ASSESSING INVASIVE ALIEN AQUATIC PLANT SPECIES' PHYTOREMEDIATION EFFECTS USING BIOLOGICAL INDICATORS IN THE SWARTKOPS RIVER SYSTEM

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Abstract

Pollution effluents in freshwater ecosystems are becoming increasingly ubiquitous as a result of cumulative anthropogenic activities, such as wastewater treatments works, and industrial, agricultural and mining activities. These activities are more noticeable in urban river catchments where there is greater human population densities and industrial developments. The ecological effects of anthropogenic activities on freshwater ecosystems include: excessive deposition of contaminants such as nutrients, pharmaceuticals, microplastics and other chemicals, which change physicochemical properties, causing a decline in aquatic biodiversity. These effects, impact negatively on the resilience of freshwater, making the systems vulnerable to invasion by alien aquatic plants. Ultimately, the loss of local biodiversity associated with the invasive alien aquatic plants (IAAP) results in a loss of some ecosystem goods and services. The Swartkops River system, Eastern Cape Province, drains most of the neighbouring formal and informal settlements, agricultural lands and industries, and hence is exposed to water pollution from human activities along the river catchment. Various water quality assessments are needed to evaluate the extent of pollutants and their impacts on the river ecosystem.

Phytoremediation is one approach employed internationally for removing harmful nutrients and chemicals in freshwater ecosystems. Most studies measure the success of phytoremediation through measuring the reduction of contaminants in water or soil chemistry in mesocosm settings, which may not take into account all the important environmental factors that exist in the field. The present study assesses the phytoremediation potential of *Pontederia* (*Eichhornia*) *crassipes* and *Salvinia molesta* by evaluating water and sediment chemistry, periphyton and aquatic macroinvertebrate community recovery along seven field sites (excluding IAAP species mats sites) located upstream and downstream IAAP species mats on the Swartkops River between April and September 2018. Water and sediment samples were collected once monthly on ten seven field sites, including the IAAP species mat sites. Periphyton and aquatic macroinvertebrates were collected on seven sites, excluding the IAAP mat sites. *Pontederia crassipes* and *S. molesta* infestation in Swartkops River showed positive phytoremediation results and improved some water and sediment chemistry in the downstream treatments as compared to upstream treatments. Although there were some fluctuations with some variables, important water and sediment chemistry were reduced downstream. By contrast, biological

assessment results did not show any response to the presence of IAAP species and phytoremediation.

Periphyton and aquatic macroinvertebrates diversity and community assemblages were more influenced by water quality. Although IAAP species did provide improvement in water and sediments chemistry, multiple effluent point and non-point sources in Swartkops outpaced phytoremediation. Taxa evenness and relative taxa abundance showed significant differences between the upstream and downstream sites, however taxa richness and Shannon's diversity showed no significant differences, indicating no relative recovery in biodiversity for either periphyton or aquatic macroinvertebrates. Similarly, the upstream and downstream sites showed similar periphyton and aquatic macroinvertebrates assemblages structure, all dominated by pollution tolerant taxa, thus indicating no functional diversity improvement down river; because of improvement in water chemistry downstream sites, it was expected that periphyton and aquatic macroinvertebrates assemblage structure would also improve at these downstream sites.

It is possible that the phytoremediation process is outpaced by effluent discharges, given the multiple sources and distance between upstream and downstream mat sites. The study showed phytoremediation potential but the results were not indicated by biological indicators. A replica study conducted on a moderately disturbed river system is recommended to measure the success and recovery of biological indicators and assemblage composition following IAAP species phytoremediation; a moderately disturbed river compared to a largely disturbed river will broaden findings and look at differences for a wider application of phytoremediation.

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List of Abbreviations and Acronyms

ANOSIM – Analysis of similarity

ANOVA – Analysis of variance

CAP – Canonical Analysis of Principal Coordinates

COD – Chemical Oxygen Demand

DBI – Dragonfly Biotic Index

db-RDA – Distance-Based Redundancy Analysis

DEFF – Department of Environment, Forestry and Fisheries

DistLM – Distance Based Linear Modelling

DO – Dissolved oxygen

DWAF – Department of Water and Environmental Affairs

DWS – Department of Water and Sanitation

EC – Electrical conductivity

FFGs – Functional Feeding Groups

IAAP – Invasive Alien Aquatic Plants

NMDS – Non-Metric Multi-Dimensional Scaling

PCA – Principal Component Analysis

PERMANOVA – Permutational Multivariate Analysis of Variance

PRIMER – Plymouth Routines in Multivariate Ecological Research

SASS – South African Scoring System

SASS5 – South African Scoring System version 5

SAWQGs – South African Water Quality Guidelines

TDS – Total Dissolved Solids

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Declaration

I declare that this thesis has not been submitted to any university other than Rhodes University (Grahamstown, South Africa). The work presented here is that of the author, unless otherwise stated.

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CHAPTER 1

General Introduction

1.1 Freshwater ecosystems

Freshwater ecosystems are comparatively less distributed (less than 1%) over the world surface (Collen *et al*., 2014); however, they are biologically diverse habitats including wetlands, lakes, springs, streams, and rivers (Wrona *et al*., 2013). This wide range of ecosystems contains numerous habitats of varying complexity, habitat diversity and freshwater species biodiversity, and their discontinuity (compared to marine systems) promote high species endemism (Moss *et al*., 2009). Freshwater resources have socio-economic and ecological value to the environment and the well-being of society (Hanemann, 2005), and they are regarded healthy if they perform or yield certain environmental and ecological values which are of benefit to humans (Liu *et al*., 2009). In return, the freshwater systems support both plant and animal life and provide ecosystem goods and services to society (Wang *et al*., 2012). Many of these streams have deep-rooted riparian vegetation that provides resistance to erosion of riverbanks, assimilates nutrients, and supplies the system with a continuous source of energy (Alberti *et al*., 2007). These systems ensure adequate water supply to the downstream environment, support a biologically and functionally diverse system, and thus provide ecosystem resilience (Liu *et al*., 2009). According to Vannote *et al*. (1980), river systems are hydrologically and ecologically connected; however, in the presence of humans, external factors such as anthropogenic activities alter this connectivity. Anthropogenic activities are believed to alter a river system’s physical, chemical and biological characteristics to an extent that the river is unable to self-reorganise (or becomes less resilient), thus leading to complete degradation. The physical, chemical and biological characteristics of a system are important in maintaining a healthy and resilient ecosystem that can supply goods and services. In the context of river restoration, assisting the river system to an alternative, stable state with desirable physical, chemical and biological characteristics is known to help the ecosystem to recover and self-reorganise effectively.

1.1.1 Socio-economic and ecological importance of aquatic ecosystems

As freshwater systems perform their ecosystem funtions, they provide multiple benefits to humans, known as ecosystem services, these are divided into provision, support, regulation, and cultural services that have long been linked into a complex structure providing goods and services (Costanza *et al*., 2017). Freshwater ecosystems are capable of minimising our vulnerability to natural hazards, such as floods and drought (Acreman, 2004); produce storm protection, pest control, water purification, and air quality maintenance (Costanza *et al*., 2017). These ecosystems also play an important role in regulation of temperature and climate (Bolund and Hunhammar, 1999). Freshwater ecosystems also offer support services; these are basic ecosystem services that serve an important role in biogeochemical processes, natural cycling of nutrients, and spreading of seeds for plant productivity (Costanza *et al*., 2017). This may also include provision of habitat to a variety of organisms, which include photosynthetic microalgae (e.g. phytoplankton and periphyton), aquatic macroinvertebrate and vertebrate species, and macrophytes (Acreman, 2004; Jiang *et al*., 2015). Macrophytes serve as oxygen sources, provide with food, fibre, timber, have medicinal properties and serve as natural water filters to reduce pollution (Sanilkumar and Thomas, 2007); provide clean water for domestic use, and for agricultural and industrial activities (provision services) (Malmqvist and Rundle, 2002). Freshwater ecosystems play a significant role in the generation of hydroelectricity (Hadwen *et al*., 2012); produce recreational units, aesthetic, and cultural identities which results in attraction of tourists and driving other economic activities (cultural activities) (Costanza *et al*., 2017).

1.2 Freshwater threats

Through hydrological connectivity, freshwater ecosystems are a major link between the atmosphere and the biosphere, which makes them highly vulnerable to anthropogenic activities and external pressures occurring at catchment level (Sala *et al*., 2000; Castello *et al*., 2013). Having said that freshwater ecosystems have been significantly transformed to a point where little or none of their original biodiversity is noticeable; these alterations are much more evident after the ecosystem has degraded (Dudgeon *et al*., 2006). These ecosystem alterations usually have adverse effects, such as the replacement of key functional and biological organisms, leaving tolerant and generalist taxa dominating the ecosystem (King and Brown, 2010). As areas continue to urbanise, destruction of aquatic habitats increases (e.g. building of dams for more water supply to enhance living conditions), reducing and shifting species compositions, so leading to the loss of ecosystem services to the point where the system is unable to rehabilitate itself (Carpenter *et al*., 2011). Meybeck (2003) explained how these external disturbances (as discussed below) have become all but irreversible and threaten the socio-economic and ecological well-being of freshwater ecosystems.

1.2.1 Land transformation and flow modification

Habitat transformation for urban development and agricultural activities is reported as major factor for biodiversity loss in both terrestrial and aquatic environments (Hooke *et al*., 2012). Sand mining on riverbanks transforms the habitat, exacerbates riverbank erosion, increases turbidity, disrupts allochthonous processes and the connectivity between riparian and aquatic ecosystems, thus affecting adult Odonata species' ecology, and compromising the flood buffering provided by riparian ecosystems (Dudgeon *et al.*, 2006). Artificial channelling of rivers and impoundments restricts natural flow and limits water for downstream environments; this disrupts the ecology of Plecoptera, Trichoptera and other instream invertebrates with flow requirements. In addition, the dilution of river flow disturbs migration patterns and refugia for aquatic species (Dudgeon, 2012). These modifications impact species' breeding patterns, particularly seasonal breeders who receive either less, more, or no water flow for their developmental requirements (Limburg and Waldman, 2009; Dudgeon, 2012). Some areas and species require a perennial, steady and uniform flow, and thus flow modifications may result in a direct loss of habitat. Species such as amphibians, some odonates with amphibiotic life cycles as well as various aquatic insects, particularly filter feeders and those with gills, are highly dependent on river flow for food and oxygen (Rowley *et al*., 2010; Dudgeon, 2012).

1.2.2 Water pollution

Water pollution is the introduction of foreign substances or contaminants into the river system through anthropogenic activities. These contaminants change the chemical composition of water, creating unfavourable conditions for sensitive aquatic organisms and leading to a decline and shift in biological and functional diversity (Alberti *et al*., 2007; Harding, 2015). Overuse of pesticides and fertilizers in agricultural practices, together with poor wastewater management, are considered the main sources of surface water pollution for developing countries such as South Africa (Ansara-Ross *et al*., 2012). These landscape activities contribute to excessive nitrogen, ammonium and phosphate concentrations within the river systems to an extent where the system is unable to break down or turn over excess nutrients (Luo *et al*., 2012). As a result, algae blooms occur, causing a further decline in water quality by blocking sunlight to submerged aquatic organisms, reducing dissolved oxygen concentration and causing the accumulation of toxins from cyanobacteria algae blooms (Landsberg, 2002). The cyanobacteria toxin is incorporated into the aquatic food web through bioaccumulation, reaching tertiary predators and threatening human and wildlife health (Ghosh and Singh, 2005). For example, a study by Khansari *et al*. (2005) reports chemical bioaccumulation in freshwater fish species (*Tuna* sp.), which is considered one of the largest food sources for humans (ecosystems goods). Khansari *et al*. (2005) further reported that *Tuna* sp. directly accumulate high concentrations of mercury in their bodies through water contamination, and indirectly, via the aquatic food web. Mercury is easily transferred from *Tuna* sp. to humans through consumption; its toxicity causes various health problems (Khansari *et al*., 2005). Most of South African rivers have lost the capacity to assimilate nutrients and other toxic substances due to extreme pollution of the waterways. Over the past 20 years, pollution in freshwater ecosystems has accelerated, leaving about 60% of South African rivers threatened, and 25% critically endangered, particularly by eutrophication (DWA, 2013). De Villiers (2007) reported on the deteriorating water quality status of the Berg River, Western Cape, South Africa, as a result of agricultural activities taking place along the river catchment. The study also reported that the river had high concentrations of nutrients (nitrogen and phosphorus) that were altering the trophic levels in the river, threatening aquatic organisms, ecosystem services and human health. Another case study by Xu *et al*. (2014) reported a drastic decline in aquatic macroinvertebrate taxa assemblages (scrapers, shredders and predators) in rivers in China as a result of increased phosphorus and nitrogen concentrations, while collector-gatherers became dominant, resulting in an uneven distribution of functional feeders.

1.2.3 Invasive alien plant species

South Africa has a high diversity and density of invasive alien species in terrestrial and aquatic ecosystems (Richardson and Van Wilgen, 2004). Invasive alien plant species are defined as non-native plant species that are introduced out of their native range through the global plant trade as ornamental or medicinal plants (Richardson and Van Wilgen, 2004). After habitat loss, invasive alien species are considered the second leading cause of aquatic biodiversity loss and ecosystem degradation, globally (Srivastava *et al*., 2014). Simberloff (2006) reports that invasive alien species pose a serious threat to natural ecosystems since they lack natural enemies, and thus proliferate in their introduced range. These plant species have highly competitive traits (i.e. asexual reproduction); their timing of growth also gives them a competitive advantage and they can therefore outcompete native plant species (Bremner and Park, 2007).

Invasive alien aquatic plant (IAAP) species modify their invaded ecosystems by altering the physical structure, hydrology and species composition (Tobias *et al*., 2019). These plants species use invaded systems resources excessively by utilising more surface water through evapotranspiration; they sequester nutrients, block sunlight for underwater life, and reduce dissolved oxygen concentration, which in turn, increases carbon dioxide and causes anoxic conditions, which affect ecosystem structure and functioning (Van Wilgen, 1996; Richardson *et al*., 2000; Masifwa *et al*., 2001). Invasive alien aquatic plants alter the water quality, biodiversity (Masifwa *et al*., 2001), reduce water velocity (Champion and Tanner, 2000) and are also capable of restricting the production and distribution of ecosystem goods and services (Richardson and Wilgen, 2004). For example, *Pontederia* (*Eichhornia) crassipes* Mart. Solms-Laub (Pontederiaceae) (water hyacinth) and *Pistia stratiotes* L. (Araceae) (water lettuce) proliferate in tropical and subtropical regions where temperatures are high (Lu *et al*., 2010). These plants form dense mats that affect the distribution and composition of biodiversity (Ndimele *et al*., 2011), displacing national endemic and keystone organisms from the ecosystem (Hornoy *et al*., 2011). With all these threats facing freshwater environments, there is a need to implement ways in which freshwater ecosystems can be monitored to avoid further deterioration and biodiversity loss.

1.3 Freshwater ecosystem biological monitoring

1.3.1 Physicochemical analysis

Physicochemical monitoring of aquatic ecosystems includes the direct measurement of water chemistry variables, for example dissolved oxygen (DO), total dissolved solids (TDS), water temperature, hydrogen potential (pH), chemical oxygen demand (COD), biological oxygen demand (BOD) and water nutrients (nitrate (NO_3), ammonia (NH_4), and phosphate (PO_4)

(Palmer, 2004). Physicochemical monitoring is a widely used, traditional water quality spot analysis method, which has been used to trace and spot water chemistry concentrations through time and space (Ritchie *et al*., 2003). Water quality assessment may also involve the use of remote sensing techniques, such as mounting sensors of various spatial and temporal resolutions on moving aircraft and satellites to evaluate chemical pollutants, suspended solids, water temperature and chlorophyll-*a* concentrations. However, this method is rarely used because of costs associated with capturing and analysing the images (Ritchie *et al*., 2003). Physicochemical analysis remains an inexpensive method, although it has some disadvantages: results are not time integrated and they differ with the time of the day that is, DO and water temperature, and cannot be used as reliable measurements for management of river systems because of their high variability (Ritchie *et al*., 2003; Arimoro *et al*., 2015). However, with changing variables, physicochemical analysis is an efficient assessment when used in conjunction with other biological data as they help to explain assemblage variation.

1.3.2 Aquatic organisms

Freshwater biological monitoring involves the use of aquatic biota, which includes aquatic microalgae (phytoplankton and periphyton), aquatic macroinvertebrates, adult dragonflies, freshwater fish and macrophytes, to assess the health of aquatic ecosystems (Bonada *et al*., 2006; Holt and Miller, 2010; Odume, 2017). The use of the above-mentioned organisms as biological indicators is often based on their responsiveness to changes in physical, chemical, and biological characteristics of the system in question, where individual taxa will show measurable indications of any external influence and trophic interactions (Bonada *et al*., 2006). Responsiveness of a biological indicator focuses on the organism's taxon abundance, richness, evenness and biodiversity, which reflects the environmental conditions (Jacob and Manju, 2013; Odume, 2017). Organisms used for biomonitoring depend on freshwater ecosystems for most of their lifespan, thus a shift in their habitat preference will result in the discomfort of specific individuals (Dallas, 2013). Such external disturbances can be water chemistry, flow regime, physical aspects of water and biological interactions (Ollis *et al*., 2006). Biological indicators help to track ecosystem changes and can also be used to determine the ecological state of river systems and predict changes in water quality (Navarro-Ortega *et al*., 2015), providing significant information needed to classify water resources, restoration and sustainable management (Hughes *et al*., 2012).

1.3.2.1 Aquatic microalgae

Aquatic microalgae are diverse aquatic micro-organisms found growing on natural substrates (macrophytes, stones, sediments) and swimming in the water column in aquatic ecosystems (Stevenson, 2014). Aquatic microalgae form the basis of the aquatic ecosystem food web as primary producers, responsible for driving net ecosystem productivity (Stevenson, 2014). However, excessive water contamination has the potential to alter aquatic microalgae physiological processes. For example, a case study by Dao and Beardall (2016) reported the effect of lead (Pb) from mining and other industrial activities on the photosynthetic activities and development (biomass) of aquatic microalgae. The study identified *Scenedesmus* sp. and *Chlorella* sp. (Chlorophyta: Green algae) as indicator species that were sensitive to high Pb concentrations, which affected their chlorophyll content and drastically reduced photosynthetic ability.

Aquatic microalgae have high species diversity, and are regarded as reliable ecological and environmental biological indicators of water quality (Lane and Brown, 2007). Aquatic microalgae have been widely used as reliable indicators for biological assessment of aquatic ecosystems by various authors, including McCormick and Cairns (1994), Taylor *et al*. (2007a), Lane and Brown (2007), Kumari *et al*. (2008), Berthon *et al*. (2011), Chakraborty *et al*. (2014), because they are highly responsive to environmental changes, rendering the topmost indication of change in aquatic systems. Aquatic microalgae are abundant and diverse (widely distributed) in both freshwater and marine ecosystems, are easy to sample and identify (Dalu *et al*., 2014a). In addition, aquatic microalgae are both sensitive and tolerant to various environmental conditions: acidic, nutrient-rich waters, metal-polluted waters, or any organically polluted waters (Taylor *et al*., 2007a). Some aquatic microalgae species tolerate heavily polluted streams, while others are sensitive to even the slightest environmental changes (Kilonzo *et al*., 2014). For example, diatom (Bacillariophyta) species including *Achnanthes minutissima* and *Surirella angusta* have been reported to tolerate heavy metal pollution such as copper (Cu), zinc (Zn), lead (Pb) and cadmium (Cd) in a mine-polluted river system in Japan. *Achnanthes minutissima* abundance increased with an increase in heavy metal concentration in a river system (Nakanishi *et al*., 2004). By contrast, aquatic microalgae taxa such as *Euglenophyceae* sp. (Kumari *et al*., 2008) *Cocconeis* sp., and *Encyonema* sp. decreased in number when organic and nutrient loads increased in pesticide-contaminated river water in France (Rimet and Bouchez, 2011).

1.3.2.2 Aquatic macroinvertebrates

Aquatic macroinvertebrates are boneless organisms found within freshwater bodies that can be seen with the naked eye. Some of them spend their entire life cycle in water (e.g. Ancylidae, Physidae, Hirudinea, Oligochaete, Belostomatidae, Nepidae) while others transform to terrestrial organisms as adults (Coenagrionidae, Chironomidae, Baetidae, Hydropsychidae) (Bonada *et al*., 2006). Aquatic macroinvertebrates are found in different biotopes such as stones, vegetation, sand, gravel, and mud (Bonada *et al*., 2006), and they serve as energy sources for higher trophic organisms in aquatic ecosystems. Most importantly, aquatic macroinvertebrates are used in biomonitoring; these organisms are excellent indicators of change used in water quality because they have varying sensitives to various environmental changes in water bodies, and have short life cycles that enable them to respond quickly to both organic and inorganic pollution (Azrina *et al*., 2006; Arimoro and Ikomi, 2008). In addition, biomonitoring also provide important information needed for biological conservation; for example, the Dragonfly Biotic Index (DBI) which uses adult male dragonfly and damselfly species to assess the health of freshwater ecosystems (Simaika and Samways, 2012; Thompson *et al*., 2017). The DBI employs three sub-indices, namely: geographical distribution, habitat sensitivity and the red list status of each Odonata species (Thompson *et al*., 2017). Based on the three sub-indices, male Odonata species are individually assigned a DBI score between 0-9; the score will differ between Odonata species, based on their preferred habitat and their conservation status. The DBI score of 9 indicates a sensitive species that is threatened by environmental disturbances with a small distribution, usually an endemic species. By contrast, Odonata species with low DBI score of 0 represent a highly tolerant and a generalist Odonata species that has a wide distribution range (Thompson *et al*., 2017). According to Chutter (1998), in South Africa, aquatic macroinvertebrates can be used to assess water quality because of the different sensitivity scores assigned to each family taxa, scores which range from 1 to 15. Aquatic macroinvertebrates with a sensitivity score closer to 1 indicate a severely disturbed freshwater ecosystem and are thus a tolerant taxa; those with a sensitivity score towards 15 are the taxa most sensitive to any physical or chemical alternation.

In southern Africa, freshwater river systems have been assessed using the South African Scoring System (SASS) protocol developed by Chutter (1994) and further modified by Dickens and Graham (2002) to its current SASS version 5 (SASS5). The SASS5 is used nationally to evaluate the ecological health of aquatic ecosystems. Although there have been attempts to test SASS5's applicability across borders (Zimbabwe) (Bere and Nyamupingidza, 2014) and within standing water bodies (Bowd *et al*., 2015), the SASS5 technique is only designed for rivers and streams and is not applicable to wetlands, estuaries and other lentic habitats (Dickens and Graham, 2002). According to Dallas (2000), when this technique is applied, the outcome and the interpretation of results should take into consideration the biotope diversity on site, flooding events, and/or water level, and any general physical alteration. The South African Scoring System is a cost-effective biological monitoring technique, time integrated, widely used, and easy to apply; it is a rapid technique that requires identification of aquatic macroinvertebrates to family level (Dallas, 1997).

The advantages of using aquatic macroinvertebrates for biological monitoring are that they (a) respond rapidly to various physical and chemical water body alterations in terms of their abundance, absence or presence, and composition (Chatzinikolaou *et al*., 2006); (b) are sedentary (limited mobility), which qualifies them as excellent candidates for biological monitoring (Carlisle *et al*., 2007); (c) occur in almost all aquatic habitats; (d) depend on an aquatic environment for most or part of their life cycle (Odume, 2017); (e) are large enough to be seen with the naked eye, and their size makes them easy to handle; (f) sampling does not require sophisticated equipment (collection and identification to family level), and they provide an instant measure of biological water quality.

However, aquatic macroinvertebrates are selective in the type of pollution they respond to and they are not capable of pinpointing the type of pollution the system is experiencing (Mahler and Barber, 2010). For example, during heavy flowing currents or post-flooding, Mahler and Barber (2010) reported inconsistent results when using aquatic macroinvertebrates for biological monitoring. Although most biological monitoring techniques involve aquatic macroinvertebrate family level identification, there has been some controversy around biological monitoring taxonomic resolution (i.e. species level, or family level identification). Barber-James and Pereira-da-Conceicoa (2016) highlight that using family level identification in aquatic mayflies, Ephemeroptera (i.e. Baetidae), underestimates the sensitivity of some species within the group because within a family, there can be various species with different pollution sensitivities, and dietary preferences that may affect their response to water chemistry. In addition, the study reported that identification at family level limits the interpretation to responses because some species and genera are associated with particular habitats and flora. Tampering with these habitats has a negative impact and causes the extinction of some species, which cannot be picked up when identification is aimed at family level. Thus, authors recommend that species-level identification should be incorporated in biological monitoring techniques of river systems. However, studies by Midgley *et al*. (2006), Coetzee *et al*. (2014), Kayde and Booth (2012) demonstrated that aquatic macroinvertebrate family level-identification was sensitive to alien invasive macrophytes and fish in South Africa. Thus, family-level identification still provides useful rapid monitoring assessment for freshwater ecosystems.

1.3.2.3 Macrophytes

Macrophytes play an important role in aquatic ecosystems as energy sources; they provide habitats for fish, macroinvertebrates, and other aquatic organisms (Bakker *et al*., 2013). Macrophytes drive ecosystem services, such as nutrient cycling and regulation of oxygen (Sood *et al*., 2012), and apart from their structural and functional role, macrophytes are widely used for bio-assimilation of contaminates within freshwater ecosystems; an environmentally friendly technique commonly used in restoring nutrient-enriched aquatic systems (Ojuederie and Babalola, 2017). Altering macrophyte communities' forms and traits, particularly in the case of alien invasion, can affect water quality and ecosystems' structure and functions. However, free-floating macrophytes, such as *Spirodella* sp. or *Lemna* sp., are known for effective nutrient assimilation in freshwater systems (Motitsoe *et al*., 2020). These macrophytes have a high biomass and exhibit complex root structures, thus they are able to extend their root length to compete for nutrients in the water column. For example, plants in the genus *Azolla* have been reported as excellent candidates for the removal of heavy metal pollutants in aquatic ecosystems (Arora *et al*., 2006) because *Azolla* sp. thrive in several freshwater and wastewater systems, possess a nitrogen-fixing cyanobacterium, *Anabaena* sp., have high biomass productivity, and can tolerate high concentrations of toxic pollutants (Arora *et al*., 2006). This is true for macrophytes with phytoremediation properties, which lack a cuticle (e.g. *Fontinalis antipyretica*) for easy absorption of pollutants by roots and distribution throughout the entire plant (Díaz *et al.*, 2012b). Plants used for phytoremediation are able to reduce the solubility of contaminants in their environment, breaking them down to less harmful chemicals and absorbing them with the help of mutualistic bacteria, thus providing an ecosystem service role to aquatic environments (Harguinteguy *et al.*, 2015; Ojuederie and Babalola, 2017). Lastly, these plants are static and very easy to identify, which makes them excellent candidates for phytoremediation (Rai, 2008).

1.4 The use of phytoremediation to remove pollutants in freshwater systems

Phytoremediation is the use of green plants and associated rhizospheric micro-organisms to remove concentrations of harmful contaminants in the environment to safer concentrations or render them harmless (Ali *et al.*, 2013). The approach was introduced, tested and validated for contaminate bio-assimilation in freshwater ecosystems, attracting the attention of many scientists (Baker *et al.*, 1994). Phytoremediation is an environmentally friendly "green technology" process that is less costly and uses macrophytes' natural abilities to remove contaminants from the environment (Cho-Ruk *et al.*, 2006; Tangahu *et al.*, 2011). Macrophytes use physiological processes with the help of microbial species and break down metals into usable cellular elements without any negative effect on the plant (Ojuederie and Babalola, 2017). The phytoremediation technique extracts heavy metals such as iron (Fe), Zn, uranium (U), chromium (Cr), Cu, manganese (Mn), Pb, mercury (Hg) and arsenic (As) from water and sediments; some of these metals are essential for plant growth in small quantities, while the biological function of others remains unknown (Prasad 2004; Cho-Ruk *et al.*, 2006).

Plants have evolved to accumulate contaminants from very low to high concentrations; contaminants are absorbed from the contaminated media (soil or water) by roots and transported by a process known as translocation from the roots to the shoots. The different sub-categories of phytoremediation are: phytoextraction, phytofiltration, phytostabilisation, phytovolatilisation, phytodegradation (Table 1.1) (Tangahu *et al.*, 2011).

Table 1.1: Phytoremediation mechanisms that assist the assimilation of pollutants in freshwater environments.

Technique	Definition	Reference
Phytoextraction	Absorption by roots of contaminants from the medium (soil, water or sludge) to the plant biomass.	Arora *et al*. (2006) Yoon *et al*. (2006) Vanhoudt *et al*. (2018) Mishra and Maiti (2017)
Phytofiltration	Removal of contaminants from surface water, minimising their movement to groundwater.	Rezania *et al*. (2015) Vanhoudt *et al*. (2018) Díaz *et al*. (2012a) Ali *et al*. (2013)
Phytodegradation	Modification of chemical substances by plants with the aid of enzymes.	Sakakibara *et al*. (2013) Sarkar *et al*. (2017)
Phytostabilisation	Pants equipped to stabilise contaminants in soils to reduce their mobility to any part of the food chain.	Singh (2012) Rezania *et al*. (2015) Sung *et al*. (2015)
Phytovolatilisation	Removal of contaminants from soil by plants which then release them into the air.	Ali *et al*. (2013) Priya and Selvan (2017) Mishra and Maiti (2017)

1.4.1 Advantages and limitations of phytoremediation

Phytoremediation can be used to remove heavy metals, radionuclides and organic contaminants, such as pesticides, from aquatic environments (Ali *et al*., 2013) and the terrestrial environment (Mwegoha, 2008). Phytoremediation is a green, efficient, cost effective, solar-driven remediation technique with high public acceptance worldwide, and is easily applicable over a wide range of freshwater bodies (Sarma, 2011). Phytoremediation also conserves the natural conditions of the remediated medium (Sursarla *et al*., 2002). Another advantage of phytoremediation is that the technique does not require highly trained specialists; its success depends on using it within the relevant context, with the appropriate approach and plant species which have the ability to accumulate high concentrations of contaminants (Tangahu *et al*., 2011). This success may be influenced by the type of plant species used, its biomass, concentrations of pollutants, depth, and velocity of the water body (Jutsz and Gnida, 2015). Various authors including Mishra and Tripathi (2008), Swain *et al*. (2014), Favas *et al*. (2012), Anning and Akoto (2018), have reported phytoremediation success. These studies report that different macrophytes are well equipped and adapted to assimilate and remove organic and inorganic pollutants in various water bodies, recording high concentration removal rates at different time scales, setting and pollutants.

A case study by Lu *et al*. (2010) reported the efficiency of water lettuce (*Pistia stratiotes*) in removing nutrients and improving eutrophic storm water in a pond experiment in the St. Lucie Estuary, India. For the case study, *P*. *stratiotes* was planted in two stormwater detention ponds and water samples were collected weekly for the analysis of physicochemical variables including pH, turbidity, suspended solids, EC and nutrients (PO_4 and NO_3). *Pistia stratiotes* plant samples were collected monthly for nutrient concentration analysis from control ponds (unimpacted waters) and experimental ponds (impacted waters). Results showed there was an improvement in water quality variables in experimental ponds which included suspended solids, nutrient concentrations, and water turbidity (more than 60% decrease in water turbidity). The PO_4 concentrations were reduced by approximately 14-31%, and concentrations of inorganic nutrients (NO_3 and NH_4) were reduced by more than 50%. Having said that, phytoremediation has some disadvantages as it is considered a slow rehabilitation process (Chintakovid *et al*., 2008; Tangahu *et al*., 2011), meaning the level of contamination in soil or water system is a limiting factor, and if the area is heavily contaminated, assimilation would take much longer than expected (Salido *et al*., 2003). Low plant biomass and the slow growth rate of hyper-accumulator plant species can also reduce the phytoremediation efficacy (Ramamurthy and Memarian, 2012). Furthermore, phytoremediation techniques are seasonal, thus effective only during peak summer months compared to winter months (Chintakovid *et al*., 2008). During the winter season, plants become inactive, slowing down the process; in addition, pest and disease attacks in climate-affected subtropical regions may compromise the accumulation capacity of some plants (Karami and Shamsuddin, 2010). Attacks may result in low plant biomass or decreased efficiency of the plant in accumulating pollutants, thus slowing the phytoremediation process. Phytoremediation application is more effective in static, shallow water bodies where water flow and circulation are slower, than in fast flowing rivers and deep-water bodies (Farraji *et al*., 2016).

1.5 Invasive alien aquatic plant (IAAP) species for phytoremediation

Floating IAAP species have extensively been used as a tool of phytoremediation to improve water quality in countries such as India, Zimbabwe, Nigeria and Benin as a substitute of the expensive and unpredictable traditional methods such as soil washing (Mishra and Tripathi, 2008), phoslock application (Yamada-Ferraz *et al*., 2015), immobilisation, and encapsulation (Liu *et al*., 2018). Invasive alien aquatic plant species have been successfully used for phytoremediation programmes and these include, *Salvinia molesta* D.S. Mitchell (Salviniaceae) (Giant Salvinia: salviniaceae), *P. stratiotes*, and *P. crassipes* (Mishra and Maiti, 2017). *Pontederia crassipes* has been reported effective in phytoremediation since it grows well in disturbed waters and eutrophic systems (Maine *et al*., 2001; Priya *et al*., 2013), and because *P. crassipes* relies on photosynthesis to drive the phytoremediation process (Saha *et al*., 2017). *Pontederia crassipes* is the most preferred aquatic plant for phytoremediation because it adapts quickly and tolerates various environmental conditions; it has an extensive root system and a large biomass that aids in metal accumulation (Cu, Pb and Cd) (Liao and Chang, 2004). Similarly, *Lemna* sp. have been used for phytoremediation in both standing water bodies and rivers systems for the past 30 years. *Lemna* sp. have successfully removed radioactive substances and dyes in polluted water systems (Lissy and Madhu, 2011). In addition, this plant species is capable of tolerating water temperatures between 7–35 °C and pH of about 3.5–10.5 (Newete and Byrne, 2016). *Salvinia molesta* has also been used in phytoremediation (Al-Hamdani and Sirna, 2008). Donatus (2016) reported that *S. molesta* accumulates various heavy metals, but is restricted to a few. High concentrations of heavy metals accumulated were reported more frequently in roots than leaves and this included Pb and nickel (Ni). *Pistia stratiotes* can also withstand high levels of temperature and pH (Lima *et al*., 2013), thriving well in polluted water (Rezania *et al*., 2016).

South African aquatic ecosystems have been invaded since the late 1800s by different IAAP species (Cilliers, 1991), the most common invasive aquatic plants being *P. crassipes*, *S. molesta*, *P. stratiotes, Azolla filiculoides* and *Myriophyllum aquaticum* Vell. Verdc (Haloragaceae) (Hill, 2003). Of these plants, *P. crassipes* and *S. molesta* are regarded as the most problematic IAAP species in aquatic systems in the country. These plants are all native to South America (Hill, 2003), and were introduced to South Africa through aquarium trading and horticultural activities (Martin and Coetzee, 2011). The high content of phosphate and nitrate perpetuated by urbanisation and other anthropogenic activities in the aquatic systems (Heard and Winterton, 2000; Coetzee and Hill, 2012) help these plants proliferate (Wilson *et al*., 2005; Augyte and Pickart, 2014). This proliferation is possible because South Africa's aquatic ecosystems (freshwaters to marine waters) are under stress of increasing population and development of the economy (Oberholster and Ashton, 2008). Water quality is altered and has declined, driven by an expanding economy, population growth, industrialisation, and agricultural activities around catchment areas (Azrina *et al*., 2006). Pollution has escalated, decreasing benefits acquired from these resources, with most of the rivers being nutrient-rich because of the large amount of sewage untreated before discharge. It is important to find ways to minimise the pollution and rehabilitate aquatic ecosystems, and to measure their success, is important.

1.6 Aims

This study examined phytoremediation potential of IAAP to mitigate pollution from anthropogenic activities along Swartkops River in the Eastern Cape Province of South Africa. It was hypothesised that both temporary and permanent IAAP species mats would assimilate major water and sediment chemistry variables and improve water, sediment quality and recover aquatic biodiversity diversity and assemblage composition downstream of the IAAP species mat.

1.7 Thesis overview

This study comprises six chapters. **Chapter 1** is the general introduction. **Chapter 2** provides the study site description, experimental design, and methods. **Chapter 3** looked at the environmental variability of study sites with IAAP species infestation. **Chapter 4** investigates periphyton species composition and diversity patterns upstream and downstream of the IAAP species mats. **Chapter 5** explores aquatic macroinvertebrate composition and diversity patterns upstream and downstream of the IAAP species mats. **Chapter 6** discusses the research findings and provides recommendations and future research opportunities on phytoremediation techniques and application, and shows how aquatic biota can be important biological indicators to assess environmental change.

CHAPTER 2

Study Site Description and Methods

2.1 Study area

2.1.1 Description and topography

The study was conducted in the Swartkops River system, Uitenhage, Eastern Cape Province of South Africa (Odume *et al*., 2012). The Swartkops River and its tributaries, the KwaZunga and Elands rivers, originate from the Groot Winterhoek Mountains and flow down to an estuary that supports a diversity of fish and water birds (Odume *et al*., 2012) before entering Algoa Bay, Indian Ocean (Figure 2.1). The Swartkops River is a 155–km river system that drains a 42 km-wide catchment area, most of which are the Uitenhage urban areas, neighbouring industries, agricultural lands, formal and informal settlements (DWAF, 1996a). The Swartkops River catchment serves many water-use sectors and has been subjected to water quality deterioration over the past years, caused by land transformation in and around the catchment (Maclear, 1996; Nyawo, 2017) (Figure 2.1). Protected areas dominate the upper reaches of the catchment; the middle (north west of Uitenhage) regions are agricultural, and the lower regions, from Uitenhage town further downstream, comprise informal and formal residential settlements and industries. All these activities contribute to the release of domestic effluents, industrial waste, untreated sewage and other discharges, directly and indirectly into the river system (DWAF, 2003). Other surrounding areas lying within the catchment include Kwanobuhle, Despatch, Perseverance, and Motherwell.

2.1.2 Precipitation and temperature

Rainfall occurs throughout the year and may vary due to orographic and topographic influences, with a minimum monthly average of 60–mm to the highest yearly rainfall of about 760–mm (Maclear, 1996; Odume *et al*., 2012). The natural vegetation dominating the lower catchment is Bushveld and Succulent thicket, which has been severely impacted by the introduction of alien invasive species such as *Eucalyptus* sp. (Gum trees) and *Acacia* sp. (Black Wattle and Port Jackson willow) (Odume *et al*., 2012).

2.1.3 External disturbances in the Swartkops River

Since the Swartkops River drains the urban and industrial land uses, it suffers from human-disturbances (Figure 2.1), which include agricultural run-off, sewage spills and outfalls, stormwater drainage, water abstraction and abattoir waste, which serve as point and diffuse pollution sources to the Swartkops River system (Odume *et al*., 2016; Odume, 2019). Severe pollution has driven invasion by aquatic weeds, including *P. crassipes* and *S. molesta*, in areas such as Uitenhage and Despatch, affecting the functionality and productivity of the ecosystem (Figure 2.2) (Odume *et al*., 2012). It has been reported that pollution in the Swartkops River starts immediately after the Groendal Dam, which is in the KwaZunga River, before interacting with other external disturbances indicated above. The KwaZunga and Elands rivers are the two main tributaries of the Swartkops River system and are highly polluted by anthropogenic activities in the upper areas of Uitenhage (Nyawo, 2017). In addition, over a million people are estimated to live and work along the Swartkops River catchment, and this exposure makes the river more vulnerable to various pollutants degrading the water quality (Binning and Baird, 2001).

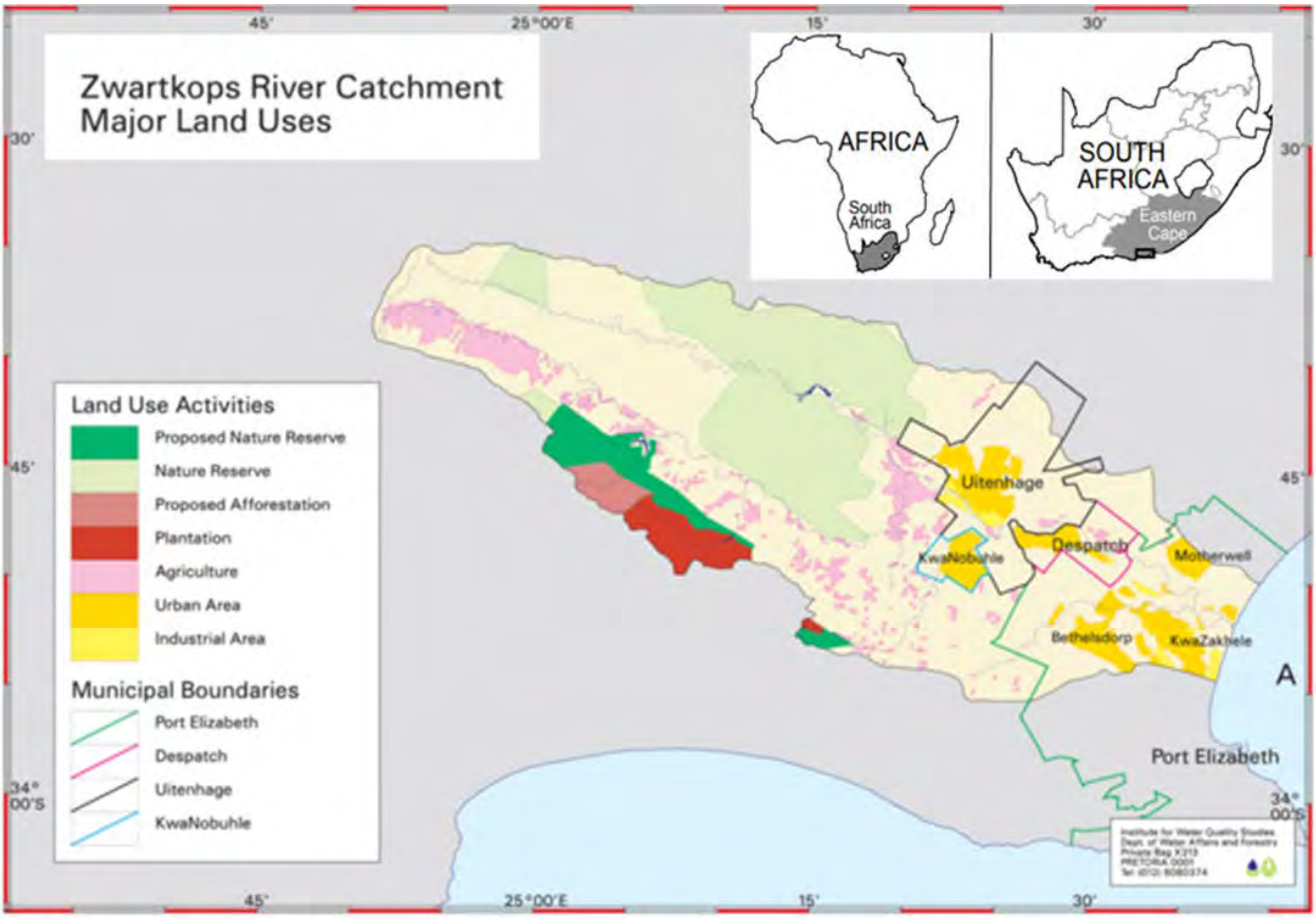

Figure 2.1: Major land-use activities within the Swartkops River catchment, Uitenhage Eastern Cape, South Africa (adopted and modified from Maclear, 1996).

2.2 Study site

2.2.1 Experimental design and sampling area

The study was conducted over a six-month period with monthly sampling intervals, from April to September 2018. Three permanent, dense IAAP species mats were identified along the river course. Sampling sites were identified upstream and downstream of each IAAP mat: Site 1; site 2 (upstream of mat 1), site 3 (mat 1), site 4 (downstream of mat 1); site 5 (upstream of mat 2), site 6 (mat 2), site 7 (downstream of mat 2); site 8 (upstream of mat 3), site 9 (mat 3), site 10 (downstream of mat 3) (Figure 2.2). Water and sediment samples were collected at all ten sites, including IAAP mats; whereas periphyton and aquatic macroinvertebrates were collected from all other sites (seven sites), except the IAAP mats because it is known from various studies that the presence of IAAP mats reduces biological diversity. Also the study aims were to compare biological diversity upstream and downstream sites of IAAP mats. In overall, study sites were chosen based on the presence of IAAP mats and accessibility (Figure 2.2).

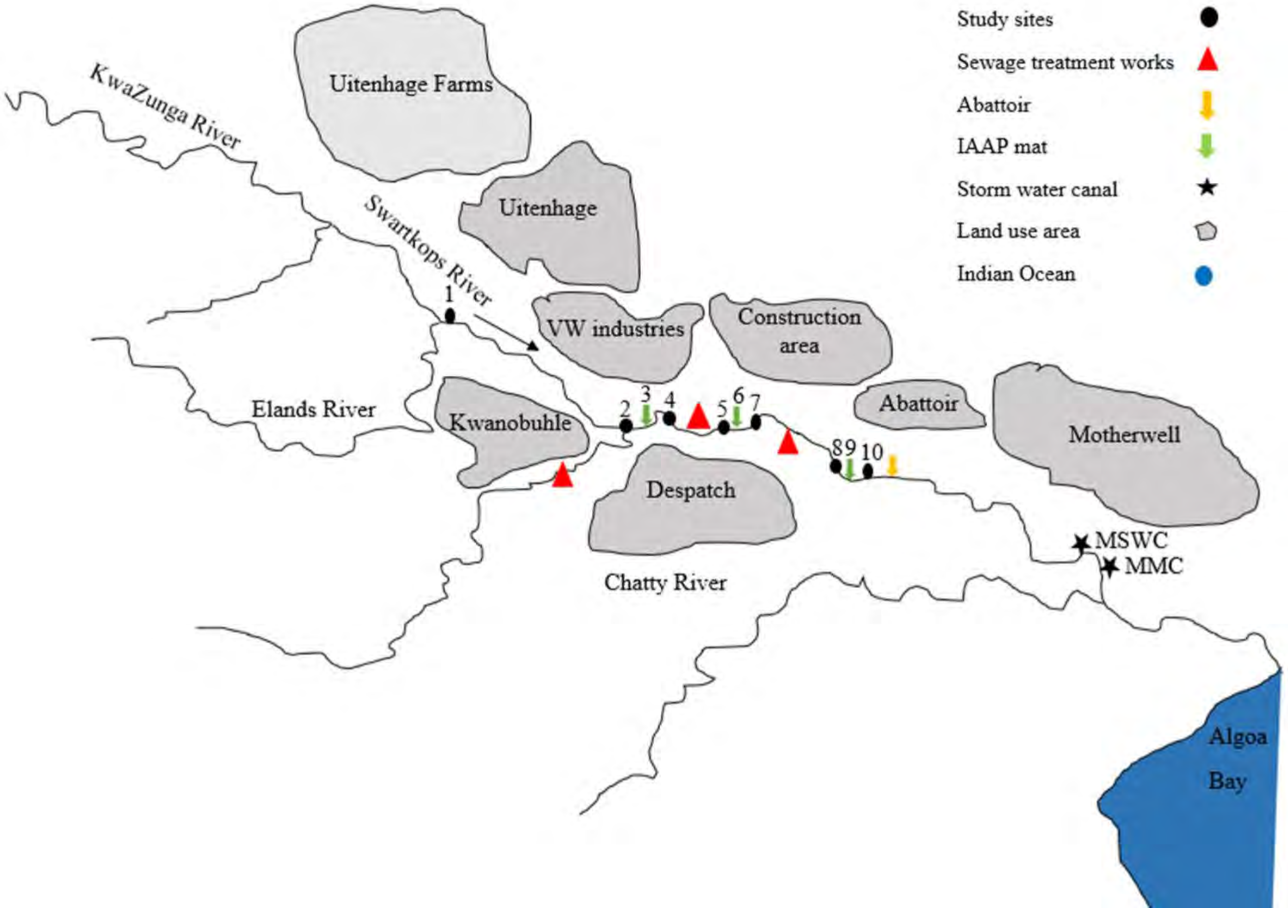

Figure 2.2: The ten study sites including invasive alien aquatic plant mat sites (green arrow) in the Swartkops River system, Eastern Cape Province, South Africa. Motherwell Storm Canal (MWSC) and Markman Canal (MMC).

2.2.2 Description of sampling treatments

Site 1 was situated among agricultural lands upstream of Uitenhage town and downstream of the protected area. This site was chosen as it represents the least modified site. It is situated upstream of industrial and urban entry points within the Swartkops catchment and it is not impacted by invasive aquatic weeds. The site is situated approximately 8.70 kilometres from site 2 (upstream mat 1). Site 1 had a shallow, narrow channel composed of cobbles and a few pebble stones, with a medium flow and high water clarity. Dominant riparian vegetation included grasses and a few shrubs. The stream vegetation, consisted of *Cyperus* sp., *Phragmites* sp. and *Typha* sp. The site had an open canopy and modifications included pipes for extracting water.

Site 2 (upstream of mat 1) and site 4 (downstream of mat 1) are situated further downriver, after the confluence of the Swartkops and Kwanobuhle rivers, below the Volkswagen factory (Figure 2.2). Site 2 (upstream mat 1) is located about 84.14 metres upstream site 3 (mat 1) (*P. crassipes*), while site 4 (downstream of mat 1) is located 60.89 metres downstream site 3 (mat 1). *Typha* sp. and *Phragmites* sp. were the dominant riparian vegetation, and *Nasturtium officinale* was the permanent stream vegetation. These sites were composed mainly of pebbles and boulders with medium turbidity, and had a fully open canopy.

Site 5 (upstream of mat 2) and site 7 (downstream of mat 2) are situated in Despatch, downstream of sewage treatment works and adjacent to a quarry (Figure 2.2). Both site 5 (upstream mat 2) and site 7 (downstream of mat 2) had a wider river channel, shallow water depth, open canopy and were composed of cobbles and a few boulders. Dominant riparian vegetation included *Phragmites* sp., *Cyperus* sp., *Typha* sp. and *Eucalyptus* sp.; stream vegetation comprised *P. crassipes* and *N. officinale*. Site 5 (upstream of mat 2) is situated approximately 2.42 kilometres upstream site 6 (mat 2) (*P. crassipes*). The site was turbid and was the most impacted of the sites. In addition, this site occasionally had higher water velocity caused by sewage effluent being released directly into the stream. Site 7 (downstream of mat 2) is situated about 487.1 metres downstream site 6 (mat 2). Both sites experienced high velocity due to sewage effluent discharge throughout the study.

Site 8 (upstream of mat 3) and site 10 (downstream of mat 3) are located further downstream of the Swartkops River system near Perseverance, which was downstream of sewage treatment works. Site 8 (upstream of mat 3) is located approximately 166 metres upstream site 9 (mat 3) (*S. molesta*), and site 10 (downstream of mat 3) is located 65.18 metres downstream site 9 (mat 3). Downriver, there was an abattoir, and a cultural site used by local traditional healers. Permanent riparian vegetation included *Eucalyptus* sp. and *Typha* sp., while stream vegetation was *N. officinale*. Dominant on-site substrates were cobbles and pebbles; evident obstructions and modifications were grazing livestock by the riverbanks, and water abstraction for dairy farms in the vicinity.

2.3 Sample collection

2.3.1 Environmental variables

To assess the phytoremediation success, water and sediment variables were collected, together with biological data, and evaluated and compared across the sites to determine if the IAAP mats had an effect on the river's ecological status.

2.3.1.1 Physicochemical parameters

Physicochemical parameters were measured every month, at all selected sites, including the three IAAP mats, over a six-month period (April to September 2018). During each sampling occasion, the following variables were measured: pH, EC, TDS, salinity, water temperature and DO *in situ* using a waterproof, handheld multi-parameter probe Eutech (PCS Tester 35 model) and a DO Pen Sper-Scientific (85004) meter. Water depth and water flow were measured using a portable flow meter MARSH McBIRNEY (2000 model). Additionally, water samples (500 ml, n=3) were collected and taken to the laboratory to determine [NO_3] and [NH_4] using Ion Specific Electrode (Range: 1.0 to 100 mg/l) (Vernier LabQuest®2) and [PO_4] concentrations using a HI 83203 Multi-parameter Bench Photometer for Aquaculture (Range: 0.0 to 30.0 mg/l) metres.

2.3.1.2 Water chemistry (nutrient and heavy metal analyses)

During each sampling occasion, water samples were collected for water chemistry and heavy metal analyses. Clean and clear 1 L (n=1) polyethylene plastic bottles were used to collect water samples; each bottle was rinsed with river water prior to water sample collection, and water samples were collected 10–15 cm below the water surface. Samples were stored on ice and transported back to the laboratory. Within 24 hours, the collected water samples (1 L) were packaged and sent to BEM-Labs, Cape Town, South Africa for water chemistry analysis, including NH_4, PO_4, and NO_3, COD and zinc (Zn), iron (Fe), cadmium (Cd), arsenic (As), chromium (Cr), lead (Pb), mercury (Hg), and copper (Cu).

2.3.1.3 Sediment chemistry

Using a gardening trowel, an integrated soil sediment sample (1 kg, n=1) was collected at five areas per site at approximately 0–10 cm in depth to compare how soil sediment chemistry differed in relation to water quality between sites. Samples were collected into plastic zip-lock bags, stored in a cooler with ice until they reached the laboratory. As with the water samples, soil sediments were sent to BEM-Labs, Cape Town, South Africa, for soil sediment analysis which included NH_4, P, NO_3, Zn, Fe, Cd, As, Cr, Zn, Pb, Hg, and Cu.

2.3.2 Biological variables

2.3.2.1 Periphyton sampling

Periphyton samples were collected at all sites except the IAAP mats, following methods described by Taylor *et al*. (2005). Using a white collecting tray, integrated periphyton samples (epiphyton and epilithon) were collected from different substrates: five submerged rocks of different sizes (e.g. cobbles and boulders), emergent, submerged and free-floating macrophyte species. Emergent macrophyte stems were sampled by cutting off the stems above the water line and above the point where they emerged from the sediments, using a field knife. A handful of submerged macrophyte material (leaves and stems) was collected, and only five mature free-floating macrophytes were collected and placed in a collecting tray. On-site water (2500 ml) was added in to the tray, and using a new toothbrush, periphyton biofilm was completely scrubbed off all substrates. The resulting 2500 ml periphyton sample was filtered on site through a 64 µm mesh net to eliminate unwanted debris and small invertebrates, and the sample was transferred into three clear polyethylene containers (n=3) of 350 ml volume for periphyton community analysis, and three opaque polyethylene containers (n=3) of 450 ml volume for periphyton chlorophyll-*a* biomass analysis. Periphyton community analysis sub-samples were further preserved with 5 ml of Lugol's iodine solution (prepared by dissolving 100 g potassium iodide and 50 g of iodine crystals in 2 litres distilled water). All samples were stored on ice and transported back to the laboratory for further treatment and preparation.

2.3.2.2 Chlorophyll-a biomass analysis

Prior to periphyton chlorophyll-*a* analysis, samples were homogenised by agitating the containers by hand for 5 seconds. Then, a standard volume of 60–70 ml of periphyton sample was filtered through nylon net filter paper (diameter of 45 cm and 20 µm mesh size) using a rocker 300 vacuum pump at 20 kPa. Thereafter the nylon filter paper was folded in half and placed into a 20 ml reaction tube with a screw, and a total volume of 10 ml 90% acetone solution was added to extract chlorophyll-*a* pigment under -20 °C for a minimum of 48 hours in the dark. This was to allow acetone to break down the chlorophyll lipid bonds and suspend the liquid in the solution for extraction (Ritchie, 2006). Chlorophyll-*a* biomass was then determined fluorometrically using a 10 AU laboratory and field fluorometer (Tuner designs). Under limited light, 8 ml of extracted samples was exposed to the fluorometer wavelength, and readings were noted before and after samples were acidified by adding 2/3 drops of 0.1 ml of hydrochloric acid. The final chlorophyll-*a* biomass was calculated using the following formula modified from Lorenzen (1967) and Daemen (1986):

$$\text{Chl-}a\ (\text{mg/m}^3) = \left(\frac{Acetone\ volume}{Filtered\ sample\ volume}\right) \times (Reading\ before\ acidificantion - Reading\ after\ acidification) \times 0.325$$

2.3.2.3 Periphyton community analysis

In the laboratory, periphyton community analysis sub-samples (350 ml, n=3) were allowed to sediment on a flat bench (stable) for 72 hours (LeGresley and McDermott, 2010). Thereafter, about 300 ml of the supernatant was drained using a top-down siphoning system, care being taken not to disturb the concentrated sediments. The remaining concentrated periphyton sample of 50 ml was moderately agitated by hand for 5 seconds to evenly dispense the algae cells. Using a Pasture pipette, 0.1 ml of the periphyton sample was drawn out and placed onto the haemocytometer counting chamber (Neubauer improved; 9 mm² total grid area and a standard depth of 0.1 mm) (LeGresley and McDermott, 2010) and covered with a glass coverslip. Identification and counting were conducted to the lowest possible taxonomic level (normally genera and/or species) using a light phase microscope (Olympus CX21) at 400X magnification using various identification guides (John *et al*., 2002; van Vuuren *et al*., 2006; Taylor *et al*., 2007a; Griffiths *et al*., 2015). Each sub-sample (each site had three replicates) was analysed by counting a haemocytometer grid with an area of 9 mm^2, thus making 3*9 mm^2 = 27 mm^2 periphyton total area per site, per sampling occasion. The relative abundance of periphyton cells was calculated using an equation by LeGresley and McDermott (2010).

$$\text{Cells/m}\ell = \frac{Cells\ counted \times Concentrated\ sample\ volume}{Area\ counted \times 0.1\ (chamber\ depth)} \times Total\ sample\ volume$$

2.3.2.4 Aquatic macroinvertebrate sampling

Aquatic macroinvertebrate sampling was conducted following the procedure outlined by Gabriels *et al*. (2010). Briefly, organisms were sampled using a standard aquatic sampling net with a square frame (30 cm x 30 cm) and 1000 µm mesh size. Distinctive biotopes, for example, stones, vegetation and gravel, sand and mud were sampled for three minutes collectively using the kick and sweep method. Aquatic macroinvertebrate samples were transferred into zip-lock bags, placed on ice, and transported to the lab for sorting and identification to family level for aquatic insects, and sub-class for non-insects, following Day *et al*. (2002), Day and de Moor (2002a;b), Gerber and Gabriel (2002), and de Moor *et al*. (2003a; b).

2.4 Data analysis

Study sites were categorised into treatments for multivariate analysis and to ease interpretation where; S1 treatment (site 1), upstream treatment (all upstream of IAAP mat sites 2, 5 and 8), the IAAP mat treatment (mat 1 (site 3), mat 2 (site 6) and mat 3 (site 9)) and the downstream treatment (all downstream of IAAP mat sites 4, 7 and 10).

CHAPTER 3

Environmental variability of the study sites selected along a section of Swartkops River with IAAP mat patches

3.1 Introduction

Environmental contamination is one of the major threats responsible for degradation of the surface environment (Sakakibara *et al*., 2011). Heavy metal contamination plays a significant role in this destruction as one of the most crucial environmental problem worldwide (Malar *et al*., 2014). This is because heavy metals are generated through both natural (geogenic) and anthropogenic activities including mining (extraction of ores and subsequent processing) and industrialisation (Li *et al*., 2009). The mobilization of heavy metals through such activities have resulted in the release of these elements into the environment contributing to a variety of toxic effects in both the environment and living organisms.

Heavy metal contaminants from anthropogenic activities have been reported to change the agricultural soil's fertility by altering biogeochemical cycles and increasing concentrations in the environment which are toxic for living organisms (Priya and Selvan, 2017; Ramachandra *et al*., 2018). Heavy metal pollutants results in deterioration of cell organs, contamination of food chains, disruption of metabolic activities, stunned growth, and cessation of photosynthetic processes in micro and macro-organisms (Moosavi and Seghatoleslami, 2013). In aquatic ecosystems, heavy metal elements adversely affect water resources by changing the chemical composition of water, creating unfavourable conditions for sensitive aquatic organisms, leading to a decline and shift in biological and functional diversity (Alberti *et al*., 2007; Priya and Selvan, 2017; Harding, 2015).

Amongst these heavy metals, lead (Pb) is one of the most hazardous pollutants and of great concern due to its impact on both human health and the environment (water, air and agricultural soil) (Malar *et al*., 2014). Fertilizers, industrial waste, mining and smelting of Pb ore are the main sources of Pb pollution in the environment (Sharma and Dubey, 2005). Lead has been reported to disturb metabolic activities in plants by affecting different cell components which disrupts growth, seed germination (Sharma and Dubey, 2005) and photosynthesis processes (Moosavi and Seghatoleslami, 2013). And unlike organic pollutants, that can be easily reduced to harmless molecules, heavy metals such as cadmium, lead, zinc, mercury, and copper are unchangeable by biochemical reactions (Malar *et al*., 2014).

In response to such, there is a growing need to address environmental contamination and develop remediation technologies that can be used to treat contaminated mediums. Various technologies including artificial membranes, precipitation, and ion-exchange have been used for reducing excessive nutrient loading and non-biodegradable elements from different mediums (Qdaisa and Moussa, 2004). These methods have both environmental and economic drawbacks (Hanif *et al*., 2015); they are expensive, may generate end waste material that requires special deposition and are not always adequate in removal of heavy metals even in low concentrations (Chandra and Yadav, 2010), and may alter sediment microbes making the soil less beneficial in organic carbon breakdown and recycling of micronutrients (Gaur and Adholeya, 2004).

Phytoremediation technique has been advocated for its environmental friendliness and cost effectiveness (Sakakibara *et al*., 2011). Besides, this technique can be applied to both small and large areas with a wide variety of contaminants (organic substances, heavy metals and radionuclides) (Skinner *et al*., 2007), and growth media (sludge, water and sediment) using hyperaccumulator plants species (Sakakibara *et al*., 2011). Various authors have conducted studies to investigate the fate of heavy metals and some organic pollutants in field and lab experiments using the phytoremediation technique (Chandra and Yadav, 2010; Lu *et al*., 2010; Hammad, 2011; Sakakibara *et al*., 2011; Moyo *et al*., 2013; Loan *et al*., 2014; Malar *et al*., 2014). Aquatic macrophytes including *Lemna minor, Azolla* sp., *S. molesta*, *P. crassipes* and *Pistia stratiotes* have shown good bioindicator as well as bioremediation success. These species are natural hyperaccumulators of different elements and can be effective in phytoextraction of pollutants in contaminated mediums (Wang *et al*., 2017). These plants are capable of accumulating pollutants including Cd, Zn, Pb, Cu, Cr, Hg and Mn from aqueous solutions, soil and sludge through various mechanism (phytoextraction, phytodegradation, phytostabilisation, phytofiltration etc.) thus acting as biofilters (Ali *et al*., 2013). In this context, *P.crassipes*, commonly known as Water hyacinth and *S. molesta* are also macrophytes with excellent phytoremediation abilities (Ali *et al.*, 2013). Their fibrous root system, enormous biomass production rate, high tolerance to pollution and perennial life cycle qualifies for their use in treatment of wastewater, allowing them to absorb large amount of pollutants including heavy metals such as Zn, As, Pb, Cr and Mn (Ali *et al*., 2013). These plant species have been successfully used by treatment of various water pollutants. Mishra e*t al*. (2008) reported that *P. crassipes* grows well in coal mining effluents and is highly effective in removal of heavy metals contaminants.

Pontederia crassipes and *S. molesta* were found growing in patches across various sections of the study area and to demonstrate their phytoremediation abilities, a study was conducted to assess the pollutants (heavy metals and nutrients) uptake. Removal of contaminants is of great importance in protection of the environment as well as lessening of heavy metal toxicity (Kim *et al*., 2004). Unlike organic waste that is easily biodegradable, heavy metal contaminants can accumulate up to harmful levels. Therefore, this study chapter aims to observe the removal efficiency of *S. molesta*, and *P. crassipes* and evaluate environmental variability between selected study sites along a polluted river system in the Eastern Cape Province.

3.2 Materials and methods

3.2.1 Study area and data collection

Details on study area and sample collection are described in Chapter 2.

3.2.2 Data analysis

To test significant differences in environmental variables (e.g. physicochemical parameters, water, and sediment chemistry) between sites and treatments and to investigate the phytoremediation effect of IAAP mats on water and sediment quality improvement, the Shapiro-Wilk and Levene tests were employed to test the data-sets for normality and homogeneity of variances. The outcome of the tests revealed that no environmental variables were normally distributed (Shapiro-Wilk, $P<0.05$), nor were the variances homogenous (Levene test, $P>0.05$). Thus, a non-parametric test, in this case Kruskal-Wallis analysis of variance (ANOVA) test, with multiple comparison test was employed to test for significant difference in environmental variables with sites and treatment as factors. All statistical analyses were conducted in R version 3.6.1 (R Core Team, 2019), except where specified.

Additionally, to examine the relationship between measured environmental variables between sites and treatments, all environmental variables (physicochemical, and water-sediment chemistry) were log transformed log(x+1) to assume normality, standardised, and resembled to Euclidean distance matrix. Thereafter, the unconstrained principal component analysis (PCA) method was fitted to visualise environmental variable patterns based on the Euclidean distance similarities between treatments. A permutational multivariate analysis of variance (PERMANOVA) test was performed to test significant differences in environmental variable patterns between treatments. Principal component analysis and PERMANOVA were conducted in PRIMER version 6.1.16 and PERMANOVA$^+$ version 1.0.6 (PRIMER-E Ltd, Plymouth; Clark and Gorley, 2006). Percentage difference in the level of water and sediment chemistry variables between sites (upstream and downstream of each mat) was calculated by multiplying the difference by 100 for each variable.

3.3 Results

3.3.1 Field measured physicochemical variables

pH was significantly different between sites (H=28.46, P<0.001) (1–2; 1–3; 1–4; 1–9; and 1–10) and treatments (H=16.78, P<0.001) (S1–upstream; S1–mats and S1–dowsntream). The highest pH value (8.57) was recorded downstream of mat 1 (site 4) and the lowest pH value (7.65) was recorded at site 1 (Table 3.1).

The EC showed significant differences between sites (H=39.55, P>0.001) (1–2; 1–3; 1–4; 1–7; 1–8; 1–9; 1–10) and treatments (H=32.47, P>0.001) (S1–upstream; S1–mats and S1–dowsntream). The EC concentrations was lowest (0.35 μS/cm) at site 1 and highest (3.44 μS/cm) at site 10 (downstream of mat 3); EC increased slightly downstream sites. Dissolved oxygen was significantly different between sites (H=18.98, P=0.025), but was not significantly different between treatments (H=1.10, P=0.776) (Table 3.1). The DO was lowest (4.43 mg/l) at site 6 (mat 2) and highest (6.35 mg/l) at site 8 (upstream of mat 3). Water temperature showed no significant differences between sites (H=12.92, P=0.16) and treatments (H=2.66, P=0.447). Nitrates showed significant differences between sites (H=23.21, P=0.006) (2–9; 3–9), but not between treatments (H=6.36, P=0.095) (Table 3.1). Nitrate concentration was highest (10.38 mg/l) at site 2 (upstream mat 1) and lowest (0.42 mg/l) at site 9 (mat 3).

Ammonium was not significantly different between sites (H=13.70, P=0.133), but was significantly different between treatments (H=11.11, P=0.011) (S1–upstream; S1–mats and

S1–downstream). The NH_4 concentrations were lowest (0 mg/l) at site 1 and highest (0.33 mg/l) upstream of mat 2, site 5. Overall, all upstream treatments had very high NH_4 concentrations when compared to all IAAP species mats and downstream treatments (Table 3.1).

Phosphate (PO_4) concentrations showed significant differences for sites (H=39.36, P<0.001) (1–5; 1–6; 1–7; 1–10; 2–5) and treatments (H=9.55, P=0.0230) (S1–upstream; S1–mats and S1–dowsntream) (Table 2.1). Downstream of mat 3, site 10 had the highest recorded PO_4 concentration (10.13 mg/l) while upstream of mat 1, site 2 had the lowest recorded PO_4 concentration (3.80 mg/l) (Table 3.1). Overall, downstream treatments had the highest recorded concentrations of PO_4 for the duration of the study, while the upstream treatments had the lowest recorded PO_4 concentrations.

Table 3.1: Physicochemical parameters mean and (± standard deviation) recorded from 10 sites including IAAP mats throughout the study (April–September 2018) in the Swartkops River system, South Africa. Bolded H-values indicate significant differences (Kruskal-Wallis ANOVA, $P<0.05$). IAAP Mat=Invasive Alien Aquatic Plant species mat, U=upstream treatments, D=downstream treatments; the number in brackets represents the site number.

Physicochemical variables		IAAP Mat 1 (*P. crassipes*)			IAAP Mat 2 (*P. crassipes*)			IAAP Mat 3 (*S. molesta*)			Sites	Treatments
	S1 (1)	U(2)	mat 1 (3)	D(4)	U(5)	mat 2 (6)	D(7)	U(8)	mat 3 (9)	D(10)	*H*-value	*H*-value
pH	7.65 (±0.44)	8.40 (±0.50)	8.54 (±0.46)	8.57 (±0.49)	8.24 (±0.50)	8.14 (±0.53)	8.17 (±0.52)	8.21 (±0.46)	8.40 (±0.50)	8.39 (±0.42)	**28.46**	**16.78**
EC (µS)	0.35 (±0.19)	2.32 (±0.53)	2.26 (±0.51)	2.29 (±0.48)	1.90 (±0.26)	2.05 (±0.41)	2.09 (±0.35)	2.21 (±0.48)	2.12 (±0.44)	3.44 (±4.24)	**39.35**	**32.48**
DO (mg/L)	5.37 (±1.65)	6.02 (±1.16)	6.17 (±1.16)	5.67 (±0.60)	4.88 (±1.26)	4.43 (±1.68)	4.47 (±1.84)	6.25 (±2.13)	5.68 (±2.47)	6.11 (±3.27)	**18.98**	1.10
Temp. (°C)	15 (±2.34)	15.08 (±2.00)	15.36 (±1.95)	15.5 (±2.06)	16.3 (±2.67)	16.89 (±3.19)	16.42 (±2.22)	17.13 (±3.14)	17.47 (±2.53)	16.94 (±2.60)	12.92	2.66
NO_3 (mg/L)	0.86 (±1.90)	10.38 (±18.06)	3.31 (±2.85)	2.74 (±2.63)	2.18 (±2.78)	1.2 (±2.27)	1.79 (±2.32)	1.58 (±2.91)	0.42 (±1.44)	1.67 (±2.31)	**23.21**	6.36
NH_4 (mg/L)	0 (±0.03)	0.28 (±0.27)	0.28 (±0.30)	0.3 (±0.3)	0.33 (±0.36)	0.23 (±0.21)	0.23 (±0.21)	0.19 (±0.19)	0.17 (±0.18)	0.18 (±0.19)	13.70	**11.11**
P (mg/L)	7.12 (±13.07)	3.80 (±1.55)	4.20 (±2.02)	4.04 (±2.54)	9.32 (±2.84)	8.95 (±3.44)	8.6 (±3.88)	7.06 (±4.56)	8.91 (±6.11)	10.13 (±7.92)	**36.96**	**9.55**

3.3.2 Laboratory analysed water chemistry (BEM-Labs)

Iron (Fe) concentration showed significant differences between sites (H=28.13, P=0.001) (1–6; 1–8; 1–9; 1–10) and treatments (H=14.10, P=0.003) (S1–mats; S1–downstream). Iron concentrations were highest (1.1 mg/l) at site 1 and lowest (0.09 mg/l) downstream of mat 3, at site 10 (Table 3.2).

There was a significant difference in Zn concentrations between sites (H=18.03, P=0.034) but not between treatments (H=1.79, P=0.616). The highest (0.12 mg/l) Zn concentration was recorded downstream of mat 3 (site 10). The lowest constant Zn concentration of 0.02 mg/l was recorded for various sites including site 1; upstream of mat 1, at site 2; site 3 (mat 1); downstream of mat 1, at site 4; site 6 (mat 2); upstream of mat 3, at site 8, and site 9 (mat 3).

The COD concentrations were significantly different between sites (H=21.89, P=0.001) (1–5; 1–6) and treatments (H=9.17, P=0.027) (S1–mats; S1–upstream). Lowest COD concentration was recorded at site 1 (14.64 mg/l) and the highest (57.4 mg/l) upstream of mat 2, site 5 (Table 3.2).

Arsenic and Cu concentrations were not significantly different between sites and treatments. Heavy metals Cd, Cr, Hg, and Pb, had constant concentrations of 2.1 µg/l; 26 µg/l; 2.1 µg/l and 6 µg/l, respectively throughout the sampling periods (Table 3.2). Thus, concentrations were not significant between sites and treatments.

Table 3.2: The mean and ± standard deviation of water chemistry parameters recorded from 10 sites, including IAAP mats throughout the study between April and September 2018 in the Swartkops River system, South Africa. Bolded H-values indicate significant differences (Kruskal-Wallis ANOVA, $P<0.05$). IAAP Mat=Invasive alien aquatic plant species mat, U=upstream treatment, D=downstream treatments; the number in brackets represents the site number. NS=not significant, $P>0.05$.

Heavy metals		IAAP Mat 1 (*P. crassipes*)			IAAP Mat 2 (*P. crassipes*)			IAAP Mat 3 (*S. molesta*)			Sites	Treatment
	S1 (1)	U (2)	mat 1 (3)	D(4)	U (5)	mat 2 (6)	D (7)	U (8)	mat 3 (9)	D (10)	*H*-value	*H*-value
Fe (mg/l)	1.1 (±0.45)	0.19 (±0.07)	0.2 (±0.05)	0.18 (±0.05)	0.24 (±0.14)	0.13 (±0.10)	0.14 (±0.06)	0.10 (±0.03)	0.13 (±0.10)	0.09 (±0.02)	**28.138**	**14.107**
Cu (mg/l)	0.02 (±0.02)	0.02 (±0.01)	0.02 (±0.01)	0.04 (±0.04)	0.4 (±0.04)	0.02 (±0.02)	0.02 (±0.01)	0.02 (±0.01)	0.02 (±0.02)	0.02 (±0.01)	1.965	0.063
Zn (mg/l)	0.02 (±0)	0.02 (±0)	0.02 (±0)	0.02 (±0)	0.04 (±0.02)	0.02 (±0)	0.03 (±0)	0.02 (±0)	0.02 (±0)	0.12 (±0.23)	**18.083**	1.794
C0D (mg/l)	14.64 (±13.39)	28.6 (±10.92)	37.2 (±7.5)	28 (±12.67)	57.4 (±22.51)	55.8 (±18.51)	46 (±25.09)	32 (±10)	36 (±17.74)	30.2 (±10.99)	**21.892**	**9.175**
As (µg/L)	4 (±0)	5.72 (±2.44)	49 (±0)	5 (±2.24)	9 (±9.40)	4 (±0)	4 (±0)	4 (±0)	6.49 (±2.73)	4 (±0)	16.532	3.276
Cd (µg/L)	2.1 (±0)	2.1 (±0)	2.1 (±0)	2.1 (±0)	2.1 (±0)	2.1 (±0)	2.1 (±0)	2.1 (±0)	2.1 (±0)	2.1 (±0)	NS	NS
Cr (µg/L)	26(±0)	26(±0)	26(±0)	26(±0)	26 (±0)	26 (±0)	26 (±0)	26 (±0)	26(±0)	26 (±0)	NS	NS
Hg (µg/L)	2.1(±0)	2.1(±0)	2.1(±0)	2.1(±0)	2.1 (±0)	2.1(±0)	2.1 (±0)	2.1 (±0)	2.1(±0)	2.1 (±0)	NS	NS
Pb (µg/L)	6(±0)	6(±0)	6(±0)	6(±0)	6 (±0)	6 (±0)	6 (±0)	6 (±0)	6(±0)	6 (±0)	NS	NS

3.3.3 Sediment chemistry

Sediment chemistry results revealed that Fe was significantly different between sites (H=24.32, P=0.004) (1–5; 3–5) and treatments (H=8.35, P=0.039) (S1–dowsntream). Fe concentration was relatively high at all sites, with the lowest concentration of 220.43 mg/kg upstream of mat 2, at site 5, to the highest concentration of 1321.25 mg/kg recorded at site 1 (Table 3.3).

Zinc was significantly different between sites (H=35.75, P<0.001) (1–2; 1–3; 1–5; 2–7; 2–10; 5–7; 5–10) and treatments (H=22.2, P<0.001) (S1–mats; S1–upstream; downstream and upstream). The highest (87.16 mg/kg) Zn concentration was recorded upstream of mat 2, at site 5 and the lowest (7.19 mg/kg) concentration downstream of mat 3, at site 10.

Arsenic (As) showed significant differences between sites (H=17.08, P=0.05) and treatments (H=9.05, P=0.029) (S1–downstream). In general, As concentrations were very low, ranging from 0.27 mg/kg downstream of mat 3 (site 10) and highest (4 mg/kg) site 1.

Chromium (Cr) concentrations were significant between sites (H=20.39, P=0.016) (5–7; 5–9; 5–10) but not significant between treatments (H=7.18, P=0.022) (Table 2.3). Chromium (Cr) concentrations were 8.15 mg/kg downstream of mat 3, at site 10 and 41.12 mg/kg upstream of mat 2, at site 5. The lowest Cr concentration was recorded downstream of mat 3, at site 10 with 8.15 mg/kg, and the highest (41.12 mg/kg) was recorded upstream of mat 2, at site 5.

Lead (Pb) showed significant differences between sites (H=26.19, P=0.002) (5–10; 9–3) and treatments (H=9.61, P=0.022) (downstream–upstream), with the lowest Pb concentration (3.67 mg/kg) downstream of mat 3, at site 10 and the highest concentration site 3 (mat 1) (21.10 mg/kg) (Table 3.3).

Copper (Cu) was significantly different between sites (H=26.46, P=0.002) (2–10; 2–7; 5–7) and treatments (H=19.39, P<0.001) (mats–downstream; upstream–downstream). Site 7 recorded the lowest Cu concentrations of 0.67mg/kg, and upstream of mat 2 (site 5) recorded the highest Cu concentration of 5.56 mg/kg (Table 3.3).

Phosphorus (P) concentrations were significantly different between sites (H=21.63, P=0.010) and treatments (H=19.33; P<0.001) (S1–upstream; downstream–upstream). Overall, P concentrations were quite high, with the highest P concentration recorded upstream of mat 2, at site 5 (2240.38 mg/kg) and the lowest downstream of mat 2, at site 7 (281.07 mg/kg) (Table 3.3). There were no significant differences between sites and treatments for heavy metals (e.g. Cd, Hg) and water nutrient NH_4 (Table 3.3).

Table 3.3: Sediment chemistry mean and ± standard deviation recorded from 10 sites, including IAAP mats through out the study (April 2018–September 2018) in the Swartkops River system, South Africa. Bolded H-values indicate significant differences (Kruskal-Wallis ANOVA, $P<0.05$). IAAP Mat=Invasive alien aquatic plant species mat, U=upstream treatment, D=downstream treatments; the number in brackets represents the site number.

Heavy metals (mg/kg)	IAAP Mat 1 (*P. crassipes*)				IAAP Mat 2 (*P. crassipes*)			IAAP Mat 3 (*S. molesta*)			Sites	Treatments
	S1 (1)	U (2)	mat 1 (3)	D (4)	U (5)	mat 2 (6)	D (7)	U (8)	mat 3 (9)	D (10)	*H*-value	*H*-value
Fe	1321,25 (±838.89)	967.37 (±641.5)	950.78 (±283.05)	520.02 (±261.10)	220.43 (±136.60)	554.19 (±230.32)	268.95 (±206.10)	769.73 (±421.7)	511.56 (±210.11)	628.36 (±514.10)	**24.32**	**8.35**
Zn	7.59 (±4.27)	62.55 (±31.04)	41.92 (±4.05)	26.92 (±15.30)	87.16 (±45.38)	19.89 (±12.44)	9.65 (±5.63)	22.4 (±15.97)	11.17 (±2.24)	7.19 (±1.92)	**35.79**	**22.2**
Cd	0.21 (±0.21)	0.12 (±0.15)	0.14 (±0.16)	0.17 (±0.32)	0.10 (±0.11)	0.02 (±0.05)	0.02 (±0.03)	0.02 (±0.04)	0.06 (±0.10)	0.06 (±0.09)	8.260	1.751
As	4 (±2.67)	0.61 (±0.68)	0.32 (±0.42)	1.20 (±1.44)	1.49 (±0.83)	1.46 (±0.10)	0.28 (±0.42)	1.55 (±1.90)	0.42 (±0.42)	0.27 (±0.43)	**17.08**	**9.04**
Cr	11.54 (±3.37)	16.23 (±6.98)	18.03 (±7.53)	16.02 (±10.28)	41.12 (±25.25)	16.14 (±7.34)	9.50 (±3.59)	11.73 (±5.21)	9.08 (±4.32)	8.15 (±2.86)	**20.39**	7.18
Pb	14.07 (±6.73)	16.36 (±7.50)	21.10 (±7.39)	10.23 (±7.24)	19.90 (±8.54)	6.43 (±4.51)	6.45 (±5.57)	8.42 (±6.79)	4.32 (±5)	3.67 (±4.41)	**26.19**	**9.61**
Hg	2.07 (±3.25)	1.77 (±2.50)	1.26 (±1.48)	0.72 (±0.88)	1.36 (±1.52)	0.87 (±1.45)	0.64 (±0.60)	0.98 (±1.19)	0.99 (±1.41)	0.83 (±1.11)	1.756	1.06
Cu	1.58 (±0.75)	4.29 (±0.67)	2.73 (±1.01)	1.83 (±1.49)	5.56 (±3.96)	3.43 (±2.47)	0.67 (±0.24)	2.34 (±0.86)	1.32 (±0.45)	0.92 (±0.14)	**26.46**	**19.39**
P	296.24 (±128.88)	978.45 (±410.0)	489.94 (±275.31)	391.60 (±543.64)	2240.83 (±1899.70)	454.77 (±333.68)	281.07 (±215.38)	1077.85 (±274.5)	863.70 (±542.90)	468.8 (±344.05)	**21.63**	**19.33**
NH_4	46.86 (±59.80)	93.15 (±155.6)	25.21 (±8.85)	29.26 (±30.22)	58.35 (±73.64)	22.15 (±9.92)	17.89 (±11.37)	111.08 (±110.7)	138.29 (±146.72)	467.2 (±898.77)	16.23	1.06
NO_3	1.87 (±1.80)	2.55 (±0.97)	3.98 (±2.17)	1.41 (±1.10)	9.67 (±5.02)	1.42 (±0.82)	6 (±7.32)	6.23 (±5.03)	9.92 (±15.69)	21.14 (±27.16)	**17.92**	7.044

3.3.4 Invasive alien aquatic plant species phytoremediation in the Swartkops River

Results of the study revealed evidence of phytoremediation by both *P. crassipes* and *S. molesta* were concentrations of various environmental variables (laboratory water nutrients and Bemlab sediments) decreased between upstream to downstream sites, thus indicating possible IAAP assimilation potential. Laboratory water nutrients showed reduction in concentrations at various sites; NO_3 concentration at IAAP mat 1 showed a 7.64 mg/l (74%) reduction between the upstream and downstream sites, and the IAAP mat 2 showed a 0.39 mg/l (18%) reduction between upstream and downstream sites. There was a total reduction of 0.1 mg/l (30%) for NH_4 concentration between the upstream and downstream sites at mat 2, and 0.01 mg/l (5%) between upstream and downstream at mat 3, and PO_4 concentrations showed a total reduction of 0.72 mg/l (8%) upstream and downstream of mat 2 (Table 3.4)

Sediment chemistry in general showed a decrease in concentrations between upstream and downstream sites, indicative of assimilation potential. Sediment Fe concentrations showed a total reduction of 447.35 mg/kg (46%) downstream of IAAP mat 1, and 141.37 mg/kg (18%) downstream of IAAP mat 3 (Table 3.5). Similarly, Zn concentration showed a total reduction of 35.63 m/kg (57%) downstream of IAAP mat 1; 77.51 mg/kg (89%) downstream of IAAP mat 2 and 15.21 mg/kg (65%) downstream of IAAP mat 3. Arsenic (As) showed a total reduction of 1.21 mg/kg (81%) and 1.28 mg/kg (83%) downstream of IAAP mat 2 and IAAP mat 3, respectively; Chromium (Cr) 0.21 mg/kg (1%) downstream of IAAP mat 1, 31.62 mg/kg (77%) downstream of IAAP mat 2 and 3.58 mg/kg (31%) downstream of IAAP mat 3. Lead (Pb) showed a total reduction of 6.13 mg/kg (37%) downstream IAAP mat 1, 13.45 mg/kg (68%) downstream IAAP mat 2 and 4.75 mg/kg (56%) downstream IAAP mat 3. Mercury (Hg) concentrations reduced by 1.50 mg/kg (59%) downstream IAAP mat 1; 0.72 mg/kg (53%) downstream IAAP mat 2 and 0.15 mg/kg (15%) downstream IAAP mat 3. Copper (Cu) concentrations were reduced by 2.46 mg/kg (57%) downstream IAAP mat 1, 4.89 mg/kg (88%) downstream IAAP mat 2 and 1.42 mg/kg (60%) downstream IAAP mat 3 (Table 2.5). Ammonium (NH_4) concentrations were reduced by 63.89 mg/kg (69%) downstream IAAP mat 1, and were also reduced by 40.46 mg/kg (69%) downstream IAAP mat 2. Phosphorus (P) concentrations were reduced by 586.85 mg/kg (60%), 1959.76 mg/kg (87%) and 609.05 mg/kg (57%) downstream IAAP mat 1, IAAP mat 2 and IAAP mat 3, respectively. Nitrate (NO_3)

concentrations were reduced by 1.14 mg/kg (45%) downstream IAAP mat 1 and 3.67 mg/kg (38%) downstream IAAP mat 2 (Table 3.5).

Table 3.4: Percentage reduction of important water nutrient concentrations; Nitrate (NO_3), Ammonium (NH_4) and Phosphate (PO_4) by *Pontederia crassipes* and *Salvinia molesta* at the Swartkops River system, Eastern Cape, South Africa.

Laboratory water chemistry (mg/l)	Percentage reduction between upstream and downstream sites		
	IAAP mat 1 (*P.crassipes*)	IAAP mat 2 (*P.crassipes*)	IAAP mat 3 (*S.molesta*)
NO_3	74	18	0
NH_4	0	30	5
PO_4	0	8	0

Table 3.5: Percentage reduction of important heavy metal and nutrient concentrations in sediments by *Pontederia crassipes* and *Salvinia molesta* at the Swartkops River system, Eastern Cape, South Africa.

Sediments chemistry (mg/kg)	Percentage reduction between upstream and downstream sites		
	IAAP mat 1 (*P.crassipes*)	IAAP mat 2 (*P.crassipes*)	IAAP mat 3 (*S.molesta*)
Fe	46	0	18
Zn	57	89	65
As	0	81	83
Cr	1	77	31
Pb	37	68	56
Hg	59	53	15
Cu	57	88	60
NH_4	69	69	0
NO_3	45	38	0
P	60	87	57

3.3.5 Spatial patterns/changes in environmental variables

Principal component analysis revealed that, of the 27 measured environmental variables, only eight variables showed a strong correlation (Pearson correlation, r>0.6) and accounted for

32.8% of the total variation in environmental characteristics between treatments. The PCA ordination illustrated a distinct clustering where each cluster represented four treatments (e.g. S1, upstream, IAAP mat and downstream) (Figure 3.3). The S1 treatment differed from the other three treatments. There was an overlap with distinguishable differences in environmental variables between the upstream, IAAP mat, and downstream treatments, indicative of shared environmental characteristics. When this was followed up, PERMANOVA results for environmental variables were significantly different between treatments (Pseudo-$F_{3, 56}$ =4.92; P=0.001), thus affirming that the presence of IAAP mats had an impact on environmental variables. Environmental variables, for example, Fe (water chemistry) and As (sediment chemistry) showed a strong positive correlation towards the S1 treatment, whereas EC showed a strong correlation towards the IAAP mat but correlated negatively to the S1 treatment. High sediment chemistry, including Pb, Cu, P, Cr, and Zn concentrations, showed a positive correlation towards the upstream and the downstream treatments (Figure 3.3).

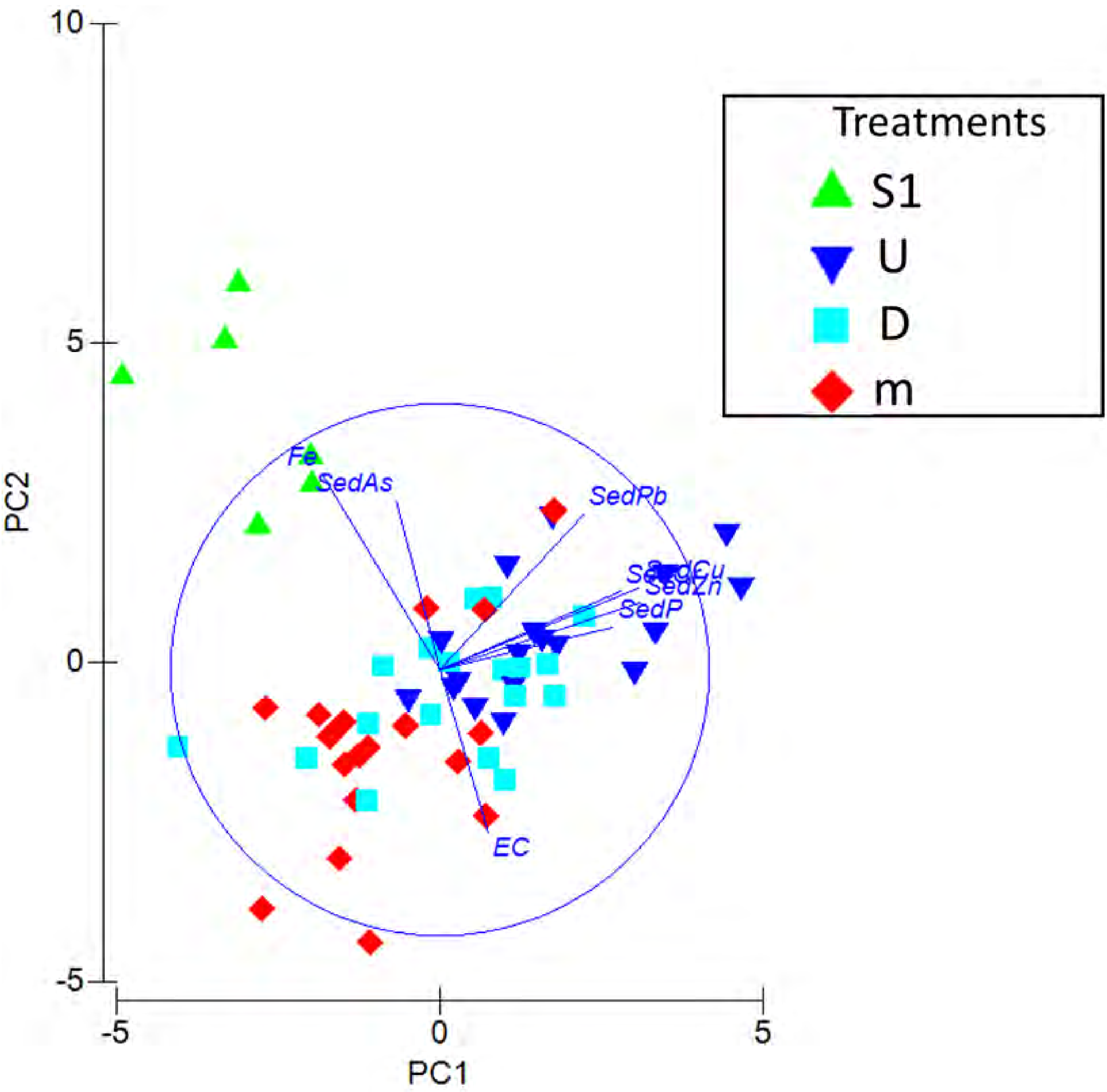

Figure 3.1: Principal Component Analysis (PCA) illustrating environmental variables (water and sediment chemistry) recorded from 10 sites (including IAAP mats) categorised as treatments: Site 1 (S1), Upstream (U), IAAP mat (M) and Downstream (D) at the Swartkops River system, Eastern Cape Province, South Africa. Only significant variables with a strong Pearson correlation (*r*>0.6) are represented on the plot.

3.4 Discussion

3.4.1 Water resource management in South Africa

Various organisations, the Department of Environment Affairs (now Department of Environment, Forestry and Fisheries) and the Department of Water Affairs (now Department of Water and Sanitation) South Africa, are responsible for providing important strategies with regard to the ecological state of South African rivers as a global mandate from the Millennium Ecosystem Assessment (2005) and the Sustainable Development Goals of 2015 (Hák *et al*., 2016). These organisations provide information on the water quality status of rivers, ensure that freshwater resources are under effective management, and that society can benefit from their well-being, as per the agreement drawn up during the Convention on Biological Diversity in 1993 (United Nations, 1992). Among others, the South African Water Quality Guidelines (SAWQG) initiated by the Department of Water Affairs previously provided water quality guidelines for surface water within South African borders (DWAF, 1996a). These guidelines are important in managing water quality, protecting wildlife, and human and environmental well-being.

3.4.2 Water quality standards for Swartkops River system

The present study revealed poor and deteriorating water quality, especially for treatments that were located near industrial activities, and sewage works in the Swartkops River system. These findings reflect different land-use activities and their impact on the water quality in the Swartkops River catchment, as is also evident from Binning and Baird (2001), Odume *et al*. (2012), Odume (2014) and Adams e*t al*. (2019).

The average measured pH values were generally neutral and slightly alkaline, between 7.65 and 8.57. These values were within the SAWQG of 5.5–73.5 for pH concentration for industrial and sewage discharge (DWAF, 1996b). Low pH values are usually the result of decomposing organic matter releasing carbon dioxide and result in the formation of organic acid, whereas high pH is usually the result of high photosynthetic activity (plankton) which lowers the carbonic acid and increases the pH (Fouché *et al*., 2013). According to Tucker and D'Abramo (2008), very low and very high pH concentrations can be stressful for aquatic organisms owing to antagonistic effects. Surprisingly, measured EC (0.35 μS/cm and 3.44 μS/cm) was within the SAWQG for industrial/sewage effluents (250000 μS). However, NH_4 concentrations exceeded the acceptable concentrations (0.007 mg/l) for the survival of aquatic organisms (DWAF, 1996b). This result is alarming for aquatic ecosystem health and aquatic biodiversity, and site 5 and 7 should be tagged as sites of management significance. These sites were located near industrial activities and sewage treatment works.

Heavy metal concentrations in sediments were found to be generally higher than heavy metal concentrations in water, possibly because concentrations in the water column are constantly washed off and change with the time of the day, while some of these concentrations slowly settle in sediments, depending on the water velocity. Sewage sludge from nearby treatment works carried rich content, thus contributing to the increase in sediment chemistry concentration (Binning and Baird, 2001). Some essential heavy metals like Cu, Zn, Fe, and Mn are required in low quantities for the functionality of aquatic organisms, mainly macrophytes (Khayatzadeh and Abbasi, 2010); with the help of bacteria/cyanobacteria, these elements can be broken down and assimilated by macrophytes (Qin *et al*., 2017). Whilst some heavy metal concentrations can be persistent and critical to aquatic organisms (Khayatzadeh and Abbasi, 2010). For example, heavy metals such as Pb, Cr, Hg and Cd, are regarded as extremely toxic to organisms (Cohen *et al*., 2001). Rashed (2001) reported high concentrations of Cd and Pb concentrations in *Tilapia nilotica* in the high dam lake, Aswan (Egypt). Results from the study showed the highest concentration in the vertebral column and in the fish scales, and were considered harmful to the fish and secondary consumers.

In the present study, essential (Fe, Zn, and Cu) and non-essential (Pb) components in water varied between treatments. Fe concentrations upstream and downstream of mat 3 were within the DWAF guideline for domestic water (0.1 mg/l), but all remaining treatments slightly exceeded SAWQG, justifying more robust monitoring management of the Swartkops River. Zinc (Zn) concentrations in the water column ranged from 0.02 mg/l to 0.12 mg/l; most Zn concentrations were within the same range of 0.02 mg/l for most sites. However, upstream of mat 2, at site 5 and downstream of mat 2, at site 7, and downstream of mat 3, at site 10 exceeded the acceptable SAWQG for aquatic ecosystem range for Zn 0.01 and 0.02 mg/l (DWAF, 1996a). Zinc concentrations reportedly limit plant growth by disturbing their photosynthetic processes (Mishra and Tripathi, 2008); Zn concentrations also change gene expressions in some fishes (Choi *et al*., 2016), leading to growth abnormalities. Sediment Zinc concentrations ranged from 7.19 mg/kg to 87.16 mg/kg and were within the DWAF limit (240 mg/kg). Similarly, Zn concentrations obtained by Gyedu-Ababio *et al*. (1999) in the Swartkops River were also within the DWAF limit; however, their concentrations were slightly higher (6.0 µg/g to 116.16 µg/g) than Zn concentrations from the present study. These findings clearly demonstrate the industrial effluent discharged to be a severe problem in the Swartkops River system, causing water deterioration. Recorded Fe concentrations ranged from 220.43 mg/kg to 1321.25 mg/kg. High concentrations of Fe ranging from 2942 µg/g to 31232 µg/g were also recorded by Gyedu-Ababio *et al*. (1999) in the Swartkops River sediments. The present study's Fe concentrations were also high at all sites, suggesting that a wide range of pollutants ranging from agricultural pollution, pesticides and industrial effluents containing Fe are being released into the Swartkops River system.

Coetzee and Hill (2012) reported that South Africa has adopted inappropriate standards for water quality and should revise their standards to match international standards, and should have effective waste management strategies in place. For example, South Africa adopted high PO_4 concentrations of about 1 mg/l, despite evidence showing that phosphate concentrations of less than 0 mg/l were necessary to minimise eutrophication impacts on aquatic ecosystems. Their study further reported how South African water trophic status was far too high compared to international standards, and resulted in more eutrophication in freshwater ecosystems as a result of increased effluent from expanding population growth. This is the case for inland river systems like Swartkops that are not monitored for agricultural run-off, industrial and sewage discharge, and which flow directly into important dams and coastal environments.

3.4.3 Phytoremediation in the Swartkops River system

The removal of heavy metals and nutrient effluents from the Swartkops River system by *P. crassipes* and *S. molesta* was apparent in the present study and in those of Mishra and Tripathi (2009), Donatus (2016), Ng and Chan (2017) and Eid *et al*. (2019). Some of these sites, including upstream of mat 2 at site 5 had very poor water quality variables (i.e. high PO_4, NO_3, NH_4 and heavy metals concentrations (Zn, Cr, Pb, Hg and Cu)) compared to site 1 and recorded the highest concentrations of pollutants because of high levels of pollutants from waste water treatment facilities and other industrial activities located near the sampled areas. In general, a decrease in pollution concentrations was observed for most of the sampled sites from upstream to downstream sites. This could be attributed to the presence of dense mats of IAAP that are able to filter and accumulate both organic and inorganic nutrients. It is also possible that some of the pollutants were accumulated by soils and not by the dense IAAP mat since sediments also recorded high concentrations of organic and inorganic pollutants (Shayler *et al*., 2009; Webb *et al*., 2019). The variation in pollutant concentrations, and a decreasing trend in the concentrations indicate that *P. crassipes* and *S. molesta* can tolerate severely modified environments, as shown in the present study and in that of Odjegba and Fasidi (2007).

Mishra and Tripathi (2009) reported on the potential of *P. crassipes* to hyper-accumulate Cr and Zn effluents, where *P. crassipes* efficiently assimilated about 84% of Cr and 95% of Zn from different concentrations during 11-day experimental period. *Pontederia crassipes* showed no signs of morphological and/or physiological stress for Zn, but showed minor morphological signs with removal levels of Cr at 10.0 mg/l and 20.0 mg/l, such as chlorosis of leaves (yellowing of leaves) and root shedding. The study concluded that *P. crassipes* can be used as a phytoremediation agent to remove heavy metal concentrations in freshwater ecosystems. In the present study, Zn concentrations showed a decreasing trend between the upstream and the downstream site for all mats; noticeably the highest Zn concentration recorded was 87.16 mg/kg upstream of mat 3, at site 8, which was reduced to the lowest concentration of 7.19 mg/kg downstream of mat 3 (*S. molesta*), at site 10. These results supported findings by Mishra and Tripathi (2009) on the high accumulation capability of *P. crassipes* for Zn and other water nutrients. Some findings from the present study contrasted with findings from Odjegba and Fasidi (2007) who reported that *P. crassipes* was a poor accumulator of Hg. The present study recorded a decreasing trend of Hg in sediments possibly as result of *P. crassipes* assimilation. Mercury (Hg) decreased from the highest concentration upstream of mat 1, at site 2 (1.77 mg/kg) to the lowest concentration recorded downstream of mat 2, at site 7 (0.64 mg/kg), a 64% reduction, showing removal efficiency of *P. crassipes*.

Another study by Moyo *et al*. (2013) evaluated the effectiveness of *P. crassipes* in the assimilation of TDS, EC, sulphates and phosphates, and reported that *P. crassipes* dense mats significantly reduced 25% of EC (from 624 µs/cm to 426 µs/cm); 26% of TDS (from 378 mg/l to 281 mg/l); 45% of sulphates (0.11mg/l to 0.06 mg/l); and 33% of phosphates (89 mg/l to 6 mg/l) before and after *P. crassipes'* presence. However, variables such as pH, total nitrogen, nitrates and nitrites showed insignificant differences, a trend similar to that reported in the present study.

Consistent with the reults of this study, river systems with IAAP species infestation are known to improve the water quality variables because IAAP species possess the ability to assimilate nutrients and other water pollutants (Ali *et al*., 2013). The overall results from my study indicate that *P. crassipes* and *S. molesta* do indeed assimilate nutrients and other water pollutants. *Pontederia crassipes* and *S. molesta* assimilated organic and inorganic pollutants from the river system, as evidenced by lower concentration of water variables such as NO_3, NH_4, PO_4, Pb, Zn, Hg, Cu and P downstream of the IAAP mats. Although there were fluctuations and inconsistencies in some variables, the overall status of results showed a decreasing trend for most of the variables. This study demonstrated the phytoremediation abilities of *P. crassipes* and *S. molesta*, confirming the findings reported by other authors (Rommens *et al*., 2003; Wang *et al*., 2012; Zhou *et al*., 2013; Kumari and Tripathi, 2014; Zhang *et al*., 2015; Victor *et al*., 2016).

The next chapters examine periphyton and aquatic macroinvertebrate diversity and community assemblage (chapter 4 and 5) as potential biological indicators for phytoremediation success. Previous studies have successfully assessed phytoremediation success, as seen for water and sediment chemistry in the present chapter and in other studies (Mahujchariyawong and Ikeda, 2001; Lu *et al*., 2010; Hammad, 2011; Lissy and Madhu, 2011; Moyo *et al*., 2013; Donatus, 2016).

CHAPTER 4

Periphyton Diversity Patterns Along an Urban River System Invaded by Invasive Alien Aquatic Plants Species, Eastern Cape, South Africa

4.1 Introduction

Aquatic microalgae species (e.g. phytoplankton and periphyton) are considered basal resources in all aquatic ecosystem food webs and, together with macrophytes, are the primary producers on which all trophic levels depend for energy (Dalu *et al*., 2014a; Stevenson, 2014). In these ecosystems, aquatic microalgae control biogeochemical recycling processes, such as carbon recycling (Cahoon, *et al*., 1999; Dalsgaard, 2003); drive the ecosystem productivity, and maintain oxygen levels to sustain aquatic processes, including photosynthesis (Stevenson, 2014). Aquatic microalgae also indicate the ecosystem's trophic status and levels of inorganic chemistry in many water bodies (Nakanishi *et al*., 2004; Lane and Brown, 2007). Any form of disturbance that would alter aquatic microalgae communities' results in the modification of primary productivity, with severe impacts on other aquatic organisms higher up the trophic level (Lürling and Roessink, 2006).

It has been reported that disturbances in the water chemistry, including contaminants (Lane and Brown, 2007; Li *et al*., 2010; Dalu *et al*., 2014a), habitat alteration (Stevenson, 2014), and invasive alien macrophytes can influence aquatic microalgae community assemblages and composition by creating conditions that are either favourable or unfavourable to aquatic microalgae species (Harding, 2015). Aquatic microalgae are increasingly being used in ecological assessment for environmental change and pollution in aquatic water bodies around the globe (Lane and Brown, 2007; Omar, 2010; Bere and Mangadze, 2014; Chakraborty *et al*., 2014; Stevenson, 2014) because aquatic microalgae are abundant and diverse (widely distributed) in both freshwater, brackish, and marine ecosystems (Taylor *et al*., 2007a). They are able to reflect various environmental disturbances occurring over extended periods of time, so providing an integrated measure of river health (Ndiritu *et al*., 2006). Additionally, aquatic microalgae are easy to sample: they do not require sophisticated equipment, and are highly responsive to sudden and continuous changes in water chemistry variables, making them excellent biological indicators (Dell'Uomo and Torrisi, 2011; Dalu and Froneman, 2016). For example, Nakanishi *et al*. (2004) and Kumari *et al*. (2008) have reported high abundance and diversity of aquatic microalgae in less polluted water than in heavily polluted waters. In disturbed aquatic systems, aquatic microalgae species, including *Oscillatoria limosa*, *O. rubens*, *Fragilaria* sp., *Lyngbya* sp., *Spirogyra* sp., and *Hormidium klebsii* are common (Nakanishi *et al*., 2004), possibly because they tolerate pollution, and can serve as indicators of deteriorating aquatic environment. Some species, such as *Fragilaria* sp., and *Oscillatoria* sp., occupy a wide range of waters and are common in mesotrophic and eutrophic waters, alkaline waters, and oxygen-rich water (van Vuuren *et al*., 2006). Diatoms, in particular, have been used to detect heavy metal pollution in heavily polluted river systems because of their tolerance of pollution. Diatom species, such as *Achnanthes minutissima*, *Surirella angusta,* have been reported to tolerate heavy metal pollution such as Copper, Iron, Zn, Pb and Cd in river systems; for example, *A. minutissima* abundance always increases with an increase in heavy metal concentrations in river systems (Nakanishi *et al*., 2004).

Aquatic systems with high organic and nutrient pollution are known to harbour pollution-tolerant diatom species, such as *Navicula sensu lato,* and their communities increase with an increase in organic and nutrient concentration (Passy, 2007). *Nitzschia palea* and *Navicula lanceolata* can be resistant to both pesticides, such as atrazine, and tolerant of various organic sources of pollution (Guasch *et al*., 1998). Heavily polluted waters are dominated by Cyanophyta and Chlorophyta phyla, which are known as pollution indicator species owing to their dominance in high-nutrient water bodies (Kumari *et al*., 2008). This dominance shows how, among other factors, aquatic microalgae can be affected by different land-use activities, such as nutrients and organic concentrations (Kelly *et al*., 2008). Aquatic microalgae assemblages are routinely used to assess environmental conditions as they are adapted to various water chemistry variables. Thus, aquatic microalgae could be used as biological indicators of river health, in combination with aquatic macroinvertebrates (see Chapter 5). This study investigates the response of periphyton diversity and assemblage structure to assess IAAP species phytoremediation success in the Swartkops River, Eastern Cape, South Africa. I hypothesis that the presence of IAAP mats will improve water quality (as seen in Chapter 3) and periphyton diversity and shift assemblages structure to pollution sensitive taxa downstream IAAP species mats.

4.2 Materials and methods

4.2.1 Study area and data collection

Details on study area and sample collection are described in Chapter 2.

4.2.2 Data analysis

To investigate the phytoremediation effect of IAAP species mats on periphyton diversity and assemblage between sites and along the urban river; periphyton biodiversity indices, namely, relative species abundance (N), species richness (S), Shannon's diversity index: H' = $-\sum^{s}_{i=1}$ *pi* ln *pi*, (where *pi* is the proportional abundance of taxa *i* in the sample given *s* taxa), and Pielou's evenness: J' = $\frac{H'}{\ln(S)}$ were computed and compared across seven sites using PRIMER version 6.1.16 and PERMANOVA^{+} version 1.0.6 using the DIVERSE function (PRIMER-E Ltd, Plymouth; Clark and Gorley, 2006).

Then to test for significant difference in periphyton biodiversity indices, the Shapiro-Wilk test and Levene's test were employed to test for normality and homogeneity of variances, respectively. The outcome of periphyton biodiversity indices variables revealed that data were normally distributed (Shapiro-Wilk, P>0.05) and the variances were homogenous (Levene test, P<0.05) except for the periphyton relative species abundance (Shapiro-Wilk, P<0.05, Levene test, P>0.05). Thus, the relative species abundance data was log (x+1) transformed and a parametric test, in this case ANOVA, was used to test for significant differences in periphyton biodiversity indices with sites as a factor.

To compare periphyton community composition between sites, periphyton species were categorised into phyla (Appendix 1), including Cyanophyta, Bacillariophyta, Euglenophyta, and Chlorophyta following John *et al*. (2002), van Vuuren *et al*. (2006) and Taylor *et al*. (2007a). The chi-square test was employed to test for significant differences in periphyton phyla percentage abundance between sites. Thereafter, to examine and visualise shifts in periphyton assemblages between treatments (derived from sites), periphyton relative taxa abundance were square-root transformed using the Bray-Curtis resemblance metrics which decreased the importance of dominant periphyton species and increased the influence of least or rare periphyton species (Clarke and Ainsworth, 1993). An unconstrained ordination analysis, non-metric multi-dimensional scaling (NMDS) was used to illustrate periphyton community assemblage shifts between treatments. Thereafter, analysis of similarity (ANOSIM) test was performed to investigate the statistical difference in periphyton assemblages between treatments in PRIMER version 6.1.16 and PERMANOVA^{+} version 1.0.6 (PRIMER-E Ltd, Plymouth; Clark and Gorley, 2005).

If the ANOSIM analysis was significant, Indicator species analysis (IndVal) was further employed to identify discriminatory periphyton species that were responsible for differences in assemblages' composition between treatments. Indicator value species varied from 0 to 100, attaining maximum value when all individuals of a species occurred at all sites of a single group (González *et al*., 2015). The significance of the indicator value for each taxon was tested by a Monte Carlo test with 9999 permutations, α=0.05, in PC-ORD version 5.13 (McCune and Mefford, 2006). Periphyton species with a significant indicator value were considered indicator species.

To determine the role of environmental variables on periphyton community assemblage, distance based linear modelling (DistLM) analysis was performed. From this, a distance based redundancy analysis (db-RDA) bi-plot was created to illustrate significant environmental variables that influenced periphyton assemblage structure between treatments (db-RDA ordination method was chosen because data was not normally distributed; plus db-RDA gave clear visual results compared to other ordination methods). All statistical analyses were performed in R version 3.6.1 (R Core Development Team, 2016), except when specified.

4.3 Results

4.3.1 Periphyton diversity and assemblage patterns

Periphyton diversity and community structure showed significant variation between sites (Figure 3.1). Pielou's evenness ($F_{(4, 35)}$=8.64, P=0.01) and Shannon diversity ($F_{(4, 35)}$=3.55, P=0.02) were significantly different between sites, whereas periphyton relative taxa abundance ($F_{(4, 35)}$=1.89, P>0.05) and taxa richness ($F_{(4, 35)}$=0.86, P>0.05) were not significantly different between sites.

Periphyton relative abundance was lowest at site 1, and site 10 (downstream mat 3) but was high downstream of mat 2 (site 7), followed by site 5 (upstream of mat 2) (Figure 4.1A). Site 2, upstream of mat 1, recorded high relative taxa abundance compared to site 4, downstream of mat 1, as was the case for the upstream (site 8) and downstream (site 10) of mat 3. Upstream of mat 3, site 8 recorded the highest taxa richness, whereas upstream of mat 2, site 5 had the lowest recoded taxa richness. Both upstream and downstream of mat 2, sites 5 and 7 had the least taxa richness compared to site 1 and the rest of the sites (Figure 4.1B). Although not significant, Pielou's evenness was high at site 1 and lowest at upstream and downstream of mat 2, at sites 5 and 7. Apart from mat 2 sites (upstream site 5 and downstream site 7), mat 1 and mat 3 evenness increased from upstream to downstream sites (Figure 4.1C). Shannon diversity was recorded as high at site 1, and gradually decreased, reaching the lowest downstream of mat 2, at site 7, and increase thereafter upstream of mat 3, at site 8 and slightly decreased downstream of mat 3, at site 10 (Figure 4.1D).

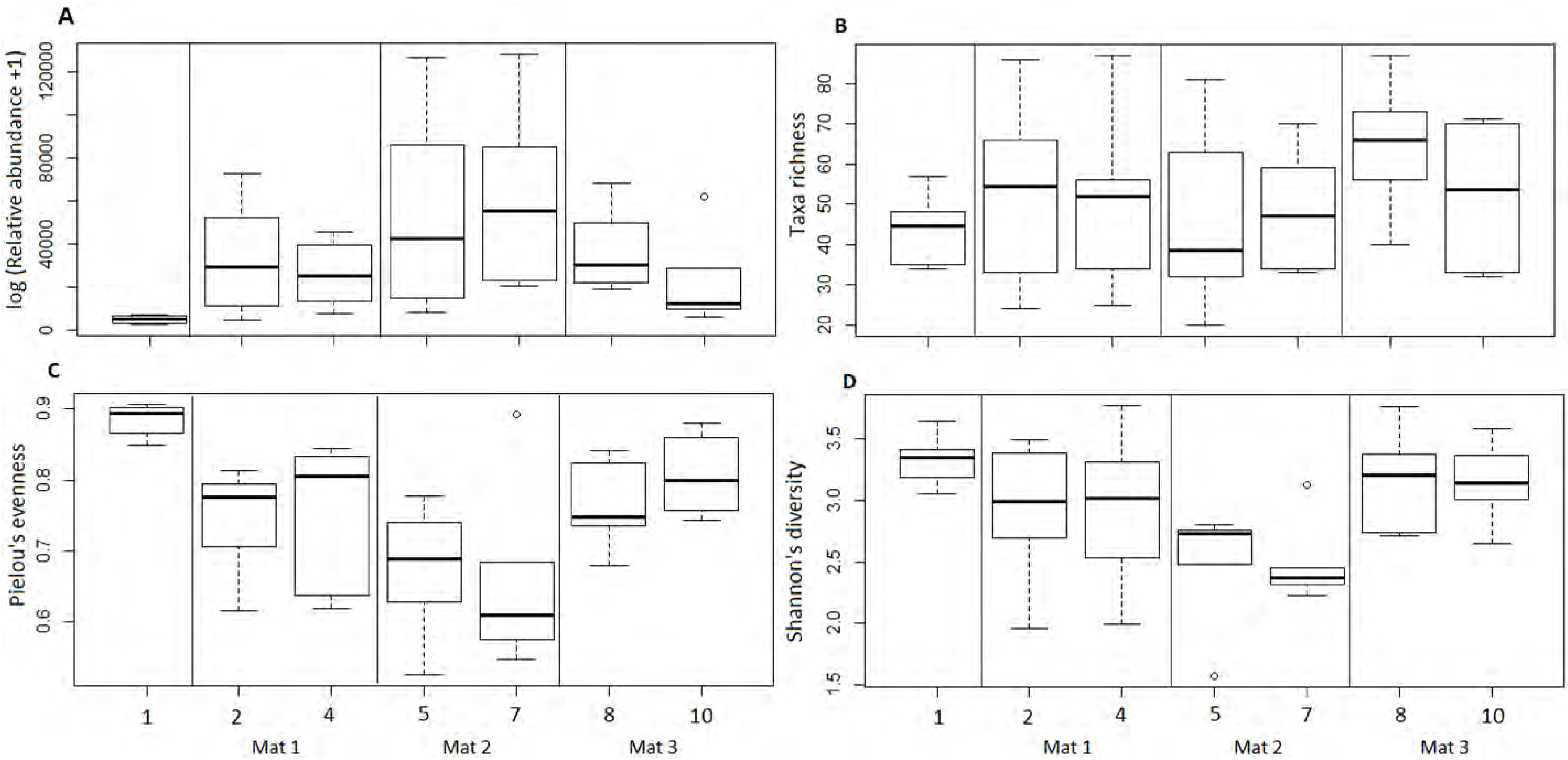

Figure 4.1: Periphyton biodiversity indices; (A) relative taxa abundance, (B) taxa richness, (C) Pielou's evenness and (D) Shannon diversity index from seven sites of three IAAP mats in the Swartkops River system, Eastern Cape, South Africa. Box plots represent median values with interquartile range. Whiskers represent maximum and minimum values.

A total of 262 periphyton species were recorded and identified during the study. Periphyton community composition was significantly different between sites: Cyanophyta (X^2= 88738, df = 41, P<0.001), Bacillariophyta (X^2= 1277848, df = 41, P<0.001), Euglenophyta (X^2= 107073, df = 41, P<0.001) and Chlorophyta (X^2= 16514, df = 41, P<0.001). Bacillariophyta were the most abundant group at all sites, contributing more than 65% abundance, followed by Cyanophyta, Euglenophyta and Chlorophyta. Bacillariophyta proportions increased from site 1, and reached the highest downstream of mat 2, at site 7, thereafter decreasing slightly (Figure 4.2). The opposite trend was demonstrated by Euglenophyta and Chlorophyta, which started decreasing from site 1 and reached the lowest abundance between the sites upstream and downstream of mat 2 and increased at the mat 3 sites. Cyanophyta was high at site 1, but showed significant reduction between site 4 downstream of mat 1, and downstream of mat 3, at site 10 (Figure 4.2).

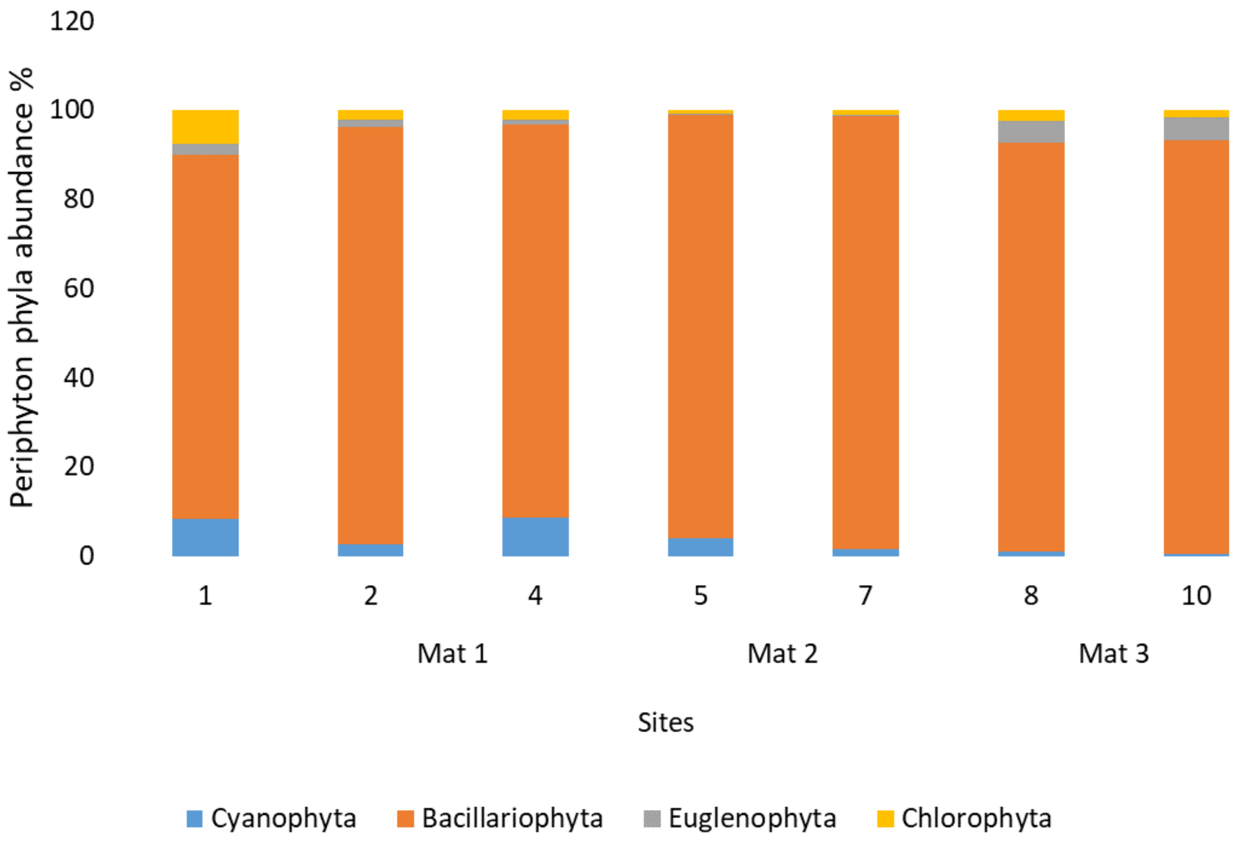

Figure 4.2: Periphyton phyla percentage composition from seven sites of three IAAP mats at the Swartkops River system, Eastern Cape, South Africa.

A NMDS revealed that there was a variation between the Site 1 (S1), and upstream (U) and downstream (D) treatments (Figure 4.3). The NMDS showed an overlap between the upstream and downstream treatments, indicating similar periphyton community assemblages between treatments, whereas the S1 clustered away from both upstream and downstream treatments (Figure 4.3). When this was investigated further, ANOSIM revealed that there was periphyton assemblage variation between the S1 and upstream treatment (Global R=0.469; *P*=0.02), and the S1 and downstream treatment (Global R=0.56; *P*=0.02). However, there was no distinguishable periphyton assemblage variation between the upstream and downstream treatments (Global R=-0.036; *P*=0.938) as visually shown by the NMDS diagram.

IndVal analysis revealed that upstream of mat 3, site 8 reported six significant indicator species, including *Cocconeis* sp., *C. pediculus*, *C. placentula var. lineata*, *Fragilaria bicep*s, and *F. ulna*. This was followed by the S1 treatment where three significant indicator species were identified, including *Cymbella aspera*, *Nitzschia gracilis*, and *Rhopalodia gibba* (Table 4.1). The upstream site 5 and downstream site 7 from IAAP mat 2 each recorded two significant periphyton indicator species: *N. gregaria*, *Pinnularia viridiformis*, and *G. parvulum* and *N. veneta*, respectively. Downstream of mat 3, site 10 and upstream of mat 1, site 2 each recorded one indicator species, namely, *Melosira varians* and *Nitzschia reversa*, respectively (Table 4.1).

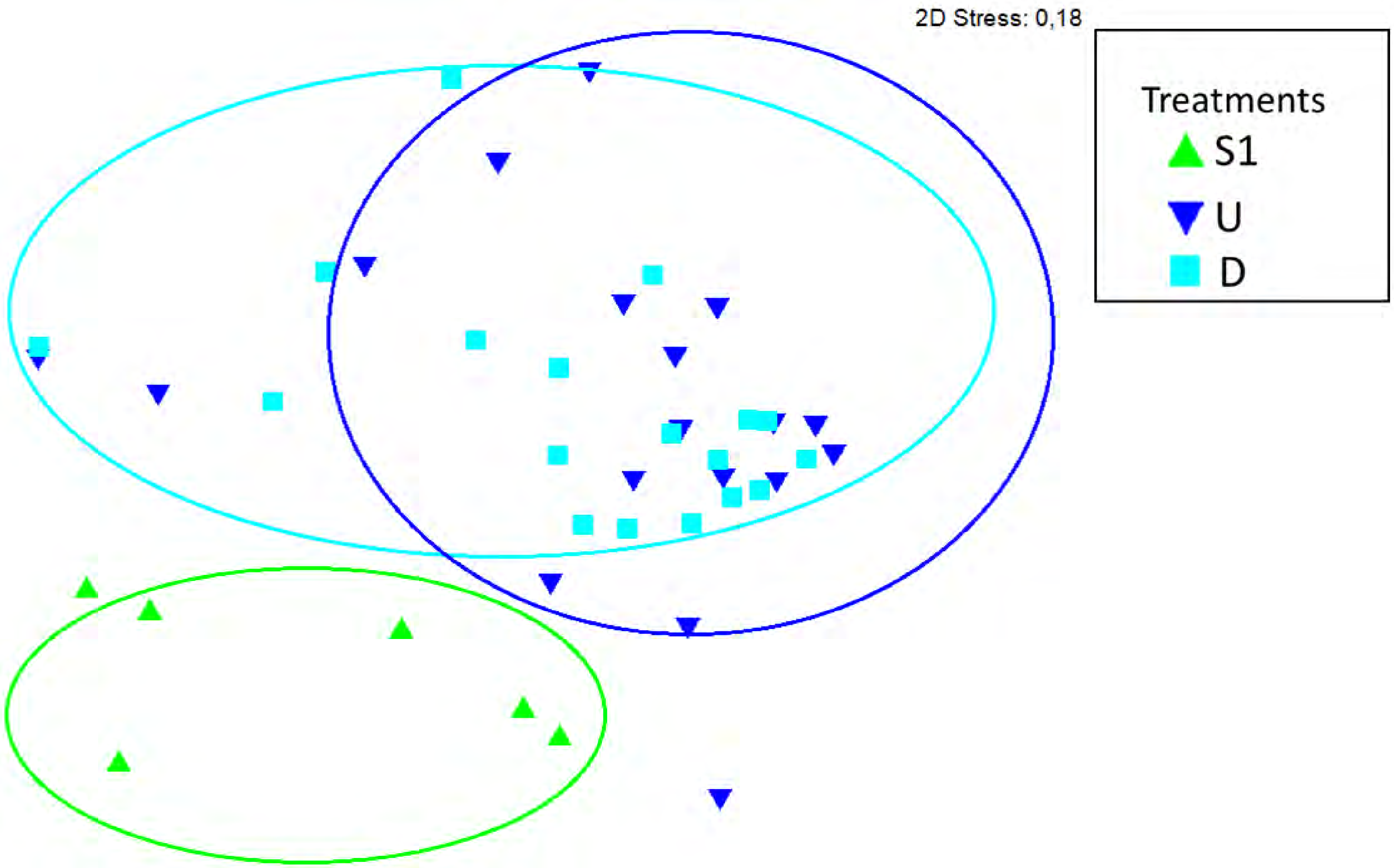

Figure 4.3: A Non-Metric Multi-Dimensional Scaling (NMDS) ordination illustrating periphyton community assemblage from seven sites (excluding mat sites) categorised into treatments along the Swartkops River system, Eastern Cape, South Africa.

Table 4.1: List of significant ($P<0.05$) indicator periphyton species from seven sites and treatments (excluding mat sites) along the Swartkops River system, Eastern Cape, South Africa.

Aquatic Microalgae species	Ind Val%	Mean	St. Dev	*P*-value	Site	Treatment	Mat
Cocconeis species	40.0	22.3	6.99	0.0242	8	Upstream	Mat 3
Cocconeis pediculus	44.7	23.1	9.17	0.0257	8	Upstream	Mat 3
Cocconeis placentula var. lineata	32.6	16.6	7.73	0.0411	8	Upstream	Mat 3
Cymbella aspera	43.3	15.1	8.30	0.0200	1	S1	S1
Fragilaria biceps	44.6	23.2	9.08	0.0265	8	Upstream	Mat 3
Fragilaria ulna	39.6	24.9	6.33	0.0267	8	Upstream	Mat 3
Gomphonema parvulum	34.5	21.7	6.72	0.0498	7	Downstream	Mat 2
Melosira varians	46.3	30.1	8.34	0.0489	10	Downstream	Mat 3
Navicula gregaria	36.9	25.3	5.65	0.0420	5	Upstream	Mat 2
Navicula veneta	52.3	29.4	6.66	0.0032	7	Downstream	Mat 2
Nitzschia gracilis	66.7	15.3	8.63	0.0010	1	S1	S1
Nitzschia reversa	45.9	28.3	10.57	0.0598	2	Upstream	Mat 1
Pinnularia viridiformis	38.5	25.6	5.41	0.0027	5	Upstream	Mat 2
Rhopalodia gibba	43.1	15.6	7.43	0.0098	1	S1	S1

The distance based redundancy analysis (db-RDA) complemented NMDS results and provided additional environmental characteristics that were specific per treatment, and assisted in periphyton assemblage composition and clustering (Figure 4.4). Four environmental variables contributed significantly (Pearson correlation, $r>0.5$) to the grouping of treatments in response to periphyton assemblage and explained a total variation of 30%. Water and sediment Fe concentration showed a strong correlation with the S1 treatment, and a negative correlation with both upstream and downstream treatments, while NO_3 and sediment Zn showed a strong correlation with the upstream and downstream treatments (Figure 4.4).

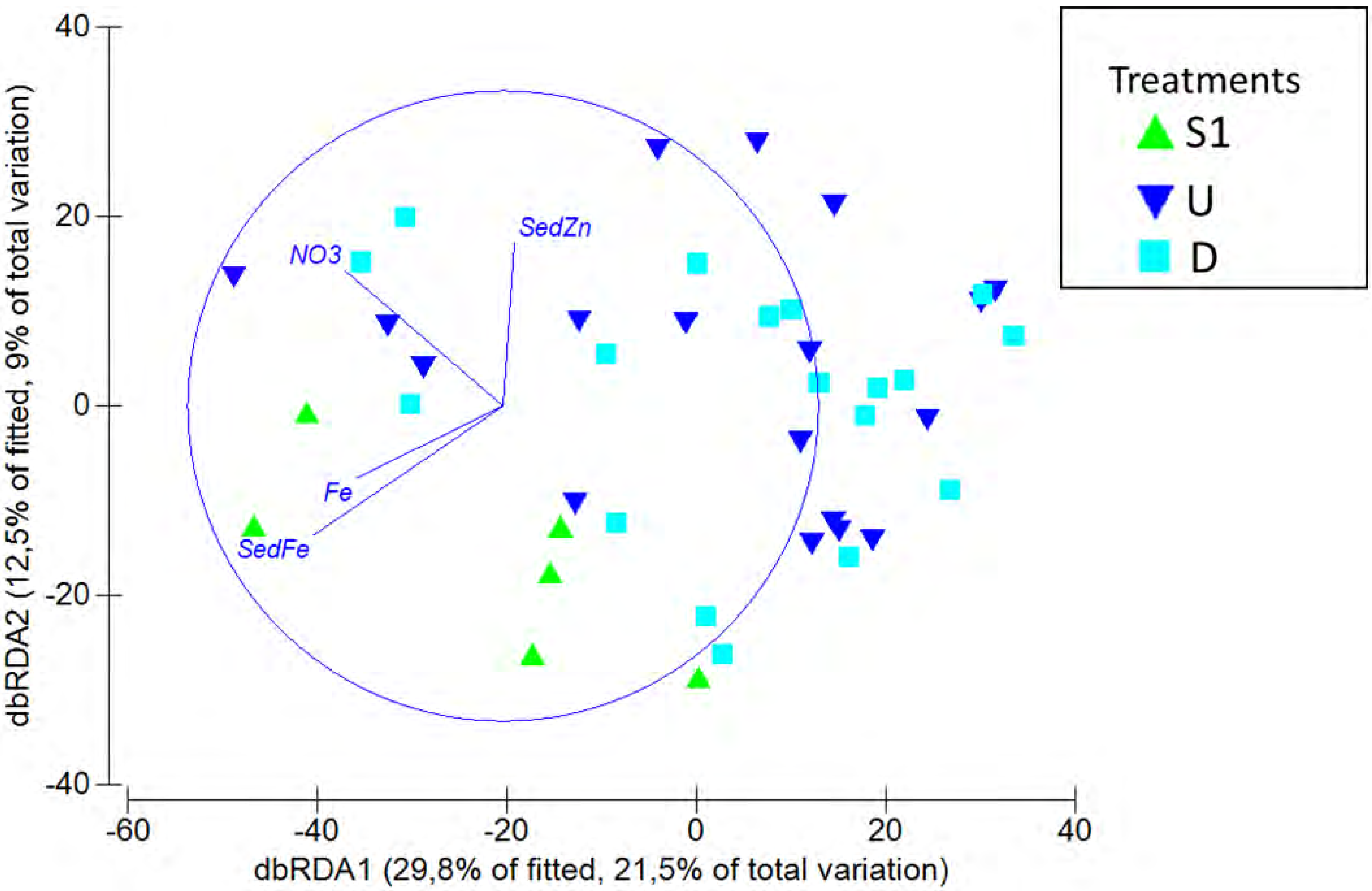

Figure 4.4: Distance based redundancy analysis (db-RDA) ordination bi-plot illustrating the relationship between environmental variables that best explained periphyton assemblage from the Swartkops River system, Eastern Cape, South Africa. SedZn – sediment Zinc; SedFe – sediment Iron. S1 – Site 1 treatment, U – Upstream mat and D – Downstream mat treatments.

4.4 Discussion

The study demonstrated that periphyton community composition in the Swartkops River was largely influenced by environmental variables, particularly nutrients and heavy metal concentrations. Results confirm that the composition of periphyton responded to both physical and chemical characteristics along the Swartkops River and that the IAAP species mats did not have any effect on biodiversity indices and assemblage composition, as seen with water and sediments chemistry in Chapter 3. The study's hypothesis was that there is variation in periphyton sampled above and below the IAAP mats; however, results proved otherwise. The study's results were not in agreement with the hypothesis on the recovery of periphyton communities' downstream.

As indicated before, periphyton are usually aquatic microalgae attached to substrates (plants and stones) and this exposes the species to various physical and chemical environmental conditions (Li *et al*., 2010). The presence of dense IAAP mats changes the river's flow pattern, traps detritus and greatly deprives aquatic biota (i.e. invertebrates, vertebrates, and microalgae) of access to sunlight and oxygen (Midgley *et al*., 2006, Coetzee *et al*., 2014). Lack of sunlight and oxygen exchange at these sites alters chemical and physical variables by preventing processes such as photosynthesis to occur, resulting in significant differences in environmental variables between invaded and non-invaded sites (Brendonck *et al*., 2003). The mats also prevent growth of submerged macrophytes, which are aquatic microalgae substrates for attachment, and lack of submerged macrophytes can be unfavourable in structuring aquatic microalgae community assemblages. This implies that invaded areas generally support low species diversity and abundance compared to non-invaded sites (Stier *et al*., 2017).

Aquatic microalgae in freshwater ecosystems are largely determined by the interaction of variables such as organic and inorganic nutrients (phosphorus, nitrates, and heavy metal concentrations) (Bere *et al*., 2014; Dalu *et al*., 2014b). For the present study, periphyton species were structured by the presence of organic and inorganic variables, with groupings of these revealing the water quality state. Various periphyton species responded well to changes in water quality variables (i.e. Fe, Cr, Pb, Zn, NH_4; (sediments); NO_3, As (water); PO_4 (sediemnts and water) as a result of their tolerances, sensitivity and adaptation to pollution. Most of the identified periphyton species as shown by IndVal analysis were pollution-tolerant species, and these included *Navicula veneta*, *Cocconeis pediculus*, *Cocconeis placentula var. lineata*, *Fragilaria biceps*, *Fragilaria ulna*, *Gomphonema parvulum*, *Cymbella aspera*, *Melosira varians*, *Navicula gregaria*, *Nitzschia gracilis*, *Nitzschia reversa*, *Pinnularia viridiformis* and *Rhopalodia gibba* (Taylor *et al.*, 2007a, Triest *et al*., 2012, Bere and Mangadze, 2014). The dominance of pollution-tolerant species showed that pollutants in water did not affect these periphyton species. According to Taylor *et al*. (2007a), these species are associated with heavily eutrophic systems that are high in electrolyte content, while some species are highly tolerant of pollution and associated with industrial pollution (Soares *et al*., 2007). The high degree of pollution-tolerant taxa in this study could be attributed to the fact that most sites, excluding site 1, were subjected to point and non-point pollution from human impacts, which were observed during the duration of the study in the Swartkops River catchment. The result was species distribution biased towards pollution-tolerant species (*Nitzschia reversa* and *N. palea*) and cosmopolitan species (e.g. *Fragilaria ulna*) (Dalu *et al*., 2016). These findings agree with studies conducted by Odume *et al*. (2012), Bere and Mangadze (2014), Bere *et al* (2014), Tan *et al*. (2014) and Dalu *et al*. (2017).

The Swartkops River catchment is highly industrialised and this increases chances of various point and non-point pollution sources throughout the river, which influence the distribution of periphyton assemblages. The impact of industrialisation and urbanisation in the Swartkops River on periphyton communities was highlighted in periphyton biodiversity indices. Results from biodiversity indices revealed that periphyton diversity and evenness decreased with an increase in pollution gradient; this was evident upstream (site 5) and downstream (site 7) of mat 2. Both sites were recorded to be the most severely impacted sites as they were located downstream of sewage treatment works and industrial areas, and thus recorded high concentrations of Fe, Cu, Zn, and COD concentrations during the study. High concentrations of Zn, Cr, Cu, and P in sediments were also recorded at the same sites. As a result, periphyton richness, Shannon's diversity, and Pielou's evenness were the lowest, but relative taxa abundance was high as a result of pollution tolerant periphyton taxa. By contrast, the less impacted site 1 registered a high Pielou's evenness, Shannon's diversity and taxa richness. These findings are in agreement with those reported by Dalu *et al*. (2017), which showed that aquatic microalgae community compositions in the Bloukrans River, South Africa, were largely influenced by water variables, especially nutrients, in comparison to sediment and physical pollution. Bere *et al*. (2014) reported that urban streams in Chinhoyi Town, Zimbabwe, subjected to different anthropogenic inputs (inadequate system of sorting and improper disposal of waste) had low aquatic microalgae species richness, and poor ecological status. Taylor *et al*. (2007b) reported that low aquatic microalgae species richness could be an indication of unfavourable growing conditions and that this could be attributed to poor water quality which limits the appearance of species that are sensitive to pollution. This poor water quality leads to species-poor periphyton communities that are largely dominated by generalist taxa, including *Cyclotella* sp., *Navicula* sp., *Nitzschia* sp., and *Gomphonema* sp. (Taylor *et al*., 2007b). For example, *Chlorella* sp. are aquatic microalgae that thrive well in heterotrophic conditions; these species are able to thrive in organic conditions that include wastewater residues (Wang *et al*., 2010). Pollution-tolerant species will thrive well even where there are few or no pollution-sensitive species and autotrophic microalgae. In the present study, the most dominant species was *N. veneta*, which was found at all sites and in the variously polluted water already discussed.

Microalgae species, including *Melosira varians*, *Fragilaria ulna*, and *Navicula gregaria* are usually found in abundance in mesotrophic to eutrophic waters, and they sometimes occur in slightly brackish waters (Taylor *et al*., 2007a). These findings concur with the present study's findings, as well as findings by Moura *et al*. (2007) who indicated that *Melosira* sp. indeed preferred systems that were highly eutrophic. In the present study, *Melosira varians* were most abundant upstream and downstream of mat 3, at sites 8 and 10, which were located further downstream of the Swartkops River. Although IAAP mat 3 was further down river and showed normal ranges of physicochemical variables with no signs of elevated eutrophic content (see Chapter 3), this finding clearly justifies the use of biological indicators as a reliable and time-integrated assessment tool in addition to water chemistry spot analysis (Motitsoe *et al*., 2020). *Gomphonema parvulum*, *Navicula trivalis, N. gregaria* and *N. veneta* are generally considered to tolerate polluted conditions, thriving in a range of waters including eutrophic, moderate to high electrolyte, and brackish waters (Taylor *et al*., 2007a). *Navicula veneta* and *Gomphonema parvulum* were recorded as indicator species downstream of mat 2, at site 7, while *N. gregaria* was recorded upstream of mat 2, at site 5. These sites (5 and 7) were located near industrial and construction activities, and were the most polluted sites. *Navicula gregaria* has been reported as an example of a good indicator of high electrolyte and high concentrations of pollutants in water, whereas *N. veneta* is strongly associated with pollutants from industrially impacted waters (Taylor *et al*., 2007a).

Zongo and Boussim (2015) also reported that aquatic microalgae assemblages in the Pendjari area (Benin West Africa) were influenced by the river's physicochemical variables. The study further reported that changes in the concentration of physicochemical parameters significantly influenced aquatic microalgae assemblages. For example, water depth and transparency (turbidity) influenced the survival of microalgae genera such as Cyanophytes and Chlorophytes. Water depth determines the intensity of sunlight penetration, which is important in the growth, development and survival of various microalgae species of different sensitives; the shallower and more transparent the water body is, the more penetration of sunlight, resulting in high microalgae photosynthetic activities (Zongo and Boussim, 2015). It is possible that most of the pollutants were being washed off from the highly impacted upstream to the downstream sites by the hydrological connectivity of river systems. It was evident that fluctuations in aquatic microalgae assemblage distribution between treatments were largely due to the deteriorating water quality in the Swartkops River system (Odume *et al*., 2012). Deteriorating water quality has been reported to affect the biodiversity (Odume *et al*., 2012) and could have influenced the abundance of pollution-tolerant periphyton species from the upper treatments to the lower sampled treatments.

The present study demonstrated that anthropogenic activities along the Swartkops River catchment have impacted the river system, with nutrients and heavy metal concentrations closely reflecting agricultural and industrial activities along the river catchment. The impacts of anthropogenic activities were reflected on the water quality, biodiversity and distribution of periphyton assemblages. Compared to site 1, both upstream and downstream sites were similar in periphyton assemblage composition showing no significant differences in biodiversity indices. Both sites were largely dominated by pollution tolerant periphyton species, these species were adapted to eutrophic, moderate and high electrolyte content, brackish and saline waters, with very few species being sensitive to such water conditions. In addition, the inability of indicator species analysis (IndVal) to pick up species that were more sensitive to pollution confirmed the observed and recorded poor water quality concentrations; this clearly indicated that the presence of *P. crassipes* and *S. molesta* dense mats in the Swartkops River did not influence periphyton recovery, but rather showed how periphyton were good biological indicators of the observed water quality.

CHAPTER 5

Aquatic Macroinvertebrate Responses to Invasive Alien Aquatic Plant Species Phytoremediation in an Urban River, Eastern Cape, South Africa

5.1 Introduction

Freshwater ecosystems are continuously being assessed for their biological distinctiveness and sustainability for aquatic species conservation (Arimoro *et al*., 2015). However, pollution of freshwater ecosystems through anthropogenic activities is a major concern and threatens environmental sustainability (Dodds *et al*., 2013). Such activities may include agriculture, industrial and domestic waste-water run-off that often result in river catchment modification, which alters the aquatic hydrological regime and water quality (Nyenje *et al*., 2010). Urbanisation is one of the key factors affecting freshwater ecosystems at multiple scales, and it poses a great threat to aquatic biodiversity, river integrity and the ecosystem services they provide (Hoyer and Chang, 2014). Urban river systems are often degraded because effluent discharge from sewage treatment works and run-off from settlements, storm water drainage alters the flow (Halstead *et al*., 2014) and influence the water quality by increasing water nutrient concentrations, reducing dissolved oxygen concentration, which results in loss of aquatic biological and functional diversity (Rosa *et al*., 2014; Odume, 2019).

Aquatic macroinvertebrate assemblages respond to various pollutants in freshwater systems; they are widely used for biological monitoring, providing a measurable response to river health (Chutter 1994; Dickens and Graham, 2002; Kenney *et al*., 2009; Balachandran *et a*l., 2012; Uherek and Pinto Gouveia, 2014; Odume *et al*., 2019). For example, the South African Scoring System (SASS) is one such technique developed to assess riverine and stream water conditions (Chutter, 1998; Dickens and Graham, 2002). The South African Scoring System assigns each taxon a sensitivity score, which is used in assessing alterations in environmental conditions and how they change over time, revealing trends in water quality. For example, Hirudinea and Chironomidae are known aquatic macroinvertebrate taxa tolerant to severely polluted freshwater systems, in contrast to Ephemeroptera, Plecoptera and Trichoptera species known to be sensitive to pollution (Dickens and Graham, 1998; Bonada *et al*., 2005; Azrina *et al*., 2006; Davies *et al*., 2010; Ratia *et al*., 2012; Olomukoro and Dirisu, 2014). Agboola *et al*. (2019) conducted a study on the effectiveness of aquatic macroinvertebrate community-based metrics to examine the health of 15 river catchments from 38 locations in KwaZulu-Natal, South Africa; samples were collected from headwaters (less impacted sites) to the lowland rivers (most impacted sites). The study reported that the total number of Plecoptera, Trichoptera, and Ephemeroptera individuals increased with improvement in overall water quality (from lowland to headwaters), as supported by Walsh *et al*. (2005), while Oligochaetes and Chironomidae dominated lowland polluted sites (agricultural activities and paper conversion industries) that had high concentrations of nutrients and organic matter. Another study by Thiere and Schulz (2004) assess the impact of agricultural-related activities on aquatic macroinvertebrate assemblages at Lourens River, Western Cape, South Africa. The study reported a significant reduction in Ephemeroptera, Helodidae (Coleoptera), and Leptoceridae (Trichoptera) taxa abundance on highly impacted sites, while Ancylidae (Grastropoda) occurred in high densities. Thus, the change in aquatic macroinvertebrate taxa assemblage patterns is an indicator of river health (Arimoro *et al*., 2012).

Aquatic macroinvertebrates are ideal biological indicators as they are easily identified to family level, easy to collect and handle, and they show a measurable response to disturbance, providing the information needed to interpret the water quality and river health; together with their sedentary nature and wide distribution, they are used across different water bodies (Lenat and Resh, 2001; Basset *et al*., 2004; Walsh, 2006; Masese *et al*., 2009; Sharma and Chowdhary, 2011; Odume *et al*., 2012; Fierro *et al*., 2017). Midgley *et al*. (2006) and Coetzee *et al*. (2014) reported reduced macroinvertebrate abundance and diversity under *P. crassipes* mats as compared to open water patches, indicating the negative impact floating IAAP species have on aquatic biodiversity. In addition, these studies reported that pollution-tolerant taxa were the most abundant taxa beneath the floating mat compared to Ephemeroptera, Trichoptera and Plecoptera, due to the unfavourable conditions created by IAAP species mats (e.g. low dissolved oxygen and light; high levels of carbon dioxide = anoxic conditions).

The negative impact of urbanisation on the biota of South African rivers has been demonstrated by previous studies. For example, Wepener *et al*. (2011) reported mass fish mortality rates in the Vaal River, South Africa due to multiple stressors associated with urbanisation. These stressors included pollutants from wastewater treatment works, mining and industrial effluents, and raw waste from informal and formal settlements within the Vaal Catchment. Loskop Dam in the Olifants River also reported fish and crocodile mortalities, which were attributed to anthropogenic activities (agriculture, industries, and coal mining) upstream, and along the tributaries (Dabrowski *et al*., 2013; Verhaert *et al*., 2017). Berg River in the Western Cape, South Africa is no exception (de Villers, 2007), and rivers in the Melbourne region in Australia suffer the same pollution, according to Walsh *et al*. (2001). It is clear that for decades, anthropogenic activities have severely affected freshwater ecosystems and the biota, and better management strategies are required.

The Swartkops River is a typical urban river system that is severely impacted by a wide range of anthropogenic activities (Odume *et al*., 2012). Major sources of pollution surrounding the river catchment include settlement run-off (Kwanobuhle, Despatch and Uitenhage), industrial facilities, municipal wastewater treatment works, agricultural activities and storm water drainage systems (Binning and Baird, 2001). These activities release raw, untreated wastewater that finds its way into the river system, and as a result, alters the physical, chemical and biological components of the river system (Odume *et al*., 2016). The extensive pollution in the river catchment has been reported as altering the physicochemical variables, negatively impacting aquatic biota diversity and richness (Odume *et al*., 2012). Pollution in the Swartkops River has also led to the excessive growth of IAAP species, forming dense mats in various sections along the river. These plants species proliferate under disturbed environments, out-competing the remaining native species, and altering the ecosystem structure and functions (Hill and Coetzee, 2017). However, various authors, including Singh *et al*. (2012), Favas *et al*. (2012) and Saha *et al*. (2017) report IAAP species' potential for assimilating excessive nutrients and heavy metal in aquatic environments as also described in Chapter 3 of this thesis.

Given the increase in alteration of natural vegetation by landscape development and the ever-worsening use of chemicals at the catchment level, there is a necessity to continuously assess the status of rivers and aquatic biota. Having learned that IAAP species form dense mats that alter water quality and reduce aquatic biodiversity thus compromising ecosystem structure (Midgley *et al*., 2006; Chamier *et al*., 2012; Coetzee *et al*., 2014), there is a need for ecologically meaningful mitigation programmes that can be easily assessed (Arimoro *et al*., 2015). Previous studies (e.g. Favas *et al*., 2012; Singh *et al*., 2012; Saha *et al*., 2017) have justified the use of floating IAAP species as reliable techniques to assimilate excessive nutrients and chemicals in urban and eutrophic systems. This chapter investigates whether the potential phytoremediation effects of IAAP species will improve the recovery of aquatic macroinvertebrate diversity and assemblage structure in the Swartkops River system. I hypothesise that IAAP mats will improve water quality downriver (as seen in Chapter 3) and in so doing, assist in the recovery of aquatic macroinvertebrate diversity and shift to pollution sensitive assemblage structure downstream of IAAP mats.

5.2 Materials and methods

5.2.1 Study area and Data collection

Details on study sites and samples collection are described in Chapter 2.

5.2.2 Data analysis

To compare the effect of IAAP species mats phytoremediation on the recovery of aquatic macroinvertebrate biodiversity indices from upstream to downstream IAAP mats; relative taxa abundance (N), taxa richness (S), Shannon's diversity index: $H' = -\sum_{i=1}^{s} pi \ln pi$, (where *pi* is the proportional abundance of taxa *i* in the sample given *s* taxa), and Pielou's evenness: $J' = \frac{H'}{\ln(S)}$, were computed between sites in PRIMER version 6.1.16 and PERMANOVA^{+} version 1.0.6 using the DIVERSE function (PRIMER-E Ltd, Plymouth; Clark and Gorley, 2006). Then, to test significant difference between biodiversity indices, the Shapiro-Wilk and Levene's tests were employed to test for normality and homogeneity of variances, respectively. Because aquatic macroinvertebrate biodiversity indices data were not normally distributed (Shapiro-Wilk, $P<0.05$) and the variances were not homogenous (Levene test, $P>0.05$), a non-parametric test, the Kruskal-Wallis ANOVA test with multiple comparisons was used to test for significant differences in aquatic macroinvertebrate biodiversity indices with sites as factor.

Additionally, to investigate aquatic macroinvertebrate functional diversity, aquatic macroinvertebrate taxa were grouped according to their functional feeding groups (FFGs): collector-filters, collector-gatherers, scrapers/herbivores, shredders and predators, following Palmer *et al*., (1996), Tomanova *et al*., (2006), Zilli *et al*., (2008), Uwadiae, (2010), Masese *et al*., (2014), Ramírez and Gutiérrez-Fonseca, (2014), and Cummins, (2016). A chi-square test was then employed to test for significant differences in FFGs proportions between sites. The patterns of aquatic macroinvertebrate assemblages between treatments (e.g. S1, upstream and downstream IAAP mat derived from sites) were evaluated using square-root transformed relative taxa abundance with Bray-Curtis similarity resemblance matrix to decrease the importance of abundant aquatic macroinvertebrate families and increase the influence of rare aquatic macroinvertebrate families (Clarke and Ainsworth, 1993). Thereafter, a constrained Canonical Analysis of Principal Coordinates (CAP) ordination was used to visualise aquatic macroinvertebrate assemblage patterns between treatments. Further, ANOSIM was performed to test aquatic macroinvertebrate assemblage patterns between treatments along the Swartkops River system using PRIMER version 6.1.16 and PERMANOVA+ version 1.0.6 (PRIMER-E Ltd, Plymouth; Clark and Gorley, 2006).

As in Chapter 4, if aquatic macroinvertebrate assemblage patterns came out significant between treatments, the indicator value species analysis method (IndVal) was further conducted to identify and highlight discriminatory aquatic macroinvertebrate taxa-influencing assemblage differences between treatments. The significance of the indicator value for each taxon was tested by a Monte Carlo randomisation test with 9999 permutations, $\alpha=0.05$, in PC-ORD version 5.13 (McCune and Mefford, 2006). Aquatic macroinvertebrate families with a significant indicator value of ($P<0.05$) were considered as indicator families. Then, to determine which environmental variables influenced aquatic macroinvertebrate assemblage composition, distance based linear modelling (DistLM) was used. The DistLM provided environmental variables that best explain aquatic macroinvertebrate assemblage, thus a distance based redundancy analysis (db-RDA) bi-plot was created to illustrate significant environmental variables that influenced assemblage variation between treatments.

5.3 Results

5.3.1 Aquatic macroinvertebrate diversity and assemblage patterns

Aquatic macroinvertebrate relative abundance (H=14.277, df=6 P=0.0007), and Pielou's evenness (H= 25.152, df=6, P=0.0007) were significantly different between sites. However, aquatic macroinvertebrate taxa richness (H=2.294, df=6, P=0.317) and Shannon diversity (H=1.9409, df=6, P=0.378) were not significantly different between sites. Aquatic macroinvertebrate relative abundance was highest upstream of mat 2, at site 5 and least at site 1. Overall, the upstream sites had greater macroinvertebrates abundance than the downstream sites (Figure 5.1A). Relative abundance started increasing from site 1, reaching high abundance upstream of mat 2, at site 5; thereafter, it showed a drastic decline downriver, upstream and downstream of mat 3 (sites 8 and 10). Aquatic macroinvertebrate taxa richness was high downstream of mat 3, at site 10 and the lowest upstream of mat 2, at site 5 (Figure 5.1B). Both aquatic macroinvertebrate Pielou's evenness and Shannon diversity showed similar trends, which were the opposite of those observed from relative abundance. Indices showed an inverse bell-shape, where site 1, site 8 and site 10 had the highest scores, whereas sites 5 and 7 were the lowest (Figure 5.1C & D).

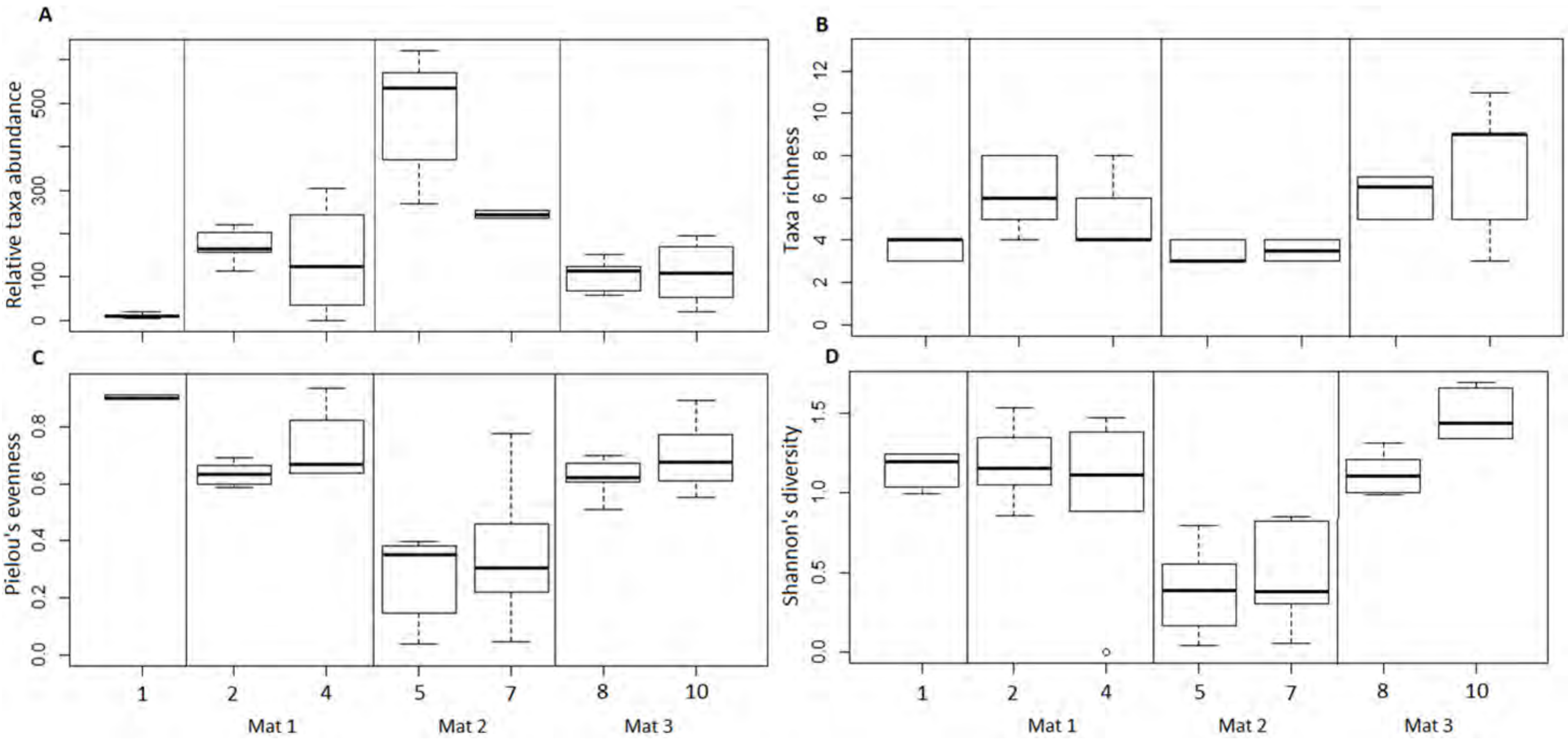

Figure 5.1: Aquatic macroinvertebrate biodiversity indices; (A) relative taxa abundance, (B) taxa richness, (C) Pielou's evenness and (D) Shannon's diversity index from seven sites of three mats at the Swartkops River system, Eastern Cape, South Africa. Box plots represent median values with interquartile range. Whiskers represent maximum and minimum values.

A total of 33 aquatic macroinvertebrate families were collected, identified and assigned to FFGs (Appendix 2). The total proportion of aquatic macroinvertebrate FFGs showed a clear, significant difference between sites: collector-gatherers (X^2=289.19, df = 41, P< 0.001), collector-filterers (X^2=10077, df = 41, P< 0.001), predators (X^2=3275.6, df = 41, P< 0.001), scrapers (X^2=84.667, df = 41, P<0.001), and shredders (X^2=65, df = 41, P=0.010). Site 1 was well represented by all five FFGs compared to other sites; this site had the highest proportion of shredders and scrapers, which drastically decreased mid-river, and returned at sites 8 and 10 in small proportions. This was similar to collector-gatherers that were represented in a small proportion (<5%) which reduced to half at sites 5 and 7 and increased upstream and downstream of mat 3 (sites 8 and 10). Collector-filterers and predators were well presented throughout the sites, with a high percentage of predators recorded downstream of mat 2, at site 7, and the lowest upstream of mat 2, at site 5; collector-filters were more dominant upstream of mat 2, at site 5 and the least downstream of mat 2, at site 7 (Figure 5.2).

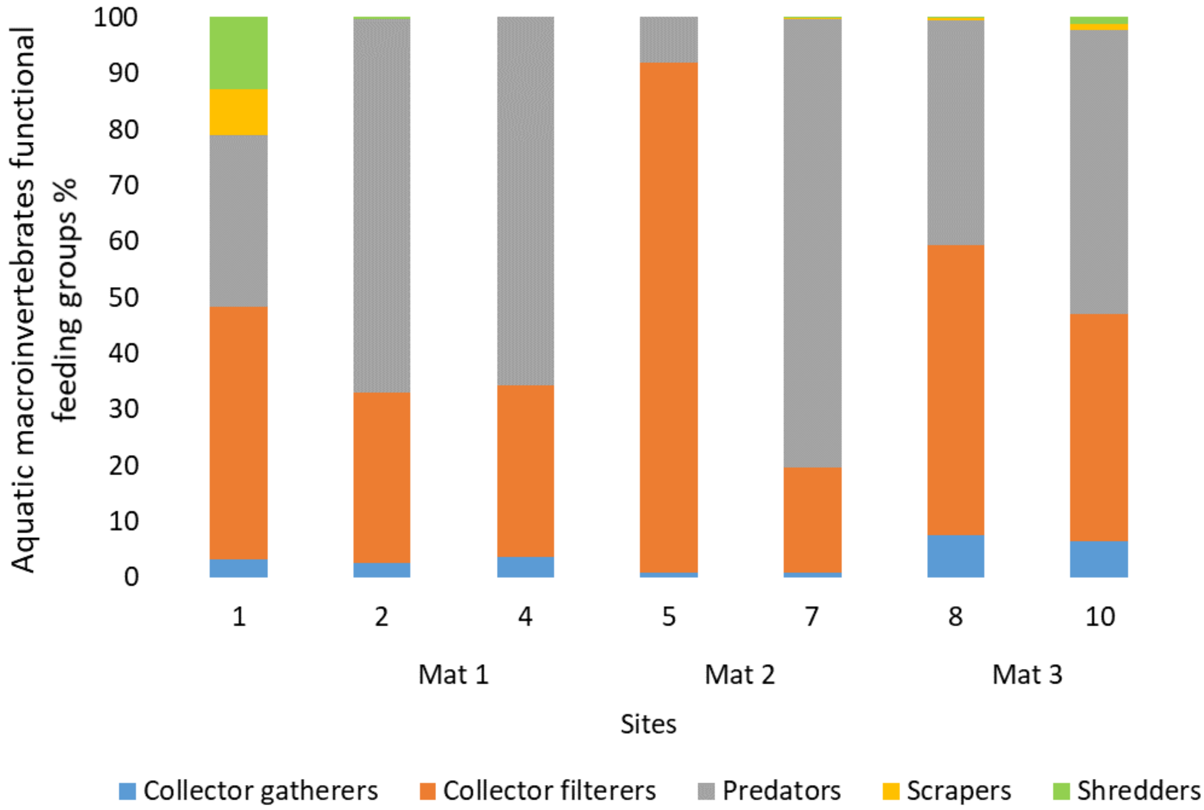

Figure 5.2: Percentage composition of aquatic macroinvertebrate functional feeding groups from seven sites in the Swartkops River system, Eastern Cape, South Africa.

Canonical Analysis of Principal Coordinates ordination revealed a clear separation between the three treatments, S1, upstream and downstream, based on aquatic macroinvertebrate assemblage composition. The S1 treatment was distinct from the upstream and downstream treatments (Figure 5.3), with some overlap between the upstream and downstream treatments. The canonical square correlation, CAP axis 1 explained 87.9% of aquatic macroinvertebrate variation (δ^2=0.88), while the CAP axis 2 accounted for 54.2% (δ^2= 0.54), indicative of complete and moderate differences in aquatic macroinvertebrate assemblages between treatments (Figure 5.3). ANOSIM revealed that aquatic macroinvertebrates showed a complete assemblage variation between the S1 and upstream treatment (Global R=0.95; *P*=0.01), and S1 and downstream treatments (Global R=0.78; *P*=0.01). Upstream and downstream treatments showed no assemblage variation (Global R=0.139; *P*=0.01), thus similar composition, as visually illustrated by CAP analysis. IndVal results revealed that Elmidae and Libellulidae taxa were identified as indicator families for the S1 treatment, Chironomidae for the upstream treatments, and Ceratopogonidae, Hirudinea and Potamonautidae as characteristic taxa for the downstream treatment (Table 5.1).

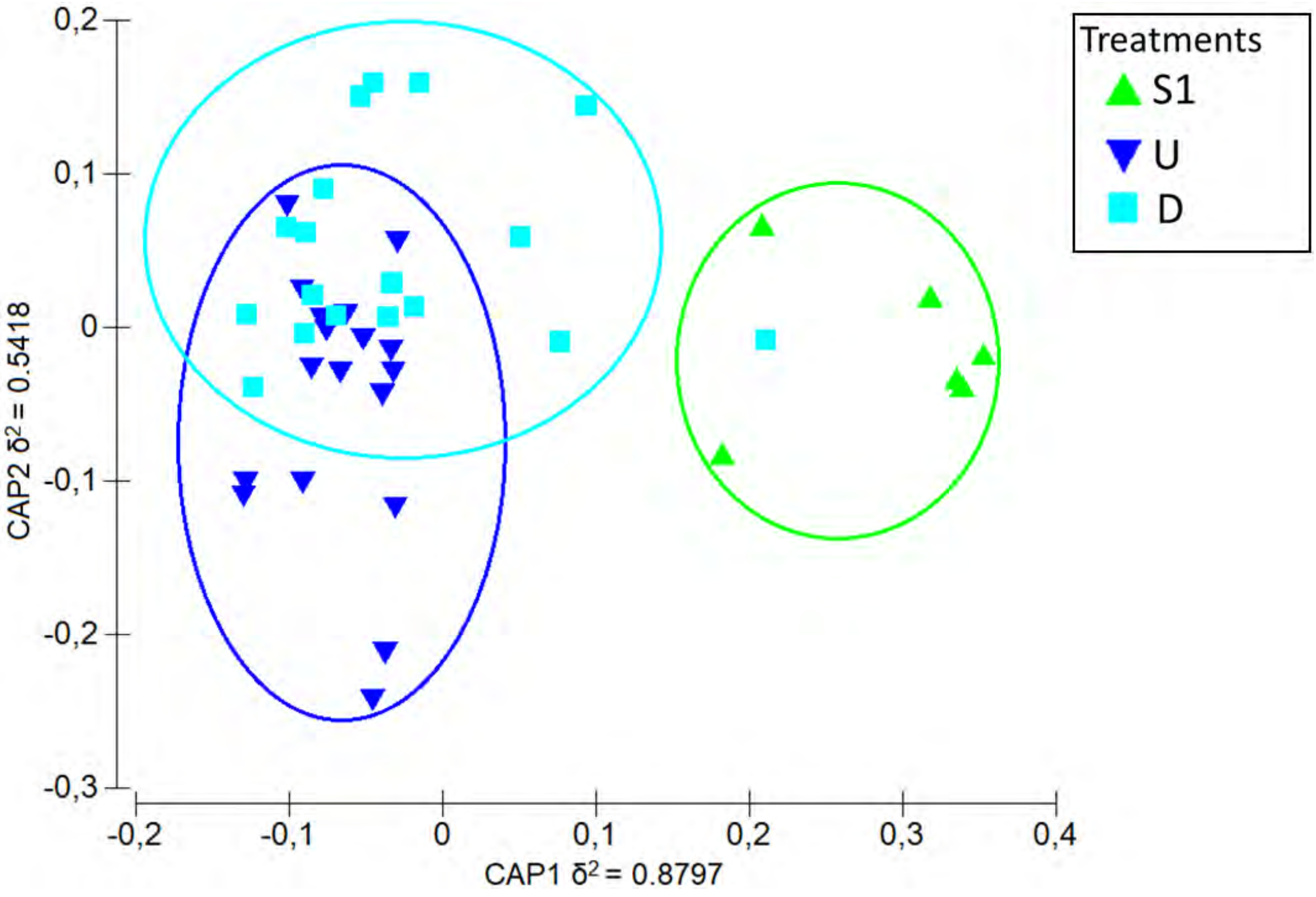

Figure 5.3: Canonical Analysis of Principal Coordinates (CAP) ordination bi-plot illustrating aquatic macroinvertebrates assemblage patterns between treatments at Swartkops River system, Eastern Cape, South Africa. The δ2 = represents total variation in aquatic macroinvertebrates explained per axis.

Table 5.1: List of significant (P<0.05) indicator values of aquatic macroinvertebrate families from seven sites in Swartkops River system, Eastern Cape.

Family	IndVal%	Mean	St. Dev	*P*-value	Site	Treatment	Mat
Elmidae	50.0	13.3	7.85	0.009	1	S1	S1
Libellulidae	36.7	15.2	8.24	0.039	1	S1	S1
Chironomidae	66.1	25.3	6.33	0.0001	4	Upstream	Mat 2
Ceratopogonidae	43.3	15.5	8.54	0.035	10	Downstream	Mat 3
Potamonautidae	53.0	15.5	7.24	0.002	10	Downstream	Mat 3
Hirudinea	45.0	22.3	4.57	0.0001	7	Downstream	Mat 2

Distance-based linear model (DistLM) analysis provided six environmental variables that contributed significantly to the total aquatic macroinvertebrate assemblage variation of 40.9% grouping between treatments (Figure 5.4). Environmental variables included sediment chemistry, for example, Zn, P, Cu, and Cr concentrations and water chemistry variables, for example, Fe and EC. Water chemistry Fe showed a strong correlation with the S1 treatment, and EC showed a negative association, whereas the sediment chemistry, for example, Zn, P, Cu, and Cr showed a strong correlation with the upstream treatment and the opposite for the downstream treatments (Figure 5.4).

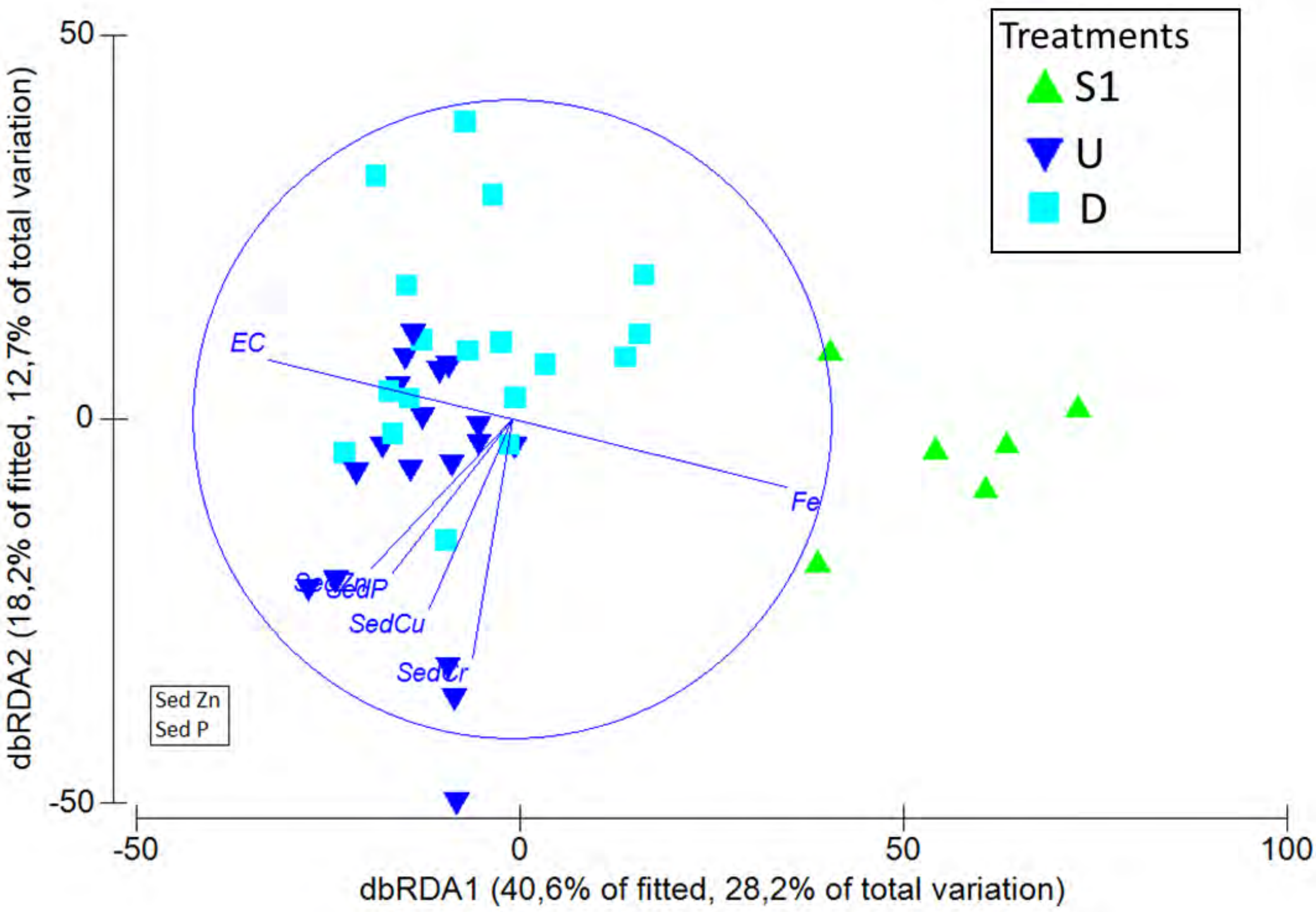

Figure 5.4: Distance based redundancy analysis (db-RDA) bi-plot illustrating relationships between environmental variables that best describe aquatic macroinvertebrate assemblages between the S1 (S1), upstream (U) and downstream (D) treatment at the Swartkops River system. Only environmental variables that influenced grouping of treatments with a strong Pearson correlation of (r>0.6) are represented on the plot.

5.4 Discussion

The study provided an opportunity to understand how the presence of dense IAAP mats species like *P. crassipes* and *S. molesta* along an urban Swartkops River improved water quality, on the assumption that recovering aquatic macroinvertebrates are biological indicators of water quality. Some authors have questioned whether the presence of *P. crassipes* in a river system serves as an additional habitat for aquatic macroinvertebrate assemblages, increasing habitat complexity and thus diversity, or whether they suffocate aquatic macroinvertebrate populations (Masifwa *et al*., 2001; Brendonck *et al*., 2003).

Results obtained from the study were in agreement with our first hypothesis that IAAP mats will improve environmental variables downstream of the IAAP mats, as seen in Chapter 2, but contradicts the assumption that aquatic macroinvertebrates will recover downstream of an IAAP mat as a sign of successful phytoremediation. The lack of support by the results for aquatic macroinvertebrate recovery could be attributed to a number of factors; it could be that IAAP mats do not modify the water chemistry enough to alter the downstream structure of macroinvertebrates assemblages. Swartkops River has multiple pollution entry point sources and other non-point sources, thus there are constant influxes of nutrient-rich and contaminated waste water; although assimilated by the IAAP mat, sufficient quantities of effluent affected aquatic macroinvertebrates negatively. Other factors, such as the distance between sampling treatments (upstream – IAAP mat - downstream), could also have contributed: some treatments were located about one kilometre (upstream of mat 2, at site 5) away from the IAAP while others were located 65 metres from the IAAP mat; this did not account for additional point sources between the upstream and the IAAP mat. Hence, sites both upstream and downstream of mat 2 showed reduced periphyton (Chapter 4), aquatic macroinvertebrate taxa richness, and Shannon diversity scores, and high relative abundances of pollution-tolerant periphyton (Chapter 4) and aquatic macroinvertebrates taxa. This further signified that even if dense IAAP mats were assimilating pollutants, a significant volume of wastewater still persisted and changed the water physicochemistry down river.

Field experiments are also considered different, dynamic, and difficult to work with because of stochastic events such as droughts and floods which can affect the outcome of results. Since the study was the first of its kind in the highly impacted Swartkops River system, it is possible that phytoremediation techniques could be more effective in moderately impacted systems than in systems largely impacted, such as the Swartkops. This is because from Chapter 3, it was evident that phytoremediation was taking place, however since the river system is under a lot of stress from different effluents flushed through out the system, phytoremediation effects were not so clear anymore downstream sites, therefore a moderately impacted water system could benefit a lot from phytoremediation. A study by Phillips's *et al*. (2015) in the Swartkops estuary investigated the metal accumulation abilities of three macrophytes: *Phragmites australis, Spartina maritima* and *Typha capensis*. Phillip's study reported a high accumulation of heavy metals (e.g. Cd, Pb, Zn, and Cu) concentrated in *P. australis*, *S. maritima* and *T. capensis* organs (roots, stems and leaves) and sediments, thus showing sign of assimilation; however, the study did not report an improvement in the physicochemical or biological variables along the estuary.

The present study revealed how deterioration of the water quality in the Swartkops River was affecting different trophic levels of aquatic biota, and how this was important in using them as biological indicators (Odume *et al*., 2012). The upstream and downstream sites were similar in assemblage composition and showed no significant difference in biodiversity indices, clearly indicating that the presence of IAAP mats did not influence aquatic macroinvertebrate recovery, but rather how biota were sensitive biological indicators of a deteriorating river system. Aquatic macroinvertebrates biodiversity indices; family richness, Pielou's evenness and Shannon's diversity showed a similar trend to periphyton assemblage, where a drastic decline was observed upstream (site 5) and downstream (site 7) of IAAP mat 2, but thereafter increased going downriver. The substantially impacted site 5, upstream of mat 2, showed a high relative abundance of Chironomidae taxa, which is known for its high tolerance of impacted sites. Chironomidae larvae possess haemoglobin within their tissue which make it uniquely tolerant of nutrient-rich environments (Masese *et al*., 2009). Considering the fact that highly impacted sites cannot provide suitably satisfactory habitats for sensitive aquatic macroinvertebrate families, Chironomidae, which are able to withstand high levels of pollution, thrive there. The recorded abundance of Chironomidae also correlated with the amount of heavy metal concentration in both water and sediment, as they are noticeably tolerant of these conditions. Heavy metals analysis in water samples revealed that upstream treatment at mat 2, site 5 had the highest concentration of Fe, Cu, and Zn water chemistry and heavy sediment metals concentration; Zn, Cr, and Cu.

Eggermont and Verschuren (2003) and Masese *et al*. (2009) reported that the abundance of Chironomidae families can be effective indicators of severe stress in water columns compared to other aquatic macroinvertebrate families. These findings concur with findings by Elias *et al*. (2014) who reported that Oligochaeta and Chironomidae in Tanzanian rivers located in the Kilimanjaro regions were unaffected by high concentrations of organic and inorganic pollutants, such as industrial and household waste, agricultural activities, and other human alterations. The results of the present study also revealed that the dominant aquatic macroinvertebrate taxa were pollution tolerant. However, this contradicts the findings of Wright and Burgin (2009) who reported that the Chironomidae family includes both sensitive and tolerant species; while some Chironomidae species are pollution tolerant, some in the same Chironomidae family are pollution sensitive. Odume and Muller (2011) study reported that Chironomidae at both genus and species level were sensitive to differences in water quality, and further indicated that Chironomidae biotic indices (taxa richness and diversity) were sensitive to differences in water quality, decreasing from site 1 to the other downstream sampled sites, and thus could be used as indicators of water quality impairment.

Ormerod *et al*. (1994) indicated that different types of land use and the geographic region usually influence the dominance of specific aquatic macroinvertebrate taxa in water bodies. A study by Kibichii *et al*. (2007) reported that Baetidae and Simuliidae mostly dominated streams on Mount Kenya and the Njoro River in Kenya. Bredenhand and Samways (2009) indicate that upstream sites of a small river in the Cape Floristic Region (South Africa) had greater abundance and variance of aquatic macroinvertebrate families (Baetidae, Simuliidae and Leptophlebiidae) than downstream sites with silt and sediments that were mainly dominated by Caenidae. Some families in the downstream region included the families Hydraenidae, Hydrophilidae and Notonemouridae (Plecoptera). In the present study, the most dominant macroinvertebrate families were Chironomidae followed by Hirudinea, Belostomatidae, Coenagrionidae and Oligochaeta. Both upstream and downstream treatments had the same families of dominant aquatic macroinvertebrates, thus showing no aquatic macroinvertebrates variance (indicating no biological difference) according to Bredenhand and Samways (2009). These findings support the indicator value analysis that revealed that the dominating aquatic macroinvertebrate taxa at all sampled treatments were pollution-tolerant taxa, including Elmidae, Libellulidae, Hirudinea and Chironomidae. These results did not show improvement in aquatic macroinvertebrate taxa between the upstream and downstream sites.

Aquatic macroinvertebrates were grouped into functional feeding groups revealing common feeding attributes which are useful in understanding the complexity of aquatic macroinvertebrates communities (Gérino *et al*., 2003). The present study revealed that site 1 was more functionally diverse and heterogeneous, thus we assume that the community structure was more robust and resilient compared to other sites. Predator were the most abundant FFGs, with shredders being the least abundant. High predator community assemblage could be due to that they prey on other invertebrates by either swimming or crawling beneath or above water surface and capturing prey. Collector-filterers were the second dominant FFGs at all sites, followed by collector-gatherers. Both groups were represented by Chironomidae (upstream of mat 2, at site 5) and Oligochaeta (upstream of mat 3, at site 8). It is possible that their dominance was influenced by suspended organic matter in the river. Scrapers and Shredders were the least abundant FFGs; this could be attributed to limited resources such as low canopy cover for shredders resources as well as deteriorating water conditions that could not have favoured a high composition of scrapers.

The ecological impacts of anthropogenic activities (farming and industrial activities, run-off from informal settlements, wastewater treatments) in the Swartkops River system were analysed through environmental and biological water quality assessments. Findings from the study revealed that the observed poor water quality affecting aquatic macroinvertebrate diversity was a result of anthropogenic activities. The study's general findings revealed that the Swartkops River system is continuously deteriorating, as indicated by other authors. It was also observed that the poor water quality conditions did not change with time but did change significantly with sites, suggesting that the ecological state of the river was strongly influenced by activities occurring at a local scale. Major water quality degradations were observed upstream of mat 2, at site 5, where the water quality was very poor because of wastewater treatment and industrial activities. This site also recorded the lowest aquatic macroinvertebrate richness, evenness, and Shannon's diversity. However, the site recorded the highest aquatic macroinvertebrate abundance, composed mostly of pollution-tolerant taxa (Chironomidae). Although the river system had patches of IAAP species dense mats that assimilated organic and inorganic nutrients, there was no recovery of aquatic macroinvertebrates at downstream sites as we had hypothesised.

The study revealed how pollutants in water from anthropogenic activities had an impact on the distribution of aquatic macroinvertebrates assemblages, permitting an establishment of high abundance of pollution-tolerant aquatic macroinvertebrates families dominating both upstream and downstream sites, indicating the deterioration of the river system over time. Upstream and downstream sites showed no significant differences in biodiversity indices and had similar aquatic macroinvertebrate assemblage composition. In addition, IndVal analysis also revealed that only pollution tolerant families were dominant with few pollution sensitive species. This response served as a clear indication of how aquatic macroinvertebrates are excellent indicators for assessing water quality and ecosystem integrity and can be used as biological indicators. This further revealed that the presence of IAAP species for phytoremediation was not effective in recovery of aquatic macroinvertebrates downstream as they were with environmental variables.

CHAPTER 6

General Discussion, Conclusions and Recommendations

6.1 Introduction

The significance of water to human life has driven settlements closer to streams and river systems (Malmqvist and Rundle, 2002), resulting in urbanisation, industrialisation and other anthropogenic activities in catchment areas (Vörösmarty *et al*., 2013). Following these activities, water pollution has been a common factor driving freshwater ecosystem degradation and loss of aquatic biodiversity (Lake, 2000; Simaika and Samways, 2012), and so altering natural processes (Khan *et al*., 2010). The Swartkops River system is no exception, and due to landscape developments within its catchment, it is subjected to significant external disturbances which have altered the water quality and the ecosystem structure as seen in the present study and that of Binning and Baird (2001), Odume *et al*. (2012), Odume *et al*. (2016), and Nyawo, (2017).

Pollution in the Swartkops River system has also driven the prevalence of IAAP species, particularly *P. crassipes* and *S. molesta*. These plants deprive aquatic biota (i.e. aquatic microalgae, invertebrates and vertebrates) of access to sunlight and oxygen, leading to lack of energy transfer or subsidies to higher trophic levels and thus causing local die-offs (Midgley *et al*., 2006; Coetzee *et al*., 2014). Authors (e.g. Rezania *et al*., 2015; Victor *et al*., 2016; Ng and Chan, 2017) have reported that dense IAAP mats reduce both organic and inorganic pollutants in aquatic systems through a process called phytoremediation. This suggests that IAAP species can be used on small and large scales to improve water quality. This study tested the effectiveness of IAAP species in assimilating water and sediment pollutants (Chapter 3). The possible use of biological indicators, such as periphyton and aquatic macroinvertebrates, to assess water quality improvement from IAAP in the Swartkops River system (Chapters 4 and 5).

Invasive alien aquatic plant species assimilated (Chapter 3) major water and sediment nutrients. The highest recorded concentration reduction in water chemistry was 74% for NO_3 concentration between the upstream (site 2) to downstream (site 4) of IAAP mat 1. This reduction was followed by PO_4 concentrations that recorded 8% reduction from the upstream (site 5) to downstream (site 7) of IAAP mat 2. Sediment chemistry; PO_4 concentration showed the highest reduction of 87% from the upstream (site 5) to downstream (site 7) at IAAP mat 2, followed by a 46% reduction in Fe concentration from upstream (site 2) to downstream (site 4) at IAAP mat 1. These findings clearly showed that IAAP species greatly improved the water and sediment quality downriver, justifying their use for phytoremediation in urban rivers.

This chapter discusses the findings from the three data chapters and places them in a broader, global context of phytoremediation. The chapter provides a review on phytoremediation, further compares biological indicators to environmental change, and looks at the implications of using multiple biological monitoring endpoints and their taxonomic resolution to assess remediation at the Swartkops River, and possibly, other freshwater ecosystems. Lastly, phytoremediation practices, application and assessments are reviewed using evidence-based case studies like the Swartkops River, together with a discussion of experimental designs, shortfalls or limitations, and future research and recommendations.

6.2 Assessing invasive alien aquatic plant (IAAP) species phytoremediation success using multiple biodiversity indices

The poor water quality in the Swartkops River system is a result of pollutants from sewage treatments, agricultural activities, industrial and domestic waste. Periphyton and aquatic macroinvertebrates were used as biological indicators to assess the ecological integrity of the Swartkops River system following phytoremediation by dominant IAAP species, *P. crassipes* and *S. molesta*. Periphyton and aquatic macroinvertebrates were sensitive and reliable biological indicators to detect water quality deterioration, as supported by Azrina *et al*. (2006), Lane and Brown, (2007), Taylor *et al*. (2007b), Arimoro and Ikomi, (2008), Berthon *et al*. (2011) and Dao and Beardall, (2016).

There has been controversy on biological assessments based on family level taxonomic resolutions. Some authors have reported that higher taxonomic identification (e.g. family and order) are less informative than lower taxonomic identification (e.g. genus and species) (Jones, 2008). Authors have argued that variation in tolerance to stressors at genus level varies significantly (Bailey *et al*., 2001; Jones, 2008). In most cases, identification of biological community assemblages to a certain taxonomic level are primarily influenced by the study's aims and objectives. Schmidt-Kloiber and Nijboer (2004) report that species taxonomic identification is often not used and sometimes impossible due to lack of taxonomic expertise, unavailability of literature or autecological information which may only be known by specialists, lack of time and human resources including costs of identification. Periphyton and aquatic macroinvertebrates have different sensitivity to water quality; they respond differently to various pollutants. For the present study, the family level taxonomic resolution was used for aquatic macroinvertebrates assessments. Findings revealed that aquatic macroinvertebrates taxonomic resolution was reliable, responding well to water quality variables in the Swartkops River. These findings were similar and as informative as lower taxonomic resolution (species level identification) that was used for periphyton. The reliability and informativeness of aquatic macroinvertebrates family level resonated with findings from studies by Riley (2008), Beyene *et al*. (2009), Elias *et al*. (2014), Xu *et al*. (2014), Dalu *et al*. (2017) and Nhiwatiwa *et al*. (2017). As noted in Reid (2015) study, assemblage composition is fundamental for the functioning of aquatic ecosystems. In the present study and that of Reynolds *et al*. (2002) and Dalu *et al*. (2014a) water quality variables were important and influenced both periphyton and aquatic macroinvertebrates assemblages.

Grouping of aquatic macroinvertebrates into FFGs reveal shared or common feeding attributes, which can be useful in understanding the complexity of aquatic macroinvertebrates communities by combining morphological characteristics and behavioural attributes (Gérino *et al*., 2003; Ramírez and Gutiérrez-Fonseca, 2014). Findings from the present study revealed that site 1 was more functionally diverse and heterogeneous compared to all other sampled downriver sites which were highly impacted. Thus we assume the community structure was more robust, resilient and more acceptable with a good representative of FFGs complexity compared to other sites. The most dominant FFGs at most sites were predators, followed by collector-filters, collector-gatherers, scrapers, with shredders being the least abundant functional group. The high assemblage of predators may be due to the fact that they prey on other aquatic and terrestrial invertebrates, thus food availability is the main factor that influences their abundance and distribution. Predators search for prey by either crawling or swimming beneath (e.g. aquatic beetles) and above (e.g. pond skaters and striders) water surfaces or flying above the water surfaces (e.g. dragonflies). In addition, predators employ strategies to capture prey; they have strong specialised teeth and modified mouth parts to assist in capturing and consuming of prey (Ramírez and Gutiérrez-Fonseca, 2014).

Collector-filters reduce floating particles to downstream reaches, this group also have special adaptations to capture particles in water; for example, Simuliidae larvae have modified mouth parts that assist in capturing food particles (Ramírez and Gutiérrez-Fonseca, 2014). Collector-filters and collector-gatherers were the second dominant feeding group represented by Chironomidae (upstream of mat 2, at site 5) and Oligochaeta (upstream of mat 3, at site 8). Their dominance was influenced by an increase in suspended organic matter from all activities around site which were washed or released into the river (Dalu *et al*., 2017). Both the upstream and downstream sites were mostly dominated by collector-filters, collector-gatherers and predators; functional diversity was homogenised over time thus there was less difference in taxa composition, but more similarity due to habitat modification (deteriorating water quality).

Scrapers and shredders were the least abundant of all FFGs. The low abundance representation of scrapers and shredders could be attributed to the water quality state in the Swartkops River system. Due to water quality deterioration the development and composition of periphyton promoted pollution tolerant taxa, which was not the preferred or palatable group to periphyton for depended organisms such as scrapers. Majority of the sites in the Swartkops river had <10% canopy cover, therefore there was not enough plant material deposited onsite, leaving the shredder community with limited resources to cut/chew, thus lead to reduced percentage abundances.

6.3 Potential use of periphyton and aquatic macroinvertebrates as bioindicators to assess phytoremediation success

As discussed before, degraded freshwater ecosystems are a result of a wide range of anthropogenic activities along river catchments. These activities modify physical habitats, alters water physicochemical variables, which often results in removal and addition of species (Malmqvist and Rundle, 2002). There is, therefore, a growing interest in assessing these river conditions, applying restoration techniques and measuring recovery of aquatic biota to solve problems that arise with degraded freshwater ecosystems. Recovery of rivers is generally defined as the improvement of river systems after sustaining alterations; that is, reversing altered river systems or sites closer to their original or alternative state with normal functioning (Geist and Hawkins, 2016). Generally recovery is dependent on measurable success mainly ecological recovery (Palmer *et al*., 2005; Jansson *et al*., 2007; Johnson *et al*., 2010; Geist and

Hawkins, 2016), these measurements address recovery in terms of physical properties (e.g. sediment dynamics and riparian vegetation restoration), chemical properties (reduction in organic and inorganic excessive amounts of contaminants), and biological properties which addresses change in biological indices assemblage patterns (Geist and Hawkins, 2016).

Recovery improves the systems ecological condition, leading to a more self-sustainable and resilient system, however, this can be challenging if the cause of river degradation is unknown (Geist and Hawkins, 2016). In the present study, recovery was measured in terms of improvement in environmental and biological variables that were sampled and analysed between various sites. Since IAAP species are known to be excellent phytoremediation agents, it was hypothesised that both environmental and biological variables would improve downstream IAAP species dense mats. Meaning that there would be a significant difference in nutrient and heavy metal concentration in upstream sites compared to downstream sites; as well as significant difference in biodiversity (periphyton and aquatic macroinvertebrates) and assemblage composition. Findings from the present study revealed that indeed there was improvement of various environmental variables on some of the downstream sites showing recovery of the system with assistance of IAAP species. However, both upstream and downstream sites were similar in terms of biological assemblage composition, showing no significant difference and improvement. This further showed how the IAAP species dense mats did not influence recovery of biological variables as they did with environmental variables.

Lack of periphyton and aquatic macroinvertebrates assemblage recovery could be attributed to a few factors. The Swartkops River water quality have been reported to be in a deteriorating state (Binning and Baird, 2001; Odume *et al*., 2012; Nyawo, 2017), and this was also observed on the present study. During the course of the study, it was observed that the Swartkops River had multiple point and non-point effluent sources, thus constant influxes of both organic and inorganic pollutants were released into the system. The constant influxes of nutrients and heavy metal pollutants could have also made it impossible for IAAP species to assimilate and show recovery of periphyton and aquatic macroinvertebrates downstream sites. In addition, the distance between the sampling sites was not consistent and this influenced more pollution sources and influxes before the next sampled site, hence no recovery. It was evident that pollutants persisted up until the last sampled downstream. Giller (2005) and Geist and Hawkins (2016) indicated that the first step to enable recovery of a degraded ecosystem is by reduction or removal of impacts causing degradation, and no continuous harm should be inflicted into a system during the recovery or restoration phase. In addition, Johnson *et al*. (2010) also indicated that successful recovery/ restoration can be expected to occur in the absence of continuous human-induced activities which are main drivers of change, thus it was impossible to see recovery of periphyton and aquatic macroinvertebrates in the Swartkops River due to constant multiple point and non-point pollution effluents into the river. Results from this clearly show that the system should be placed under strict management intervention, where sites 5 and 7 to be tagged as sites of management interest to further investigate the type and impact of pollution sources which showed to be severe on the system.

6.4 The potential use of IAAP as phytoremediation agents

Phytoremediation is an environmentally friendly technique that is solar driven, cost effective, and applicable to various water bodies, both lotic and lentic (Tangahu *et al*., 2011; Singh *et al*., 2012). Plants used for phytoremediation are generally well equipped to handle toxicity by various mechanisms that retain and stabilize inorganic compounds which can be toxic by breaking them down, reducing their concentrations and storing them in vacuoles (Weyens *et al*., 2009; Kavamura and Esposito, 2010). For example, phytoremediation has improved contaminated topsoil by conserving its fertility and utility (Ali *et al*., 2013). Some authors have reported on improved water quality after the application of phytoremediation (Lu *et al*., 2010; Singh *et al*., 2012). This technique has been regarded an excellent global method of reducing pollution caused by enhanced nutrient concentrations and organic matter (Teixeira *et al*., 2011). The convenience of phytoremediation in both soil and water has been thoroughly researched, and findings have spiked an interest in screening more, different plant species that can be used for phytoremediation (Wang *et al*., 2017).

Various studies have reported on the successful use of IAAP species for phytoremediation (Table 6.1). From the table, majority of phytoremediation experiments were lab based experiments which investigated the phytoremediation technique using IAAP species in assimilating heavy metal pollutants. Only a few field tested experiments were recorded, these experiments tested phytoremediation ability in remediating agricultural (nutrient) polluted river systems. Similarly, none of the experimental type (lab or field) investigated the response or recovery of aquatic organisms, instead focused on the improvement in water quality, nutrient and heavy metal reduction following phytoremediation application. All experiments yielded positive results with high reduction rate in both nutrient and heavy metal concentration and further supported the efficiency of phytoremediation in treating different types of pollutants in freshwater ecosystems. The present study assessed the IAAP species phytoremediation success using biological indicators. Although there were some fluctuations, the present study's findings on improvement and reduction of heavy metals and nutrient concentration supported findings by other studies in (Table 6.1). However, the study's findings did not show improvement of biological organisms (periphyton and aquatic macroinvertebrates) that were used. This showed that some chemically detectible changes may not be reflected by periphyton and aquatic macroinvertebrate responses. Based on the study's findings it is recommended that periphyton and macroinvertebrates be used as bioindicators to evaluate phytoremediation, however, it is highly advisable that assemblage level bioindicators be explored further with changes and improvement in methods, for example, increasing sampling effort or rather species suborganisational bioindicators be explored since assemblage level indicators seem to fail to pick up changes. In addition, it is also recommended that South Africa should revise their water quality standards as the present standards are too high, influencing more pollution in streams and rivers.

Table 6.1: Phytoremediation studies on the successful assimilation of organic and inorganic effluents using different invasive alien macrophytes and native macrophyte species.

Plant species	Type of disturbance	Organic, inorganic and parameters analysed	Experiment type	Findings/ highlights	Location	Reference
Pistia stratiotes	Eutrophic storm water	Water turbidity, Phosphates, Nitrates,	Lab experiment	Almost 60% reduction in water turbidity. Approximately 14-31% reduction in P; 50% reduction of N	India	Lu *et al*. (2010)
Pontederia crassipes	Heavy metal (radioisotope) contaminated waters	Caesium (Cs) and Cobalt (Co)	Lab experiment	96.4 % of Co and 33% of Cs removed from the solution at the end of 48 hours using 5 g *Pontederia crassipes* under shade lab light	Giza, Egypt	Saleh (2012)
Pontederia crassipes	Waste water from artificial wetland	Cu and Cr	Lab experiment	Nearly 65% of heavy metal contaminants were removed	India	Lissy and Madhu (2011)
Pontederia crassipes	Artificial waste water	Cd	Lab experiment	Nearly 100% removal of Cd at an initial Cd concentration of 50 mg/l	China	Zhang *et al*. (2015)
Pontederia crassipes	Agricultural drain water	Cu, Ni and Zn	Field experiment	The bio-concentration factor (BCF) for Cu, Ni and Zn in *P. crassipes* root tissues was 1344.6, 1250.0, and 22,758.6 respectively.	Egypt	Hammad (2011)

Pontederia crassipes	Eutrophic lake	Transparency, NH_4, NO_3, PO_4, TN, TP and COD	Field experiment	Improvement of water quality surrounding *P. crassipes* and reduction in most of the parameters.	China	Wang *et al*. (2012)
Pontederia crassipes	Heavy metals polluted irrigation canals	Fe, Ni, Pb, Zn, Cd, Co, Cu, Cr and Mn	Field experiment	Highest heavy metal concentration recorded in roots compared to other organs of the plant.	Egypt	Eid *et al*. (2019)
Pontederia crassipes	Polluted river water	Total hardness, TN, TDS, EC, sulphates, phosphate, pH, NO_2, and NO_3.	Field test experiment	About 25% decrease in EC, TDS (26%), Sulphates (45%), Total hardness (37%), and phosphates (33%)	Zim-babwe	Moyo *et al*. (2013)
Pontederia crassipes	Aqueous solution	Cr and Zn	Lab experiment	95% Zn and 84% Cr removal.	India	Mishra and Tripathi (2009)
Pontederia crassipes	Waste water	BOD, COD, and CN, DO, TSS, N and P.	Green house experiment	Major reduction in BOD, COD and CN concentrations recorded.	Indonesia	Nuraini and Felani (2015)
Pontederia crassipes	Heavy metal polluted water	Zn, Ag, Ni, Cr, Cu, Pb, Hg, and Cd	Lab experiment	Reduction in concentrations observed for all heavy metals with the highest tolerance of Zn and lowest tolerance of Hg.	Nigeria	Odjegba and Fasidi (2007)

Pontederia crassipes	Artificial waste water	Ni	Lab experiments	Adsorption was found to be highest during the first 24 hours. Overall, highest Ni concentration was recorded in roots.	Argentina	González *et al*. (2015)
Pontederia crassipes and *Pistia stratiotes*	Aquaculture waste water	pH, DO, COD turbidity, NO_3, BOD, nitrite phosphate, NO_2, NH_3 and TKN	Lab experiment	Considerable reduction in turbidity, recording highest percentage reduction of 87.05% from *P. crassipes;* 93.69% from *P. stratiotes* with similar reduction in NH3, TKN, COD, NO_3 and nitrate phosphate.	Malaysia	Akinbile and Yusoff (2012)
Eleocharis acicularis	Heavy metal contaminated water and sediment	Cd, Cu, As, Zn, and Pb	Field-tested experiment	Highest removal concentration of 239 mg Cd/kg, 20 200 mg Cu/kg, 894 mg As/kg, 14200 mg Zn/kg, and 1740 mg Pb/kg.	South west Japan	Sakakibara *et al*. (2011)
Pontederia crassipes and *Lolium perenne*	Polluted river water	NH_3-N, COD, TP	Field experiment	Reduction of NH_3-N, COD, TP by 48.6%, 20.0% and 63.3% respectively.	China	Wang *et al*. (2011)

Pontederia crassipes and *Ipomoea aquatica*	Domestic wastewater	NH_4, PO_3, TSS, COD	Field experiment	High values (*P. crassipes*) and low values (*I. aquatica*). Comparison between *P. crassipes* and water morning glory showed 26.8% - 32.6% for NH4; 56.7% - 61.4% for PO4; 337.8% - 53.3% for TSS and 44.4% - 53.4% for COD.	Vietnam	Loan *et al*. (2014)
Pontederia crassipes, *Azolla microphylla* and *Carica papaya* stem	Domestic sewage waste water	PO_4, NO_3, and NH_4	Lab experiment	*Pontederia crassipes*, and *Carica papaya* stem showed significant removal of NO_3 by 74% and NH_4 by 67%. *Azolla microphylla* and *Carica papaya* stem showed great removal of PO_4 by 80%.	India	Anandha Varun and Kalpana (2015)
Typha angustifolia	Phenol and melanoidin aqueous solution	Pb, Cu, Ni, Fe, Mn and Zn	Lab experiments in open environment	Decrease in concentrations of all pollutants observed with growth of *T. angustifolia*	India	Chandra and Yadav (2010)
Salvinia molesta	Treated palm oil mill effluent (POME)	NO_3, PO_4, NH_4, turbidity, and COD.	Lab experiment in raceway pond rig	About 95% PO_4 removal recorded. NO_3 concentrations reduced to 0.50 mg/l and NH_4 showed an average concentration of 2.62 mg/l. Turbidity decreased from 7.56 NTU to	Malaysia	Ng and Chan (2017)

				0.94 NTU. COD removal efficiency determined at 39%.		
Lemna minor	Sewage mixed with industrial and municipal effluent	Cu, Cd, Pb and Ni	Glass house experiment	More than 80% reduction in all heavy metal concentrations recorded; the highest (99%) concentration removal recorded for Ni	Pakistan	Bokhari *et al*. (2016)

6.5 General conclusions

The study examined the phytoremediation potential of IAAP to mitigate pollution from anthropogenic activities along of the Swartkops River in the Eastern Cape Province of South Africa. The phytoremediation potential of IAAP was tested by measuring the recovery and response of environmental variables, periphyton and aquatic macroinvertebrate biodiversity indices and community assemblage. Invasive alien aquatic plant species phytoremediation did not modify the diversity and structure of either periphyton or aquatic macroinvertebrates assemblages. Periphyton and aquatic macroinvertebrates, were largely dominated by pollution-tolerant taxa at upstream and downstream sites despite improvement of some water and sediment chemistry in downstream of phytoremediation treatments. Although there were few fluctuations with some variables, most of water and sediment variables showed a reduction in heavy metal and nutrient concentrations between upstream and downstream sites. This could have been because of IAAP species' potential for phytoremediation, although it is also possible that natural factors (river self-purification) could have played a role as the river flowed downstream. Fluctuations in some environmental variables and the lack of improvement in biological variables could have been due to constant pollution effluents from multiple sources (sewage treatment works, industries and other anthropogenic activities) along the river. It is possible that the phytoremediation process did not show changes and improvement in aquatic biodiversity because aquatic biodiversity is strongly linked to water quality. Constant influxes at Swartkops River overpowered the effect that IAAP mats had on water quality and thus on biological indicator like periphyton and aquatic macroinvertebrates which provide a more time integrated water quality indications compared to water quality spot analysis. If the river system was not receiving constant flushes of wastewater, then aquatic biota would have improved over time, aligning with water quality downstream. Various authors have proved phytoremediation success in environmental variables; however, very few studies have tested the recovery of biodiversity. The study results provided evidence that IAAP species play a role in assimilating nutrients and other pollutants in freshwater ecosystems. However, water quality spot analysis was not a reliable, time integrated assessment to monitor river health; and measure phytoremediation success in the Swartkops River because variables change with time of the day and concentration of pollutants during certain flows. Thus this study propose biological monitoring should be included in any water quality related remediation techniques to measure restoration success. The current study contributes to the literature on freshwater pollution and the efficiency of using both biological variables and environmental variables to assess phytoremediation success.

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Appendices

Appendix 1: Aquatic microalgae percentage composition in the Swartkops River system, Eastern Cape, South Africa. Bolded values show periphyton compositions that were 3% and more of the total composition.

Periphyton species	**Sampled sites**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Anabaena filaments	**3,5**	0,40	0,90	1,19	0,20	0,34	0,13
Oscillatoria species	2,83	2,03	**3,13**	1,92	0,74	0,24	0,33
Cocconeis species	0,32	0,69	0,40	0,14	0,04	2,33	2,14
Cocconeis pediculus	1,28	0,68	0,17	0	0	**3,25**	1,15
Cyclostephanos dubius	0,83	**3,61**	**3,63**	1,50	1,59	**3,52**	2,80
Cyclotella species	0,21	0,27	0,02	0,11	0,33	0,99	1,34
Cyclotella meneghiniana	1,34	**6,78**	**7,36**	**4,94**	**3,46**	**10,86**	**13**
Diadermis confervacea	1,74	0,63	**3,58**	0,42	0,76	**3,33**	**4,57**
Fragilaria ulna	1,53	0,40	0,40	0,13	1,67	**3,3**	1,97
Fragilaria ulna var. acus	**3,23**	0,20	0,08	0,05	1,31	**5,02**	**3,76**
Gomphonema species	0,10	**3,09**	1,86	1,69	1,76	0,9	0,85
Melosira varians	0,95	0,80	0,77	0,20	0,18	**7,74**	**13,99**
Navicula species	**3,16**	**9,23**	**7,8**	**18,84**	11,48	**4,53**	**5,5**
Navicula gregaria	0,41	**3,92**	2,30	**4,93**	**3,57**	0,50	0,99
Navicula veneta	**3,76**	**10,51**	12,89	**24,45**	**41,74**	**4,09**	**3,82**
Navicula viridula	0	0	0	0,006	0	0	0
Nitzschia species	**3,83**	**3,6**	**3,71**	**3,5**	2,47	1,80	2,49
Nitzschia dissipata	**4,63**	1,67	0,78	0,39	0,63	0,36	0,18
Nitzschia filiformis	**4,67**	1,37	0,88	0,67	0,65	2,01	**4,49**
Nitzschia palea	**3,61**	**4,75**	**4,22**	1,64	**3,29**	1,12	1,07
Nitzschia reversa	1,49	**5,94**	**5,18**	0,25	0,02	0,14	0
Nitzschia umbonata	1,14	**3,44**	**3,07**	**4,22**	2,43	0,72	0,75
Pinnularia viridiformis	1,69	2,69	**3,11**	**5,08**	**3,02**	1,07	1,51
Pinnularia viridis	0	0	0	0	0	0,03	0
Placoneis species	0	0,12	0,10	0,22	0,03	**3,78**	0,09
Phacus nordstedtii	0,22	0,49	0,34	0,07	0,13	**3,47**	**3,4**
Spyrogyra filaments	**3,34**	0,36	0,33	0,21	0,31	0,14	0,25

Appendix 2: Aquatic macroinvertebrates assigned functional feeding groups per sampled family in the Swartkops River system, Eastern Cape, South Africa.

Aquatic Macroinvertebrates families	**Functional feeding groups**	**Sampled sites**						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Baetidae	Collector-gatherers	0	2	11	3	0	7	0
Hydropsychidae	Collector-filters	6	0	1	0	3	0	60
Ecnomidae	Collector-filters	0	0	0	0	0	3	0
Psychomyiidae	Collector-filters	0	0	0	0	1	0	0
Dytiscidae	Predators	0	0	0	0	0	0	1
Dytiscidae larvae	Predators	0	1	0	0	0	0	0
Elmidae	Shredders	6	0	0	0	0	0	0
Naucoridae	Predators	0	0	15	0	0	0	0
Notonectidae	Predators	0	1	0	0	0	0	0
Belostomatidae	Predators	2	121	128	8	15	16	70
Gerridae	Predators	0	1	0	0	0	0	0
Corixidae	Scrapers	0	0	0	0	3	0	2
Libellulidae	Predators	11	2	2	0	0	0	0
Aeshnidae	Predators	0	2	0	0	0	1	0
Coenagrionidae	Predators	6	58	47	3	0	13	72
Chlorolestidae	Predators	0	1	0	0	0	0	0
Culicidae	Collector-filters	12	3	20	28	1	5	0
Tabanidae	Predators	0	1	0	0	0	0	1
Psychodidae	Collector-gatherers	0	0	0	0	0	2	0
Dixidae	Collector-gatherers	0	1	10	18	0	0	1
Chironomidae	Collector-filters	7	295	233	2612	282	315	205
Ceratopogonidae	Collector-gatherers	0	0	0	0	0	2	13
Syrphidae	Collector-gatherers	0	0	0	0	1	0	0
Simuliidae	Collector-filters	3	13	0	4	0	4	0
Planaria	Predators	0	0	0	0	0	1	3
Pyralidae	Shredders	2	0	0	0	1	0	1
Potamonautidae	Shredders	0	3	0	0	0	1	7
Hirudinea	Predators	0	495	356	222	1210	221	183
Oligochaeta	Collector-gatherers	2	24	10	4	13	36	28
Physidae	Scrapers	3	0	0	0	0	3	2
Lymnaeidae	Scrapers	2	0	0	0	0	0	2
Ancylidae	Scrapers	0	0	0	0	0	0	1

Appendix 3: Significant differences in field measured physiochemical parameters between sampled treatments in the Swartkops River system, Eastern Cape, South Africa.

Parameter	Treatment	Treatment		
		S1	**D**	**m**
pH	**D**	**<0.001**	-	-
	m	**0.001**	0.990	-
	U	**0.005**	0.806	0.936
EC (µS)	**D**	**<0.001**	-	-
	m	**<0.001**	0.98	-
	U	**<0.001**	0.94	1.00
DO (mg/l)	**D**	0.98	-	-
	m	0.95	0.99	-
	U	0.79	0.86	0.95
Temp (°C)	**D**	0.45	-	-
	m	0.41	1.00	-
	U	0.61	0.98	0.97
NO_3 (mg/l)	**D**	0.23	-	-
	m	0.85	0.42	-
	U	0.25	1.00	0.46
NH_4 (mg/l)	**D**	**0.04**	-	-
	m	**0.04**	1.00	-
	U	**0.01**	1.00	1.00
PO_4 (mg/l)	**D**	**0.023**	-	-
	m	**0.020**	1.00	-
	U	**0.004**	1.00	1.00

Appendix 4: Significant differences between field measured physiochemical parameters and sites in the Swartkops River system, Eastern Cape, South Africa.

Parameter		Sites								
pH		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
	2	**0.05**	-	-	-	-	-	-	-	-
	3	**0.004**	1.00	-	1.00	0.863	-	0.615	0.960	1.00
	4	**0.002**	1.00	-	-	-	-	-	-	-
	5	0.381	1.00	-	0.769	-	-	-	-	-
	6	0.814	0.897	-	0.331	1.00	-	0.995	0.600	0.446
	7	0.666	1.00	-	0.489	1.00	-	-	-	-
	8	0.213	1.00	-	1.00	1.00	-	1.00	-	-
	9	**0.015**	1.00	1.00	1.00	0.967	0.686	0.830	1.00	1.00
	10	**0.010**	1.00	-	1.00	0.94	-	0.760	1.00	-
EC (µS)	**2**	**<0.001**	-	-	-	-	-	-	-	-
	3	**<0.001**	1.00	-	1.00	0.746	-	1.00	1.00	1.00
	4	**<0.001**	1.00	-	-	-	-	-	-	-
	5	0.120	0.565	-	0.686	-	-	-	-	-
	6	0.09	0.978	1.00	0.990	1.00	-	1.00	1.00	1.00
	7	**<0.001**	0.979	-	0.993	1.00	-	-	-	-
	8	**<0.001**	1.00	-	1.00	0.850	-	1.00	-	-
	9	**<0.001**	1.00	1.00	1.00	0.913	1.00	1.00	1.00	1.00
	10	**<0.001**	1.00	-	1.00	0.676	-	1.00	1.00	-

DO (mg/l)	**2**	0.95	-	-	-	-	-	-	-	-
	3	0.93	1.00	-	1.00	0.45	-	0.44	1.00	1.00
	4	1.00	1.00	-	-	-	-	-	-	-
	5	1.00	0.51	-	0.93	-	-	-	-	-
	6	1.00	0.44	0.83	0.90	1.00	-	1.00	0.17	0.53
	7	1.00	0.49	-	0.92	1.00	-	-	-	-
	8	0.74	1.00	-	0.97	0.21	-	0.20	-	-
	9	1.00	1.00	0.75	1.00	0.80	-	0.79	1.00	1.00
	10	0.98	1.00	-	1.00	0.60	-	0.58	1.00	-
Temp (°C)	**2**	1.00	-	-	-	-	-	-	-	-
	3	1.00	1.00	-	1.00	0.98	-	0.93	0.76	0.84
	4	1.00	1.00	-	-	-	-	-	-	-
	5	0.96	0.94	-	1.00	-	-	-	-	-
	6	0.86	0.82	0.90	0.98	1.00	-	1.00	1.00	1.00
	7	0.90	0.86	-	0.99	1.00	-	-	-	-
	8	0.71	0.65	-	0.93	1.00	-	1.00	-	-
	9	0.49	0.43	0.55	0.80	1.00	1.00	1.00	1.00	1.00
	10	0.79	0.74	-	0.96	1.00	-	1.00	1.00	-
NO_3 (mg/l)	**2**	0.327	-	-	-	-	-	-	-	-
	3	0.349	1.00	-	1.00	1.00	-	0.997	0.738	0.916
	4	0.414	1.00	-	-	-	-	-	-	-
	5	0.866	0.998	-	1.00	-	-	-	-	-
	6	1.00	0.619	0.644	0.712	0.981	-	0.989	1.00	1.00
	7	0.904	0.996	-	1.00	1.00	-	-	-	-
	8	1.00	0.716	-	0.798	0.992	-	0.996	-	-
	9	1.00	**0.05**	**0.05**	0.07	0.353	0.970	0.414	0.940	0.786

	10	0.996	0.903	-	1.00	1.00	-	1.00	-	-
NH_4(mg/l)	**2**	0.16	-	-	-	-	-	-	-	-
	3	0.21	1.00	-	1.00	1.00	-	1.00	1.00	-
	4	0.26	1.00	-	-	-	-	-	-	-
	5	0.18	1.00	-	1.00	-	-	-	-	-
	6	0.37	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00
	7	0.37	1.00	-	1.00	1.00	-	-	-	-
	8	0.50	1.00	-	1.00	1.00	-	1.00	-	-
	9	0.84	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	10	0.82	0.99	-	1.00	0.99	-	1.00	1.00	-
PO_4 (mg/l)	**2**	1.00	-	-	-	-	-	-	-	-
	3	1.00	1.00	-	1.00	0.08	-	0.236	0.845	0.303
	4	1.00	1.00	-	-	-	-	-	-	-
	5	**<0.01**	**0.05**	-	0.06	-	-	-	-	-
	6	**0.03**	0.136	0.182	0.142	1.00	-	1.00	1.00	1.00
	7	**0.04**	0.180	-	0.187	1.00	-	-	-	-
	8	0.40	0.781	-	0.792	0.926	-	0.944	-	-
	9	0.07	0.291	1.00	0.301	1.00	-	1.00	1.00	0.367
	10	**0.05**	0.236	-	0.245	1.00	-	1.00	1.00	-

Appendix 5: Significant differences between Bemlabs water chemistry parameters and treatments in the Swartkops River system, Eastern Cape, South Africa.

Heavy metals	Treatment	Treatment		
		S1	**D**	**m**
Fe (mg/l)	**D**	**0.002**	-	-
	m	**0.004**	0.998	-
	U	0.015	0.851	0.926
Cu (mg/l)	**D**	1	-	-
	m	1	1	-
	U	1	1	1
Zn (mg/l)	**D**	0.90	-	-
	m	0.93	1.00	-
	U	0.79	0.99	0.97
COD (mg/l)	**D**	0.176	-	-
	m	**0.02**	0.589	-
	U	**0.05**	0.871	0.960
As (µg/l)	**D**	1.00	-	-
	m	0.92	0.93	-
	U	0.81	0.78	0.99
Cd (µg/l)	**D**	1	-	-
	m	1	1	-
	U	1	1	1
Cr (µg/l)	**D**	1	-	-
	m	1	1	-
	U	1	1	1
Hg (µg/l)	**D**	1	-	-
	m	1	1	-
	U	1	1	1
Pb (µg/l)	**D**	1	-	-
	m	1	1	-
	U	1	1	1

Appendix 6: Significant differences in Bemlabs water chemistry between sites in the Swartkops River system, Eastern Cape, South Africa.

Heavy metals	**Site**	**Site**								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Fe (mg/l)	**2**	0.678	-	-	-	-	-	-	-	-
	3	0.841	1.00	-	1.00	1.00	-	0.986	0.501	0.308
	4	0.625	1.00	-	-	-	-	-	-	-
	5	0.812	1.00	-	1.00	-	-	-	-	-
	6	**0.022**	0.881	0.735	0.910	0.769	-	1.00	1.00	1.00
	7	0.173	0.998	-	1.00	0.991	-	-	-	-
	8	**0.007**	0.693	-	0.742	0.540	-	0.989	-	-
	9	**0.028**	0.906	0.775	0.931	0.806	1.00	1.00	1.00	1.00
	10	**0.002**	0.486	-	0.540	0.341	-	0.943	1.00	-
Cu (mg/l)	**2**	1	-	-	-	-	-	-	-	-
	3	1	1	-	1	1	-	1	1	1
	4	1	1	-	-	-	-	-	-	-
	5	1	1	-	1	-	-	-	-	-
	6	1	1	1	1	1	-	1	1	1
	7	1	1	-	1	1	-	-	-	-
	8	1	1	-	1	1	-	1	-	-
	9	1	1	1	1	1	1	1	1	1
	10	1	1	-	1	1	-	1	1	-
Zn (mg/l)	**2**	1.00	-	-	-	-	-	-	-	-
	3	1.00	1.00	-	1.00	0.76	-	1.00	1.00	1.00
	4	1.00	1.00	-	-	-	-	-	-	-

	5	0.42	0.42	-	0.42	-	-	-	-	-
	6	1.00	1.00	1.00	1.00	0.76	-	1.00	1.00	1.00
	7	0.99	0.99	-	0.99	0.99	-	-	-	-
	8	1.00	1.00	-	1.00	0.42	-	0.99	-	-
	9	1.00	1.00	1.00	1.00	0.76	-	1.00	1.00	1.00
	10	1.00	1.00	-	1.00	0.84	-	1.00	1.00	-
COD (mg/l)	**2**	0.977	-	-	-	-	-	-	-	-
	3	0.532	0.996	-	0.996	0.898	-	1.00	1.00	1.00
	4	0.975	1.00	-	-	-	-	-	-	-
	5	**0.013**	0.321	-	0.327	-	-	-	-	-
	6	**0.017**	0.375	0.928	0.382	1.00	-	0.992	0.640	0.471
	7	0.277	0.951	-	0.954	0.985	-	-	-	-
	8	0.871	1.00	-	1.00	0.579	-	0.995	-	-
	9	0.862	1.00	1.00	1.00	0.594	0.655	0.996	1.00	1.00
	10	0.951	1.00	-	1.00	0.411	-	0.977	1.00	-
As (µg/l)	**2**	0.99	-	-	-	-	-	-	-	-
	3	1.00	0.99	-	1.00	0.98	-	1.00	1.00	1.00
	4	1.00	1.00	-	-	-	-	-	-	-
	5	0.98	1.00	-	1.00	-	-	-	-	-
	6	1.00	0.99	1.00	1.00	0.98	-	1.00	1.00	1.00
	7	1.00	0.99	-	1.00	0.98	-	-	-	-
	8	1.00	0.99	-	1.00	0.98	-	1.00	-	-
	9	0.85	1.00	0.85	0.99	1.00	0.85	0.85	0.85	0.85
	10	1.00	0.99	-	1.00	0.98	-	1.00	1.00	-
Cd (µg/l)	**2**	1	-	-	-	-	-	-	-	-

	3	1	1	-	1	1	-	1	1	1
	4	1	1	-	-	-	-	-	-	-
	5	1	1	-	1	-	-	-	-	-
	6	1	1	1	1	1	-	1	1	1
	7	1	1	-	1	1	-	-	-	-
	8	1	1	-	1	1	-	1	-	-
	9	1	1	1	1	1	1	1	1	1
	10	1	1	-	1	1	-	1	1	-
Cr (μg/l)	**2**	1	-	-	-	-	-	-	-	-
	3	1	1	-	1	1	-	1	1	1
	4	1	1	-	-	-	-	-	-	-
	5	1	1	-	1	-	-	-	-	-
	6	1	1	1	1	1	-	1	1	1
	7	1	1	-	1	1	-	-	-	-
	8	1	1	-	1	1	-	1	-	-
	9	1	1	1	1	1	1	1	1	1
	10	1	1	-	1	1	-	1	1	-
Hg (μg/l)	**2**	1	-	-	-	-	-	-	-	-
	3	1	1	-	1	1	-	1	1	1
	4	1	1	-	-	-	-	-	-	-
	5	1	1	-	1	-	-	-	-	-
	6	1	1	1	1	1	-	1	1	1
	7	1	1	-	1	1	-	-	-	-
	8	1	1	-	1	1	-	1	-	-
	9	1	1	1	1	1	1	1	1	1
	10	1	1	-	1	1	-	1	1	-

Pb (µg/l)	**2**	1	-	-	-	-	-	-	-	-
	3	1	1	-	1	1	-	1	1	1
	4	1	1	-	-	-	-	-	-	-
	5	1	1	-	1	-	-	-	-	-
	6	1	1	1	1	1	-	1	1	1
	7	1	1	-	1	1	-	-	-	-
	8	1	1	-	1	1	-	1	-	-
	9	1	1	1	1	1	1	1	1	1
	10	1	1	-	1	1	-	1	1	-

Appendix 7: Significant differences in sediment chemistry between treatments in the Swartkops River system, Eastern Cape, South Africa.

Heavy metals (mg/kg)	**Treatment**	**Treatment**		
		S1	**D**	**m**
Fe	**D**	**0.035**	-	-
	m	0.505	0.248	-
	U	0.197	0.734	0.842
Zn	**D**	0.702	-	-
	m	**0.008**	0.243	-
	U	**<0.001**	**<0.001**	0.182
Cd	**D**	0.65	-	-
	m	0.66	1.00	-
	U	0.71	1.00	1.00
As	**D**	**0.031**	-	-
	m	0.158	0.787	-
	U	0.519	0.208	0.741
Cr	**D**	0.985	-	-
	m	0.934	0.537	-
	U	0.445	**0.05**	0.585
Pb	**D**	0.189	-	-
	m	0.644	0.649	-
	U	1.00	**0.02**	0.325
Hg	**D**	0.94	-	-
	m	0.94	1.00	-
	U	1.00	0.84	0.85
Cu	**D**	0.680	-	-
	m	0.815	**0.02**	-
	U	0.221	**<0.001**	0.456
P	**D**	1.00	-	-
	m	0.663	0.322	-
	U	**0.022**	**<0.001**	0.071
NH_4	**D**	1.00	-	-
	m	0.95	0.85	-

	U	0.94	0.84	1.00
NO_3	**D**	0.624	-	-
	m	0.750	0.992	-
	U	0.086	0.363	0.221

Appendix 8: Significant differences in sediment chemistry between sites in the Swartkops River system, Eastern Cape, South Africa.

Heavy metals (mg/kg)		Sites								
Fe		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
	2	1.00	-	-	-	-	-	-	-	-
	3	1.00	1.00	-	0.788	**0.03**	-	0.06	1.00	0.80
	4	0.762	0.963	-	-	-	-	-	-	-
	5	**0.023**	0.104	-	0.824	-	-	-	-	-
	6	0.845	0.984	0.866	1.00	0.735	-	0.885	1.00	1.00
	7	0.06	0.203	-	0.937	1.00	-	-	-	-
	8	0.995	1.00	-	1.00	0.283	-	0.463	-	-
	9	0.762	0.963	0.788	1.00	0.824	1.00	0.937	1.00	1.00
	10	0.775	0.967	-	1.00	0.813	-	0.931	1.00	-
Zn	**2**	**0.01**	-	-	-	-	-	-	-	-
	3	**0.05**	1.00	-	0.988	1.00	-	0.144	0.974	0.06
	4	0.509	0.902	-	-	-	-	-	-	-
	5	**<0.001**	1.00	-	0.824	-	-	-	-	-
	6	0.800	0.663	0.894	1.00	0.540	-	0.958	1.00	0.857
	7	1.00	**0.05**	-	0.788	**0.03**	-	-	-	-
	8	0.602	0.846	-	1.00	0.749	-	0.857	-	-
	9	0.993	0.216	0.463	0.981	0.144	1.00	1.00	0.993	0.997
	10	1.00	**0.017**	-	0.587	**0.009**	-	1.00	0.678	-
Cd	**2**	1.00	-	-	-	-	-	-	-	-
	3	1.00	1.00	-	1.00	1.00	-	0.96	0.85	0.99

	4	1.00	1.00	-	-	-	-	-	-	-
	5	1.00	1.00	-	1.00	-	-	-	-	-
	6	0.84	0.98	0.90	0.96	0.97	-	1.00	1.00	1.00
	7	0.93	1.00	-	0.99	0.99	-	-	-	-
	8	0.78	0.96	-	0.93	0.95	-	1.00	-	-
	9	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	10	0.98	1.00	-	1.00	1.00	-	1.00	1.00	-
As	**2**	0.63	-	-	-	-	-	-	-	-
	3	0.36	1.00	-	1.00	0.65	-	1.00	0.94	1.00
	4	0.93	1.00	-	-	-	-	-	-	-
	5	1.00	0.88	-	0.99	-	-	-	-	-
	6	1.00	0.95	0.80	1.00	1.00	-	0.59	1.00	0.59
	7	0.19	1.00	-	0.96	0.43	-	-	-	-
	8	0.99	0.99	-	1.00	1.00	-	0.81	-	-
	9	0.52	1.00	1.00	1.00	0.79	0.91	1.00	0.98	1.00
	10	0.19	1.00	-	0.96	0.42	-	1.00	0.81	-
Cr	**2**	0.991	-	-	-	-	-	-	-	-
	3	0.979	1.00	-	1.00	0.902	-	0.8	0.979	0.524
	4	1.00	1.00	-	-	-	-	-	-	-
	5	0.206	0.846	-	0.602	-	-	-	-	-
	6	0.997	1.00	1.00	1.00	0.775	-	0.918	0.997	0.707
	7	1.00	0.866	-	0.977	**0.05**	-	-	-	-
	8	1.00	0.991	-	1.00	0.206	-	1.00	-	-
	9	1.00	0.846	0.902	0.97-	**0.04**	-	1.00	1.00	0.775
	10	0.995	0.617	-	0.856	**0.013**	-	1.00	0.995	-

Pb	**2**	1.00	-	-	-	-	-	-	-	-
	3	0.997	1.00	-	0.721	1.00	-	0.254	0.587	**0.04**
	4	0.995	0.958	-	-	-	-	-	-	-
	5	0.998	1.00	-	0.750	-	-	-	-	-
	6	0.806	0.579	0.232	1.00	0.254	-	1.00	1.00	1.00
	7	0.830	0.610	-	1.00	0.277	-	-	-	-
	8	0.982	0.902	-	1.00	0.617	-	1.00	-	-
	9	0.540	0.308	0.087	0.979	0.10	1.00	1.00	0.995	1.00
	10	0.375	0.187	-	0.931	**0.05**	-	1.00	0.974	-
Hg	**2**	1	-	-	-	-	-	-	-	-
	3	1	1	-	1	1	-	1	1	1
	4	1	1	-	-	-	-	-	-	-
	5	1	1	-	1	-	-	-	-	-
	6	1	1	1	1	1	-	1	1	1
	7	1	1	-	1	1	-	-	-	-
	8	1	1	-	1	1	-	1	-	-
	9	1	1	1	1	1	1	1	1	1
	10	1	1	-	1	1	-	1	1	-
Cu	**2**	0.501	-	-	-	-	-	-	-	-
	3	0.968	0.996	-	0.958	1.00	-	0.116	1.00	0.411
	4	1.00	0.463	-	-	-	-	-	-	-
	5	0.792	1.00	-	0.762	-	-	-	-	-
	6	0.975	0.995	1.00	0.967	1.00	-	0.130	1.00	0.440
	7	0.830	**0.006**	-	0.857	**0.03**	-	-	-	-
	8	0.998	0.958	-	0.996	0.998	-	0.271	-	-
	9	1.00	0.334	0.902	1.00	0.633	0.917	0.931	0.984	0.999

	10	0.988	**0.05**	-	0.993	0.156	-	1.00	0.670	-
P	**2**	0.49	-	-	-	-	-	-	-	-
	3	1.00	0.94	-	0.98	0.82	-	0.99	0.85	1.00
	4	1.00	0.28	-	-	-	-	-	-	-
	5	0.30	1.00	-	0.14	-	-	-	-	-
	6	1.00	0.78	1.00	1.00	0.57	-	1.00	0.60	1.00
	7	1.00	0.31	-	1.00	0.16	-	-	-	-
	8	0.32	1.00	-	0.16	1.00	-	0.18	-	-
	9	0.86	1.00	1.00	0.66	1.00	0.98	0.69	1.00	0.98
	10	1.00	0.80	-	1.00	0.60	-	1.00	0.63	-
NH_4	**2**	1.00	-	-	-	-	-	-	-	-
	3	1.00	1.00	-	1.00	1.00	-	1.00	0.84	0.94
	4	1.00	1.00	-	-	-	-	-	-	-
	5	1.00	1.00	-	1.00	-	-	-	-	-
	6	1.00	1.00	1.00	1.00	1.00	-	1.00	0.62	0.79
	7	1.00	0.98	-	1.00	1.00	-	-	-	-
	8	0.88	0.96	-	0.59	0.65	-	0.33	-	-
	9	0.69	0.85	0.63	0.36	0.42	0.39	0.17	1.00	1.00
	10	0.96	0.99	-	0.76	0.81	-	0.51	1.00	-
NO_3	**2**	0.997	-	-	-	-	-	-	-	-
	3	0.931	1.00	-	0.846	0.925	-	1.00	1.00	1.00
	4	1.00	0.986	-	-	-	-	-	-	-
	5	0.137	0.648	-	0.077	-	-	-	-	-
	6	1.00	0.995	0.902	1.00	0.110	-	0.918	0.463	0.663
	7	0.943	1.00	-	0.867	0.910	-	-	-	-

8	0.524	0.967	-	0.375	1.00	-	1.00	-	-
9	0.979	1.00	1.00	0.937	0.824	0.967	1.00	0.995	1.00
10	0.721	0.995	-	0.571	0.994	-	1.00	1.00	-