# ASSESSMENT OF THE ECOLOGICAL INTEGRITY OF THE ZAALKLAPSPRUIT WETLAND IN MPUMALANGA (SOUTH AFRICA) BEFORE AND AFTER REHABILITATION:
## The Grootspruit Case Study

Report to the
**WATER RESEARCH COMMISSION**

by

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# EXECUTIVE SUMMARY

**BACKGROUND**

By virtue of their positions in the landscape and relationship to drainage networks, wetlands are frequently impacted by coal mining activities, especially opencast methods. These impacts will be ongoing, since coal is a strategic resource and will continue to be mined extensively to support the country's development. At the same time, however, regulatory authorities and the public now have an improved understanding of the range of economic, social, ecological and hydrological costs of wetland loss and degradation. The rules of the game have changed, with regulators increasingly insisting that mines avoid, minimise and mitigate their impacts on wetlands, and internalise the true costs of wetland loss into their balance sheets. Many mining proposals entailing large-scale wetland loss have encountered delays in licence approvals, unrealistic rehabilitation commitments and unwelcome public and media attention. As a result, the coal mining sector has realised that it needs to proactively and systematically address the business risk posed by its impact on wetlands.

**AIMS**

The primary objective of the research is to provide the coal sector with an understanding of how its impacts on wetlands can be limited and mitigated, as well as what can be practically executed. The research study aimed to address various gaps in the current body of knowledge as expressed by various mining companies as well as South African National Biodiversity Institute (SANBI) and the CSIR. Not only will this help the decision-making support system of the coal sector, but it can also be utilised by other sectors in the country.

This component of the research that is the focus of this report examined the extent to which rehabilitation of a degraded wetland that was receiving pollution derived from coal mining improved the wetland's ability to reduce the levels of these particular pollutants in the water flowing through it. The project team, in consultation with Coaltech members, Working for Wetlands and other stakeholders identified a wetland that met all the criteria necessary to make it a suitable case study. Rehabilitation was conducted by the Working for Wetlands programme (SANBI), CSIR, Coaltech and the WRC in accordance with standard procedures.

Wetlands provide more ecosystem services per hectare than any other ecosystem, being sites of intense biogeochemical activity that play an important role in improving water quality. Various levels of wetland functionality that were studied, include:

***Water Quality***

The purpose of the water quality component is to describe the changes in water chemistry observed for the rehabilitated area compared to the values described in the baseline assessment. The primary objective is therefore to determine to what extent the rehabilitation of the Grootspruit has improved the downstream water quality when compared to upstream.

***Bacterial Consortium***

Not much is known about the key microbial processes present in wetlands, despite the fact that microorganisms play a major role in acid mine drainage remediation, especially in unimpacted wetlands. For example, microorganisms play a vital role in cycling and transformation of nutrients and removal of pollutants in water. Hence, a better understanding of these processes is crucial in the ability to rehabilitate functional wetlands and to determine their potential use in assessing wetland degradation. The aim of this part of the study was to determine the changes in diversity and richness of the microbial consortium in the Grootspruit within the Zaalklapspruit Wetland that received mining effluent before and after rehabilitation.

***Freshwater Algae***

Freshwater algae are known to be excellent indicators of water quality and are useful organisms for biomonitoring purposes. This is due to the fact that they give a time-integrated indication of specific water quality components and are known to respond rapidly to water quality changes. Thus, determining diversity of the algal assemblages in the wetland before and after rehabilitation will give a good indication of the succession of the algal community after rehabilitation. The objectives of the study were to employ freshwater algae to differentiate between conditions before and after wetland rehabilitation.

***Vegetation Assessment***

Field spectroscopy provides an easy and more affordable method of assessing plant health and a number of health indices have been developed in this regard. Thus, the objective for this part of the study is to determine the use of the spectral properties of selected vegetation as an affordable, non-destructive way of tracking the improvement of wetland vegetation after rehabilitation.

***Teratogenic Potency and Embryotoxicity***

The aim of this part of the study is to evaluate the teratogenic potential and embryotoxicity of surface water collected from the Grootspruit upstream and downstream of the rehabilitated area.

***Resource Economics***

The aim of the socio-economic component of the project is to internalize the costs of the ecosystem services delivered by wetlands, which are impacted by coal mining activities. In this case the focus is on acid mine drainage and the purpose of the work is to evaluate the role of wetlands in treating and thereafter to develop recommendations for cost benefit analysis applications in mine planning.

**RESULTS**

***Water Quality***

Monitoring the water quality is an efficient means of determining the change rehabilitation efforts are making in a wetland which is severely compromised by mining activities. Rehabilitation of the Grootspruit involved the increase of surface area to allow for increased gravitational drainage. Increased soil contact increased the filtration and suspension of pollutants as well as biologically mediated processes which strongly affect the water chemistry, resulting in the removal of contaminants from the water column. The water quality downstream of the rehabilitated area improved significantly after rehabilitation. The pH and alkalinity were increased to levels in the natural fresh water range, in which many of the metals become insoluble and precipitated out of the water column. Sulphate concentration decreased by 65% and the total dissolved solids decreased by 50% compared to pre-rehabilitation values. It is evident that the rehabilitation efforts have started to restore the water chemistry to that which resembles the reference site (un-impacted by mining). The chlorophyll a concentration increased in the rehabilitated area, but was still significantly lower compared to the reference site, indicating a slow return of a functional ecosystem service. The longevity of the positive response of the water quality will rely strongly on the continued presence of sulphate reducing bacteria in oxygen rich water.

***Bacterial Consortium***

The biogeochemical recycling of nutrients in wetlands is facilitated largely by microorganisms, with the microbial consortium playing a fundamental role in the removal of pollutants in these systems. An understanding of the nutrient cycling in wetlands is critical in the rehabilitation and protection of wetland systems. Pyrosequencing was used to obtain an understanding of the diversity of the microbial consortium present in the water fraction of the wetland and the bacterial composition was examined. High resolution melt real-time polymerase chain reaction was used to detect three functional groups of microorganisms, namely sulphate reducing bacteria, denitrifying bacteria and methanogens, due to the important role that they play in regulating the cycling of major nutrients and carbon in freshwater wetlands. The low pH sites generally had low levels of *Escherichia coli* and total coliforms, possibly due to the very acidic pH of the water which is unfavourable. Where high levels of indicator organisms were present upstream of the wetland, effective removal of faecal pollutants by the wetland was obtained after rehabilitation. Bacteria responsible for sulphate reduction were detected mainly at the sites with acidic pH and high iron or sulphate content. Denitrifying bacteria were detected at the majority of the sites before rehabilitation. Methanogens were only detected on two occasions. The bacterial composition of the water fraction of the wetland revealed bacteria present before rehabilitation exhibited the capacity to survive under extreme conditions in polluted environments. The downstream sites after rehabilitation resembled that of the reference site. A comparison of sites downstream of the wetland prior to rehabilitation and after rehabilitation revealed a decrease in bacterial diversity with a shift in the community structure towards an increase in abundance of bacteria from the phylum Firmicutes. Bacteria that are unclassified or bacteria poorly described in literature were the most abundant followed by members belonging to the phylum Proteobacteria. Bacteriodetes and Actinobacteria that play an important role in biopolymer degradation were also well represented. As these bacterial populations are important participants in wetland environments, this suggests the healthy functioning of the wetland after rehabilitation.

***Freshwater Algae***

A study was conducted to use freshwater algae in conjunction with water quality to differentiate between conditions before and after wetland rehabilitation and to determine if epilithic phytoplankton can be used as bioindicator for wetland rehabilitation. The Grootspruit, which forms part of the Zaalklapspruit Wetland, was rehabilitated by the redirection of water flow in order to enlarge the surface area of the wetland. Water and algae were sampled at sites upstream and downstream from the rehabilitation area to determine the effect of wetland rehabilitation on water quality and algal assemblage. Univariate statistical analysis was used to determine the diversity and richness of the algae at the different sites before and after rehabilitation. With regard to the water quality, pH levels, as well as chlorophyll a and alkalinity concentrations increased after rehabilitation downstream from the area which has been rehabilitated, while electrical conductivity, sulphate, total suspended solids, aluminium and iron decreased. It was also found that the species diversity and richness of the algae have increased at the downstream sites after rehabilitation. Overall, the results indicated an improvement in both water quality and algal diversity after the rehabilitation of this wetland.

***Vegetation Assessment***

The condition of the wetland vegetation exposed to mine wastewater and acid mine drainage was assessed using field spectroscopy and spectral vegetation indices. Green leaves of *Phragmites australis* and *Typha capensis* were sampled during the growth seasons before and after the implementation of rehabilitation structures. Firstly, the sites mostly affected by the mining wastewater and acid mine drainage were identified, using indices of leaf pigments (carotenoids and chlorophyll) as indicators. Secondly, changes in vegetation conditions after rehabilitation were compared to conditions prior to the rehabilitation. Vegetation indices for carotenoids (the Carotenoid Reflectance Index Red edge) and chlorophyll (Red-edge Position Linear Extrapolation method) were derived from leaf-level field spectroscopy. Significant differences between sites and years for carotenoid and chlorophyll index values were assessed using a one-way Analysis of Variance. Though agricultural land use are predominant in the study area, the sites downstream of the mining land uses had lower mean chlorophyll index values compared to the reference site upstream of the mining sites. No consistent change in vegetation condition for both species could be noted between the two years following the implementation of rehabilitation structures. Chlorophyll values of *Typha capensis* showed a significant increase from Year 1 to Year 2 for all sites, whereas chlorophyll values for *Phragmites australis* showed a significant increase at Site 1 (reference, upstream) and a significant decrease at Site 7 (downstream). The contradictory responses between the species could be further investigated using detailed measurements of water and soil contaminants to determine whether the relationships between particular trace metals and the vegetation indices are significant.

***Teratogenic Potency and Embryotoxicity***

It was found that larval survival was markedly higher after rehabilitation at the downstream site. It was also observed that the incidence of malformations was higher upstream from the rehabilitation area than the reference site, the downstream site as well as the negative control. After rehabilitation, the proportions of malformed individuals were lower in the downstream treatment than both the reference site and the negative control treatment. In contrast to this, before rehabilitation, the downstream locality was characterized by a higher teratogenic potency than the reference site.

### *Resource Economics*

The work demonstrates that wetlands are valuable and in acid mine drainage affected environments, the water purification and waste assimilation service provided by wetlands are especially valuable. Wetland valuation starts with defining the wetland ecosystem services, as defined by the Millennium Ecosystems Assessment and The Economics of Ecosystems and Biodiversity. Ecosystem services are defined as the benefits provided to humans (i.e. to the economy) by ecosystems. Three categories of ecosystem services are of interest here, namely provisioning, cultural and regulating services. A range of wetland valuation tools exist and are typically applied in the specialist field of resource economics. The particular ecosystem service of interest analysed here is the regulating service of water purification and waste assimilation. Practically, this service means that wetland ecosystems provide natural water treatment and waste removal systems, which is an alternative to water treatment plants.

In order to demonstrate the value of this service, we applied the primary water chemistry monitoring data of the CSIR, before and after rehabilitation of the Zaalklapspruit Wetland by SANBI's Working for Wetlands Programme, and compared the resultant natural water treatment ability of the wetland to the alternative of treatment through reverse osmosis. Data for reverse osmosis treatment was sourced via the Water Resource Classification System data provided by Golder Associates for a reverse osmosis acid mine drainage treatment scenario in the Olifants Water Management Area. Thus, by rehabilitating wetlands, we have an alternative for building reverse osmosis plants. The resulting water purification and waste assimilation value must be viewed in the context of the other wetland ecosystem services. We used the wetland valuation method used in the Olifants Water Resource Classification System to demonstrate the values of the other wetland ecosystem services; this was therefore based on secondary data.

Thus, based on the Olifants Water Resource Classification System wetland ecosystem service valuation, the value of the provisioning and cultural services delivered by the Zaalklapspruit Wetland is estimated at R21,300/ha/annum (or R2.9 million per year for the 135 ha Zaalklapspruit Wetland); and the value of the regulating services delivered by the wetland, excluding water purification and waste assimilation, is R22,940/ha/annum (or R3.1 million per year). The water purification and waste assimilation service of the Zaalklapspruit Wetland has a value ranging between R20 000-R85 000/ha/annum or R2.6-R11.4 million per year. This is based on monitoring data of the CSIR taken within less than a season after rehabilitation and this value can therefore likely be expected to increase as the rehabilitated wetland stabilises and matures.

The production of ecosystem services relates closely to wetlands' inherent 'asset' value, which is also referred to as "ecological infrastructure". Based on the estimates done in this study, the asset (or ecological infrastructure) value of the Zaalklapspruit Wetland ranges between R501-R763 million, of which the water purification and waste assimilation service contributes R130-R560 million. Thus, by rehabilitating the Zaalklapspruit Wetland at a cost of R1.7 million, we have been able to produce R130-R560 million on the natural asset balance sheet of South Africa. This is a very significant result and it strongly supports investment in wetland rehabilitation, especially in acid mine drainage affected areas. It also demonstrates that wetland rehabilitation may form a very important part of wetland impact mitigation strategies.

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**Reference Group**

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# CHAPTER 1: BACKGROUND

## 1.1 INTRODUCTION

In exploring mutually acceptable solutions, the Council for Scientific and Industrial Research (CSIR) and the South African National Biodiversity Institute (SANBI) have built constructive relationships with the coal mining sector. Thus, in 2011 the CSIR and SANBI embarked on a three-year cooperative applied research project, funded by the Coaltech Research Association. Supplementary funding is also being provided by the SANBI Grasslands Programme and Working for Wetlands Programme, as well as the Water Research Commission (WRC), for particular components of the work. The project's focus is on developing mechanisms for limiting and mitigating the impact of coal mining on wetlands and providing guidelines to the coal mining industry and regulators in this regard. Wetlands provide more ecosystem services per hectare than any other ecosystem, being sites of intense biogeochemical activity that play an important role in improving water quality. It was therefore important to obtain an in depth understanding of the changes with regard to key variables by studying various levels of wetland functionality.

One of the components of the project focused on rehabilitation of a degraded wetland that was receiving pollution derived from coal mining, in order to determine whether rehabilitation would improve the wetland's ability to reduce the levels of these particular pollutants in the water flowing through it. The project team, in consultation with Coaltech members, Working for Wetlands and other stakeholders identified a wetland that met all the criteria necessary to make it a suitable case study. Factors taken into account included the type of wetland, type and extent of degradation, level of coal mining impact, rehabilitation feasibility and landowner willingness. The wetland had been impacted upon physically by previous agricultural activities that had drained portions of its temporarily and seasonally saturated zones and incised the channel running through the system, thereby substantially concentrating flow and reducing the retention time of water within the wetland.

Working for Wetlands, a wetland rehabilitation programme funded by the Department of Environmental Affairs and housed within SANBI at the time of the project, together with the CSIR, Coaltech and the WRC conducted the rehabilitation of the wetland in accordance with standard procedures. A rehabilitation plan was compiled that set specific objectives for improving the ability of the wetland to mitigate the levels of mining-related pollutants it received (SANBI, 2013).

## 1.2 PROJECT AIMS

The following aims were set for the project:

1. Water Quality: The aim of the water quality component was to determine to what extent the rehabilitation of the Grootspruit has improved the downstream water quality when compared to upstream.
2. Bacterial Consortium: The aim of this part of the study was to determine the changes in diversity and richness of the microbial consortium in the Grootspruit that received mining effluent before and after rehabilitation.
3. Freshwater Algae: Due to their nutritional requirements, position in the food chain and their rapid response to various toxicants, they represent a very promising indicator of wetland degradation; therefore, the aim of this component was to employ freshwater algae to differentiate between conditions before and after wetland rehabilitation.
4. Vegetation Assessment: The aim for this part of the study was to determine the use of the spectral properties of selected vegetation as an affordable, non-destructive way of tracking the improvement of wetland vegetation after rehabilitation.
5. Teratogenic Potency and Embryotoxicity: The aim of this part of the study was to evaluate the teratogenic potential and embryotoxicity of surface water collected from the Grootspruit upstream and downstream of the rehabilitated area.
6. Resource Economics: The aim of the socio-economic component of the project was to internalize the costs of the ecosystem services delivered by wetlands, which are impacted by coal mining activities.

# CHAPTER 2: WATER QUALITY ASSESSMENT OF THE GROOTSPRUIT WITHIN THE ZAALKLAPSPRUIT WETLAND IN MPUMALANGA

## 2.1 INTRODUCTION

Water quality assessment is an essential tool with which to determine the impact and sustainability of ecological restoration. Baseline parameters are determined prior to restoration, and the changes in water chemistry after restoration are monitored to determine the reduction in contamination, increase in pH as well as increased water quality conditions for healthy aquatic ecosystems.

Previously, a baseline assessment upstream and downstream of the Grootspruit, earmarked for rehabilitation, was completed. Herein, the water chemistry was determined which would assist in describing the extent to which rehabilitation of the area could improve the overall water quality. These water quality conditions were compared to that of a reference site, which was relatively unimpacted by mining practices. The impacted area showed very low chlorophyll a (Chl a), alkalinity and pH values compared to that of the reference site. The Zaalklapspruit project area also showed elevated metal, sulphate and total dissolved solids (TDS) concentration, all indicative of an area impacted by acid mine drainage (AMD). The area was low in nutrients and the electrical conductivity (or TDS) increased downstream.

With remediation of the wetlands, the area for drainage of the AMD impacted water is increased to allow for gravitational drainage. This allows for metal removal and neutralization, or increased alkalinity, due to increased sulphate reducing bacterial (SRB) activity (Gazea *et al*., 1996). Improvement of the water quality therefore relies on biologically mediated processes, supported by filtration of suspended and colloidal materials (Johnson and Hallberg, 2005). The Grootspruit consists of both anaerobic and aerobic conditions, containing both water logged soil with conditions favourable to SRB as well as oxygen-rich overland flowing water.

Changes in the water quality will probably be due to:

1) Changes in the properties of the contaminants;
2) Changes in the environment; and
3) Changes to properties in the sediment

The purpose of this report is to describe the changes in water chemistry observed for the area which has been rehabilitated compared to the values described in the baseline assessment. The primary objective is therefore to determine to what extent the rehabilitation in the Grootspruit has improved the downstream water quality (compared to upstream). Improvement in the water quality will primarily be determined by changes in pH, alkalinity, metals, TDS and Chl a.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Study Area

The Grootspruit is located in the Mpumalanga Province (South Africa) on a tributary of the Zaalklapspruit Wetland which flows into the Wilge River. In total 11 sites were samples over two years which have been grouped together as the reference site, upstream and downstream of the rehabilitated area (Figure 2.1). Downstream site evaluation is also classified according to Pre- and post-rehabilitation data.

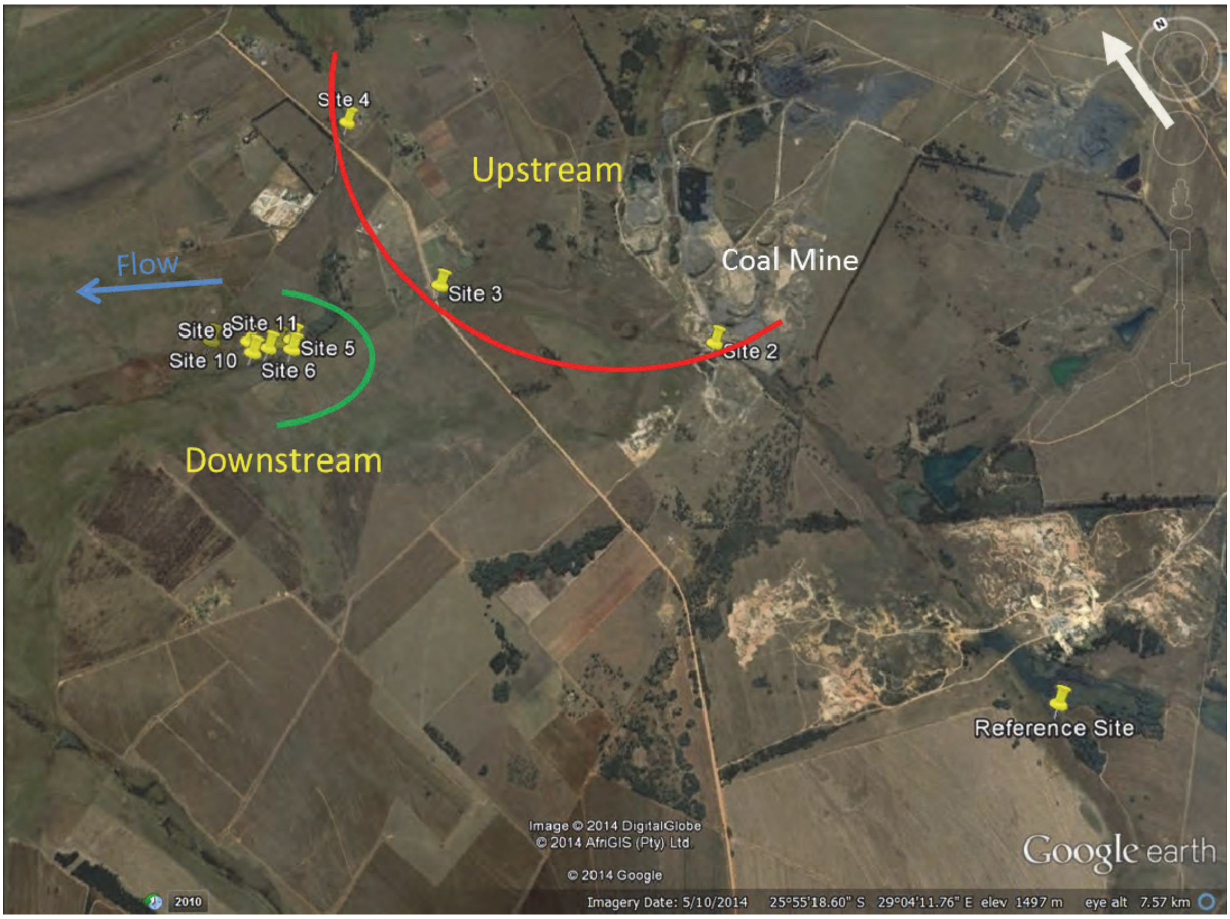



**Figure 2.1 Sampling sites at the Grootspruit with overall data grouping. The upstream area includes Sites 2, 3 and 4 while the downstream area includes Sites 5, 6, 7, 8, 9, 10 and 11 and then the reference site.**

### 2.2.2 Water Analysis

The *in situ* water quality parameters (i.e. pH, conductivity and dissolved oxygen) were measured using a Thermo Five star handheld water quality meter. Turbidity was also determined on site using a Hach 2100P Turbidity meter (Loveland, USA). Water samples were collected in 1-litre bottles and kept on ice according to the sampling method of Shelton (1994). Samples were sent to the CSIR's accredited laboratory within 48 hours of collection for chemical analysis according to (APHA, AWWA and WPCF, 1992). Table 2.1 lists the variables analysed for in the collected water samples.

**Table 2-1 Physical parameters, major ions, metals and nutrients analysed for in the water**

| Physical parameters | Major ions | Metals | Nutrients |
|---|---|---|---|
| Electrical conductivity (EC)<br>pH<br>Dissolved oxygen (DO) | Potassium (K)<br>Sodium (Na)<br>Calcium (Ca)<br>Magnesium (Mg)<br>Sulphate ($SO_4$)<br>Chloride (Cl)<br>Alkalinity (as $CaCO_3$)<br>Silica (Si)<br>Fluoride (F) | Aluminium (Al)<br>Arsenic (As)<br>Cadmium (Cd)<br>Copper (Cu)<br>Iron (Fe)<br>Lead (Pb)<br>Manganese (Mn)<br>Nickel (Ni)<br>Selenium (Se)<br>Zinc (Zn)<br>Boron (B)<br>Vanadium (V) | Nitrate + Nitrite as N<br>Nitrate<br>Nitrite as N<br>Ortho-phosphate ($PO_4$-P)<br>Dissolved organic carbon (DOC)<br>Total organic carbon<br>Phosphorous (P)<br>Ammonium-N ($NH_4$-N) |

The water chemistry was described using a piper diagram. A piper diagram is a graphical representation of the chemistry of water samples. The cations and anions are shown by separate ternary plots. The main purpose of the piper diagram is to show clustering of data points to indicate samples that have similar compositions. A piper diagram plots the major ions as percentages of milliequivalents on two different triangles. These triangles show the relative composition of the cations and anions. Piper diagrams are useful in showing distinct water quality populations (Appelo and Postma, 1993), as well as likely drainage scenarios. Piper diagrams were made using Aquachem version 5.1 software (Schlumberger Water services, Copyright 200).

Principal Component Analysis (PCA) biplots were constructed to assess spatial trends of the water quality variables at the surveyed sites. These plots were derived using XLSTAT version 2014.4.10 (Addinsoft, 1995-2014) software.

## 2.3 RESULTS AND DISCUSSION

The water quality parameters were grouped and compared to establish the level of improvement, if any, in the water quality after rehabilitation of the wetland. The most significant parameters associated with AMD determined for the sampling areas are summarised in box and whisker plots in Figure 2.2. The SO4, TDS, pH and alkalinity determined for the reference site remained relatively unchanged during the sampling period and therefore un-impacted by mining practices or AMD. Upstream of the rehabilitation area, however, impact was ongoing at varying degrees during the monitoring period. Upstream of the rehabilitation area the median SO4 concentration was 573 mg/L. The SO4 and TDS concentrations decreased significantly after rehabilitation of the wetland, median SO4 concentration before rehabilitation was 1210 mg/L which decreased to 473 mg/L and the median TDS decreased from 1048 mg/L to 525 mg/L. The pH for the water increased after rehabilitation to within the optimal range for healthy aquatic ecosystems (DWAF, 1996).

In Figure 2.2, arsenic serves as an indicator parameter for the presence of toxic heavy metals (metalloids), due to its widely known toxic impacts on human health (USEPA, 1984). Upstream of the rehabilitation area, samples have been found with arsenic concentrations which may pose a human health risk. Downstream in the rehabilitated area, the arsenic concentrations have been reduced to below the detection limit of 0.1 µg/L.

The measured alkalinity increased significantly after rehabilitation of the area to a median value of 22 mg/L which has become comparable to that of the reference site. The alkalinity upstream of the rehabilitated area remains low at a median value of 0.25 mg/L which improves slightly during the wetter season. Alkalinity is increased due to the bacterial reduction of oxyanions (sulphates, nitrates, selenates, etc.) and of metal oxides ($Fe_2O_3$, $MnO_2$, etc.) during mineralization of organic carbon generally which result in the production of alkalinity (Ehrlich, 1993) as well as the dissolution of accumulated carbonates.

The chl a concentrations, which are indicators of organic biomass activity in the water, remained relatively high for the reference site at 14.1 µg/L. Upstream, the chl a concentration remained low at 0.3 µg/L. After rehabilitation of the downstream wetland the chl a concentration improved significantly to 1.0 µg/L.

The metals primarily associated with the impacts of AMD are aluminium, iron and manganese. These metal concentrations pre and post rehabilitation are shown in Figure 2.3. The median aluminium concentration decreased from 4 770 µg/L to below the detection limit after remediation of the downstream area. Iron concentrations decreased by more than 90% (Figure 2.3) and median manganese concentrations decreased from 8 675 µg/L to 417 µg/L after rehabilitation. Decreases in the metal concentrations are most likely due to the observed increase in pH as well as alkalinity. The solubility of aluminium decreases significantly above pH 5 (Nordstrom and Ball, 1986). Iron precipitation is more intricate, being governed by water pH, oxidation/reduction potential, and the presence of potential ligands such as sulphate and carbonate anions. As with aluminium, removing the ferric iron (Fe3+) component of the total iron load simply involves increasing water pH and allowing sufficient retention. Removing ferrous iron requires that it first be oxidized to ferric iron or that it reacts with hydrogen sulphide in a reducing environment which is catalysed by bacteria at pH lower than 5 (Hedin *et al*., 1994; Robbins and Norden, 1994;), whereas chemical hydrolysis predominates Fe2+ removal in neutral or alkaline waters.

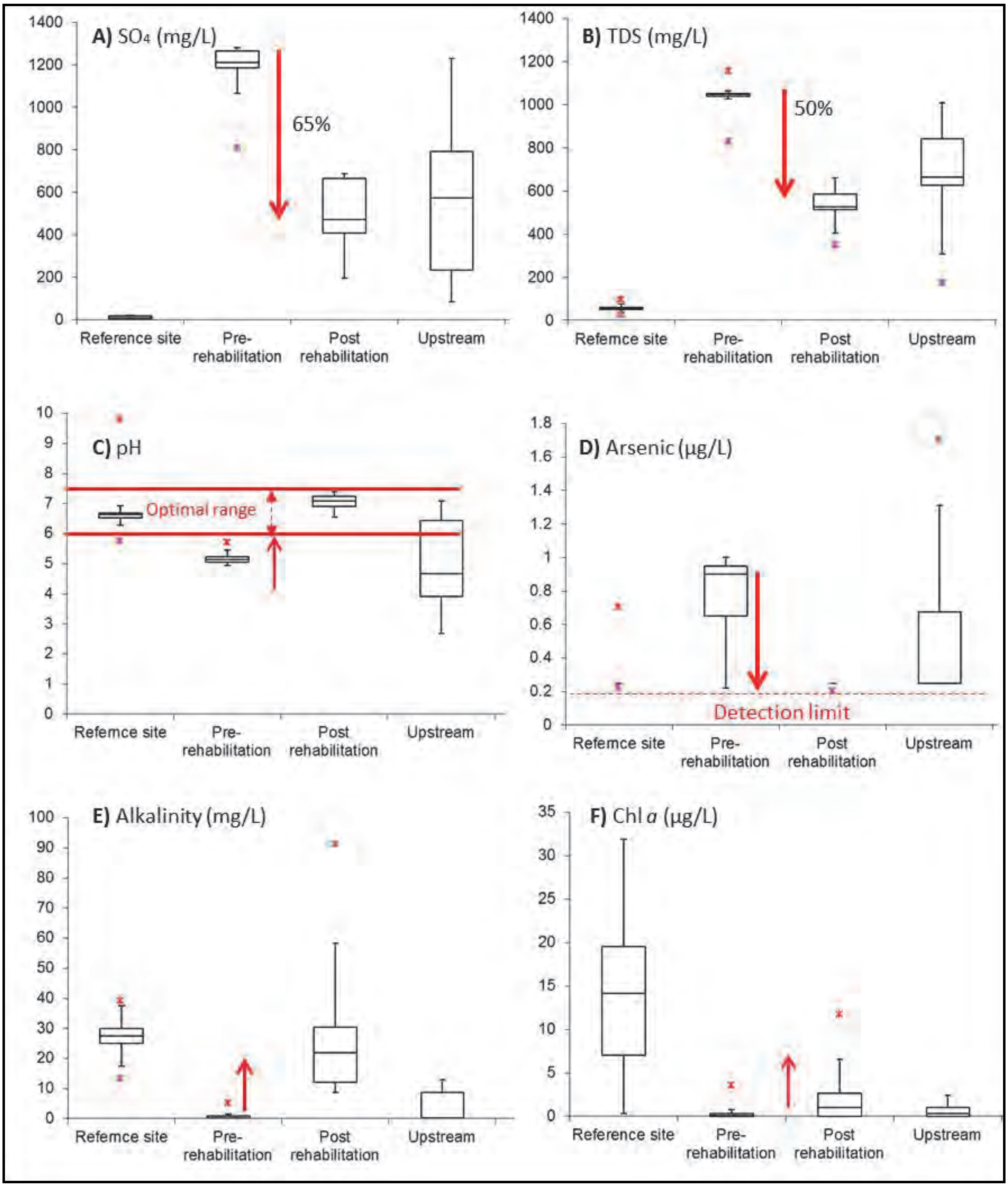



**Figure 2.2 Box and whisker plots for A) sulphate ($SO_4$), B) total dissolved solids (TDS), C) pH, D) arsenic, E) alkalinity and F) chlorophyll a (Chl a) concentrations determined for the upstream, reference and downstream sites before and after rehabilitation. Red arrows indicate increase (up) and decrease (down) of the specific variable after rehabilitation. The box indicates the median line with the third and first quartile indicated above and below, respectively. The ends of the whiskers are set at 1.5x above the third quartile (Q3) and 1.5x below the first quartile. The * indicates the lowest and highest outlier in the data series.**

The removal of manganese in wetlands is also quite complex. Manganese removal is facilitated through oxidation and/or hydrolysis reactions which are generally assisted by the drastic reduction of ferrous iron, as this metal cation redissolves manganese precipitates (Hedin *et al*., 1994). Manganese oxidation typically proceeds slowly in aerobic wetlands. The Grootspruit, however, consists of anaerobic regions as well as oxygen-rich overland surface water flow, which largely contributed to the significant reduction in manganese after rehabilitation.

The chloride concentrations determined before and after rehabilitation are markedly similar. Surface flow wetlands have been proven to be ineffective in the treatment of elevated chloride concentrations (Vidales-Contreras *et al*., 2010). Chloride is considered to be very stable in most terrestrial environments (Kadlec and Knight, 1996) due to the high solubility of chloride and because the uptake thereof into plant tissues is negligible (Hayashi *et al*., 1998). It is for this reason that chloride has often been used to estimate evapotranspiration in wetland ecosystems (Hayashi *et al*., 1998).

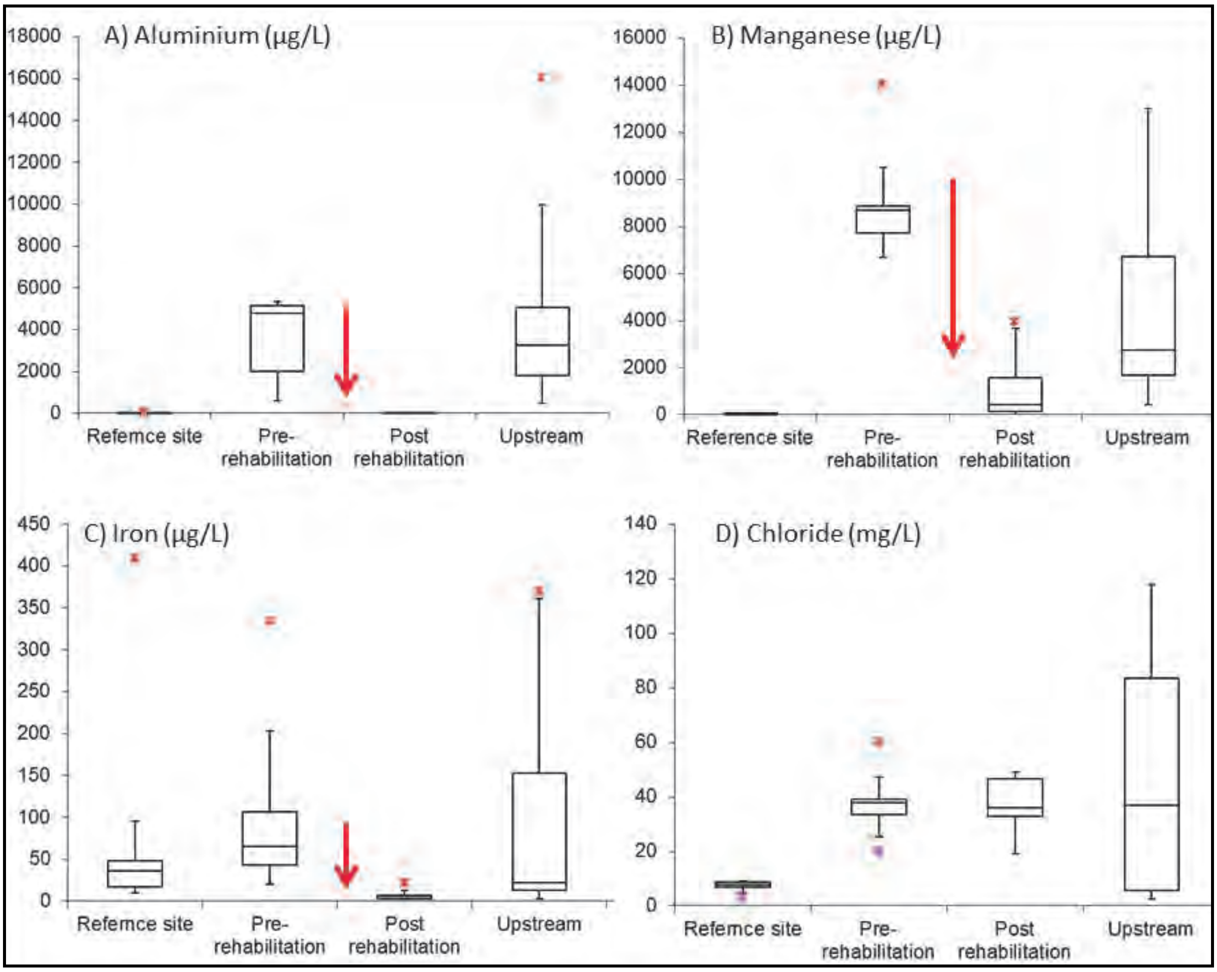



**Figure 2.3 Box and whisker plots for A) aluminium, B) manganese, C) iron and D) chloride concentrations determined for the upstream, reference and downstream sites before and after rehabilitation. Red arrows indicate increase (up) and decrease (down) of the specific variable after rehabilitation. The box indicates the median line with the third and first quartile indicated above and below, respectively. The ends of the whiskers are set at 1.5x above the third quartile (Q3) and 1.5x below the first quartile. The * indicates the lowest and highest outlier in the data series.**

The concentrations for most metals determined for the reference site were very low, and often below the detection limit of the detection techniques (Figure 2.4). The median lead and vanadium concentrations were significantly higher upstream of the rehabilitated area compared to all of the sites monitored. The median vanadium and lead concentration were 1.4 and 0.6 µg/L, respectively, which were determined at the highest site closest to the mining activities. Vanadium in AMD is prone to the formation of oxalates when suddenly exposed to aerated conditions, which is why the highest concentration of vanadium would be close to the source of AMD entering the natural water resource (Smith *et al*., 2001). Similarly, lead has a high affinity for organic components in wetland sediment and is rapidly removed from the water column when introduced (Pickering, 1986).

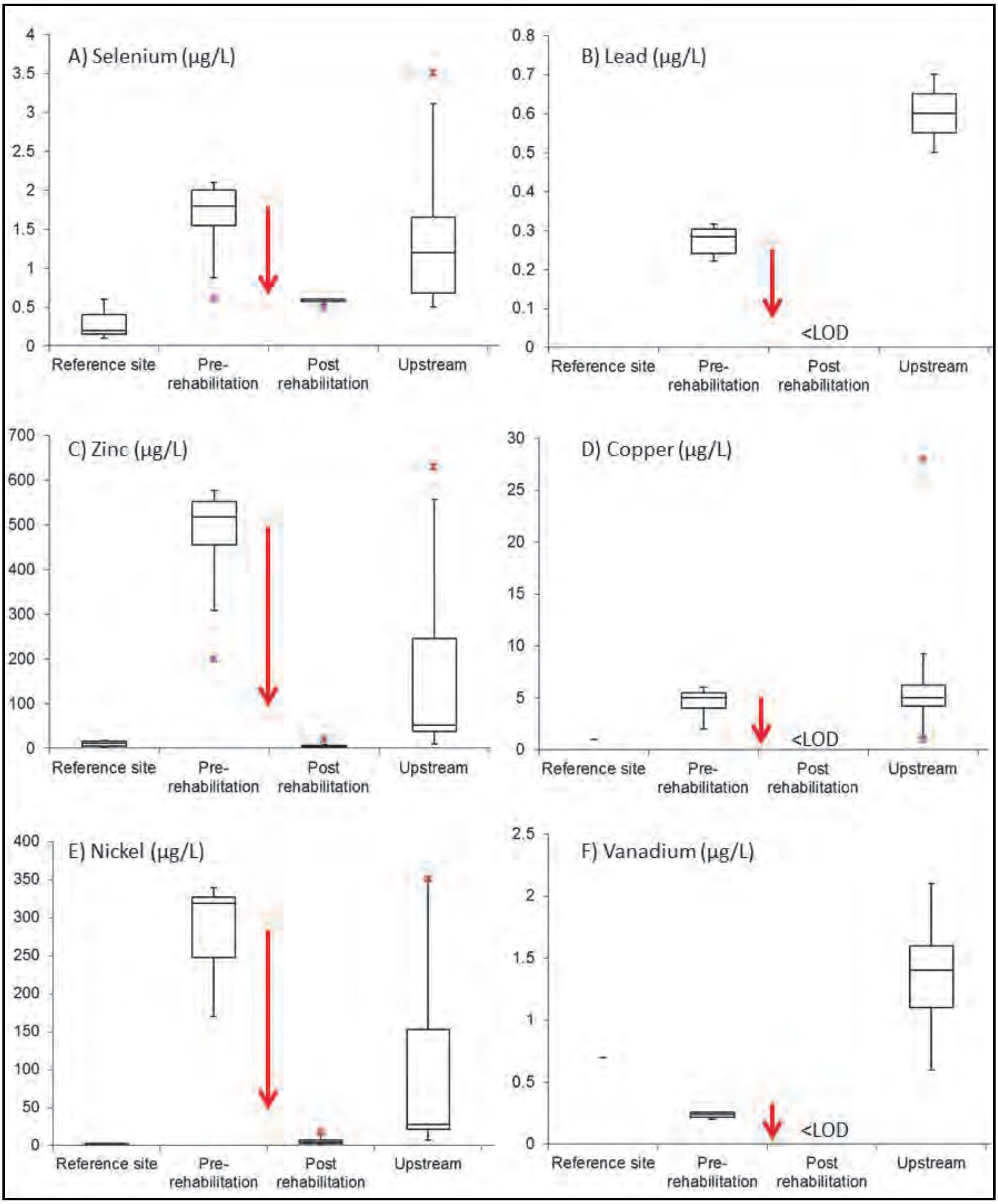



**Figure 2.4 Box and whisker plots for A) selenium, B) lead, C) zinc, D) copper, E) silica and F) vanadium concentrations determined for the upstream, reference and downstream sites before and after rehabilitation. Red arrows indicate increase (up) and decrease (down) of the specific variable after rehabilitation. <LOD = Below level of detection. The box indicates the median line with the third and first quartile indicated above and below, respectively. The ends of the whiskers are set at 1.5x above the third quartile (Q3) and 1.5x below the first quartile. The * indicates the lowest and highest outlier in the data series.**

All of the metals shown in Figure 2.4, reduced significantly in the water column post rehabilitation. This is likely due to the formation of highly insoluble sulphide compounds with copper, lead, nickel and zinc (Ettner,1999; Smith *et al*., 2001;). Hydrogen sulphide (a by-product of sulphate reducing bacterial activity) can react with transition metals to form highly insoluble compounds (Stumm and Morgan, 1981). The reduction in selenium is likely due to increased biological volatilization of selenium in plant-rich sediment (Hansen *et al*., 1998). The median concentration of vanadium before rehabilitation was 0.2 µg/L which was reduced to below the detection limit of 0.2 µg/L. The extent of reduction is therefore unclear and the mechanism for vanadium removal is also not evident. As oppose to most metals contained in AMD, vanadium precipitation and removal is decreased in more alkaline and neutral conditions. Nutrient and silica concentrations did not vary significantly from their baseline values and also no remediative effects were observed for these parameters after rehabilitation.

Figure 2.5 clearly shows the distribution of the sampling sites. The ordination plot describes 92.48% of the variation in the data, with 70.66% on the first axis and 21.82% on the second axis. The upstream and pre-rehabilitation sites were associated with increases in arsenic, iron, aluminium, manganese, TDS, sulphate and chloride concentrations. It is further evident that the post rehabilitation area showed increases in alkalinity and pH as well as decreased metal, sulphate, TDS and chloride concentrations compared to pre-rehabilitation conditions. The reference site had significantly higher Chl a concentrations which were associated with decreased chloride, sulphate, TDS and manganese concentrations. The PCA plot also indicated the inverse relationships between alkalinity and pH and the aluminium, arsenic and iron concentrations. Increases in pH and alkalinity resulted in decreased concentration of these metals. Figure 2.5 also confirmed that the reference site was not impacted by activities similar to those upstream or downstream of the rehabilitation area.

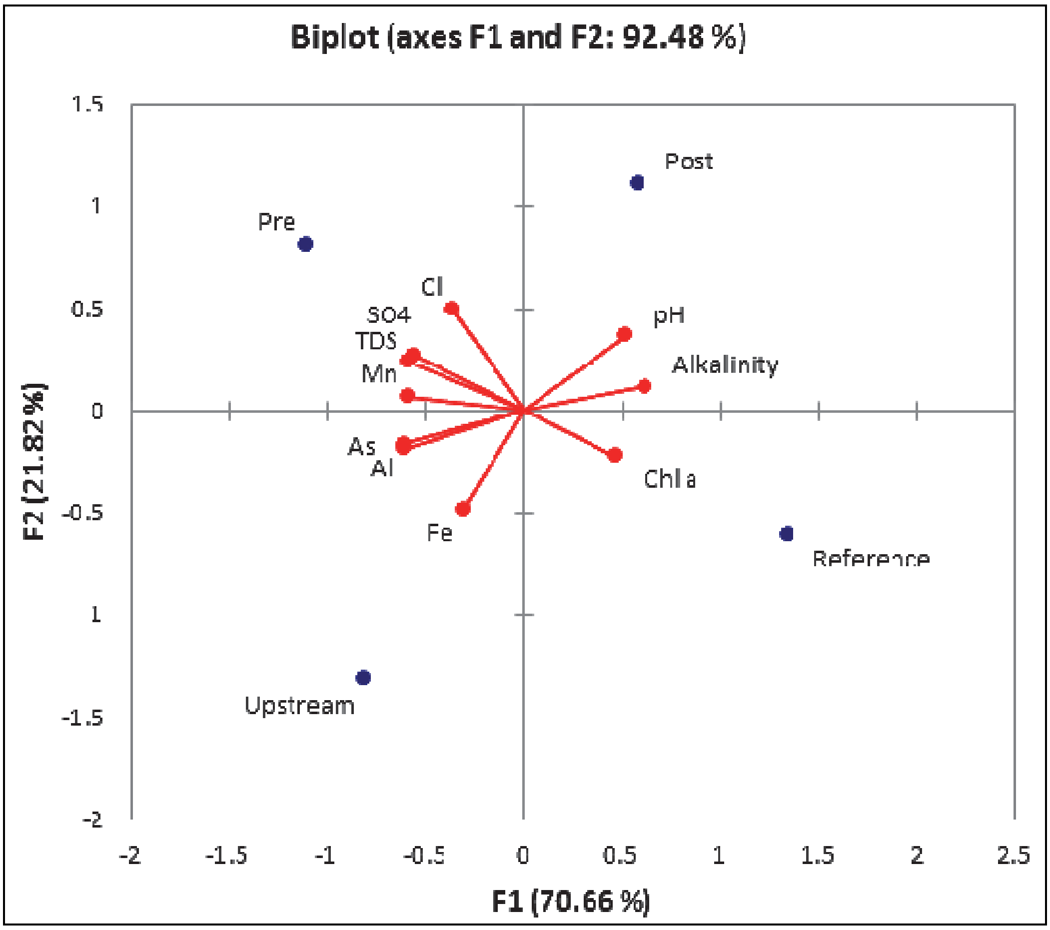



**Figure 2.5 Principal component analysis plot for the median water quality variables measured for the reference, upstream and downstream sites before and after rehabilitation**

The piper diagram in Figure 2.6 is based on the ionic data determined from the median concentration in water samples collected from the various sampling sites. The reference site is clearly not as impacted by mining activities as the sites in the Zaalklapspruit area. Although the area of drainage has been extended in the rehabilitated area, the geology allowing for the cationic exchange, primarily Ca+, has remained near identical. From the ionic distribution, it is clear that the sites are less impacted by AMD, which is a result of decreased sulphate concentrations and increased alkalinity. It is evident that the rehabilitated area remains significantly impacted by AMD, although a small degree of ionic water quality improvement is apparent.

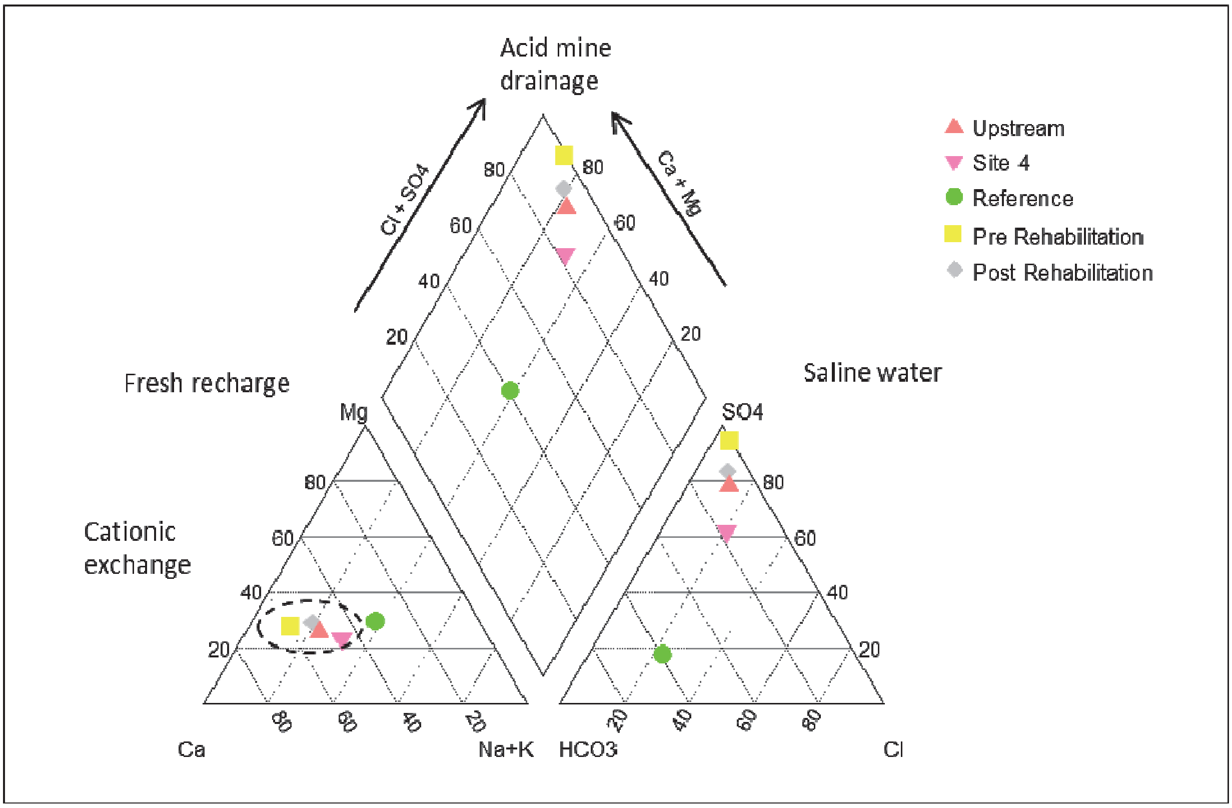



**Figure 2.6 A piper diagram based on the ionic data determined for the Upstream, Reference and Downstream site before and after rehabilitation**

## 2.4 CONCLUSIONS

Rehabilitation of the wetland area improved the water quality by:

i) Increasing the pH, alkalinity and Chl a concentrations;
ii) Decreasing the metal concentrations in the surface water; and
iii) Reducing the sulphate and TDS concentrations.

This was achieved by increasing the sediment/soil contact area which created an environment with a combination of anaerobic and aerobic conditions. Herein, optimal conditions for SRB to thrive were combined with oxidative states made available through the flow of oxygen-rich surface water. Bacterial sulphate reduction accounts for much of the improved alkalinity observed after rehabilitation. It can also contribute significantly to metal removal by formation of insoluble sulphides.

The rehabilitated wetland will continue to remove the metals and increase the pH of the surface water as long as the SRB remain active. For the bacteria to survive they require anaerobic conditions, adequate supply of sulphate and smaller organic compounds provided by the bed substrate. Another consideration is the formation of $H_2S$, which may increase due to a lack of metals and a highly oxidative environment, and can be converted back to sulphate. Care should thus be taken that no further upstream interventions are applied which may compromise the sulphate inflow to or slow the overall flow rate to the rehabilitated area.

# CHAPTER 3: AN ASSESSMENT OF THE BACTERIAL CONSORTIUM BEFORE AND AFTER REHABILITATION WITHIN THE WETLAND WATER FRACTION

## 3.1 INTRODUCTION

Microorganisms play a vital role in the biogeochemical recycling of nutrients in wetlands. The microbiology of wetlands is the most important factor influencing the removal of pollutants in these environments (Faulwetter *et al*., 2009). Therefore, an understanding of the nutrient cycling in wetlands is critical in the rehabilitation and protection of wetland systems. The dominant microbial processes in wetlands include denitrification and nitrification, methanogenesis and methanotrophy, sulfate and iron oxidation/reduction (Gutknecht *et al*., 2006). Research has shown that the presence of microbial functional groups such as denitrifiers and sulphate reducing bacteria are responsible for the removal of specific pollutants in wetlands (Faulwetter *et al*., 2009). The water table height, depth from the surface, and distance from plant roots create oxic to anoxic gradients. This results in a complex relationship between anaerobic and aerobic settings, which causes a wide range of processes to occur in wetlands (Gutknecht *et al*., 2006). Many bacterial species are facultative anaerobes, capable of functioning under both aerobic and anaerobic conditions in response to changing environmental conditions. Other factors that affect wetland microbial activities, that result in biogeochemical changes include temperature, hydrology, substrate availability, pH and plant community structure (Gutknecht *et al*., 2006).

The recent application of new molecular microbial techniques provides an immense advantage in the study of wetlands. Pyrosequencing is one of the recently developed high-throughput sequencing approaches and was used in this study in order to obtain an understanding of the diversity of the microbial consortium present in the water fraction of the wetland.

Real-time polymerase chain reaction (RT-PCR) is a highly sensitive culture independent method for the detection of microorganisms in environmental samples. Functional genes generally have more sequence variation than 16S rRNA genes and can therefore be used as biomarkers to discriminate between closely related but ecologically different populations (Palys *et al*., 1997). Three functional groups of microorganisms were selected to be quantified in the wetland due to the important roles that they play in regulating the cycling of major nutrients and carbon in freshwater wetlands. These include sulphate reducing bacteria (SRB), methanogens and denitrifying bacteria.

Sulphate reducing bacteria (SRB) are anaerobic, gram negative bacteria that use sulphate as an electron acceptor. Sulphate is taken up as a nutrient and reduced to sulphide, which is incorporated into sulphur-containing amino acids and enzymes (Muyzer and Stams, 2008). The reduction of sulphate can directly influence the carbon cycle in wetlands (Pester *et al*., 2012). Sulphate reducing bacteria have been detected in habitats such as acid-mine drainage sites where the pH can be as low as 2 (Sen and Johnson, 1999). Quantitative real-time PCR has been used to quantify the number of SRB in rice field soils (Stubner, 2004), soda lakes (Foti *et al*., 2007), and industrial wastewater (Ben-Dov *et al*., 2007).

Methanogens belong to the domain Archaea and play an integral role in carbon cycling (Steinberg and Regan, 2008). Their habitats include freshwater sediments, soils and wetlands, marine sediments, rice paddies, geothermal environments, animal gastrointestinal tracts and anaerobic digesters (Barber and Ferry, 2001). Methanogens are anaerobic, although several species have been shown to be relatively tolerant to oxygen (Barber and Ferry, 2001). Methanogens are only able to use a limited number of basic organic compounds for their carbon and energy requirements. Most methanogens generate energy by the reduction of $CO_2$ to methane ($CH_4$) using $H_2$ as an electron donor.

Denitrification is one of the important processes of the global nitrogen cycle carried out by bacteria (Zumft, 1997). Denitrifying bacterial habitats include soil, sediment and aquatic environments (Gamble *et al*., 1977; Ward, 1996; Zumft, 1997). Bacterial denitrification has been shown to remove significant amounts of nitrogen from the wetland waters (Ingersoll and Baker, 1998; Willems, 1997; Søvik and Mørkved, 2008). Denitrification is the stepwise reduction of nitrate by denitrifying bacteria to dinitrogen ($N_2$).

The wetland of focus in this study is the Zaalklapspruit Wetland in the Upper Olifants catchment of the Mpumalanga Province. This wetland receives coal mine effluent which impacts water quality severely. The wetland was determined to be functioning poorly as permanent channel incision had caused concentrated channelized water flow; therefore, rehabilitation of the wetland was required. Microbial populations can undergo swift changes in composition and function in response to changing environmental conditions and bacteria are also highly sensitive to small fluxes of contaminants in the environment (Sims *et al*., 2013). As changes in the wetland ecosystem will be reflected by a shift in the microbial consortium, with the aid of molecular techniques, the aim of this study was to determine the composition and diversity of the microbial consortium in the Zaalklapspruit Wetland before and after rehabilitation in order to acquire an indication of wetland status.

The aim of this part of the study was to determine the composition and diversity of the bacterial consortium in the Zaalklapspruit wetland before and after rehabilitation in order to obtain an indication of wetland status. The following objectives were set for the study:

1. To quantify the indicator organisms present in the water fraction of the wetland.
2. To determine the composition and diversity of the bacterial consortium in water fraction of the wetland using pyrosequencing.
3. To detect sulphate reducing bacteria, denitrifiers and methanogens by RT-PCR.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Sampling

Samples were collected during two separate trips during both the pre-rehabilitation period (2013) and post-rehabilitation period (2014) in sterile polyethylene bottles. Sites both upstream and downstream of the wetland were sampled as well as a reference site. Care was taken to ensure that samples collected during these two years were taken during the same season/hydrological extreme.

### 3.2.2 Indicator Organisms

Quantification of *Escherichia coli* and total coliforms was carried out using the Colilert™ Most Probable Number (MPN) method (IDEXX, USA).

### 3.2.3 Pyrosequencing

#### *3.2.3.1 DNA Extraction*

A volume of 2 L of water was sampled from each site and filtered through 0.45 µm cellulose nitrate filters (Sartorius Stedium). The remaining cell debris was gently scraped from the filter and resuspended in 2 mL of 1 × phosphate buffered saline (PBS) (137 mM NaCl; 2.7 mM KCl; 10 mM $Na_2HPO_4$, 1.8 mM $KH_2PO_4$), pH 7.4. The suspension in 1 × PBS was centrifuged at 13 000 rpm to pellet the cells and the supernatant was removed. Deoxyribonucleic acid (DNA) was extracted using the DNeasy blood and tissue kit (Qiagen) and the manufacturer's protocol for extraction of Gram-positive bacteria was followed.

#### *3.2.3.2 Pyrosequencing Reaction*

Amplification, pyrosequencing and the data analysis was carried out by Inqaba Biotec™ (Pretoria, South Africa). The universal 16S rRNA primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') (Weisburg *et al*., 1991) and 518R (5'-ATTACCGCGGCTGCTGG-3') (Muyzer *et al*., 1993) were used to amplify the V1, V2 and V3 hypervariable regions of the gene. Each amplicon was gel purified in equimolar amounts for sequencing. Sequencing was carried out on a 454 GS FLX Titanium sequencing platform (Roche 454 Life Sciences). The sequencing files were exported as FASTA files and BLAST searches were carried out using the 16S Ribosomal RNA sequences (bacteria and archaeal) database. Using Microsoft Excel, the BLAST data was ordered according to percentage identity and from here, the hits for each query were identified. The species identified in the top hits were quantified and a graph representing the diversity on read counts was drawn. Read counts in this text refer to the amount of times or frequency that a sequence identity is assigned to a query sequence. The bacteria identified were assigned to their known bacterial phyla. For the purposes of this report, undescribed bacteria and 'no hits' were not included in the graphs for ease of reference.

### 3.2.4 Polymerase Chain Reaction

#### *3.2.4.1 Primers and Positive Controls*

For the detection of SRB, denitrifiying bacteria and methanogens, four PCR assays were adapted or modified from literature. The primer sequences used in the study are given in Table 3.1.

**Table 3-1 The polymerase chain reaction primers used in this study for the detection of sulphate reducing bacteria, denitrifying bacteria and methanogens**

| Target Gene | Primer Sequences | Source |
|---|---|---|
| *dsrA*<br>Sulfite Reductase Subunit A | **dsr1f** 5' ACSCACTGGAAGCACG 3'<br>**rh3-dsr-r** 5'GGTGGAGCCGTGCATGTT3' | Wagner *et al*. (1998); Ben-Dov *et al*. (2007) |
| *nirK*<br>Copper-containing nitrite reductase | **nirK876** 5' ATYGGCGGVCAYGGCGA 3'<br>**nirK1040** 5' GCCTCGATCAGRTTRTGGTT 3' | Henry *et al*. (2004) |
| *nirS*<br>Dissimilatory reductase | **nirSWF** 5' GTCGTGCAGCCSGAGTWCAA 3'<br>**nirS6R(m)** 5' AGTCGTTGAACTTRCCGGTC 3' | Modified from Braker *et al*. (1998) |
| *mcrA*<br>T7 methyl coenzyme M reductase alpha and beta-subunit | **Mlas** 5 'TAYTGKGTGAAHCCKACACCACC 3'<br>**mcrA-rev** 5'CGTTCATBCGTAGTTVGGRTAGT3' | Steinberg and Regan (2008) |

Bacteria that metabolize sulphates and nitrates, or produce methane, are difficult to culture in laboratory conditions, precluding the use of live cultures as PCR amplification controls (and quantitation standards). Amplification controls were prepared as follows: the target region for each assay was identified using the BLAST function hosted online by the National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov). The target gene sequences were downloaded from NCBI and the primer binding sites were located within the sequences. The primer binding sites, indicated by the framed areas, are given below:

*dsrA*: Sulfite Reductase Subunit A (Amplicon size: 222 bp)
5'ACGCACTGGAAGCACGGAGGTATTGTTGGCGTCCTGGGCTATGGCGGCGGCGTCATCGGCCGCTAC
AGCGACATGGGCGACCGATTCCCGAATGTCGCGCACTTCCATACCATCCGCGTCAACCAGCCGT
CCGGGTGGTTCTACACGAGCGAGGCGATCAGGACGCTGTGCGACATCTGGGAGAGGCACGGGT
CGGGCCTCACCAACATGCACGGCTCCACC 3'

*nirK*: copper-containing nitrite reductase (Amplicon size: 164 bp)
5'ATCGGCGGGCACGGCGACTGGGTCTGGCCGTACGGGAAGTTCGGCAACAAGCCCGACCAGGGGCT
CGAGTCGTGGGACGTCGTACCCGGCGGGACGGCCGCCGCGGTGTACACCTTCGCGCAGGACG
GCCTGTACGTCTACCTGAACCACAACCTGATCGAGGC 3'

*nirS*: dissimilatory reductase (Amplicon size: 166 bp)
5'GTCGTGCAGCCCGAGTACAACGCGGCGGGCGACGAAGTGTGGTTCTCCGTGTGGAACGGCAAGGAG
CAGCGCTCGGCCATCGTCGTGGTGGACGACAAGACGCTCAAGCTGAAGGCCGTCATCGACGAC
AAGCGCATCATCACGCCGACCGGCAAGTTCAACGACT3'

*mcrA*: T7 methyl coenzyme M reductase alpha and beta-subunit (Amplicon size: 458bp)
5'TACTGTGTGAACCCTACACCACCACACGGACGACATACTCGACGACTTCTGCTACTACATTGCAGACT
ACGTGAAGAAGAAGTACGGCGGATTTGCAAAGGCCCCAAGAACGATGGATACAGTTCTCGACGT
GGCAACTGAAGCAACGCTATACGGCCTGGAGCAGTACGAGCGCTTCCCGGCACTGATGGAGAC
ACACTTCGGCGGTTCGCAGAGGGCTTCAGTTCTTGCAGCAGCTTCCGGTGTTGGAACAGCTATC
GCGACTGGAGATGCCCAGGCAGGAGTTAACGGCTGGTATCTGTCGATGATTCTCCACAAGGAGC
ACATGGGCAGGCTCGGATTCTACGGTTACGACCAGCAGGATCAGCTCGGTATGACGAACAGCTT
CTCCTACAGAAGCGACGAGGGCTTGCCGCTGGAGCTGAGAGGCGTGAACTATCCCAACTACGCC
ATGAACG3'

The amplification control sequences shown above were synthesized by Inqaba Biotec™ (Pretoria, South Africa) and cloned into the pJET1.2 Blunt vector (separately) using *E. coli* as the host. The amplification target embedded in the cloning vectors (a plasmid contained in the transformed *E. coli* culture) was extracted and used as an amplification control. Plasmid extraction and purification was performed using the Zyppy plasmid miniprep kit (Zymo Research, USA).

#### *3.2.4.2 High Resolution Melt Polymerase Chain Reaction Assay Conditions*

Real-time PCR amplification of the targets was performed in 0.1 mL thin walled PCR tubes (Corbett Research, Australia / Qiagen, Germany). Each reaction contained 1x SensiMix HRM reaction buffer containing dNTP's, MgCl2 (6 mM), heat activated DNA polymerase and EvaGreen dye (Quantace, UK), 0.2 µM of each primer and 5 µL of DNA extract as template. Nuclease free water (Applied Biosystems, USA) was used to make up the reaction to a final volume of 25 µL. Amplification was performed in a RotorGene 6000 rotary thermal cycler (5-plex) with high resolution melt (HRM) capability (Corbett Research, Australia/Qiagen, Germany). A heat activation step of the DNA polymerase was performed at 95°C for 10 minutes, followed by 45 cycles of a DNA denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds. A final extension step was performed at 72°C for five minutes after cycling. To differentiate and identify amplification products formed, HRM curve analysis was performed by lowering the temperature to 60°C for five minutes, followed by an increase in temperature to 90°C at increments of 0.1°C per second. Fluorescence was measured continuously and melting temperature (Tm) peaks were calculated based on the initial fluorescence curve (F/T) by plotting the negative derivative of fluorescence over temperature versus time (-dF/dT versus T).

## 3.3 RESULTS

A summary of the *E. coli* and total coliform most probable number (MPN) counts, pH values and the pyrosequencing reads obtained for upstream and downstream sites during both sampling trips before and after rehabilitation is given in Table 3.2. Site 1 was a reference site not impacted by polluted water. Sites 2 and 3 upstream of the wetland received polluted water. The downstream sites referred to were located downstream of the wetland. The qualitative results for RT-PCR analysis for detection of SRB, denitrifying bacteria and methanogens from upstream and downstream sites before and after rehabilitation are given in Table 3.3.

**Table 3.2 *Escherichia coli* and total coliform most probable number (MPN) counts, pH values and number of pyrosequencing reads obtained for upstream and downstream sites**

| Site Name | *E. coli* MPN per 100 mL | Total Coliform MPN per 100 mL | pH | Number of Pyrosequencing Reads Obtained |
|---|---|---|---|---|
| **Pre-rehabilitation** | | | | |
| Reference Site 1 (Trip 1) | 120.1 | 38730 | 5.74 | 515 |
| Upstream Site 2 (Trip 1) | <1 | <1 | 2.69 | 374 |
| Upstream Site 3 (Trip 1) | 21.3 | 5650 | 3.93 | 1384 |
| Downstream Site1 (Trip 1) | 58.1 | 3990 | 5.02 | 781 |
| Reference Site 1 (Trip 2) | 4.2 | 2870 | 6.63 | 789 |
| Upstream Site 2 (Trip 2) | 88.8 | 275.5 | 3.88 | 564 |
| Upstream Site 3 (Trip 2) | 6.3 | 435.2 | 4.51 | 848 |
| Downstream Site 1 (Trip 2) | <1 | 85.5 | 5.19 | 794 |
| **Post-rehabilitation** | | | | |
| Reference Site 1 (Trip 1) | 276.4 | 1986.3 | 9.77 | 296 |
| Upstream Site 2 (Trip 1) | 0 | 248.1 | 3.89 | 332 |
| Upstream Site 3 (Trip 1) | 3022.1 | >2419.6 | 4.1 | 1113 |
| Downstream Site 5 (Trip 1) | 69.2 | 727 | 6.55 | 643 |
| Reference Site 1 (Trip 2) | 1.0 | 1986.3 | 6.53 | 1328 |
| Upstream Site 2 (Trip 2) | 14.8 | 286.4 | 6.52 | 1795 |
| Upstream Site 3 (Trip 2) | 8.2 | 1076.6 | 7.09 | 2744 |
| Downstream Site 1 (Trip 2) | 8.6 | 417.8 | 7.4 | 1664 |

**Table 3.3 The qualitative polymerase chain reaction results for five targets [dsrA (sulphate reducing bacteria), nirK, NirS (denitrifying bacteria) and mcrA (methanogens)] from upstream and downstream sites before and after rehabilitation**

| Site and Month Sampled | ***dsrA* PCR results (sulphate reducing bacteria)** | ***nirK* PCR results (denitrifying bacteria)** | ***nirS* PCR results (denitrifying bacteria)** | ***mcrA* PCR results (methanogens)** |
|---|---|---|---|---|
| **Pre-rehabilitation** | | | | |
| Reference Site 1 (Trip 1) | - | + | + | - |
| Upstream Site 2 (Trip 1) | + | + | + | - |
| Upstream Site 3 (Trip 1) | + | + | + | - |
| Downstream Site 1 (Trip 1) | - | + | - | - |
| Reference Site 1 (Trip 2) | - | + | + | - |
| Upstream Site 2 (Trip 2) | + | - | + | - |
| Upstream Site 3 (Trip 2) | + | - | + | - |
| Downstream Site 1 (Trip 2) | - | - | - | + |
| **Post-rehabilitation** | | | | |
| Reference Site 1 (Trip 1) | - | - | - | - |
| Upstream Site 2 (Trip 1) | - | - | - | - |
| Upstream Site 3 (Trip 1) | - | - | - | - |
| Downstream Site 1 (Trip 1) | - | - | - | - |
| Reference Site 1 (Trip 2) | - | - | + | - |
| Upstream Site 2 (Trip 2) | - | - | - | - |
| Upstream Site 3 (Trip 2) | - | - | + | + |
| Downstream Site 1 (Trip 2) | - | - | + | - |

Detected targets are as depicted as +, while targets not detected are depicted as -.

### 3.3.1 Pre-rehabilitation

#### *3.3.1.1 Reference Site 1*

The pH at the reference site which was not affected by polluted water ranged from 5.74-6.63 (Table 3.2). The *E. coli* counts ranged from 88.8-120.1 MPN/100 mL, while the coliforms ranged from 275.5-38730 MPN/100 mL. The majority of the bacteria present are poorly described in literature. For the purposes of clarity, these bacteria were excluded from the graphic representation. The majority of described bacteria during Trip 1 belonged to the Proteobacteria with a relative abundance of 75.36%, followed by Bacteriodetes (14.49%) and Actinobacteria (7.97%) with a small representation of bacteria from the phylum Firmicutes (0.72%) and Deinococcus-Thermus (0.72%) (Figure 3.1A). During Trip 2 the most abundant bacteria are poorly described in literature, followed by Proteobacteria with a relative abundance of 95.65%, bacteria belonging to the phylum Actinobacteria (2.46%) and Firmicutes (0.19%) (Figure 3.2A). Real-time PCR analysis showed that this site harboured denitrifying bacteria (both the cytochrome enzyme (NirS) and copper containing enzyme (NirK) types). No sulphate reducing bacteria or methanogens were present (Table 3.3).

#### *3.3.1.2 Upstream Site 2*

The pH was found to be highly acidic and ranged between 2.69 during Trip 1 and 3.88 during Trip 2 (Table 3-2). No viable *E. coli* or coliforms could be detected at this site during Trip 1, however, 88.8 *E.coli* MPN/100 mL and 275.5 MPN/100 mL were detected during Trip 2 (Table 3.2). The acidic conditions present at this site may also explain the low bacterial composition observed with pyrosequencing analysis as this site had the lowest number of reads on average compared to the rest of the sites. The majority of the bacteria present are poorly described in literature. The next most abundant bacteria present at this site were the Cyanobacteria with a relative abundance of 71.15% and bacteria belonging to the phyla Proteobacteria (26.92%) and Acidobacteria (1.28%) and a small contingent belonging to the phylum Aquificae (0.64%) (Figure 3.1B). During Trip 2 the most abundant bacteria are poorly described in literature, followed by Proteobacteria with a relative abundance of 77.63% bacteria belonging to the phylum Actinobacteria (10.28%) and Firmicutes (7.97%), with a small abundance of 2.06% belonging to the phylum Deinococcus-Thermus and Tenericutes (2.06%) (Figure 3.2B). Real-time PCR analysis showed that this site harboured denitrifying bacteria (both the cytochrome enzyme (NirS) and copper containing enzyme (NirK) types) as well as SRB (detected by the presence of the dsrA gene (Table 3.3). During Trip 2 no NirK denitrifying bacteria were present.

*3.3.1.3 Upstream Site 3*

This site also exhibited a low pH (3.93-4.51), however, viable *E. coli* and coliforms could be detected here (Table 3.2). The *E. coli* counts ranged from 6.3-21.3 MPN/100 mL, while the coliforms ranged from 435.2-5650 MPN/100 mL. The read count obtained for pyrosequencing analysis was (on average) higher than at other sites (Table 3.2). A higher diversity of bacteria was also observed at this site, with the majority of bacteria poorly described in literature. The pyrosequencing analysis revealed that the most abundant phyla during Trip 1 were the Proteobacteria (70.24%) followed by Actinobacteria (6.23%), Firmicutes (5.88%), Cyanobacteria (5.54%), Bacteriodetes (4.5%), Planktomycetes (2.77%), Chloroflexi (2.08%), Acidobacteria (1.73%) and Verrucomicrobia (1.04%) (Figure 3.1C). During Trip 2 the most abundant described phyla were again the Proteobacteria with a relative abundance of 87.74%, followed by Tenericutes (5.19%), Firmicutes (2.36%), Bacteriodetes (1.65%), Fusobacteria (2.36%) and Cyanobacteria (0.47%) (Figure 3.2C). Real-time PCR analysis for the detection of certain functional groups revealed a similar picture to that observed upstream at Site 2. Denitrifying bacteria (both the cytochrome enzyme (NirS) and copper containing enzyme (NirK) types) and SRB (dsrA) were present. During Trip 2 no NirK denitrifying bacteria were present (Table 3.3).

*3.3.1.4 Downstream Site 1*

*Escherichia coli* and coliforms were present in water from the downstream sites during both sampling trips at fairly low levels with *E. coli* ranging from <1 to 58.1 MPN/100 mL and coliforms from 85.5-3990 MPN/mL. The read count obtained for pyrosequencing analysis was (on average) higher than at other sites with the exception of Upstream Site 3. The pH was found to be slightly acidic (5.02-5.19) (Table 3.2). The pyrosequencing results showed that the most abundant bacteria are poorly described or not described in literature. The majority of described bacteria belonged to the Proteobacteria (57.66%), followed by the phyla Cyanobacteria (26.58%), Actinobacteria (4.05%), Acidobacteria (3.6%), Firmicutes (2.25%), Verrucomicrobia (1.8%), Planktomycetes (1.8%), Bacteriodetes (0.9%), Chloroflexi (0.9%), and Gemmatimonadetes (0.45%) (Figure 3.1D). During Trip 2 the most abundant bacteria were from phylum Proteobacteria (96.83%) followed by representatives from the phyla Fusobacteria (1.3%), Actinobacteria (0.93%), Bacteriodetes (0.37%), Firmicutes (0.37%) and Cyanobacteria (0.19%) (Figure 3.2D). The RT-PCR assays showed that denitrifying bacteria and sulphate reducers were absent from this site. A very low concentration of methanogens were detected at this site during Trip 2 (Table 3.3).

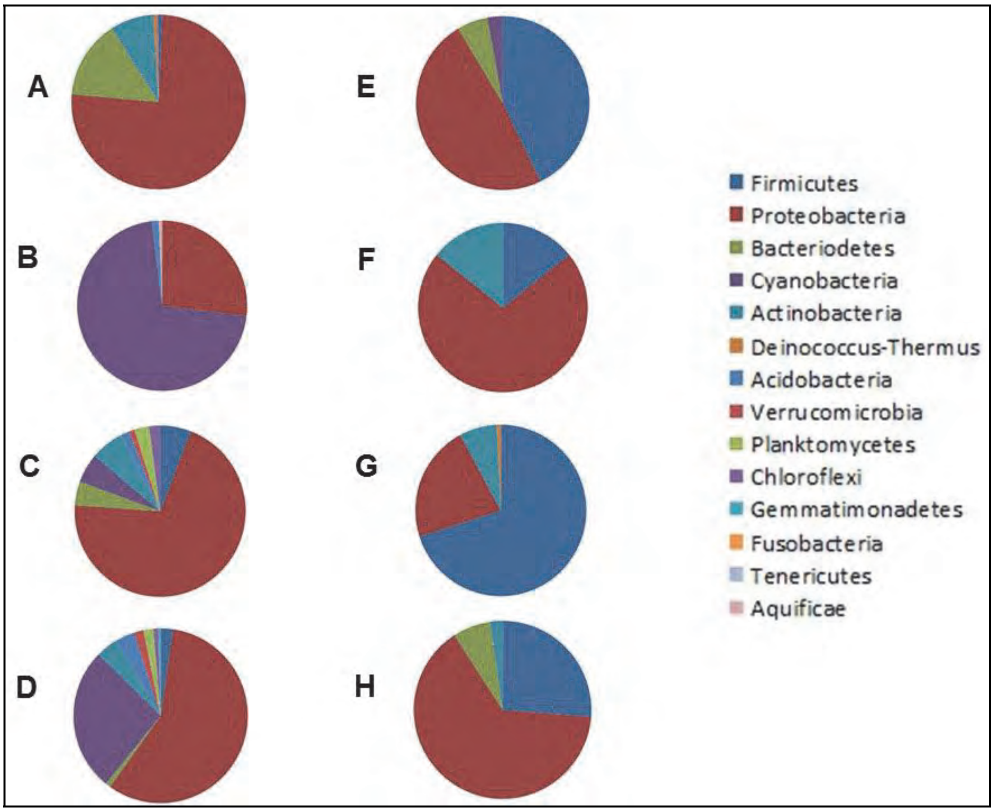



**Figure 3.1 Bacterial composition before and after rehabilitation during Trip 1, A) Reference Site 1 pre-rehabilitation, B) Upstream Site 2 pre-rehabilitation, C) Upstream Site 3 pre-rehabilitation, D) Downstream Site 1 pre-rehabilitation, E) Reference Site 1 post-rehabilitation, F) Upstream Site 2 post-rehabilitation, G) Upstream Site 3 post-rehabilitation, H) Downstream Site 1 post-rehabilitation**

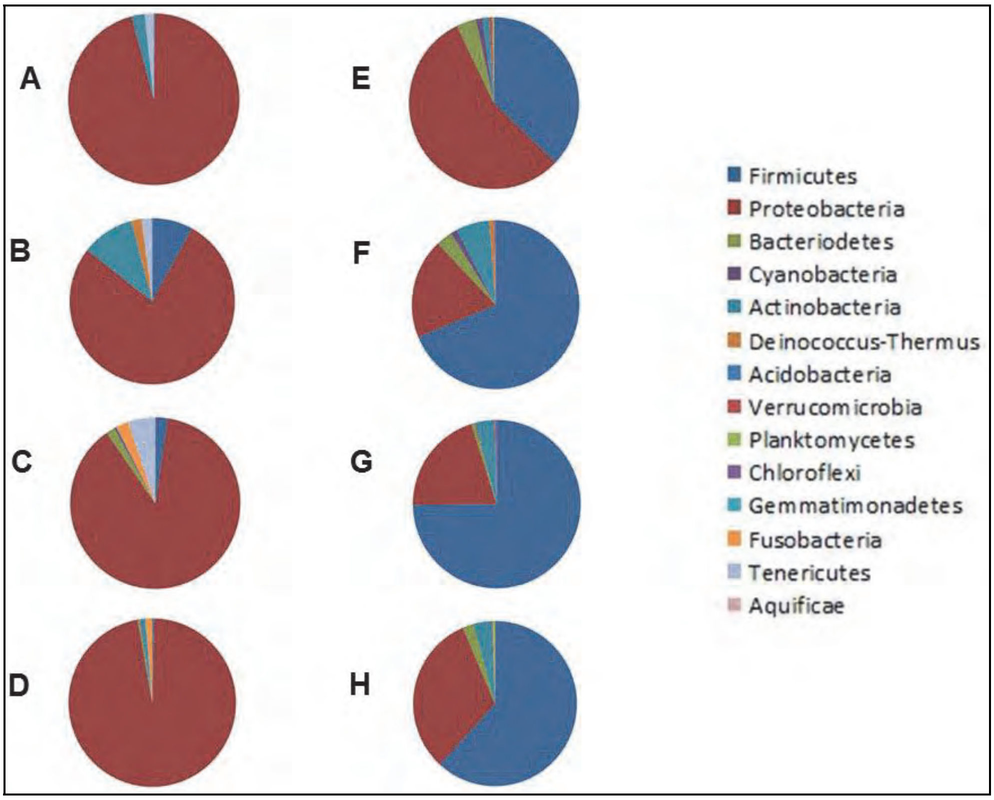



**Figure 3.2 Bacterial composition before and after rehabilitation during Trip 2, A) Reference Site 1 pre-rehabilitation, B) Upstream Site 2 pre-rehabilitation, C) Upstream Site 3 pre-rehabilitation, D) Downstream Site 1 pre-rehabilitation, E) Reference Site 1 post-rehabilitation, F) Upstream Site 2 post-rehabilitation, G) Upstream Site 3 post-rehabilitation, H) Downstream Site 1 post-rehabilitation**

### 3.3.2 Post-rehabilitation

#### *3.3.2.1 Reference Site 1*

In the second year the pH at the reference site ranged from 6.93-9.77 (Table 3.2). The *E. coli* counts ranged from 276.4 MPN/100 mL during Trip 1 to 1.0 MPN/100 mL during Trip 2, while the coliforms remained stable at 1986.3 MPN/100 mL. The pyrosequencing analysis revealed that the most abundant bacteria were those that are not described or poorly described in literature. The majority of described bacteria during Trip 1 belonged to the Proteobacteria with a relative abundance of 48.57%, followed by Firmicutes with 42.86%. The next most abundant phylum was Bacteriodetes (5.71%) and Cyanobacteria (2.86%) (Figure 3.1E). During Trip 2 the most abundant bacteria were unclassified, followed by Proteobacteria with a relative abundance of 55.63% and bacteria belonging to the phylum Firmicutes (37.19%), followed by Bacteriodetes (3.75%), Actinobacteria (1.25%), Cyanobacteria (1.25%), Verrucomicrobia (0.63%) and Planktomycetes (0.31%) (Figure 3.2E). Real-time PCR analysis showed that this site harboured denitrifying bacteria (both the cytochrome enzyme (NirS) and copper containing enzyme (NirK) types). The real-time PCR assays showed that denitrifying bacteria possessing the NirS gene were detected during Trip 2, however, no NirK denitrifying bacteria was detected. Sulphate reducers and methanogens were absent from this site (Table 3.3).

#### *3.3.2.2 Upstream Site 2*

In the second year after rehabilitation had taken place, Upstream Site 2 during Trip 1 still had an acidic pH of 3.89 with 0 MPN/100 mL of *E. coli* detected and only 248.1 MPN/100 mL of coliforms detected here (Table 3.2). The read count obtained for pyrosequencing analysis was also very low. During Trip 2 the pH had increased to 6.52 with 14.8 MPN/100 mL of *E. coli* detected and 286.4 MPN/100 mL of coliforms. The pyrosequencing read count also increased (Table 3.2). The pyrosequencing analysis again revealed that the most abundant bacteria were those that are poorly described in literature. During Trip 1 the most described bacteria were those from the phylum Proteobacteria with a relative abundance of 71.43%. A small representation of bacteria from the phyla Actinobacteria (14.29%) and Firmicutes (14.29%) were also present (Figure 3.1F). During Trip 2 the most abundant described bacteria were from the phylum Firmicutes (68.75%), followed by the Proteobacteria (19.17%), Actinobacteria (6.46%) Bacteiodetes (3.13%), Deinococcus-Thermus (0.83%), Acidobacteria (0.21%) and Verrucomicrobia (0.21%) (Figure 3.2F). The RT-

PCR assays showed that denitrifying bacteria and sulphate reducers and methanogens were absent from this site (Table 3.3).

#### *3.3.2.3 Upstream Site 3*

During the post rehabilitation phase Upstream Site 3 had a pH of 4.1 with high levels of *E. coli* detected at 3022.1 MPN/100 mL and >2419.6 MPN/100 mL of coliforms detected (Table 3.2). During Trip 2 the pH increased to 7.09 and the *E.coli* and coliforms were lower at 8.2 and 1076.6 MPN/100 mL, respectively, and the pyrosequencing read count increased. The pyrosequencing analysis revealed that the most abundant bacteria were those that are not described or poorly described in literature. The next most abundant bacteria during Trip 1 were from the phylum Firmicutes with a relative abundance of 70.08% followed by Proteobacteria (22.05%), Actinobacteria (7.09%) and Deinococcus-Thermus (0.79%) (Figure 3.1G). During Trip 2 the most abundant described bacteria were Firmicutes (74.87%) followed by the Proteobacteria (20.21%), Actinobacteria (3.51%), Bacteriodetes (0.7%), Chloroflexi (0.35%), Cyanobacteria (0.18%) and Verrucomicrobia (0.18%) (Figure 3.2G).The RT-PCR assays showed that denitrifying bacteria, sulphate reducers and methanogens were absent from this site during Trip 1, however, methanogens and denitrifying bacteria with the nirS gene were present during Trip 2 (Table 3.3).

#### *3.3.2.4 Downstream Site 1*

The downstream sites after rehabilitation had an improved neutral pH range of 6.55-7.4 (Table 3.2). *Escherichia coli* and coliforms were present in water from the downstream sites during both sampling trips at low levels with *E. coli* ranging between 8.6-69.2 MPN/100 mL and coliforms from 727- 417.8 MPN/100 mL. With the exception of Upstream Site 3, the highest number of reads (on average) was obtained at the downstream sites after rehabilitation, indicating a high bacterial composition (Table 3.2). The bacterial community downstream of the wetland after rehabilitation was comprised largely of bacteria that are not described or are poorly described in literature. The most abundant described bacteria were mainly from the phylum Proteobacteria (64.64%) followed by Firmicutes (26.24%). Bacteria belonging to the phylum Bacteriodetes and Actinobacteria were also present with 6.84% and 2.28%, respectively (Figure 3.1H). During Trip 2 bacteria from the phylum Firmicutes had the largest representation (61.8%), followed by the Proteobacteria (31.76%), Actinobacteria (3.65%) and Bacteriodetes (2.15%). There was also a small occurrence of bacteria from the phylum Planktomycetes (0.43%) and Verrucomicrobia (0.21%) (Figure 3.2H). The RT-PCR assays revealed that the more widely distributed denitrifying bacteria possessing the nirS gene were detected at the downstream site during Trip 2, however, no nirK denitrifying bacteria was detected. Both methanogens and sulphate reducers were absent in all downstream sites after rehabilitation (Table 3.3).

## 3.4 DISCUSSION

Total coliform bacteria are frequently used to assess the general hygienic quality of water and in some instances they may indicate the presence of pathogens responsible for the transmission of infectious diseases, however, many of the bacteria in this group may originate from growth in the aquatic environment. *Escherichia coli* is a specific indicator of faecal pollution that originates from humans and warm-blooded animals (DWAF, 1996). It is important to note that it is widely accepted that the total coliform group of bacteria is diverse and they can be considered normal inhabitants of many soil and water environments, which have not been impacted by faecal pollution (Stevens *et al*., 2003). At upstream sites prior to rehabilitation relatively low levels of both *E. coli* and total coliforms were obtained when compared to the reference site, especially at the sites where the pH of the water was particularly acidic (pH 2.69,) which created unfavourable conditions for the indicator organisms. The rate of reduction of *E. coli* on contact with acid mine waters has been determined to be rapid, however, a small number can sustain these acid conditions (Joseph and Shay, 1952). In the second year after rehabilitation of the wetland had taken place, the *E. coli* and total coliform counts were particularly high at Upstream Site 3 with >2419.6 MPN/100 mL total coliforms and 3022.1 MPN/100 mL of *E .coli* where the pH had increased to 4.1. This represents a significant and increased risk of infectious disease transmission. However, the downstream site showed a significant decrease in both total coliforms and *E. coli* counts during Trip 1 to 69.2 MPN/100 mL *E. coli* and 727 MPN/100 mL of total coliforms. The pH of the water also improved at the downstream sites and was close to neutral post-rehabilitation. In addition to the removal of organic and inorganic contaminants, functional wetlands also play a role in the removal of pathogens from the water (Kivaisi, 2001). The effectiveness of wetlands depends on a number of mechanisms that influence bacterial removal efficiency such as the rate of effluent flow and quality, die-off rate, rate of removal by filtration (by plant roots) and sedimentation, rate of addition from transient (or resident) animal sources and the rate of predation (Perkins and Hunter, 2000). The reduction of indicator organism counts downstream of the wetland indicates that the wetland was effectively reducing faecal pollutants post-rehabilitation.

Three functional groups of microorganisms were selected to be analysed in the wetland due to the important role that they play in regulating the cycling of major nutrients and carbon in freshwater wetlands. The use of molecular techniques to detect and quantify micro-organisms that have sulphate reduction, methane producing and denitrifying ability has several advantages. The techniques are rapid, sensitive and accurate (Bustin, 2009) and also circumvents many of the problems associated with culturing this diverse and fastidious group of micro-organisms. High resolution melt PCR is particularly well suited for the detection of gene targets that contain sequence variability, as is the case with SRB, methanogens and denitrifiers. Four PCR methods were adapted (or modified) from literature, and converted into HRM RT-PCR assays. The assays target the following genes: dsrA (sulfite reductase subunit A), nirK (copper-containing nitrite reductase), nirS (dissimilatory reductase) and mcrA (T7 methyl coenzyme M reductase alpha and beta-subunit) (Braker *et al*., 1998; Wagner *et al*., 1998; Henry *et al*., 2004; Ben-Dov *et al*., 2007; Steinberg and Regan, 2008).

Real-time PCR analysis showed that prior to rehabilitation, the upstream wetland sites, which exhibited low pH, harboured sulphate reducing bacteria, detected by the presence of the dsrA gene. Microbial sulphate reduction can take place in environments with a pH below 5 (Koschorreck, 2008) and SRB have been detected in habitats such as acid-mine drainage sites with a pH of 2 (Sen and Johnson, 1999). Chemistry data showed that water from Upstream Site 3 contained high sulphate levels, while Upstream Site 2 contained high levels of iron. The sulphate reducers were absent from the reference site and downstream prior to rehabilitation, where the sulphate concentration remained high, but the iron concentration was lower and no SRB were present post-rehabilitation at both upstream and downstream sites where the sulphate and iron concentrations were reduced.

Real-time PCR analysis showed that before rehabilitation the reference site harboured denitrifying bacteria (both the cytochrome enzyme (NirS) and copper containing enzyme (NirK) types). At the upstream wetland sites, which exhibited low pH, both NirS and NirK denitrifying bacteria were present, however, no NirK denitrifying bacteria were present during Trip 2 at the upstream sites. Denitrifiers utilize nitrate and are a very diverse group of organisms that occur in a wide variety of environments (Zumft, 1997). The nirS gene was more readily detected and this gene has been shown to be more widely distributed, while nirK is found in only 30% of the denitrifiers studied. The nirK gene is, however, found in a wider range of physiological groups (Coyne *et al*., 1989). The RT-PCR assays showed that at the downstream sites prior to rehabilitation, denitrifying bacteria were only detected on one occasion (copper containing enzyme nirK), while denitrifying bacteria, were absent during Trip 2. After rehabilitation had taken place, denitrifying bacteria were mostly absent with the exception of those ascertained by the presence of the nirS gene, detected at the reference site and Upstream Site 3, as well as downstream of the wetland During Trip 2. The absence of denitrifiers at the majority of sites after rehabilitation may be due to the lack of nutrients in the water to sustain their growth.

Real-time PCR analysis showed that the methanogens were only detected on one occasion at the downstream site before rehabilitation during Trip 2 and after rehabilitation at Upstream Site 3. In environments where sulphate is present, SRB compete with methanogens for common substrates such as hydrogen, acetate and methanol, and SRB are much more versatile than methanogens in their ability to utilize organic acids such as propionate and butyrate (Stams *et al*., 2003). Acetate-utilizing sulphate reducers have been found to out-compete acetoclastic methanogens (Schönheit *et al*., 1982; Oude Elferink *et al*., 1994). In the absence of sulphate, some SRB have the ability to grow fermentatively or in synthrophic association with methanogens (Oude Elferink *et al*., 1994). In anaerobic environments SRB compete with homoacetogens and methanogens for hydrogen (Muyzer and Stams, 2008). The methanogens rely on other metabolic groups such as fermentative microorganisms that decompose cellulose and other complex molecules to volatile carboxylic acids and H2 (Barber and Ferry, 2001). As bacteria that degrade complex molecules were present after rehabilitation, it would be expected that more methanogens would be present. Although few studies on the structure and function of methanogens in wetlands have been undertaken (Sims *et al*., 2013), in a study of the restoration of a highly disturbed freshwater wetland ecosystem a general decline in methanogenic activity in soil was observed with restoration age (Smith *et al*., 2007).

Pyrosequencing was chosen as a high throughput sequencing technique which allowed for the sequencing of a large number of partial mixed 16S rRNA sequences in parallel (Claesson *et al*., 2009). The lowest number of reads (on average, 469) was obtained from Upstream Site 2 before rehabilitation, where the pH was particularly low, indicating low bacterial composition. With the exception of Upstream Site 3, the highest number of reads was obtained at the downstream sites after rehabilitation (on average, 1153.5), indicating a high bacterial composition. A seasonal influence was shown before rehabilitation with a reduction in diversity observed during Trip 2, possibly due to lower temperatures. The pyrosequencing analysis of the bacterial composition in the wetland indicated that at all sites the most abundant organisms found were poorly described or not described in literature (unclassified). This provides a challenge in analysing the data and for purposes of clarity these organisms were excluded from the graphic representation. The bacterial composition exhibited great variability and diversity between sites and at the same site sampled on different occasions. This highlights the adaptability of the bacterial community and the swift changes that bacterial populations undergo in response to environmental changes. For this reason further research is required in order to distinguish specific bacterial phyla as indicators for wetland efficiency.

With the exception of Upstream Site 2, where the Cyanobacteria dominated, the Proteobacteria were the next most abundant phylum in the wetland before rehabilitation. The Proteobacteria are the largest and phenotypically most diverse phylogenetic lineage and several representatives are ecologically important as they play key roles in the carbon, sulphur and nitrogen cycles (Kersters *et al*., 2006). Cyanobacteria (also called blue-green algae) were prevalent at upstream and downstream sites before rehabilitation during Trip 1. Cyanobacteria produce potent toxins that affect human and animal health and occur in nutrient loaded waters (Codd, 2000). Aquificae were present at Upstream Site 2 pre-rehabilitation and bacteria belonging to this phylum can survive harsh environmental conditions. Although most species of the Aquificae are hydrogen-oxidizing bacteria, thiosulfate or sulphur can also be used as alternative energy sources (Griffiths and Gupta, 2006). Members of the phylum Deinococcus-Thermus that are highly resistant to environmental stressors (Tian and Hua, 2010) were present at Upstream Site 2 before rehabilitation and Upstream Site 3 after rehabilitation. Acidobacteria were present at Upstream Site 3 and Downstream during Trip 1 before rehabilitation. Acidobacteria are diverse and ubiquitous in soil environments (Quaiser *et al*., 2003), but have been detected in freshwater habitats (Barns *et al*., 1999). Some of the members of this phylum are acidophilic, which could explain the presence of these bacteria at low pH sites. No Acidobacteria were detected after rehabilitation downstream of the wetland. Hartman *et al*. (2008) found a strong increase in the abundance of Acidobacteria with lower pH in freshwater wetlands.

Tenericutes, that lack cell walls, were present in Upstream Sites 2 and 3 during Trip 2. With the exception of Upstream Site 2 during Trip 1, all pre-rehabilitation sites had a small representation of Firmicutes. Firmicutes are saprophytic microorganisms that survive on decaying organic matter and play a large role in cellulose degradation. Many Firmicutes produce endospores, which are resistant to desiccation and can survive extreme conditions. Firmicutes have been found to constitute bacterial communities present in intertidal wetland sediments (Wang *et al*., 2012). Actinobacteria were found at most upstream and downstream reference sites and these bacteria are abundant in freshwater and soil and play an important role in the decomposition of organic materials. While no bacteria from the phylum Bacteriodetes were present at Upstream Site 2, a small percentage of them were present at Upstream Site 3 and the downstream reference site before rehabilitation. Bacteroidetes strains are saprophytic organisms widely distributed in the environment. Upstream Site 3 appeared to have the greatest diversity pre-rehabilitation with a small representation of organisms from the phyla Planctomycetes, Chloroflexi and Verrucomicrobia during Trip 1 and Fusobacteria during Trip 2. Verrucomicrobia are ubiquitous in most soil environments (Zhang and Xu, 2008) and the relative abundance of Verrucomicrobia and has been found to be higher in natural wetland soils than in the constructed wetlands (Ansola *et al*., 2014). A number of genera of the Planctomycetes, which were once thought to occur primarily in aquatic environments, have been discovered in wetlands (Prasanna *et al*., 2011). Members of the Chloroflexi have been found in sediments and moderately acidic wetlands (Wilhelm *et al*., 2011; Yergeau *et al*., 2012), while Fusobacteria are obligate anaerobes considered to be human and animal pathogens. A similar composition of bacteria was present at the downstream sites before rehabilitation with a small representation of bacteria during Trip 1 from the phylum Gemmatimonadetes, which are widespread in soil environments. An examination of the bacterial population of the wetland after rehabilitation revealed far less diversity than that detected prior to rehabilitation, with Proteobacteria, Actinobacteria and Firmicutes present at upstream reference sites and the presence of environmentally resistant members of the phylum Deinococcus-Thermus.

A comparison of sites downstream of the wetland before and after rehabilitation revealed some vast changes in the bacterial composition. While the Proteobacteria still represented a large proportion of the bacteria present, there was a shift in the community structure towards an increase in abundance of bacteria from the phylum Firmicutes. Bacteria belonging to the phylum Bacteriodetes and Actinobacteria were also represented. The downstream reference site during Trip 2 also showed a small representation of bacteria belonging to the phyla Verrucomicrobia and Planktomycetes. The bacterial composition of the downstream sites was similar to that of Reference Site 1 in the post-rehabilitation year, with the exception of the presence of Cyanobacteria at the reference site. In a study examining the bacterial diversity from a worldwide range of wetlands soils and sediments, the Proteobacteria were the largest and most diverse phylum. Bacteria belonging to the phyla Bacteroidetes, Chloroflexi, Firmicutes and Actinobacteria, were well represented and these microorganisms are likely to be important participants in the wetland environment (Lv *et al*., 2014). The shift from a minimal presence of Firmicutes to a large representation post-rehabilitation is reiterated in a study which showed that although Firmicutes are known to be key players in cellulose degradation in neutral habitats, they were not detected in low pH Sphagnum peatlands (Pankratov *et al*., 2011). Bacteria belonging to the phyla Firmicutes, Bacteroidetes and Actinobacteria are all key players in biopolymer degradation, which indicates that representatives of bacteria from these phyla may primarily need to be present and abundant for healthy wetland functioning. The relatively few bacterial phyla that were present post-rehabilitation corresponds to the work of Hartman *et al*. (2008) where the composition of wetland soils was examined and it was found that wetland rehabilitation decreased bacterial diversity, which is an opposite response to that in terrestrial ecosystems.

## 3.5 CONCLUSION

To evaluate the efficiency and performance of a wetland, it is vital to be able to measure how pollutants are broken down or created by micro-organisms within the wetland system. The bacterial composition of the water fraction of the wetland before and after rehabilitation was examined. A reduction of indicator organism counts downstream of the wetland revealed that the wetland was effectively reducing the faecal pollutants after rehabilitation. The pH of the water improved at the downstream sites and was close to neutral after rehabilitation. Sulphate reducing bacteria, methanogens and denitrifying bacteria represent important classes of bacteria and Archaea that play an important role in a wetland's remedial functioning. The functional gene analysis indicated that SRB were detected mainly at the sites with acidic pH and high iron and sulphate contents, while denitrifying bacteria were detected in the majority of the pre-rehabilitation sites, which may be due to the presence of nutrients to support their growth. Methanogens, which rely on other metabolic groups to decompose cellulose and other complex molecules to usable compounds, were only detected on two occasions, namely downstream pre-rehabilitation and upstream post-rehabilitation. Pyrosequencing analysis indicated that bacterial composition and diversity corresponded strongly with the water pH. The most abundant bacteria present at all sites in the wetland were poorly described or not described in literature (unclassified). Many bacteria present at wetland sites prior to rehabilitation were found to have the ability to survive under extreme conditions in harsh environments. Although the bacterial richness (number of individuals per taxa) increased at the downstream sites, a decrease in bacterial diversity (number of different taxa) was obtained post-rehabilitation. A comparison of sites downstream of the wetland prior to rehabilitation and post-rehabilitation revealed a shift in the community structure towards an increase in abundance of bacteria from the phylum Firmicutes. Bacteria belonging to the phylum Proteobacteria had a large representation. Members of the phylum Bacteriodetes and Actinobacteria were also well represented and the downstream post-rehabilitation site also resembled that of the reference site. As the members from these bacterial phyla are key players in biopolymer degradation, these bacteria may provide the foundation for healthy wetland functioning.

# CHAPTER 4: FRESHWATER ALGAL COMMUNITIES AS INDICATORS OF PRE- AND POST-REHABILITATION CONDITIONS

## 4.1 INTRODUCTION

Wetlands are highly sensitive ecosystems which make them vulnerable to degradation by land use activities (Danielson, 2002). Ecological wetland rehabilitation focuses on improving wetland ecosystem structure and function by improving the ecological integrity of the system. This approach incorporates the physical, chemical and biological components of a wetland ecosystem. The most common and cost effective ecological approach is to reduce contaminants in the system and to let the wetland system recover on its own. However, as in most wetland rehabilitation projects, a pre- and post-rehabilitation monitoring program must be implemented by using indicator species to determine if ecological integrity has improved and continues to improve. Although a variety of organisms can be employed as biological indicators in conjunction with physico-chemical parameters, algae were selected as biological indicators of this wetland before and after rehabilitation. The latter was done on the basis that (1) algae are stationary, and therefore directly indicative of the physico-chemical conditions of their immediate habitat (Stevenson and White, 1995); (2) algae have a short life cycle and will respond quickly to rehabilitation; and (3) algae are good indicators of acid mine drainage (AMD) impacts in lentic systems for the purpose of rehabilitation (Oberholster *et al*., 2014).

Acid mine drainage is formed when sulphide minerals are exposed to atmospheric, hydrological and biological elements (oxygen, water and chemo-autotrophic bacteria), resulting in sulphuric acid that imparts a low pH and net acidity to water containing elevated sulphate, dissolved metal concentrations, low alkalinity and high conductivity (Hogsden and Harding, 2012). Due to the toxic effects of AMD, sensitive species are systematically reduced or eliminated. Because the properties of AMD render receiving natural wetlands less habitable to various biota, wetlands that receive AMD are often characterized by very low biodiversity and the fauna and flora become dominated by highly resistant organisms and acidophilic biota (Oberholster *et al*., 2014). In these acidic environments one of the earliest and most reliable bioindicators of changes to algal communities is the proliferation of the filamentous green algal families. In contrast, alkalinisation on algal communities has previously been studied, generally as part of lake experiments using lime to neutralize acid water (Hörnström, 1999). Under these conditions the reduction of the bloom forming green algal families was reported by Jackson *et al*. (1990) and Fairchild and Sherman (1992). According to data of earlier wetland surveys by Pan and Stevenson (1996) and Stevenson and Bahls (1999), pH is an important factor regulating algae composition in wetlands. Oberholster *et al*. (2013) reported that benthic green filamentous algae can act as good indicators of wetland degradation by evaluating pH values and electrical conductivity in relationship with different types of wetlands.

However, research on wetland algal communities as indicators of pH changes due to wetland conditions before and after rehabilitation is scarce. To the authors' knowledge, only one previous study in literature tested the response of periphytic algal communities to a wide range of pH values in a boreal wetland using a mesocosm experiment (Wyatt and Stevenson, 2010). In the latter treatment study the authors reported that alkalinisation (pH 9) significantly alters algal community structure and slackens nutrient restrains on algal production, while acidification (pH 5) reduces algal diversity.

The objectives of the study were to employ freshwater algae to differentiate between pre- and post- wetland rehabilitation conditions and to determine if these algae can be used as bioindicators for wetland rehabilitation. The purpose of the wetland rehabilitation was to improve the water quality and enhance functions of the wetland by addressing impacts associated with upstream land use activities (e.g. AMD from coal mining).

## 4.2 MATERIALS AND METHODS

### 4.2.1 Study Area

The Grootspruit (latitude -25.906480; longitude 29.052827) (Figure 4.1), which forms part of the Zaalklapspruit Wetland is situated in the Mpumalanga Province of South Africa. This wetland covers an area of 135.3 ha. Six sites were selected that have been sampled before and after rehabilitation. Four of these sites are situated upstream from the rehabilitation area and were used as relative reference sites to indicate the effect of the immediate environment on the wetland. The other two sites are situated downstream from the rehabilitation area. The sites are referred to as follows: upstream sites before rehabilitation (Pre-U1, Pre-U2, Pre-U3 and Pre-U4); upstream sites after rehabilitation (Post-U1, Post-U2, Post-U3 and Post-U4);

downstream sites before rehabilitation (Pre-D1 and Pre-D2); and downstream sites after rehabilitation (Post-D1 and Post-D2).

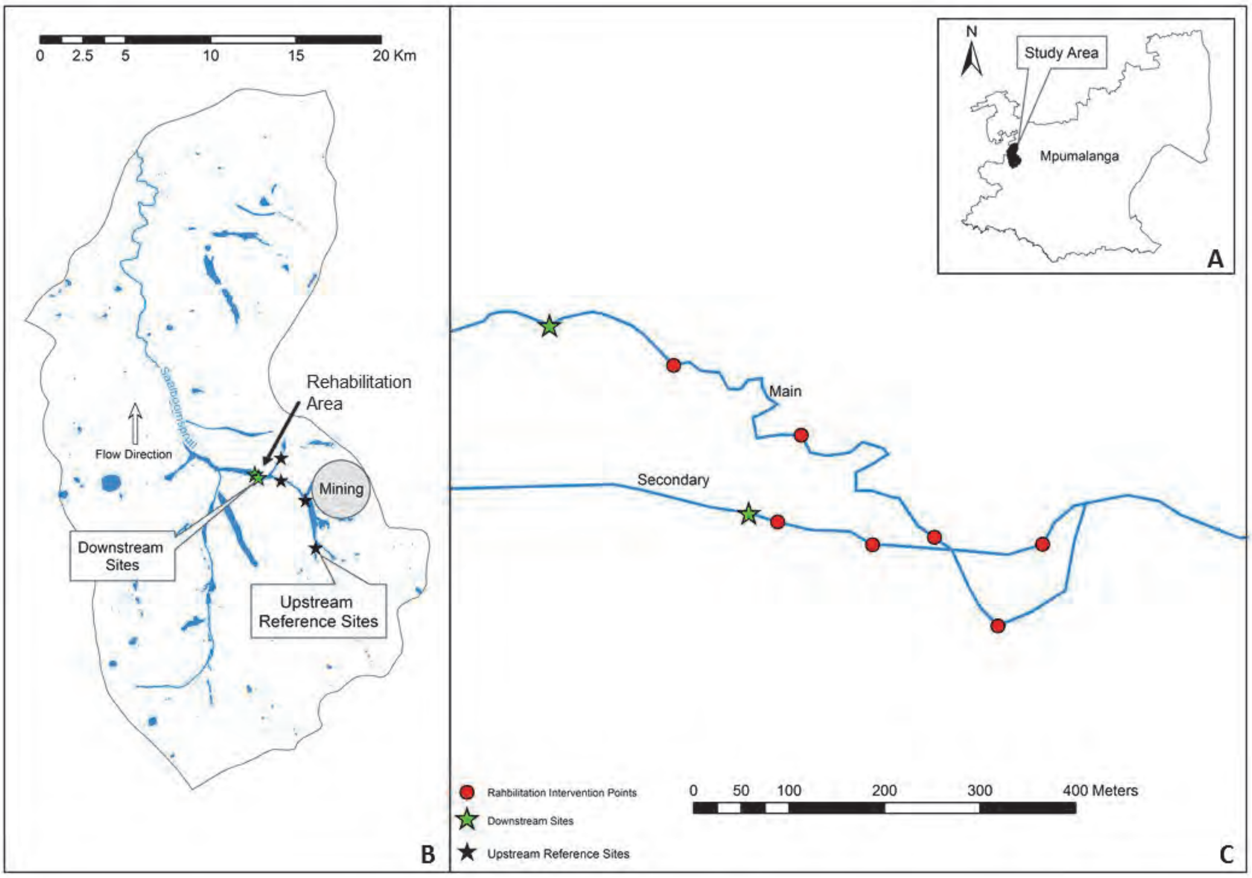



**Figure 4.1 Map of the study area, indicating its location within the Mpumalanga Province (A); sampling sites on the Grootspruit within the larger Zaalklapspruit Wetland (B), and a closer view of the rehabilitation area in the Grootspruit (C). The relative location of the various intervention points constructed during rehabilitation is also indicated in (C).**

Rehabilitation of the wetland was done by expanding the existing wetland area through the redirection of surface flow. The benefits of such a practise are that the additional wetland vegetation area enhances nearly all aspects of the functions typically associated with wetlands. The main channel of the rehabilitated area had four intervention points, while the secondary incised channel was rehabilitated at three points (Figure 4.1C). These intervention points were strategically selected to prevent further erosion and to deactivate channelization of both the main and secondary incised channels. By slowing the velocity of the water through the wetland and redirecting the flow by using man-made concrete structures (Figure 4.2), the wetland's surface area was expanded at the downstream sites. The purpose of the redirection of the wetland flow was to increases contact time between water and sediment by spreading it out over a larger area. Of the total cover area of 135.3 ha a total of 94.4 ha were identified for rehabilitation. This area of 94.4 ha was expanded to 103.8 ha by widening the wetland and thus gaining a total of 9.4 ha during the rehabilitation phase.

### 4.2.2 Sampling Sites

The substrate type of each survey site and in-stream substrate cover (i.e. macrophytes) was determined visually according to the method of Stevenson and Bahls (1999). An assessment of the degree of bank erosion was made to distinguish between the adverse effects of AMD and other land use activities according to Spencer *et al*. (1998). The bank stability was divided into the following categories: Stable = the banks or edges of the stream are stable and protected by good vegetation cover; good = evidence of minor localised erosion without damage to bank structure or vegetation; moderate = some erosion evident, with minor damage to bank structure and vegetation; poor = significant areas of erosion evident with little vegetation present; and unstable = extensive erosion evident, where bare, steep and sometimes undercut banks are present. The bank stability assessment indicated whether stress originated from abandoned mined land or outside sources (e.g. agriculture activities) following the index of Spencer *et al*. (1998). Table 4.1 gives a short description of the sites.

**Figure 4.2 The concrete structures that were built to regulate flow during the rehabilitation of the wetland**

### 4.2.3 Physico-chemical Parameters

At each site the pH and electrical conductivity (EC) were measured and water was collected in a one-litre sample bottle for the analysis of the following variables at the CSIR's accredited laboratory: sulphate ($SO_4^{2-}$), alkalinity (as $CaCO_3$), total suspended solids (TSS), aluminium (Al), iron (Fe), chloride (Cl-), magnesium (Mg), calcium (Ca), sodium (Na) and potassium (K). Water was also collected in two-litre sample bottles for the analysis of chlorophyll a. All samples were kept on ice and in the dark during transportation to the laboratory.

For the determination of chlorophyll a in water, the protocol recommended by Sartory and Grobbelaar (1984) was used. The samples were filtered through 0.45 µm pore-size glass fibre filters. The filter paper was carefully removed and placed inside a 15 ml Pyrex screw-capped tube to which 9.8 ml 91.8% ethanol was added. The tube was closed and placed in a water bath at 78°C for five minutes. The tube was then removed and placed in the refrigerator for 24 hours to cool to room temperature and to allow the extraction to complete. The extract was decanted into a centrifuge tube and centrifuged for 10 minutes at 4 000 rpm. An aliquot of 4 ml of the supernatant was then decanted into a 1 cm path-length cuvette and the absorbance was read at 665 nm and 750 nm on a spectrophotometer. A blank containing 91.8% ethanol was used. The extract was acidified by adding 100 µl 0.3 mol/l HCl. The solution was mixed and allowed to stand for two minutes. The absorbencies were then re-read at 665 nm and 750 nm. Mathematical equations were then used to determine the chlorophyll a concentrations in each sample. Benthic chlorophyll a concentrations of the algal mats were used as surrogate for algal biomass according to Biggs (1996). Benthic chlorophyll a was measured using the methodology of Porra *et al*. (1989).

**Table 4-1 Description of the sites upstream and downstream from the rehabilitation area**

| Sites | Coordinates | | Bottom substrate | Canopy cover (%) | In-stream macrophytes | Average flow regime (cm S-1) | Geology | Average stream channel depth (cm) | Source of impact | Bank stability |
|---|---|---|---|---|---|---|---|---|---|---|
| | Latitude | Longitude | | | | | | | | |
| Upstream 1 | -25.944406 | 29.083638 | Clay, silt | 0 | *Typha capensis* | >10 | Dolomite | 72 | Agriculture | Good |
| Upstream 2 | -25.919270 | 29.078197 | Clay, cobbles | 0 | *Typha capensis* | >10 | Dolomite | 22 | AMD from coal mining | Poor |
| Upstream 3 | -25.908724 | 29.065338 | Sand, bedrock | 0 | *Typha capensis*, *Phragmites australis* | >10 | Dolomite | 23 | AMD from coal mining | Poor |
| Upstream 4 | -25.896916 | 29.065335 | Clay, sand | 0 | *Typha capensis* | >10 | Dolomite | 14 | Industry | Poor |
| Downstream 1 | -25.907202 | 29.053360 | Clay, sand | 0 | *Typha capensis* | >10 | Dolomite | 11 | Agriculture, mining, industry | Stable |
| Downstream 2 | -25.907375 | 29.054311 | Clay, sand | 0 | *Typha capensis* | >10 | Dolomite | 9 | Agriculture, mining, industry | Stable |

### 4.2.4 Periphytic Phytoplankton Sampling

Sampling of the algae was conducted before and after rehabilitation. At each site the presence of epilithic filamentous algae (algae growing on gravel, stones and bedrock) was first defined with the naked eye, since these types of algae have a distinct structure (Sheath and Cole, 1992). The percentage cover of filamentous algae was estimated using the method of Sheath and Burkholder (1985). If present, an area of substrate surface (5 cm in diameter) was isolated for epilithic filamentous algal sampling using a syringe extended with a tygon tube (Hauer and Lamberti, 2006). The algal abundance in the samples was evaluated by counting the presence of each species (as cells in a filament or equal number of individual cells).

Diatoms were sampled from various biotopes, namely sediment (epipelic diatoms), on and between the sand particles (episammic diatoms), on gravel, stones and bedrock (epilithic diatoms) and attached to macrophytes (epiphytic diatoms) according to Taylor *et al*. (2007a). They were preserved with ethanol to a final concentration of 20% for microscopic analyses. The diatom samples were cleared of organic matter by heating it in a potassium dichromate and sulphuric acid solution and the cleared material was rinsed, diluted and mounted in Pleurax medium for microscopic examination.

All algae were identified using a compound microscope at 1 250 times magnification (Van Vuuren *et al*., 2006; Taylor *et al*., 2007b). The samples were sedimented in an algae chamber and were analysed using the strip-count method (APHA, AWWA and WPCF, 1992).

### 4.2.5 Statistical Analysis

The Berger-Parker dominance index (Berger and Parker, 1970) was used to measure the evenness or dominance of phytobenthos at each sampling site:

$$D = Nmax/N \qquad \text{Eqn 1}$$

Where Nmax = the number of individuals of the most abundant species present in each sample, and N = the total number of individuals collected at each site.

The density of the most abundant species (cell/l) was calculated using the following formula:

$$\text{Density} = Xn / M \times 44\ \mu l$$

Where Xn = number of individuals in species X; M = number of drops observed under the microscope. A drop of the sampled water taken up by a micropipette = 44 µl (Moser *et al*., 2004).

Univariate statistical analysis, namely Shannon diversity index (H') (Shannon, 1948) and Margalef's species richness (d) (Margalef, 1951), was used to determine the diversity and richness of the algae at the different sites before and after rehabilitation. For this purpose, the software program PRIMER version 6.0 was used (Clarke and Gorley, 2006) was used. The normality and homogeneity of variance of the data were evaluated using the Shapiro-Wilk's W test, as well as Levene's test, respectively. Non-parametric data were transformed using boxcox or rank transformations in order to meet analysis of variance (ANOVA) assumptions. Spatial differences were determined using a one-way ANOVA in combination with the Fisher's LSD post hoc test (Statistica 12, Statsoft, US). Significant differences were set at $p < 0.05$ for all statistical tests.

## 4.3 RESULTS

### 4.3.1 Physico-chemical Parameters Before and After Wetland Rehabilitation

From the results of the water quality fingerprinting (Figure 4.3) the sites downstream from the rehabilitation area, showed a progressive decrease of $SO_4^{2-}$ and an increase in $CaCO_3$. From this type of analysis it can also be confirmed that Site U2, which occurred close to the mining activities, was more impacted by AMD as reflected by the higher $SO_4^{2-}$ concentration, especially before rehabilitation.

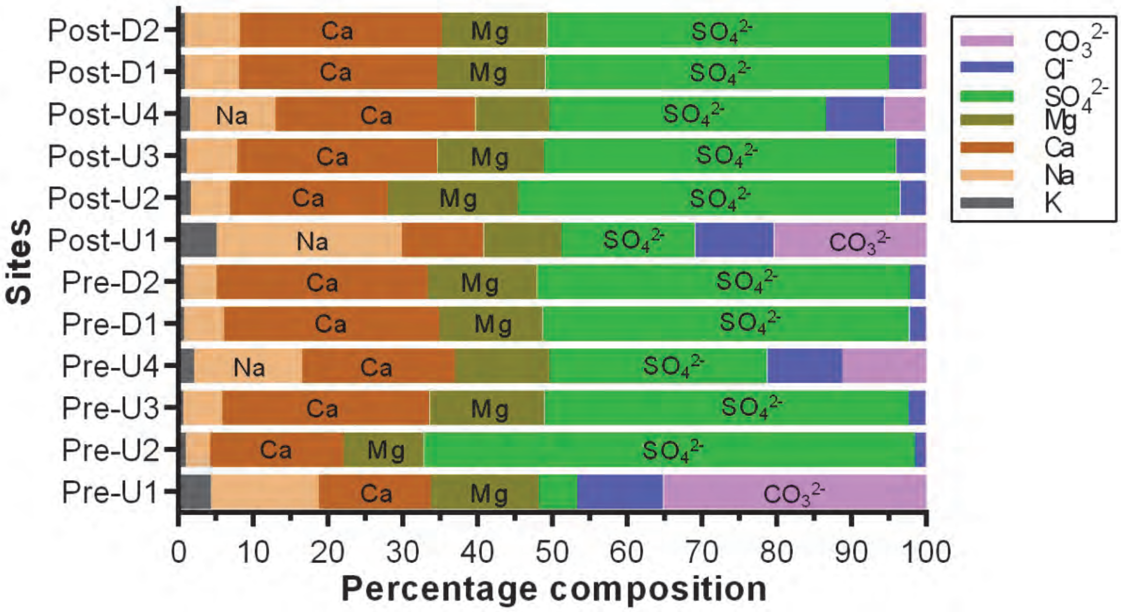



**Figure 4.3 The water quality fingerprint of all the sites before and after rehabilitation, indicating the percentage composition of $CO_3^{2-}$, $Cl^-$, $SO_4^{2-}$, Mg, Ca, Na and K. The three most dominant elements at each site are shown.**

The effect of the rehabilitation process can be seen in the different water quality variables presented in Chapter 2. The pH, Chl-a (in the water column) and alkalinity increased over time downstream from the rehabilitation area, while EC (or total dissolved solids), $SO_4^{2-}$, TSS, Al and Fe decreased. Turbidity has increased both upstream and downstream from the rehabilitation area.

The major pattern in benthic chl-a and diatom species diversity reflected a generalised gradient of disturbance associated with the decanting of AMD at the upstream sites. A very low biomass of epilithic filamentous algae (benthic chl-a >0.8 mg/m) and diatom diversity was observed at the upstream sites when compared to the downstream sites. At the upstream sites a strong relationship between the low benthic algae (chl-a average of >1 mg/m) and the low average pH of 2.85 was observed.

### 4.3.2 Algal Dynamics in the Pre-rehabilitation Phase

In the water column, average concentrations of Al and Fe at Pre-U2 were the highest in comparison to all the other sampling sites, which could also have an adverse effect on the benthic algae as indicated by the low benthic chl-a of >1 mg/m measured at this site in comparison to the reference site. A strong relationship was observed between the average pH of 7.95 and the average benthic chl-a of 11.25 mg/m at Pre-U1 in comparison to Pre-U2 impacted by AMD and a low pH value. Turbidity of the water column at all survey sites were similar in comparison to Pre-U1 (NTU <5). The highest epilithic filamentous algae biomass of chl-a of 13.4 mg/m was measured at Pre-U3. The epilithic filamentous algae mats at Pre-U2 and Pre-U3 were dominated (Berger-Parker index 0.101 and 0.461, respectively) by the algal species *Klebsormidium acidophilum* (Novis) which is known to be dominant in AMD impacted water. A decrease of diatom diversity between Pre-U1 and Pre-U2 was also observed during the study period, while Pre-U4 was dominated (Berger-Parker index 0.301) by the diatoms *Gyrosigma rautenbachiae* (Cholnoky) which is a good indicator of industrial pollution (Taylor *et al*., 2007b). The thickest layers of hydroxide precipitates (± 2 mm) were observed at Pre-U2, and reduced substantially to the downstream sites (Pre-D1 and Pre-D2), with very little substrate deposits present at these two sites (± 1 mm). According to the Berger-Parker dominance index the following algae were dominant at each of the sampling sites before rehabilitation: Pre-U1 [the diatom *Navicula cryptotenella* (Lange-Bertalot) (0.352) and the filamentous green algal Spirogyra reinhardi (Chmiel) (0.271)]; Pre-U2 [the diatom *Craticula buderi* (0.179) and the filamentous green algal *Klebsormidium acidophilum* (Noris) (0.182)]; Pre-U3 [the diatom *Craticula buderi* (Brebisson) (0.278) and the filamentous green algae *Klebsormidium acidophilum* (Noris) (0.491) and *Mougeotia cf. laevis* (Archer) (0.271)]; Pre-U4 [the diatom *Gyrosigma rautenbachiae* (Cholnoky) (0.289), and the filamentous green algal *Spirogyra reinhardi* (2.81)]. The filamentous blue-green algal *Oscillatoria tenuis* al so occurred at Pre-U1 in large numbers (3025 cells/l). The occurrence of the latter species which is found in nutrient rich aquatic environments can possibly be related to the agricultural activities surrounding Pre-U1 (Douterelo *et al*., 2004). Pre-D1 and Pre-D2 were dominated by the green filamentous algal *Mougeotia cf. laevis* (0.274) and the diatoms *Craticula buderi* and *Nitzschia clausii*.

### 4.3.3 Algal Dynamics in the Post-rehabilitation Phase

After rehabilitation of the wetland benthic Chl-a and diatom species diversity downstream from the rehabilitation area showed a distinct difference with data generated from the pre-rehabilitation phase. The downstream sites were dominated by the green filamentous algal *Klebsormidium rivulare* (Kutzing), which replaced *Mougeotia cf. laevis*, as well as the diatoms *Tabellaria flocculosa* (Roth) and *Nitzschia nana* (Grunow), which replaced *Craticula buderi* and *Nitschia clausii* (Hantzsch). A strong relationship was observed between the average increase in pH and benthic chl-a at the downstream sites during the post rehabilitation phase.

Figure 4.4 gives an indication of how the diversity (H') and richness (d) of the algae changed before and after rehabilitation at the different sites. The diversity at Site D1 remained the same before and after rehabilitation, but increased at Site D2, while the species richness increased at both downstream sites after rehabilitation.

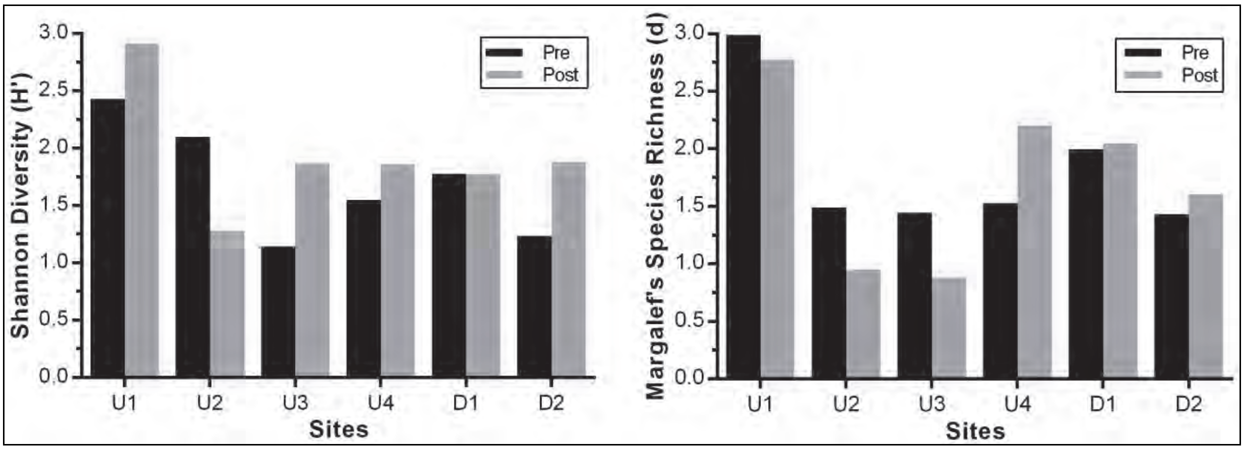



**Figure 4.4 Shannon diversity and Margalef's species richness at the sampling sites before and after rehabilitation**

From the redundancy analysis plot in Figure 4.5 it can be clearly seen how the grouping of the sites downstream from the rehabilitation area have shifted after the rehabilitation process. The references sites (upstream sites) grouped together before and after rehabilitation, while there is a clear split in the grouping of the downstream sites before and after rehabilitation. The variation explained through Figure 4.3 can be seen in the data (Table 4.2) and data summary presented in Table 4.3.

**Table 4.2 Composition of the periphytic phytoplankton communities before and after rehabilitation. The relative abundance of each periphytic phytoplankton taxa was grouped as follows: + = ≤ 50 (rare); ++ = 51- 250 (scarce); +++ = 251-1000 (common); ++++ = 1001-5000 (abundant); and +++++ = 5001-25 000 (predominant) cells/l.**

| Species | Pre-U1 | Pre-U2 | Pre-U3 | Pre-U4 | Pre-D1 | Pre-D2 | Post-U1 | Post-U2 | Post-U3 | Post-U4 | Post-D1 | Post-D2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bacillariophyta** | | | | | | | | | | | | |
| *Achnanthidium exiguum* | | | | | | | | | | | + | ++ |
| *Amphora coffeaeformis* | | | | | | | | | | ++ | | ++ |
| *Cocconeis pediculus* | ++ | | | | | | | | | | ++ | +++ |
| *Craticula buderi* | | +++ | ++ | | ++++ | +++ | | +++ | ++ | | + | + |
| *Craticula cuspidate* | | | | ++ | ++ | + | | | | | | |
| *Ctenophora pulchella* | | + | | | | | | + | | | + | |
| *Cymbella kappii* | + | + | + | | | | | ++ | | | | |
| *Cymbella neocistula* | | | | | | | | | | ++ | | |
| *Cymbella tumida* | ++ | | | | | | + | | | | | |
| *Diatoma vulgaris* | ++ | | | + | + | | + | | | | | |
| *Flagilaria ulna* | ++ | | | + | + | + | | | ++ | ++++ | +++ | +++ |
| *Frustulia rhomboids var. crassinerva* | | | | | | | | | | | ++ | |
| *Frustulia saxonica* | | ++ | ++ | | ++ | + | + | | | + | | |
| *Frustulia vulgaris* | | | + | | ++ | | | | | | | |
| *Gomphonema aff. Gracile* | | | ++ | ++ | ++ | ++ | | +++ | ++ | + | | |
| *Gomphonema parvulum* | | | | + | + | + | | | | | | |
| *Gomphonema pseudoaugur* | ++ | | | | | | ++ | | | | | |
| *Gryrrosigma scalproides* | ++ | ++ | ++ | + | ++ | + | | | | | | |
| *Gyrosigma acuminatum* | | | | | | | | | | ++ | | |
| *Gyrosigma attenuatum* | | | | | | | | | | ++ | | |
| *Gyrosigma rautenbachiae* | | | | ++++ | ++ | + | | | | +++ | | |
| *Melosira variance* | ++ | | | | | | ++ | | | | | ++ |
| *Navicula cryptotenella* | +++ | | | ++ | + | | ++ | | | | | |
| *Navicula microcari* | | | | | | | | | | + | | |
| *Navicula pupala* | ++ | | | | | | + | | | | | |
| *Navicula tripunctata* | ++++ | | | | | | ++ | | | | | |
| *Nitschia clausii* | | | | +++ | +++ | +++ | | | | ++ | ++ | |
| *Nitschia nana* | | | + | | | | | | ++ | | ++++ | ++++ |
| *Nitschia sublinearis* | | | | | | | | | + | | | |
| *Nitzschia intermedia* | ++ | | | | | | | | | | | |
| *Nitzschia pura* | + | | | | | | | | | | | |
| *Nitzschia reversa* | ++ | ++ | ++ | +++ | ++ | | | + | | | ++ | ++ |

| Species | Pre-U1 | Pre-U2 | Pre-U3 | Pre-U4 | Pre-D1 | Pre-D2 | Post-U1 | Post-U2 | Post-U3 | Post-U4 | Post-D1 | Post-D2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pinnularia viridiformis* | +++ | | | | | | | | | | | +++ |
| *Pinnularia viridis* | | | | | | | + | | | | + | |
| *Placoneis placentula* | | | | | | | | | | + | | |
| *Rhopalodia gibba* | | | | | | | | | | | ++ | |
| *Synedra ulna* | ++ | + | + | ++ | ++ | ++ | | | | | + | ++ |
| *Tabellaria flocculosa* | | | | | | | | | | ++ | ++++ | ++++ |
| **Chlorophyta** | | | | | | | | | | | | |
| *Cladophora glomerata* | | | | | | | ++ | | | | ++ | |
| *Closterium margaritiferum* | +++ | | | | | | ++ | | | | | |
| *Closterium peracerosum* | | | | | | | ++ | | | | | + |
| *Closterium spinosporum* | | | | | | | | | | + | | |
| *Cosmarium hammeri* | | | | | | | | | | +++ | ++ | +++ |
| *Cosmarium pseudopraemorsium* | ++ | | | | | + | ++ | | | | | |
| *Microspora quadrata* | | ++ | | | ++ | ++ | | ++ | | | | |
| *Pandorina sp.* | | | | | | | ++ | | | | | |
| *Scenedesmus armatus* | ++ | | | | | | +++ | | | + | | |
| *Spirogyra africana* | ++ | | | | | | | | | | | |
| *Spirogyra reinhardi* | +++ | | | +++ | | | +++ | | | | | |
| *Staurastrum anatinum* | ++ | | | | | | ++ | | | + | | |
| **Streptophyta** | | | | | | | | | | | | |
| *Klebsormidium acidophilum* | | +++ | ++++ | | ++ | | | +++ | | | | |
| *Klebsormidium rivulare* | | | | | | | | | ++ | +++ | ++++ | ++++ |
| *Mougeotia cf. laevis* | | | ++ | | ++++ | ++++ | | | | | | |
| *Zygnema cf. cylindrospermum* | | + | | | | | | ++ | | | | |
| **Euglenophyta** | | | | | | | | | | | | |
| *Euglena sociabilis* | ++ | | | | | | +++ | | | | | |
| *Phacus pleuronectes* | +++ | ++ | + | + | + | | ++ | | | | | |
| *Trachelomonas intermedia* | + + | | | | | | +++ | | | | | |
| **Cyanophyta** | | | | | | | | | | | | |
| *Batrachospermum atrum* | | | | | | | | | | | | |
| *Cylindrospermopsis raciborskii* | | | | | | | + | | | | + | |
| *Glaucospira* sp. | +++ | | | | | | | | | | | |
| *Merismopedia punctata* | ++ | + | + | ++ | ++ | | | | | | | |
| *Oscillatoria princeps* | ++ | | | | | | ++ | | | + | ++ | |
| *Oscillatoria tenuis* | ++++ | | | | | | | | | | | |
| **Rhodophyta** | | | | | | | | | | | | |
| *Batrachospermum atrum* | | | | | | | | ++++ | ++ | ++ | + | + |

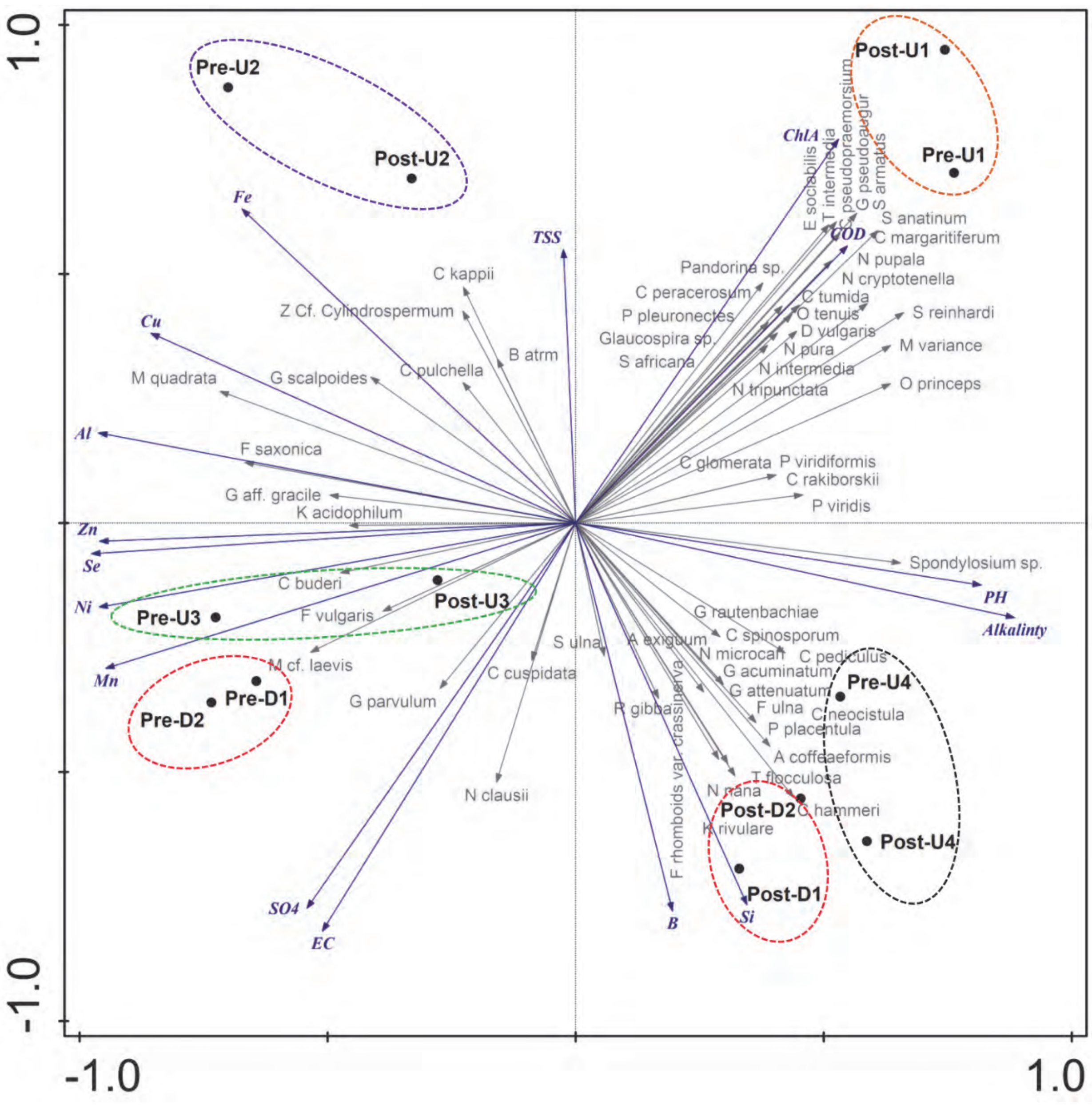



**Figure 4.5 Redundancy analysis plot showing the (dis)similarity of the respective sites before and after rehabilitation. This plot is based on algal diversity with water quality variables overlain. The shift that occurred at the downstream sites after rehabilitation is highlighted by the red circle.**

**Table 4.3 The analysed data summary table for the redundancy plot**

| Statistic | Axis 1 | Axis 2 | Axis 3 | Axis 4 |
|---|---|---|---|---|
| Eigenvalues | 0.7091 | 0.1799 | 0.045 | 0.029 |
| Explained variation (cumulative) | 70.91 | 88.89 | 93.4 | 96.3 |

## 4.4 DISCUSSION

Algae can be found in all aquatic habitats. In most streams, rivers and wetlands, algae are the most diverse assemblage of organisms (Dixit and Smol, 1989). They are excellent indicators of water quality and environmental changes due to land use activities (Dixit and Smol, 1989). The autecology, or physiological requirements and tolerance of algal species to nutrients, organic enrichment, dissolved oxygen, major ions, temperature or pH can be classified qualitatively from literature accounts.

In a series of stream experiments done by Elwood and Mulholland (1989) and Baker and Christensen (1991) it was found that benthic mats of green algae greatly increased as pH decreased to 5.0, while an increase in the availability of Al caused by acidification can also result in an increase in green algae (e.g. *Mougeotia* sp. and *Temnogametum* sp.) (Turner *et al*., 1991). This observation was in agreement with the current study where the replacement of *Mougeotia cf. laevis* by *Klebsormidium rivulare* as the dominant filamentous green algal species at the downstream sites after rehabilitation possibly related to the decrease of Al concentrations in the water column during the post-rehabilitation phase. It was also evident from our study that a distinct shift in diatom assemblage occurred after rehabilitation when dominant diatoms tolerant to strong pollution conditions, such as mine and industrial effluent (e.g. *Craticula buderi* and *Nitschia clausii*) were replaced by the diatom species *Tabellaria flocculosa* (Roth) and *Nitzschia nana*. According to Taylor *et al*. (2007b), Tabellaria flocculosa indicates oligotrophic circumneutral or slightly acidic conditions, while *Nitzschia nana* is only found in moderately polluted water. This correlates with the improvement in water quality noticed at the downstream sites after rehabilitation as can be seen in Figure 4.3. The pH levels increased and the buffering/neutralising capacity of the water (as represented by alkalinity) also increased after rehabilitation. Simultaneously, the changes in the algal assemblages correlated with the decrease in certain variables, namely SO42-, Al, Fe and EC, which resulted in improved water quality. The lower TSS in the water also meant that more light was able to penetrate the water column, thus increasing the productivity (as represented by Chl-a). This highlights the improved functioning of the wetland after rehabilitation.

According to Dixit and Smol (1989) and Bækken *et al*. (2006), the greatest changes in phytoplankton usually occur at a pH range of 4.7 to 5.6, just beyond the interval of a pH of 5.5 to 6.5 where carbonates, which is a key source of both acid neutralizing capacity and inorganic carbon for photosynthesis, becomes rapidly depleted and then lost. However, the reduced availability of dissolved inorganic carbon stimulates the proliferation of certain filamentous green algae as observed in our study (Turner *et al*., 1987). The low diatom and filamentous green algal biomass observed at Pre-U2 and Post-U2 in comparison to all the other selected sites can possibly be related to the higher rate of oxide deposition at this site. Oxide deposition may smother algae and inhibit photosynthesis (Anthony, 1999). However, many studies suggest that green filamentous algae often dominate algal communities in streams affected by AMD (Verb and Vis, 2001, 2005). This finding was consistent with our study. Our findings indicated that very low pH, high concentrations of dissolved metals (also represented by high conductivity) and higher rates of metal oxide deposition were associated with a reduction of the algal diversity and altered the benthic community structure in the Grootspruit at the sites upstream from the rehabilitation area.

## 4.5 CONCLUSION

Through this study we found that rehabilitation of the wetland through the expansion of the existing wetland area via the redirection of surface flow, slowing the velocity of the water through the wetland and increasing the contact time between water and sediment improved the water quality downstream from the rehabilitated area. This was indicated through the increase in pH levels, chl-a and alkalinity concentrations, as well as the decrease in EC, $SO_4^{2-}$, TSS, Al and Fe. We also found that species diversity and richness of the algae have increased after rehabilitation and that freshwater algae can be used as bioindicator for wetland rehabilitation.

# CHAPTER 5: ANNUAL ASSESSMENT OF VEGETATION IMPACTED BY MINE WASTEWATER USING LEAF SPECTRAL PROPERTIES OF *PHRAGMITES AUSTRALIS* AND *TYPHA CAPENSIS* IN EMALAHLENI, SOUTH AFRICA.

## 5.1 INTRODUCTION

Several studies have revealed signs of leaf pigment stress, reduction of nutrients and growth inhibition of vegetation exposed to mine water and/or acid mine drainage (AMD) or contaminated soil (Delgado *et al*., 1993; Kooistra *et al*., 2001; Batty and Younger, 2004; Kooistra *et al*., 2004; Gardea-Torresdey *et al*., 2005; Peralta-Videa *et al*., 2009). Changes in foliar chemistry are highly correlated with changes in absorption features in the electromagnetic spectrum (Curran, 1989). Field spectroscopy and derived spectral vegetation indices (SVIs) therefore provide a quick and affordable means of determining vegetation condition, compared to labour intensive sampling and expensive laboratory analysis of contaminated soil and vegetation (Wu *et al*., 2007).

Macrophytes such as *Phragmites australis* and *Typha capensis*, often occur naturally in wetlands of South Africa, and are tolerant to degraded sites exposed to mine and agricultural effluent (Van Deventer and Cho, in press). Both *Phragmites* and *Typha* species are capable of growing in water and soil with elevated levels of trace and heavy metals, low pH and high sulphates associated with mining wastewater and AMD (Hecker, 2003; Bragato *et al*., 2009; Tian *et al*., 2009; Bonanno and Lo Giudice, 2010; Bonanno, 2011; Rufo *et al*., 2011; Klink *et al*., 2013). World-wide, these species have shown potential for bioremediation of contaminated soils, are efficient at extracting metals from water and soil and hence have been considered in passive treatment systems (Gazea *et al*., 1996; Tian *et al*., 2009). Regardless of their ability to grow rather profusely under these harsh conditions, leaf spectra still show signs of chlorophyll stress using leaf-level field spectroscopy (Van Deventer and Cho, in press). Mining wastewater impacts leaf chlorophyll through trace and heavy metal accumulation in the soil and plant parts. Leaf chlorophyll reduction is visible through the increase of reflection in the red band, more particularly the red edge which has proven to be more sensitive to both chlorophyll and nutrient stress and is hence mostly used in vegetation condition monitoring (Horler *et al*., 1983; Miller *et al*., 1990; Guyot *et al*., 1992; Gitelson and Merzlyak, 1998; Zarco-Tejada *et al*., 2003; Cho and Skidmore, 2006). Exposure to heavy metals associated with mining indicated a reduction in wavelength of the shift in red edge reflectance slope towards the blue region and an increase of the NIR region (Davids and Tyler, 2003; Hecker, 2003; Clevers *et al*., 2004; Sridhar *et al*., 2007; Liu *et al*., 2011). Leaves may also show early signs of dying off and an increase in carotenoid levels, similar to senescent leaves (Adams *et al*., 1999). A number of SVIs related to carotenoids and chlorophyll could therefore be useful in assessing vegetation condition in wetlands impacted by mining wastewater.

The rehabilitation programme aimed at improving water quality through improving wetland function. Ridge and furrow cultivation as well as artificial drainage in the central reaches of the Grootspruit caused channel incision and an increased concentrated flow (Green Door Environmental, 2013). Diffusing flow is expected to improve water quality and restore natural vegetation to the wetland.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Study Area

The study area is located along the Grootspruit (±25.90°S and 29.06°E) situated east of Pretoria, south of the N4 freeway, and about 15 km south-west from Emalahleni in South Africa (Figure 5.1). The Grootspruit (which forms part of the Zaalklapspruit Wetland) originates in the upper reaches of the Olifants River catchment, running northwards for about 16 km up to its confluence with the Saalboomspruit. The predominant land-use in the sub-quaternary catchment is natural and cultivated land, except for coal mining towards the north-east of the sub-quaternary catchment (Figure 5.2). The area receives mean annual rainfall of 668 mm per annum with characteristically wet summers and dry winters (Middleton and Bailey, 2008).

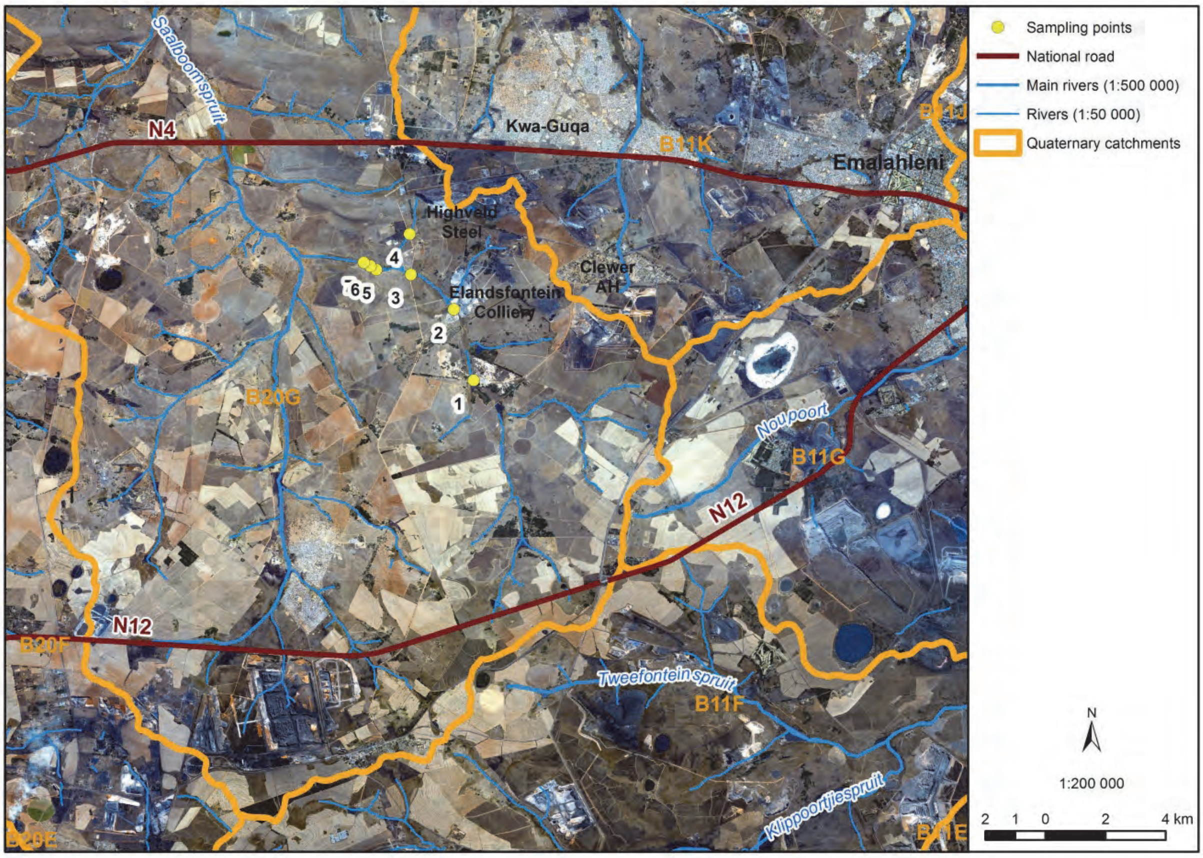



**Figure 5.1 Location of sampling sites on the Grootspruit**

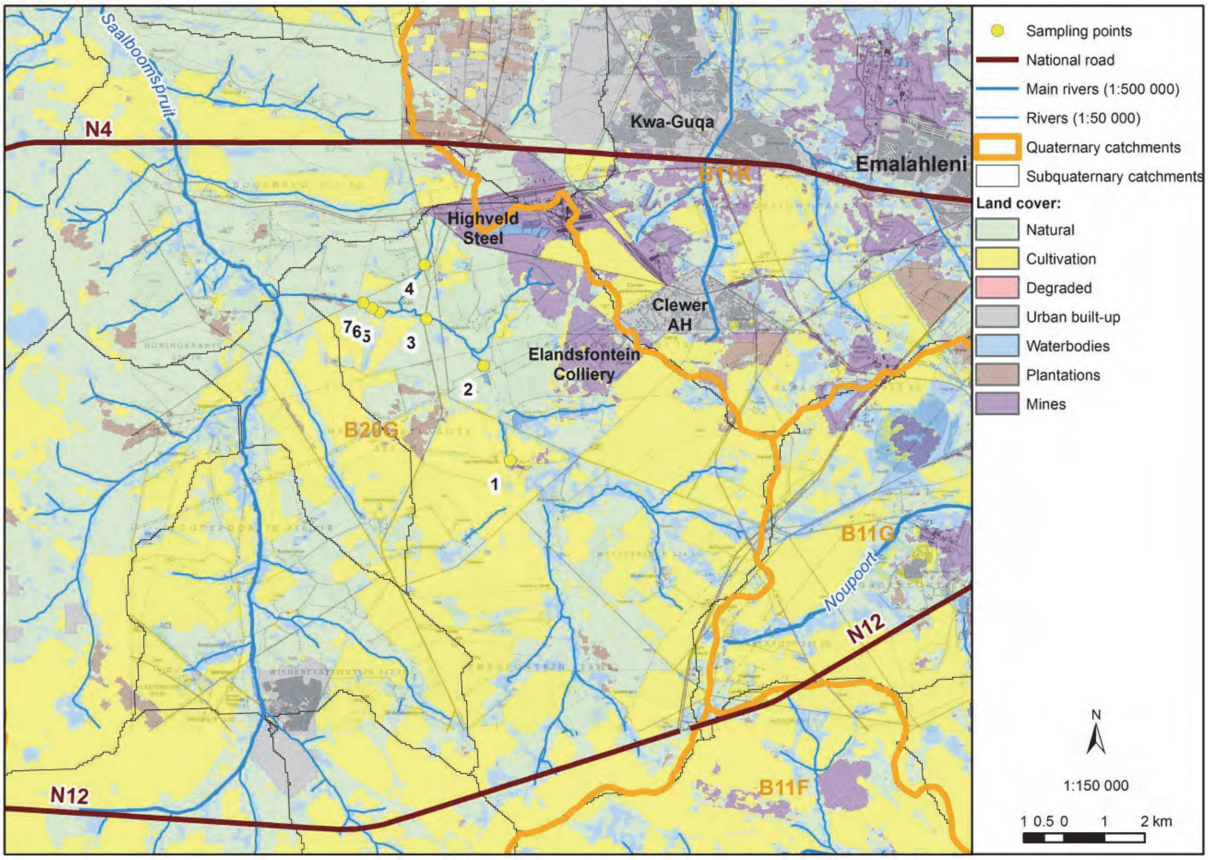



**Figure 5.2 Land cover categories (SANBI, 2009) displayed on topographical maps of the study area for the extent of the Grootspruit's sub-quaternary catchment (Topographical 1:50 000 2529CC dated 2003 and 1:50 000 river line data dated 2006 from Department of Rural Development and Land Reform)**

### 5.2.2 Sampling

Seven sites were identified for sampling the macrophytes *Phragmites australis* and *Typha capensis* along the Grootspruit (Figure 5.2; Figure 5.3; Table 5.1). Sites 1 and 2 were situated directly upstream of the Highveld Steel and Elandsfontein Colliery coal mine operations, Sites 3 and 4 received direct drainage from the mine and Sites 5 to 7 were situated downstream of the mine. All sites received runoff from agricultural practices.

Green leaves of *P. australis* and *T. capensis* were sampled during the peak growth season before and after rehabilitation. At each site and for each species, five green leaves were randomly sampled along the wetland with a minimum distance of about 1 m from one another.

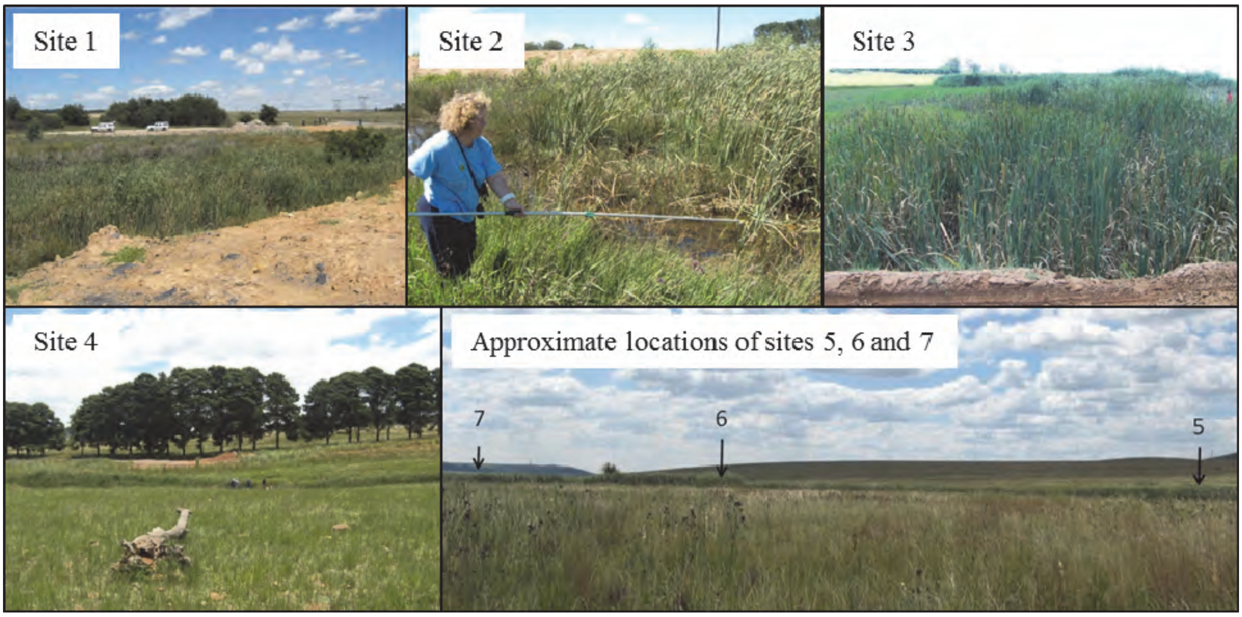



**Figure 5.3 Vegetation at the sampling sites**

**Table 5.1 Vegetation and coordinates of the sampling sites**

| Site | Vegetation | Site description | Coordinates |
|---|---|---|---|
| **1** | *Phragmites australis* *Typha capensis* | Site 1 is situated about 8 km from the origin of the Grootspruit on the watershed. This is an artificial wetland where a gravel road runs across the dam wall. Sand mining operations take place directly east of the wetland. The site receives primarily runoff from agricultural lands located on the watershed. Both macrophytes are predominantly found on the eastern slopes of the wetland, as well as north and south of the dam wall. The majority land use in a 1 km radius is cultivation. | ± 25.9404°S; 29.0843°E |
| **2** | *Phragmites australis Typha capensis* | Site 2 is situated approximately 2.4 km north of Site 1. This small artificial wetland is located in a severely disturbed, topographically modified and disturbed environment, even though the land cover indicates it as natural. This site receives runoff from mining (the Elandsfontein Colliery) and agricultural land uses. | ± 25.9193°S; 29.0783°E |
| **3** | *Phragmites australis Typha capensis* | Site 3 is a narrow, shallow channelled wetland draining underneath the dirt road through a culvert towards the west. It is approximately 1.7 km downstream and north-west from Site 2. Site 3 receives runoff from mining (the Elandsfontein Colliery) and agricultural land uses. | ± 25.9089°S; 29.0656°E |
| **4** | *Typha capensis* | A narrow channelled wetland drains water from Highveld Steel at a steep slope for 1 km into an artificial wetland. Site 4 is situated on the 260 m stretch of the channel between the artificial wetland and the dirt road. The land immediately to the east of the wetland is tilled. | ± 25.8969°S; 29.0652°E |
| **5** | *Phragmites australis* | Site 5 is about 600 m west of the confluence of the Site 4 tributary to the Grootspruit and therefore receives runoff from natural, agricultural and mining land uses. It is a channelled wetland with *Phragmites australis* on the banks of the wetland. Rehabilitation structures were implemented at this site. | ± 25.9075°S; 29.0553°E |
| **6** | *Typha capensis* | Site 6 is approximately 200 m west of Site 5. It receives runoff from natural, agricultural and mining land uses. It is a channelled wetland with *Typha capensis* on the banks of the wetland. Rehabilitation structures were implemented at this site. | ± 25.9065°S; 29.0535°E |
| **7** | *Phragmites australis Typha capensis* | Site 7 is situated approximately 250 m west of Site 6. It receives runoff from natural, agricultural and mining land uses. It is a channelled wetland with large stands of both *Phragmites australis* and *Typha capensis* on the banks of the wetland. | ± 25.9054°S; 29.0515°E |

### 5.2.3 Leaf Level Spectral Reflectance Measurements

An Analytical Spectral Device (ASD), the FieldSpec Pro spectroradiometer (Analytical Spectral Devices Inc, Boulder CO, USA) was used to record the leaf spectra of green leaves of the macrophytes. The spectrometer has a spectral range extending from 350 to 2 500 nm and a bandwidth of 3 nm resampled to 1 nm (http://www.asdi.com). A contact probe was used for spectral reflectance measurements to avoid external factors (light illumination differences and influence from soil background). The instrument was regularly optimised against a white Spectralon reference panel in the contact probe. The radiance values were converted to reflectance values using the ViewSpecPro software.

A number of spectral vegetation indices for carotenoids and chlorophyll were chosen as non-destructive means of determining pigment content (Table 5.2). For carotenoids, the Carotenoid Reflectance Index (CRI) was selected based on its maximum sensitivity to carotenoid content with the least contribution by chlorophyll pigment content; ability to determine carotenoid content across a wide variety of non-related species, across various climatic regions and a range of carotenoid pigment content, and in particular for carotenoid content in green leaves (Zur *et al*., 2000; Gitelson *et al*., 2002; Gitelson *et al*., 2002; Gitelson and Merzlyak, 2004; Gitelson *et al*., 2006). The chlorophyll Red-edge Linear Extrapolation method (REP Le) index was chosen because of its high predictive capability and low error results using a wide variety of crops and tree species (Cho and Skidmore, 2006; Main *et al*., 2011).

The SVIs were derived from the leaf spectral measurements (Table 5.2). The increase of values of both SVIs implied an increase in pigment values. We hypothesised that sites affected by mine wastewater and AMD could potentially have, on average, higher levels of carotenoids, therefore higher CRI values compared to the other sites, and lower levels of chlorophyll, thus lower REP Le values compared to the other sites. Similarly, sites with lower levels of carotenoids and higher levels of chlorophyll were assumed to show less impact of mine wastewater and AMD compared to the other sites.

**Table 5.2 Vegetation indices used to assess vegetation condition for macrophytes exposed to mine water**

| Leaf characteristic investigated | Index name | Equation | Reference |
|---|---|---|---|
| **Carotenoids** | Carotenoid Reflectance Index Red edge (CRI RE) | $Car_{Red\ edge} = [(R_{510\text{-}520})^{-1}\text{-}(R_{690\text{-}710})^{-1}]*R_{NIR}$<br><br>(In this study reflectance at 510 nm, 710 nm and 800 nm were used to replace the above values.) | Gitelson *et al*. (2002); Gitelson and Merzlyak (2004); Gitelson *et al*. 2006) |
| **Chlorophyll** | Red-edge Position Linear Extrapolation method (REP Le) | See Cho and Skidmore, 2006 | Cho and Skidmore (2006) |

### 5.2.4 Data Analysis

For each SVI, the minimum, maximum, mean and standard deviation values were determined for the two species sampled. Sites with the highest mean carotenoid values and lowest mean chlorophyll values were listed as possible indication of sites impacted by mine wastewater and AMD.

For each species and year, the differences between sites were assessed using a one-way Analysis of Variance (ANOVA) and post-hoc Tukey Honest Significant Difference (HSD) test in Statistica v. 8 (StatSoft, Inc, 1984-2007). The Bonferroni correction for comparable pairs was done for both species: for *P. australis* the p-value were divided by ten comparable pairs ($p = 0.005$), whereas for *T. capensis* the p-value were divided by 15 comparable pairs ($p = 0.003$). Differences between years for each site per species were assessed using a one-way ANOVA with a p-value < 0.05).

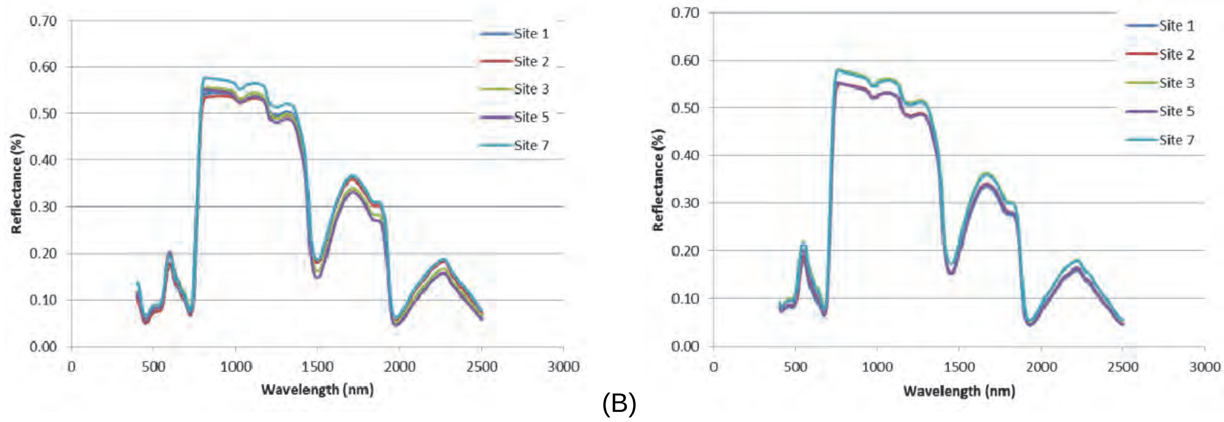
## 5.3 RESULTS

### 5.3.1 Results for Phragmites australis

*5.3.1.1 Green Leaf Spectra of Each Sampling Site per Species*

During Year 1, Site 7 showed a higher average reflectance of leaf spectra of *P. australis* in most regions of the electromagnetic spectrum, compared to the other sites (Figure 5.4A). The reflectance of Sites 3 and Site 5 is slightly higher in the region of the spectrum associated with carotenoids (±515 nm) and chlorophyll (±720 nm) though lower in the NIR associated with leaf structure (±800 nm) for Year 1 compared to Site 7.

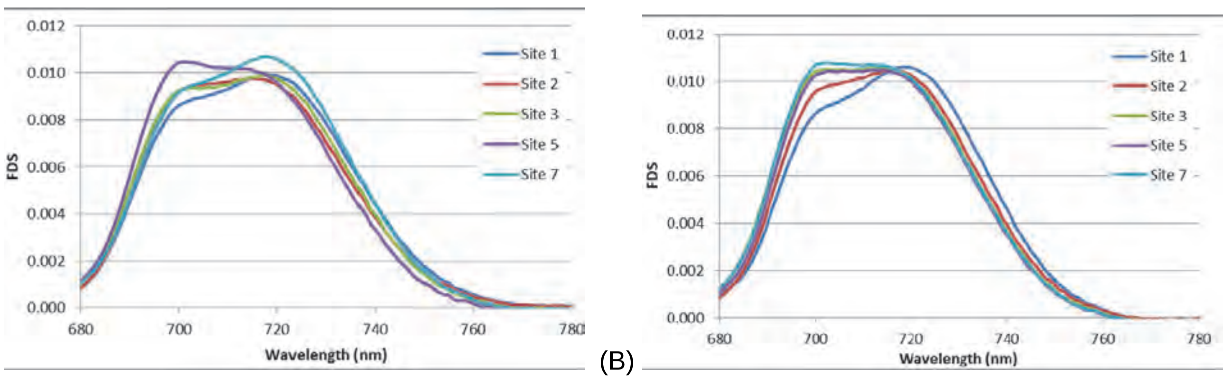
During Year 2, Sites 3 and 7 showed very similar patterns with a higher average reflectance of leaf spectra compared to the other sites (Figure 5.4B). Site 3 showed a slightly higher average leaf spectra values in the pigment regions, compared to Site 7.




(A) (B)

**Figure 5.4 Average reflectance for *Phragmites australis* per site during (A) Year 1 and (B) Year 2. Water absorption bands are noticeable at 970, 1200, 1400 and 1940 nm.**

During Year 1, the First Derivative Spectra (FDS) of *P. australis* spectra showed noticeable differences around the chlorophyll red edge (680-730 nm). Site 5 showed a dominance of the first derivative peak at 700 nm (Figure 5.5A), indicating low chlorophyll levels compared to the other sites where the second peak of the chlorophyll red edge was more dominant (towards 717 nm). During Year 2, only Sites 1 and 2 showed a second dominant peak of the red edge near 717 nm (Figure 5.4B). The two peaks of the chlorophyll red edge seems to be equally dominant (703 and 714 nm) for Sites 3 and 5, whereas Site 7 showed a clear dominance of the first derivative peak at 702 nm (Figure 5.5B).




(A) (B)

**Figure 5.5 First derivative for *Phragmites australis* per site during (A) Year 1 and (B) Year 2**

Variation of Carotenoid Indices per Site for *Phragmites australis*

Sites 1 and 2 showed significantly (p = 0.005, Bonferroni corrected) higher mean CRI values for *P. australis* compared to Sites 5 and 7 for both years (Table 5.3, Figure 5.6; Table 5.4). The average carotenoid values for Site 5 were significantly lower compared to Site 2 (p = 0.005, Bonferroni corrected).

**Table 5.3 Carotenoid Reflectance Index, minimum, maximum, mean and standard deviation values for each site for *Phragmites australis* for the two years**

| **Year:** | **Year 1** | | | | **Year 2** | | | |
|---|---|---|---|---|---|---|---|---|
| **Parameter:** | **Min** | **Max** | **Mean** | **Stdev** | **Min** | **Max** | **Mean** | **Stdev** |
| **Site 1** | 2.41 | 3.77 | **3.21** | 0.32 | 1.86 | 3.48 | **2.89** | 0.37 |
| **Site 2** | 2.72 | 4.23 | **3.28** | 0.36 | 2.32 | 3.90 | **3.03** | 0.37 |
| **Site 3** | 1.55 | 3.64 | **2.77** | 0.45 | 1.88 | 2.81 | **2.34** | 0.25 |
| **Site 4** | | | | | | | | |
| **Site 5** | 2.09 | 3.48 | **2.84** | 0.39 | 2.15 | 3.06 | **2.74** | 0.26 |
| **Site 6** | | | | | | | | |
| **Site 7** | 2.14 | 3.52 | **2.70** | 0.31 | 1.68 | 2.96 | **2.50** | 0.30 |

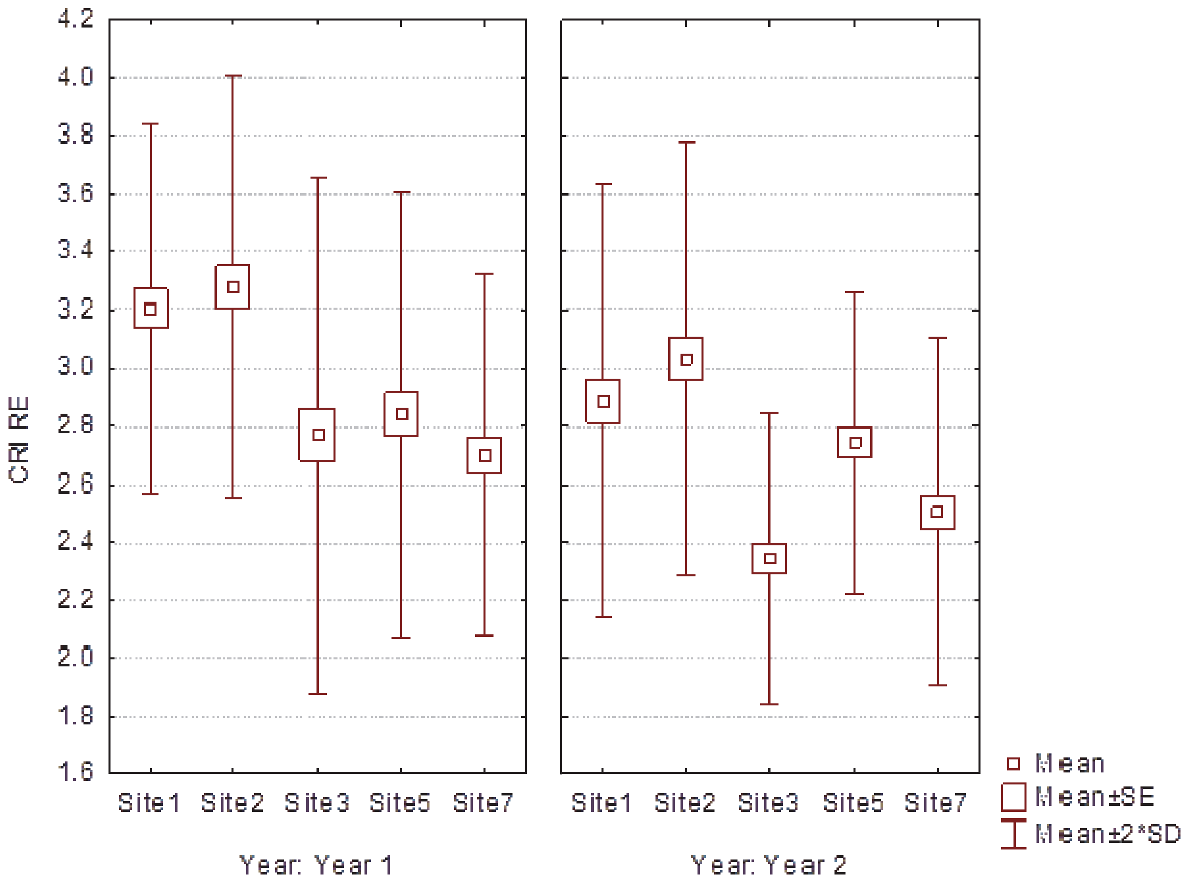



**Figure 5.6 Boxplots of Carotenoid Reflectance Index values for *Phragmites australis* per site over the two years**

**Table 5.4 One-way Analysis of Variance (Tukey's Honest Significant Difference post-hoc test) for Carotenoid Reflectance Index values of *Phragmites australis* between sites for each year**

| Year 1 | | | | | | Year 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sites:** | **Site 1** | **Site 2** | **Site 3** | **Site 5** | **Site 7** | **Sites:** | **Site 1** | **Site 2** | **Site 3** | **Site 5** | **Site 7** |
| **Site 1** | | | | | | **Site 1** | | | | | |
| **Site 2** | 0.954 | | | | | **Site 2** | 0.477 | | | | |
| **Site 3** | 0.001* | 0.000* | | | | **Site 3** | 0.000* | 0.000* | | | |
| **Site 5** | 0.005 | 0.001* | 0.966 | | | **Site 5** | 0.481 | 0.012 | 0.000* | | |
| **Site 7** | 0.000* | 0.000* | 0.956 | 0.660 | | **Site 7** | 0.000* | 0.000* | 0.380 | 0.068 | |

* - significant $p < 0.05$, Bonferroni corrected $p = 0.005$

Carotenoid Reflectance Index values have significantly ($p = 0.05$) decreased from Year 1 to Year 2 for all sites except Site 5 (Figure 5.7, Table 5.5).

**Table 5.5 One-way Analysis of Variance (Tukey's Honest Significant Difference post-hoc test) for Carotenoid Reflectance Index Red edge values of *Phragmites australis* between years for each site**

| Year 2 | | | | | |
|---|---|---|---|---|---|
| **Sites:** | **Site 1** | **Site 2** | **Site 3** | **Site 5** | **Site 7** |
| Year 1 | 0.002* | 0.022* | 0.000* | 0.301 | 0.030* |

* - significant $p < 0.05$

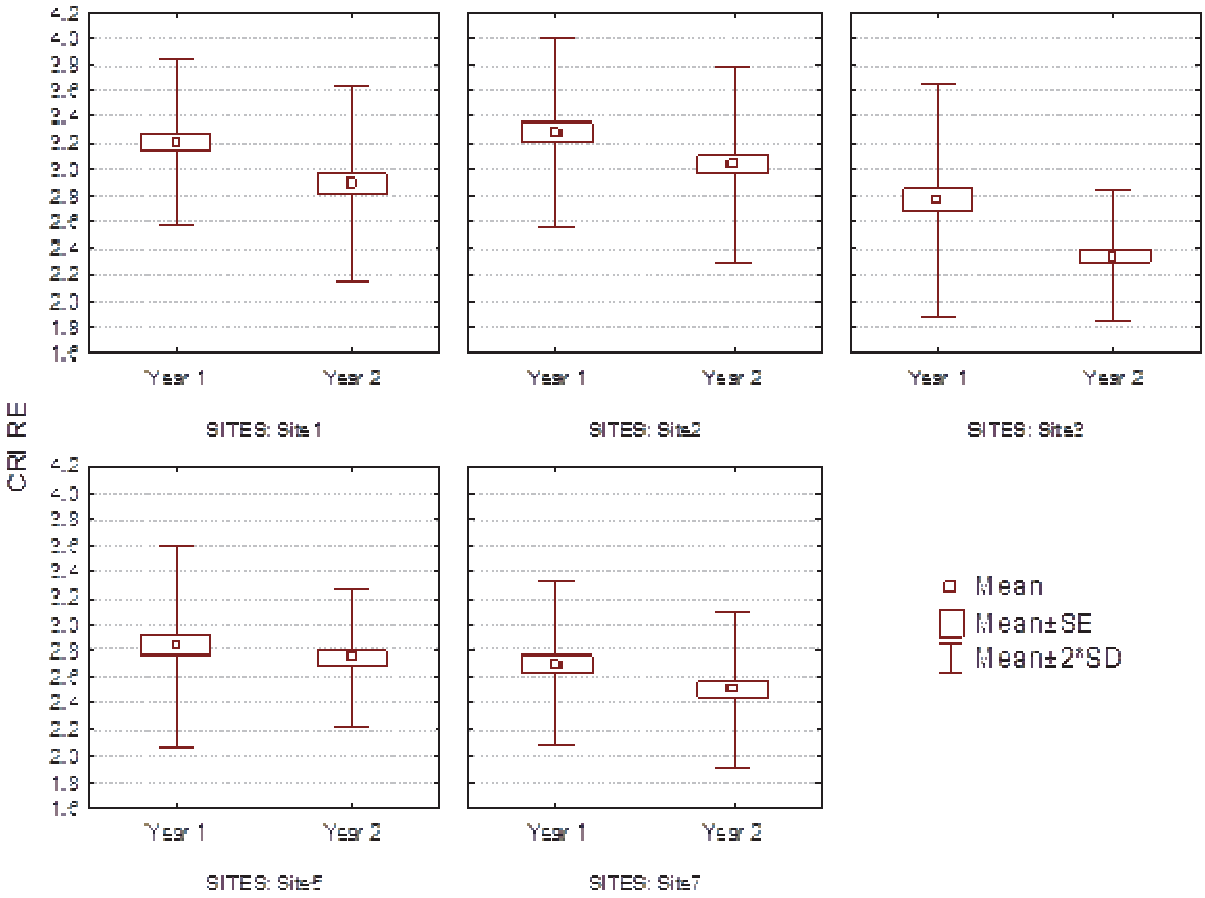



**Figure 5.7 Boxplots of Carotenoid Reflectance Index values for *Phragmites australis* between years for each site**

*5.3.1.2 Variation of Chlorophyll Indices per Site for Phragmites australis*

Site 1 showed the highest average REP Le values for *Phragmites australis* for both years when compared to the other sites (Table 5.6; Figure 5.8). During Year 1, Site 5 had significantly lower (p = 0.005, Bonferroni corrected) REP Le values compared to Sites 1 and 7, whereas the average REP Le values for Site 1 was significantly higher than Sites 3, 5 and 7 for Year 2 (Table 5.7).

**Table 5.6 Red-edge Position Linear Extrapolation minimum, maximum, mean and standard deviation values for each site for *Phragmites australis* for two years**

| **Year:** | **Year 1** | | | | **Year 2** | | | |
|---|---|---|---|---|---|---|---|---|
| **Parameter:** | **Min** | **Max** | **Mean** | **Stdev** | **Min** | **Max** | **Mean** | **Stdev** |
| **Site 1** | 702.36 | 718.89 | **709.60** | 3.90 | 695.40 | 722.67 | **713.06** | 5.93 |
| **Site 2** | 697.64 | 718.50 | **707.34** | 5.15 | 700.82 | 715.01 | **708.73** | 3.71 |
| **Site 3** | 688.66 | 714.44 | **707.46** | 5.51 | 698.70 | 712.80 | **705.73** | 3.98 |
| **Site 4** | | | | | | | | |
| **Site 5** | 691.77 | 713.33 | **704.40** | 5.49 | 697.22 | 713.43 | **706.36** | 4.42 |
| **Site 6** | | | | | | | | |
| **Site 7** | 704.73 | 715.10 | **710.24** | 2.75 | 692.24 | 717.94 | **705.70** | 5.94 |

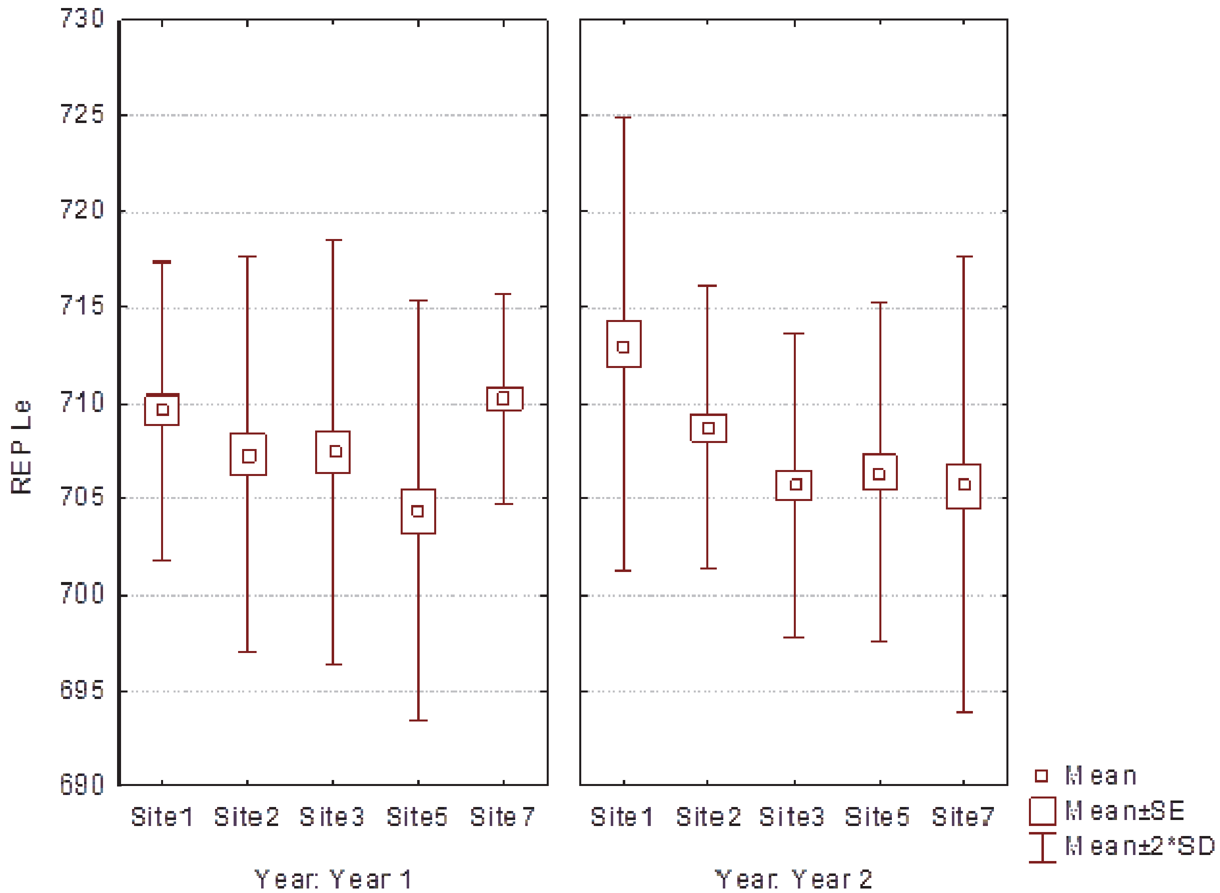



**Figure 5.8 Boxplots of Red-edge Position Linear Extrapolation values for *Phragmites australis* per site over two years**

**Table 5.7 One-way Analysis of Variance (Tukey's Honest Significant Difference post-hoc test) for Red-edge Position Linear Extrapolation values of *Phragmites australis* between sites for each year**

| Year 1 | | | | | | Year 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sites:** | **Site 1** | **Site 2** | **Site 3** | **Site 5** | **Site 7** | **Sites:** | **Site 1** | **Site 2** | **Site 3** | **Site 5** | **Site 7** |
| **Site 1** | | | | | | **Site 1** | | | | | |
| **Site 2** | 0.433 | | | | | **Site 2** | 0.019 | | | | |
| **Site 3** | 0.488 | 0.999 | | | | **Site 3** | 0.000* | 0.199 | | | |
| **Site 5** | 0.001* | 0.179 | 0.149 | | | **Site 5** | 0.000* | 0.427 | 0.991 | | |
| **Site 7** | 0.989 | 0.192 | 0.228 | 0.000* | | **Site 7** | 0.000* | 0.190 | 1 | 0.989 | |

* - significant $p < 0.05$, Bonferroni corrected $p = 0.005$

When comparing the average REP Le chlorophyll index values of Year 2 to Year 1, Site 1 showed a significant increase ($p < 0.05$) in values between the two years, whereas Site 7 showed a significant decrease ($p < 0.05$) of index values (Figure 5.9; Table 5.8). Sites 2, 3 and 5 showed no significant increase in average REP Le values, though a slight decrease in variability was visible (Figure 5.9).

**Table 5-8 One-way Analysis of Variance (Tukey's Honest Significant Difference post-hoc test) for Red-edge Position Linear Extrapolation values of *Phragmites australis* between years for each site**

| | Year 2 | | | | |
|---|---|---|---|---|---|
| **Sites:** | **Site 1** | **Site 2** | **Site 3** | **Site 5** | **Site 7** |
| Year 1 | 0.019* | 0.278 | 0.211 | 0.171 | 0.001* |

* - significant $p < 0.05$

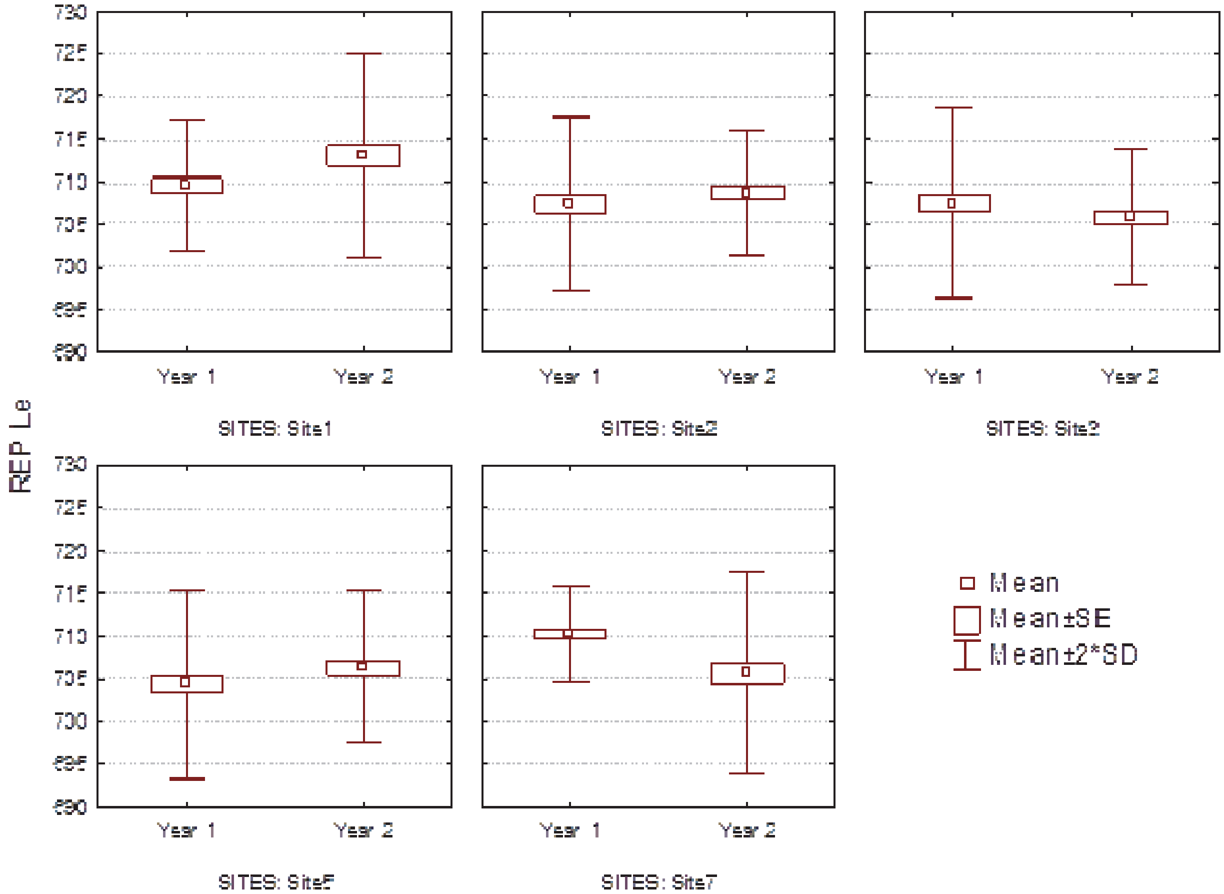
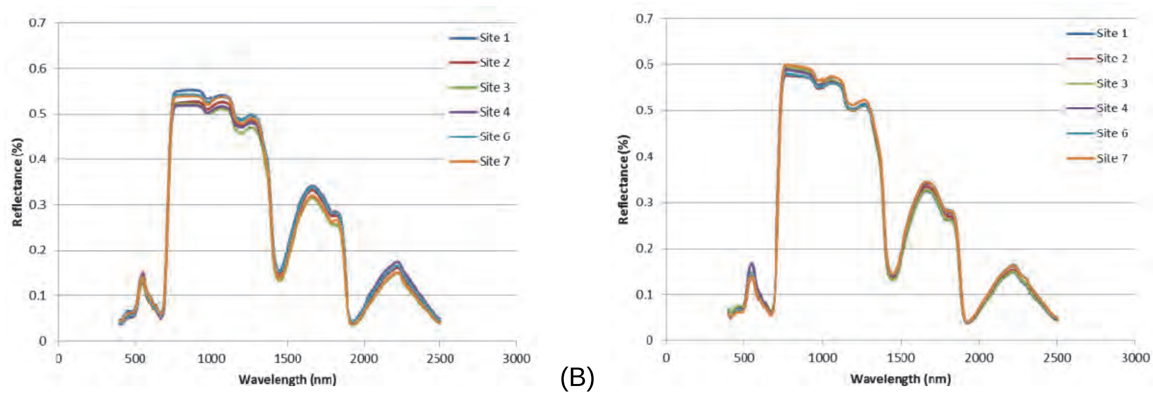



**Figure 5.9 Boxplots of Red-edge Position Linear Extrapolation values for *Phragmites australis* between years for each site**

### 5.3.2 Results for *Typha capensis*

For *T. canpensis*, the average leaf spectra per site during Year 1 (Figure 5.10A) showed variation in reflectance in different parts of the electromagnetic spectrum. Site 1 showed the highest and Site 2 the lowest reflection in both the carotenoid (±515 nm) and chlorophyll (660 nm) ranges of the electromagnetic spectrum during Year 1. In the NIR, Site 1 showed the highest reflection during Year 1 and Site 4 the lowest reflection during Year 1.

During Year 2, Site 4 showed the highest reflection in the carotenoid region and Sites 2 and 7 the lowest reflection (Figure 5.10B). For chlorophyll, Site 3 showed the highest reflection, whereas Sites 2 and 7 showed the lowest reflection during Year 2. In the NIR, Site 7 showed the highest reflection and Site 2 the lowest reflection during Year 2 for *T. capensis*.




(A) (B)

**Figure 5.10 Average reflectance for *Typha capensis* per site during (A) Year 1 and (B) Year 2. Water absorption bands are noticeable at 970, 1200, 1400 and 1940 nm**

The FDS for *T. capensis* during Year 1 showed most sites to have a single peak between 715 to 717 nm except for Site 4 which has a dominant peak at 712 nm (Figure 5.11A). During Year 2 most sites showed a single peak between 716 and 718 nm, with Site 4 showing a dominant peak at 715 nm (Figure 5.11B).

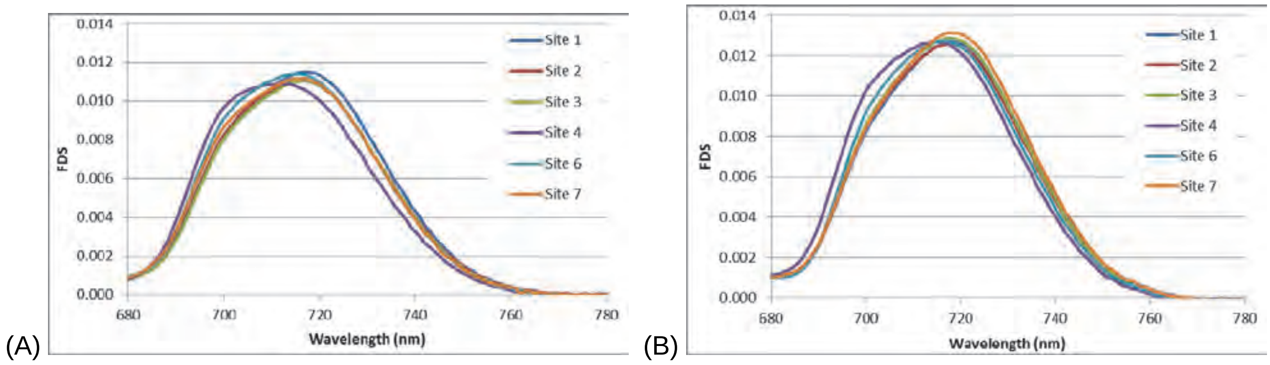



**Figure 5.11 First derivative for *Typha capensis* per site during (A) Year 1 and (B) Year 2**

#### *5.3.2.1 Variation of Carotenoid Indices per Site for Typha capensis*

During Year 1 the CRI values of *T. capensis* showed that Site 1 had the lowest average index values, whereas Sites 1 and 3 had the lowest average index values during Year 2 (Table 5.9; Figure 5.12). Sites 2 and 6 had the highest average index values in Year 1, and Sites 2 and 7 during Year 2 (Table 5.9). During Year 1, Site 1 had significantly lower ($p = 0.003$, Bonferroni corrected) average carotenoid index values compared to Sites 2 and 6 (Table 5.10), whereas during Year 2, Site 3 had significantly lower index values compared to Site 7.

**Table 5.9 Carotenoid Reflectance Index minimum, maximum, mean and standard deviation values for each site for *Typha capensis* for two years**

| Year: | Year 1 | | | | Year 2 | | | |
|---|---|---|---|---|---|---|---|---|
| **Parameter:** | **Min** | **Max** | **Mean** | **Stdev** | **Min** | **Max** | **Mean** | **Stdev** |
| **Site 1** | 3.18 | 5.71 | **4.23** | 0.76 | 2.92 | 5.34 | **4.26** | 0.61 |
| **Site 2** | 3.54 | 6.84 | **5.23** | 0.97 | 3.32 | 6.12 | **4.76** | 0.72 |
| **Site 3** | 3.85 | 5.47 | **4.70** | 0.51 | 2.61 | 5.47 | **4.19** | 0.70 |
| **Site 4** | 3.49 | 6.31 | **4.97** | 0.71 | 3.10 | 6.20 | **4.53** | 0.71 |
| **Site 5** | | | | | | | | |
| **Site 6** | 3.82 | 6.52 | **5.02** | 0.66 | 3.63 | 5.77 | **4.72** | 0.53 |
| **Site 7** | 3.42 | 6.18 | **4.53** | 0.78 | 3.80 | 5.99 | **4.90** | 0.59 |

**Table 5.10 One-way Analysis of Variance (Tukey's Honest Significant Difference post-hoc test) for Carotenoid Reflectance Index values of *Typha capensis* between sites for each year**

| Year 1 | | | | | | Year 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sites:** | **1** | **2** | **3** | **4** | **6** | **Sites:** | **1** | **2** | **3** | **4** | **6** |
| **1** | | | | | | **1** | | | | | |
| **2** | 0.000* | | | | | **2** | 0.071 | | | | |
| **3** | 0.235 | 0.120 | | | | **3** | 0.998 | 0.021 | | | |
| **4** | 0.007 | 0.816 | 0.801 | | | **4** | 0.684 | 0.810 | 0.412 | | |
| **6** | 0.003* | 0.916 | 0.660 | 0.999 | | **6** | 0.130 | 0.999 | 0.044 | 0.915 | |
| **7** | 0.724 | 0.012 | 0.968 | 0.303 | 0.191 | **7** | 0.007 | 0.974 | 0.001* | 0.333 | 0.916 |

* - significant $p < 0.05$, Bonferroni corrected $p = 0.003$

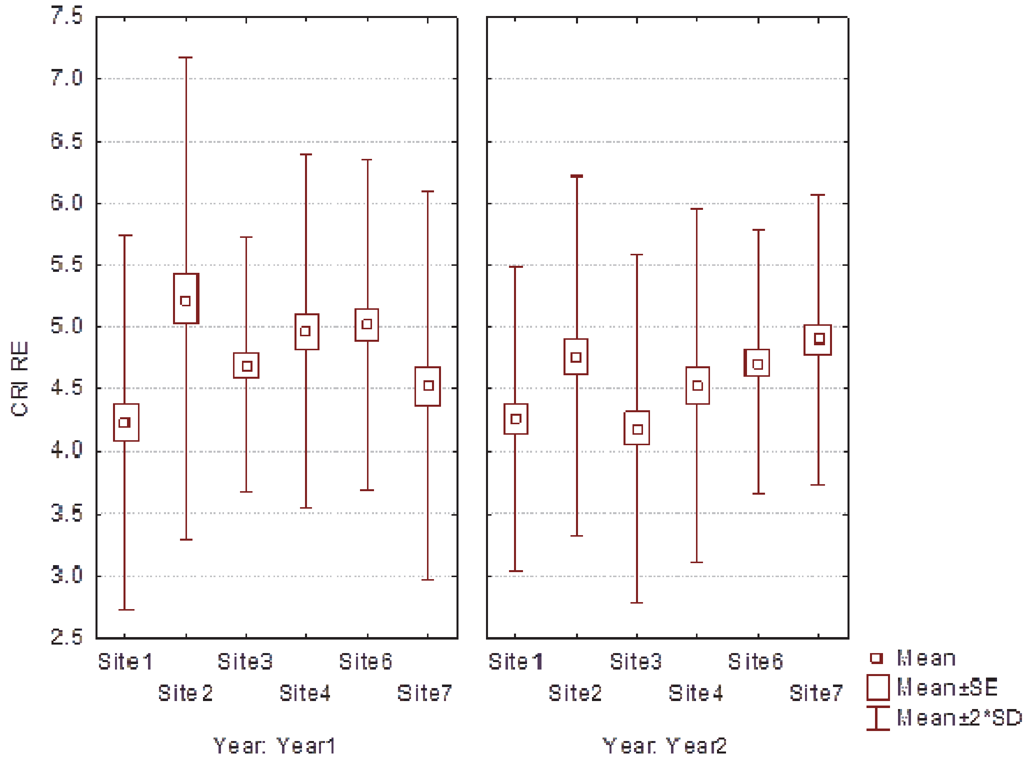



**Figure 5.12 Boxplots of Carotenoid Reflectance Index values for *T.capensis* per site for two years**

Between Year 1 and Year 2 the average CRI values for *T. capensis* at Sites 3 and 4 significantly (p = 0.05) decreased (Figure 5.13; Table 5.11).

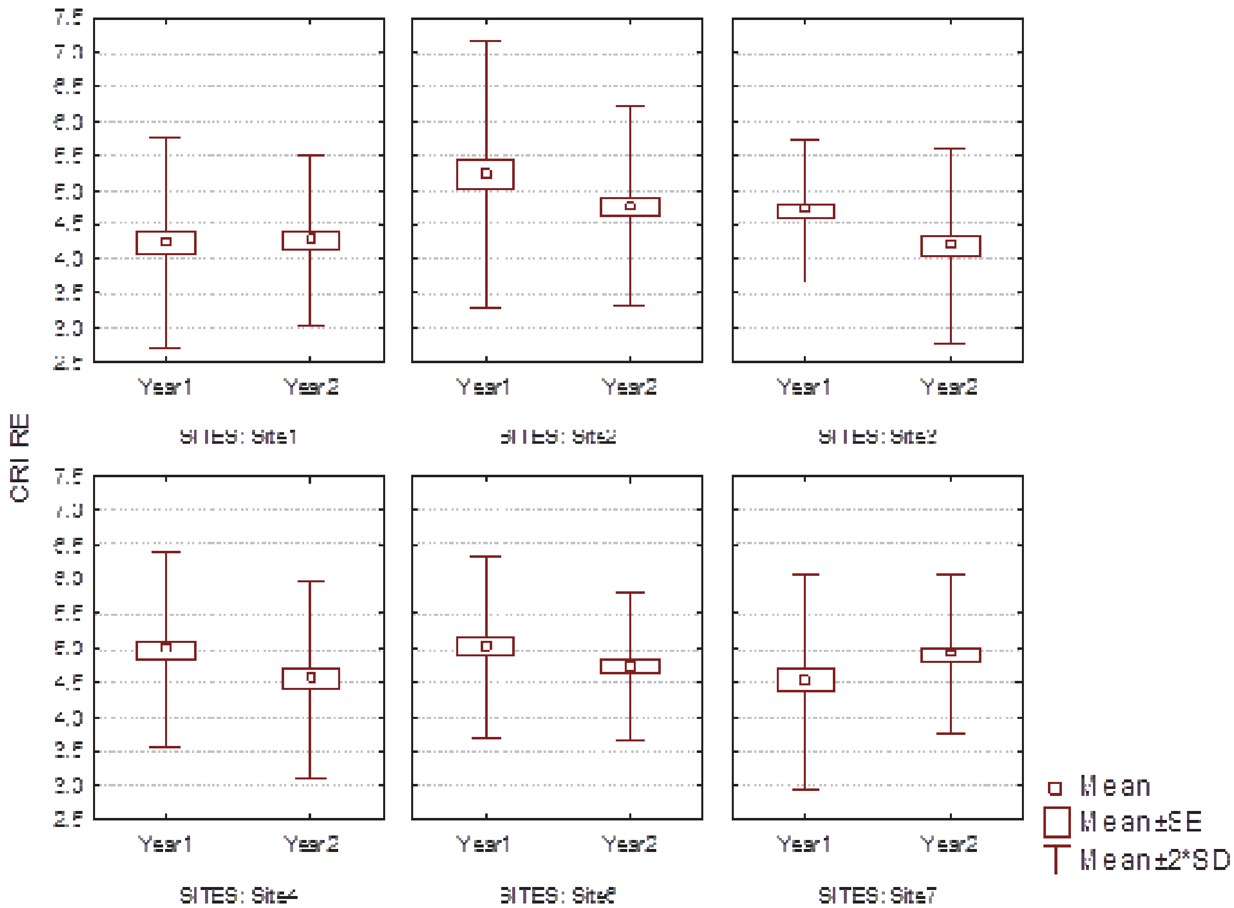



**Figure 5.13 Boxplots of Carotenoid Reflectance Index values for *T. capensis* between years for each site**

**Table 5.11 One-way Analysis of Variance (Tukey's Honest Significant Difference post-hoc test) for Carotenoid Reflectance Index values of *T. capensis* between years for each site**

| | Year 2 | | | | | |
|---|---|---|---|---|---|---|
| **Sites:** | **Site 1** | **Site 2** | **Site 3** | **Site 4** | **Site 6** | **Site 7** |
| Year 1 | 0.877 | 0.061 | 0.005* | 0.036* | 0.086 | 0.062 |

* - significant $p < 0.05$

*5.3.2.2 Variation of Chlorophyll Indices per Site for Typha capensis*

Sites 1, 2 and 3 showed higher average chlorophyll REP Le index values for *T. capensis* during Year 1 (Table 5.12; Figure 5.14). During Year 2, the first three sites, as well as Site 7 showed higher average REP Le index values compared to Sites 4 and 6. During Year 1, average REP Le values at Site 4 was significantly ($p = 0.003$, Bonferroni corrected) lower compared to those of Sites 1 and 3, whereas during Year 2, the average index values at Site 4 were significantly lower compared to Sites 1, 2, 3 and 7 (Table 5.13).

**Table 5.12 Red-edge Position Linear Extrapolation minimum, maximum, mean and standard deviation values for each site for *T. capensis* for two years**

| **Year:** | **Year 1** | | | | **Year 2** | | | |
|---|---|---|---|---|---|---|---|---|
| **Parameter:** | **Min** | **Max** | **Mean** | **Stdev** | **Min** | **Max** | **Mean** | **Stdev** |
| **Site 1** | 702.68 | 725.08 | **717.76** | 5.38 | 706.75 | 733.35 | **722.51** | 6.31 |
| **Site 2** | 693.38 | 730.96 | **717.36** | 8.42 | 705.62 | 734.13 | **722.18** | 7.14 |
| **Site 3** | 708.70 | 730.53 | **717.67** | 6.04 | 695.11 | 734.03 | **722.44** | 7.83 |
| **Site 4** | 691.98 | 724.92 | **710.07** | 8.50 | 700.73 | 727.34 | **714.47** | 6.81 |
| **Site 5** | | | | | | | | |
| **Site 6** | 700.43 | 728.69 | **714.65** | 8.44 | 710.14 | 730.07 | **719.17** | 5.23 |
| **Site 7** | 703.28 | 723.48 | **714.98** | 6.29 | 713.97 | 732.22 | **722.76** | 5.62 |

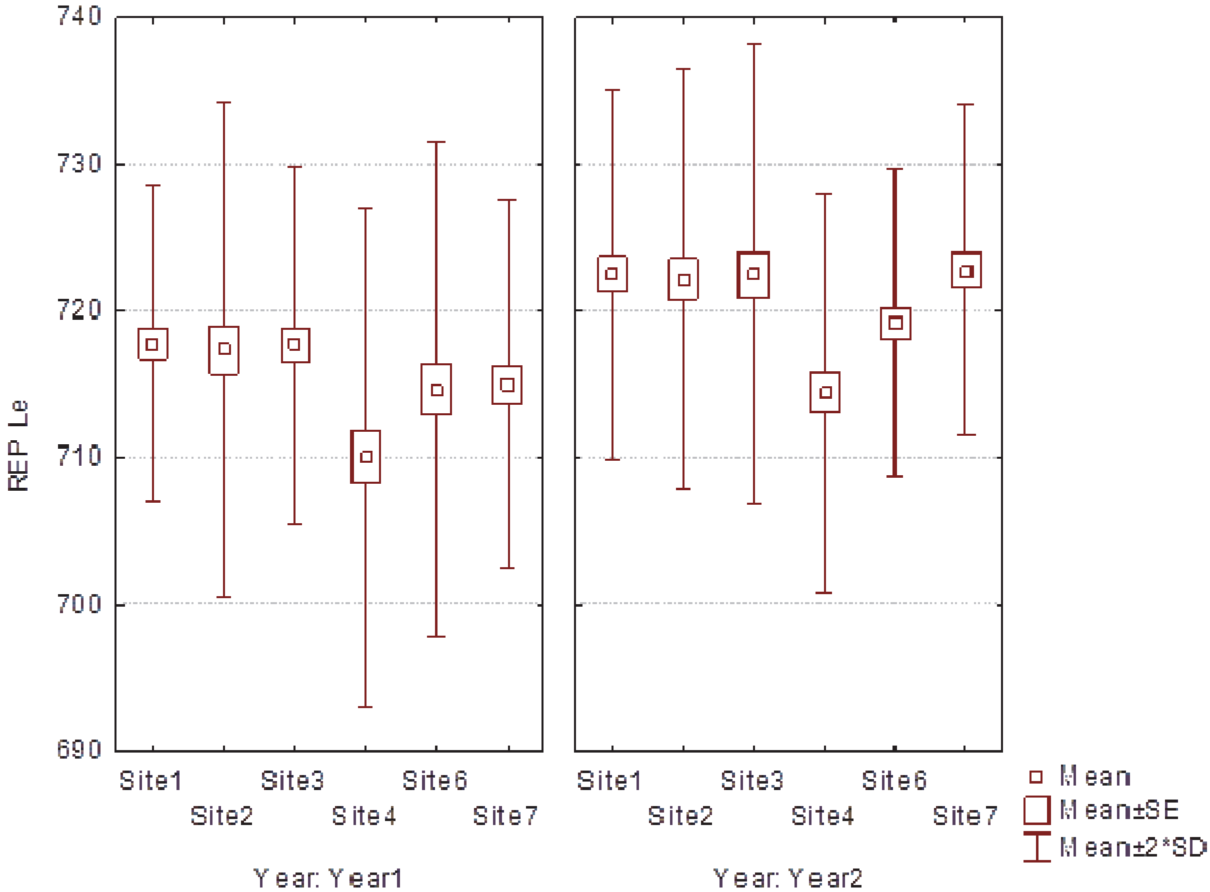



**Figure 5.14 Boxplots of Red-edge Position Linear Extrapolation values for *T. capensis* per site for two years**

**Table 5.13 One-way Analysis of Variance (Tukey's Honest Significant Difference post-hoc test) for Red-edge Position Linear Extrapolation values of *Typha capensis* between sites for each year**

| Year 1 | | | | | | Year 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sites:** | **1** | **2** | **3** | **4** | **6** | **Sites:** | **1** | **2** | **3** | **4** | **6** |
| **1** | | | | | | **1** | | | | | |
| **2** | 0.999 | | | | | **2** | 0.999 | | | | |
| **3** | 1 | 0.999 | | | | **3** | 1 | 0.999 | | | |
| **4** | 0.003 * | 0.005 | 0.003 * | | | **4** | 0.000 * | 0.000 * | 0.000 * | | |
| **6** | 0.660 | 0.778 | 0.689 | 0.227 | | **6** | 0.464 | 0.581 | 0.490 | 0.113 | |
| **7** | 0.759 | 0.859 | 0.785 | 0.162 | 0.999 | **7** | 0.999 | 0.999 | 0.999 | 0.000 * | 0.378 |

* - significant $p < 0.05$, Bonferroni corrected $p = 0.003$

A significant increase ($p = 0.05$) in average chlorophyll REP Le index values were noted for *T. capensis* at all sites between the two years (Figure 5.15; Table 5.14).

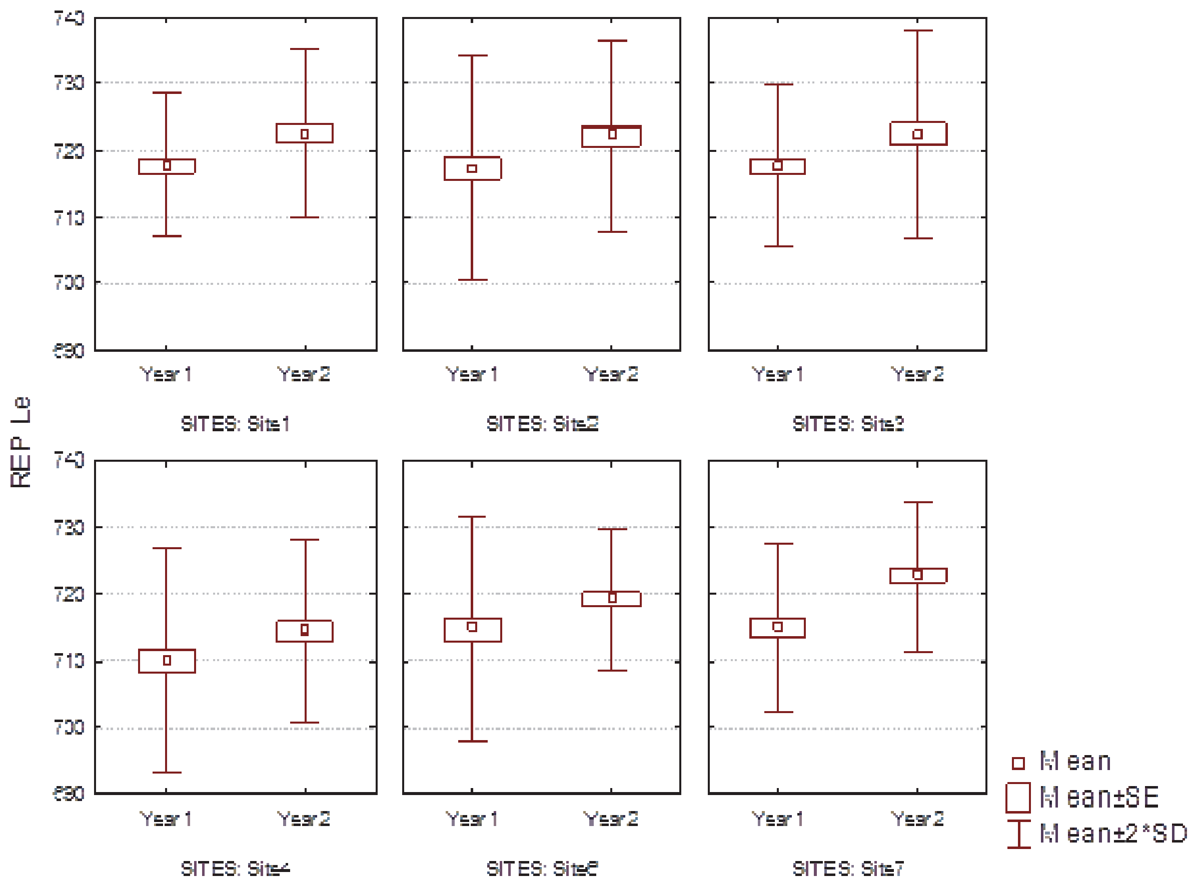



**Figure 5.15 Boxplots of Red-edge Position Linear Extrapolation values for *Typha capensis* between years for each site**

**Table 5-14 One-way Analysis of Variance (Tukey's Honest Significant Difference post-hoc test) for Red-edge Position Linear Extrapolation values of *Typha capensis* between years for each site**

| | Year 2 | | | | | |
|---|---|---|---|---|---|---|
| **Sites:** | Site 1 | Site 2 | Site 3 | Site 4 | Site 6 | Site 7 |
| **Year 1** | 0.006* | 0.034* | 0.020* | 0.049* | 0.027* | 0.000* |

* - significant $p < 0.05$

### 5.3.3 Identifying Impacted Sites

#### *5.3.3.1 Identifying Sites Showing Signs of Vegetation Impacted by Mining Wastewater and Acid Mine Drainage*

Sites with high carotenoid index values did not coincide with sites with low chlorophyll index values, and therefore the hypothesis could not be used to indicate the most impacted sites (Table 5.15). Sites 1 and 2 showed higher mean carotenoid index values for *P. australis* compared to the other sites during both Years 1 and 2. The mean chlorophyll index values for *P. australis* were low for both years, and the lowest values were recorded at Site 5 for Year 1, and Sites 3, 5 and 7 for Year 2.

Mean carotenoid index values for *T. capensis* were also high for Sites 2 and 7 during Year 1 and Sites 2, 6 and 7 during Year 2 (Table 5.15). The lowest mean chlorophyll index value for *T. capensis* was recorded at Site 4 during both years. Compared to *P. australis*, *T. capensis* showed higher REP Le chlorophyll index values on average for both years. The mean REP Le chlorophyll index values for Site 4 was mostly significantly lower during each year compared to the other sites.

**Table 5.15 Sites with the most average index values showing potential impact by acid mine drainage (high for carotenoids, lowest for chlorophyll) per species for the two years**

| Leaf index type | SVI | *Phragmites australis* | *Typha capensis* |
|---|---|---|---|
| Carotenoids (highest) | CRI RE | 1, 2 | 2 & 7 (yr 1); 2, 6 & 7 (yr 2) |
| Chlorophyll (lowest) | REP Le | 5 (yr 1); 3, 5 & 7 (yr 2) | 4, 6 & 7 (yr 1); 4 & 6 (yr 2) |

### 5.3.4 Assessing Condition Following Rehabilitation

Changes in vegetation conditions following the implementation of rehabilitation structures occurred. *Phragmites australis* showed a significant decrease in mean carotenoid index values between Years 1 and 2 for all the sites except Site 5 where the decrease was not significant. On the other hand, mean chlorophyll index values for *P. australis* significantly increased at Site 1, and significantly decreased at Site 7, while changes in values for the other sites were not significant. While the carotenoid index showed similar responses between the sites, the chlorophyll response for *P. australis* varied per site, showing a decrease in mean REP Le values for Site 7, and therefore a decrease in vegetation health at this site between the two years.

*Typha capensis* showed a significant increase in mean index values for chlorophyll at all sites along the Grootspruit between the two years. No significant change was observed for the mean carotenoid index values of *T. capensis* along this tributary between the two years, though a significant decrease was observed at Sites 3 and 4 from Year 1 to Year 2. In general, the condition of *T capensis* showed improvement between Years 1 and 2 at all sites.

## 5.4 DISCUSSION

Sites downstream of the mining land uses at Elandsfontein Colliery and Highveld Steel showed lower mean chlorophyll index values, and therefore poorer vegetation health conditions compared to Site 1 (reference site). The lowest REP Le chlorophyll mean index values of *P. australis* were recorded during Year 1 at Site 5 and Year 2 at Sites 3, 5 and 7. The lowest chlorophyll mean index values for *T. capensis* were observed during both years at Site 4, immediately downstream of Highveld Steel. Though agricultural land uses dominate in the study area and are particularly prevalent at Site 1, the vegetation health indices indicate that vegetation downstream of the mining areas is in poorer condition compared to Site 1 (reference site).

On average, *T. capensis* had higher mean chlorophyll index values compared to *P. australis* for both years. A significant increase in mean REP LE chlorophyll index values of between 4.4 and 4.8 nm were observed for *T. capensis* at Sites 1 to 6 and a significant increase of 7.8 nm at Site 7. Mean chlorophyll index values of *P. australis*, on the other hand, only increased significantly at Site 1 (reference site) by 3.5 nm, showed no significant changes for Sites 2, 3 and 5 and decreased significantly by 4.5 nm at Site 7. The contradictory response of the two species downstream of the rehabilitation structures during Year 2 may be attributed on the one hand to difference in leaf structure of the two species, as well as to varying degrees of environmental and particularly soil conditions at the sites. Both water quality and soil parameters will influence the vegetation response patterns and extensive sampling will be required to relate the changes in vegetation indices to particular contaminants in the soil or water at each site. To assess whether any trend in vegetation improvement prevail downstream of the rehabilitation site in future, further monitoring over a longer period of time is recommended.

Vegetation conditions can easily be derived from leaf spectra measured with a portable field spectrometer. This is a non-destructive and relatively fast method of deriving vegetation conditions, compared to field surveys and costly laboratory analysis. Vegetation indices, known for the quantification of carotenoids and chlorophyll leaf pigments, have been used to derive vegetation indices. Many studies have shown the chlorophyll red edge region to be sensitive to trace and heavy metals.

## 5.5 CONCLUSION

Wetland vegetation of the Grootspruit showed poorer conditions downstream of the mining land uses compared to the reference site upstream of the mining sites. No consistent change in vegetation conditions between the two years was visible following the implementation of rehabilitation structures. Further assessments will be required to determine whether a trend in improvement downstream of the rehabilitation structures can be observed. This study used field spectroscopy to measure the reflectance of a plant's leaves and derive vegetation indices as indicators of health. This method provides a relatively quick non-destructive assessment of leaf condition, compared to extensive field surveys and costly laboratory analysis.

# CHAPTER 6: TERATOGENIC POTENCY AND EMBRYOTOXICITY OF CONTAMINATED SURFACE WATER FROM THE GROOTSPRUIT

## 6.1 INTRODUCTION

The impact of acid mine drainage (AMD) on the wetlands in the Olifants River Water Management Area (WMA) originated from a time before the promulgation of the 1956 Water Act of South Africa. Detrimental environmental consequences of polluted mine drainage and discard dumps were not fully realized and mines, at closure of operations, were abandoned without adequate pollution control measures being implemented. Abandoned mines can cause a decrease in the quality of runoff, resulting in severe water quality problems in rivers and wetlands. This can have detrimental effects on biota, for example at a pH of ≥4.0 precipitation of metal hydroxides (e.g. ferric hydroxides, or FeOH3), commonly known as yellow boy, can smother biota, whereas at lower pH the dissolved metal ions can penetrate the membranes of biota and cause toxicity to specimens (De Nicola and Stapleton, 2002). It is generally agreed that measuring only the physical and chemical attributes of water cannot provide the complete assessment of the health of an aquatic system under study (Oberholster *et al*., 2008). A major reason for this is our limited knowledge of the effects of toxic variables on aquatic biota. Physical and chemical information is furthermore biased towards the momentary condition that exist in space and time when the samples are collected, often missing short-term events, which may be critical to ecosystem health. On the other hand, biota are accurate indicators of overall environmental conditions, since they are subject to the totality of adverse effects of chemical and physical influences.

The Frog Embryo Teratogenicity Assay Xenopus (FETAX) is a well-established biological assay applied to detect the teratogenic potential (i.e. drive towards malformation) of chemicals or surface water samples (Hoke and Ankley, 2005). Effects observed using FETAX have been shown to correlate highly with those observed in mammalian model organisms (Leconte and Mouche, 2013) and the assay therefore generate valuable information which can be utilized in risk assessments for a diversity of organism classes (Hoke and Ankley, 2005). The FETAX assay was applied to evaluate water quality in the Grootspruit during 2013 and an extremely high teratogenic potency and toxicity was observed at some of the sampling localities (Oberholster *et al*., 2014).

The aim of this study was to evaluate the teratogenic potential and embryotoxicity of surface water collected from the Grootspruit upstream and downstream of the rehabilitated area.

## 6.2 MATERIALS AND METHODS

### 6.2.1 Frog Breeding

In preparation for facilitated spawning, adult female and male *Xenopus laevis* were primed daily with 100 iu human chorionic gonadotropin (hCG) injected into the dorsal lymph sack. Priming continued until nuptial pads were prominent in males and cloacal labia red and swollen in females. Spawning was subsequently induced using boosting injections of 300 and 500 iu of hCG in male and female frogs, respectively. Breeding occurred overnight in 15 litre tanks containing FETAX solution (625 mg/L NaCl, 96 mg/L $NaHCO_3$, 30 mg/L KCl, 15 mg/L $CaCl_2$, 60 mg/L $CaSO_4.\cdot 2H_2O$, 75 mg/L $MgSO_4$). Each tank housed a single breeding pair and was fitted with a plastic grid, restricting the frogs from their eggs.

### 6.2.2 Frog Embryo Teratogenicity Assay – *Xenopus laevis*

The FETAX technique was applied according to the ASTM (1998) standard, with slight modifications. In short, fertilized eggs were de-jellied by gentle swirling a 2% w/v l-cystein (Sigma, ZA) (pH 8.1). Viable larvae at Niewkoop and Faber (NF) stage 8 (mid-blastula) to 11 (early gastrula) were identified using a stereo microscope and assigned to exposure vessels. During Phase 1, each treatment group was represented by two replicates, each containing 10 individuals, whereas two replicates, each containing 25 individuals were included during Phase two.

Surface water samples were diluted 0% (pure), 25%, 50% and 75% in FETAX solution. Two negative control replicates, each containing 10 larvae in pure FETAX solution were furthermore included during Phase 1, whereas four replicates each containing 25 larvae were included in Phase 2. The larvae were exposed in 1 litre glass tanks containing 250 mL of test medium during Phase 1, whereas 100 mm × 15 mm glass petri dishes containing 40 mL of liquid was employed during Phase 2. The test medium was replaced daily during Phase 2, whereas Phase 1 comprised of a static exposure. The experiment was performed subject to a 14:10 light:dark cycle at 24 ±2°C. The larvae were monitored daily for mortalities, which were confirmed using a stereomicroscope or tactile stimuli, and deceased individuals were immediately removed.

All animals were euthanized after 96 h exposure using 0.1% benzocaine (Heynes Mathew, Ltd., ZA), and transferred to buffered 10% formalin. Developmental malformations were classified according to Bantle *et al*. (1990) under Leica EZ4 and ES2 stereo microscopes and photographed. Teratogenicity was quantified as the 50% effective concentration for malformation (EC50), malformation incidence (%) as well as through the Teratogenic Index (TI), which expresses teratogenicity in context of acute toxicity. Moreover, the minimum concentration for growth inhibition (MCGI) was determined by comparing the total lengths of individuals from the respective concentrations of the different exposure groups with that of the control group. In addition, the developmental stages of tadpoles were classified according to Nieuwkoop and Faber (1994).

### 6.2.3 Data Analyses

Hatching success between the different wetland sites was compared using one-way ANOVA. All analyses were deemed significant with significance assumed at a probability level of $P < 0.05$. All sampling sites were evaluated for their lethal concentration at which point 50% of test animals die (LC50), the half maximal effective concentration (EC50) for malformation and TI, which is based on the ratio between LC50/EC50 values, using the USEPA PROBIT analysis software (Version 1.5). Normality and homogeneity of variance of the data were assessed using the respective Shapiro-Wilk's and Levene's tests. For non-parametric data Kruskall-Wallis ANOVA analysis was used followed by multiple comparisons of mean ranks to assess pairwise differences, whereas for parametric data, a one-way ANOVA was used followed by Dunnet's post-hoc test. All analyses were performed using the software program Statistica Version 11 (StatSoft, Inc., 2012).

## 6.3 RESULTS AND DISCUSSION

Larval survival was markedly higher during the phase two (2014, after rehabilitation) laboratory exposure (Figure 6.1). As a result, LC50 and TI could not be calculated for the upstream and downstream sites, and the comparison was therefore simply based on the incidence of malformation upstream and downstream. There was no significant variation in larval length among any of the treatments investigated. As a result, the MCGI could not be calculated for the respective sites.

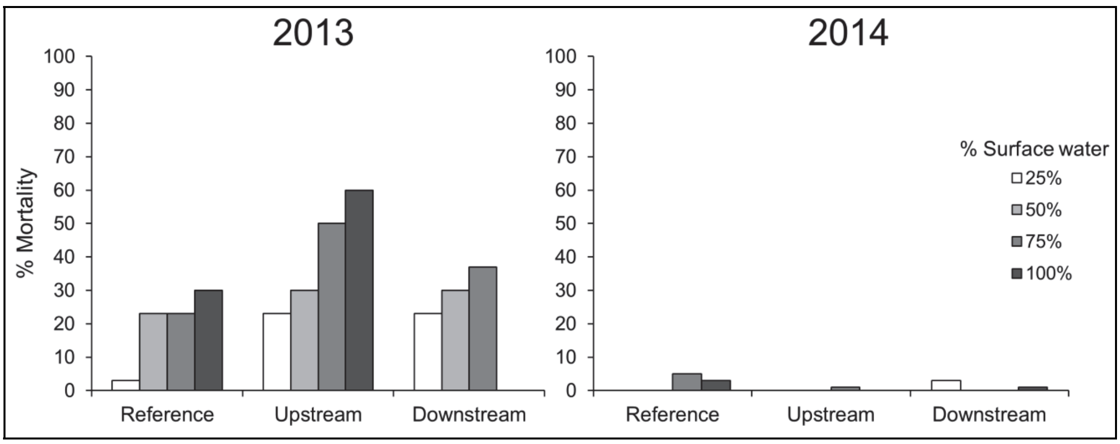



**Figure 6.1 Mortality of *Xenopus laevis* larvae after 96 h exposure to a series dilution of surface water collected from a reference site upstream and downstream of the rehabilitated area in the Grootspruit system (100% signifies pure surface water)**

Generally, low frequencies of malformations were observed for all the surface water samples evaluated during 2014, apart from the upstream site (Figure 6.2). In particular, the incidence of malformations was higher in the upstream treatment (21.74%), than the reference site (8.89%), the downstream site (8.33%) as well as the negative control (9.78%). The proportions of malformed individuals were lower in the downstream treatment than both the reference site and the negative control treatment (Figure 6.2), suggesting that the wetland rehabilitation improved the quality of the water in terms of teratogenic potential. In contrast, during 2013 the downstream locality was characterized by a higher teratogenic potency than the reference location, providing further evidence that the wetland rehabilitation employed in 2014 improved the surface water quality.

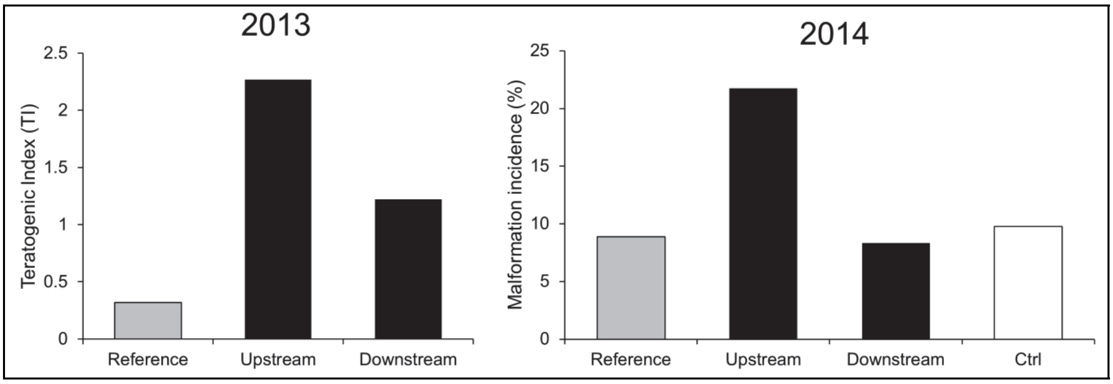



**Figure 6.2 The incidence of malformations in *Xenopus laevis* larvae exposed to a series dilution of surface water collected from a reference site, as well as sites upstream and downstream of the rehabilitated area in the Grootspruit system (100% signifies pure surface water), as well as a negative control containing FETAX solution**

Some of the most common malformations seen in the tadpoles exposed to the different test waters collected during the pre-rehabilitation phase in 2013 were photographed and presented in Figure 6.3. For example, the body length of the control tadpoles (Figure 6.3A) and tadpoles exposed to water from the reference site (Figure 3.B) differed greatly from those exposed to 75% concentration water from one of the impacted sites upstream of the rehabilitation area (Figure 6.3C). Most of the tadpoles that survived in water from the reference site were normal, although hatching success was not high. Blistering, incomplete gut coiling and oedema were common malformations in tadpoles exposed to three of the impacted sites upstream of the rehabilitation area (Figure 6.3C, D, E). Narrowing of the eyes, undeveloped mouth parts, improper gut coiling, oedema, blistering, as well as a sloping forehead were seen in tadpoles exposed to water from the site downstream of the rehabilitation area (Figure 6.3F).

After rehabilitation, a high proportion of larvae exposed to water upstream from the rehabilitated area suffered from malformations in the digestive tract (Figures 6-4 and 6-5). These gut malformations included improper coiling of the gut and diverted gut. Previous studies have shown gut malformations in association with metal exposure (Plowman *et al*., 1994; Bacchetta *et al*., 2012; Peltzer *et al*., 2013), which may explain the high proportion of gut malformations observed in the upstream treatment. A combination of a malformed gut and abdominal oedema was furthermore observed in a number of larvae exposed to surface water collected from the upstream locality.

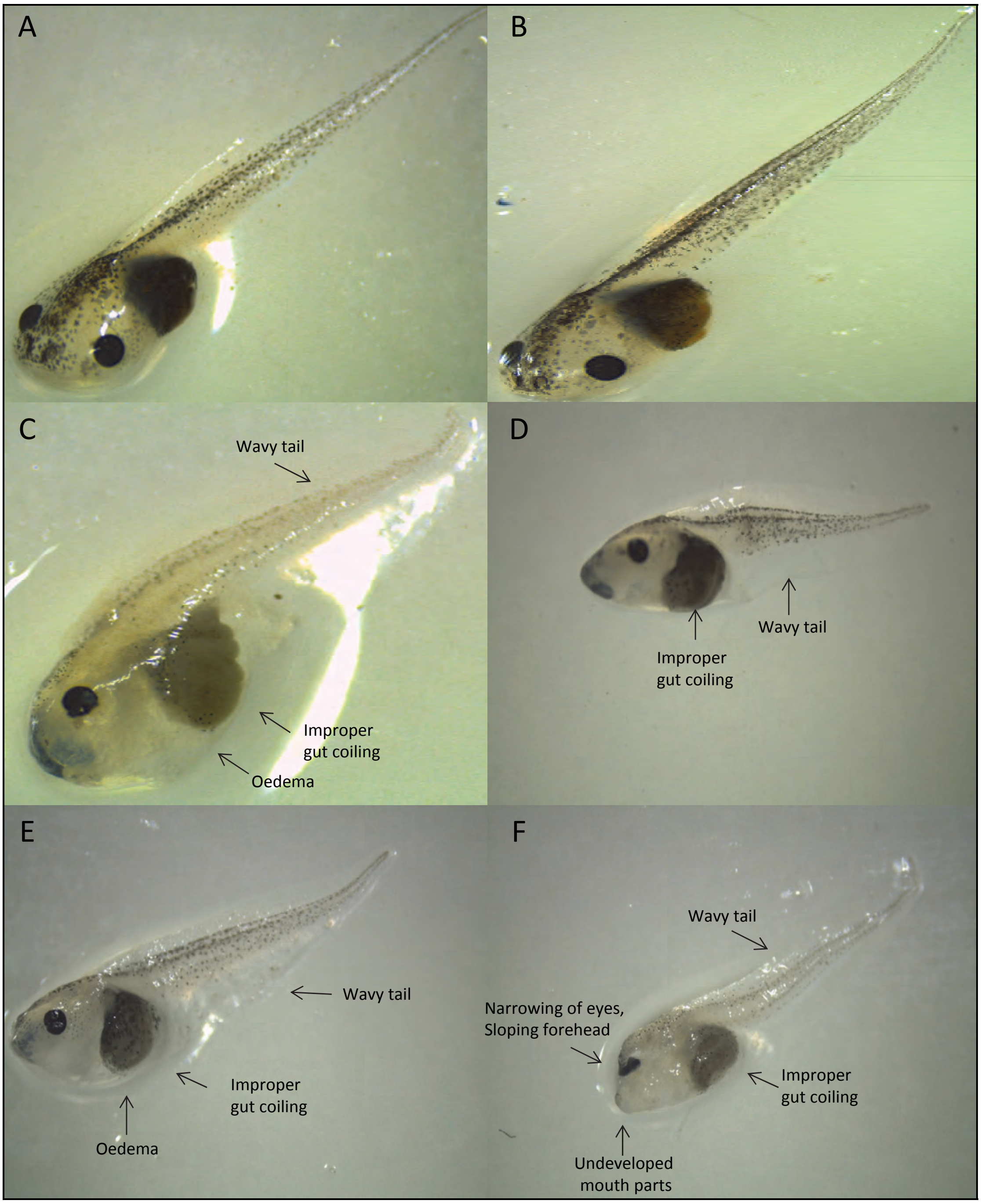



**Figure 6.3 Typical malformations seen in *Xenopus laevis* tadpoles exposed to different test waters collected during 2013 (before rehabilitation). (A) A healthy control tadpole (FETAX solution only); (B) normal tadpole exposed to 100% water from the reference site; malformed tadpoles exposed to (C) 75% water from a site upstream of the rehabilitated area; (D) 10% water form a site upstream of the rehabilitated area; (E) 100% water from a site upstream of the rehabilitated area; and (F) 10% water from a site downstream of the rehabilitated area.**

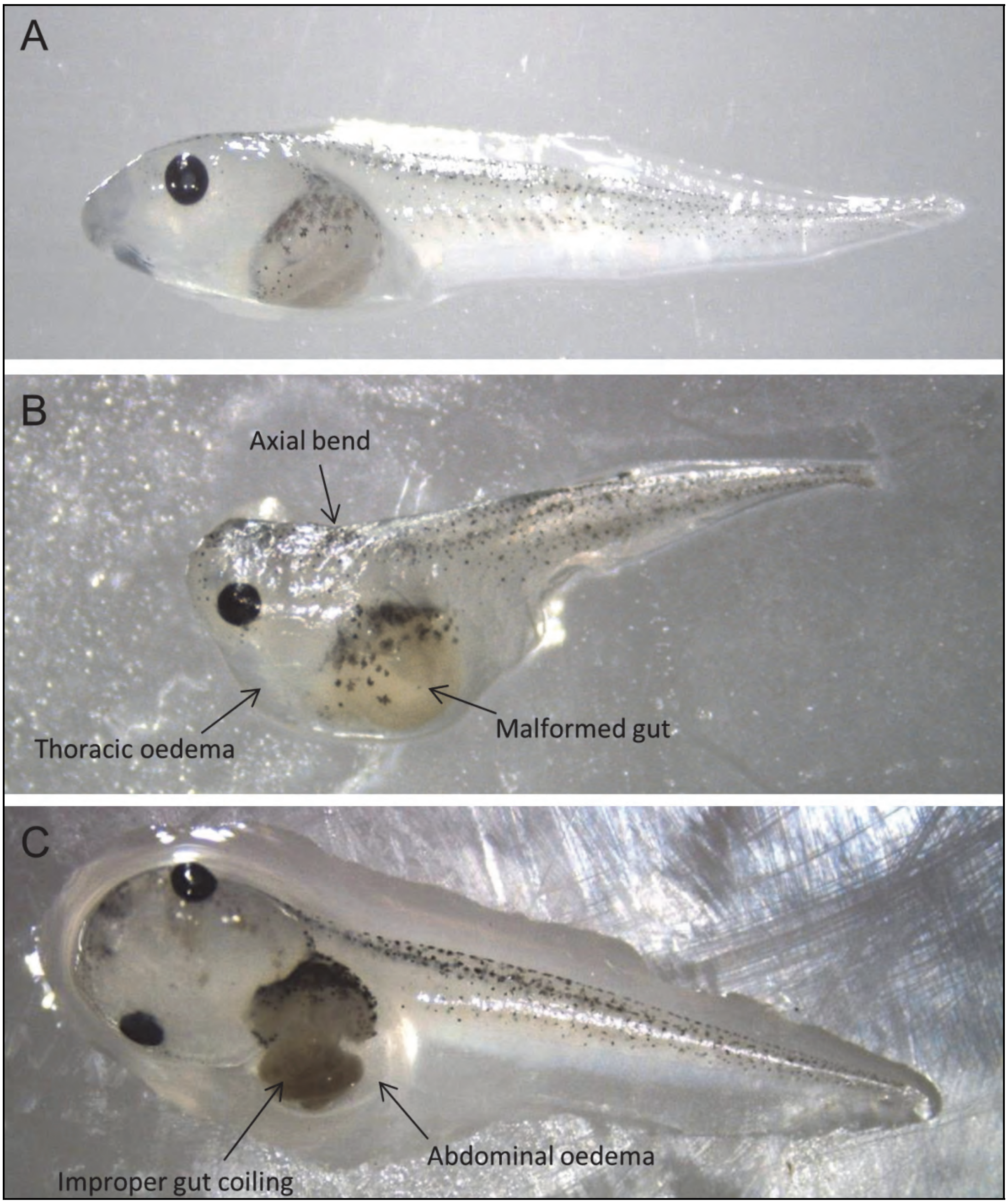



**Figure 6.4 Typical malformations observed in tadpoles exposed to surface water samples collected during 2014 (after rehabilitation). (A) A normally developed tadpole exposed to FETAX solution (negative control); (B) an example of thoracic oedema, gut malformation and an axial deformity in a tadpole exposed to surface water collected from one of the upstream sites, diluted 75% with FETAX solution; (C) an example of improper gut coiling and abdominal oedema in a tadpole exposed to surface water collected from one of the upstream sites, diluted 50% with FETAX solution. The results for the tadpoles exposed to water downstream from the rehabilitated area were similar to that of the tadpoles in the negative control.**

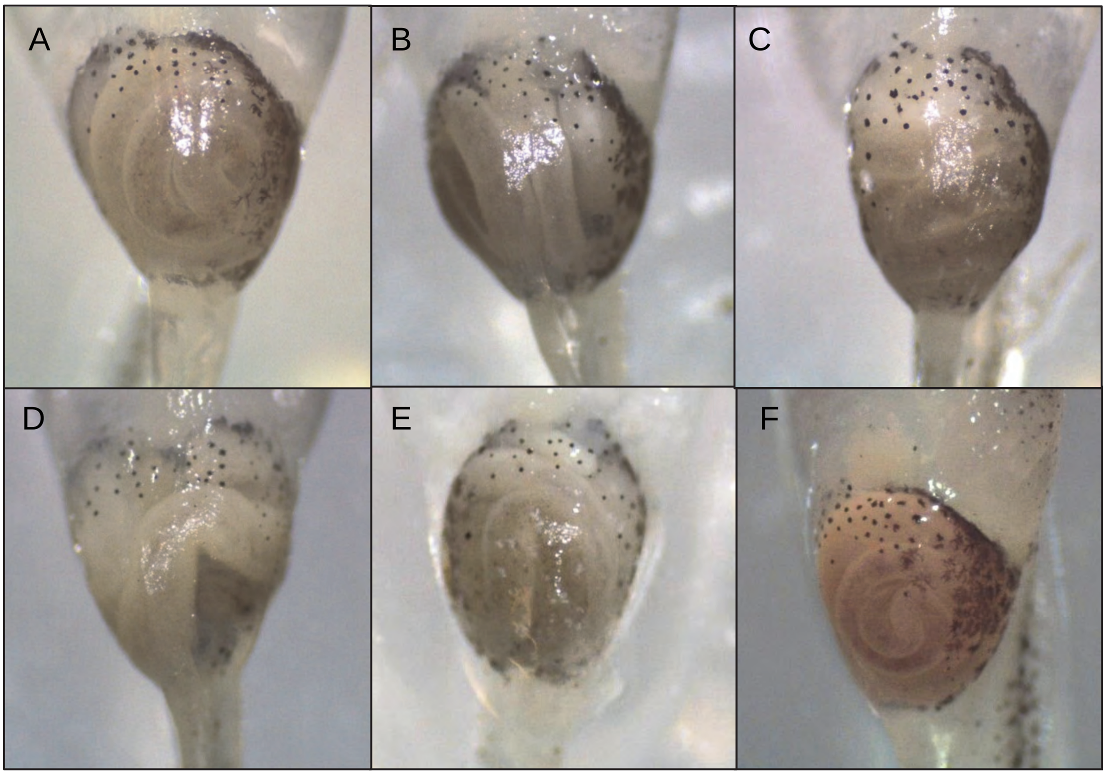



**Figure 6.5 Macroscopic imagery of the gastro-intestinal tracts (gut) of (A) a normally developed tadpole exposed to FETAX solution (negative control); (B-E) tadpoles exposed to surface water collected upstream of the rehabilitated area; (F) tadpoles exposed to surface water collected downstream of the rehabilitated area during 2014 (after rehabilitation)**

## 6.4 CONCLUSION

The FETAX assay is a well-established biological assay applied to detect the teratogenic potential of surface water. The aim of this part of the study was to evaluate the impact of the wetland rehabilitation efforts on the teratogenic potential and embryotoxicity of surface water collected from the Grootspruit upstream and downstream of the rehabilitated area. It was found that larval survival was markedly higher after rehabilitation at the downstream site. It was also observed that the incidence of malformations was higher upstream from the rehabilitation area than the reference site, the downstream site as well as the negative control. After rehabilitation, the proportions of malformed individuals were lower in the downstream treatment than both the reference site and the negative control treatment. In contrast to this, before rehabilitation, the downstream locality was characterized by a higher teratogenic potency than the reference site. This suggests that the rehabilitation of the wetland improved the water quality in terms of teratogenic potential.

# CHAPTER 7: RESOURCE ECONOMICS OF THE ZAALKLAPSPRUIT WETLAND

## 7.1 INTRODUCTION

Coal is the major primary energy source for South Africa. More than 90% of the country's electricity, approximately 30% of the liquid fuel and approximately 70% of its total energy needs are produced from coal (SANEDI, 2011). Historically, the coal industry has not had a good reputation when it comes to environmental performance. Environmental impacts resulting from the coal industry range from water, air, biodiversity and land use transformation. However, increasingly the industry is responding to these issues and challenges through careful planning, implementation of effective pollution control strategies and increased monitoring and management of impacted areas (SANEDI, 2011).

Of particular concern to the coal sector, is the impact on wetlands. Wetlands, by virtue of their positions in the landscape and relationship to drainage networks, are frequently impacted by coal mining activities, especially opencast methods. As coal-mining activities are likely to continue in the future to support South Africa's developmental needs, impacts on wetland resources are likely to continue.

This realization, along with the continued destruction of wetlands in the upper Olifants Catchment, has caused consternation with conservation authorities, society and the mining industry. To this end, Coaltech has commissioned the Council for Scientific and Industrial Research (CSIR), the South African National Biodiversity Institute (SANBI) and Prime Africa Consultants to develop mechanisms for limiting and mitigating the impacts of coal mining on wetlands and provide guidelines to the coal mining industry and regulators in this regard.

At a meeting convened by Coaltech and CSIR in December 2010, the coal-mining sector present outlined their particular wetland-related research needs, which have been consolidated and summarised under the following broad thematic areas:

1. The identification of high risk wetlands that should be avoided;
2. Guidelines for offsite mitigation;
3. The potential of natural and artificial wetlands to mitigate mining pollutants;
4. Reconstruction of wetlands in post-mining landscapes; and
5. Rehabilitation of different wetland types (De Klerk *et al*., 2013).

In 2011, Coaltech and the CSIR approached Prime Africa Consultants to add a further component, which dealt with developing a framework for the valuation of the ecosystem services delivered by wetlands and internalizing these values in a modified cost benefit analyses (CBA) to fully account for wetland value.

### 7.1.1 Objectives

Traditionally, the CBA methodology has been used by mining companies to make decisions regarding the financial viability of potential mining projects. This methodology, however, does not take ecosystem services thinking into consideration. The aim therefore of the socio-economic component of the project is to internalize the costs of the ecosystem services delivered by wetlands, which are impacted by coal mining activities and develop a CBA tool that addresses both financial and environmental factors. The adapted CBA tool will also take into account the cost of various wetland mitigation measures including offsite mitigation. The proposed methodology is referred to as a corporate ecosystem valuation (CEV) and is based on the principles advocated by the World Business Council for Sustainable Development (WBCSD).

## 7.2 THE IMPORTANCE OF WETLANDS AND COAL

Wetlands are important components of South Africa's rich biodiversity heritage and contribute greatly to the livelihoods of many vulnerable communities and underpin many of South Africa's economic sectors. Coal is a strategically important resource, which provides for 90% of South Africa's energy production and is a large contributor to export revenues. Wetlands and coal mining are intricately linked and nowhere can this be seen more clearly than in the Mpumalanga Highveld. The purpose of this section is to articulate the importance of both wetlands and coal mining to South African society.

### 7.2.1 The Importance of Wetlands

Wetlands are defined by the National Water Act (NWA), Act 36 of 1998, as a key component of the water resources of South Africa. They are important, because water resources produce ecosystem services. Ecosystem services are real quantifiable benefits that people receive from ecosystems. Wetlands are highly productive ecosystems and due to their ecological complexity provide a variety of goods and services that are of value to society. These goods and services can be described as services of nature, directly enjoyed, consumed, or used to yield human well-being (Boyd and Banzhaf, 2007).

Wetlands in Southern Africa have been shown to contribute to the livelihoods of rural communities by providing valuable grazing land, cultivation area, building materials and medicinal goods (Turpie, 2000; Masiyandima *et al*., 2004; McCartney and Van Koppen, 2004; Masiyandima *et al*., 2005). In addition to these services, wetlands provide a host of other services, which are often indirectly used by society, and are therefore undervalued in economic markets. These services include the provisioning of fresh water, flood attenuation and water purification, among others.

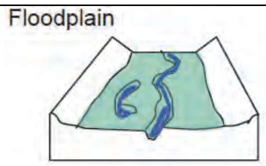
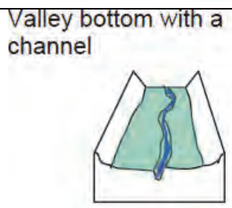
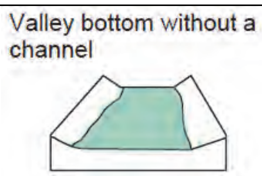
The definition of wetlands used for this report is taken from the NWA (1998) and is as follows: 'Wetlands are land which is transitional between terrestrial and aquatic systems where the water table is usually at or near the surface, or the land is periodically covered with shallow water, and which land in normal circumstances supports or would support vegetation typically adapted to life in saturated soil.'

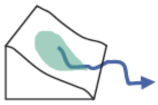
#### *7.2.1.1 Wetland Types*

Wetlands in South African have been classified in various ways (Breen and Begg, 1989; Cowan, 1995; Kotze *et al*., 2008) and are generally based on hydrogeomorpic (HGM) characteristics (Table 7.1). The HGM approach to wetland classification uses hydrological and geomorphological characteristics to distinguish primary wetland units. The HGM approach is therefore based on factors that influence how wetlands function.

Various wetland types occur in the study area and in particular, valley bottoms with channels, hillslope seepages and pans/depressions.

**Table 7.1 Wetland hydrogeomorphic (HGM) types typically supporting inland wetlands in South Africa (Source: Kotze *et al*., 2007)**

| Hydrogeomorphic Types | Description |
|---|---|
|   | Valley bottom areas with a well-defined stream channel, gently sloped and characterized by floodplain features such as oxbow depressions and natural levees and the alluvial (by water) transport and deposition of sediment, usually leading to a net accumulation of sediment. Water inputs from main channel (when channel banks overspill) and from adjacent slopes. |
|   | Valley bottom areas with a well-defined stream channel, but lacking characteristic floodplain features. May be gently sloped and characterized by the net accumulation of alluvial deposits, or may have steeper slopes and be characterized by the net loss of sediment. Water inputs from main channel (when channel banks overspill) and from adjacent slopes. |
|   | Valley bottom areas with no clearly defined stream channel, usually gently sloped and characterized by alluvial sediment deposition, generally leading to a net accumulation of sediment. Water inputs mainly from channel entering the wetland and also from adjacent slopes. |

| Hydrogeomorphic Types | Description |
|---|---|
| Hillslope seepage linked to a stream channel  | Slopes on hillsides, which are characterized by the colluvial (transported by gravity) movement of materials. Water inputs are mainly from sub-surface flow and outflow is usually via a well-defined stream channel connecting the area directly to a stream channel. |
| Isolated hillslope seepage  | Slopes on hillsides, which are characterized by the colluvial (transported by gravity) movement of materials. Water inputs mainly from sub-surface flow and outflow either very limited or through diffuse sub-surface and/or surface flow but with no direct surface water connection to a stream channel. |
| Depression (includes Pans)  | A basin shaped area with a closed elevation contour that allows for the accumulation of surface water (i.e. it is inward draining) and/or intersection of groundwater. It may also receive sub-surface water. An outlet is usually absent, therefore this type is usually isolated from the stream channel network. |

### *7.2.1.2 Wetlands at Risk*

The goal for wetland protection is underpinned by the understanding that wetland ecosystems are at risk from degradation and transformation, and without conservation efforts the loss of wetland function and subsequent loss of the delivery of ecosystem services will continue unabated. The recent National Biodiversity Assessment (NBA) showed that of South Africa's 791 wetland ecosystem types, 48% are critically endangered, 12% are endangered, 5% are vulnerable and only 35% being classified as least threatened (Nel *et al*., 2011) (Figure 7.1).

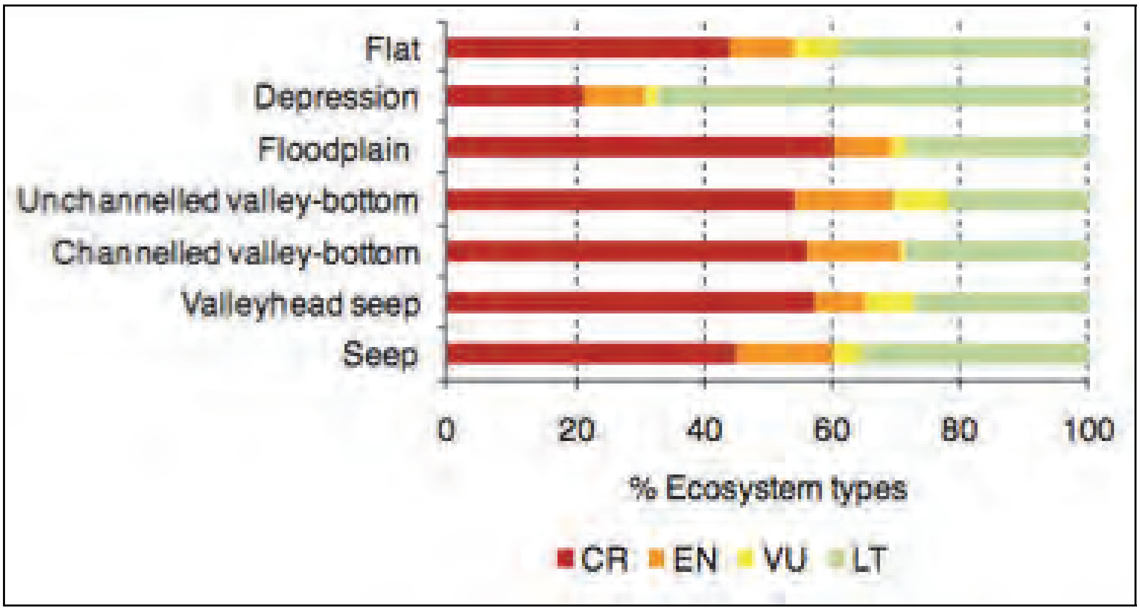



**Figure 7.1 Summary of National ecosystem threat status within each wetland hydro-geomorphic unit. CR, EN, VU and LT refer respectively to critically endangered, endangered vulnerable and least threatened ecosystems (Source: Nel *et al.*, 2011)**

### *7.2.1.3 Wetland Mitigation and Rehabilitation*

As wetlands and coal mining often exist within the same landscape matrix, it is inevitable that developmental trade-offs will have to be made. The mitigation of negative environmental impacts on wetland function and process is a requirement that coal-mining companies are legally obliged to follow. Successful mitigation requires proactive planning which is guided by following the mitigation hierarchy (Figure 7.2).

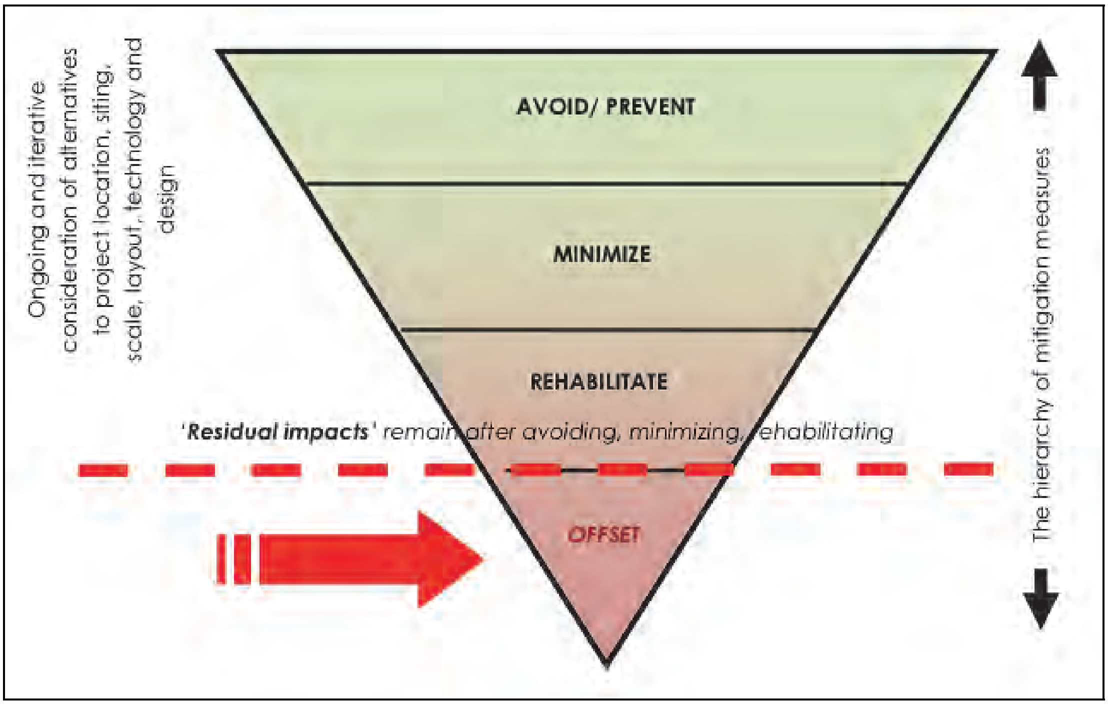



**Figure 7.2 The mitigation hierarchy (Source: SANBI 2012)**

Under this hierarchy, four steps are presented (adapted from DEA, 2013):
1. **Avoid/Prevent:** Refers to considering options in project location, sitting, scale, layout, technology and phasing to avoid impacts on biodiversity;
2. **Minimise:** Refers to considering alternatives in the project options in project location, sitting, scale, layout, technology and phasing that would minimise impacts on biodiversity and ecosystem services;
3. **Rehabilitate:** Refers to rehabilitation of areas where impacts are unavoidable and measures are provided to return impacted areas to near-natural state or an agreed land-use after mine-closure. However, rehabilitation may fall short of replicating the diversity and complexity of the natural system; and
4. **Offset:** Refers to measures over and above rehabilitation to compensate for the residual negative effects on biodiversity after every effort has been made to minimise and then rehabilitate impacts.

The goals of wetland rehabilitation will differ according to the scale and impact of the mining development. It is, however, likely that there would be a requirement to show an improvement in functioning from the degraded/impacted wetland. Improvement in functioning could be measured in an increased capacity to treat polluted water or an increase in functional wetland hectare equivalents.

### 7.2.2 The Importance of Coal

#### *7.2.2.1 Economic Indicators*

Coal is considered the primary energy source for South Africa. Approximately 90% of South Africa's energy and approximately 30% of liquid fuel are produced from coal (SANEDI, 2011). Coal also plays an important role in the chemical and steel manufacturing businesses and in 2012 provided R52,2 billion is export revenues (SACOM, 2013).

In 2012, the mining sector accounted for 8.3% of Gross Domestic Product (GDP) directly, on a nominal basis. If the indirect multiplier and induced effects of mining are included, then the overall contribution to GDP is closer to 17%. Nominal mining GDP of R262.7 billion was recorded in 2012 (SACOM, 2013).

In 2012, total coal sales by value increased by 10% to R96.1 billion. Approximately 71% of production was sold locally at a value of R43.9 billion, whilst the balance of production (29%) was exported at a value of R52.2 billion. The coal sector accounted for 19% of mining exports and 7% of total merchandise export in 2012, making it the third largest mineral export after gold and the platinum group metals (SACOM, 2013).

South Africa produced 261.6 MT of coal in 2013, an increase of 2% from 2012. Local sales accounted for 71% (185.6 MT) while exports accounted for 29% (76.1 MT) of the total coal produced (SACOM, 2013). The majority of coal is exported through the Richards Bay Coal Terminal (RBCT), while the remainder is exported through the Matola Coal Terminal in Mozambique and small amounts through Richards Bay and Durban harbours (SANEDI, 2011). The RBCT has plans in the pipeline to increase the terminal's capacity. Phase VI of the expansion plan will increase current capacity from 91MT to 110MT. Total exports through the RBCT increased by 4% to 68MT in 2012, still short of its full potential. Transnet Freight Rail (TFR) improved rail performance and railed 68.5 MT of coal to RBCT, an increase of 4% year-on-year (SACOM, 2013).

In 2012, The South African coal mining sector directly employed 83 240 people, which is a 6% increase from 2011 and paid R17.4 billion in salaries and wages (SACOM, 2013). Employment down the value chain is also significant: at the end of the 2009/10 financial year Eskom reported that it had 36 547 direct employees and Sasol directly employed 28 978 people in its South African operations at the end of the 2010 financial year (SANEDI, 2011). Both of these enterprises depend on coal for the bulk of their primary inputs. Direct employment by coal mining, Eskom and Sasol is therefore more than 139 000, with many more indirect employment numbers. In 2009, the coal mining sector accounted for about 1.8% of GDP directly, or 4.5% if indirect multipliers are added (StatsSA, 2010).

#### *7.2.2.2 Coal Reserves*

South Africa has an estimated 32 billion tonnes of coal reserves and approximately 70% of these resources are found in the Waterberg, Witbank, Highveld and Ermelo coalfields, with the remainder in the Sasolburg, Springbok Flats and other smaller fields (Figure 7.3). In Limpopo, there is a single large colliery near Lephalale in the Waterberg Coalfield and a small colliery in the Soutpansberg East area (SANEDI, 2011).

#### *7.2.2.3 Major Coal Producers*

The main coal producers in South Africa are Anglo American's Thermal Coal business unit, Exxaro, Sasol Mining, BHP Billiton and Xstrata, as demonstrated in Table 7.2.

**Table 7.2 Major coal producers in South Africa (Source: SACOM, 2013)**

| Coal Producer | Sales (Tonnes) | Sales Value (R) |
|---|---|---|
| Anglo Inyosi Coal | 12 808 626 | 4 285 571 133 |
| Anglo Operations Limited | 42 401 962 | 13 798 611 109 |
| BHP Billiton | 33 826 722 | 13 030 221 309 |
| Coal of Africa | 3 394 968 | 1 394 010 711 |
| Continental Coal | 1 568 098 | 558 630 447 |
| Exxaro (Eyesiswe) | 15 893 657 | 3 685 331 751 |
| Exxaro (Kumba Resources) | 21 639 938 | 6 242 157 951 |
| Kangra | 3 177 901 | 1 837 738 640 |
| Kuyasa | 1 627 919 | 330 326 629 |
| Optimum Coal | 13 745 661 | 5 946 050 646 |
| Sasol Coal | 44 108 249 | 11 782 001 177 |
| Shanduka | 6 708 200 | 2 692 796 860 |
| Total Coal SA | 4 044 734 | 2 569 184 425 |
| Xstrata Coal | 15 433 568 | 7 714 200 592 |
| **Grand Total** | **220 380 203** | **R75 866 833 380** |

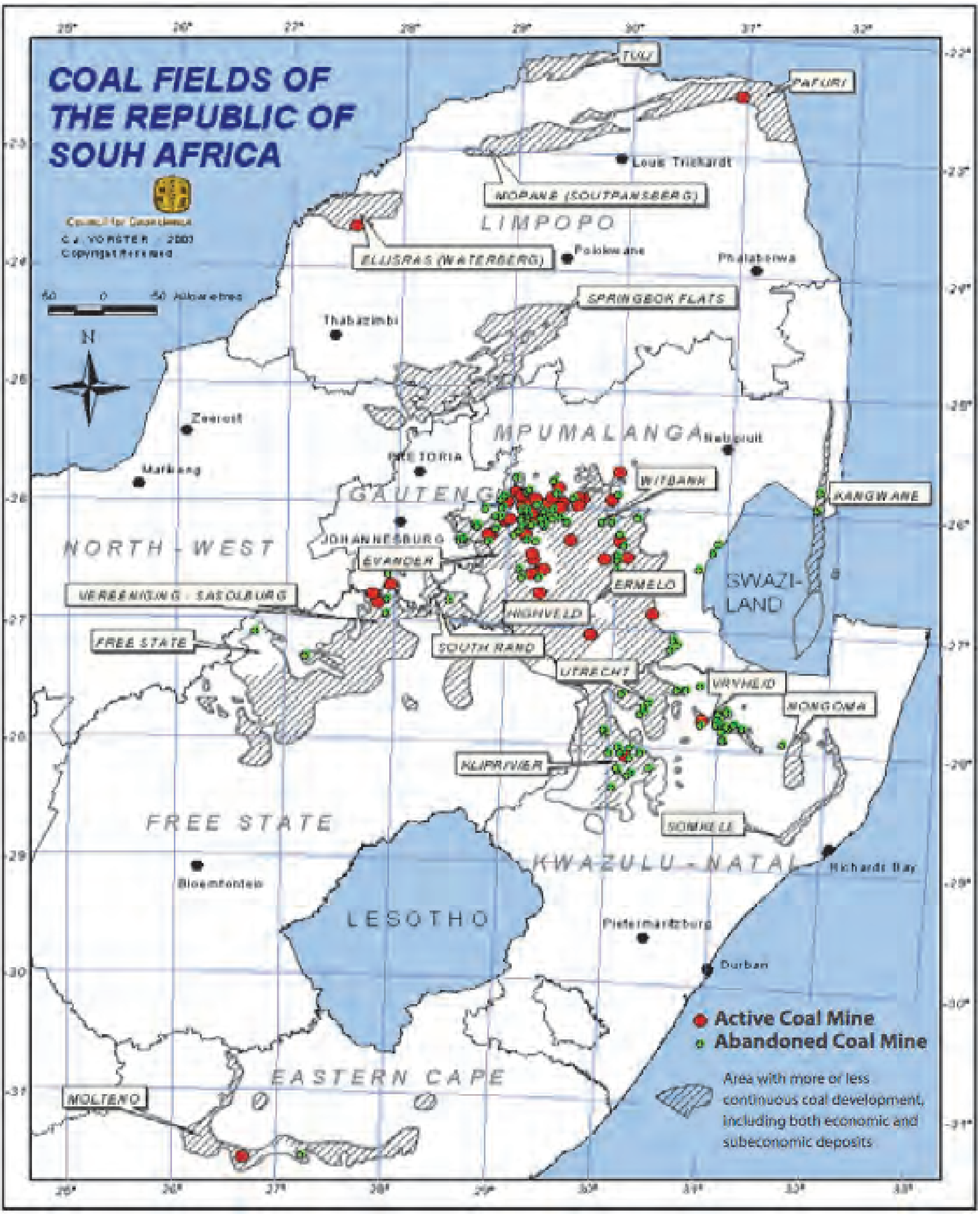



**Figure 7.3 Coal fields, active and abandoned coal mines in South Africa (Source: Council for Geoscience, 2010)**

### *7.2.2.4 Future Coal Growth*

Generally, short and medium term growth in coal production has been predicted to be positive. In the next five years, production in South Africa is predicted to increase from 260 Mtpa in 2010 to 333 Mtpa in 2015, and beyond 2019 new ventures are anticipated to continue this trend (SANEDI, 2011). The positive growth trend is a response to the increased growth in domestic power requirements and export demand (SANEDI, 2011).

South African coal companies will aim to increase exports amidst demand from growing markets in Asia and India. According to Anglo America (2010), demand for imported thermal coal in 2010 rose by 40% and 15% year-on-year in China and India, respectively, in the 2009-2010 period. Thermal coal exports are predicted to increase from 72 Mtpa in 2010 to 93 Mtpa in 2017, while metallurgical coal exports (including anthracite fines for export PCI market) are anticipated to rise to 7.8 Mtpa in 2020 from 1.3 Mtpa in 2010 (SANEDI, 2011).

Growth in coal production will largely take place in the thermal coal contingent, while metallurgical coal will represent 12% of coal production from new projects by 2020 (SANEDI, 2011). Although the Waterberg coalfields will be mined increasingly in the future, the Witbank and Highveld coalfields are predicted to maintain their position as the major producing regions (SANEDI, 2011). Of the six new metallurgical coal projects planned (including Vele, Makhado and Chapudi), five will be in the Limpopo Province.

The South African National Energy Development Institute (SANEDI) has developed more detailed future growth scenarios for the South African coal industry through the South African Coal Roadmap (SANEDI, 2011). The four scenarios developed, are:

1. More of the same;
2. Lags behind;
3. Low carbon world; and
4. At the forefront.

The main determinants and drivers of the scenarios are given in Figure 7.4.

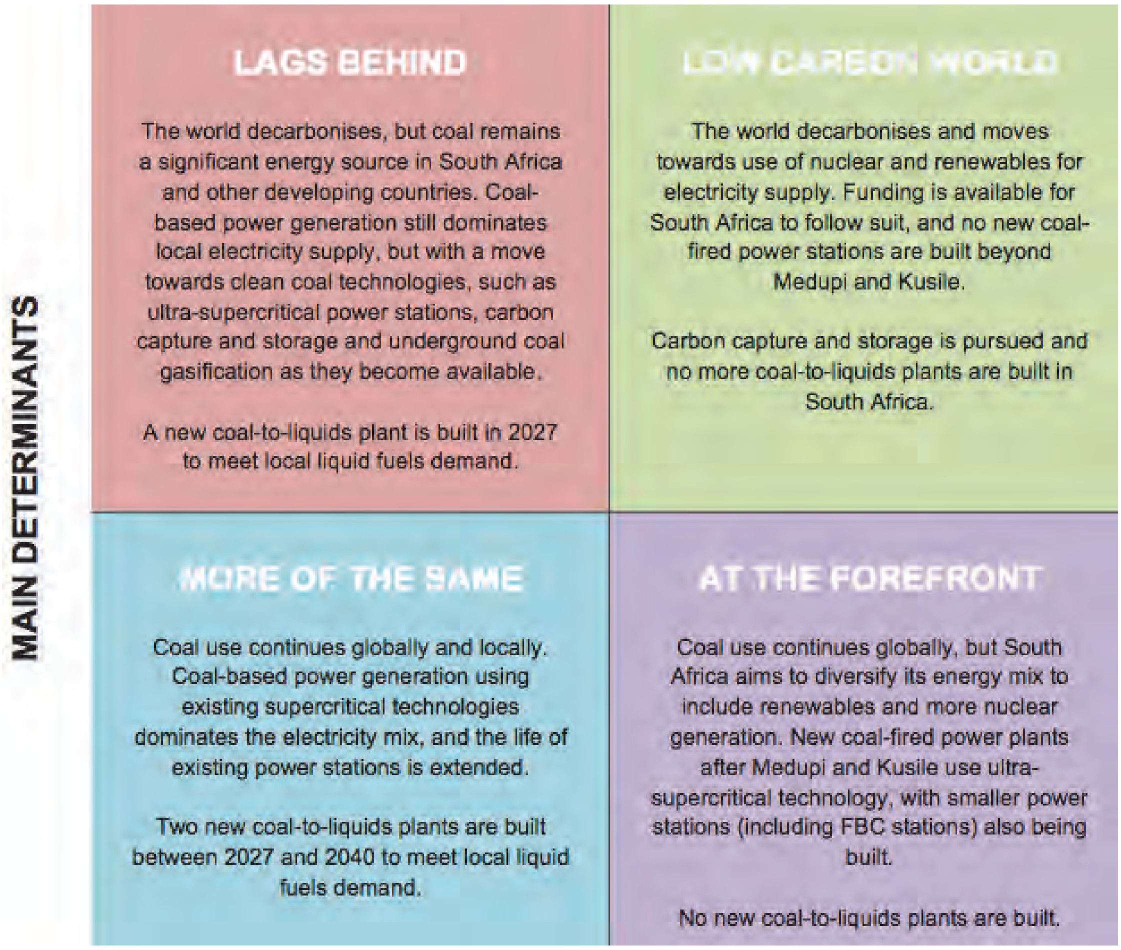



**Figure 7.4 The four scenarios developed by the South African National Energy Development Institute (SANEDI) for the South African coal industry (Source: SANEDI. 2011)**

## 7.3 WETLAND ECOSYSTEM SERVICES

### 7.3.1 Valuation Frameworks

The valuation of ecosystem services has rapidly gained traction in the development and natural resources fields and is increasingly utilized by decision makers when assessing the impacts of development on ecological systems (MEA, 2005; TEEB, 2010). This is due primarily to the realization that biodiversity and its associated ecosystem services can no longer be treated as inexhaustible and free ‘goods’ and their true value to society as well as the costs of their loss and degradation, need to be properly accounted for (TEEB, 2010; De Groot *et al*., 2012).

The “values” of the ecosystem services provided by wetland ecosystems can be expressed in a number of ways and methods. It can be expressed qualitatively (i.e. which cities benefit from which wetland for water purification or flood control), or quantitatively (i.e. the number of people benefitting from clean water) and it can also be expressed in monetary terms (i.e. the monetary value of sequestered carbon, avoided costs of water pre-treatment and supply or avoided costs of potential flood damage) (TEEB 2013). When interpreting ecosystem service values, it is important to note that it is only a tool of analyzing trade-off options and decisions should not be made in isolation of other societal values and needs.

### 7.3.2 The Millennium Ecosystem Assessment

The Millennium Ecosystem Assessment (MEA) (2005) defines ecosystem services as the benefits that people receive from ecosystems and makes the link between ecosystem services and human well-being. The MEA classifies ecosystem services into supporting (basic ecosystem functions and processes that underpin all other services), regulating (covering the absorption of pollutants, storm buffering, erosion control and the like), provisioning services (covering the production of foods, fuels, fibre, etc.) and cultural services (covering non-consumptive uses of the environment for recreation, amenity, spiritual renewal, etc.) (Figure 7.5).

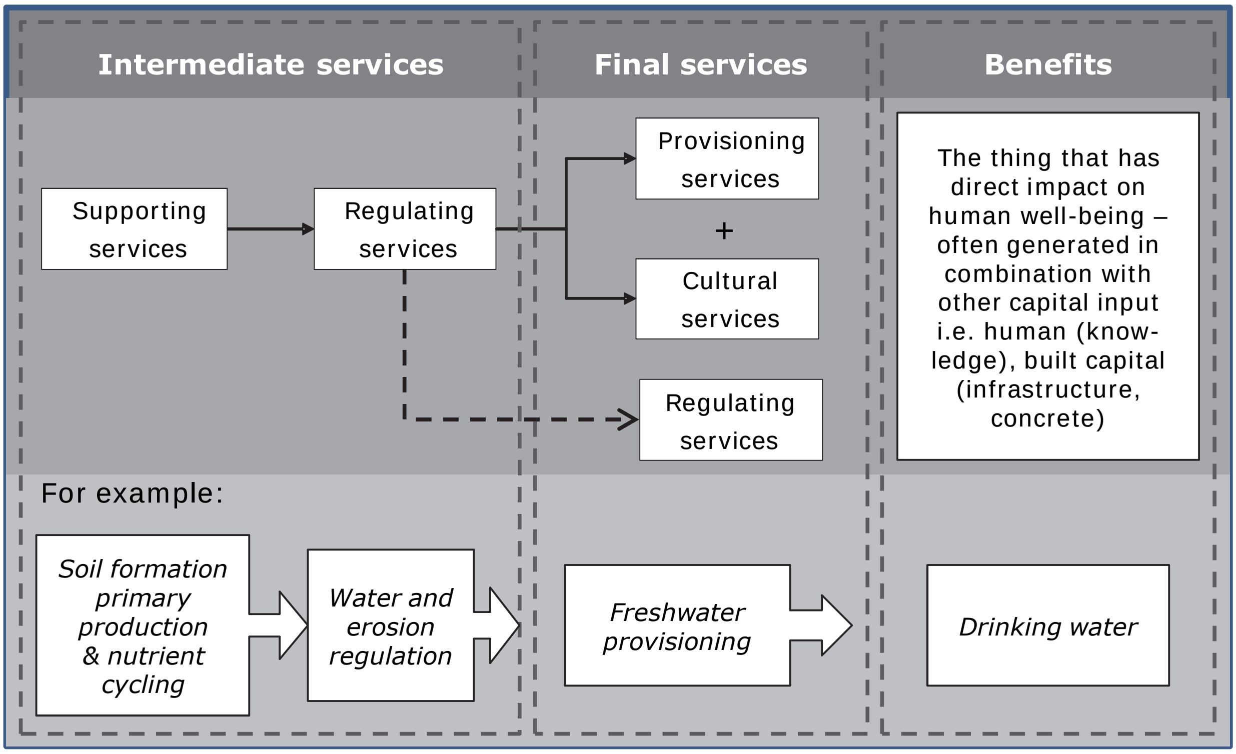



**Figure 7.5 The distinction between intermediate services, final services and benefits (adapted from Fisher *et al*., 2008) illustrated by the stylised relationship between supporting, regulating, provisioning and cultural services as defined by the Millennium Ecosystem Assessment (MEA) (Hassan 2007; Perrings 2006)**

It is important to recognize that the utilitarian values (the benefits consumed, used or enjoyed) of these services are not additive. Supporting and regulating services can be considered to be similar to intermediate consumption in the economic sense. Provisioning and cultural services are those that enter final consumption. In order to avoid double accounting, only the final consumption services should be valued. That is, the services inventory for a given evaluation case must be benefit specific and the service units that depend on these services must be mutually exclusive and expressed in "final ecosystem service units" (Boyd and Banzhaf, 2007). To achieve this, we begin with the definition of ecosystem services employed by Boyd and Banzhaf, in turn developed from the MEA definition: "Ecosystem services are components of nature, directly enjoyed, consumed or used to yield human well-being.

#### *7.3.2.1 The Economics of Ecosystems and Biodiversity*

The Economics of Ecosystems and Biodiversity (TEEB) is an international initiative to draw attention to the benefits of biodiversity. It focuses on the values of biodiversity and ecosystem services, the growing costs of biodiversity loss and ecosystem degradation and the benefits of action addressing these pressures. The TEEB initiative has brought together over five hundred authors and reviewers from across the continents in the fields of science, economics and policy (TEEB, 2013).

The TEEB initiative can be viewed as the next step in ecosystem service understanding and builds on the MEA by providing a focussed approach for dealing with the costs of biodiversity loss and how this impacts society.

#### *7.3.2.2 WET-Management*

While not a wetland valuation tool, the Wet-Management series nevertheless provides guidance on wetland ecological condition as well as the identification of wetland ecosystem services. Both of which are important for wetland valuation.

The purpose of the Wet-Management series of tools is to provide assistance to those wishing to undertake wetland rehabilitation in a well-informed and effective way against a backdrop where loss and degradation of wetlands is increasing (Kotze *et al*., 2007). Of particular importance to the valuation of wetland ecosystem services are the WET-Health (Macfarlane *et al*., 2008) and WET-Ecoservices (Kotze *et al*., 2008) tools.

WET-Health assists in assessing the health of wetlands using indicators based on geomorphology, hydrology and vegetation. For the purposes of rehabilitation planning and assessment, WET-Health helps users understand the condition of the wetland in order to determine whether it is beyond repair, whether it requires rehabilitation intervention, or whether, despite damage, it is perhaps healthy enough not to require intervention. WET-Health is tailored specifically for South African conditions and has wide application, including assessing the Present Ecological State (PES) of a wetland for purposes of Ecological Reserve determination in terms of the National Water Act and for environmental impact assessments (Macfarlane *et al*., 2008).

WET-EcoServices is used to assess the goods and services that individual wetlands provide, thereby aiding informed planning and decision- making. It is designed for a class of wetlands known as palustrine wetlands (i.e. marshes, floodplains, vleis or seeps). The tool provides guidelines for scoring the importance of a wetland in delivering each of 15 different ecosystem services (including flood attenuation, sediment trapping and provision of livestock grazing) (Figure 7.6).

| WETLAND HYDRO-GEO-MORPHIC TYPE | REGULATORY BENEFITS POTENTIALLY PROVIDED BY WETLAND | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Flood attenuation | | Stream flow regulation | Enhancement of water quality | | | | |
| | Early wet season | Late wet season | | Erosion control | Sediment trapping | Phos-phates | Nitrates | Toxicants[2] |
| 1. Floodplain | ++ | + | 0 | ++ | ++ | ++ | + | + |
| 2. Valley-bottom - channelled | + | 0 | 0 | ++ | + | + | + | + |
| 3. Valley-bottom - unchannelled | + | + | +? | ++ | ++ | + | + | ++ |
| 4. Hillslope seepage connected to a stream channel | + | 0 | + | ++ | 0 | 0 | ++ | ++ |
| 5. Isolated hillslope seepage | + | 0 | 0 | ++ | 0 | 0 | ++ | + |
| 6. Pan/ Depression | + | + | 0 | 0 | 0 | 0 | + | + |

**Figure 7.6 Regulating ecosystem services provided by wetland hydro-geomorphic type**

### *7.3.2.3 Valuation of Wetlands and Ecological Infrastructure*

The concept of ecological infrastructure has recently gained traction among conservation biologists and can be seen as an additional lens in which to view the valuation of wetland ecosystem services. According to SANBI (2012), ecological infrastructure refers to functioning ecosystems that deliver valuable services to people (e.g. fresh water, climate regulation, storm protection and soil formation). It is the nature-based equivalent of built or hard infrastructure.

The SANBI definition goes further and describes five attributes of ecological infrastructure:

1. Ecological infrastructure is a public good;
2. Ecological infrastructure enhances built infrastructure;
3. Ecological infrastructure supports rural development;
4. Ecological infrastructure helps us cope with climate change; and
5. Ecological infrastructure creates jobs.

With the addition of ecological infrastructure, one can develop a wetland valuation model that takes the value of natural assets into consideration and is not constrained by ecosystem service valuation only. When ecosystem service valuation is added to the model, we have the beginnings a fully integrated model, which is in line with conventional economic balance sheet and income statement thinking.

The relationship between the ecological asset (balance sheet item) and the delivery of ecosystem services (income state items) can be described as the annual rent received from an asset (i.e. a house for example (Figure 7.7)). The wetland ecosystem services are delivered every year (more or less in the same quantity) and are dependent on the condition of the ecological infrastructure as well as external factors such as rainfall, land use change, etc. If the condition of the ecological infrastructure is modified, there may be a corresponding change in delivery of ecosystem services.

In subsequent sections a valuation is given for the ecosystem services as well as an ecological infrastructure value (asset).

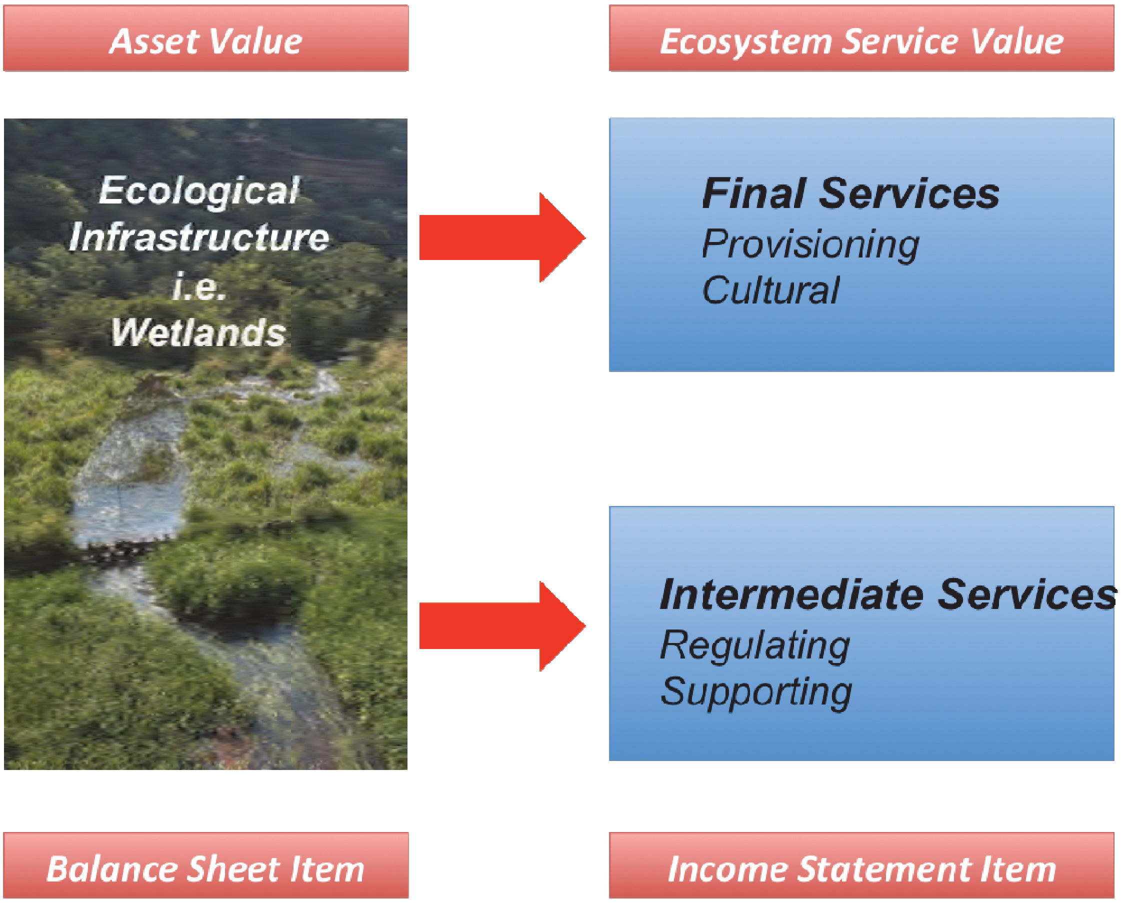



**Figure 7.7 The relationship between ecological infrastructure and the delivery of wetland ecosystem services**

### 7.3.3 Wetlands and Ecosystem Services

List of Wetland Ecosystem Services
Wetlands are important aquatic ecosystems, which produce ecosystem services. These ecosystem services are real benefits provided to people and the economy.

The Millennium Ecosystem Assessment (2005) Framework and the TEEB Assessment classify ecosystem services into four categories: supporting, regulating, provisioning and cultural services. The supporting and regulating services produced by wetlands originate from wetlands' role in the biogeochemical cycling and storage of nutrients, organic material and metals and its role as a sink or a source of these compounds depending on the wetland's state and oxygen levels. Sediments are also retained by wetlands. Normal hydrological flux within a wetland and wetland functioning, therefore, has great value in the control of water quality and erosion (Kotze *et al.*, 2008).

A comprehensive list of ecosystem services provided by wetlands is given in Table 7.3.

#### *7.3.3.1 Water Purification Services of Wetlands*

The water purification potential of wetlands has long been seen as a viable option for the treatment of a wide range of environmental and water quality problems (Sheoran and Sheoran, 2006). On the Mpumalanga Highveld, the high level of acid mine drainage (AMD) polluted waters pose a significant risk to surface and groundwater resources. While AMD poses a significant risk to wetland functioning, wetlands (natural or constructed) may also play an important role in treating AMD from mining operations.

**Table 7-3 List of wetland ecosystem services provided by wetlands (Source: TEEB 2013)**

| Ecosystem Service Category | Ecosystem Service | Ecosystem Structure and Function |
|---|---|---|
| Regulating Services | Coastal protection | Attenuates and/or dissipates waves, buffers winds |
| | Erosion control | Provides sediment stabilisation and soil retention |
| | Flood protection | Water flow regulation and control |
| | Water supply | Groundwater recharge/discharge |
| | Water purification | Provides nutrient and pollution uptake as well as retention, particle deposition |
| | Carbon sequestration | Generates biogeochemical activity sedimentation, biological productivity |
| | Maintenance of temperature, precipitation | Climate regulation and stabilisation |
| Provisioning Services | Raw materials and food | Generates biological productivity and diversity |
| | Maintains fishing, hunting and foraging activities | Provides suitable reproductive habitat and nursery grounds, sheltered living space |
| | Grazing opportunities | Provides grazing area for livestock during winter and dry months |
| Cultural Services | Tourism, recreation, education and research | Provides unique and aesthetic landscape, suitable habitat for diverse fauna and flora |
| | Cultural, spiritual and religious benefits, bequest values | Provides unique and aesthetic landscape of cultural, historic or spiritual meaning |

Acid mine drainage is produced when sulfide-bearing material is exposed to oxygen and water. The production of AMD usually, but not exclusively, occurs in iron sulfide-aggregated rocks (Akcil and Koldas, 2006). The contaminants caused by AMD of concern are sulphate, manganese, iron and heavy metal concentrations.

Wetlands have several functions that aid in the removal of metals in drainage and ameliorate AMD. These characteristics are required for certain processes to occur: adsorption and ion exchange, bioaccumulation, bacterial and abiotic oxidation, sedimentation, neutralization, reduction, and dissolution of carbonate minerals (Perry and Kleinmann, 1991).

Key to the resource economic component of this study is to understand the potential of a wetland to treat AMD. The Zaalklapspruit Wetland receives AMD from the adjacent coal mine (De Klerk *et al*., 2103). Understanding the baseline water quality parameters prior to rehabilitation and after rehabilitation will provide insight into the amount of AMD that a wetland is able to treat. The information will allow us quantify financially the value of the water quality treatment service delivered by the wetland.

## 7.4 SYSTEMS ANALYSIS

### 7.4.1 Location

The Zaalklapspruit Wetland is situated approximately 15 km east of Emalahleni in the Mpumalanga Province. The wetland is in the Olifants Water Management Area (WMA) and is found in the B20G Quaternary Catchment. The wetland is a moderate sized (135 ha) naturally unchannelled valley bottom wetland system located along the Grootspruit (Figure 7.8).

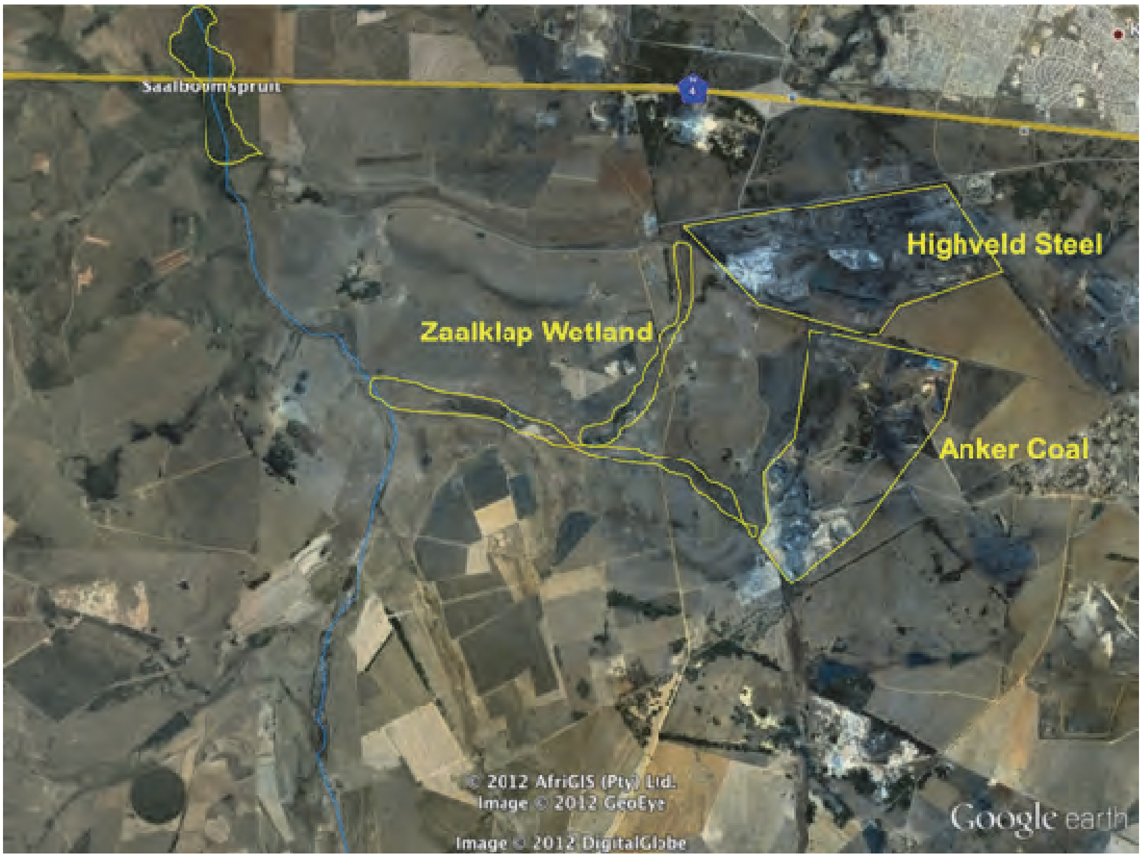



**Figure 7.8 Location of the Zaalklapspruit Wetland as well as the location of the Highveld Steel Facility and the Anker Coal Elandsfontein Colliery**

### 7.4.2 Impacts from Surrounding Land Uses

The Zaalklapspruit Wetland is heavily impacted by a variety of sources and as a result many of the ecosystem services delivered by the wetland such as water purification and water provisioning have been compromised. The major sources of impact include:

- Agricultural runoff from farming activities;
- Overgrazing from commercial cattle and dairy operations;
- Modification and alteration of physical components of the wetland (i.e. stream and bank modification);
- Pollution from the adjacent ash dumps of Highveld Steel;
- Pollution from the Adjacent Anker Coal Elandsfontein Colliery; and
- Pollution from other upstream mining operations.

The wetland health assessment suggests that wetland hydrology is most severely impacted followed by wetland vegetation and wetland geomorphology (Figure 7.9). Given these changes, the current state of the wetland can be described as moderately modified as reflected by a PES Category of “C” (EcoPulse, 2013).

### 7.4.3 The Impact of Acid Mine Drainage on the Wetland

Understanding the baseline water quality attributes of the Zaalklapspruit Wetland is an important constituent of the resource economic component of the study. The CSIR has completed the baseline water quality sampling for the wetland and has measured the following water quality parameters: pH, electrical conductivity and dissolved oxygen (De Klerk *et al*., 2013). The following metals were analysed: aluminium (Al), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). Five sampling points were identified and are shown in Figure 7.10.

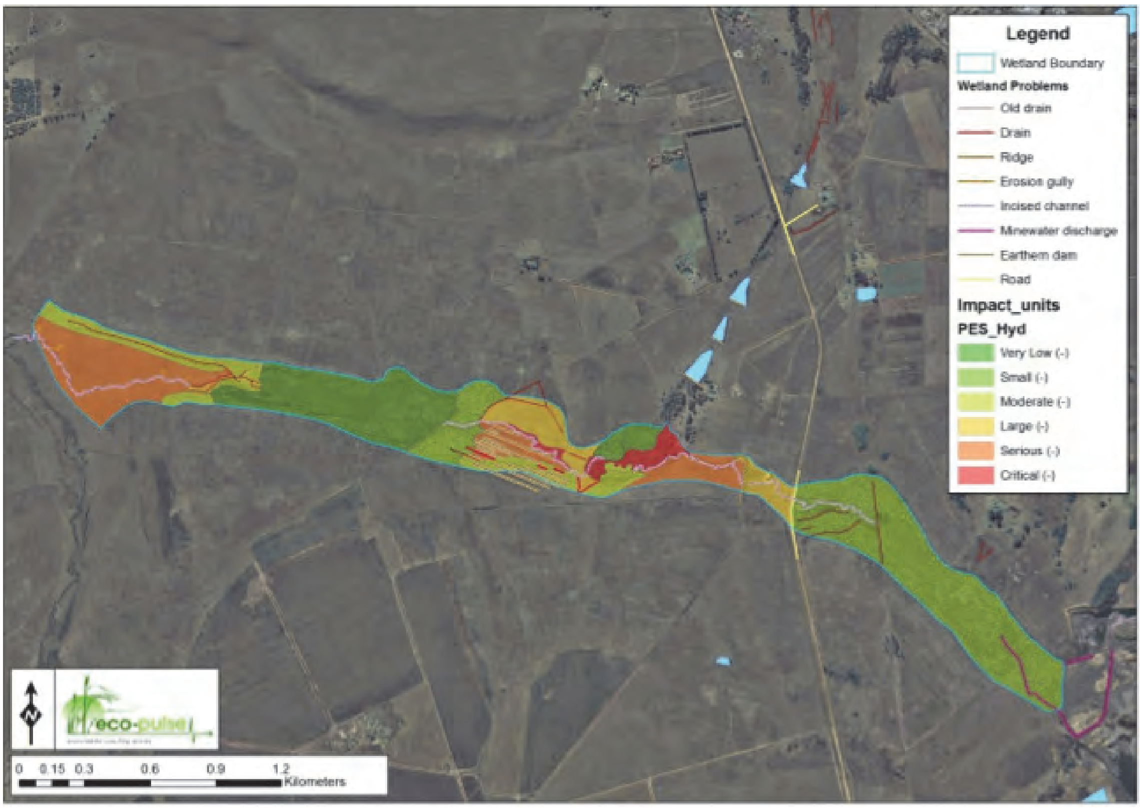



**Figure 7.9 A map of the Zaaklapspruit Wetland showing impacts to water distribution and retention patterns within assessment unit (Source: EcoPulse, 2013)**


**Figure 7.10 The five sampling sites at the Zaalklapspruit Wetland (Source: De Klerk *et al.*, 2013)**

From the results of the study by De Klerk *et al*. (2013) it is evident that the AMD impact observed at Site 2 significantly impacted the water quality of this wetland system. The tributary (Site 4), which flows into the Grootspruit, contributes little in terms of the dissolved metal loads and the cumulative effect thereof seen at Site 5 is more a function of the impact seen at Site 2 (De Klerk *et al*., 2013).

### 7.4.4 Zaalklap Wetland Ecosystem Services

For the purposes of the valuation of the ecosystem services delivered by the Zaalklapspruit Wetland, the following table was prepared, highlighting the ecosystem services to be valued for the Zaalklap Wetland (Table 7.4).

**Table 7.4 List of ecosystem services delivered by the Zaalklapspruit Wetland to be valued**

| Ecosystem Service Category | Ecosystem Service | Relevance to Zaalklap Wetland |
|---|---|---|
| Regulating Services | Coastal protection | Not applicable |
| | Erosion control | It is possible that the wetland does contribute to maintenance of bank integrity, but this service would be fairly small. |
| | Flood protection | It is possible that the wetland would contribute to flood control during particularly high rainfall events. However, due to the fact that the wetland is highly channelized, it is unlikely that the contribution would be significant. The contribution would likely increase after rehabilitation as the channelizing would be lessened. |
| | Water regulation and supply | Although the wetland catchment area is relatively small, it comprises roughly 17% of the total area of the B20G quaternary catchment and would provide a significant component of the total flow. |
| | Water purification | The wetland plays an extremely important role in treating water from the adjacent coalmines. |
| | Carbon sequestration | Due to the current ecological state and size of the wetland, it is unlikely to contribute significantly to carbon sequestration. |
| | Maintenance of temperature, precipitation | The size of the wetland would mean that it is unlikely to contribute to either temperature or rainfall maintenance. |
| Provisioning Services | Raw materials and food | There are no reports of local communities harvesting raw materials or food from this wetland. |
| | Maintains fishing, hunting and foraging activities | There are no reports of local communities hunting, fishing or foraging from this wetland. |
| | Grazing opportunities | The wetland is utilised by farmers in the area for grazing during winter months. |
| Cultural Services | Tourism, recreation, education and research | There is no record of tourism activities on this wetland. |
| | Cultural, spiritual and religious benefits, bequest values | There is no record of spiritual or cultural use of this wetland. |

## 7.5 VALUE OF THE ZAALKLAPSPRUIT WETLAND

### 7.5.1 Wetland Value in the Olifants Water Management Area

#### *7.5.1.1 Background*

Prime Africa Consultants, under contract to the Department of Water Affairs (DWA), recently conducted a large-scale valuation study of the aquatic ecosystem service values of the Olifants WMA. This study was an 18-month peer-reviewed study, conducted as part of the Olifants Water Resources Classification System project. This work is summarised in http://www.dwaf.gov.za/rdm/WRCS/.

The combined provisioning and cultural aquatic ecosystem services produced by water resources were valued at approximately R2,559 million in 2012. This represents more than 2% of the contribution to GDP generated within the catchment. Moreover, more than 55% of the GDP contributing sectors in the Olifants

River Catchment are directly dependent on water use licences. The economy and people of the Olifants River Catchment are thus highly dependent upon the water resources of the catchment.

Wetlands form a key part of the ecological infrastructure of the Olifants aquatic ecosystem. According to SANBI's National Freshwater Ecosystem Priority Areas (NFEPA) database, the Olifants WMA has more than 126 000 ha of wetland area. These wetlands play an indispensable role in delivering the above ecosystem services. Wetland's ecological infrastructure is thus indispensable and non-substitutable inputs into ecosystem services production.

At this point it is important to reiterate again the relationship between wetland ecological infrastructure and wetland ecosystem services. Wetland ecological infrastructure is a natural asset and could be thought of as a balance sheet item, whereas wetland ecosystem services are the benefits people get from wetlands, which could be thought of as income statement items. When we think of the value of wetlands we need to distinguish between these two concepts and carefully consider the values of each. From a theoretical point of view, the value of the wetland ecological infrastructure is equal to the discounted value of wetland ecosystem services into perpetuity.

In order to address these issues, the MEA and TEEB have introduced a new way of thinking about the value of biodiversity as a life supporting system underlying the benefits provided by ecosystem services to human society. As stated above, the MEA and TEEB distinguish between four types of ecosystem services: provisioning, cultural, regulating and supporting services. Provisioning services describe the material or energy outputs from ecosystems. Cultural services include the non-material benefits people obtain from contact with ecosystems. Regulating services are the services that ecosystems provide by acting as regulators. Supporting Services underpin almost all other services through its function of providing living spaces for humans, plants and animals. Regulating and supporting services constitute those ecosystem services that are indirectly consumed in the economy, and that mitigate many environmental risks.

#### *7.5.1.2 Value of Provisioning and Cultural Aquatic Services*

The values for the directly used provisioning and cultural services delivered by wetlands in the Olifants WMA are given below in Table 7.5. It is important to note that the values given are based on the larger ecosystem service value calculated for the Olifants WMA and therefore some of the values may not be applicable (i.e. the tourism and recreation values). The total value for the Provisioning and Cultural services is R2 559 million in 2012, which equates to R20 286/ha/annum for wetlands in the Olifants WMA.

**Table 7.5 Value of the wetland provisioning and cultural services in the Olifants Water Management Area (2012)**

| Ecosystem Service | Value R'million |
|---|---|
| ***Provisioning services*** | |
| Harvested products | 274 |
| Resource-poor farmers | 1,169 |
| Resource rent to agriculture | 332 |
| **Sub-total** | **2,014** |
| ***Cultural services*** | |
| Aesthetic value | 26 |
| Recreation | 70 |
| Tourism | 449 |
| **Sub-total** | **545** |
| **Grand-total** | **2,559** |
| **R/ha/annum** | **20,286** |

#### *7.5.1.3 Value of the Regulating Services*

Regulating services are more difficult to value, because it is not consumed directly in the economy. Current consensus among the professional community of resource economists is that a production function approach is best suited as a valuation method for intermediate ecosystem services. Production functions would quantify values for ecosystem services that contribute at least part of the value of those resources. They would apply knowledge of ecosystem functioning and processes to derive the value of supporting and regulating ecosystem services. They do this through deriving the value of ecosystems and the services they provide as intermediate inputs into goods and services that are produced and consumed by economic agents (Mäler, 1991; Barbier, 2000; Barbier, 2003; Perrings, 2006; Kinzig *et al*., 2007; Barbier *et al*., 2009). An ecological production function would have an ecosystem service as dependent variable (or response variable), and one or more ecosystem component and/or process indicators as independent variables (or influencing factors/determining variables).

Understanding the concept of biodiversity is key to formulating production functions. A useful characterization of biodiversity is provided by Noss (1990) and describes biodiversity as the composition, structure and function of an ecosystem: "…composition has to do with the identity and variety of elements in a collection, and includes species lists and measures of species diversity and genetic diversity. Structure is the physical organization or pattern of a system, from habitat complexity as measured within communities to the pattern of patches and other elements at a landscape scale. Function involves ecological and evolutionary processes, including gene flow, disturbances, and nutrient cycling."

One way to analyse the value of aquatic ecosystem services in the Olifants WMA would be to estimate the values for the water flow, water purification and other regulating services delivered by wetlands and other aquatic ecological infrastructure in the Olifants WMA. Table 7.6 below attempts to do this, and the total value for the regulating services are R3 643 million in 2012, which equates to R28 880/ha/annum for wetlands in the Olifants WMA.

This does not represent the full value of aquatic ecological infrastructure as it is also known that approximately 55% of GDP in the Olifants WMA depend directly on water use licences of some form, and water use licences in turn depend on well-functioning aquatic ecosystem services. The GDP of economic sectors directly dependent upon Water Use Licenses in the Olifants WMA in 2010 was R72 billion (DWA, 2012).

**Table 7.6 Value of the wetland regulating services in the Olifants Water Management Area (2012)**

| Ecosystem Service | Value (R'million) |
|---|---|
| Regulating services | |
| Water flow regulation | 2,733 |
| Water purification / waste assimilation | 876 |
| Flood attenuation | 23 |
| Carbon sequestration | 11 |
| **Grand-total** | 3,643 |
| **R/ha/annum** | **28,880** |

### 7.5.2 Ecosystem Services and Ecological Infrastructure

#### *7.5.2.1 Wetlands are Ecological Infrastructure*

At this point it becomes clear that it is not sufficient to express the value of wetlands as a single indicator, and that a basket of indicators are required to understand wetland value.

Over the past two decades, there has been an increasing awareness worldwide that ecosystems are more than "nice-to-haves" and that they play an important role in the economy. In South Africa, wetland ecosystems are under pressure from a wide range of impacts, and authorities, companies and the public are becoming increasingly concerned about wetland health. Coupled with this increasing awareness, legislation and the penalties for wetland degradation are becoming increasingly stringent.

Of particular interest to wetland practitioners in South Africa are the following developments:

- The National Water Act: Wetlands form part of ecological infrastructure and deliver wetland ecosystem services. Any form of wetland impacts in South Africa is governed by the National Water Act which, in Section 21, defines wetlands as a component of a water course and which regards wetland impacts as a water use activity, requiring a water use licence: "21. For the purposes of this Act, water use includes - … (c) impeding or diverting the flow of water in a watercourse; … (i) altering the bed, banks, course or characteristics of a watercourse…" In our experience, DWA is becoming increasingly strict on the awarding of Section 21 (c) and (i) water use licences.
- In 2010, an Environmental Assessment Practitioner was found guilty of failing to disclose the presence of a wetland at the site of the proposed Pan African Parliament in Midrand and received a sentence (North Gauteng Regional Court, Case number 14/1740/2010).
- Ecological infrastructure: In South Africa, SANBI published in November 2012, a discussion document on ecological infrastructure. Ecological infrastructure is defined to be the natural capital underlying the supply of ecosystem services to the economy (http://www.grasslands.org.za/document-archive/category/15-dialogue-on-ecological-infrastructure). This is a continuation of work by the Development Bank of Southern Africa (DBSA) (http://www.dbsa.org/Confr/Presentations/Ecological%20Infrastructure%20by%20J%20Manuel.pdf).
- WET-Management Series: The Department of Water Affairs and the Water Research Commission (WRC) have, over the past five years, developed wetland assessment methodologies for delineation, classification and importance assessment and ecosystem services identification of wetland systems.
- Wetlands mitigation and offset guidelines: The South African National Biodiversity Institute and Working for Wetlands in 2012 published a draft Wetlands Offset Guideline, which categorises all wetlands in South Africa into different wetland infrastructure categories and assign scarcity indicators to them.

Underlying all of the above initiatives is the understanding that wetlands are ecological infrastructure, which produces ecosystem services that have real benefits to people. In some cases, these ecosystem service benefits are highly tangible and large, and in other instances the benefits are indirect, intangible or smaller. When thinking of these wetlands values, two economic concepts are of interest, commodities and substitution. These are discussed below.

#### *7.5.2.2 Ecological Commodity Characteristics and Substitution*

An interesting phenomenon arising from the concept of ecological infrastructure is the emergence of ecological commodity properties of wetlands. An early example of this is the Wetland Mitigation Banking system in the US (Robertson, 2004). The Clean Water Act gave the Corps of Engineers the power to issue developers with permits to allow the damage of wetlands in exchange to their commitment to create or restore larger wetlands elsewhere. From the system it turned out that the average cost of wetland mitigation was approximately of $45,000 an acre, putting in practice a market price on preserved wetlands (Bayon, 2004).

In economics, a commodity can be defined as a basic good used in commerce that is interchangeable with other commodities of the same type. Commodities are most often used as inputs in the production of other goods and services. The quality of a given commodity may differ slightly, but it is essentially uniform across producers. Commodities often show the following characteristics:

- Clear demand and supply for a natural resource;
- Limited geographic distribution and supply;
- Global or regional market depends on it for providing benefits;
- Uniformity and fungibility – commodities are graded and classified as a specific type so that their characteristics are fairly uniform; and
- Has a per unit value.

The above-mentioned characteristics identified, are applicable to wetlands (i.e. ecological commodities) to some extent. The unit value is especially difficult to quantify and as yet no all-encompassing value exists. The subsequent section shows a first attempt at developing a per unit value for wetlands.

### *7.5.2.3 Wetland "Commodity" Value Demonstrated by the South African National Biodiversity Institute Working for Wetlands Data*

Wetland Supply Curve

Earlier in this section we demonstrated the increasing societal demand for wetlands and wetland ecosystem services. At the same time the proposed Wetlands Offset Guidelines of SANBI indicates a willingness to substitute between wetland ecosystems. All of this provides some level of evidence of an increasing demand for wetland ecosystem services. Furthermore, the work of Working for Wetlands (WfWet) provides evidence of a potential to supply wetland habitats. The rehabilitation work done by WfWet is captured in a series of wetland rehabilitation reports, which contain information regarding the size of the project, the cost of rehabilitation and several other ecological indictors such as the PES of the wetland prior to rehabilitation and the projected PES after rehabilitation.

Using the WfWet database, we can use wetland rehabilitation costs as a proxy for estimating the commodity value of wetlands, which expresses a form of regulating service provided by wetland ecological infrastructure. The resulting supply curve for wetlands is given in Figure 7.11 below.

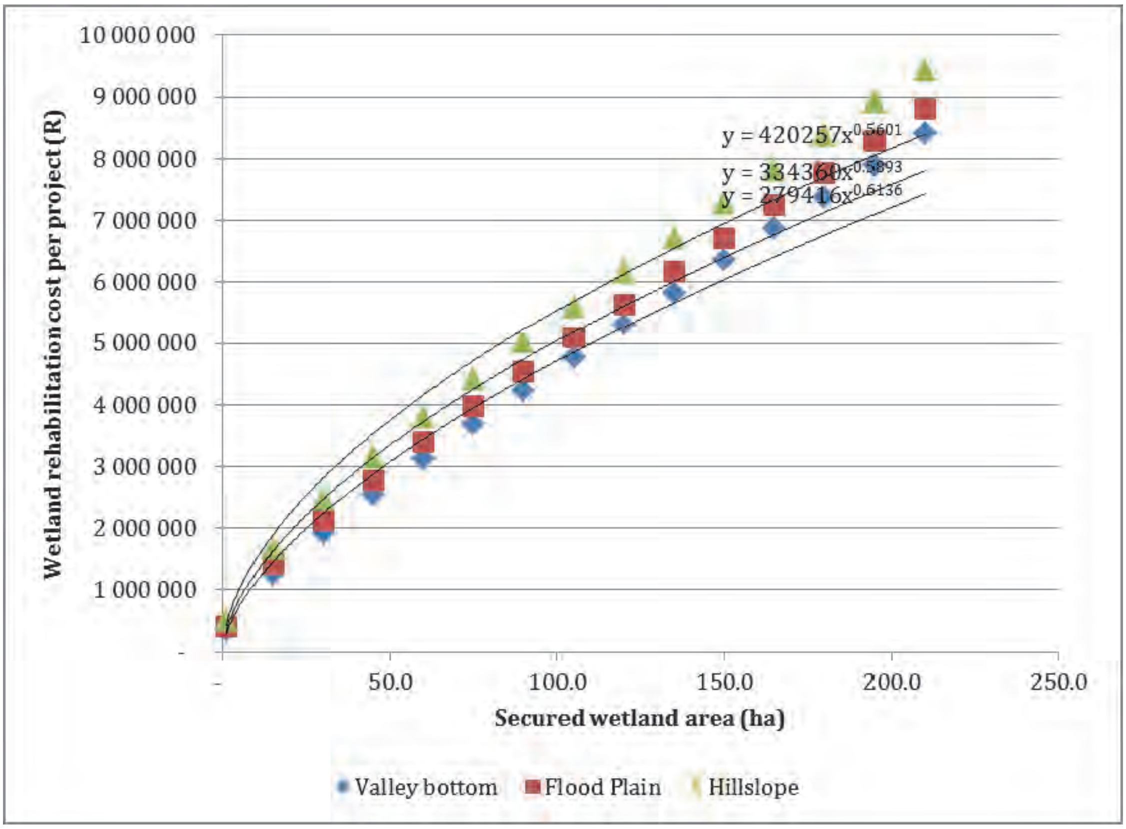



**Figure 7.11 Supply curve for wetlands based on the rehabilitation costs provided by Working for Wetlands**

### *7.5.2.4 Criticism against "Commodification" and Substitution*

The perception of "commodification" of the natural environment has received criticism (Kosoy and Corbera, 2010). Such criticism is based on:

- An ethical consideration which regards ecological assets fundamental to life and therefore invaluable; and
- A view that environmental assets are public resources, which should not be traded for profit.

The response to this criticism lies in the concept of substitution. Substitution is the process of purchasing one commodity in place of another. This definition, however, fails to account for commodities that are not perfectly substitutable. Many commodities are closely related; yet in the eyes of the consumer not perfect substitutes. It is useful to think of ecological commodities as lying on a continuum according to how closely they are related. At one extreme are perfect substitutes, at the other perfect complements. In the middle are independent commodity pairs. In between either extreme is a range of closely related ecological commodities with some degree of substitutability.

There may therefore exist cases where some wetlands are not substitutable (i.e. become no-go areas). In other instances, wetlands may be substitutable with other wetlands. The degree of substitutability and the cost of substitution are not primarily determined in a market, but through scientific and engineering cost assessments.

### 7.5.3 The Zaalklapspruit Wetland's Water Purification and Waste Assimilation Value

Sulphate content is a key water chemistry indicator of AMD. After rehabilitation of the Zaalklapspruit Wetland, sulphate levels decreased from levels varying between 509-662 mg/L upstream of the wetland, to levels varying between 195-477 mg/L downstream of the wetland. The reduction in sulphate levels therefore potentially varied between 110-467 mg/L.

Thus, the Zaalklapspruit Wetlands has become, in a very short period after rehabilitation, a very effective sulphate treatment system. It is likely that the effectiveness of this system will further improve with time as the rehabilitated wetland system stabilises and matures.

The value of this water purification and waste assimilation service can be estimated through evaluating the alternative cost of reverse osmosis. The Olifants WMA Water Resources Classification System (WRCS) of the Department of Water and Sanitation (DWS) have identified reverse osmosis as a suitable technology for large scale and wide-spread treatment of AMD in the Olifants WMA, and have developed a future water management scenario based on reverse osmosis (RO). The cost of water treatment with RO was evaluated in the Olifants WRCS, based on analysis by Golder Associates. The marginal cost of RO treatment is presented in Figure 7.12.

No water flow data was measured and only Mean Annual Runoff (MAR) was available for estimating the load of sulphate removed by the wetland. The resultant load of sulphate was then converted to an equivalent RO plant treatment volume, upon which the cost of RO treatment was based. This cost, which is also the water purification and waste assimilation value of the Zaalklapspruit Wetland, varied between R2.6-R11.4 million per year for the data measured by the CSIR. This is equivalent to a wetland ecological infrastructure value ranging between R130-R560 million, or an annual water purification and waste assimilation value ranging between R20 000-R85 000/ha.

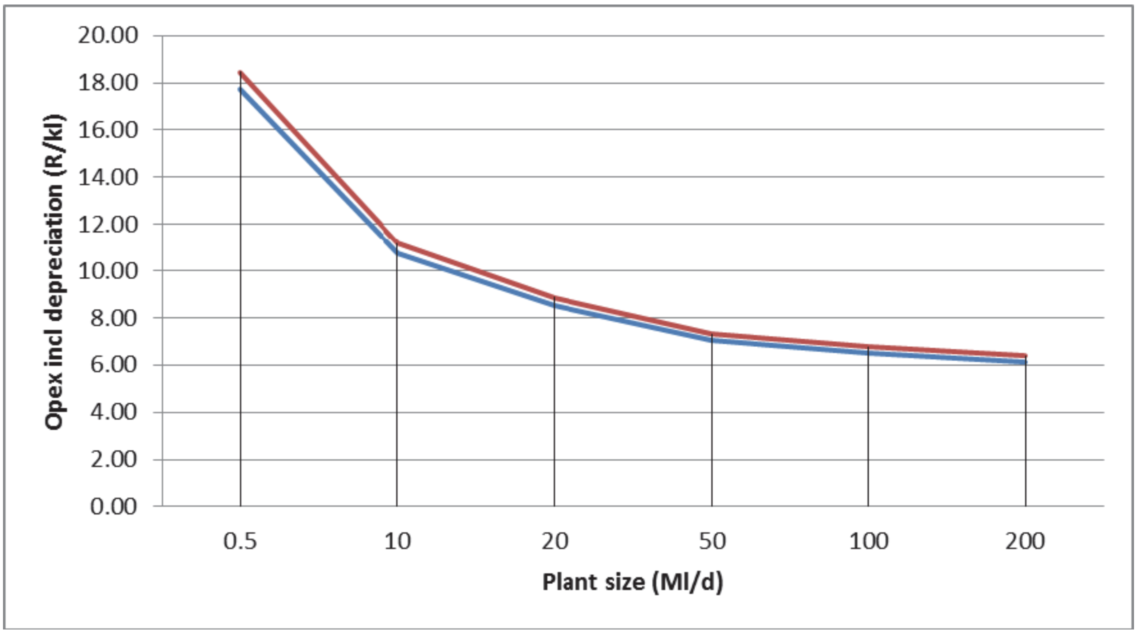



**Figure 7.12 Marginal costs of reverse osmosis water treatment for acid mine drainage**

### 7.5.4 Conclusion

The Zaalklapspruit Wetland produces valuable wetland ecosystem services and, through these production activities, is a valuable component of ecological infrastructure.

Based on the Olifants WRCS wetland ecosystem service valuation discussed in Section 5.1, the value of the provisioning and cultural services delivered by the Zaalklapspruit Wetland is estimated at R21 300/ha/annum (or R2.9 million per year for the 135 ha Zaalklapspruit Wetland); and the value of the regulating services delivered by the wetland, excluding water purification and waste assimilation, is R22 940/ha/annum (or R3.1 million per year).

The water purification and waste assimilation service of the Zaalklapspruit Wetland has a value ranging between R20 000-R85 000/ha/annum or R2.6-R11.4 million per year. This is based on monitoring data of the CSIR taken within less than a season after rehabilitation and this value could therefore likely be expected to increase as the rehabilitated wetland stabilises and matures.

This demonstrates that the Zaalklapspruit Wetlands' water purification and waste assimilation ecosystem service is possibly larger than the other wetland ecosystem services values based on the Olifants WRCS estimates of other wetland ecosystem services together.

The production of ecosystem services relates closely to wetlands' inherent 'asset' value, which is also referred to as "ecological infrastructure". Based on the estimates done in this study, the asset (or ecological infrastructure) value of the Zaalklapspruit Wetland ranges between R501-R763 million, of which the water purification and waste assimilation service contributes R130-R560 million. Thus, by rehabilitating the Zaalklapspruit Wetland at a cost of R1.7 million, we have been able to produce between R130-R560 million on the natural asset balance sheet of South Africa.

The results therefore very strongly support investment in wetland rehabilitation in general and for wetland rehabilitation in AMD affected areas specifically. It also demonstrates that wetland rehabilitation may form a very important part of wetland impact mitigation strategies.

The evidence have also shown that wetlands are taking on some form of commodity value, although its degree of substitution is limited by scientific determination (i.e. some wetlands may not be substitutable at all, while others may be substituted through wetland rehabilitation projects). Thus, a cost-benefit framework needs to internalise the effects of wetland impacts and substitution costs.

## 7.6 VALUING WETLAND SUSTAINABILITY

### 7.6.1 Overall Approach and Associated Methodologies

The proposed "Valuing Wetland Sustainability" approach attempts to value the ecosystem services delivered by wetlands and their inherent ecological infrastructure value and incorporate them into a format with which decision makers are able to make better-informed decisions.

The approach is based on four steps:

1. Systems analysis;
2. Generate alternatives;
3. Analysis of alternatives; and
4. Recommendation of preferred alternative(s).

Integral to performing each of these steps and sub-steps is the concept of ecosystem services. Ecosystem services are defined formally by two United Nations led initiatives, the MEA and TEEB. Furthermore, the WBCSD, of which many coal-mining operators are members, adopts the ecosystem services framework in their CEV Guideline.

Moreover, the DWS in South Africa have adopted an aquatic ecosystem services approach to analysing, valuing and planning in water resource management. Ecosystem services form the basis for trade-off analysis of the DWS's WRCS, which in turn is the basis for setting resource quality objectives (RQOs) for water resources.

Ecosystem services are defined as the benefits that ecosystems provide to humans. In the case of the wetlands, we are interested in aquatic ecosystem services related to wetlands. According to South African water law, these water resources are public property. They are fundamental to the production of ecosystem services, and any negative impacts on them therefore have legal and public liability consequences. In addition, and in support of the DWS processes, the South African WRC has developed a Guideline and Manual for the evaluation of aquatic ecosystem services (Ginsburg *et al*., 2009).

### 7.6.2 Supporting the Valuing Wetland Sustainability Approach

The Valuing Wetland Sustainability Approach provides a thorough and internationally relevant standard for sustainability analysis. The Approach makes use of several international best practice methodologies, which include:

- Adopting the MEA and TEEB ecosystem services framework as a basis for explicitly identifying and defining sustainability impacts. These are internationally accepted frameworks and have also been adapted by the WBCSD.
- Applying a comparative risk assessment (CRA) approach to identifying, prioritising and quantifying project risks. This technique is an internationally accepted risk assessment technique and is very effective in a workshop environment. This technique will be applied during Step 2 and will integrate the outcomes of Step 1.
- Applying best international practise in resource economics. These studies typically quantify the value of ecosystem services, within a project financial and economic analysis context.
- Demonstrating the calculation of net present values (NPV).

### 7.6.3 Step 1: Systems Analysis

#### *7.6.3.1 Identify Key Sustainability Challenges and Opportunities*

This sub-step will take the form of an evidence-based systems description. A system description of this nature combines available scientifically credible data and information into a detailed description of how the natural and human systems in the study area function and interact. The system description takes the definition of biodiversity as a point of departure and thus defines and describes the species diversity as well as the components and functionality of the affected system. The system description further defines the dependence of humans on the ecosystem and thus identifies the beneficiaries of ecosystem services.

The objectives of this step are all about obtaining an understanding of the nature of the opportunity the resource presents (e.g. size and scope, location, ecological infrastructure, human infrastructure and timelines). This includes:

- Understanding the project context, in relation to both external conditions (e.g. regulatory environment) and internal conditions;
- Identifying how the project could impact, or be impacted by, sustainability value drivers;
- Understanding the major sustainability challenges and opportunities for the project; and
- Identifying the key project decisions.

This step will thus be executed primarily as a desk-based analysis, supported by a series of structured conversations with members of the project team and other subject-matter experts.

#### *7.6.3.2 Identify Key Project Decisions*

Following from the systems description the next sub-step is to develop a sufficiently detailed engineering design description through which to identify key project decisions. This will focus for the most part on defining the production objectives within a plausible time frame.

These decisions relate to project design and project implementation processes that could significantly influence the project's outcomes. This would include mining method, project footprint and the location of mine and associated infrastructure, source and quantity of project inputs, waste disposal methods and volumes, decisions regarding logistics and project implementation processes. As in the preceding step, this step will thus be executed primarily as a desk-based analysis, supported by a series of structured conversations with members of the project team and other subject-matter experts.

### 7.6.4 Step 2: Generate Alternatives

#### *7.6.4.1 Generate Alternatives*

Following closely from the previous sub-step, the aim of this sub-step is to define options for each key project decision. A number of alternative solutions may be considered for addressing a key project decision. Following from Step 1, an 'options generation workshop' should be held with the entire project owners' team together with various subject matter experts and external advisors.

This workshop will serve to:

- Confirm the key project decisions identified in Step 1;
- Generate options for each key project decision; and
- Identify the specific sustainability risks and opportunities associated with each option.

#### *7.6.4.2 Identify Sustainability Risks and Opportunities for Each Alternative*

This step will take the form of a CRA workshop methodology. The CRA methodology will assist us to integrate the outputs of the preceding steps into a framework that will enable the calculation of sustainability value in Step 3. The CRA methodology:

- Identifies sustainability risks (i.e. risks to ecosystem services provided by the natural systems); and
- Identifies opportunities for mitigation (i.e. avoid, minimise and compensate).

The CRA method describes the risks through the quantification of consequence and likelihood of each project decision and option on each system component, and thus prioritises each risk component. This will help the project team to conduct a detailed discussion to map specific sustainability risks and opportunities against each of the options that have been generated.

### 7.6.5 Step 3: Analysis of Alternatives

#### *7.6.5.1 Define Scenarios and Assumptions*

The objectives of this step are to define scenarios to determine how sustainability risks and opportunities could materialise over the life of a mine.

The workshop will guide the workshop participants to generate a number of future scenarios. This would likely include defining the best case, likely case and worst-case scenarios, but will be adjusted according to the project scope as appropriate.

The scenarios workshop will use the outcomes of the CRA process (see above) and assess the engineering and financial risks as well as the sustainability risks. By their nature, sustainability risks are far less certain than other risk types and there is often insufficient historic data to support a statistical analysis of probability. This is where the systems analysis (Step 1) and the CRA (Step 2) become useful, as these steps help to contextualise and quantify sustainability risks in an evidence-based manner sufficient for environmental regulatory purposes. With this evidence-base as background, the scenario workshop participants, who will include sustainability experts, will apply their expert judgement, along with their collective knowledge of the project and its context, as a basis for defining each scenario and assessing the sustainability risks and opportunities in the context of a range of business impacts, such as:

- Cost increases or decreases including CAPEX and OPEX;
- Revenue loss or increase including revenue lost due to production stoppages, transportation interruptions, supply shortages, etc., or additional revenue streams (e.g. provision of water treatment services);
- Impacts on project delivery timeline including delays or a shortening of timelines; or
- Reputational damage or enhancement.

Following the scenarios we will collect data on the various aspects and inputs required to quantify the relevant aspects of each of the scenarios.

#### *7.6.5.2 Calculate Sustainability Value for Each Scenario*

The sustainability value can be calculated using a discounted cash flow analysis combined with a cost-benefit analysis approach. Several internationally accepted methodologies provide guidelines for the best practices of performing such analysis.

The analysis should proceed through two phases. First, a financial cash flow analysis for the life of the project should be conducted. Thereafter, the financial analysis will be converted into an economic cash flow analysis. The economic cash flow analysis transfers financial flows and ecosystem services effects into an economic analysis, which estimates the project life cycle effects in society, for each scenario. The economic analysis, with internalised ecosystem services effects, therefore provides a metric for societal sustainability impacts. Internalising the ecosystem services requires the identification and valuation of wetland ecosystem services using best practice resource economic techniques.

The financial and economic cost-benefit analysis will enable comparative assessment of different scenarios. The assessment may be done using a range of financial and economic indicators.

#### *7.6.5.3 Calculate the Net Present Value*

There is a requirement to balance the theoretical NPV and the sustainability considerations throughout the project development life cycle. Ideally, both profitability and risk should be analysed for a project in order to understand the long-term impact on future performance.

The calculation of NPV (and related project and environmental sustainability indictors) is calculated as a logical next step to the calculation of sustainability values for each scenario (preceding step).

### 7.6.6 Step 4: Recommendation of the Preferred Alternative(s)

This step proceeds through three sub-steps, including:

1. Comparison of NPVs and sustainability value at stake and recommend the preferred alternatives;
2. Define business case for preferred alternatives; and
3. Recommend preferred alternatives to key decision makers.

The alternative(s) that represents the optimal business case for a key project decision should now be recommended. This will be based on, amongst other considerations: maximising value; balancing NPV, sustainability risks and opportunities; and technical, commercial, legal and strategic considerations.

# CHAPTER 8: CONCLUSIONS AND RECOMMENDATIONS

## 8.1 CONCLUSIONS

Various levels of wetland functionality were assessed during this study, which include water quality, the bacterial consortium, freshwater algae, a vegetative spectral assessment, teratogenic potency and embryo toxicity, as well as a resource economics component. The rehabilitation of the Zaalklapspruit Wetland involved the increase in surface area to allow for increased gravitational drainage. Increased soil contact increased the filtration and suspension of pollutants as well as biologically mediated processes which strongly affect water chemistry, resulting in the removal of contaminants from the water column and overall improved functioning of the wetland. This is based on monitoring data of the CSIR taken within less than a season after rehabilitation and this value can therefore likely be expected to increase as the rehabilitated wetland stabilises and matures.

The main conclusions found during this study are as follows:

- The water quality downstream of the rehabilitated area improved significantly after rehabilitation. The pH and alkalinity were increased to levels in the natural fresh water range, in which many of the metals become insoluble and precipitate out of the water column. Sulphate concentrations decreased by 65% and the total dissolved solids decreased by 50% compared to pre-rehabilitation values. It is evident that the rehabilitation efforts have started to restore the water chemistry to that which resembles the reference site.
- The changes in the bacterial diversity highlighted a shift in the community structure downstream after rehabilitation, which resembled that of the reference site.
- The rehabilitation of the wetland resulted in an increase in the species diversity and richness of the algae after rehabilitation and also confirmed the usefulness of freshwater algae as bioindicators for valley bottom wetland rehabilitation.
- With regard to the vegetation assessment, no consistent change in vegetation conditions for the two species studied, could be noted after rehabilitation.
- It was found that larval survival of *X. laevis* was markedly higher after rehabilitation at the downstream site. It was also observed that the incidence of malformations was higher upstream from the rehabilitation area. After rehabilitation, the proportions of malformed individuals were lower in the downstream treatment in contrast to the same site before rehabilitation, where it was characterized by a higher teratogenic potency. The rehabilitation work demonstrated that wetlands are valuable and in acid mine drainage affected environments the water purification and waste assimilation service provided by wetlands are especially valuable. The increase in productivity in the rehabilitated area is an indication of the slow return of functional ecosystem services.
- Based on the estimates done in this study, the improvements in water quality provided by the rehabilitated wetland translate to an economic value of between R2.6-R11.4 million per year. The benefit is considered high when compared with the R1.7 million invested in rehabilitation of the wetland. It also demonstrates that wetland rehabilitation may form a very important part of wetland impact mitigation strategies.
- During this study various minor issues of structural design, etc. was observed, but can easily be resolved by making very minor adjustments, for example to the spillway heights and undertaking further earthworks to encourage water to spread out further during low flow periods. This illustrates the importance of adaptive management or a learning-by-doing approach in ensuring that rehabilitation benefits are optimised.

## 8.2 RECOMMENDATIONS

From the results obtained during this study it was evident that the function of the test wetland improved after rehabilitation, especially with regard to improved water quality. From the above outcomes the following recommendations can be drawn:

- This study showed that the rehabilitated wetland was able to improve the water quality of the water received from mining operations within a short period of time. It is thus, important to consider the longer term effectiveness of the rehabilitation so as to understand the assimilative capacity of such a rehabilitated wetland under these circumstances. This will also allow researchers to determine how sustainable rehabilitation is within such a scenario in the long term.
- The contradictory responses between the species studied during the vegetation assessment could also be further investigated using detailed measurements of water and soil contaminants to determine whether the relationships between particular trace metals and the vegetation indices are significant.
- Proper monitoring of rehabilitation activities under these impacted scenarios is very important and should be done sufficiently to improve the efficiency of the rehabilitation efforts and in so-doing promote adaptive management which is extremely important under these circumstances.
- Innovative and purpose built engineering designs for the rehabilitation structures is also needed to improve the efficiency of the rehabilitation efforts, especially during low flows.

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