, which in turn influence their spectral characteristics (Schweiger *et al.*, 2017). With this understanding, it follows that wetland vegetation exposed to AMD pollution may show different spectral reflectance when compared to the same species grown in AMD-free conditions. The extent to which the morphological and physiological adaptations in a PFT are linked to changes in environmental conditions in predictable ways determine the ability to detect PFTs with remote sensing (Ustin and Gamon, 2010). This is because functionally important compounds like pigments, water, and nitrogen-containing compounds can be identified and quantified in plant spectra. Thus, in some instances, it is necessary and possible to map vegetation at the level of PFTs, rather than the discrete plant taxonomic types. The aim for this study was to discriminate between *A. donax* and *P. australis* plant species under healthy (control) and copper + acid (treatment) conditions. The specific objectives for the study were (i) to determine if copper + acid treatment had significant effect on the spectral reflectance properties of plant leaves, (ii) to discriminate between *A. donax* and *P. australis* species both under control and copper + acid treatment conditions and, (iii) to compare the Random Forest (RF) and Support Vector Machines (SVM) models in discriminating the two plant species using features selected from the GRRF.

4.2 Materials and Methods

4.2.1 Glasshouse experiment setup

A glasshouse experiment was setup at the University of the Witwatersrand, Johannesburg, South Africa. Rhizomes of *A. donax* (giant reed) and *P. australis* (common reed) were collected from Lake Florida, Johannesburg, South Africa, in January 2018, and were then planted in rooting beds filled with sand. After 21 days rhizome cuttings with one plant of about 12 cm in height each were selected, and transplanted into 26 tubs for each of the two plant species (*A. donax* and *P. australis*). Two rhizomes were planted into each tub containing layers (4 cm each) of gravel at the bottom followed by sand and top soils at the top. The tubs were irrigated frequently to compensate for the loss and maintain water levels at about 8 centimeters above the top soil. Each tub was equipped with a fish tank pump (flow rate 400 l/h model PH400; power head pump) to agitate all treatments as indicated in Newete *et al.* (2016).

After being transferred to the experimental tubs, the rhizome cuttings were allowed to acclimatize and continue to grow in biomass for three months before the treatments were added. Half (13) of the tubs for each of the reed species were then treated with 5mg/L of copper from a stock solution prepared using cupric sulphate pentahydrate ($CuSO_4.5H_2O$) and nitric acid to bring the medium to a pH of 3-4, to create a simulated acid mine drainage condition as characterized in Tutu *et al.* (2008). The remaining half (13) of the tubs for each of the reed species were kept as controls, free of Cu + acid treatment. At the end of the experiment, that is, after two month of the plants' exposure to the treatments, leaf spectral reflectance and chlorophyll were measured. Leaf, water and soil samples were prepared from all tubs with surviving plants and sent to the Agricultural Research Council-Institute for Soil Climate and Water (ARC-ISCW, Pretoria) Laboratory for copper analysis. At this stage, all the tubs for *A. donax* controls, 12 for *A. donax* treatments, 11 for *P. australis* controls and 11 for *P. australis* treatments had surviving plants.

4.2.1.1 Spectral reflectance measurements:

Leaf spectral reflectance for *P. australis* and *A. donax* was measured at the end of the experiment, in June 2018. It was measured from all the tubs with surviving plants using the Analytical Spectral Device (ASD) FieldSpec 4 (Wide-Res model). This device is a compact, field-portable, and precision instrument with a rapid data collection time of 0.2 second per spectrum. It measures spectral reflectance from 2151 bands over the 350 -2500 nm spectra range, and spectral resolution configuration of 3nm in the visible and near-infrared (350 - 1000 nm) and 30nm in the short wave infrared (1001 - 2500 nm) (ASD Inc., 2016). Using the ASD leaf clipper, a total of 12 spectral reflectance measurements were recorded from four randomly selected leaves from each tub with three repeat measurements per leaf. ViewSpec-Pro software was used to view and process the spectral reflectance measurements. The three repeat measurements from each leaf were then averaged to one sample. A total of 188 leaves were sampled and for more on this refer to table 4.1 below.

Table 4.1 Spectral reflectance samples from *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. plants

Class	**Training Samples (70 %)**	**Test Samples (30 %)**	**Total Samples**
A. donax controls (AD_C)	37	15	52
A. donax treatments (AD_T)	34	14	48
P. australis controls (PA_C)	31	13	44
P. australis treatments (PA_T)	31	13	44

4.2.1.2 Chlorophyll measurements:

Chlorophyll measurements were done at the end of the experiment. The Minolta chlorophyll meter, SPAD-502, was used to measure chlorophyll content from four randomly selected leaves from each tub, for both control and treatment tubs. The four measurements from each tub were then averaged to make one sample per tub. In total 47 chlorophyll samples were collected, 13 from the controls and 12 from the treatments of *A. donax*, and 11 each from the *P. australis* controls and treatments. The SPAD meter is a non-destructive hand held and self-calibrating device that provides an index of chlorophyll concentrations. The meter measures leaf absorbance at two wavelengths, 400-500 nm and 600-700 nm, and converts it into digital signals, and then into a SPAD value which is proportional to extractable chlorophyll concentration in the leaf (Minolta, 1989; Süß *et al.*, 2015). SPAD values range from 0 to 99. The higher the chlorophyll content, the healthier the plant and the higher the SPAD value.

4.2.2 Analysis of spectral reflectance data

4.2.2.1 Variable selection using Guided Regularised Random Forest (GRRF)

Due to the significantly high number of dimensions (2151 spectral bands) compared to limited number of samples, the data was prone to problem of ‘curse of dimensionality’, which leads to multi-collinearith in the data (Mureriwa *et al.*, 2016). Thus, dimensionality reduction process (feature selection) was conducted to reduce the number of independent variables (spectral bands) before performing the classification process. The Guided Regularized Random Forest (GRRF) model was employed for feature selection. The GRRF utilizes the feature importance scores produced from the ordinary random forest model to select the key number of variables from the regularized random forest (RRF) algorithm that give the lowest classification error rate (Deng and Runger, 2013). Outlined below is how the GRRF model uses importance scores from RF for feature selection, summarized from Breiman (2001) and Deng and Runger (2013).

For a training data set with N observations, P features where X_i(i = 1,…,P) and the class Y∈{1.2,…,C}, a feature selection process will select a compact feature subset without loss of predictive information about Y.

From the RF model and for a particular tree, the Gini index at node ʋ, Gini(ʋ) is given as:

$$Gini(\upsilon) = \sum_{C=1}^{C} \acute{\mathrm{P}}_C^{\upsilon}\,(1 - \acute{\mathrm{P}}_C^{\upsilon})$$

Where $\acute{\mathrm{P}}_C^{\upsilon}$ is the fraction of observations in class C at node ʋ. The Gini information gain of feature X_i for splitting node ʋ, Gain(X_i, ʋ), is define as below:

$$\mathrm{Gain}(\mathrm{X}_i, \upsilon) = \mathrm{Gini}(\mathrm{X}_i, \upsilon) - w_L \mathrm{Gini}(\mathrm{X}_i, \upsilon^L) - w_R \mathrm{Gini}(\mathrm{X}_i, \upsilon^R)$$

Where υ^R and $\upsilon^L$are the right and child nodes of node ʋ respectively, and w_R and w_L are fractions of observations alloted to the right and left child nodes, respectively. At each node, a random set of features out of P is evaluated, and the one with maximum $\mathrm{Gain}(\mathrm{X}_i, \upsilon)$ is used for splitting node ʋ. The RF importance score for feature X_i is calculated as:

$$Imp_i = (1/ntree) \sum_{\upsilon \in \mathrm{S}_{\mathrm{X}_i}} \mathrm{Gain}(\mathrm{X_i}, \upsilon)$$

Where S_{X_i} refers to a set of all nodes over all trees (*ntree*) in the RF model where X_i was used for splitting.

Using the raw feature importance score from RF, the GRRF model gives the normalised feature importance scores (Imp'_i) as:

$$Imp'_i = Imp_i / max_{i=1}^{p} Imp_i$$

Where $0 \leq Imp'_i \leq 1$, and the corresponding information gain is computed as:

$$\text{Gain}_R(X_i, \upsilon) = \begin{cases} \lambda_i \text{Gain}(X_i, \upsilon) & X_i \notin F \\ \text{Gain}(X_i, \upsilon) & X_i \in F \end{cases}$$

Where F is the set of indices of features used for splitting in previous nodes, and F is an empty set for the root node in the first tree. λ_i is the co-efficient for feature X_i, calculated as:

$$\lambda_i = (1 - \gamma)\lambda_o + \gamma Imp'_i$$

Where $\lambda_o \in [0,1]$is the base co-efficient, which controls the degree of regularisation. $\gamma \in [0,1]$ is the importance co-efficient, which controls the weight of the normalised importance score. Given λ_o and γ a feature with a larger importance score has a largerλ_i, and is therefore penalised less.

The size of the selected feature subset is controlled by changing values of either λ_o and γ. Changes in the value of γ are more important in determining performance in terms of classification accuracy. To reduce the number of parameters, the GRRF model fixes λ_o at 1 and thus γ remains as the only parameter. The corresponding equation for calculating λ_i becomes:

$$\lambda_i = (1 - \gamma) + \gamma Imp'_i$$

4.2.2.2 Random Forest Classification

A Random Forest is a group of many decision tree classifiers. It consists of "a collection of tree-structured classifiers {$h(\mathbf{X}, \Theta_k)$, k=1,…} where the {Θ_k} are independent identically distributed random vectors and each tree casts a unit vote for the most popular class at input x" (Breiman, 2001). Random Forest employs bootstrapping (also called bagging), that is sampling with replacement, to select data for each $\{\Theta_k\}$ from the original data. Cases in each $\{\Theta_k\}$ become the training set for building the *k*th tree, and the trees are grown without pruning. The RF classifier does not have the problem of over-fitting. The accuracy of RF is determined by the power of the individual tree classifiers in the forest and the degree to which the trees are independent of each other (Breiman, 2001). Part of the original data that is not selected when sampling the training set for the *k*th tree is referred to as Out Of Bag (OOB) data. Out Of Bag data is one third (about 30 %) of the original data, and is used as the test set to calculate the classification error, also called the OOB error for the *k*th tree, and variable importance (Breiman, 2001). Two user defined parameters, *mtry* and *ntree*, are important in the RF algorithm. *Mtry* is the number of input variables or features randomly selected at each node to grow a tree, and *ntree* is the number of trees to be grown. The default value for *ntree* is 500, and that for mtry is $\sqrt{P}$, where P is the number of predictor variables in the original data set. However, the two parameters need to be optimized to find the combination that yields the best classification accuracy. The best combination for *mtry* and *ntree* for this study was determined from a grid search based on the OOB error. Accuracy was assessed using the overall accuracy, based on misclassification error computed from the OOB error estimates, the user accuracy and the producer accuracy, and the Kappa index (Breiman, 2001; Tso and Mather, 2009).

4.2.2.3 Support Vector Machines classifiers

The Support Vector Machines non-linearly transforms the input vectors into a high dimensional feature space, where the hyper-plane that best separate the data set classes is selected (Vapnik, 2000). An infinite number of hyper-planes separating data categories can be constructed in the feature space. However, the SVM selects one that has the highest margin between the classes, and produces minimum generalization error (Pal and Matther, 2005). The algorithm uses support vectors to separate data into classes. Support vectors are data points or observations from each class, which lie closest to the hyper-plane or the classification boundary. Accuracy assessment for SVM was done using the 30 % hold out sample (Table 4.1). Like with RF, the Kappa index, Producer Accuracy (PA), User Accuracy (UA), and the Overall Accuracy (OA) were used to assess classification ability for the classifier. Overall accuracy is the number of correctly classified samples as a fraction of the total number of test samples expressed as a percentage, while UA is the likelihood that a sample assigned to a specific class by a classifier belongs to that class, and PA expresses the probability of a certain class being correctly recognized.

4.2.2.4 Statistical Analysis

The paired-samples t-test, using SPSS, was performed on spectral data to determine if there were significant mean differences in leaf spectral reflectance between control and contaminated plants for *A. donax* and *P. australis*, respectively. This was to determine if copper + acid treatment had any significant effect on the spectral characteristics of the two plant species. One-way ANOVA was conducted on soil, water and leaf copper concentrations, and on SPAD chlorophyll readings to determine if there were differences among means of different treatments. Turkey post hoc tests were performed to determine the sources of differences among the means.

4.3. Results

4.3.1 Significance of copper and acid treatment on chlorophyll content and spectral reflectance

Results for chlorophyll content and copper concentration for soil, water and leaves for *A. donax* and *P. australis* in the control and treatment plants are summarized in Table 4.2. Soil and water from treated plant tubs for both species, had significantly higher mean copper concentrations than the respective control samples ($p < 0.05$). Mean leaf copper concentration for treated *A. donax* was significantly higher than that of the control plants.

Table 4.2 Mean chlorophyll content and copper concentrations for soil, water and leaves, compared by one-way ANOVA. Means in the same column followed by same superscript are not significantly different ($p > 0.05$)

Species	**Treatment**	**SPAD Chlorophyll content ± S.E**	**Mean copper concentration ± S.E**		
			Leaf (mg/kg)	**Soil (mg/kg)**	**Water (ppb)**
Arundo donax	Control	36.050 ± 0.444^{a}	4.826 ± 0.176^{a}	1.798 ± 0.073^{a}	4.645 ± 0.513^{a}
	Treatment	36.317 ± 0.468^{a}	7.701 ± 0.318^{b}	9.341 ± 0.280^{b}	33.330 ± 3.297^{b}
Phramites australis	Control	36.010 ± 4.012^{a}	6.611 ± 0.593^{a,b}	1.547 ± 0.97^{a}	4.832 ± 0.743^{a}
	Treatment	41.344 ± 2.466^{b}	8.268 ± 0.702^{b}	12.833 ± 1.097^{c}	29.887 ± 3.236^{b}

Although chlorophyll content increased for both plant species between control and Cu + acid treated plants respectively, the Turkey post hoc test revealed that significant difference ($P < 0.05$) was recorded between control and treated plants of *P. australis* only. Post hoc analysis also revealed that for treated plants, the mean chlorophyll content was significantly higher for *P. australis* (41.3 ± 2.5) than *A. donax* (36.3 ± 1.4). Increases in chlorophyll content due to Cu + acid treatment resulted in statistically significant ($P < 0.05$) decreases in mean leaf spectral reflectance for both plants respectively, as was found from the paired-samples t-test. Thus, copper + acid treatment had significant effects on the spectral reflectance of both *P. australis* and *A. donax* plant species. The changes in reflectance due to Cu + acid treatment were not uniform across the spectrum for both plants (Figure 4.1). For *A. donax*, the Cu + acid treatment resulted in reflectance increase in the visible region and decrease in the near infrared region, while in the mid infrared, the reflectance decreased between wavelengths 1567 and 1801 nm, and increased between 1903 and 2500 nm. For *P. australis* Cu + acid treatment resulted in decreased spectral reflectance in the visible and the mid infrared regions, while it increased in the near infrared region.

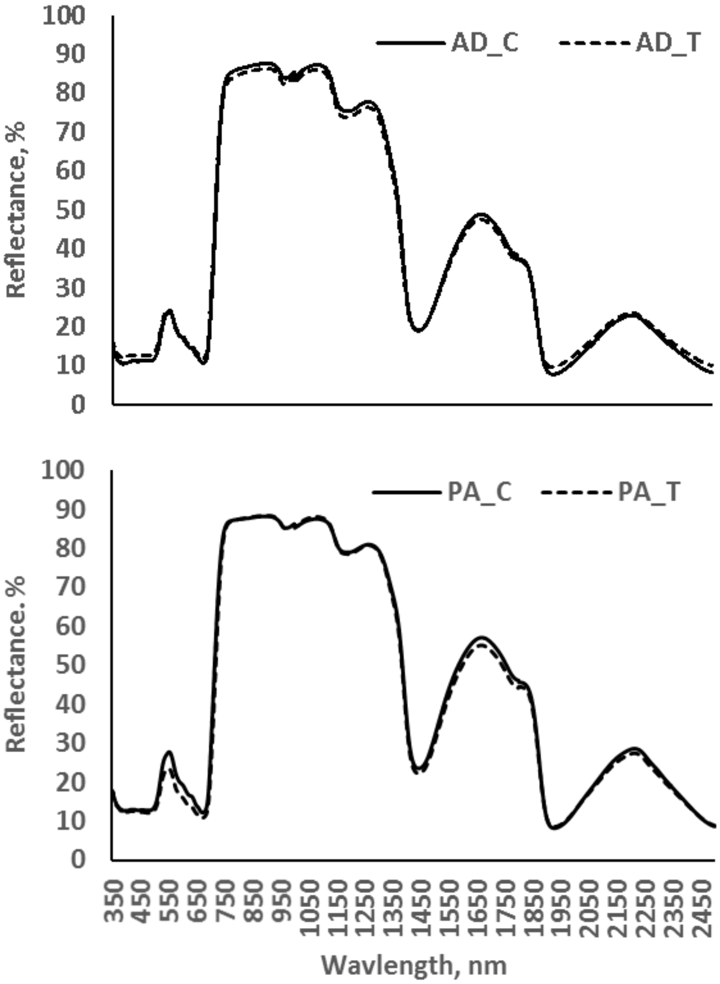

Figure 4.1 Comparison of the mean spectral reflectance between the controls and treatments of *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. respectively. NB: AD denotes *Arundo donax* PA denotes *Phragmites australis*, C denotes control and T denotes treatment.

Under control conditions, mean reflectance of *A. donax* was lower than that of *P. australis* for all the wavelengths (Figure 4.2). For plants grown under Cu + acid treatment, spectral reflectance of *A. donax* was higher than that of *P. australis* in the visible region, and lower in all the other wavelengths.

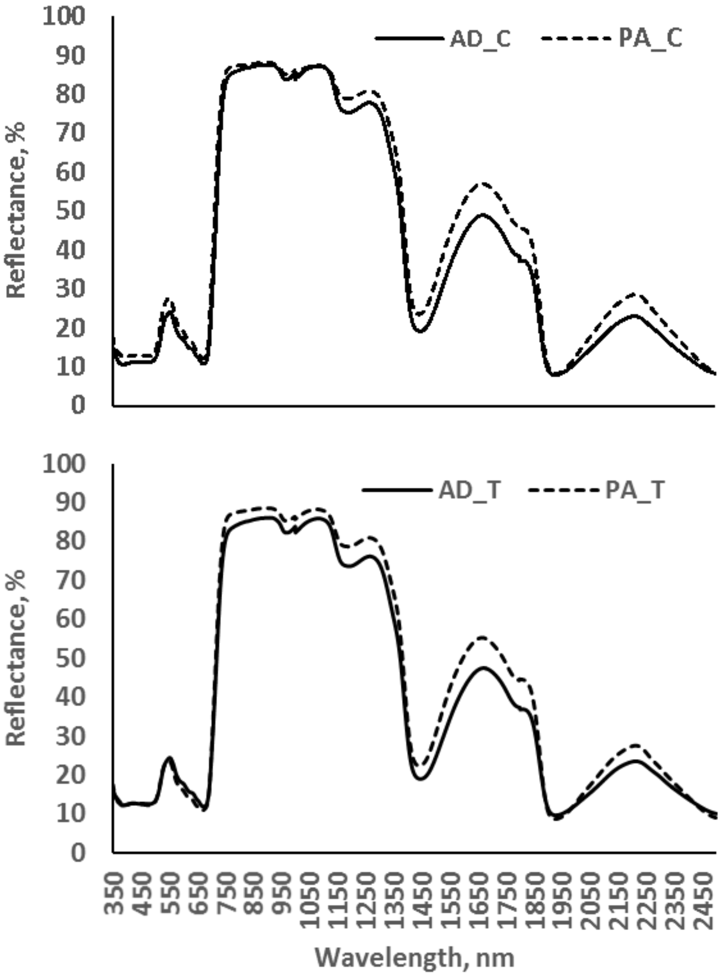

Figure 4.2 Comparison of the mean spectral reflectance between the controls and the treatments of *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud.. NB: AD denotes *Arundo donax* PA denotes *Phragmites australis*, C denotes control and T denotes treatment

4.3.2 Spectral discrimination between Arundo donax and Phragmites australis

Results described here are for feature selection process by the GRRF and the subsequent classification processes using RF and SVM. Feature selection and classification were done to discriminate between the following sets of categories:

A. donax species under control conditions (AD_C), and *P. australis* species under control conditions (PA_C)

A. donax plants under copper + acid treatment conditions (AD_T), and *P. australis* plants under copper + acid treatment conditions (PA_T)

4.3.2.1 Feature importance measurement and selection

The ordinary RF classifier was used to determine the importance of each wavelength in discriminating between respective classes as shown in Figure 4.3. The mean decrease in Gini index was used for feature importance measurement, and the most important variables are those with the highest index.

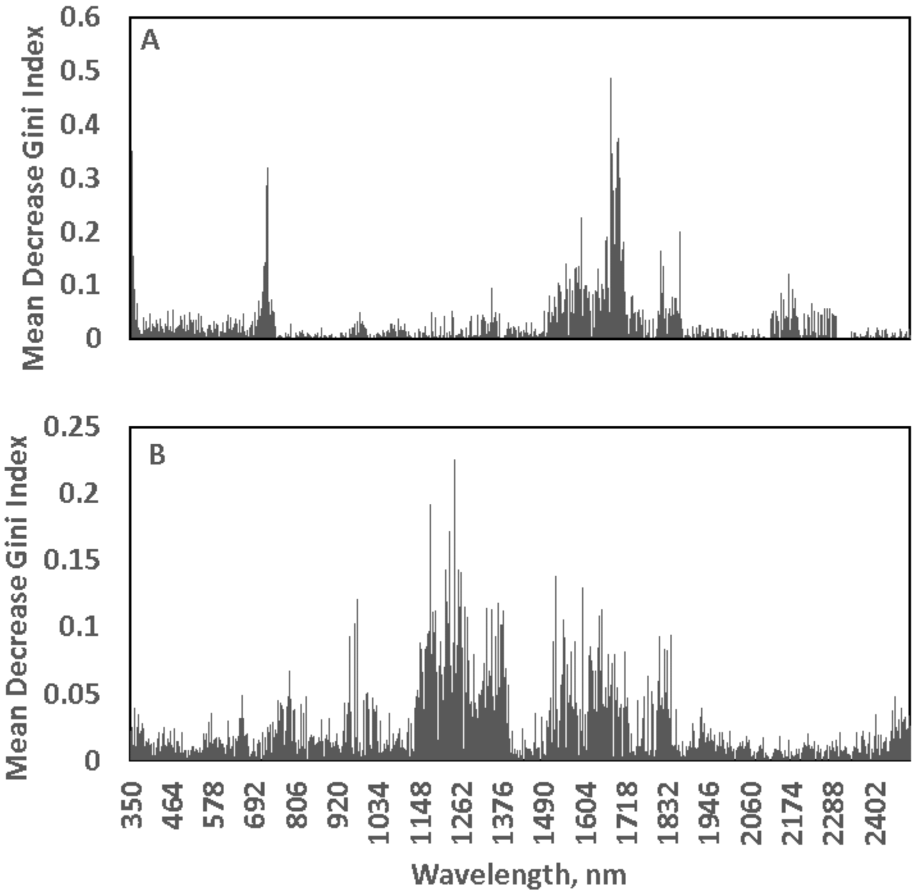

Figure 4.3 The importance of wavelengths as measured by ordinary RF using the mean decrease in Gini index in the discrimination between controls (A) and treatments (B) of *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud..

For discrimination between *A. donax* and *P. australis* species grown under control conditions the most important wavelengths were concentrated in the visible, the red edge and the mid infra-red regions. Wavelengths 353, 726 and 1674 nm were the most important in the visible, the red edge and mid-infrared regions, respectively. On the other hand, for discrimination between *A. donax* and *P. australis* grown under contaminated environment the most important wavelengths were relatively spread across the electromagnetic spectrum, and wavelengths 657, 1246 and 1363 nm were the most important in the visible, the near-infra-red and the mid-infrared regions, respectively.

The measured importance scores from the RF were used to enable the GRRF to select subset wavelengths that best discriminate between the respective classes. Through the GRRF, three optimal wavelengths were identified that yielded the lowest OOB error for each of the discrimination processes. These wavelengths were 703, 1585 and 1699 nm for discrimination of the two species under control conditions, and 1238, 1247 and 1550 for discrimination under contaminated conditions (Figure 4.4).

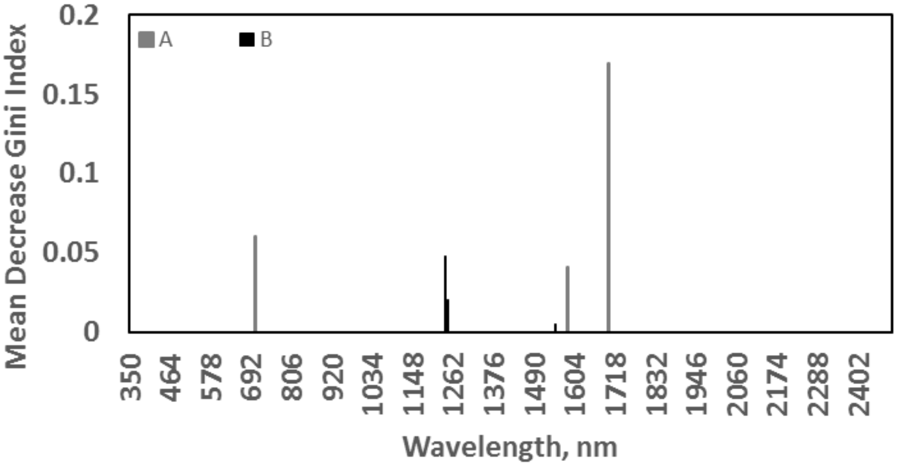

Figure 4.4 Wavelengths selected by GRRF based on the importance scores as measured by the traditional RF for the discrimination between controls (A) and treatments (B) of

4.3.2.2 Random forest (RF) and support vector machine (SVM) classification

The optimal wavelengths selected by the GRRF for each data set were subjected to SVM and RF classifiers respectively, and the performances were measured using the Kappa index, overall accuracy, the Producer's accuracy and the User's accuracy. The accuracy results of both RF and SVM algorithms were higher when discriminating between the treatment plants than between the control plants. The SVM was however, relatively better in discriminating between the two species under control conditions than the RF model, with overall accuracies of 75 % and 64.2 % (OOB error = 35.8 %), respectively. For both the RF and SVM classification processes, the User's accuracies and Producer's accuracies were higher in the discrimination of plants grown under control conditions than of plants grown under treatment conditions. Highest separability was found in the classification of *P. australis* under treatment conditions, where only one sample was misclassified as *A. donax* by the RF mode (PA = 92.3) and two samples misclassified as *A. donax* by the SVM model (PA = 84.7). The overall accuracy for discriminating between the treatments of the two species (AD_T and PA_T) using RF was 85.2 % (OOB error = 14.8 %), while for SVM it was 81.4 %. Tables 4.3 and Table 4.4 show the overall accuracy, Kappa index, the producer's and user's accuracies of the RF and SVM classifiers in discriminating the data sets.

Table 4.3 Summary of accuracy assessment results showing the overall accuracy (OA), User's accuracy, Producer's accuracy, and Kappa index for discrimination between control plants of *A. donax* (AD_C) and *P australis* (PA_C), using the RF and SVM models.

Random forests model						**Support Vector Machines model**					
CLASS	AD_C	PA_C	Total	Producer's Accuracy, %	User's Accuracy, %	CLASS	AD_C	PA_C	Total	Producer's Accuracy, %	User's Accuracy, %
AD_C	9	4	13	60	69.2	AD_C	13	5	18	86.6	72.2
PA_C	6	9	15	69.2	60	PA_C	2	8	10	61.5	80
Total	15	13	28			Total	15	13	28		
OA = 64.2 %		Kappa = 0.2				OA = 75 %		Kappa = 0.48			

Table 4.4 Summary of accuracy assessment results showing the overall accuracy (OA), Producer's accuracy, User's accuracy and Kappa index for discrimination between treatment plants of *A. donax* (AD_T) and *P. australis* (PA_T), for the RF and SVM models.

Random forests model						**Support Vector Machines model**					
CLASS	AD_T	PA_T	Total	Producer's Accuracy, %	User's Accuracy, %	CLASS	AD_T	PA_T	Total	Producer's Accuracy, %	User's Accuracy, %
AD_T	11	1	12	78.6	91.7	AD_T	11	2	13	78.6	84.6
PA_T	3	12	15	92.3	80	PA_T	3	11	14	84.6	78.6
Total	14	13	27			Total	14	13	27		
OA = 85.2 %		Kappa =	0.7			OA = 81.4 %		Kappa = 0.63			

4.4 Discussion

Accumulation of heavy metal contaminants by plants influences the plants' leaf spectral reflectance (Liu *et al.*, 2010b; Shakya *et al.*, 2008). In this study, significant mean differences in spectral reflectance between control and treatment plants for both *P. australis* and *A. donax* were found. Spectral differences in the visible region were caused by changes in chlorophyll content due to increased copper accumulation by treatment plants. The higher the chlorophyll content, the less the reflectance in the green region and the more the absorption in the red region (Everitt *et al.*, 2004). This explains why in both plant species, the treated plants, which also had higher chlorophyll content, had lower reflectance than the control ones, and why *A. donax* plants had lower reflectance under control conditions and higher reflectance under Cu + acid conditions, in the visible region than *P. australis*, respectively. This agrees with Everitt *et al.* (2004), where plants with dark green foliage color, and have more chlorophyll, had lower visible green reflectance than plants with lighter green color. Chlorophyll, among other plant pigments, has the most influence on plant photosynthetic potential, and hence primary productivity, and its concentration decreases when plants are under stress (Blackburn, 2006). Thus, higher chlorophyll content in the treated plants compared to control plants of both species shows that the two species were tolerant to the simulated acid treatment (Cu + acid). In the near infrared region (700 to 1299 nm) reflectance is influenced by leaf internal structure and in the mid infrared (1300 to 2500) by water and other bio-chemicals.

Accumulated copper in plant leaves is involved in chloroplast formation, cell metabolism and maintaining the membrane structure of thylakoids in the plant (Adrees *et al.*, 2015; Henriques, 1989). Excess copper may destroy the leaf internal structure for non-tolerant plants (Su *et al.*, 2017). Although for both control and treatment plants, leaf reflectance for *P. australis* in the NIR region was higher than that of *A. donax*, the difference was greater for plants under treatment conditions (Figure 4.2). Thus, under polluted environments, the two plants tend to belong to different Plant Functional Types (PFTs) from their respective control plants. This is in line with the PFTs theory as described by Ustin and Gamon (2010). The biochemical and structural traits of the plants as determined by their PFTs influence their spectral characteristics in the NIR and the mid infrared region (Schweiger *et al.*, 2017; Ustin and Gamon, 2010).

Using the GRRF, different sets of wavelengths were selected as important for discrimination of control plants (703, 1585 and 1699 nm) and treatment plants (1238, 1247 and

1550) between *A. donax* and *P. australis*, respectively. This shows that the uptake of copper under acidic conditions had a significant effect on the spectral properties of the plants (Liu *et al.*, 2010b). Our results showed a narrower window of band selection in the electro-magnetic spectrum for the discrimination of controls and treatments between *A. donax* and *P. australis* respectively, as compared to other studies (Adam and Mutanga, 2009; Mureriwa *et al.*, 2016; Prospere *et al.*, 2014). This is because the two reeds are morphologically and ecologically similar and thus their reflectance properties overlap (Everitt *et al.*, 2004; Saltonstall *et al.*, 2010). Many other wavelengths with high importance scores could not be selected due to regularization, the GRRF only selects new features if they provide substantially new predictive information in the classification model (Deng and Runger, 2012), and that reduces multi-collinearity and training time for classification process. The findings in this study also agree with those in Fernandes *et al.* (2013) where above four important wavelengths were selected in discrimination processes between *A. donax* and other plant species, but only three were selected for discrimination between *A. donax* and *P. australis* under vegetative period. No wavelengths were selected in the visible region for both discrimination processes. Such results are comparable to Everitt *et al.* (2004) where reflectance in the visible green (0.52 to 0.60 µm) visible red (0.63 to 0.69 µm) and near-infrared (0.76 to 0.90 µm) spectral bands were used to assess canopy spectra of giant reed and associated vegetation. They found that the best wavelengths to differentiate *A. donax* from associated vegetation, which included *P. australis*, were the near infrared, and not the visible wavelengths. The selected wavelengths differed for the two discrimination processes, one near infrared and two mid infrared wavelengths for discrimination between control plants, and two near infrared wavelengths and one mid infrared for treatment plants. This is because copper accumulation by treatment plants resulted in changes on leaf biochemical, structural and physiological traits and these functional changes in the leaves altered the spectral characteristics of the treatment plants (Ustin and Gamon, 2010). The different sets of wavelengths selected were all in the near infrared and the mid infrared regions, and reflectance in these regions is influenced by plant biochemical and leaf internal structure (Fernandes *et al.*, 2013; Kumar *et al.*, 2002).

Classification results showed that the two plant species, *A. donax* and *P. australis* were more separable under treatment conditions than under control conditions. The discrimination between *A. donax* and *P. australis* under control conditions was better achieved using the SVM classifier with an overall accuracy of 75 % compared to the RF which only resulted in an overall accuracy of 64.2 %. However, the Kappa indices were still very low for both algorithms (0.48 and 0.2 respectively). On the other hand, for discrimination between treated plants, classification accuracy was better with the RF (overall accuracy =85.2 %, Kappa = 0.7) than with SVM (overall accuracy = 81.4 %, Kappa = 0.63). This suggests, that the two species are morphologically similar and as a result discrimination under normal conditions is confounded by spectral mixture (Saltonstall *et al.*, 2010). However, the effective separability between the two under treatment conditions suggests that they respond differently to the heavy metal contaminants (copper and acid contamination in this case), which is also reflected in their spectral reflectance response. These results are in agreement with findings by Fernandes *et al.* (2013) where the two species, *A. donax* and *P. australis* were spectrally separable at senescence and not at vegetative stage. This can be explained by the PFTs theory described above. After examining the overall accuracies for the two classifiers on the different treatments, we recommend the use of RF classifier for discrimination of *A. donax* and *P. australis* species in wetlands. This is because RF can discriminate the plants under polluted environment better, and most of the wetlands in South Africa where the two plants generally grow have various levels of organic and inorganic pollutants (Newete and Byrne, 2016; Papazoglou *et al.*, 2005; Tutu *et al.*, 2008; Vymazal and Březinová, 2016). Other advantages of RF over SVM is that where the sample size is too small for independent accuracy assessment, the internal OOB error of RF could be used for accuracy assessment. The RF requires optimization of two parameters only (ntree and mtry), whereas SVM requires a selection of a suitable kernel function first and then setting of the specific parameters (Abdel-Rahman *et al*., 2014; Breiman, 2001; Vapnik, 2000). Other studies also recommended RF over SVM for hyperspectral data discrimination, for example Abdel-Rahman *et al*. (2014).

4.5 Conclusion

Copper uptake by *A. donax* and *P. australis* in an acidic wetland environment has a significant effect on the spectral properties of the plants. Spectral discrimination between the two species was less efficient for health plants compared to plants affected by copper and acid pollution. The RF produced the higher level of separability (overall accuracy = 85.2 % and Kappa = 0.7) between *A. donax* and *P. australis* compared to SVM (overall accuracy = 81.4 %, Kappa = 0.63). Overall, there is potential for using the GRRF coupled with either RF or SVM classification in discriminating and mapping the two plants in wetlands affected by heavy metal pollution from acid mine drainage. The results in this study can be up scaled to the freely accessible Sentinel-2 data, with resolution of up to 10m, revisit time of five days, as well as 13 spectral bands. The red-edge, near infrared and mid infrared bands of Sentinel-2 imagery can be tested for this purpose.

CHAPTER FIVE

5. Mapping *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. using Sentinel-2 data in Johannesburg, South Africa.

This chapter is based on:

Mabhungu, L., Adam, E. and Newete, S.W. (*In preparation*). Mapping *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. using Sentinel-2 data in Johannesburg, South Africa.

Abstract

This chapter investigated the effectiveness of Sentinel-2 data in mapping *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. reeds in wetland areas of Johannesburg, South Africa using two classiffication algorithms, the Random Forests (RF) and the Support Vector Machines (SVM). The results showed that *P. australis* exists along, and covers, most water bodies in the study area. More *P. australis* exists in the study area than *A. donax*, which appear to emerge in areas already covered by *P. australis*. The classification accuracy assessment showed that RF and SVM have the same efficiency (overall accuracy = 91.2 and kappa coefficient = 0.89) in mapping the distribution of *A. donax* and *P. australis* and other land coverers. It was concluded that the green, SIWR_2 and the red bands of Sentinel-2 data with RF or SVM classification models can be used to identify and map *A. donax* and *P. australis* in wetlands.

Key words: *Arundo donax* ; *Phragmites australis*; Random Forest; Sentinel-2; species mapping; support vector machines.

5.1 Introduction

Arundo donax L. and *Phragmites australis* (Cav.) Trin. ex Steud. cover large areas of wetlands in South Africa. They are widely distributed in the Eastern Cape, Gauteng, KwaZulu-Natal, Mpumalanga and Western Cape provinces of South Africa (De Villiers *et al.*, 2011). Other aquatic plants common in wetlands of South Africa include *Phalaris arundinacea, Scirpus* spp. and *Typha* spp. (Papazoglou *et al.*, 2005; Newete and Byrne, 2016; Vymazal and Březinová, 2016). *Arundo donax* and *P. australis* are aggressive invaders outside their native ranges (Lambert *et al.*, 2010), and they have a great morphological resemblance between them. They are both tall, robust and perennial grass-like plants with hollow stems (De Villiers *et al.*, 2011). The only visible differences between the two is that *A. donax* is relatively taller (2 – 7m tall) with broader leaves (10 – 80mm wide) than *P. australis* (1.5 – 3m tall and 10 – 35mm wide) (Lusweti, 2011; Mabhungu *et al.*, 2019). Together with other Large-Structured Invasive Grasses (LSIGs), the two reeds belong to a plant functional group that grow and aggressively colonize aquatic ecosystems (Lambert *et al.*, 2010). This causes wide range of negative impacts on aquatic systems (Herrera and Dudley, 2003). Other plant species belonging to LSIGs are *Neyraudia reynaudiana* (Kunth) Keng ex Hitchc., *Pennisetum purpureum* Schumach., *Miscanthus sinensis* Andersson, *Phalaris arundinacea* L. *S. spontaneum* L. and *Saccharum ravennae* L. (Lambert *et al.*, 2010).

Generally, control of *A. donax* and *P. australis* include herbicide treatment, cutting and removing biomass and prescribed fire (Bell, 1997; Lambert *et al.*, 2010). *Phragmites australis* is a cosmopolitan species and is native in South Africa (Henderson and Cillier, 2004; Packer *et al.*, 2017). New populations of *P. australis* establish from stem fragments, rhizomes and seeds, while established populations expand through clonal growth of the horizontal rhizome system and ground-surface stolons (Packer *et al.*, 2017). *Arundo donax* is a naturalized alien species in South Africa and was introduced in the late 1700s for soil erosion control (Milton, 2004; Canavan et al., 2017). It invades riparian areas across South Africa and its spread is facilitated by human activities such as building of dams and soil stabilization (Canavan *et al.*, 2017). Along with other aquatic macrophytes, the two reeds are popular in highly polluted wetland systems of South Africa (Rouget *et al.*, 2004; Nel, 2015). A number of studies conducted worldwide have proved the effectiveness of *A. donax* and *P. australis* in phytoremediation of wetlands (Sheoran and Sheoran, 2006; Yang *et al.* 2006), thus they are often planted in constructed wetlands (Bonanno, 2012; Aminsharei *et al.*, 2017; Bello *et al.*, 2018). Despite their role of phytoremediation in wetland systems, the two species also have negative impacts on wetlands. They form dense stands which shade other wetland vegetation, and thus negatively impact on biodiversity of indigenous communities, which affect food-webs, and hence change ecosystem processes (Milton, 2004). Invasive alien plants have severely degraded most riparian habitats of South Africa and biological control, for example the *Rhizaspidiotus donacis* (Leonardi) (Hemiptera: Diaspididae) insect, is one of the methods considered for control of *A. donax* (Holmes, 2005; Pillay, 2016; Canavan *et al.*, 2017).

Effective control and management of the two reeds requires up-to-date information about their spatial and temporal distribution in ecosystems. Traditional methods of mapping the distribution of vegetation species, which involve visual observation and identification of species quality and quantity, demand intensive fieldwork. This is time consuming, relatively expensive and sometimes impossible due to poor accessibility or large coverage (Barry *et al.*, 2002). Remote sensing, on the other hand, has the potential to improve knowledge and management of riparian vegetation by providing cost-effective and spatially continuous data over wide extents (Huylenbroeck, 2020). Mapping and discrimination of plant species using remote sensing is possible because different vegetation have different biophysical and biochemical properties which respond to spectral reflectance differently (Kumar *et al.*, 2001). Vegetation spectral response is influenced by leaf pigments in the visible region, leaf internal structure in the near infrared region (750 to 1299nm), and by water and other bio-chemicals in the mid infrared (1300 to 2500) (Fernandes *et al.*, 2013; Kumar *et al.*, 2002). However, wetland plants and their properties are not as easily detectable as terrestrial plants. This is because of high spectral and spatial variability among wetland vegetation that produces short ecotones and sharp demarcation between the vegetation units (Adam *et al.*, 2010). Moreover, *A. donax* and *P. australis* are morphologically and ecologically similar (Saltonstall *et al.*, 2010), which increases spectral overlapping, and it makes it more challenging to spectrally discriminate between them (Everitt *et al.*, 2004; Fernandes *et al.*, 2013). Thus, the selection of the appropriate spatial and spectral resolution and the best process to use to extract the spectral information is of paramount importance (Elhadi *et al.*, 2009). Both multispectral and hyperspectral remote sensing have been used for mapping wetland vegetation. However, species discrimination using hyperspectral data yields more accurate results than that of multispectral data (Smith and Blackshaw, 2003; Adam *et al.*, 2010). Vegetation mapping at species level using multispectral sensors like SPOT and Landsat has problems of spectral overlap between vegetation species due to the sensors' low spectral resolution (Mansour *et al.*, 2012). However, most high resolution satellite images are expensive and so not available to everyone, and this limits their use by many researchers (Dube *et al*, 2014). The high resolution Sentinel-2 data with a short revisit time (between 5 and 10 days) is freely accessible (Ienco *et al*., 2019). A number of machine learning classifiers have been developed for use in remote sensing. Most commonly used machine learning classifiers are K-Nearest Neighbours (KNN), Random Forest (RF) and Support Vector Machine (SVM), owing to simplicity in their hyper-parameterization and their relatively high performances (Richard *et al*., 2020). In a study by Fernández-Delgado *et al*. (2014), 179 classifiers were evaluated and the RF and SVM outperformed all the other classifiers. Both RF and SVM do not require any assumption about the distribution of data and have no problem of over-fitting the test data (Abdel-Rahman *et al*., 2014). Amani *et al*. (2017) compared the KNN, SVM, Decision Tree (DT), and RF for wetland mapping, and found that RF had the highest classification accuracy. While other studies have concluded that RF and SVM have comparable performances in vegetation classification, for example Abdel-Rahman *et al*. (2014), where RF and SVM produced overall accuracies of 74.5 and 73.50, respectively, other researchers found differences in the performances of the two classifiers. The purpose of the current study is to test the effectiveness of Sentinel-2 data in mapping *A. donax* and *P. australis* reeds using the Random Forests (RF) and the Support Vector Machines (SVM).

5.2 Materials and methods

5.2.1 Study area

The study area is located in Johannesburg, South Africa, and covers parts of Soweto, Roodepoort and Randburg. It is situated between latitudes 26° 09’ S to 26° 17’ S and longitudes 27° 51’ E to 27° 59’ E. Johannesburg has a subtropical highland climate characterized by hot, rainy days and cool evenings in summer and relatively dry sunny days and cold nights in winter. Wetlands in Johannesburg, for example the Klip River wetland areas, receive pollution from industrial and mining fields (active and abandoned) in the area (McCarthy *et al.*, 2007). Although *Phragmites australis* is the major vegetation cover in Johannesburg wetlands *Arundo donax* is also found in wetlands around the study area (McCarthy *et al.*, 2007). Figure 5.1 shows location of the study area.

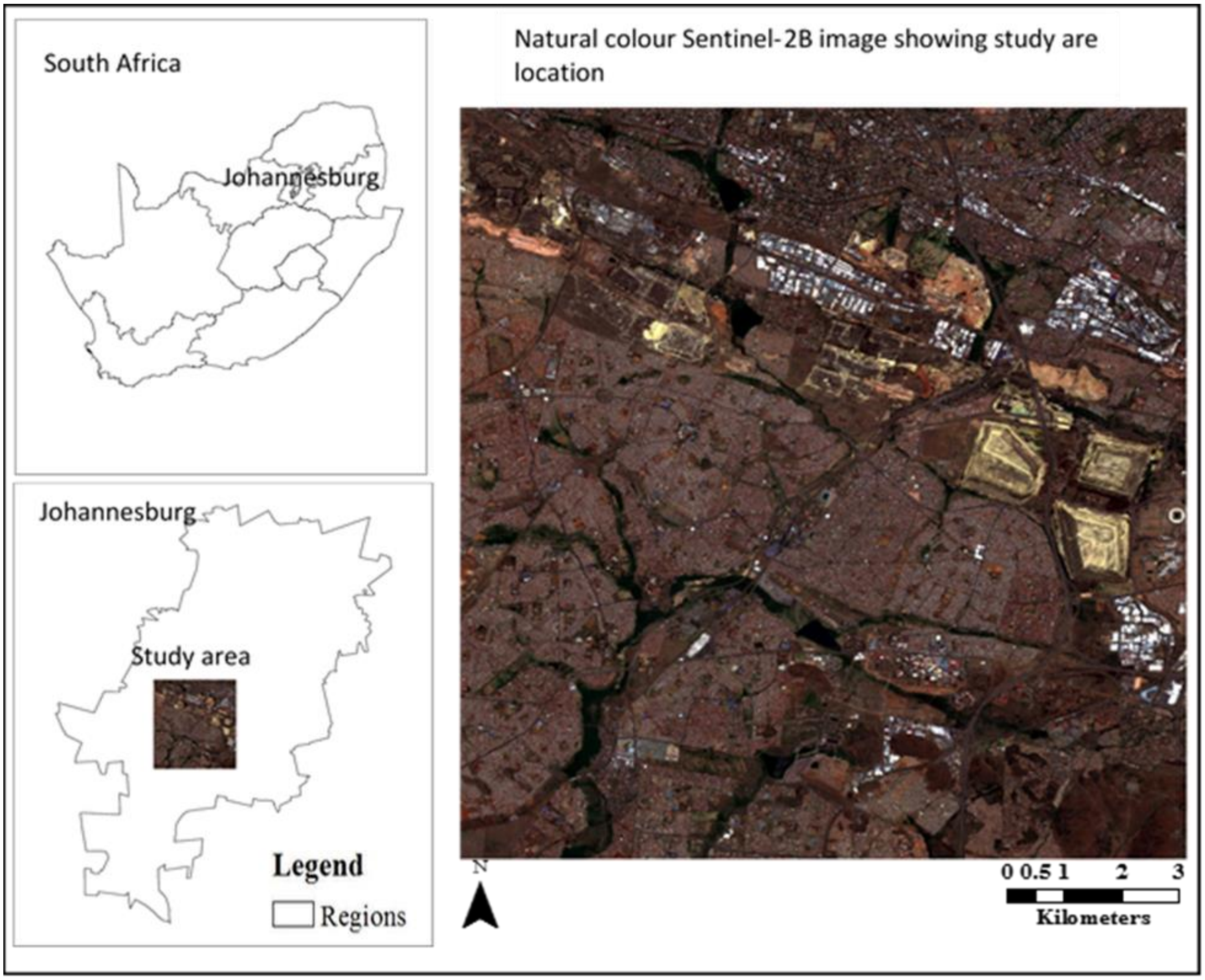

Figure 5.1 Map of South Africa showing the location of the study area in Johannesburg and the natural color Sentinel-2 satellite image of the study area. The study area covers parts of Soweto, Roodepoort and Randburg regions in Johannesburg.

5.2.2 Remote sensing data

The spatial distribution of *Arundo donax* and *Phragmites australis* was mapped using sentinel-2 satellite imagery, which was acquired from Copernicus Open Access Hub (https://scihub.copernicus.eu/), accessed free of charge. Sentinel-2 data has resolution of up to 10 m, revisit time of five days, and 13 spectral bands (Drusch *et al.*, 2012). The data comprise of three red edge bands, which enhances the capability to capture the strong reflectance of vegetation in the near infrared portion of the electromagnetic spectrum (Abdi, 2019). Satelite images covering the study area, with 0% cloud cover and acquisation date between 1 November 2019 and 31 December 2019 were included in the search criteria. The image for this study was downloaded on 30 January 2020. The selected Sentinel-2 scene was from Sentinel-2B, acquired on 11 November 2019 and was on Level-1c processing format, which requires atmospheric correction. Atmospheric correction was performed using the Sen2Cor (v2.5.5) plugin in the Sentinel Application Platform software (SNAP) (Pflug *et al.*, 2016; Sola et al., 2018). Table 5.1 shows the characteristics of the Sentinel-2B bands used in this study. All the bands at 20 m spatial resolution were resampled to 10 m using bilinear interpolation to facilitate integration and consistency.

Table 5.1 Characteristics of the sentinel-2B bands used in this study

No.	Band name	Central wavelength (nm)	Bandwidth (nm)	Resolution (m)
2	Blue	492.1	98	10
3	Green	559	46	10
4	Red	665	39	10
5	Vegetation Red edge_1	703.8	20	20
6	Vegetation Red edge_2	739.1	18	20
7	Vegetation Red edge_3	779.7	28	20
8	NIR	833	45	10
8a	Narrow NIR	864	32	20
11	SWIR_1	1610.4	141	20
12	SWIR_2	2185.7	238	20

5.2.3 Field data collection

Major wetlands positions were identified using inventory data and visual interpretation of high spatial resolution images for Johannesburg. A field survey was conducted on 4 December 2019 to acquire reference data for the classification processes. The two reeds, *P. australis* and *A. donax*, and other surrounding land covers, were visually identified during the survey, and their position coordinates were recorded in a Global Positioning System (GPS). Other land cover types recorded were built-up area, open water bodies, bare ground and other vegetation. These were included in the survey to facilitate image classification accuracy and to understand the relationship between the two reeds and the other land cover types in the study area. Purposive sampling was used to identify the different land covers, whose location data was recorded. For *P. australis* and *A. donax*, areas with a dominance in each species of at least 90 % were considered for sampling. Some of the data were used as training sites for the classification of Sentinel-2 imagery, and the other data were used for validating the classification process. The total number of GPS points recorded was 425 and of these 70 % was used for training while 30 % was used for validation purposes (Table 5.2).

Table 5.2 Summary of land cover positions recorded.

Class	Training Samples (70 %)	Test Samples (30 %)	Total Samples
A. donax	27	11	38
P. australis	66	28	94
Bare ground	55	23	78
Buildings	71	30	101
Water bodies	48	20	68
Other vegetation	33	13	46

5.2.4 Image classification

The location data points from the field survey were overlaid on the Sentinel-2 satellite image of the study area in Arc Map 10.6. Supervised classification was used to map the areas covered by the two reed species and other land cover types, using Random Forest (RF) and Support vector Machines (SVM) algorithms. Classified maps using the RF and SVM were produced.

5.2.4.1 Random Forest Classification

Random Forest is an ensemble of many decision tree classifiers. It consists of "a collection of tree-structured classifiers $\{h(\mathbf{X}, \Theta_k), k=1,\ldots\}$ where the $\{\Theta_k\}$ are independent identically distributed random vectors and each tree casts a unit vote for the most popular class at input x" (Breiman, 2001). Random Forest employs bootstrapping (also called bagging), that is sampling with replacement, to select data for each $\{\Theta_k\}$ from the original data. Cases in each $\{\Theta_k\}$ become the training set for building the *k*th tree, and the trees are grown without pruning. RF classifier does not have the problem of over-fitting. The accuracy of RF is determined by the strength of the individual tree classifiers in the forest and the degree to which the trees are independent of each other (Breiman, 2001). Part of the original data that is not selected when sampling the training set for the *k*th tree is referred to as Out Of Bag (OOB) data. Out Of Bag data is one third (about 30 %) of the original data, and is used as the test set to calculate the classification error, also called the OOB error for the *k*th tree, and variable importance (Breiman, 2001). Two user defined parameters, *mtry* and *ntree*, are important in the RF algorithm. *Mtry* is the number of input variables or features randomly selected at each node to grow a tree, and *ntree* is the number of trees to be grown. The default value for *ntree* is 500, and that for mtry is $\sqrt{P}$, where P is the number of predictor variables in the original data set. However, the two parameters need to be optimized to find the combination that yields the best classification accuracy. Grid search, based on the OOB error, was done to determine the optimal combination for *mtry* and *ntree*. Accuracy was assessed using the Overall Accuracy (OA), based on misclassification error computed from the OOB error estimates, the User's Accuracy (UA), and the Producer's Accuracy (PA) and the Kappa coefficient (Breiman, 2001; Tso and Mather, 2009).

5.2.4.2 Support Vector Machines classifiers

The Support Vector Machines (SVM) non-linearly maps the input vectors into a high dimensional feature space, where an optimal separating hyper-plane is selected (Vapnik, 2000). An infinite number of hyper-planes separating data categories can be constructed in the feature space. However, the SVM selects one that has the highest margin between the classes, and produces minimum generalization error (Pal and Matther, 2005). The algorithm uses support vectors to separate data into classes. Support vectors are data points or observations from each class, which lie closest to the hyper-plane or the classification boundary. Accuracy assessment for SVM was done using the 30 % hold out sample (Table 5.2). Like with RF, OA, UA, PA and the Kappa coefficient were used to assess classification ability for the classifier (Congalton and Green, 2008). User's accuracy refers to the probability that any pixel labelled as a specific land cover class in the mapped area belongs to its actual class while the Producer's accuracy shows the probability that specific land cover types of an area on the ground is classified as such (Adam *et al.*, 2017). The overall accuracy, usually expressed as a percentage, gives the proportion of reference sites that were mapped correctly. The Kappa coefficient evaluates how well the classification performed as compared to just randomly assigning values.

5.3 Results

5.3.1 Performance of Random Forest and Support Vector machines

Spatial distribution maps for *A. donax*, *P. australis* and other land covers in the study area were produced using RF and SVM classifiers (Figure 5.2). Results from both classifiers show that other land cover types occupy larger areas compared to open water and the two reed species (Table 5.3). *Arundo donax* covered the least area from both RF and SVM (2.7 % and 1.1 %) respectively. Distribution pattern of *P. australis* shows that it exists along water bodies. The study area has substantial populations of *P. australis* compared to the alien *A. donax*. There is evidence of *A. donax* species emerging in areas already covered by *P. australis* (Figure 5.2).

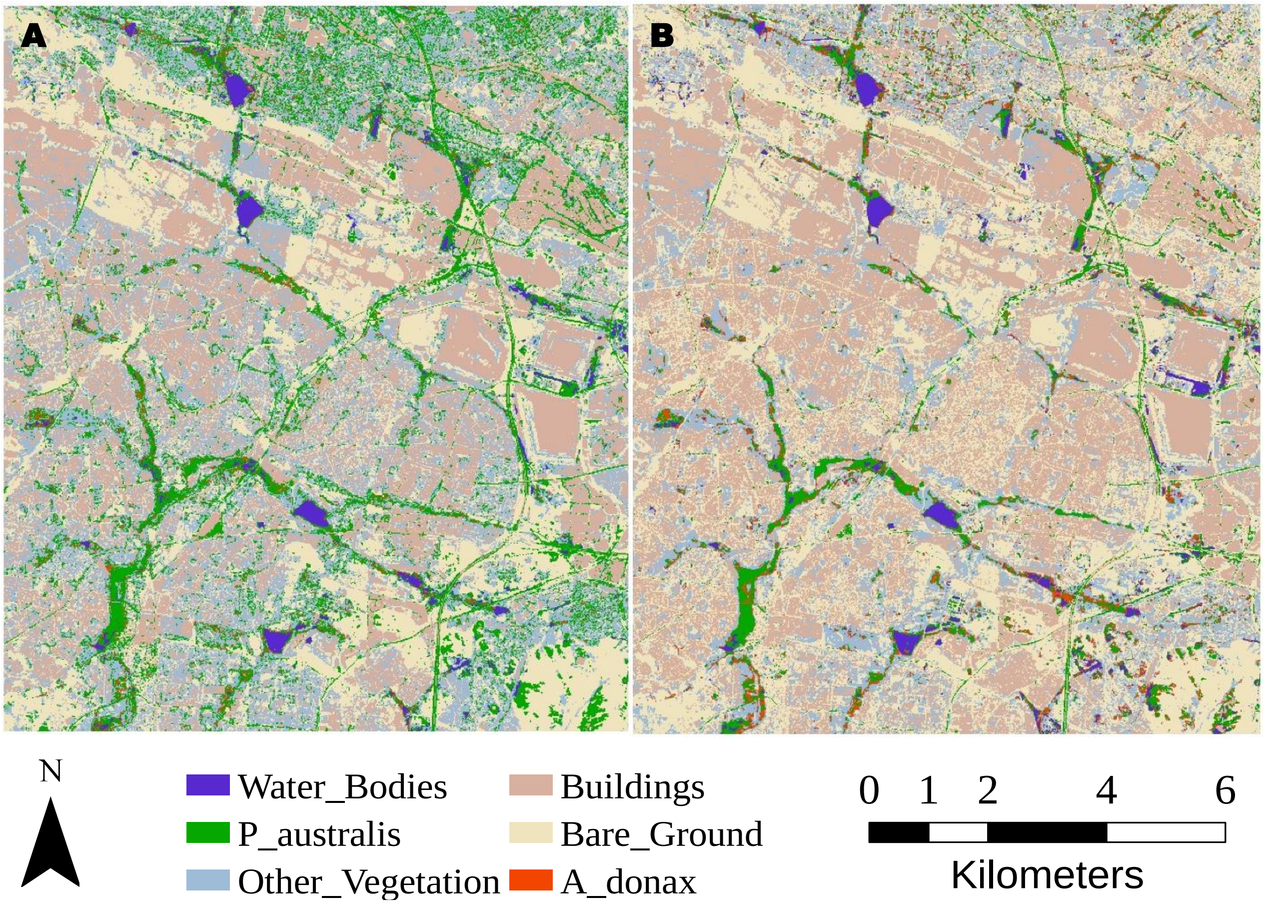

Figure 5.2 Classification maps of *Arundo donax* L., *Phragmites australis* (Cav.) Trin. ex Steud. and other land cover types obtained from Support vector machines (A) and Random forests (B) classifiers with Sentine-2 data. The study area covers parts of Soweto, Roodepoort and Randburg, Johannesburg, South Africa.

Table 5.3 Estimated areas and percentage coverage for *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. and other land cover types obtained from Sentinel-2B image using Random Forest and Support Vector Machines classifiers

	Random forest		Support Vector Machines	
Land cover class	Area (ha)	Area (%)	Area (ha)	Area (%)
A. donax	490	2.7	206	1.1
Bare_ground	6639	36.5	5079	27.9
Built_up_area	6254	34.4	4928	27.1
Other_vegetation	3645	20.0	5318	29.3
P. australis	868	4.8	2390	13.2
Open_water	285	1.6	260	1.4

From the RF classifier, it was possible to determine the role of each band of the Sentinel-2 image in the classification process. In this study, the importance of each band was determined using the mean decrease in accuracy score, which indicates how much the classification model accuracy decreases if that particular band was to be dropped. Bands with the highest mean decrease in accuracy are the most important. The most important bands were the green, SIWR_2 and the red band (Figure 5.3).

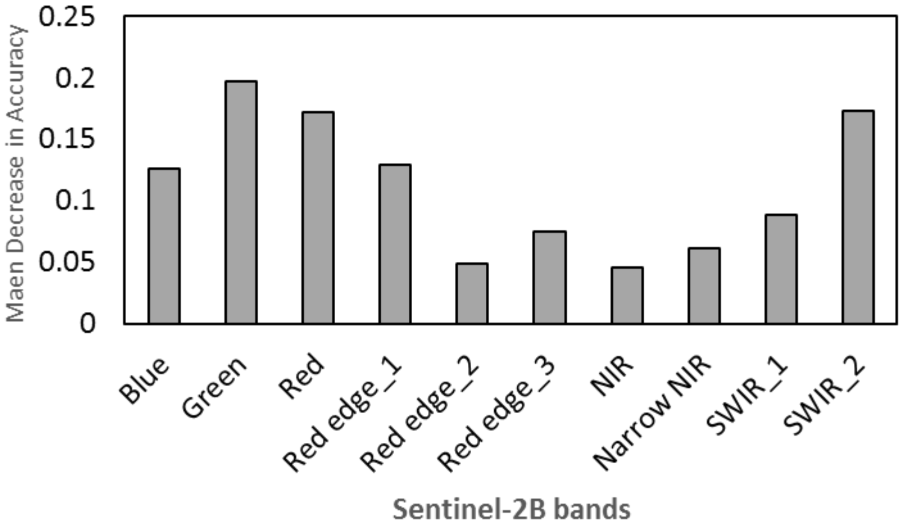

Figure 5.3 Importance of Sentinel-2B bands in the classification process using mean decrease in accuracy score.

5.3.2 Accuracy assessment

The overall accuracy and Kappa coefficient for RF and SVM were exactly the same (91.2 and 0.89, respectively) (Table 5.4). The user's accuracy values ranged between 64 % (*A. donax*) and 100 % (bare ground, built up area and open water, respectively) for RF and between 75 % (*A. donax*) and 100 % (bare ground, built up area and open water classes respectively), for SVM. The producer's accuracy for the RF classifier ranged between 62 % (other vegetation class) and 100 % (bare ground, *P. australis* and open water classes respectively), while that for SVM was between 54 % (*A. donax*) and 100 % (bare ground and open water classes respectively). There was spectral confusion of 36 % between the AD and 'other vegetation' which largely affected the accuracy level for AD producing a User's accuracy of only 64 % compared to the 86 % and 73 % for the *P. australis* and other vegetation classes respectively, when RF classifier is used. The user accuracy levels, however, seems to be slightly higher (75 %, 82 % and 77 % respectively) for the SVM classifier than for the RF except for *P. australis.* The least producer's accuracy for both classifiers was obtained in the classification of *A. donax*, which was confused with *P. australis* and other vegetation by the RF classifier, and with *P. australis* by the SVM classifier.

5.4 Discussion

Remote sensing plays a very crucial role in riparian land cover mapping at various scales (Ghorbanian *et al.*, 2020). Accurate and reliable information on the location, type and extent of wetland covers is important for proper wetland management (Sterling *et al.*, 2013; Chen *et al.*, 2015). In this study, Sentinel-2 data was used to map the distribution of *A. donax* and *P. australis* among other land covers using two machine learning algorithms, RF and SVM. High and equal overall accuracies and kappa coefficients (91.2 % and 0.89, respectively) obtained from both classifiers confirm the utility of Sentinel-2 data for discriminating and mapping of *A. donax* and *P. australis*. The high Kappa index (0.89) obtained was in agreement with the overall accuracy (91.2 %) of the classification processes. This is in contradiction with findings by Pontius Jr and and Millones (2011), who concluded that the Kappa indices are useless, misleading and/or flawed for the practical applications in remote sensing. Using the mean decrease in accuracy score, the most important Sentinel-2 bands in the classification process were green, SWIR_2, red, red edge_1 and blue, with the NIR being the least important band. This agrees with the findings indicated by

Adam *et al.* (2017) where the Blue, Green and Red bands of WorldView-2 imagery were found among the most important bands for land cover classification, and the NIR band was the least important band.

Table 5.4 Confusion matrices obtained using Random Forest classifier (left) and the Support Vector Machines classifier (right) for land cover types, *A. donax* (AD), bare ground (BG), built up area (BUA), *P. australis* (PA), open water (OW) and other vegetation (OV) classification processes.

Random Forests

	Ground reference classes						
Classes	AD	BG	BUA	OV	PA	OW	Row total
AD	7	0	0	4	0	0	11
BG	0	23	0	0	0	0	23
BUA	0	0	28	0	0	0	28
OV	1	0	2	8	0	0	11
PA	3	0	0	1	28	0	32
OW	0	0	0	0	0	20	20
Column total	11	23	30	13	28	20	125
Producer's accuracy, %	64	100	93	62	100	100	
User's Accuracy, %	64	100	100	73	86	100	

Overall accuracy = 91.2 %
Kappa coefficient = 0.89

Support Vector Machines model

	Ground reference classes						
Classes	AD	BG	BUA	OV	PA	OW	Row total
AD	6	0	0	2	0	0	8
BG	0	23	0	0	0	0	23
BUA	0	0	28	0	0	0	28
OV	0	0	2	10	1	0	13
PA	5	0	0	1	27	0	33
OW	0	0	0	0	0	20	20
Column total	11	23	30	13	28	20	125
Producer's accuracy, %	54	100	93	76	96	100	
User's Accuracy, %	75	100	100	77	82	100	

Overall accuracy = 91.2 %
Kappa coefficient = 0.89

The similar performances by the RF and SVM classifiers agrees with other studies, for example Abdi (2019) where RF and SVM performances were found to be close (overall accuracies = 73.9 % and 75.8 % respectively) in the classification of a boreal landscape using sentinel-2 data, as well as Adam *et al.* (2017) and Jombo *et al*. (2020) where the performance by RF was higher than that of SVM by just 1 % and 3 %, respectively. However, other studies reported significant differences between the performance of RF and SVM classifies in land cover classification. For example, Mukarugwiro *et al.* (2019) investigated the use of sentinel-2 data using RF and SVM to map water hyacinth in Rwandan water bodies. Their research concluded that the RF classifier had a higher overall accuracy and kappa coefficient (84 % and 0.81 respectively) than the SVM (65 % and 0.54 respectively). In our study, the RF and SVM classifiers produced 100 % accuracy on mapping of bare ground, built up area and open water. Low producer's accuracies were obtained for *A. donax* from both RF (64 %) and SVM (54 %) due to misclassification between this class and *P. australis* and other vegetation classes. The spectral characteristics for all vegetation is determined by their biochemical content (e.g. chlorophyll) and anatomical (e.g. canopy architecture) structure of their leaves or crowns (Curran, 1989; Kumar *et al.*, 2002; Fernandes *et al.*, 2013). All vegetation has a general spectral response curve, which is unique to each species. The misclassification between *A. donax* and *P. australis* classes was because the two reeds are physically and morphologically similar, and hence their spectral properties overlap (Everitt *et al.*, 2004; Saltonstall *et al.*, 2010; Fernandes *et al.*, 2013).

5.5 Conclusion

The availability of relatively high resolution sentinel-2 data provides cost effective opportunity for mapping *A. donax* and *P. australis* reeds in wetlands. The results demonstrated the ability of Sentinel-2 imagery and the RF and SVM classifiers in identifying and mapping the two species. The green, SIWR_2 and the red bands were the most important bands in the classification process. The coverage of *P. australis,* was greater than that of the alien *A. donax* species in both RF and SVM classifications. Although high overall accuracies were obtained for the classifications, there were also some misclassifications mainly due to spectral overlap between *A. donax* class and *P. australis* and other vegetation classes.

CHAPTER SIX

6. General Summary

A remote sensing-based approach for detecting and mapping the accumulation of heavy metal in and *Arundo donax L.* and *P. australis* (Cav.) Trin. ex Steud. in wetlands influenced by gold mining.

6.1 Introduction

Arundo donax L. and *Phragmites australis* (Cav.) Trin. ex Steud. play an important role in phytoremediation of wetlands. The two are morphologically similar reed species widely distributed in wetlands of South Africa (De Villiers *et al.*, 2011; Canavan *et al.*, 2018). Their capacities to uptake heavy metals from polluted wetland systems have been extensively studied worldwide. Wetlands that exist in gold mining areas of South Africa are subjected to acid mine drainage (AMD) pollution, which is characterised by low pH and high concentrations of heavy metals, which include copper, manganese, aluminum, iron, nickel, zinc, cobalt, and cadmium (Bell *et al.*, 2001; Coetzee *et al.*, 2006). Low pH and high heavy metal concentrations negatively affect wetland species composition (Ochieng *et al.*, 2010). Acid mine drainage pollution from wetlands can also end up in streams, rivers and dams, which supply potable water for humans (McCarthy and Humphries, 2013). *Arundo donax* and *P. australis* grow naturally in wetlands in South Africa, and among other emergent macrophytes, *P. australis* is the most commonly used and studied plant species for heavy metal uptake (Vymazal and Březinová, 2016). *Phragmites australis* is native to South Africa (Adams and Bate, 1999), whereas *A. donax* is a naturalised alien invasive species in South Africa and is declared as Category 1b weed in South Africa (Henderson, 2001; Department of Environmental Affairs, 2016). There is need to determine which of the two species (*A. donax* and *P. australis*) is more effective in phytoremediation under controlled experimental conditions so as to make the right choice between them for potential use in wetlands. The information will also help promote the native *P. australis* for phytoremediation in constructed wetlands, should it be found more effective.

Effective wetland management requires a robust monitoring system on the performance of phytoremediating plants to determine the appropriate time for biomass harvest before they die and release contaminants taken up in plant tissues back into the source through decomposition. Up-to-date and accurate spatial and temporal information on the plants' distribution and abundance is also crucial. Remote sensing, which is time- and cost- effective, up-to-date and relatively accurate (Lee and Yeh, 2009) can be used for mapping and monitoring the performance of phytoremediating plants in wetland systems. However, *A. donax* and *P. australis* morphologically and ecologically resemble each other and thus their reflectance properties overlap, and it becomes difficult to spectrally discriminate between them (Everitt *et al.*, 2004; Saltonstall *et al.*, 2010; Fernandes *et al.*, 2013).

The objectives of the study were:

1. To measure heavy metal concentrations in sediments and plant tissues of *A. donax and P. australis* planted in healthy medium (control), and in Cu + acid treated medium (treatment), under glasshouse conditions.
2. To compare the spectral reflectance of *A. donax* and *P. australis* plants growing under both control and treatment greenhouse conditions.
3. To model the relationships between plant copper concentration, chlorophyll and leaf spectral reflectance for *A. donax* and *P. australis*.
4. To map the spatial distribution of *A. donax* and *P. australis* reeds in wetlands in Johannesburg, South Africa using Sentinel-2 data.

6.2 Comparison of copper uptake between *Arundo donax* and *Phragmites australis*

The capacities of *A. donax* and *P. australis* to uptake copper were assessed in a glasshouse experiment (Chapter 2). The results showed that both *A. donax* and *P. australis* are capable of removing significant amounts of copper from acid mine drainage polluted wetland systems. This was evidenced by higher copper concentrations in plant tissues exposed to the contaminant compared to those of control plants. The distribution pattern of copper concentrations was in the order of roots>leaves>stems for *A. donax* and root>stems>leaves for *P. australis*. The distribution patterns from both reeds show that the roots are the main bioaccumulator organs, and this is explained by the compartmentalization tolerance strategy, common in wetland plants. The compartmentalization strategy is when the highest level of heavy metals are stored in below ground tissues as opposed to above ground tissue, and is a deffence mechanisim to protect the highly sensitive photosyntetic sites from the toxic effects of heavy metals (Weis *et al.*, 2004; Bonanno *et al.*, 2017). Despite being ecologically and morphologically similar, *A. donax* accumulated more copper in leaves than in the stems, while *P. australis* showed the opposite, which indicates that the two species respond differently to metal inputs. This is in line with observations from previous works, that heavy metal uptake patterns from different species is independed of ecology, biomass and anatomy (Bonanno *et al*., 2017).

Although the two plants have proved ability to be used for phytoremediation, they are not good candidates for phytoextraction of copper. Phytoextraction is when pollutants are significantly transported and concentrated in the above ground plant tissues and involves subsequent harvest of aerial plant biomass, and is possible when both the BCF and Translocation Factor (TF) for a plant is above one, which was not the case in this study. Between the two species, *P. australis,* which had a higher TF (0.4) than *A. donax* (0.2), is the preferred plant for phytoremediation. Thus, also considering that *A. donax* is a declared category '1b' weed under the National Environmental Management: Biodiversity Act (NEM:BA, Act No. 10 of 2004), requiring its immediate removal, *P. australis* must be promoted for phytoremediation of wetlands contaminated with copper from AMD in South Africa as opposed to *A. donax*. The mean chlorophyll content for both *A. donax* and *P. australis* was higher in treatment plants than in the control plants. The higher the chlorophyll content the healthier the plant. Regression analysis showed a significantly positive relationship ($p < 0.05$) between leaf copper concentration and chlorophyll content. However, the low R^2 (0.29) obtained meant that the independent variable (chlorophyll), could explain only a small percentage of the variation in the dependent variable. Even though the treated plants accumulated higher copper concentrations in their tissues, they did not accumulate it to phytotoxic levels. Hence, the two plant species have potential to be used for phytoremediation of wetland systems contaminated with copper concentrations above 5 mg/L.

6.3 Spectral discrimination between *Arundo donax* and *Phragmites australis* grown in copper contaminated conditions using in situ field spectral measurements

The Analytical Spectral Device (ASD) was used to determine the potential of remote sensing in discriminating between *A. donax* and *P. australis* and to evaluate their copper uptake performances (chapter four). The effect of copper uptake on leaf chlorophyll content was determined. The ASD data and two classification models, the random forest (RF) and the support vector machines (SVM) were used to compare the efficiencies in discriminating between *A. donax* and *P. australis*.

6.3.1 Influence of leaf copper concentration on chlorophyll content and spectral reflectance

Results in this study showed that copper uptake by plants exposed to copper (5 mg/L) + acid treatment in both species increased their leaf chlorophyll content. Generally, increased leaf chlorophyll content resulted in increased plants' mean spectral reflectance. The changes in spectral reflectance due to changes in chlorophyll were not uniform across the spectrum for both plants. For *A. donax* treatment resulted in a reflectance increase in the visible region and decreased reflectance in the near infrared region, while in the mid infrared, the reflectance decreased between wavelengths 1567 and 1801 nm, and increased between 1903 and 2500 nm. For *P. australis* Cu + acid treatment resulted in overall decreased spectral reflectance in the visible and the mid infrared regions, while it increased in the near infrared region. The mean reflectance for *A. donax* was lower than that of *P. australis* for all wavelengths. For plants grown under Cu + acid treatment, spectral reflectance for *A. donax* was higher than that of *P. australis* in the visible region, and lower in all the other wavelengths. Also, although for both control and treatment plants, leaf reflectance for *P. australis* in the NIR region was higher than that of *A. donax*, the difference was greater for plants under treatment conditions. This divergence in behavior between the control and treatments of both species can be explained by the principle of Plant Functional Types (PFTs), as described by Ustin and Gamon (2010). Under polluted environments, the two plants tend to belong to different Plant Functional Types (PFTs) from their respective control plants. Remote sensing is able to discriminate vegetation according to their PFTs.

6.3.2 Spectral discrimination between Arundo donax and Phragmites australis

Feature selection using the GRRF, followed by supervised classification was tested in the discrimination between *A. donax* and *P. australis* species grown under control, and Cu + acid treatment. Two classification algorithms, the RF and SVM were used for classification and compared. Using the feature importance measurements produced by the ordinary random forest, the GRRF was able to select optimum subsets that yielded the least OOB error in the classification between the two species both under control and under treatment environment. The mean decrease in Gini index was used for feature importance measurement. Different sets of wavelength were selected for the two classification processes, 703, 1585 and 1699 nm for discrimination of the two species under control conditions, and 1238, 1247 and 1550 for discrimination of treatment plants. This is because copper accumulation by treatment plants resulted in changes on leaf biochemical, structural and physiological traits and these functional changes in the leaves altered the spectral characteristics of the treatment plants (Ustin and Gamon, 2010). The different sets of wavelengths selected were all in the near infrared and the mid infrared regions, and reflectance in these regions is influenced by plant biochemical and leaf internal structure (Fernandes *et al.*, 2013; Kumar *et al.*, 2002). Classification results showed that under control or healthy conditions, the SVM (overall accuracy = 75 % and Kappa = 0.48) better discriminated the two species than the RF (overall accuracy = 64.2 % and Kappa = 0.2). For plants under treatment, the Random forest (overall accuracy = 85.2 % and Kappa = 0.7) discriminated the two reed species better than the SVM model (81.4 %; 0.63). The differences in classification efficiencies by the RF and SVM classifiers between the control and treatment plants for both species can be attributed to the PFTs theory described above.

Since most wetlands in South Africa where the two plants generally grow have various levels of organic and inorganic pollutants (Newete and Byrne, 2016; Papazoglou *et al.*, 2005; Tutu *et al.*, 2008; Vymazal and Březinová, 2016), the RF which discriminates the two reeds better under polluted environment is recommended in the discrimination of *A. donax* and *P. australis* in wetlands, over the SVM.

6.4 Use of Sentinel-2 data to map *A. donax* and *P. australis* in wetlands.

It is important to map the distribution and abundance of *A. donax* and *P. australis* in wetlands in order to properly monitor their role and function in the phytoremediation of wetlands. In the current study (chapter four), it was found that discrimination of the two plants using hyperspectral data yielded recommendable results. Other researchers have also concluded that hyperspectral data yields more accurate results than that by multispectral data in species discrimination (Smith and Blackshaw, 2003; Adam *et al.*, 2010). However, mapping of wetland plants over large areas is not easy for many researchers because most high resolution satellite images are expensive and so not available to everyone (Dube et al, 2014). Sentine-2 data provides freely accessible high resolution data with a short revisit time of 5 days. The effectiveness of Sentinel-2 data in mapping *A. donax* and *P. australis* reeds in wetland using the RF and the SVM was assessed (chapter five). The two reed species and other land cover classes in the study area (bare ground, buildings, water bodies and other vegetation) were mapped.

High and equal performances (overall accuracy = 91.2 and kappa coefficient = 0.89) were obtained from the RF and SVM classification models, confirming the utility of sentinel-2 data for discriminating and mapping of *A. donax* and *P. australis* using either of the two classification models. *Arundo donax* covered the least percentage area from both RF (2.7 %) and SVM (1.1 %) produced maps. The distribution pattern of *P. australis* showed that it covers most water bodies, and that explains the low percentage of land covered by water bodies in the study area. From both RF and SVM, the study area has substantial populations of *P. australis* compared to the alien *A.*

donax. The land cover map showed evidence of *A. donax* species emerging in areas already covered by *P. australis*.

6.5 Conclusions

The aim of this research was to investigate the use of remote sensing to monitor the uptake of copper by *Phragmites australis* and *Arundo donax* plants grown in a simulated acid mine drainage experiment. The findings of the study contribute to research in general and confirms feasibility of applying remote sensing technologies in mapping and monitoring phytoremediation of AMD polluted wetlands by *A. donax* and *P. australis.* The main conclusions based on findings from the different objectives of the study are as follows:

1. *Phragmites australis,* which is a native species to South Africa, is a better candidate for copper uptake in AMD contaminated wetlands, than the alien and invasive *A. donax*. Thus, *P. australis* can be promoted for use in contracted wetlands eventually replacing the existing invasion of wetlands by *A donax*.
2. Hyperspectral data can be used to discriminate between ecologically and morphologically similar species like *A. donax* and *P. australis*. The RF produced higher level of separability (overall accuracy = 85.2 % and Kappa = 0.7) between *A. donax* and *P. australis* compared to SVM (overall accuracy = 81.4 %, Kappa = 0.63). Due to the different efficiencies for phytoremediation between the two plants, and the subsequent response of the plants to the level of heavy metals sequestered in their tissues which affects the plant health status and more particularly their leaf chlorophyll content, it was more possible to discriminate the between *P. australis* and *A. donax* when grown in contaminated environments.
3. Copper uptake by both *A. donax* and *P. australis* resulted in increased leaf chlorophyll content in the plants. This means the plants can tolerate copper concentrations of 5 mg/L in acid mine drainage contaminated wetlands. Copper is both an essential and a toxic element to plants. At elevated levels copper impairs the photosynthetic processes in plants, which result in chlorophyll degradation. However, in this study, the leaf copper concentration accumulated in both *A. donax* and *P. australis* plants did not get to toxic levels, thus the observed increase in chlorophyll content in treatment plants. There was a significantly positive relationship between leaf copper concentration and their respective chlorophyll content, which is modelled by the following regression equation:

Leaf_copper_concentration = –6.39 + (0.35 x Chlorophyll_content)

Despite the positive relationship between leaf copper concentration and chlorophyll content, the relationship was weak on its capacity to predict copper concentration from chlorophyll content values, as depicted by the low R-squared value (0.29). Generally, higher chlorophyll content resulted in decreased mean leaf spectral reflectance.

4. The relatively high resolution and freely available Sentinel-2 data provided a cost effective way to identify and discriminate *A. donax* and *P. australis* and other land covers in wetland areas in Johannesburg, South Africa. Although there were some spectral confusion in the discrimination between *A. donax* and *P. australis*, both the RF and SVM produced a high and similar efficiency in the classification of land covers in the study area (overall accuracy = 91.2 % and kappa = 0.89 for both). According to feature importance scores from the RF model, the green, SIWR_2 and the red bands were the most important bands in the classification process.

6.6 Recommendation

The results in this study present the remote sensing technology as an alternative way for mapping and monitoring wetland plants and their role in phytoremediation of copper contaminated wetland systems. High resolution satellite image data and in-situ hyperspectral data coupled with machine learning algorithms, RF and SVM, not only are capable of detecting heavy metal-induced plant stresses, but also can effectively discriminate between morphologically similar species like *A. donax* and *P. Australis*, which facilitate wetland managment. Findings from this research contributes in building the spectral libraries for different wetland plant species which will help in discriminating between wetland species. The research concluded that *P. australis*, which is native to South Africa is the better option phytoextraction of copper uptake from wetland systems than *A. donax* which is an alien and invasive species in the country. Thus, in addition to the fact that *A. donax* is also a declared invasive species in South Africa and is subject to strict environmental regulations, the native *P. australis* must be promoted for phytoremediation of heavy metal polluted wetlands as opposed to *A. donax*. This research focused on copper uptake by the reed species from acid mine drainage polluted wetland systems. To the best of our knowledge, no previous research has assessed the capacity of *A. donax* and *P. australis* to uptake heavy metals under controlled or experimental conditions (Mabhungu *et al.*, 2019). Thus, future researchers must investigate the ability of the two plants species in up taking other heavy metal contaminants carried in AMD pollution, and whether the phytoremediating plants can be discriminated and mapped using remote sensing techniques.

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