# DEVELOPMENT OF A RELATIVE RISK ASSESSMENT FRAMEWORK TO ASSESS MULTIPLE STRESSORS IN THE KLIP RIVER SYSTEM

**Report to the**

**WATER RESEARCH COMMISSION**

**by**

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# EXECUTIVE SUMMARY

## BACKGROUND

Whilst demand for water is rapidly increasing due to population expansion, industrialization and urbanization, water supplies are increasingly coming under pressure due to resource depletion and pollution. The Klip River, its tributaries and associated wetlands have been described as one of the most heavily impacted rivers in the country being subject to every type of conceivable pollution. These included mining, urban, industrial as well as agricultural impacts. In addition to an influence on water quality there is an impact on the water level, flow regime and stream morphology of the River. Despite this, the Klip River system must still provide the necessary ecological infrastructure to satisfy basic human needs and maintain ecological processes and then also serve the other user groups, i.e. agricultural, recreational and industrial. To achieve sustainability of water resource use, sufficient protection measures must be implemented to ensure the wellbeing and availability of key ecosystem can be maintained. The National Water Act (NWA) prescribes a number of protection measures for water resources including; the establishment of a societal vision to direct the level use and or protection of resources, the classification of water resources by establishing a Management Class to represent the vision, establish the Ecological Reserve that provides for the ecological requirements and then determine Resource Quality Objectives (RQO) for water resources, which gives effect to the Management Classes. The narrative and or numeric RQOs that relate to the quantity, quality, habitat and biota of water resources, establish clear goals for the desired quality of the resources to achieve a balance between the need to use water resources and protect them. To implement RQOs the Department of Water and Sanitation (DWS) needs to characterise the risks to the achieving RQOs on multiple spatial scales, within the context of existing socio-ecological systems, and use a robust validated measure/s to evaluate the socio-economic and ecological consequences of alternative management options which can provide the information required to achieve RQOs using Source Directed Control measures, such as Water Licences.

## RATIONALE

The RQO determination procedures for the Upper Vaal Water Management Area (WMA) was recently been completed. The Upper Vaal RQOs includes numerous quantity, quality, habitat and biota subcomponent objectives for regional and prioritised river, groundwater, wetland and dam ecosystems. The Klip River, which forms a part of the greater Vaal River WMA is one of South Africa's most economically valuable aquatic ecosystems, and with the Vaal River, one of South Africa's hardest working rivers. Although the ecological importance of the Klip River is noteworthy, a wide range of socio-economic services are provided by the ecosystem to various local and regional stakeholders, including the provision of water for basic human needs and natural products to local communities, and the removal of waterborne wastes from Gauteng. As a result, the pressure on the ecological infrastructure of the Klip River is currently excessive and unsustainable. To address this, the Water Resource Classification procedure for the Upper Vaal WMA established a Heavily Used

(Class III) class for the Upper Vaal IUA UI which includes the Klip River. Therefore the main aim of this study was to demonstrate the development and application of the Regional Scale Relative Risk Assessment approach incorporating the Relative Risk Model (RRM) framework, as a suitable measure to evaluate the risks of achieving riverine RQOs for water resources in the Klip River (RUs 63-65) portion of the Vaal River Catchment. In addition the study aims to demonstrate the use of the RRM to evaluate the socio-economic and ecological consequences of alternative management options to provide the information for Source Directed Control measures, to achieve RQOs.

## AIMS

### AIM 1

To integrate the available information (grey and published literature) on the status of the Klip River Catchment together with data from two field surveys to inform subsequent components of the project.

### AIM 2

Develop and apply the Relative Risk Model (RRM) to determine the risks based on perceptions of local communities, conservation authorities, municipal authorities and other stakeholders along the Klip River system on the value of the river systems and its associated resources and impacts of contamination.

### AIM 3

Validate the risks modelled through the RRM process by linking effects assessment endpoints, i.e. biomarkers (genetic diversity in selected taxa, etc.), fish communities, to pollutant exposure (e.g. heavy metal and organic contamination in tissues of organisms, sediment and water).

### AIM 4

To use the outcomes of the RRM and validation study for incorporation into the newly proposed water quality management plan for the Klip River.

## METHODOLOGY

All available bio-physical data on the Klip River system was collated from the grey and published literature. To supplement the available data, two sampling surveys during the low flow (June 2013) and high flow (January 2014) were conducted to an additional eight sampling sites.  Five of the sites were situated on the Klip River, two on the Riet Spruit and one on the Natal Spruit. The latter three sites are situated on the two major tributaries of the Klip River. All of the sites were selected to include habitats where relevant information could be gathered to meet all the aims set for the study.

*In situ* physicochemical water quality variables were measured at each sampling site and an additional water sample was collected for chemical and microbiological analyses in the laboratory. These data were compared to the extensive historical database that was sourced. Sediment samples were collected at the same sites and the physico-chemical characteristics were determined. The physical parameters measured were organic carbon content and particle size distributions. Metal concentrations were determined in the samples using standard inductively coupled plasma – ass spectroscopy (ICP-MS) techniques.

Diatoms were sampled at all the sites using standard protocols. The diatom community structure was assessed using a range of indices to provide insight into the pollution status of the system. Macroinvertebrate sampling followed the standard SASS5 protocol. Changes in the macroinvertebrate community structure during the surveys and sites were analysed using multivariate statistical techniques such as principal component analysis and redundancy analyses. The SASS5 data were used to calculate the macroinvertebrate response assessment index (MIRAI) scores.

Two surveys were undertaken to collect fish samples for both community and ecotoxicological analyses. The available fish habitat was classified based on the velocity and depth parameters and in each habitat type, or velocity-depth class, the extent of the potential cover for the fish, namely substrate, overhanging vegetation and undercut banks, was estimated and scored. A variety of sampling techniques were applied to sample the different habitats, ranging from still-standing water to fast-flowing rivers. The sharptooth catfish (*Clarias gariepinus*) was used as indicator species in this study. The health of *C. gariepinus* was determined by using a macroscopic health assessment (fish health assessment index, FHAI). Muscle and liver samples were also collected for bioaccumulation, biomarker and genetic.

Levels of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Sr, U, V and Zn in muscle tissue were determined with ICP-MS using standard techniques for sample preparation and analysis. A 10 g muscle tissue sample was homogenised with anhydrous sodium sulphate for organic pollutant analysis. The DDT congeners - p,p'-DDE, o,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, o,p' and p,p'-DDT (the sum expressed as ΣDDTs), hexachlorobenzene (HCB), α-, β-, γ and δ-hexachlorocyclohexane (HCH) isomers (the sum expressed as ΣHCHs), the chlordanes (ΣCHLs) – cis- and trans chlordane (cChl, tChl) and its oxidised form, i.e. oxychlordane (OxC) and heptachlor (HC) and its break down products cis- and trans.

Approximately 1 g each of sharptooth catfish liver and muscle were placed in cryotubes mixed with Hendrickson stabilising buffer and placed in liquid nitrogen for biomarker analysis. A subsample of approximately 0.2 g of collected liver tissue was used for biomarker activity analyses. The remaining portions of the axial muscle were stored frozen in cryotubes for further analysis. Values were obtained for acetylcholinesterase, cytochrome P450 and metallothioneins as biomarkers of exposure as well as superoxide dismutase, catalase activity, lipid peroxidation, protein carbonyls and cellular energy allocation as biomarkers effect. Liver samples were also analysed to assess population genetic structures.

The spatial scope of the RRM selected was the lower catchment of the Klip River system, just before it flows into the Vaal River. The rationale for this delineation was that the lower portion of the river would represent all activities in the upper catchment. The management endpoints that were applied for this study were directly related to the RQOs that were set for the Klip River. An integrated conceptual model based on stake holder input during the RQO determination process was developed.  This model formed the basis to create the Bayesian Network (BN) belief model. This model was parameterised using conditional probability tables, which relied extensively on the physico-chemical data that were generated during the field work phase of the study.

**RESULTS AND DISCUSSION**

The water quality assessment revealed that all of the sites had excessive nutrient concentrations with associated microbiological contamination. These were greatest in the upper catchment adjacent to the sprawling urban area of Soweto / Lenasia. The metal concentrations showed the same trend and for most metals the target water quality guideline for aquatic ecosystems was exceeded. The sediment consisted of mainly low-medium organic material and the predominant particle size was medium to course sand.  The metal concentrations increased further down the catchment with the sediment displaying higher concentrations during the low flow period when compared to the high flow. This is indicative of the sediment and therefore concomitant metal transport function that the higher flows during the rainfall period plays.

The diatom results indicated a moderate to poor water quality with the Natal Spruit tributary the worst during both surveys. The %PTV indicated the presence of organic pollutants at most sites, and this was supported by the water quality data. The percentage of deformed cells was also highest at the Natal Spruit site during the low flow. The SASS5 results of the Klip River and its tributaries (Riet- and Natal Spruit) indicated that the macro-invertebrate community was severely degraded. It was only at Klip River site 3 that the macroinvertebrates were in a moderate condition. There was no difference between surveys and this indicated a sustained input of stressors into the system. When compared to historical data there was a noticeable in the lower reaches and this was attributed to habitat destruction in the form of sand mining.

The modification of the available habitat as well as the increased pollution levels has resulted in a decrease in the fish diversity and abundances when compared to historical studies.  These are factors that contributed to the absence of *Austroglanis sclateri* and *Labeobarbus kimberleyensis (*largemouth yellowfish) during this survey period. The fish response assessment index scores for the entire study period indicated that the fish community structures were in an impaired state. However, the overall fish condition assessment using the FHAI indicated that the fish are in a good condition with minimal organ alteration present.

The levels of hexachlorobenzenes (HCBs) and chlordanes were higher in fish from the Klip River, whereas the other OCPs were higher in the Riet Spruit.  Dieldrin levels in the Riet Spruit were similar to those recorded in tigerfish from the Luvuvhu River but higher than the tigerfish levels from Lake Pongolapoort. All OCP levels with the exception of hexachlorocyclohexanes (HCHs) were lower than those measured in *Labeo capensis* from the Vaal River. No *o,p'*-DDT levels were measured in any of the fish samples indicating only historic levels remaining in the environment.  However of concern are the high levels of γ-HCH (lindane) that were measured in the Riet Spruit. Metal bioaccumulation levels were very similar to those reported in previous studies conducted on the Klip River. When compared to metal concentrations in *C. gariepinus* from the Olifants River, the levels in the Klip River were lower. The biomarker results indicated that the fish were subjected to oxidative stress as was evident from the stimulation of the anti-oxidant biomarkers (CAT, GSH and MDA). This was particularly evident during the high flow periods. There was also an increase in the biomarker of organic pollutant exposure, CYP450, which was also reflected by the increased OCP levels measured in the fish. The population genetics analysis did not reveal any changes from other populations in nearby catchments. This indicated that genetic adaptation (divergence) due to pollutant exposure has not occurred in the Klip River system.

During this study we successfully demonstrated how the causal structure of a risk assessment tool such as the relative risk methodology (RRM) can be translated into a graphical BN models. The tiered nodal structure of the BN allowed for the causal linking of sources of stressors, habitats and endpoints of the Klip River. For the construction of the BN models multiple data types were applied *a priori*. These data were all generated during this project as well as integrated from the published and grey literature as well as expert knowledge. The BN was used to assess the management implications of different scenarios. It most instances it was evident that even in the event of drastic interventions, there remains great risk to the system.

**CONCLUSIONS**

In this study we demonstrated that the BN approach could effectively be used as a tool for water resource and conservation managers. We were able to demonstrate that the water resource management goals can be assessed against the backdrop of different scenarios. The trade-offs of cost and benefits can be evaluated in this way, e.g. it was demonstrated that even with mitigation of AMD in the Klip River, there would still not be any change to the macro-invertebrate status. The graphic nature of the interface and outputs coupled to the ability of the BN models to generate and evaluate alternative scenarios makes it a useful tool for resource management. The form of the risk distribution curves serves as a sensitivity analysis of the BN model outputs. It therefore provides an indication of where additional information will be required to reduce uncertainty. The generation of information to reduce the uncertainty in the risk predictions will in essence drive the structuring of future monitoring programmes, i.e. the monitoring becomes hypothesis driven. The new information generated by the monitoring can be used to update the input node descriptions and if necessary the rank scores making the BN model ideal for adaptive management application. The application of RRM-BN models can contribute to greater application of adaptive management practices in water resource and conservation management of the Klip River and Upper Vaal WMA. The application value within an adaptive management framework is due to the RRM-BN model communicating uncertainty in a quantifiable manner. The interactions of disparate ecological values are visually observed through the graphical interface, and once the model has been developed it can easily be updated and refined by the resource manager, thereby increasing ownership in the adaptive management process.

## RECOMMENDATIONS FOR FUTURE RESEARCH

The broad base risk distribution patterns are indicative of the degree of uncertainty related to the data used for scoring the input parent nodes, as well as the input distributions used to set up condition probabilities. These uncertainties can only be reduced by filling the knowledge gaps through hypothesis-driven fundamental research projects. Further reduction in uncertainty in particularly the Klip River catchment can be decreased through focused monitoring and field surveys. The focus of this study was primarily on ecological endpoints and since the RRM framework was based on both ecological as well as human health aspects it was not surprising that there was still overall high risk even when the factor contributing to the ecological risk were mediated. It is therefore essential that future studies should focus on the aspects that relate to both human health risk as well as economical risks. For example, what is the health risk associated with the consumption of fish from the Klip River system or consumption of products irrigated from surface and ground water from the system. Further what financial risks are associated when irrigation from Klip River water resources is stopped?  The RRM would allow for the evaluation of trade-offs be between reducing human health risks by stopping irrigation and the loss of income through irrigation based agriculture.

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# 1 INTRODUCTION AND AIMS

## 1.1 Background

Whilst demand for water is rapidly increasing due to population expansion, industrialization and urbanization, water supplies are increasingly coming under pressure due to resource depletion and pollution. Younger (2001) describes pollution as one of many pressures impacting freshwater systems and resources in South Africa. The Klip River has been described as one of the most heavily impacted rivers in the country being subject to every type of conceivable pollution (DWAF, 1999). Included here are impacts from mining, urban, industrial as well as agricultural activities. In addition to affecting water quality, these factors have also impacted on the water level, flow regime and stream morphology of the River. Despite this, the Klip River must still serve all five recognized user groups identified by the Department of Water Sanitation (DWS), i.e. domestic, agricultural, recreational, industrial uses as well as the environment (DWAF, 1999). The Klip River is an important contributor to the Vaal Barrage both in terms of flow volume as well as pollution load (Howie & Otto, 1996).

The Klip River falls within the Upper Vaal Water Management Area (Upper Vaal WMA) which covers an area of 55 565 km$^2$. More than 80% of the WMA is underlain with sedimentary rocks of the Karoo system. Soil depths are generally moderate to deep (DWAF, 2004). The Klip River, together with the Vaal River upstream of the Vaal Dam contribute the largest portion (46%) of the surface flow in the Upper Vaal WMA, which in turn supplies the bulk of the water required in Johannesburg, South Africa's largest metropolitan area as well as Pretoria and surrounding areas as shown in Figure 1. The Upper Vaal WMA contributes nearly 20% of the Gross Domestic Product (GDP) of South Africa making it the second most important Water Management Area (WMA) in terms of economy in the country (DWAF, 2003). This WMA is also the most populous in the country, with more than 80% of the population in the area residing downstream of Vaal Dam, nearly 97% of which is urban (DWAF, 2003).

Mining is the most important industrial activity in the upper catchment of the Klip River. The predominant minerals in the Upper Vaal WMA include gold, uranium, base metals, semi-precious stones and industrial minerals. A number of tributaries of the Klip River originate from the Central Basin of the Witwatersrand. The Natal Spruit provides drainage to the central portion of the basin whilst the eastern portion of the basin is drained by the Elsburgspruit. The Klipriver and Klipspruit drain the western portion of the Central Basin (DWA, 2012).

Over a period of about 80 years, the Witwatersrand basin where the Klip River originates has produced in excess of 18 000 tonnes of gold, more than half the weight of gold mined in the world (Tutu et al., 2003). This legacy has however not come without a cost. For the Klip River Catchment, this together with other anthropogenic factors previously mentioned has led to significant deterioration of the water quality and ecological integrity of the catchment.

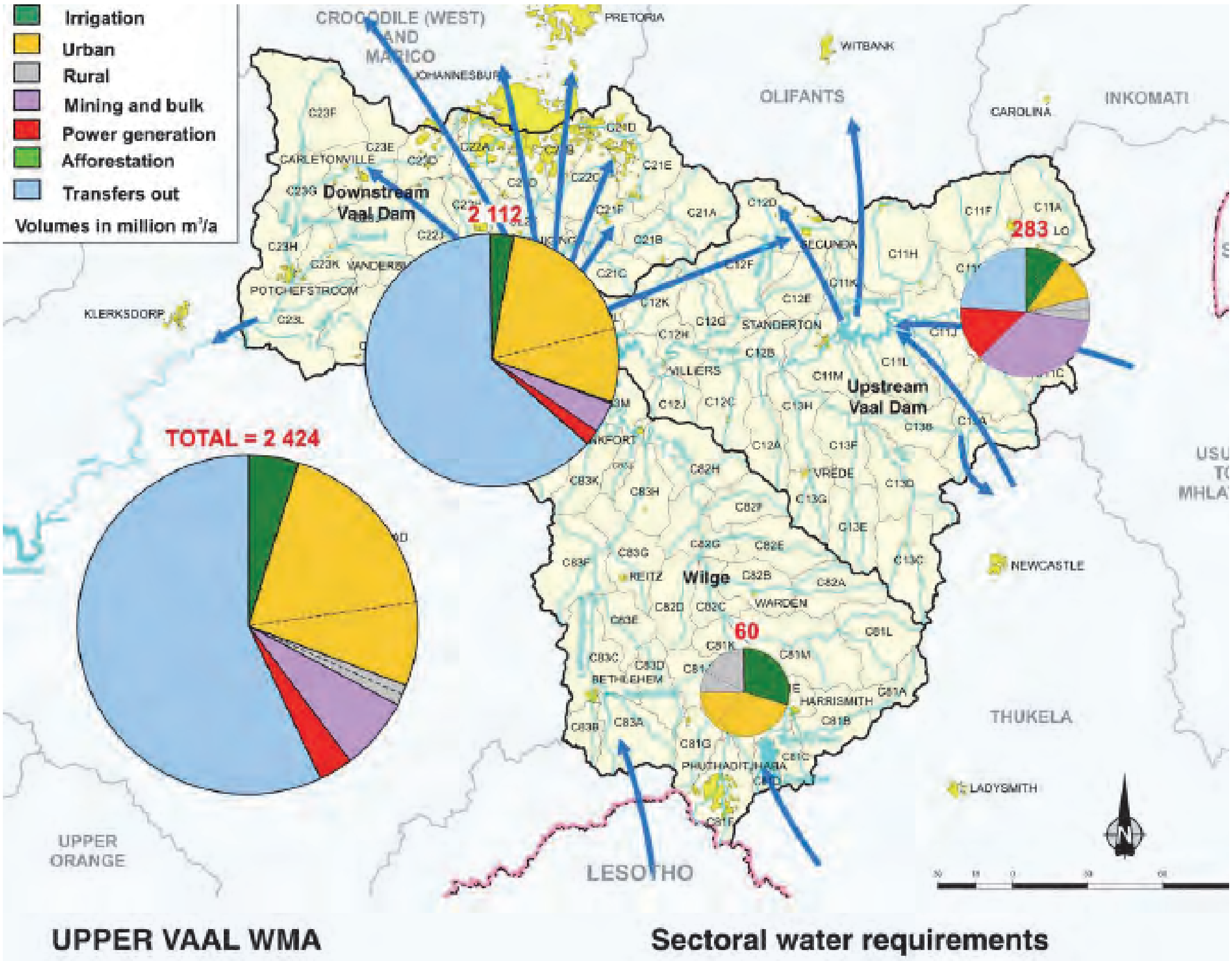


Figure 1 : Water resources utilization in the Upper Vaal WMA (From DWAF, 2003a)

Whilst a significant amount of work has been done on the water quality of the Klip River leading to a lot of knowledge on this aspect, insufficient biomonitoring data including genotoxic impacts on biota in the catchment has been generated. This study seeks to build on work done in previous studies to enhance understanding on this component.

The main aim of this project was to develop a Relative Risk Model as a water quality management tool in the Klip River system. The objectives of the project are to:

i. To integrate the available information (grey and published literature) on the status of the Klip River Catchment together with data from two field surveys to inform subsequent components of the project.
ii. Develop and apply the Relative Risk Model (RRM) to determine the risks based on perceptions of local communities, conservation authorities, municipal authorities and other stakeholders along the Klip River system on the value of the river systems and its associated resources and impacts of contamination.
iii. Validate the risks modelled through the RRM process by linking effects assessment endpoints, i.e. biomarkers (genetic diversity in selected taxa, etc.), fish communities, to pollutant exposure (e.g. heavy metal and organic contamination in tissues of organisms, sediment and water).
iv. To use the outcomes of the RRM and validation study for incorporation into the newly proposed water quality management plan for the Klip River.

## 1.2 Background on the Klip River Catchment

The Klip river catchment is located in the Gauteng Province of South Africa. It covers an area of 3000 $km^2$. The river originates in the range of hills and ridges, which run across the Witwatersrand urban complex in an east-west alignment for approximately 60 km (DWAF, 2009).

### 1.2.1 Sub-catchments

The Catchment is divided into three sub-catchments as shown in Figure 2. These are as follows:

i. **The Upper Klip sub-catchment:** This sub-catchment extends from the eastern Krugersdorp and western Roodepoort to the confluence with the Riet Spruit near Henley-on Klip. Tributaries in this catchment include the Klipspruit, Fordsburg Canal, Robinson Canal, Diepkloofspruit, Harringtonspruit, Russel stream, Bloubosspruit and the Glenvistaspruit.
ii. **The Riet Spruit sub-catchment:** This sub-catchment covers the area from the confluence with the Riet Spruit near Henley-on Klip. Tributaries in this sub-catchment include the Riet Spruit, Natal Spruit, Elsburgspruit, Withospruit and the Valsfontein.
iii. **The Lower Klip sub-catchment:** This is the natural drainage area south of the confluence of the Riet Spruit with the Klip River to the confluence with the Vaal Barrage at Vereeniging.


Figure 2: The Klip River Catchment (Source: http://ceroi.net/reports/johannesburg/csoe/html/nonjava/pollution/water/overwievwaterdpsir.htm)

### 1.2.2 Topography and Geology

The catchment is generally characterized by a gently undulating topography except for several small hill ranges in the northwest, e.g. Klipriviersberg (Klip River Forum, 2004; DWAF, 2009).

The geology of the Upper Klip has been described as complex with formations dipping steeply towards the south and striking in an east-westerly direction. In the north, the basement complex granites are overlain by Hospital Hill sequence interbedded with quartzites and shales. These are in turn covered by Witwatersrand sequence shales, quartzites and conglomerates, which contain the gold-bearing reefs (DWAF, 1999; Kotze, 2002). Tutu et al. (2003 & 2008) describe the typical mineralogical composition of the gold bearing conglomerates of the Witwatersrand region as:

- 70-90% Quartz
- 10-30% Hyllosilicates, which are mostly composed of sericite, $KAl_2(AlSi_3O_{10})(OH)_2$ and,
- 1-5% accessory and minor minerals: Of the more than 70 minor minerals that have been identified in the reefs, pyrite ($FeS_2$) is the most common.

Hard dolomites are encountered at depths of 0-50m below ground throughout most of the Catchment (Kotze, 2002). Mining activities have resulted in sinkholes and other subsidence related landforms, sometimes resulting in loss of human lives due to collapse of mine shafts and or buildings (van Niekerk & van der Walt, 2006).

Wetlands are well developed along the course of the Klip River (Klip River Forum, 2004; Kotze, 2002) especially where it flows on dolomite of the Transvaal Supergroup. Smaller wetlands also occur along its tributaries (Tutu *at al*., 2008). The soil profile changes from rock pinnacles to soft or firm clayey silts over short horizontal distances. Sandy loams are dominant in the upper reaches while clayey loams are more common the lower reaches. Both types are derived from weathering of the dolomites and sandstones (DWAF, 1999; Kotze 2002).

### 1.2.3 Climate

Most of the Klip River Catchment falls under the Highveld eco-region characterized by a warm to hot summer and a short, mild winter dominated by cool to warm days and cold nights. The bulk of the rainfall occurs in summer (October-March) often accompanied by intense thunderstorms. Annual precipitation ranges from 600-732 mm whilst annual potential evaporation is about 1700 mm (Weather Bureau, 1998; Tutu et al., 2008). Based on available data from October 1921 to October 2010, a recent study determined the average mean annual precipitation over the Witwatersrand basin where the Klip River and most of her tributaries originate at 694mm/annum as shown in Figure 3.

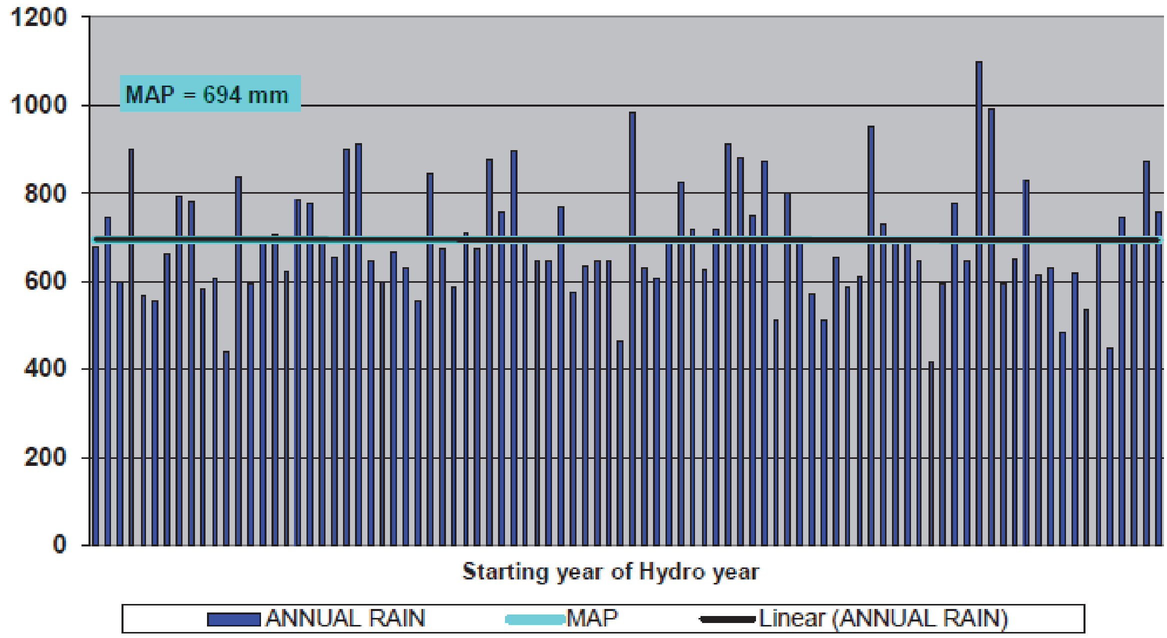


Figure 3: Rainfall pattern (October 1921-October 2010) over the Witwatersrand basin (From DWA, 2012)

### 1.2.4 Vegetation

Most of the Klip river catchment falls under the Bankenveld type vegetation the climax of which is *Acacia caffra* in the north with sour bushveld commonly occurring in the hills and rocky outcrops. Where these habitats support bushveld vegetation, it is dominated by *Protea caffra*, *A. caffra, Celtis africana* and occasionally *P. welwitchii* subspecies *glaberescens*. Traces of temperate or transitional forests with *C. africana, Kigelia africana, Halleria lucida, Leocisidea sericea, Buddlei salvifolia, and Carsinopsis ilicifolia* can be found in sheltered valleys and sinkholes (Kotze, 2002).

The grassveld is sour and wiry and is frequently burnt. Species commonly found are *Trachypogon spicatus, Tristachya hispida, Heteropogon contortus, Panicum nataliensis, Loutetia simplex, Digutaria monodactyla, D. tricholaenoides, Setaria flabellata, Eragrostis racemosa* and *Themeda triandra* (Kotze, 2002). Common forbs include *Spenostylis angustifolia, Senecio coronatus, Helichrysum acutatum, Indigofera hilaris, Jucicia anagalloides* and *Veronia natalensis*.

The lower reaches of the catchment are dominated by *Cymbopogon-Themeda* veld with a mixed to sour grassveld climax. Sparser and more tufted variations are dominated by *S. flabellate. T. traindra, H. contortus* and *Eragrostis* species are found towards the north (DWAF, 1999; Kotze, 2002).

Encroachment by exotics, mainly *A. mearnsii* (Black Wattle) in the upper reaches and *Eucalytus grandis* (Blue Gum) along the river (DWAF, 1999; Kotze, 2002) has been reported. The wetlands are dominated by *Phragmites* and *Typha* spp. reeds (Kotze, 2002; Tutu et al., 2008).

### 1.2.5 Land Use on the Klip River Catchment

The discovery of gold in the Witwatersrand area in the late 1800s led to a gold rush and the establishment of the early mines in the Johannesburg area which provided the nucleus for the gold-mining industry, which subsequently played a dominant role in the economy of South Africa. Gold mining began in 1886 (Winde & Sandham, 2004) initially with gold being extracted from coarsely crushed ore using Mercury amalgam. The tailings were deposited in large dumps that are now a common landscape feature in the Klip River Catchment (Tutu et al., 2008) especially in the headwaters of the catchment. With this method becoming increasingly inefficient, mercury amalgam extraction was followed in 1915 by cyanide extraction which required finer milling of the ore. The tailings of this process were piped to deposit sites called slime dumps (Tutu et al., 2008). Approximately two hundred and forty (240) mine tailings dumps have been registered in the Witwatersrand Basin, with one hundred and three (103) in the Central Rand (Ndasi, 2007). The total river catchment area includes 6 $km^2$ of sand dumps and 33 $km^2$ of slime dams concentrated in the upper reaches (Howie & Otto, 1996).

The Witwatersrand basin auriferous ore bodies commonly referred to as reefs also contain uranium ($U_3O_8$) estimated at 100-300 ppm. Though somewhat of low grade when compared to uranium ores in Australia and Canada at 5 000-500 000 ppm (0.5-50%), uranium has been produced mainly as a by-product of gold by mines in the Far West Rand, Klerksdorp and Free State goldfields since 1952 (Winde & Sandham, 2004).

The headwaters and the downstream reaches of the catchment are dominated by urban development with the city of Johannesburg and neighbouring satellite towns lying at the head of the catchment, whilst Vereeniging, an industrial town, located at the confluence of the Klip River and the Vaal Barrage (DWA, 2009), is the main development in the lower reaches.

Agricultural and market gardening activities dominate the catchment in between the urban centres although urban expansion and informal settlements from both the Johannesburg and Vereeniging directions are encroaching towards one another. An estimated 200 ha in the catchment is under irrigation in the area downstream of the Waste Water Treatment Works

(WWTWs) where vegetables including cabbages, carrots, pumpkin, squash and potatoes are grown (Howie & Otto, 1996). An additional 1000ha is used for instant lawn cultivation in the Eikenhof area. A 3 500ha farm owned by Rand Water is used for cultivation of maize, lucerne and wheat. Watering of livestock including cattle, sheep and pigs also occurs on the catchment.

### 1.2.6 Water Use

Although the Klip River may have been a perennial river before discovery of gold in the Witwatersrand, discharge in the river is now dominated by water from treated sewage, treated and untreated discharges from industrial sources, water pumped from mines in addition to surface water from rain. The natural run-off in the Klip River catchment has been estimated at 111 x $Mm^3$/annum (Stewart Scott, 1996). Kaffri & Foster (1989) estimate the annual recharge of the aquifer due to effluent to be three times the natural recharge by rainfall. Water use in the catchment is as follows:

- **Domestic water use:** The bulk of potable water in the Catchment is supplied by Rand Water through municipalities. Domestic use of Klip River water is thought to be largely restricted to laundering of clothes in the informal settlements that occur along the Klip River and its tributaries (Howie & Otto, 1996, Klip River Forum, 2004; Kotze, 2002).

- **Agricultural water use:** It is currently estimated that up to 11 $Mm^3$/annum of water is abstracted from the Klip River for agricultural activities mainly for crop irrigation and livestock watering (Klip River Forum, 2004).

- **Waste water treatment works:** Four WWTWs owned by the East Rand Water Care Company (ERWAT) on the East Rand and three WWTWs in southern Johannesburg (Randwater) are some of the sources of point pollution in the Klip River. 27 Ml/day of treated effluent from the Klipspruit together with 45 Ml/day of effluent from the Goudkoppies WWTW have previously been used to cool the Orlando Power Station, which is no longer in operation. Another 65 Ml/day was used for pasture irrigation and the surplus of 260 l/day discharged into the Klip River (Stephenson, 1998). Further down, the Meyerton WWTW and its industrial area, together with the town of Vereeniging, contribute to the pollution load in the Klip River to varying extents (DWAF, 2009).

- **Industrial Water use:** Following the closure of gold mines and the use of purified water instead of river water for industrial processing, the East Rand Proprietary Mines (ERPM) gold mine on the East Rand now remains the only significant point pollution source in the middle reaches of the Klip River system. It is estimated that industrial use of Klip River water has declined to less than $7.5 \times 10^6$ $Mm^3$/annum (Klip River Forum, 2004).

- **Industrial water use** is now restricted to use by NAMPAK, EVERITE and other processing industries in the middle reaches. Industrial water is now also supplied by Rand Water.

- **Recreational use:** Recreational water use in the Klip River Catchment includes swimming, fishing, canoeing and tubing downstream of the Klipspruit. Full immersion baptisms also occur along parts of the river.

Table 1 shows a summary of sources of pollution in various regions of the Klip River.

### 1.3 Water Quality and Biomonitoring Studies on the Klip River Catchment

The ecological integrity of the Klip River is considered to be impaired due to the following factors as identified by a report of the Department of Water Affairs (DWAF, 2003)

- A modified hydrological regime resulting from an altered seasonal flow regime linked with return flows from WWTWs and agricultural activities in the catchment,
- Changes in water quality as a result of surface runoff from urban areas and mine effluents,
- Changes in stream morphology and in-stream flow arising as a result of construction of numerous weirs and bridges,
- Removal of the natural riparian vegetation due to urbanisation, agriculture and industrial development, and,
- Erosion within the river channel.

Table 1: Potential sources of point and diffuse pollution in various regions of the Klip River

| Sources of Point Pollution | Diffuse Pollution |
|---|---|
| **1. Klip River upstream of the Klipspruit confluence** | |
| • Durban Deep Roodepoort Mine (pumping ceased in 1998) | • Slimes dams/ rock dumps and old waste sites at mines<br>• Informal settlements at Durban Deep Roodepoort Mine, Kagiso and Soweto including extensive Doornkop settlement<br>• Leaking sewers mainly in Soweto area<br>• Industrial areas of Chamdor<br>• Closed Solid Waste at Dobsonville |
| **2. Klipspruit tributary** | |
| • Orlando Power Station (now not operating) | • Slimes dams/ rock dumps and old waste sites at mines<br>• Central Gold recovery mine slimes<br>• Informal settlements in CBD and<br>• Leaking sewers mainly in CBD and Soweto area<br>• Industrial areas of Main Reef Road, Industria, Newtown and Selby<br>• Marie Louise and Robinson Deep solid waste sites and the now closed solid waste site at Meredale |
| **3. Klip River between Klipspruit and Riet Spruit confluence** | |
| • Goudkoppies, Olifantsvlei, Bushkoppies and Watervaal WWTWs | • Informal settlements in Lenasia, Eikenhof and Eldorado Park<br>• Leaking sewers mainly in the Eldorado Park Area<br>• Industrial areas of Kliprivier<br>• Goudkoppies solid waste site<br>• Agricultural run-off |
| **4. Riet Spruit tributary** | |
| • ERPM Gold Mine<br>• Rondebult, Dekema and Vlakplaats WWTWs | • Slimes dams/ rock dumps and old waste sites at mines<br>• ERGO & Central Gold Recovery slimes dam reclamation<br>• Informal settlements in central Johannesburg along Main Reef Road in Germiston, Katorus, kwa-Thema and Zonkizizwe<br>• Leaking sewers mainly in the Katorus Area<br>• Industrial areas of Village Deep, Alrode, Boksburg, etc.<br>• Agricultural run-off |
| **5. From Rietspruit confluence to Vaal Barrage confluence** | |
| • Meyerton WWTW<br>• Glen Douglas Dolomite Mine | • Industrial areas of Daleside, Meyerton and Iscor<br>• Old Springfield Colliery<br>• Solid waste site on Henley-on-Klip, Walkerville & Waldrift and closed site on Meyindustria<br>• Agricultural run-off |

(Based on Kotze, 2002)

### 1.3.1 Physico-chemical properties

Water quality refers to the physical, chemical and biological characteristics of water and determines its suitability to support and sustain various uses or processes for which it may be used as well as human health and ecosystem concerns. Water quality takes into account the physical characteristics of the water as well as the concentration and state of organic and inorganic material present in the water (Osman & Klaus, 2010). Water quality monitoring enables two main aspects to be determined:

a. The actual physical and chemical characteristic of water for a time period and,
b. Changes in the properties of water over time through multiple monitoring events.

Kotze 2002 carried out a study aimed at determining the ecological integrity of the Klip River. The study was based on ten sites as shown in Table 2.

Table 2: Sites used in ecological integrity study

| SITE | LATITUDE | LONGITUDE | DESCRIPTION |
|---|---|---|---|
| **SITE 1** | -26° 10.154’ | +27° 50.015’ | Located in the upper catchment close to the source below Princess and Skinners dams in a built up area with informal settlements. |
| **SITE 2** | -26° 12.837’ | +27° 48.776’ | Below gold mining and informal settlement areas before entering western boundary of Soweto. |
| **SITE 3** | -26° 17.912’ | +27° 50.538’ | In main stream of Klip river below wetland and informal settlement areas of Soweto before entering Lenasia |
| **SITE 4** | -26° 20.234’ | +27° 54.185’ | Middle reaches below confluence with Klipspruit and discharge from Olifantsvlei WWTW |
| **SITE 5** | -26° 22.855 | +28° 04.296’ | After a number of wetlands in an area of high agricultural activity at Rand Water Zwartkoppies Farm |
| **SITE 6** | -26° 27.396’ | +28° 05.173’ | Lower catchment below confluence with Riet Spruit |
| **SITE 7** | -26° 33.000’ | +28° 03.869’ | Lower catchment directly below Henley-on-Klip Weir |
| **SITE 8** | -26° 36.508’ | +28° 00.162’ | Below Meyerton town and industrial activity, Rothdene area |
| **SITE 9** | -26° 38.965’ | +28° 57.146’ | 2Km before confluence with Vaal Barrage |
| **SITE 10** | -26° 37.547’ | +28° 27.927’ | Reference site on the Suikerbosrand upstream of the confluence with the Blesbokspruit. |

(From Kotze, 2002)

Ferreira et al. (2010) report on a study conducted by Econ@uj, a multi-disciplinary consortium of environmental specialists based in the Zoology Department of the University of Johannesburg. The team carried out a baseline biomonitoring survey of the Klip River along with a Fish Health Assessment with the objective of informing development of a water quality management plan for the Klip River system. The group selected eight sites based on the study previously carried out by Kotze (2002), but also selected to ensure representation of the various sources of impacts on the River system throughout its length. The sites selected are described briefly in Table 3 and shown in Figure 4.

Table 3: Description of sites selected for biomonitoring survey in Econ@uj study of Klip River

| | |
|---|---|
| **Site 1:** Located near Roodepoort. Located near the source. River less than 2m wide and shallow. Water clear. Habitat integrity largely natural. Habitat consists of different flow types resulting in riffles and pool areas. Cobble beds and a variety of different types of marginal vegetation are also present. | **Site 5:** Positioned in the middle reaches below confluence with the Riet Spruit. Serious erosion of the stream banks at this site resulting in banks being near vertical. River is more than 10 meters wide and is very deep in some sections. In-stream habitat consists of a variety of different stones, gravel sand and mud and different marginal vegetation. |
| **Site 2:** Located near Lenasia above a large wetland. Site lies below impacts of Western Soweto and the gold mining activities (Durban Roodepoort Deep and ERPM). River now wider with a variety of flow types. Gravel, sand and mud, different sized stones and a variety of marginal vegetation is also present at this site. Evidence of a recent fire, litter dumping and there are numerous roads and railway lines near the site. | **Site 6**: Positioned in the lower reaches near the weir at Henley on Klip. Flow alterations due to artificial island constructed just above the weir leading to siltation above the weir and growth of reeds. Below the weir the habitat resembles the in-stream habitat of most of the other sites. Abundance of stones, different marginal vegetation and gravel also present. Surrounding land use largely in the form of residential areas. |
| **Site 3:** Positioned near the Olifantsvlei Waste Water Treatment Works (WWTW). River about 10 m wide and much deeper. Variety of stones, gravel, sand and mud and different marginal vegetation available as habitat. Altered flow regime due to effluent from WWTW, dam upstream of site. Large quantities of litter at the site. Site is frequently visited by the local communities for fishing purposes. | **Site 7:** Located in the lower reaches of the river, below Meyerton and the various industrial activities near the town. Site is also located near an obstruction in the form of a man made weir. Above weir river is very deep with very little and below the weir it resembles other study sites in terms of habitat. The flow below the weir is very strong and both the stream banks are near vertical due to serious erosion. Land use largely consists of natural vegetation although a pump station is located above the weir. |
| **Site 4**: Located in the middle reaches of the river downstream of agricultural activities and large wetlands. In-stream habitat consists of stones, gravel sand and mud and different marginal vegetation. Flow relatively uniform with very few pooled areas. Major erosion of the stream | **Site 8:** Positioned in the lower reaches of the river. The river at this point represents a typical mature river-. wide, deep and the water is very turbid. The habitat at this site has also changed. Decreased erosion of the stream banks. Loss in riffle habitat and the most dominant habitat is in the form of marginal vegetation. |

(Source Ferreira et al., 2010).

The study analysed a number of water quality attributes over the eight sites. The variables analysed were pH, conductivity, temperature, oxygen saturation, oxygen content, ammonium, phosphate, nitrites, nitrates, sulphates, chemical oxygen demand (COD), chloride, total hardness and turbidity. The results of the analysis together with Target Water Quality Requirement (TWQR) for Aquatic Ecosystems are shown in Table 4.

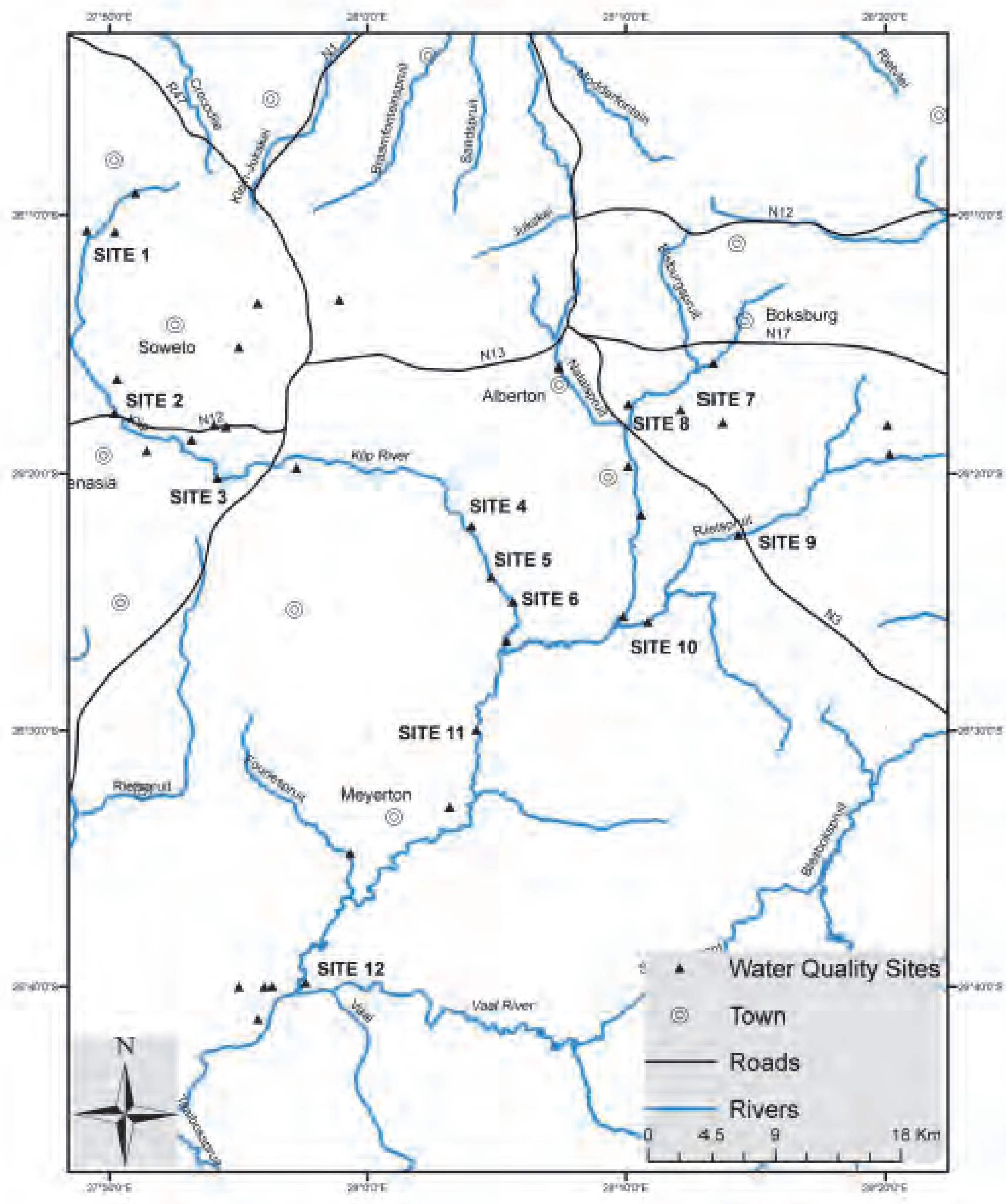


Figure 4: Location of sampling sites in Econ@uj biomonitoring study

Table 4 shows that most of the water quality parameters fall within the Target Water Quality Requirement (TWQR) for most sites. The exception here is the oxygen saturation, which dropped below the required 80% to 120% at sites 3, 5 and 8.

Table 4: Results of water quality analysis studied in Econ@uj study

| VARIABLES | TWQR | SITES | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| **pH** | 6.0-9.0 | 7.57 | 7.73 | 7.59 | 7.78 | 7.71 | 7.77 | 7.76 | 7.67 |
| **Conductivity (μS/cm)** | 0-70 mS/m | 270 | 647 | 606 | 604 | 648 | 668 | 659 | 591 |
| **Temperature (°C)** | > 2°C /10% of reference value | 24 | 17.5 | 20.5 | 19.3 | 19.6 | 22.6 | 20.8 | 19.4 |
| **Oxygen saturation** (%) | 80-120% saturation | 93 | 82.4 | 73.3 | 97.2 | 78 | 90.8 | 83 | 75 |
| **Oxygen content** (mg/l) | | 6.4 | 6.54 | 5.26 | 7.55 | 6.01 | 6.49 | 6.25 | 5.82 |
| **Ammonium** (mg/l) | 0-1 | 0.25 | 0.40 | 0.89 | 0.26 | 0.35 | 0.46 | 0.52 | 0.30 |
| **Phosphate** (mg/l) | | 0.13 | > 5.00 | 1.10 | 0.39 | 0.46 | 1.35 | 3.36 | 0.65 |
| **Nitrites** (mg/l) | 0-6 | 0.20 | 0.04 | 0.16 | 0.29 | 0.25 | 0.27 | 0.31 | 0.23 |
| **Nitrates** (mg/l) | | < 0.25 | < 0.25 | 7.0 | 10.9 | 11.0 | < 0.25 | < 0.25 | < 0.25 |
| **Sulphates** (mg/l) | 0-200 | > 300 | > 300 | > 300 | 75 | < 1 | < 1 | < 1 | < 1 |
| **COD** (mg/l) | N/A | 16.3 | 16.5 | 21.5 | > 40.0 | > 40.0 | 34.2 | > 40.0 | 26.9 |
| **Chloride** (mg/l) | 0-100 | 18.8 | 20.1 | > 25.0 | > 25.0 | > 25.0 | > 25.0 | < 10 | < 10 |
| **Total Hardness** (mg/l) | N/A | > 70 | > 120 | > 120 | > 120 | > 120 | > 120 | > 120 | > 120 |
| **Turbidity** (FAU) | 0-1 | 14 | 21 | 18 | 36 | 38 | 32 | 20 | 29 |

(Source: Ferreira et al., 2010)

Other variables of concern noted included:

- **Sulphates**: Three sites displayed concentrations in excess of 300 mg/l.
- **Turbidity:** All the sites had turbidity above 20 FAU associated with severe aesthetic effects (appearance, taste and odour). At this level, the water carries an associated risk of disease due to infectious disease agents and chemicals adsorbed onto particulate matter as well as a chance of disease transmission at epidemic level exists at high turbidity (DWAF, 1996).
- **Phosphate:** The concentrations of phosphates measured all of the sites were well above the 0.13 mg/l, which is considered to be the level above, which serious eutrophication risks are likely to occur (Van Ginkel, 2011).

The study also used historical monitoring data to conduct a multivariate analysis with intent of determining the relative importance of temporal and spatial variations on the water quality.

This analysis was based on Ca, Cl, COD, EC, Faecal coliforms, Total Hardness, K, Na, $NH_3$, $NH_4$, $NO_3$, $NO_2$, pH, $PO_4$, and $SO_4$. The findings of the multivariate analysis have a number of implications for the water quality of the Klip River. Amongst these are the following:

- Mean flow rate within the Klip River for each month based analysis carried out with data for the period 1996 to 2004 was 32.23 ± 7.02 $m^3$ /sec. The low variance shows that there is little variation in the flow rate across different seasons.
- Sites downstream of WWTWs clustered together with other sites indicating that other sites also show high levels of faecal coliforms. This implies that WWTWs are not the only source of faecal coliforms on the Klip River.
- There was also little variation in water quality between sites upstream and downstream of mining activities due to little variation in variables like electrical conductivity.
- Sites on the Elgspruit appeared distinct from other sites due to sodium and potassium salts but the source of these was not identified.

Other components of the study are shown in Figure 5.

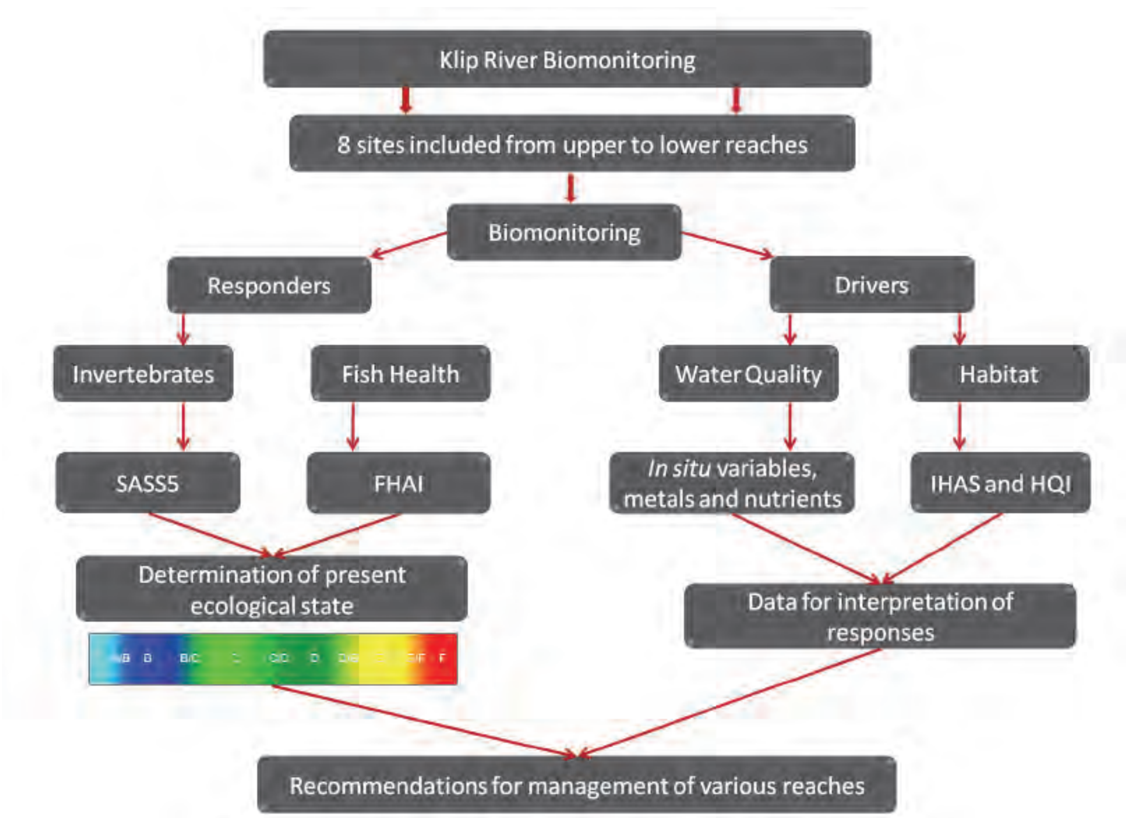


Figure 5: Components of the Econ@uj study (Ferreira et al., 2010)

Dzawiro et al. (2011a) used ideal catchment background (ICB) values for the Vaal dam, Vaal barrage, Klip River and Blesbokspruit/Suikerbosrand Rivers sub-catchments to develop a harmonised in-stream water quality guideline for the Upper and Middle Vaal Water WMAs. The study used data for the period 2003 to 2009, which was then interpolated to a daily time-step over 2526 days for 21 monitoring sites covering both WMAs shown in Table 5, which also shows the ecological functionality (EF) for each site based on their analysis.

Table 5: Sites used in Upper & Middle Vaal WMA ecological integrity study

| CATCHMENT | CODE | STRATEGIC POSITION | EF |
|---|---|---|---|
| **Blesbokspruit/ Suikerbosrand Sites** | B1 | On the Blesbokspruit River, a tributary which enters the Suikerbosrand River between S1 and S4 | |
| | S1 | Most upstream point considered on Suikerbosrand River | |
| | S4 | Located upstream of the confluence with the | |
| **Klip River Sites** | K1 | Waterval River, a tributary of Riet Spruit at Waterval. It enters the Klip between K2 and K4. | |
| | K2 | Most upstream point considered on the Riet Spruit. | |
| | K3 | On CT, a tributary of RwR. It flows into RwR between K2 and K4 | |
| | K4 | Most downstream point on RwR before confluence Klip River with the Riet Spruit | |
| | K6 | On Klip River and downstream of confluence with the Riet Spruit | |
| | K9 | Most downstream point on Klip River before confluence with the Vaal Barrage | |
| | K10 | Most upstream point considered for Klip River | |
| | K12 | On the Natal Spruit | |
| **Vaal River Sites** | T1 | Most downstream point on Taaibospruit River before the confluence with the Vaal River | |
| | L1 | Most downstream point on Leeuspruit River before the confluence with the Vaal River | |
| | R1 | Most upstream point considered on RvR | |
| | R2 | Most downstream point on RvR before confluence RvR/VR | |
| Vaal Barrage Sites | V2 | Upstream of confluence Suikerbosrand/Vaal Rivers and just downstream of the Vaal dam wall | |
| | V7 | Vaal barrage at 37 km from the Barrage wall | |
| | V9 | Vaal barrage at 24 km from the Barrage wall | |
| | V12 | Barrage wall and just downstream of the confluence with the Vaal River | |
| | V17 | Midvaal Water Board raw water intake works | |
| | V19 | Sedibeng Water Board raw water intake works | |

| Median EC (mS/m) | Colour Code | Description |
|---|---|---|
| 10-18 | | Good quality |
| 19-45 | | Fairly good quality |
| 46-80 | | Intermediate impact |
| 80 < | | Highly impacted |
| 81-100 | | Fairly highly impacted |

(From Dzawiro et al., 2011a)

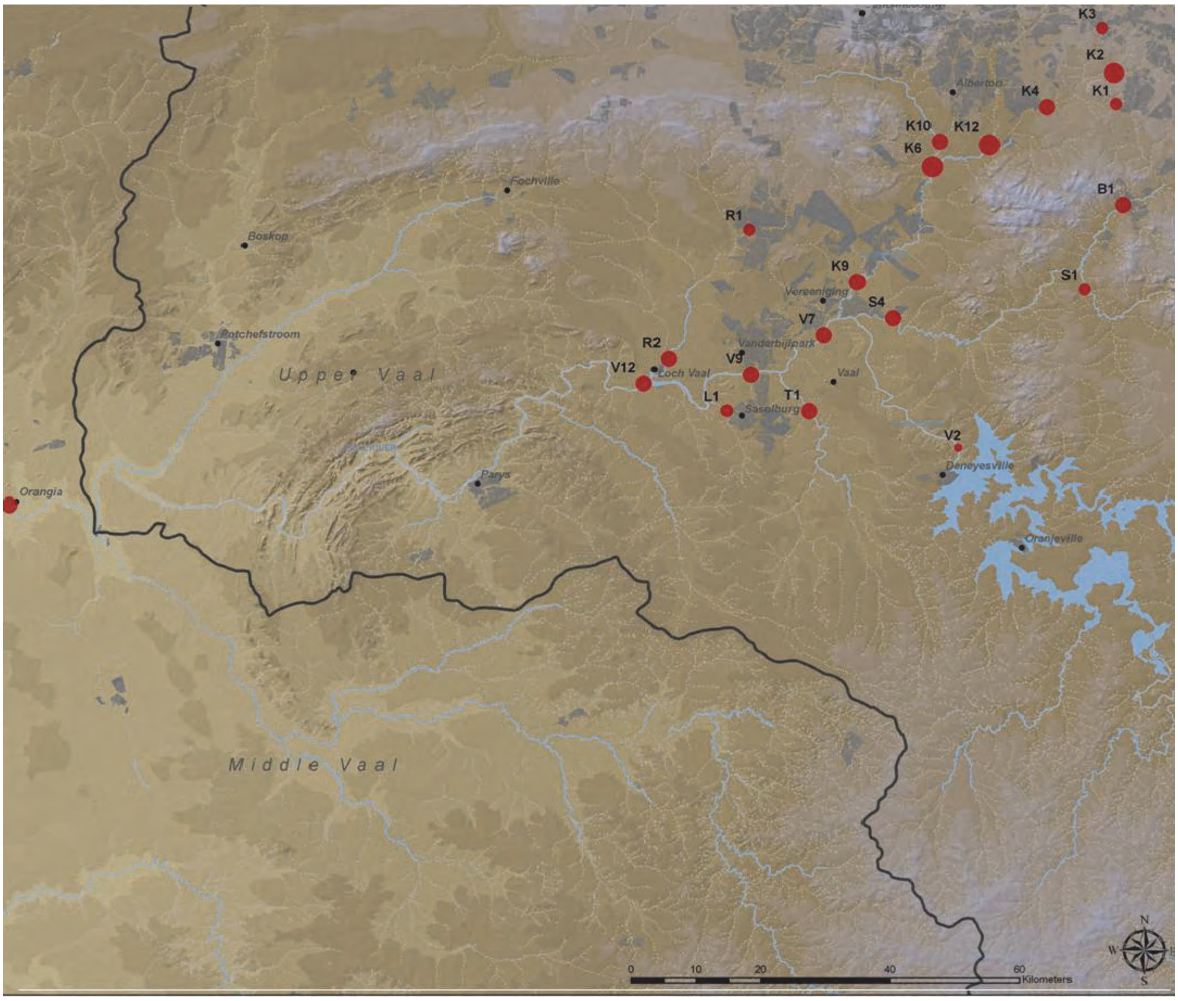


Figure 6: Location of sites used for the Upper & Middle Vaal WMA study

(From Dzawiro et al., 2011b)

The study used conductivity as a surrogate to capture the variability in water quality. The daily interpolated EC values were compared to guideline values of the ideal catchment background raw water quality objectives for each sub-catchment involved in the study and applicable to the Upper and Middle Vaal WMAs (Dzawiro et al., 2011a). Key findings of the study include:

- Mine dumps around Riet Spruit were having a huge impact on through K2 and K4 as K1 and K3 values were within acceptable guideline values.
- K12 was above the upper limit for EC whilst K10 was above the limit for some sampling periods.

### 1.3.2 Sediment Analysis

The Econ@uj study also carried out sediment analysis to determine a number of variables including water content, organic content as well as sediment grain size. The results of this analysis are tabulated in Table 6. The highest organic content was recorded at Site 2 thought to be due to effects of the WWTWs located upstream from the site. The other sites showed moderate organic content (Ferreira et al., 2010).

Table 6: Results of the sediment analysis

| Site | Water content (%) | Organic content (%) | Organic content (classification) | Sediment grain size | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | > 4000 µm | 4000-2000 µm | 2000-500 µm | 500-212 µm | 212-53 µm | < 53 µm |
| 1 | 30.02 | 3.25 | Medium | 3.05 | 6.50 | 28.91 | 31.29 | 19.66 | 10.60 |
| 2 | 49.12 | 28.81 | High | 5.96 | 7.53 | 25.27 | 37.20 | 8.01 | 16.04 |
| 3 | 20.80 | 3.99 | Medium | 24.04 | 21.88 | 27.13 | 17.24 | 8.73 | 0.98 |
| 4 | 26.67 | 2.53 | Medium | 0.67 | 1.98 | 6.37 | 44.76 | 34.14 | 12.08 |
| 5 | 27.60 | 2.08 | Medium | 0.11 | 0.85 | 12.12 | 64.24 | 17.36 | 5.33 |
| 6 | 28.09 | 4.46 | High | 39.92 | 10.18 | 7.88 | 13.44 | 16.22 | 12.36 |
| 7 | 20.12 | 1.63 | Moderately low | 2.31 | 2.78 | 19.64 | 48.30 | 20.77 | 6.20 |
| 8 | 33.69 | 5.58 | High | 1.22 | 4.93 | 12.38 | 19.46 | 28.38 | 33.63 |

The classification of percentage organic content was based on United States Environment Protection Agency (1991) as follows:

| Classification of organic matter | |
|---|---|
| Very low | < 0.05% |
| Low | 0.05-1% |
| Moderately low | 1-2% |
| Medium | 2-4% |
| High | > 4% |

(Based on Ferreira *et al*., 2010)

Sediment grain size classification followed Cyrus et al. (2000) as follows:

| | |
|---|---|
| > 4000 µm | Gravel |
| 4000 µm-2000 µm | very coarse sand |
| 2000 µm-500 µm | coarse sand |
| 500 µm-212 µm | medium sand |
| 212 µm-53 µm | very fine sand |
| < 53 µm | Mud |

### 1.3.3 Acid Mine Drainage & Salination

Acid mine drainage (AMD) has been described as the single most significant threat to the water resources in the mining areas of central South Africa (Younger, 2001). AMD refers to metal ion-rich water formed as a result of a chemical reaction between water and rocks containing sulphur-rich minerals, which leads to acidic run-off. Whilst this oxidation does occur in undisturbed rocks, mining has the effect of accelerating it due to an increase in the surface area exposed which in turn leads to excess generation beyond the natural buffering capacity of water (Ochieng et al., 2010).

In 1985, the total salt load of the Vaal Barrage catchment due to mining activity was estimated at 50 000 tonnes/annum out of a total of 400 $x10^3$ tonnes/annum due to all sources. For the Klip River catchment alone, Howie & Otto (1996) estimated this to be 20 x $10^3$ tonnes/annum largely attributed to a mine dewatering programme at the head of the catchment. AngloGold Ashanti (2004) estimated that there are 270 tailing dams covering 400 $km^2$ in the Witwatersrand region. Most of these are unlined and not vegetated and thus are a source of dust, soil and water pollution (AngloGold Ashanti, 2004; Oelofse et al., 2010). Potential environmental impacts of these dams identified by AngloGold Ashanti (2004) in Oelofse et al. (2010) include:

- Contamination of streams as a result of surface run-off from the impoundment area,
- Air and water pollution as a result of wind erosion of dried-out tailings,
- Potential for dam failure and release of slimes,
- Physical and aesthetic modification to the landscape and the environment,
- Difficulty of establishing vegetative cover to permanently stabilize the tailings due to unfavourable soil conditions in the presence of pyritic tailings.

The majority of dams have remained undisturbed for more than a century and as a result they have been exposed to oxidation by rainwater. It has been suggested that this oxidation has reached a depth of 5m in slimes dams whilst in slimes dumps this reached a depth of 2m (Marsden, 1986; Oelofse et al., 2010). Some impacts of AMD cited by Oelofse et al. (2010) include:

- Reduced pH which can be as low as 2.5,
- Increased electrical conductivity,
- Elevated ion concentrations,
- Increased mobilization and concentration of toxic heavy metals,
- Reddish brown colouration of water,

- Reduced soil quality,
- Sedimentation, and,
- Disruption of the flora and fauna.

Oelofse et al. (2010) analysed the hydrochemistry of water of a 50 m high slimes dam complex located in Randfontein in the West Rand area. Groundwater drainage in the area is dominated by a spring that rises 380 m to the NNW of the dam. In the study the hydrochemistry of water, from the spring and that of water from a borehole 590 m from the dam in the vicinity, was compared to natural dolomitic groundwater in the wider region. In March 2007, discharge was estimated at 25 l/s. The results of the water quality analysis are shown in Table 7.

Table 7: The Groundwater chemistry in the vicinity of a slimes dam

| **Parameter** | **Spring water** | | **Borehole** | | **Natural** | | **SANS 241: Class 1** |
|---|---|---|---|---|---|---|---|
| pH | 3.9 | NC | 6.1 | ✓ | 7.2 | ✓ | 5.0-9.5 |
| Electrical conductivity (ms/m) | 265 | NC | 111 | ✓ | 17 | ✓ | < 150 |
| Calcium (mg/*l*) | 262 | NC | 101 | ✓ | 16 | ✓ | < 150 |
| Magnesium (mg/*l*) | 133 | NC | 57 | ✓ | 10 | ✓ | < 70 |
| Sodium (mg/*l*) | 111 | ✓ | 60 | ✓ | 4 | ✓ | < 200 |
| Potassium (mg/*l*) | 7.8 | ✓ | 3.6 | ✓ | 0.5 | ✓ | < 50 |
| Chloride (mg/*l*) | 98 | ✓ | 70 | ✓ | 2.5 | ✓ | < 200 |
| Sulphate (mg/*l*) | 1516 | NC | 447 | NC | 22 | ✓ | < 200 |
| Total alkalinity | 2.5 | ✓ | 16 | ✓ | 56 | ✓ | < 400 |
| Nitrate (mg/*l*) | 4.1 | ✓ | 6.5 | ✓ | 1.6 | ✓ | Unspecified |
| Flouride (mg/*l*) | 0.1 | ✓ | 0.1 | ✓ | 0.1 | ✓ | < 10 |
| Iron (mg/*l*) | 0.103 | ✓ | 0.102 | ✓ | 0.031 | ✓ | < .0 |
| Manganese (mg/*l*) | 100 | **NC** | 0.035 | ✓ | 0.012 | ✓ | < 0.1 |
| Zinc (mg/*l*) | 0.433 | ✓ | 0.102 | ✓ | 0.012 | ✓ | < 5.0 |

**NC denotes non-compliance with SANS 241 for drinking water quality**

(from Oelofse et al., 2010).

The findings of the study revealed that the spring water had compromised drinking water quality on six parameters (i.e. pH, electrical conductivity, calcium, magnesium, sulphate and manganese) compared to one (sulphate) in the ground water although when compared to the natural spring water the groundwater showed elevated levels for most of the parameters measured, as shown in Table 8 (Oelofse et al., 2010).

Rosner et al. (2000) reported elevated concentration of heavy metals in top soil exposed after reclamation of mine dumps. Though not labile, these metals can potentially be leached into ground water in the long term.

Reports of salination in the Witwatersrand date back decades. Wintroupe (1933) reported high levels of salination resulting from elevated levels of chlorides, sulphates and carbonates of sodium, calcium and magnesium. Other studies have shown similar results in the gold mining areas of the Klip River Catchment. Harrison (1958) conducted a study on the Klip River and the Klipspruit near their confluence at the Olifantsvlei.  Results of various parameters measured in the study are shown in Table 8.

Table 8: Results of water quality analysis of samples from the Klipspruit and the Klip River

| Parameter Measured | Locality | |
|---|---|---|
| | **Klipspruit** | **Klip River** |
| pH (wet season) | 5.2-6.8 | 3.7-4.3 |
| pH (dry season | 6.1-7.8 | 4.0-4.8 |
| Total Dissolved Solids | 1377-1450 mg/l | 930-1530mg/l |
| Sulphates | 570-1100 mg/l | 405-1660 mg/l |
| Chlorides | 120-130 mg/l | 20-30 mg/l |
| Calcium | 380-516 mg/l | 358-580 mg/l |
| Magnesium | 235-240 mg/l | 162-221 mg/l |
| Turbidity | Low | Nil |
| Saline ammonia nitrogen | 0.06-3.0 mg/l | Undetectable-0.45 mg/l |
| Nitrate as nitrogen | 1.4-5.6 mg/l | 0.1-0.2 mg /l |

(From Harrison, 1958)

Table 8 shows that the pH is generally lower over the wet summer months compared to the drier months. This was ascribed to high run-off over the mining areas as a result of rainfall in summer. The higher levels of nitrogen in the Klipspruit were thought to be due to sewage effluent entering the Klipspruit. The study also reported differences in faunal and floral composition at these two sites as will be discussed later.

More recent studies aimed at investigating the water quality in samples collected from sites in the vicinity of mining activity related sites showed significant changes in water quality parameters at these sites. Tutu et al. (2008) analysed samples from 47 sites located in the upper reaches of the Klip River.  Of these, 30 were associated with streams, 14 with dams and three with wetlands.

The analysis was based on four groups of parameters, i.e. field parameters (pH, Electrical conductivity), major anions including sulphates, nitrates, chlorides; major cations such as sodium, potassium, calcium and magnesium as well as trace elements.

The results of the analysis are shown in Figures 7-10, which show results obtained for pH, EC, sulphate and iron.

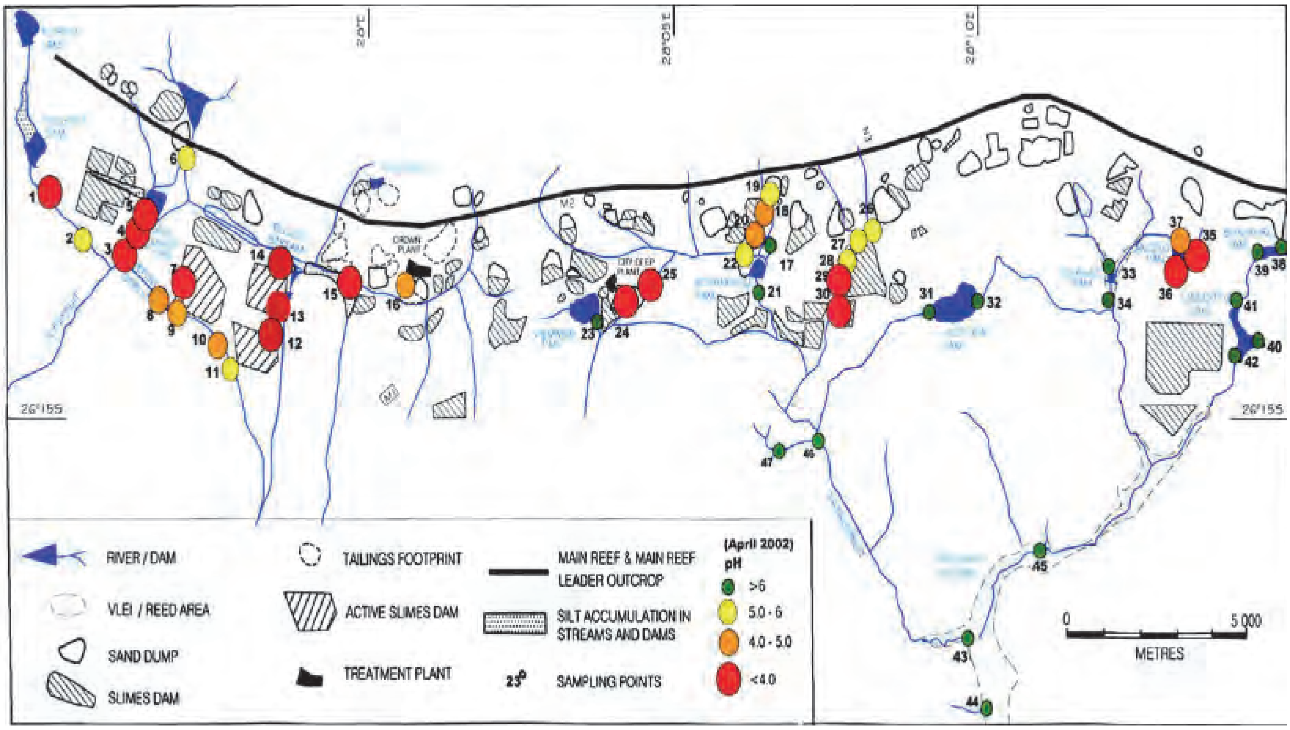

Figure 7: pH values recorded at various sites

Of the sites involved in the study, 14 sites had pH values lower than 5, 7 were in the 4.5-5.0 range whilst 8 were in the 5.0-6.0 range.

- Points 15 and 24 had the lowest pH recorded at 2.5 and 2.3 respectively.  Site 24 is located immediately south of a reprocessing plant, thus the authors suggest that oxidation of remnant tailings left a footprint, which may have resulted in acidification of the nearby stream.
- pH of > 6.0 is observed only in paddocks where tailings had been rehabilitated some time previously and away from reprocessing activities. Hence most dams and lakes, e.g. Boksburg Dam (sites 38 and 39), Cinderella Dam (sites 40-42) and Victoria Lake (sites 31 and 32) also showed pH > 6.
- The Klipspruit and Natal Spruit drainages leaving the area generally have pH values of < 5.0 (Tutu et al., 2008).

- The Wetland sites had high pH values, ranging between 7.1-8.5. This is an indication that wetlands play an important role in the regulation of water quality. Dam samples also had a high pH, all having values > 7.0.

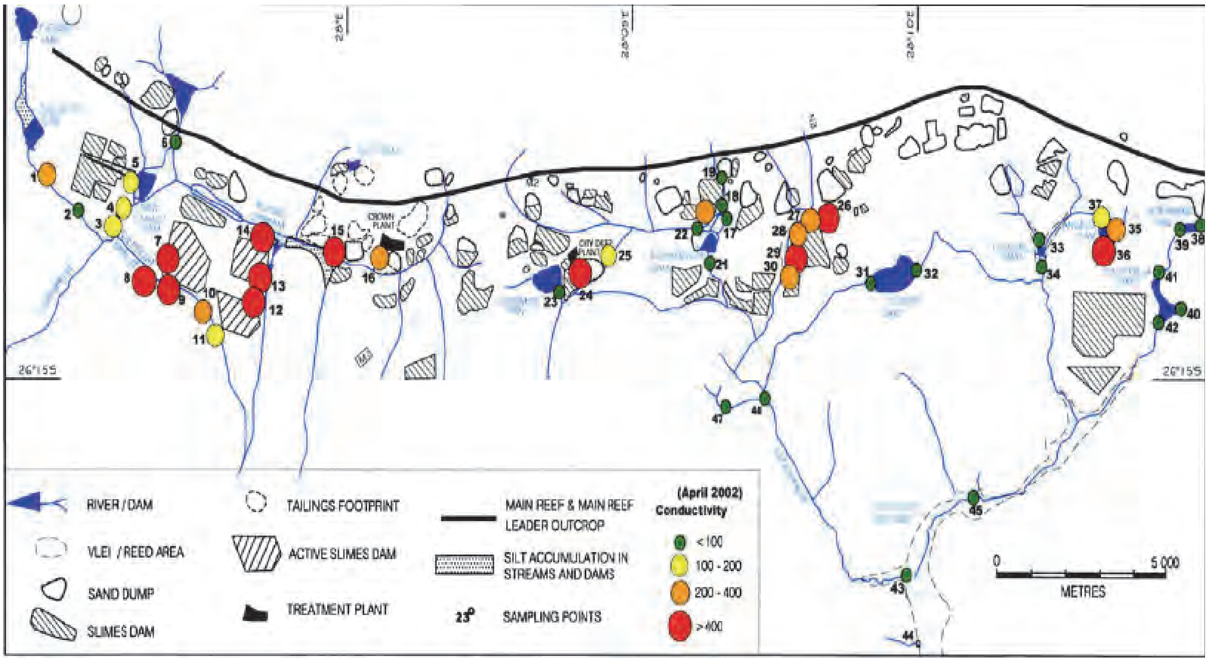


Figure 8: Electrical conductivity (from Tutu et al., 2008)

The highest EC measured was at site 24 with a value of 10.65 mS/cm followed by site 12 at 9.47 mS/cm. Again, streams generally showed higher EC values compared with dams (0.3-0.7 mS/cm) and wetlands mostly at < 0.2 mS/cm (Tutu et al., 2008).

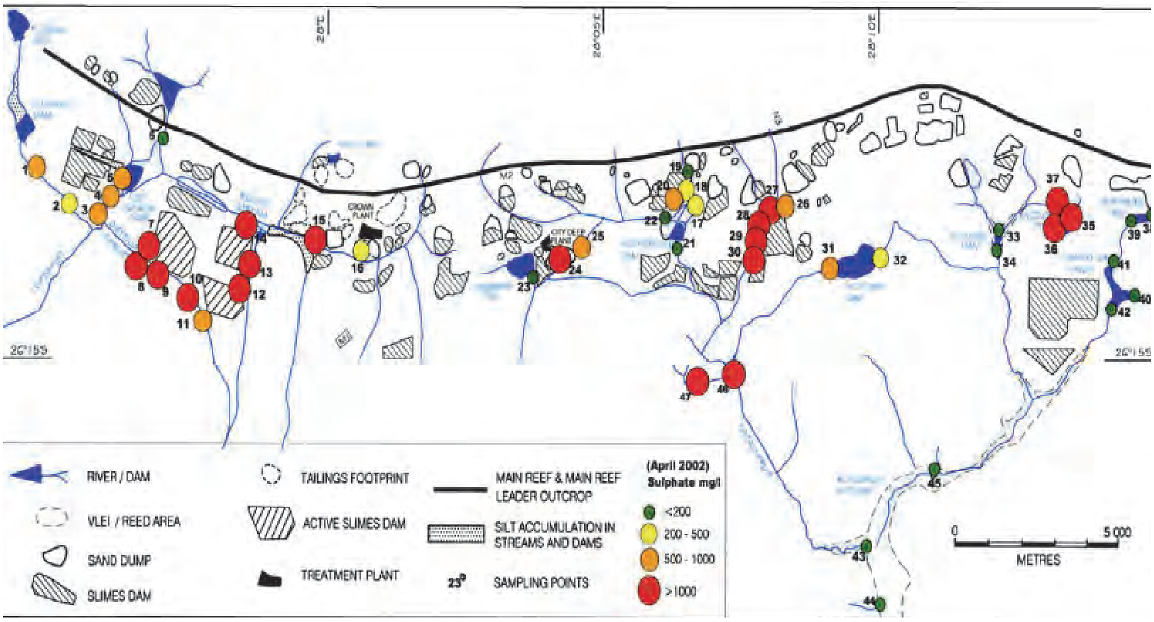


Figure 9: Total sulphate (from Tutu et al., 2008)

Sampling site 24 again showed the highest concentration of sulphates at 7571 mg/l. The next highest was site 36 at 5080 mg/l, followed by site 37 at 3210 mg/l and site 12 at 3110 mg/l. The other stream sampling sites ranged between 108 and 2906 mg/l. Dam samples ranged between 13.08 and 325.9 whilst wetlands were much lower ranging between 18.33 and 23.04 mg/l.

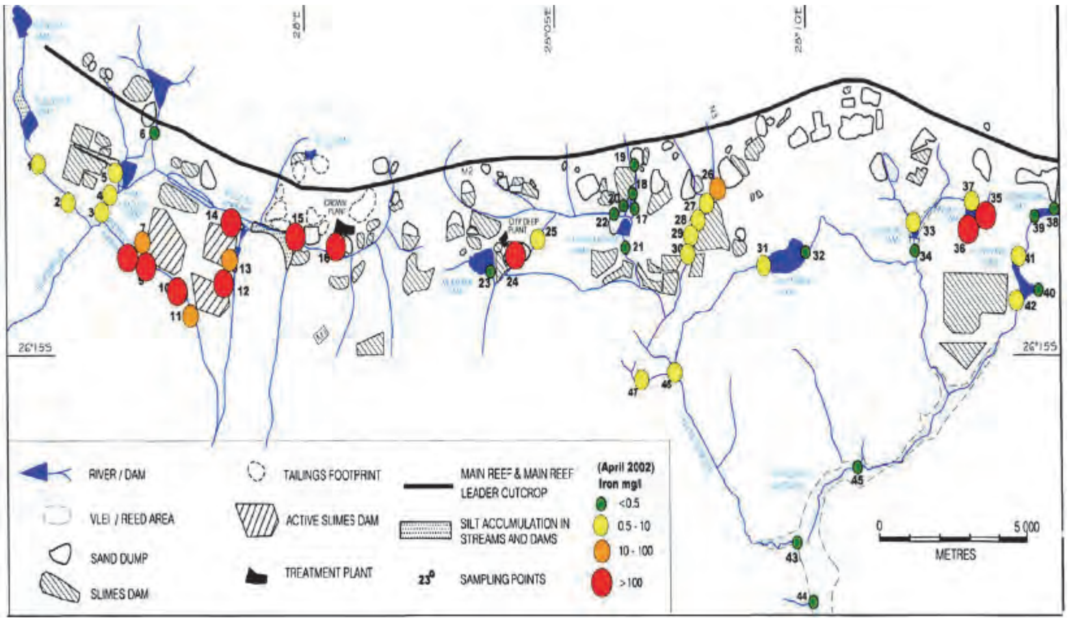

Figure 10: Total iron (from Tutu et al., 2008)

The highest concentration of iron (1010 mg/l) was measured at site 10. Wetland sites ranged at 0.050-0.080 mg/l. For Uranium, the highest concentrations were recorded at site 25, 10, 35, 20 and 1.

The study also showed that pollution levels are generally higher at the end of the wet season compared to the dry season, possibly due to increased discharge of polluted groundwater into streams during the rainy season (Tutu et al., 2008). The value of wetlands in reducing water pollution was also demonstrated by the improved water quality parameters in wetland sampling sites compared to both dam and stream sites as indicated previously.

The value of wetlands, in particular the Johannesburg wetlands, in reducing pollution levels has been studied for decades (e.g. Harrison et al., 1960). More recently, this has been discussed extensively by McCarthy et al. (2006) and McCarthy et al. (2007) who have also shown the extent of degradation of the Klip River wetlands due to excessive accumulation of pollutants as well as impacts from other anthropogenic factors including agriculture.

### 1.3.4 Radioactivity

Preliminary screening surveys of radioactivity in water sources conducted by the Institute for Water Quality Studies (IWQS – now Resource Quality Information Management (RQIM)) between 1995 and 1996 indicated elevated levels of the radioactive elements uranium and radium in streams in the vicinity of gold mining activities. The conclusion from this work was that in some cases, such streams may be regarded as unsuitable for continuous lifetime use as sources of drinking water (DWAF, 2003[2]). A routine radiological monitoring programme was carried out in the Klip River Catchment from 1998-1999 by IWQS. The results are shown in Table 9.

Table 9: Results of Radioactivity Monitoring along the Klip River

| Site | Description of site | Latitude (S) | Longitude (E) | Lifetime average annual dose mS/m | Radio-activity class | Uranium-238 chemical toxicity mg/l |
|---|---|---|---|---|---|---|
| 1 | Klip River at R41 upstream of Durban Deep Mine | 18<br>26°10'36" | 27°49'07" | 0.037 | 0 | 0 |
| 2 | Klip River at Durban Deep Mine downstream of Discharge from No 5 shaft | 26°10'39" | 27°50"13" | 0.584 | I | II |
| 3 | Klip River at N12(old R29) downstream from Durban Deep Mine | 26°17'39" | 27°50'11" | 0.056 | 0 | 0 |
| 4 | Klip River at Golden Highway (R553) downstream From Soweto and Eldorado Park | 26°20'10" | 27°54'11' | 0.073 | 0 | 0 |
| 5 | Russel Stream (tributary of Klipspruit ) at Nasrec Road (R5) | 26°13'13" | 27°58'55" | 0.121 | 1 | I |
| 6 | Russel Stream ( tributary of Klipspruit ) at New Canada Road (R10) | 26°12'37" | 27°57'09" | 0.264 | 1 | II |
| 7 | Russel Stream ( tributary of Klipspruit )at Xavier Road (R17) | 26°13'37" | 28°00'11" | 0.180 | 0 | I |
| 8 | Klipspruit at Soweto Highway (M70) | 26°13'21" | 27°55'44" | 0.043 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 10 | Stream past City Deep Gold Mine at Lower Germiston Road (R33) | 26°13’37" | 28°06’24” | 0.040 | 0 | 0 |
| 11 | Stream downstream from Simmer and Jack Gold Mine at Rand Airport Road (R46) | 26°14’16" | 28°07’27" | 0.124 | 1 | I |
| 11A | Stream past Simmer and Jack Gold Mine at Smith Avenue | 26°13’03" | 28°08’05" | 0.122 | 1 | I |
| 12 | Natal Spruit downstream from Alberton at Heidelberg Road (R554) | 26°17’31" | 28°08’31" | 0.204 | 1 | II |
| 13 | Elsburg Spruit upstream from Elsburg Dam at Lower Boksburg road (R46) | 26°12’46" | 28°11’42" | 0.029 | 0 | 0 |
| 14 | Elsburg Spruit downstream from Elsburg Dam at Brugstreet (R39) | 26°14’55" | 28°12’18" | 0.144 | 1 | I |
| 15 | Tributary of Elsburg Spruit d/s of Cinderella Dam At Germiston/ Heidelberg Road (R35) | 26°15’41" | 28°13’20" | 0.084 | 0 | I |
| 16 | Riet Spruit past Mapleton Agricultural Holdings at R103 | 26°22’15" | 28°14’40" | 0.096 | 0 | I |
| 17 | Klip River, at Riviera Golf Course, upstream From confluence with Vaal River | 26°39’50" | 26°57’20" | 0.034 | 0 | 0 |

(From DWAF, 2003) Some groundwater sampling sites have been removed from the Table

The classification of the sites used in the study from the radiological site of view for drinking is as follows:

- 10 of the 17 sites were classified as ideal (dose 0 to 0.1 mSv/a),
- Only 7 sites were found to be in the good class (dose 0.1 to 1mSv/a)
- No sites fell in the yellow class (class 2) or higher (> 1 mSv/a) implying that there is no indication that intervention is necessary.

From the viewpoint of the highest risk group, i.e. infants under 1 year of age, two sites were classified in the yellow marginal class. These were site 2 (C2H219, which is on the Klip river at Durban Deep Mine, downstream of discharge from No 5 shaft) and site 6, i.e. at Russel stream tributary of Klipspruit at New Canada Road on the R10.

Despite the low yearly mean dose on which health effects of radioactivity in drinking water are based at all sites in the Klip River catchment, isolated incidences of transient high levels of radioactivity were observed at several sites. These were however not sustained over time and, as a result did not affect the average dose significantly (DWAF, 2003). A further finding of the study was a good linear correlation between total radiation dose from all radionuclides and the uranium concentration in the water (DWAF, 2003). The Results of the Uranium toxicity determination are summarized in Table 10.

Table 10: Summary of Uranium toxicity determination

| Class | Uranium-238 chemical toxicity (mg/l) | Number of sites in category |
|---|---|---|
| 0 | 0-0.02 | 9 |
| I | 0.02-0.07 | 9 |
| II | 0.07-0.284 | 3 |
| III | 0.284-1.42 | 0 |
| IV | More than 0.284 | 0 |

Total radiation dose at the lower end of the Klip River was very low, and did not differ significantly from the natural background dose value (DWAF, 2003).

### 1.3.5 Biological Monitoring

In addition to concerns about the suitability of water for intended uses, monitoring has evolved to include trends in the quality of the aquatic environment as well as the response to the environment to anthropogenic factors (Osmon & Klaus, 2010). Environmental management is based on the knowledge of the ecological status of a given area. Exposure of natural communities to chemical contaminants presents one of the most serious problems affecting the health of aquatic ecosystems. Monitoring living organisms at all levels of biological organization is the most important tool for investigating the health of an ecosystem (Pretti & Cognetti-Varriale, 2001). Spellerberg (1991) defines monitoring as the systematic measurement of all the variables and processes related to the specific issue under consideration over time (Pretti & Cognetti-Varriale, 2001). Biological monitoring often referred to as biomonitoring refers to the regular and systematic use of living organisms to evaluate changes in environmental or water quality (Cairns & van der Shalie, 1980 in Pretti & Cognetti-Varriale, 2001). The analysis can be done at the level of individuals, species, populations and communities. The intent of monitoring is to understand changes that may occur as a result of chemical exposure over short or long periods of time. The significance of biomonitoring is that biological effects may be detected at chemical concentrations below analytical detection limits or even long after chemical exposure has ceased (Pretti & Cognetti-Varriale, 2001).

#### 1.3.5.1 Water Quality and Ecological Integrity

Pollution can be expected to have an impact on the biota within an aquatic ecosystem. The effect can be small enough to cause sub-lethal effects which may translate into small changes in ecological integrity, or, in extreme cases, be so high as to result in complete eradication of biota leading to the systems being sterile (Kotze, 2002).

Kotze (2002) carried out a study aimed at determining the ecological integrity of the Klip River. Water quality was assessed using a combination of historic data from 1973 and data taken during the course of the study. A water quality Guidance Compliance Index (GCI) was used to assess the compliance of water quality at some sampling sites. The results of the analysis are summarised in Table 11.

Table 11: GCI scores recorded at each site in various surveys

| SURVEY | SITE | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| **AUGUST 1997** | 79.20 | 58.46 | 77.04 | 77.11 | 73.33 | 71.20 | 74.40 | 68.00 | 76.20 | 74.55 |
| **NOVEMBER 1997** | 95.56 | 54.62 | 62.22 | 82.31 | 75.38 | 67.69 | 69.23 | 73.85 | 74.62 | 90.00 |
| **JANUARY 1998** | 76.30 | 62.96 | 76.30 | 76.30 | 77.78 | 74.81 | 77.14 | 77.14 | 70.71 | 83.70 |
| **AUGUST 1998** | 93.57 | 79.29 | 77.86 | 78.57 | 77.86 | 65.19 | 68.14 | 68.15 | 68.46 | 94.29 |
| **NOVEMBER 1998** | 91.85 | 83.70 | 77.04 | 67.41 | 66.67 | 66.67 | 65.19 | 65.19 | 65.93 | 82.22 |
| **JANUARY 1999** | 88.70 | 85.22 | 71.30 | 71.30 | 69.57 | 66.40 | 65.60 | 66.40 | 58.40 | 89.57 |
| **MAY 1999** | 96.80 | 93.60 | 81.60 | 81.60 | 77.60 | 70.00 | 76.92 | 76.92 | 76.92 | 88.46 |
| **MEDIAN** | **91.9** | **79.30** | **77.00** | **76.30** | **75.40** | **67.70** | **69.2** | **68.2** | **68.5** | **88.5** |
| **CATEGORY** | **A** | **C** | **C** | **C** | **C** | **D** | **D** | **D** | **D** | **B** |

Explanation of GCI categories (From Kotze, 2002):

| CATEGORY AND GCI (%) | GENERAL DESCRIPTION OF WATER QUALITY |
|---|---|
| **EXCELLENT (90-100)** | Most water quality parameters assessed fall within ideal range for protection of aquatic ecosystems. Water quality should be able to sustain a healthy ecosystem. |
| **GOOD (80-89)** | The majority of water quality parameters assessed fall within ideal range for protection of aquatic ecosystems. Water quality should be more than appropriate to sustain a healthy ecosystem. |
| **FAIR (70-79)** | Most water quality parameters assessed fall within acceptable range for protection of aquatic ecosystems though some variables may be of concern. |
| **POOR (50-69)** | The majority of water quality parameters assessed fall within ideal range for protection of aquatic ecosystems but some are only tolerable and not ideal for biota. The prevailing water quality can be seen as being poor and could be a limiting factor for integrity. |
| **VERY POOR (25-49)** | Most water quality parameters assessed are only tolerable and not ideal for the sustainability of the aquatic ecosystem. The prevailing water quality can be seen as very poor and could be a limiting factor for integrity. |
| **CRITICAL (0-25)** | Most water quality parameters assessed fall within the unacceptable range for protection of the aquatic ecosystem. The water quality can be seen as critical and unable to sustain any biota. |

The study noted the following with respect to ecological integrity of the Klip River:

- Most Klip River sites exceeded water quality guidelines for the aquatic environment. The water quality guidelines for the environment with attributes of concern are the metals and ammonia.
- Most sites also exceeded water quality guidelines for domestic water, especially with regards to most metal ions with Ca and Mg in particular, TDS, turbidity and $SO_4$. Turbidity and metals apart from copper were of concern at the reference site.
- With the exception of pH, TDS, Cd, Mn, Na and arsenic, guidelines for livestock and irrigation were met at most sites on the Klip River. For the reference site, Cd and Pb were of some concern (Kotze, 2002).

Figure 11 shows the GCI scores for each site.

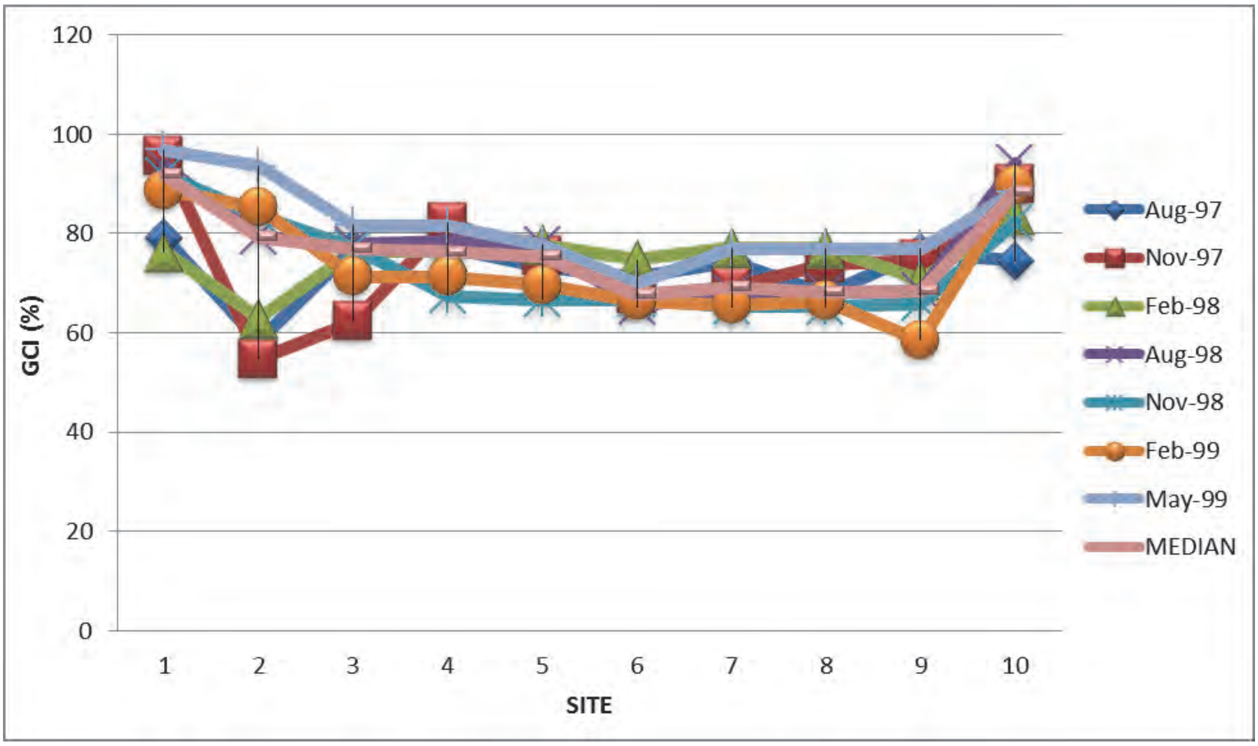


Figure 11: GCI for selected sites (August 1997- May 1999) (Prepared with data from Kotze, 2002)

Examination of Figure 11 shows site 1 had the highest GCI ranging from Fair to Excellent over the sampling period. Site 2 showed the widest variation within the sampling period, which showed some improvement from poor to excellent over the sampling period. This site was included in the analysis to investigate the impacts of mining and informal settlements on water quality.

Site 9 also showed more variation over the sampling period with deterioration from the fair category to poor by the end of the sampling period. The majority of the other sites had a narrower variation remaining in the fair and poor range throughout the sampling period. Another observation that can be made is a general deterioration down the Klip River catchment.

The Econ@uj study assessed the habitat quality and diversity at eight sites using the Habitat Quality Index (HQI) and the Integrated Habitat Assessment System (IHAS) based on McMillan (1998). The classification system used was as shown in Figure 12 whilst the results of the analysis are summarised in Table 12.

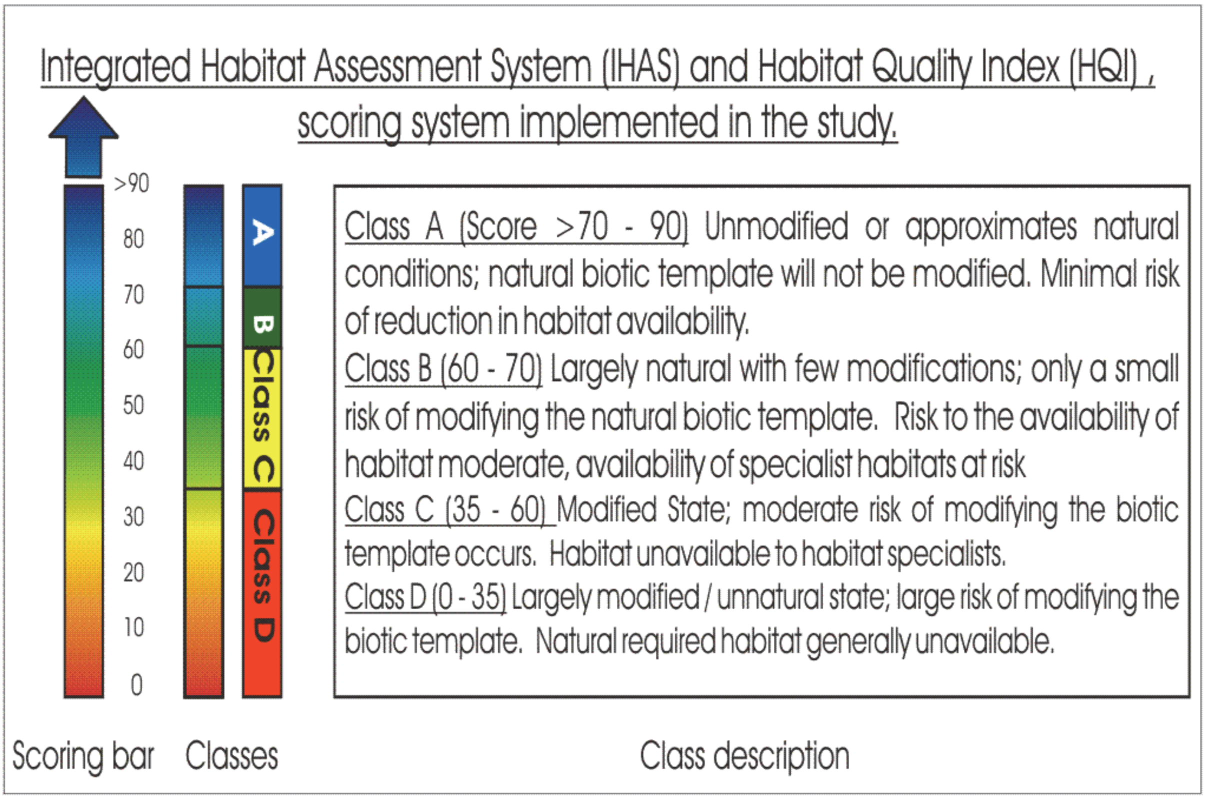


Figure 12: Scoring and classification system used to assess habitat quality and integrity in the Econ@uj biomonitoring study (Ferreira et al., 2010).

Table 12: Results of the habitat quality and diversity analysis in Econ@uj biomonitoring study. (From Ferreira et al., 2010).

| INDEX | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 |
|---|---|---|---|---|---|---|---|---|
| **IHAS** | 67 | 69 | 73 | 72 | 72 | 75 | 66 | 44 |
| **HQI** | 58 | 71 | 65 | 72 | 65 | 67 | 75 | 40 |
| **IHAS Class (Nov 2009)** | B | B | A | A | A | A | B | C |
| **HQI Class (Nov 2009)** | C | A | B | A | B | B | A | C |

Table 13 shows that the ecological integrity of the in-stream habitat ranged from natural (A class) to a modified state (C class). This shows that the habitat quality in the rivers system is generally in a near natural condition whilst only one site (site 8) appeared to be in a modified state.

### 1.3.5.2 Vegetation analysis

Weintroub (1933) investigated the aquatic and sub-aquatic plant species in the area from Florida to Brakpan. Some of the water bodies investigated in the study are the Canada and Florida lakes, and the Natal Spruit which are a tributary of the Klip River. The aquatic communities studied and the species recorded in each category are shown in Table 13.

Table 13: Vegetation Species Recorded in the Witwatersrand region

| TYPE | SPECIES RECORDED |
|---|---|
| **Communities of entirely submerged rooted plants** | *Chara braunii* C.C.Gmelin *(Braun's stonewort,* no record in PRECIS database*)* |
| | *Chara fragilis* Desvaux (no record in PRECIS database) |
| | *Chara stachymorpha* Ganterer (no record in PRECIS database) |
| | *Nitella doidgeae* (no record in PRECIS database) |
| | *Nitella dregeana* Kutz. (no record in PRECIS database) |
| | *Nitella hyalina* Ag (no record in PRECIS database) |
| | *Lagarosiphon muscoides* Harvey (Fine oxygen weed, Babergras, Fynebabergras, Waterblommetjie). |
| | *Lagarosiphon major* Moss** (Coarse oxygen weed, bobbenjantau) |
| | *Potamogeton pectinatus* Linn. [Accepted name: *Stuckenia pectinata]* (Fennel-leaved pondweed, sago pondweed, fonteingras) |
| | *Potamogeton badius llagstrom* |
| | *Scirpus fluitans* Linn. [*Isolepsis fluitans*] |
| **Communities of rooted and submerged plants with floating leaves** | *Ilysanthes conferta* Hiern [***Lindernia conferta* Hiern Philcox.]** |
| | *Limnanthemum thunbergianum* Grisebach [*Nymphoides thunbergianum* (Griseb.) Kuntze] |
| | *Marsilea macrocarpa* Presl (Waterklawer) |
| | *Polygonum amphibium* Linn [*Persicaria amphibi*] |
| | *Potamogeton javanicus* Hasskarl [*Potamogeton octandrus* ssp. o*ctandrus*.] Common names- pondweed, fonteingras |
| | *Potamogeton richardii* Solms-Laubach |
| | *Phragmites communis* Linn. [*Phragmotes australis*]. Common names- Common reed, fluitjiereed, sonquasriet, vaderlandsriet, vinkriet |
| | *Potamogeton richardii* Solms-Laubach, |
| | *Typha australis* Sch. and Thon [*Typha domingensis*] Common names Bulrush, paapkuil, palmiet |
| | *Cyperus fastigiatus* |
| | *Scirpus corymbosus* |
| **Reed swamp commu** | *Juncus effusus* Linn.*,* |
| | *J. oxycarpus* E. Meyer, |
| | *Cyperus umbrosus* Nees, [*Cyperus laxus* ssp. *laxus*] |

| | |
|---|---|
| | *C. macrant* hus Boeck |
| | *Polygonum limbaturn* Meisn. [*Persicaria limbata*] |
| | *P. meisnerianum* Cham. and Schlecht |
| **Sub-aquatic communities** | *Spergularia salina* |
| | *Spergularia marginata* Kitt. |
| | *Scirpus fluitans* Linn. |
| | *Polygonum amphibium* Linn. |
| | *Limnathemum thunbergianum* Grisebach |
| | *Marsilia macrocarpa* Presl, |
| | *Limosella tenui, folia* Wolf |
| | *Crotalaria distans* Bentham |
| | *Aponogeton spathaceus* E. Meyer |
| | *Polygonum limbatum* Meisn', |
| | *Cynodon transvaalensis* Burtt Davy, African Bermudagrass; African dogstooth grass, |
| | *Limosella tenuifolia* Wolf, [*Limosella australis*] |
| | *Cyperus isocladus* Kunth.,[*Cyperus prolifer*] |
| | *Epilobium villosum* Curt., [*Epilobium hirsutum*] |
| | *Juncus effusus* Rottboell, Soft rush |
| | *Polygonum limbatum* Meisn., [*Persicaria limbata]* |
| | P. serrulatum Lag., [*Persicaria decipiens*] |
| | P. glandulosum R. Br., No record in PRECIS |
| | P. glutinosum [*Persicaria senegalensis*] |

From Wintroup (1933) (Some species names have been revised from synoms used in Wintroup, 1933)

Harrison (1958) observed some differences in aquatic vegetation between the two sites sampled at the Klipspruit and Klip River. At the Klipspruit site, profuse growth of *Scirpus fluitans* was observed. The study also reported *Juncus exsertus, J. oxycarpus* and *Dryopteris thelypteris* in addition to *P. communis and T. latifolia* in a bog at the Klip River site.

### 1.3.5.3 Faunal studies

#### 1.3.5.3.1 Invertebrates

In addition to some differences in vegetation reported earlier, Harrison (1958) also reported differences in invertebrate species composition, which persisted throughout the sampling period. The taxa recorded at the two sites are shown in Table 14.

Table 14: Invertebrate taxa recorded at two sites on the Klip River Catchment

| Taxa recorded | October | | January | | April | | July | |
|---|---|---|---|---|---|---|---|---|
| | Klipspruit | Klip | Klipspruit | Klip | Klipspruit | Klip | Klipspruit | Klip |
| *Chaetogata* | - | 6.6 | - | - | 0.6 | 5.8 | 5.8 | - |
| *Simocephalis* | 1.2 | - | - | - | - | - | - | - |
| *Cyclops* spp. | 64 | - | 7.8 | 5.6 | 30.6 | - | 54 | - |
| *Platycyclops* sp. | - | 11.5 | - | - | - | P | - | 1.9 |
| Other | 5.2 | 23 | 7.1 | 22.2 | 33.8 | 66.9 | 23.8 | 34 |
| *Culecidae* | - | 18 | P | 11.1 | - | P | - | - |
| *Pisidium georgeanum* | - | - | P | - | - | - | - | - |

(from Harrison, 1958)

De Kock (2001) carried out a study aimed at understanding the general biology of the fresh water crab (*Potomateus warrenii*) as well as bioaccumulation of metals in the Klip River system. Four sites were selected for the study as shown in Table 15.

Table 15: Sites used in Freshwater crab bioaccumulation study

| SITE | LATITUDE | LONGITUDE | DESCRIPTION |
|---|---|---|---|
| **SITE 1** | -26° 17.912’ | +27°50.538’ | Lenasia area selected to investigate possible impacts of the Nancefield industrial area as well as greater Soweto. |
| **SITE 2** | -26° 20.228’ | +27° 54.181’ | Olifantsvlei area after the confluences of discharge from the Goudkoppies and Olifansvlei WWTWs |
| **SITE 3** | -26° 22.923’ | +27° 04.307’ | Zwartkoppies area below the confluence with the Riet Spruit. |
| **SITE 4** | -26° 39.321’ | +27° 57.443’ | In the built up areas of Vereeniging near the confluence with the Vaal. |
| **REF.** | -26° 30.708’ | +28° 17.413’ | Reference site selected at Sedaven Dam, Suikerbosrand Nature Reserve. |

(Based on de Kock, 2002)

Crabs collected from these sites were analysed for aluminium, cadmium, chromium, iron, lead, manganese and zinc over the period August 1997-August 1998 during which bimonthly sampling was carried out. The highest levels of all the metals analysed were recorded for Site 1 for the August 1997 sampling. Observations from the study include:

- **Aluminium:** Aluminium concentrations of 5-70 µg/g wet weight were reported. Sites showed fluctuation in concentrations of aluminium in crab tissue across seasons. Site 1 showed significant variation across all seasons whilst Site 4 showed significant variation between winter and summer. No significant variations were recorded at the other sites. There were also significant differences between Site 1 and Sites 2, 3 and 5 whilst Site 4 did not differ significantly from the other sites (de Kock, 2002).

- **Cadmium:** The highest mean cadmium concentration recorded was 0.25 µg/g wet weight recorded at Site 1 during the August 97 sampling. Site 3 was reported to be significantly different from the other sites and had significantly higher load than other sites. The study also reported significant differences between Sites 1 and 4 as well as between Sites 1 and 5 (de Kock, 2002).
- **Chromium:** Chromium concentrations ranging from about 0.02-1.6 µg/g wet weight were recorded. Most sites averaged below 0.4 µg/g wet weight over most seasons. Overall, chromium levels were higher at site 2 than at site 1 but no significant differences could be found between sites. However, sites showed some significant seasonal variations.
- **Iron:** Average iron concentrations in crab tissue were highest at site 1, falling in the range 8-70 µg/g wet weight, during the August 97 sampling period. The average was within the 12-16 µg/g wet weight over four of the seven sampling periods. For sites 2-4 the averages were below 10 µg/g wet weight whilst at site 5 averages remained in the 10-20 µg/g wet weight for six of the seven sampling sites. Significant differences were reported between sites 1, 2 and 3 but not between sites 1, 4 and 5 (de Kock, 2002).
- **Lead:** Apart for an average of 2.5 µg/g wet weight recorded for October 97, and an average of 0.5 µg/g wet weight recorded at Site 3 for June 98, all other sites were below 0.5 µg/g wet weight throughout the study period. No significant differences were detected between sites (de Kock, 2002).
- **Manganese:** Average concentrations in the range 10-150 µg/g wet weight reported for all sites with significant differences reported between spring and autumn at sites 1 and 4, as well as between spring and summer at sites 1 and 3. Significant differences were found between spring and autumn at site 4. No significant differences were observed at sites 2 and 5. Samples from site 5 had the lowest concentrations clustering around 5-15 µg/g body weight whilst samples from site 2 clustered around 20 µg/g body weight (De Kock, 2002).
- **Zinc:** The highest concentration was again recorded at Site 1 for the August 97 sample period at 8 µg/g wet weight. The other sites averaged about 2 µg/g wet weight for the same period. October 97 results also measured at about 2 µg/g wet weight at all sites, except from Site, 5 which was at 12 µg/g wet weight.

The Econ@uj biomonitoring study used the South African Scoring System (SASS) based on the presence of aquatic macroinvertebrate families and their perceived sensitivity to water quality changes as an index of water quality. The index has gone through several upgrades and version 5 was used for the study. SASS results are expressed both as an index score (SASS score) and the average score per recorded taxon (ASPT value). The scoring system is shown in Table 15.

Table 16: The SASS 5 scoring system and methodology used to assign ecological classes in the study

| SASS 5 Score | ASPT | Condition | Class | Colour |
|---|---|---|---|---|
| > 140 | > 7 | Natural / unmodified | A | |
| 100-140 | 5-7 | Minimally modified | B | |
| 60-100 | 3-5 | Moderately modified | C | |
| 30-60 | 2-3 | Largely modified | D | |
| < 30 | < 2 | Seriously modified | E | |

(From Ferreira et al., 2010)

The study recorded 25 taxa across the eight sampling sites during their biomonitoring survey as shown in Table 16. The highest numbers of taxa were sampled at Site 1 and Site 3. Other observations from the study include:

- Very few taxa belonging to the order Ephemeroptera, Odonata or Trichoptera, which are generally accepted as indicators of good water quality due to their sensitivity were recorded in the study.
- No major differences were observed in invertebrate communities between the reference site and the other sites downstream.
- The majority of taxa sampled were from the order Diptera, which are considered indicators of poor water quality (Ferreira et al., 2010).

Low SASS scores are often a reflection of alterations to habitat, while ASPT is a good reflection of water quality (Ferreira et al., 2010). ASPT scores ranged from 3.63 (Moderately modified) to 5.11 (minimally modified). The highest SASS 5 score was recorded at site 1 which also had the highest ASPT score placing it in class B (minimally modified). These results would seem to suggest that the habitat integrity of most of the study sites is in a reasonably good state. Sites 3 and 4 fell into class C (Moderately modified). The lowest SASS 5 score was recorded at site 2. Site 8 also had a relatively low SASS 5 score.

Table 17: Invertebrate taxa recorded in Econ@uj biomonitoring survey

| TAXON | SITE 1 | SITE 2 | SITE 3 | SITE 4 | SITE 5 | SITE 6 | SITE 7 | SITE 8 |
|---|---|---|---|---|---|---|---|---|
| Ancylidae | - | - | X | - | - | X | - | - |
| Baetidae | X | X | X | X | X | X | X | X |
| Belostomatidae | - | - | X | - | - | - | - | - |
| Caenidae | X | - | - | X | X | | X | - |
| Ceratopogonidae | X | - | - | X | - | X | - | - |
| Chironomidae | X | - | X | X | X | X | X | X |
| Coenagrionidae | - | - | X | | X | | X | X |
| Corixidae | X | - | X | X | X | X | - | - |
| Culicidae | - | - | - | - | - | X | X | - |
| Dixidae | X | - | - | - | - | | - | - |
| Dytiscidae | X | X | - | - | - | X | - | - |
| Elmidae | X | - | X | X | | - | - | - |
| Gomphidae | - | - | | X | | - | - | - |
| Gyrinidae | X | X | X | X | X | - | - | - |
| Hirudinea | - | - | X | - | - | - | - | - |
| Hydracarina | X | - | - | - | - | - | - | - |
| Hydropsychidae | X | - | X | X | - | X | X | X |
| Leptoceridae | X | - | - | X | - | - | - | - |
| Oligochaeta | X | - | X | - | - | X | X | |
| Physidae | - | - | X | - | - | - | - | - |
| Pleidae | - | - | X | - | X | - | - | - |
| Potamonautidae | X | X | X | - | X | X | X | X |
| Simuliidae | X | | | - | - | - | - | - |
| Turbellaria | X | | X | - | X | X | - | - |
| Veliidae | X | | X | X | X | X | X | X |
| **SASS SCORE** | **87** | **17** | **70** | **64** | **34** | **38** | **40** | **27** |
| **NO. TAXA** | **17** | **4** | **17** | **13** | **9** | **10** | **11** | **17** |
| **ASPT** | **5.11** | **4.25** | **4.12** | **4.92** | **3.77** | **3.8** | **3.63** | **3.86** |
| **ECOLOGICAL CLASS** | **B/C** | **D** | **C** | **C** | **C/D** | **C/D** | **C/D** | **D** |

(From Ferreira et al., 2010)

### 1.3.5.4 Fish studies

Fish species recorded by Kotze at sites in the Klip River Catchment are shown in Table 17. Table 17 shows that no fish were caught at site 1. This site was located in a built up area. Site 2, located in an area associated with both gold mining activities and informal settlements was also very poor in fish numbers, with only four individuals of *T. sparrmanii* being caught. Site 3, located downstream of a wetland had a large number of fish but these represented only three species.

Generally, sampling sites at the lower reaches were richer in both numbers of individuals as well as number of species represented.

Table 18: Species of fish by number caught by number at each sampling site

| **Species** | **Indigenous (I) / Exotic (E)** | **Locality/ Sampling site** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10*** | |
| *Austroglanis sclateri* | I | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 5 | 0 | 6 | **12** |
| *Labeobarbus aenus* | I | 0 | 0 | 0 | 48 | 76 | 37 | 194 | 65 | 57 | 88 | **565** |
| *Labeobarbus kimberlyensis* | I | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | **4** |
| *Barbus anoplus* | I | 0 | 0 | 301 | 1 | 35 | 13 | 126 | 18 | 0 | 5 | **499** |
| *Barbus pallidus* | I | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 5 | **6** |
| *Barbus paludinosus* | I | 0 | 0 | 2 | 0 | 28 | 0 | 52 | 2 | 0 | 84 | **168** |
| *Clarius gariepinus* | I | 0 | 0 | 2 | 5 | 21 | 2 | 4 | 1 | 4 | 48 | **87** |
| *Labeo capensis* | I | 0 | 0 | 0 | 0 | 13 | 10 | 70 | 256 | 116 | 245 | **710** |
| *Labeo umbratus* | I | 0 | 0 | 0 | 15 | 46 | 3 | 13 | 2 | 3 | 58 | **140** |
| *Pseudocrenilabrus philander* | I | 0 | 0 | 2 | 65 | 41 | 5 | 55 | 30 | 1 | 16 | **215** |
| *Tilapia sparrmanii* | I | 0 | 4 | 0 | 6 | 34 | 2 | 81 | 57 | 0 | 1 | **185** |
| *Cyprinus carpio* | E | 0 | 0 | 0 | 13 | 13 | 2 | 3 | 5 | 38 | 12 | **86** |
| *Micropterus salmoides* | E | 0 | 0 | 4 | 0 | 1 | 0 | 0 | 1 | 5 | 0 | **11** |
| *Gambusia affinis* | E | 0 | 0 | 0 | 13 | 13 | 0 | 0 | 0 | 0 | 0 | **26** |
| **Total number of individuals caught at site** | | **0** | **4** | **307** | **166** | **322** | **74** | **599** | **442** | **224** | **488** | |
| **No. species** | | **0** | **1** | **2** | **8** | **12** | **7** | **7** | **10** | **7** | **12** | |

(From Kotze, 2002)

Site 10 was a reference site located at the Suikerbosrand River.

Figure 13 shows that the largest number of species was caught at Site 7 followed by Site 10 which also had a large diversity at 12 species caught. Sites 8 and 5 also had large numbers of fish caught and also recorded a large number of species at 10 and 5 respectively. In terms of the species caught, some seem to show a narrow range of preference in habitat. *Austroglanis sclateri* was only caught at Sites 7, 8 and 10 only in small numbers. *Labeobarbus kimberlyensis* was only caught at Site 10. Similarly, *B. palidus* was only caught at Sites 5 and 10 in small numbers. *Barbus anoplus was* one of the most highly recorded species but appears to show a preference for Sites 3 and 8 where it was caught in the largest numbers.

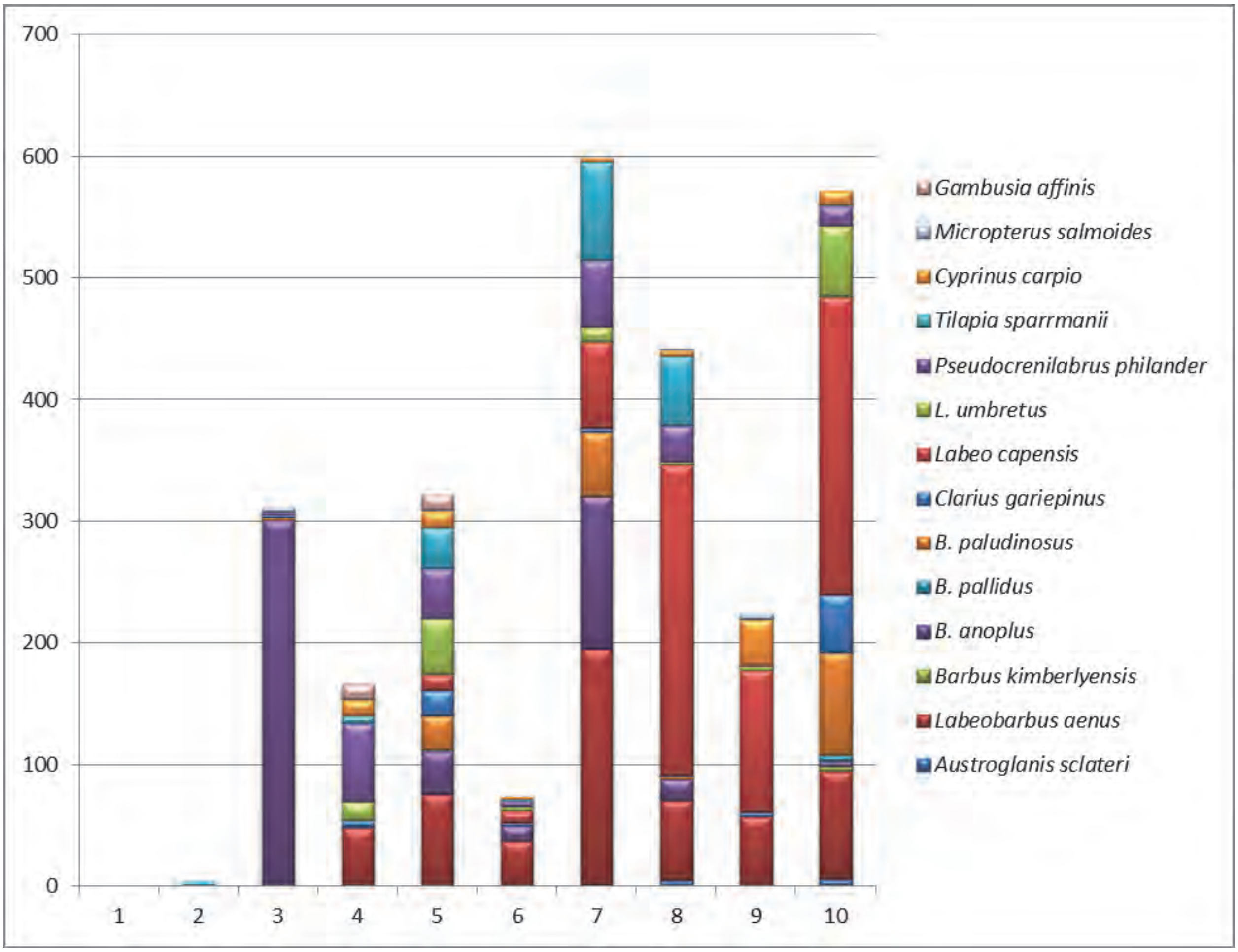


Figure 13: Number of fish by species caught at each sampling site (prepared with data from Kotze, 2002)

The Econ@uj biomonitoring study used *Clarias gariepinus* as a test organism to assess the impact of water quality on fish health. The study used the Health Assessment Index (HAI) as described by Heath et al. (2003) which considers a range of criteria including parasitic infection, gross tissue structure condition and the physiological state of the organism's blood (Ferreira et al., 2010). The HAI was combined with a histological examination.

The HAI was carried out on fish samples collected from three of the eight sites used in the study. The selected fish species for the three selected sites was *Clarias gariepinus*, the

Sharptooth catfish collected from Sites 3, 6 and 7. Major observations from the assessment include:

- An increase in average weight and length of fish from site 3 to site 7 from 1.5 kg to 2.3 kg and 59 cm to 69 cm, respectively. The team however noted that the sample sizes for Sites 3 and 7 were very small.
- Except for gill discoloration among 50% of samples collected from Site 7, the external features including skin, gills, opercula, eyes and fins were mostly normal with some erosion of the fins occurring in some fish specimens.
- The condition of the internal organs such as the spleen, hindgut and kidney were normal for all fish at the three sites.
- The liver condition of fish at site 3 showed slight discolouration for all three specimens captured at that site while only 40% of the specimens at sites 6 and 7 showed slight discolouration.
- Only two specimens at site 8 contained some parasites within the intestine
- 70% of the hematocrit values at site 3 and site 6 indicated variations from the normal values while only 20% had a lowered hematocrit at site 7.
- The white blood cell percentages of all fish specimens were normal except one specimen where a slightly higher value was present.
- The blood protein levels were all normal at site 3 but 50% of individuals at site 6 indicated a slightly higher than normal blood protein level.

Kotze (2002) previously reported some abnormalities in fish captured in the Klip River. Among these were abnormal opercula, gill damage, severe active fin erosion, skin damage, cysts and skin necrosis in specimens of *C. gariepinus* and *Labeo capensis.*

The Econ@uj team also used a range of biomarkers of exposure and some biomarkers of effect to analyse the fish caught at the selected sites (Sites 3, 6 and 7). The selected biomarkers and the key findings of the study were:

- **Acetylcholine esterase (AChE) assay:** All three sites were reported to show some level of esterase inhibition. Site 3 averaged at 2 Abs/min/mg protein whilst sites 6 and 7 both averaged 3.5 Abs/min/mg protein. The team thus concluded an increasing gradient in AchE inhibitors down the river. The study also reported significant differences in response to esterase inhibition. *C. garipienus* appeared to be more sensitive averaging at 3.5 Abs/min/mg protein whilst *L. capensis* captured at the same site averaged 3.0 Abs/min/mg protein.

- **Metallothionein (MT) assay:** Results of the MT assay showed a similar trend to the AChE assay. Site 3 averaged at about 5 µM/g wet lever weight whilst Site 6 and 7 averaged at 6 and 7.55 µM/g wet lever weight, respectively. No differences in response between species were observed.
- **Ethoxyresorufin-O-deethylase (EROD):** No significant differences were reported between sites and only a minor increase in gradient downstream with Site 3 averaging 0.12 mM/mg protein and Sites 6 and 7 averaging 0.15 mM/mg protein each. Species showing similar exposure to organometallic pollutants did not show differences in response to EROD inhibitors (Ferreira et al*.,* 2010).
- **Catalase (CAT):** The study reported significant differences in catalase response between the three sites. Site 3 averaged at less than 50 $\mu MH_2 0_2$/min/mg protein whilst Site 7 averaged at 250 $\mu MH_2 0_2$/min/mg protein. Site 6 on the other hand averaged at more than 3060 $\mu MH_2 0_2$/min/mg protein which may indicate increased levels of stress at site 6 as compared to the other two sites (Ferreira et al*.,* 2010). *C. garipienus* at an average of about 250 $\mu MH_2 0_2$/min/mg protein also showed a significantly higher response than *L. capensis* at just less than 100 $\mu MH_2 0_2$/min/mg protein.
- **Malondialdehyde (MDA):** At 500, 550 and 400 nM/mg protein reported for Sites 3, 6 and 7 respectively, showing only slight differences between responses in the different sites. The two species also showed only slight differences in response at 400 nM/mg protein for *C. garipienus* and 500 nM/mg protein for *L. capensis*.
- **Protein carbonyl content (PC):** Sites 3 and 6 averaged about 2300 nmol carbonyls/mg protein each whilst Site 7 at 1000 nmol carbonyls/mg was significantly lower. No significant differences were seen between species at about 1000 nmol carbonyls/mg for both species (Ferreira et al., 2010).
- **Reduced glutathione content (GSH):** The study reported no differences between sites as each site averaged at 4.5 µg/g tissue and only minor differences were seen between species with *L. capensis*, showing a slightly higher response at an average of 5.5 µg/g tissue compared to 4.5 µg/g tissue in *C. garipienus.*
- **Superoxide dismutase (SOD):** The study reported similar results observed to those reported for CAT with Site 3 averaging at about 25 ng SOD/mg protein and Sites 6 and 7 averaging about 200 ng SOD/mg protein and 600 ng SOD/mg protein respectively. *C. garipienus* showed a significantly higher response averaging 600 ng SOD/mg protein compared to 10 SOD/mg protein in *L. capensis* (Ferreira et al., 2010).

The team also analysed fish samples collected from the three sites for heavy metal accumulation. Metals with concentrations that could be detected by the selected methodology include Strontium, Zinc, Copper, Manganese, Cobalt and Chromium. Key findings from this analysis include:

- **Cobalt:** A slight difference was recorded at Site 7 at 0.04 µg/g compared to the other two sites, which were similar at 0.08 µg/g for both. The study also reported lower concentrations in *L. capensis* compared to *C. gariepinus.*
- **Chromium:** Samples from Site 6 showed much higher chromium concentration in muscle tissue at an average of 0.6 µg/g compared to samples from both Sites 3 and 7 at less than 0.05 and 0.1 µg/g, respectively (Ferreira et al., 2010). The study however notes that the values reported for Site 6 are lower than those reported in a study by Coetzee et al. (2002) where *C. gariepinus* and *L. umbratus* in the Olifants River which ranged from 11-56 µg/g.
- **Copper:** Sites averaged from 1.0-1.5 µg/g and also showed an increase from Site 3 to Site 7. The study also reports that *L. capensis* concentrations were higher.
- **Manganese:** The trend observed for manganese is similar to that reported for chromium with this site averaging at 1.06 µg/g compared to 0.4 and 0.66 µg/g respectively.
- **Strontium:** No differences were observed for strontium across sites. All three averaged at about 2 µg/g.
- **Zinc:** The results reported for zinc show a slight increasing gradient down the river. Averages recorded are 9 µg/g, 11 µg/g and 12 µg/g, respectively (Ferreira et al., 2010).

In addition, the study compared results for samples of *L. capensis* collected from Site 7 to those of results obtained for similar analysis carried out for samples collected from the Vaal for cobalt, chromium, copper, manganese and zinc across three sampling periods carried out in 2008-2009. Samples from the Vaal River showed higher muscle concentrations for all metals analysed (Ferreira et al., 2010).

### 1.4 Biomonitoring of Wetlands on the Klip River

Durgapersad (2005) studied the effects of wetlands on water quality and invertebrate diversity in the Klip River and Natal Spruit. The study was conducted at two sites, i.e. at Olifantsvlei and Lenasia, on the Witwatersrand (26°20'S &27°55'E). The wetland is estimated to be 800 ha in area. Before entering the wetland, the Klip River is impacted by mining, industries, and informal settlements as well as three WWTWs, i.e. the Olifantsvlei, Goudkoppies and Bushkoppies. The input site selected for this wetland was K6 (located on the Potchefstroom Road) at coordinates, 26°17.36' S and 27°50.15' E. The output site chosen was at K21 (located at the weir at Zwartkoppies Farm) with coordinates 26°24.02' S and 28°04.48' E (Durgapersad, 2005).

The wetland chosen on the Natal Spruit is 400 ha in area with coordinates 26°25'8 & 28°10'E. Before it enters the wetland, the Natal Spruit River is impacted by mining (e.g. ERPM) and three of ERWAT's sewage disposal sites, i.e. Rondebult, Dekema and Vlakplaats. The wetland does not run continuously from the input to the output point but has a break in the wetlands downstream of the Vlakplaats WWTWs, with a clearly defined stream running through the wetland. The input site chosen was E7 located in the headwaters of the Elsburgspruit at Elsburg town, downstream of the Elsburg Dam. The site is impacted by mining activities from ERPM in the form of effluent discharge from two sources. Firstly, water from the South West vertical shaft is pumped to a plant where it is reused and some overflow is discharged to the Elsburgspruit. Secondly, overflow water from the Hercules shaft is discharged into Angelo Pan. The site is also impacted on by industries in the Germiston area. Among these are City Deep, Benrose, Denver, Heriotdale, Rosherville, Driehoek and Alrode. The output site selected was N8, located on Heidelberg Road. This site is impacted on by storm water from Boksburg, which flows into the Riet Spruit sub-catchment just downstream of E7. Impacts are caused by industries such as Scaw Metals as well as formal and informal settlements of Alberton, Germiston, Vosloorus, Katlehong and Tokoza (Durgapersad, 2005).

### 1.4.1 Physico-chemical and microbial properties

The study analysed conductivity, pH, dissolved oxygen, suspended solids, temperature, suspended solids, aluminium, chloride, fluoride, iron, manganese, sodium, nitrate, ammonia, sulphates, phosphates, chemical oxygen demand (COD) and faecal coliforms in samples of water collected from the inflow and outflow sites associated with the two wetlands. The means for these variables and the percentage differences between inflow and outflow are shown in Table 19.

The majority of attributes measured fell within the ideal, acceptable and tolerant ranges. The exceptions were conductivity, sodium and sulphate at the input site on the Natal Spruit. Faecal coliforms were also far above the tolerable range at both input sites.

Table 19: Percentage differences between input and output means of the Klip River and Natal Spruit Wetlands for selected physical characteristics.

| Ideal | Acceptable | Tolerable | Unacceptable | Source (Durgapersad, 2005) |
|---|---|---|---|---|

| Variable measured | Classification | | | | Klip River Wetland | | | | Natal Spruit Wetland | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Input K6 | Output K21 | % Diff | I/D | Input E7 | Output N8 | % Diff | I/D |
| Conductivity (mS/m) | 80 | 80-100 | 100-150 | >150 | 70.00 | 63.34 | 9.52 | I | 305.48 | 112.56 | 63.35 | I |
| pH | 6-9 | | | <6 or >9 | 7.94 | 7.86 | 0.92 | NSD | 7.36 | 7.50 | 6.89 | I |
| Temperature °C | No range determined | | | | 22.62 | 22.68 | 0.28 | NSD | 22.62 | 22.77 | 0.63 | NSD |
| DO (mg/lO$_2$) | | >6 | 5-6 | <5 | 5.55 | 6.48 | 14.27 | I | 6.50 | 6.60 | 1.53 | NSD |
| Suspended solids (mg/l) | 20 | 20-30 | 30-55 | >55 | 9.93 | 39.47 | 74.84 | D | 20.52 | 22.57 | 9.09 | NSD |
| Aluminium(mg/l) | | <0.3 | 0.3-0.5 | >0.5 | 0.08 | 0.08 | 2.91 | NSD | 0.12 | 0. 08 | 34.99 | NSD |
| Chloride (mg/l) | <50 | 50-75 | 75-100 | >100 | 24.63 | 49.78 | 50.53 | D | 98.63 | 80.46 | 18.43 | I |
| Fluoride (mg/l) | 0.19 | 0.19-0.7 | 0.7-1.0 | >10 | 0.21 | 0.22 | 7.97 | NSD | 0.23 | 0.35 | 32.85 | D |
| Iron (mg/l) | <0.5 | 0.5-1.0 | 1.0-1.5 | >1.5 | 0.06 | 0.06 | 7.64 | NSD | 0.30 | 0.06 | 80.00 | I |
| Manganese (mg/l) | <1 | 1-2 | 2-4 | >4 | 0.33 | 0.15 | 53.80 | I | 2.24 | 0.35 | 84.22 | I |
| Sodium (mg/l) | <50 | 50-80 | 80-100 | >100 | 26.87 | 38.00 | 29.28 | D | 135.32 | 63.87 | 52.80 | I |
| Nitrate (mg/l) | <2 | 2-4 | 4-7 | >7 | 2.06 | 4.78 | 56.99 | D | 2.25 | 3.34 | 32.60 | D |
| Ammonia (mg/l) | <0.5 | 0.5-1.5 | 1.5-4.0 | >4.0 | 0.76 | 0.18 | 76.49 | NSD | 1.04 | 1.22 | 4.69 | NSD |
| Sulphate (mg/l) | <200 | 200-350 | 350-500 | >500 | 171.4 | 105.91 | 38.19 | I | 1660.6 | 320.37 | 80.71 | I |
| Phosphate (mg/l) | <0.2 | 0.2-0.5 | 0.5-1.0 | >1.0 | 0.26 | 0.39 | 34.30 | D | 0.03 | 0.49 | 93.09 | D |
| COD (mg/l) | <15 | 15-30 | 30-40 | >40 | 11.95 | 27.12 | 55.92 | D | 15.57 | 17.78 | 12.45 | NSD |
| Faecal coliforms (Counts/100ml) | <1000 | 1000-5000 | 5000-10000 | >10000 | 28482 | 1985 | 93 | I | 7500705 | 5071 | 99 | I |

I = Improvement D = Deterioration NSD = No Significant Difference
% Difference = [(Maximum value - Minimum value)/Maximum value] x 100

Table 19 shows that for many of the attributes measured, there was an improvement at the output site. The most significant changes include:

- An improvement in conductivity from the unacceptable range to within the tolerable at the Natal Spruit wetland.

- An improvement from the unacceptable range to within the acceptable range in sodium and sulphates at the Natal Spruit wetland.
- An improvement from the unacceptable range to within the acceptable range in the case of the Klip River wetland and to the tolerable range in the case of the Natal Spruit wetland.
- Deteriorations were observed between input and output sites in suspended solids, chlorides, sodium, nitrates, phosphates and COD in the Klip River Wetland. Similarly, deteriorations were observed in fluorides, nitrates, and phosphates at the Natal Spruit wetland.
- Both wetlands had extremely high levels of faecal coliforms that far exceeded the unacceptable range. The high FC load was attributed to the presence of WWTWs as well as informal settlements upstream of the wetlands.
- In both wetlands, there was an improvement in the FC load to the acceptable range in the case of the Klip River Wetland and to the tolerable range in the Natal Spruit wetland.

Based on the number of attributes for which improvements were recorded, the researcher concluded that the Natal Spruit wetland had a higher efficiency for improving water quality than the Klip river wetland. Reasons suggested for this include the higher impacts from mining and other activities that the Klip River wetland is exposed to as well as the presence of a diversion canal on the Klip River wetland which means some of the water does not pass through the wetland (Durgapersad, 2005).

### 1.4.2 Biomonitoring

Durgapersad (2005) also used the SASS4 index to determine the habitat integrity at the four study sites as well as a reference site located in the Suikerbosrand Nature Reserve. This analysis was carried out in summer (January-March, 2001) and in winter (June-August, 2001). The presence of invertebrates in various biotypes including stones in current, stones out of current, sand, gravel, mud, marginal vegetation as well as aquatic vegetation was determined, using identification keys and manuals. Table 19 shows the taxa recorded at each site.

The highest numbers of taxa were recorded at the Reference site during both the summer and winter. In both summer and winter the output sites had a smaller number of taxa recorded than the input site. This appears to be consistent with the improvement in water quality at output sites reported earlier.

Table 20: Invertebrate diversity at the study sites

| TAXON | | Sample Sites: Summer REF | Summer K6 | Summer K21 | Summer E7 | Summer N8 | Winter REF | Winter K6 | Winter K21 | Winter E7 | Winter N8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TURBELLARIA | • *Planarians* | P | - | - | - | - | P | - | P | - | - |
| ANNELIDA | • *Oligochaeta* | P | P | | P | P | P | P | P | P | P |
| CRUSTECEA | • Crabs | P | P | P | P | P | - | P | P | - | P |
| | • Shrimps | - | - | P | - | - | - | - | P | - | - |
| EPHEMEROPTERA | • *Boetida* | P | P | P | - | P | P | P | P | - | - |
| | • *Leptophlebiidae* | P | - | - | - | - | P | - | - | - | - |
| | • *Tricorythidae* | P | - | - | - | - | - | - | - | - | - |
| | • *Caenidae* | P | P | P | - | P | - | P | P | - | P |
| ODONATA | • *Protoneuridae* | - | - | - | - | - | P | - | - | - | - |
| | • *Coenogroridae* | P | - | P | P | P | P | - | P | P | P |
| | • *Gamphidae* | - | - | - | P | - | - | - | P | - | - |
| | • *Aesteriadae* | - | - | - | P | - | P | - | - | P | - |
| HEMIPTERA | • *Notonectidae* | P | - | - | P | - | - | - | - | - | - |
| | • *Pleidae* | - | - | - | - | - | P | P | - | - | - |
| | • *Naucaridae* | P | P | P | - | P | - | P | P | - | - |
| | • *Nepidae* | - | - | - | - | - | - | P | - | - | - |
| | • *Beloetomatidae* | P | - | P | - | - | - | - | P | - | - |
| | • *Corbcidae* | P | P | P | - | P | P | P | P | - | - |
| | • *Gerridae* | P | P | P | - | - | - | - | - | - | - |
| | • *Velidae* | P | - | - | - | P | - | P | - | - | - |
| TRICOPTERA | • *Hydropsychidae* | P | - | - | - | P | P | P | P | - | P |
| | • *Dityscidae* (adults) | P | P | - | - | - | P | - | - | - | - |
| | • *Elmidae / Dryopidae* | - | - | - | P | - | - | - | P | - | - |
| | • *Gyrinidae* | P | P | - | - | P | - | P | P | - | P |
| DIPTERA | • *Tipulidae* | - | - | - | P | - | P | - | - | P | - |
| | • *Cullicidae* | - | - | - | - | - | P | - | - | - | - |
| | • *Doidae* | - | - | - | - | - | P | - | - | - | - |
| | • *Simulidae* | P | P | P | - | P | P | P | P | - | - |
| | • *Chironomidae* | P | P | P | P | P | P | P | P | P | P |
| | • *Ceratopogoniadae* | - | - | P | - | - | P | - | P | - | - |
| GASTROPODA | • *Lymnaeidea* | - | - | - | P | - | P | - | - | P | - |
| | • *Planorbidae* | - | - | - | - | - | - | - | - | P | - |
| | • *Ancylidae* | - | - | - | - | - | P | - | - | - | - |
| PELECYBODA | • *Sphaeriidae* | - | - | - | - | - | P | - | - | - | P |
| **NO. taxa recorded** | **34** | **19** | **11** | **12** | **10** | **12** | **20** | **13** | **17** | **7** | **9** |

(From Durgapersad, 2005)

The following were also determined:

- **The SASS4 score:** SASS4 scores were determined for each sampling site based on the sensitivity of families recorded, at each site, to poor water quality summed over all taxa recorded at that site.

- **The average score per taxon (ASPT):** This was determined by dividing the SASS4 score for the site by the number of taxa recorded at that site,
- **The Integrated Habitat Assessment System (IHAS):** This was determined by considering the quality and quantity of sampling biotopes in terms of potential as habitat for invertebrates as well as the stream characteristics (width, depth and velocity) and comparing these scores with the maximum possible score expressed as a percentage.

The results of this analysis are shown in Table 21.

Table 21: Results of Habitat Integrity Assessments for selected wetland sites

| VARIABLE | SAMPLE SITES | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SUMMER | | | | | WINTER | | | | |
| | REF | K6 | K21 | E7 | N8 | REF | K6 | K21 | E7 | N8 |
| **SASS4** | 108 | 46 | 57 | 43 | 51 | 106 | 60 | 87 | 26 | 34 |
| **ASPT** | 5.7 | 4.2 | 4.8 | 4.3 | 4.3 | 5.3 | 4.6 | 5.1 | 3.7 | 3.8 |
| **IHAS** | 77 | 74 | 49 | 83 | 79 | 70 | 71 | 76 | 78 | 68 |

(Durgapersad, 2005)

| Good | Fair | Poor |
|---|---|---|

## 1.5 Socio-economic implications of the water quality status of the Klip River

The Klip River is of socio-economic importance in a number of ways. The river forms part of the Upper Vaal WMA, which is of importance in terms of its contribution to the GDP, employment as well as generation of household incomes. Figure 14 shows the contribution of the Klip River to the total GDP, employment and household income in the Upper Vaal WMA.

Despite its importance, the Klip River catchment raises a number of concerns. DWA (2011) for example notes that the Riet Spruit and the Klip Rivers are severely impacted and that improvements in the present state cannot occur without addressing water quality related problems and proposes implementation of the Integrated Water Quality Management Plan developed for the Vaal, as one of the possible strategies (DWA, 2011). One of the more serious concerns about the Klip River is Acid Mine Drainage.

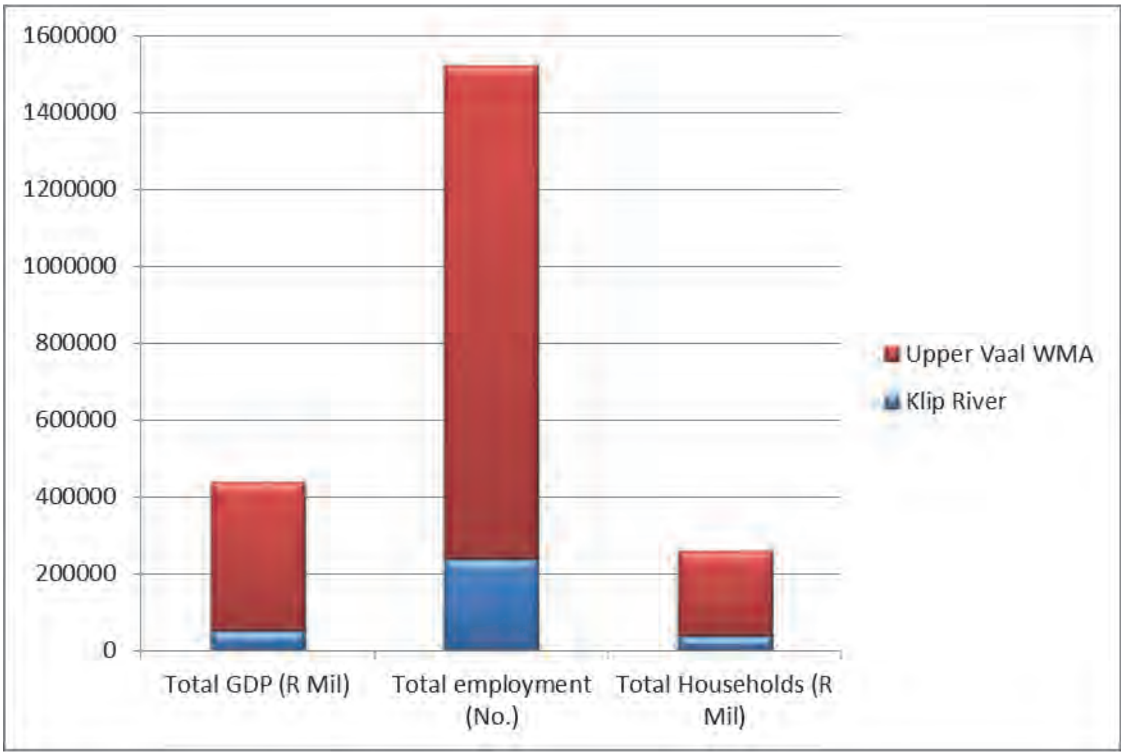


Figure 14: Contribution of the Klip River to the Upper Vaal WMA in terms of GDP, employment and household income (Prepared with data from DWA, 2011)

AMD associated with mining activities in the Witwatersrand Gold Fields has a major impact on South Africa's major river systems including the Klip River and subsequently the Vaal System as previously discussed. In addition to water that is decanting from the Western Basin, that is already causing serious concern, water that will decant from the Central Basin unless action is taken, is also likely to add significantly to the saline load of the Vaal system. The implication of the increased and possible further increase in salinity will require larger volumes of clean water from upstream sources, including the Lesotho Highlands Water Project, to maintain water quality at acceptable levels. The discolouration and potential effects on taste of the water was reported to be an important concern to residents residing in the decant area who relied on borehole water (Oelofse, 2010).

A study aimed at determining the economic effects of increasing salinity of Vaal river water showed that an additional amount of R 252 Million annually will be required for an increase in salinity of 500 mg/l, i.e. from 300 mg/l to 800 mg/l (Howie & Otto, 1996). This illustrates the high costs of maintaining water quality.  Another factor that must be considered is the human and animal health risk that of are of even greater concern.

## 1.6 Conclusion

Although there is evidence that many sites on the Klip River remain within the acceptable range, there are areas of clear concern. One of these is the altered flow regime and the dominance of return flows. Another issue of concern relates to water quality especially with regards to the nutrient load, heavy metals, etc. Studies done on crabs and fish sampled from the river reveal that there may be impacts on various taxa as indicated by bioaccumulation studies for example. This then raises questions on impacts on human health as well as other taxa in the food chain. This raises the need for more detailed studies with a view towards improvements in water quality management in the catchment.

# 2 MATERIAL AND METHODS

## 2.1 Site selection

The proposed risk regions, state of the Klip River deliverable and personal knowledge were used to identify possible sites on the Klip River within each of the proposed risk regions. The stakeholder engagement workshop identified five risk regions for the study (Figure 15), which corresponded to the quaternary catchment in the Klip River basin. The risk regions also corresponded to the municipal boundaries of the City of Johannesburg. The proposed risk regions and survey sites (Figure 15) that were found to be suitable for the study, was selected as it met all the following requirements:

- Sites were easily accessible;
- safe;
- instream habitat was sufficient to sustain diatom, macro-invertebrate and fish communities;
- positioning of sites made it possible to determine the various endpoints for the RRM;

A short description of each site is presented in the following sections. The descriptions focused on the available habitat and the current impacts present at the sites. Site photographs were taken during the field visit in June 2013 while observations were combined from both surveys.


Figure 15: Risk regions and sites on the Klip River, Natal Spruit, Riet Spruit and Vaal River.

### 2.1.1 Klip River 1 (KR1)

The most upstream site on the Klip River is situated close to Roodepoort and is upstream of the Soweto and mining areas. However, recently sand mining has increased substantially upstream and at the site. This has caused significant damage on the instream and riparian habitat (Figure 16 A & B). Presently no habitat diversity exists in the river and it is mostly dominated by coarse sediment. The riparian zone has been destroyed and many of the canopy cover have been removed. Previously this site had a very diverse habitat with a complete canopy cover that resulted in a diverse aquatic biota composition. There is evidence of recent fire, litter dumping and there are numerous roads near the site. There were no changes at the site during the field survey in January 2014.

**A** **B**

Figure 16: Site photographs of Klip River 1 showing (A) upstream and (B) downstream.

### 2.1.2 Klip River 2 (KR2)

The Klip River 2 site is situated at the Olifantsvlei Waste Water Treatment Works near Lenasia. The river is much wider (approximately 10 m) and deeper with numerous flow classes and habitat biotopes present. A small impoundment is situated upstream of the site that creates very good habitat for numerous water bird species. The habitat present at the site includes various stones sections, gravel, sand, mud and abundant marginal vegetation (Figure 17 A & B). There are large volumes of return flow from the WWTW that does enter the Klip River and the flow changes have increased the presence of erosion. The banks of the river are near vertical and many other indications of erosion are present at the site including an old road bridge that has been washed away. Apart from the WWTW and the impoundment that is disrupting flow conditions, there are also large quantities of litter at the site that can affect the water quality. It was also evident that this site is frequently visited by the local communities for fishing, recreational and religious purposes. No changes in habitat quality were noted during the January 2014 survey.

A B

Figure 17: Site photographs of Klip River 2 showing (A) upstream and (B) downstream.

### 2.1.3 Klip River 3 (KR3)

Klip River site 3 is situated in the Zwartkoppies area downstream of all the various impacts of Soweto, Lenasia and Alberton with the immediate landuse being mostly agriculture or open grassland. The instream habitat is very similar to site KR2 with a variety of stones habitats, grave, sand, mud and marginal vegetation present (Figure 18). The flow was found to be strong with very little break in the flow even, in pooled areas. The strong flows have caused significant erosion at the site and the bridge crossing the river at the sites are being slowly eroded away. Both banks are very steep and near vertical at most places due to the erosion. In some places marginal vegetation are decreased due to the erosion and steep banks. No changes in habitat were seen during the January 2014 survey but stabilising of the banks and the road bridge has been made since June 2013.

A B

Figure 18: Site photographs of Klip River 3 showing (A) upstream and (B) downstream.

### 2.1.4 Klip River 4 (KR4)

The Klip River site 4 is situated at the Henley-on-Klip Weir (Kidson Weir) in the town of Henley-on-Klip. The weir is approximately 4 m high and 50 m long and represents a significant barrier in the system. The weir has an abundance of marginal vegetation and some severe siltation has occurred on the western bank (Figure 19 A). Downstream of the weir there are rapids and some riffles that contain stones in various sizes and it resembles the habitat at site KR3. However, the strong flows have caused severe damage due to erosion downstream as well as at the weir itself (Figure 19 B). The weir is in the process of being upgraded and authorisation is awaited from the Department of Water and Sanitation. Residential areas dominate the banks of the weir and upstream land uses are predominantly agriculture. The habitat diversity at the site has not changed since the field survey during June 2013.

**A** **B**

Figure 19: Site photographs of Klip River 4 showing (A) upstream and (B) downstream.

### 2.1.5 Klip River 5 (KR5)

Klip River site 5 is positioned in the lower reaches of the river to represent the effects of Meyerton and Vereeniging on the Klip River. The river at this site is wide, deep and the clarity of the water is low (Figure 20 A & B). This site does not have any riffle habitat and the dominant habitat is the marginal vegetation. The erosion of the stream banks are still present but less severe than upstream, due to the decreased flow in the river. The habitat diversity at the site did not change from June 2013 to the January 2014 field survey.

A B

Figure 20: Site photographs of Klip River 5 showing (A) upstream and (B) downstream.

### 2.1.6 Upper Riet Spruit (RS1)

The Riet Spruit originates on the East Rand of Johannesburg in the Ekurhuleni Metropolitan Municipality (EMM). The Upper Riet Spruit site was selected on the outskirts of Katlehong and was situated in open grassland. Evidence of recent fires was present and the riparian zone appeared disturbed while litter was also evident at the site. The instream habitat comprised of larger boulders and cement bricks in the riffle sections while the slower sections had various types of marginal vegetation (Figure 21). The presence of organic matter and siltation was also evident in the pooled areas. The river was very narrow at this point compared to the downstream site. No changes in the habitat at site RS1 were noted during the January 2014 survey.

A B

Figure 21: Site photographs of Upper Riet Spruit showing (A) upstream and (B) downstream.

### 2.1.7 Lower Riet Spruit (RS2)

The Lower Riet Spruit site is situated at the ERWAT Waterval Waste Water Treatment works before the confluence with the Klip River (Figure 22). This site was selected to qualify and quantify the impact of EMM and the Riet Spruit catchment on the Klip River. This catchment is similar in size when compared to the upper regions of the Klip River. It was noted that a significant increase in the river size was seen downstream of the confluence of the Natal Spruit with the Riet Spruit, giving an indication of the amount of return flow that enters the Natal Spruit from EMM. Instream habitat was mostly deeper pooled areas while downstream of the weir (Figure 22) some riffle sections were present. Riverbanks were mostly very steep due to erosion and marginal vegetation was limited to shallower sections. The SASS and fish sampling was limited to upstream of the weir to avoid movement of fish from the Klip River itself. One dead *Lb. aeneus* was seen at the site. No changes of the available habitat were noted during the January 2014 survey.


Figure 22: Site photographs of Lower Riet Spruit showing (A) upstream and (B) downstream.

### 2.1.8 Natal Spruit (NS1)

The Natal Spruit also originates in the EMM and then flows through Katlehong and enters the Riet Spruit. Many places within the Natal Spruit are characterised by an abundance of reed growth as can be seen in Figure 23 A. This indicates that historically much of the Natal Spruit was valley bottom wetlands or floodplains that have become increasingly inundated due to the urbanisation. That has resulted in a channelized system as can be seen in Figure 23 A. The instream habitat was mostly cobbles and boulders at the site, which was situated at a road crossing. Upstream the river was smaller in size and more densely vegetated (Figure 23 B). The water at the site smelled strongly of chlorine giving an indication that

WWTW effluent is entering the system. Marginal vegetation was mostly reeds and shrubs. Habitat at the site did not show any significant changes during the January 2014 field survey.


Figure 23: Site photographs of Natal Spruit showing (A) upstream and (B) downstream.

## 2.2 Water quality

Surface water samples were taken directly below the water surface with a clean scoop bucket, transferred to a set of bottles, and transported to the laboratory in a cooler box. Two litres of water were taken for general analysis in pre-washed plastic bottles and 500 ml of water were taken in a glass bottle for bacteriological analysis. The bacteriological analyses were carried out in the laboratories of the North West University. The analyses of the other water parameters were completed at the laboratories of the University of Johannesburg. The water analysis included nutrients, salts, metals and bacteriological variables. Results were compared to the South African Water Quality Guidelines, where Target Water Quality Requirements (TWQR) is available for aquatic ecosystems (DWAF, 1996a). Table 22 provides all of the specific parameters that will be analysed for the water samples.

Table 22: The water quality variables that were analysed at the sampling localities.

| | |
|---|---|
| Nutrients | Phosphates, nitrites, nitrates, ammonium, ammonia, chlorophyll a |
| Metals | Ag, Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Si, Sr, Ti, U, V, Zn |
| Bacteriological | Heterotrophic plate count, total coliforms, faecal coliforms, E. coli |
| Ions | Cl, Ca, Na, Mg, K, F, sulphates, alkalinity, total hardness, |

### ***In situ* analysis**

The *in situ* physico-chemical variables that were sampled during the survey included temperature, pH, dissolved oxygen concentration ([DO]) and saturation (DO%), total dissolved solids (TDS) and electrical conductivity (EC). This *in situ* analysis was undertaken using a pre-calibrated Extech multi-parameter hand-held water quality meter.

### 2.3 Sediment

#### 2.3.1 Sampling and physical characteristics

A sediment sample was collected in a 500 ml polyethylene jar at each site during the survey. The samples were kept on ice during transport and frozen till analysis in the University of Johannesburg laboratories. Each sample were analysed for sediment grain size, organic matter, moisture content and metal concentrations. Additionally, a sediment sample was placed in a glass jar and frozen until analyses of organic pollutant concentrations could be undertaken.

The ASTM (2000) and USEPA (1991) standardised methods were followed for physical sediment analysis. A known amount of sediment (accurate to 0.01 g) from each site was oven dried for 96 hours at 60°C. Once dried, sediment was reweighed to determine moisture content. A known amount of sediment (accurate to 0.0001 g) was transferred to pre-weighed crucibles for organic content determination. The crucibles were placed into a furnace for a minimum of six hours at 600°C, until all organic matter was incinerated. The crucibles were allowed to cool before it is re-weighed to calculate percentage organic matter lost. The moisture and organic content of each sample will be determined in triplicate and reported as an average. The organic content can be classified according to the USEPA (1991) classification scheme in Table 23.

Table 23: Classification of organic content in sediment samples (USEPA, 1991).

| Percentage | Classification |
|---|---|
| < 0.05% | Very low |
| 0.05-1% | Low |
| 1-2% | Moderately low |
| 2-4% | Medium |
| > 4% | High |

The grain size of each sample was determined using an Endecott sieve system with mesh sizes from 4000 µm to 53 µm. Each sample was shaken for 15 minutes before each fraction is weighed and expressed as a percentage of the total sample. The grain size of each sample was determined in triplicate and reported as a fraction of total sample weight. The grain sizes present will be categorised according to Table 24 (Wentworth, 1922; Cyrus et al., 2000).

Table 24: Classification of grain size categories for sediment samples (Wentworth, 1922; Cyrus et al., 2000).

| Grain Size | Classification |
|---|---|
| > 4000 µm | Gravel |
| 4000 µm-2000 µm | Very coarse sand |
| 2000 µm-500 µm | Coarse sand |
| 500 µm-212 µm | Medium sand |
| 212 µm-53 µm | Very fine sand |
| < 53 µm | Mud |

### 2.3.2 Metal analysis

The sediment samples were received by the WRG as frozen samples after collection in PET jars. The samples were kept frozen until analysis at the laboratory facilities at the University of Johannesburg could take place. The methodology followed for the analysis was for the total digestion of sediments according to Hassan et al. (2007). Each sediment sample was oven dried for 2-4 days at 70°C. A known amount of each sample (approximately 0.5 g) was digested with Suprapur nitric acid (HNO3) in an Ethos Microwave digester for 20 min. The samples were then diluted and filtered with 0.45 µm cellulose nitrate under vacuum pressure. The filtered extract was then analysed by Inductively Coupled Plasma – Optical Emission Spectrophotometer (ICP- OES) and an Inductively Coupled Plasma – Mass Spectrophotometer (ICP-MS). The results are expressed as mg/kg. The concentrations for each of the metals are then compared to international standards (Table 25) and other local studies (Greenfield et al., 2007).

Table 25: Sediment guideline values used internationally for sediment metal pollution. Guideline values derived from Australia-New Zealand (ANZECC, 2000), Netherlands (Friday, 1998), Canada (Friday, 1998), Hamilton (2004) and Sheppard et al. (2005).

| | | **Unit** | **Guideline Value** | **Olifants River#** |
|---|---|---|---|---|
| Aluminium | Al | mg/kg | n/a | - |
| Titanium | Ti | mg/kg | n/a | - |
| Vanadium | V | mg/kg | n/a | 47.46 |
| Chromium | Cr | mg/kg | 26 | 38.83 |
| Manganese | Mn | mg/kg | 460 | 249.1 |

| | | Unit | Guideline Value | Olifants River# |
|---|---|---|---|---|
| Iron | Fe | mg/kg | n/a | 16090 |
| Cobalt | Co | mg/kg | 20 | 7.57 |
| Nickel | Ni | mg/kg | 18 | 10.89 |
| Copper | Cu | mg/kg | 16 | BD |
| Zinc | Zn | mg/kg | 200 | BD |
| Arsenic | As | mg/kg | 5.9 | 3.33 |
| Selenium | Se | mg/kg | 0.08 | - |
| Strontium | Sr | mg/kg | n/a | - |
| Molybdenum | Mo | mg/kg | 10 | BD |
| Silver | Ag | mg/kg | 1 | - |
| Cadmium | Cd | mg/kg | 0.57 | BD |
| Lead | Pb | mg/kg | 35 | BD |

### 2.4 Diatoms

Diatom communities are able to grow in many habitat areas of aquatic ecosystems like stones and marginal vegetation. Diatoms were sampled from mostly stones when available. The sampling of diatoms has been described in detail by Taylor et al. (2007) and the methods that were used in this study are based on those methods. The sampling of macrophytes was done by carefully cutting the vegetation approximately 10 cm below the surface. The cut vegetation was transferred into a plastic bag. This was repeated until 5-6 length of macrophyte has been sampled. A small amount of water was added to the bag, the bag was vigorously rubbed, taking care not to puncture it, to transfer the diatoms into the water. The water was transferred into a plastic container for transport and storage until analysis. The stones were sampled with the use of a toothbrush and a small flat container. Four or five stones were collected from the sites where it was available and then placed into the container. The stones were then vigorously scrubbed with the toothbrush to remove all of the available biofilm. Once removed it was carefully poured into the sampling container for transport to the laboratory.

Each diatom sample was preserved with ethanol to reach a final concentration of approximately 20% by volume. The diatom samples were taken to the laboratory of the University of Johannesburg for cleaning and slide preparation using the potassium permanganate and hot hydrochloric acid method as described by Taylor et al. (2007). The prepared slides were then used to enumerate and identify between 300-600 valves per slide. Matlala et al. (2011) has shown that 300 were the minimum required count for use in index calculations with the ideal count set at 400 valves.

## 2.5 Macro-Invertebrates

The index used in South Africa to assess the state of macro-invertebrates is known as the South African Scoring System (SASS5). The index makes use of the presences of macro-invertebrate families and their perceived sensitivity to water quality changes of these families. Different families show different sensitivities to pollution, these sensitivities range from highly tolerant families (e.g. Muscidae and Psychodidae) to highly sensitive families (e.g. Oligoneuridae). SASS is an accredited protocol that has been tested and widely used in South Africa as a biological index of water quality. SASS results are expressed both as an index score (SASS score) and the average score per recorded taxon (ASPT value). From this data it is possible to establish the integrity or health of a river. The standard SASS-5 protocol (Dickens & Graham, 2002) was followed to collect invertebrate samples, and various biotopes in which macro-invertebrates may occur were sampled. Three biotopes were sampled including: stones (in current, out of current and bedrock), vegetation (marginal and aquatic) and gravel, sand and mud (GSM). After sampling each biotope, using the standard SASS net (1 mm mesh and dimensions of 30 x 30 x 30 cm), the samples were placed in an identification tray and the macro-invertebrates were identified. Identification took place on site for the set period of 15 minutes. If no new taxon was identified for 5 minutes, identification was stopped.

SASS5 results are often analysed based on the biological bands method developed by Dallas (2007). These methods are, however, still under development and care should be taken using Figure 24 to interpret ecological categories. In this instance, Figure 24 was used to determine the ecological categories, as sufficient data points were present to identify the various categories. The descriptions of the ecological categories are provided in Table 26. It should be noted that SASS5 complies with international accreditation protocols and a SASS5-accredited practitioner from NWU/UJ undertook the SASS5 assessments on the field survey. For a high confidence assessment, results must be obtained for various seasons. This is because aquatic macro-invertebrate communities may often display seasonal variation in community structure. Identification of the organisms was made to family level (Dickens & Graham, 2002; Gerber & Gabriel, 2002).

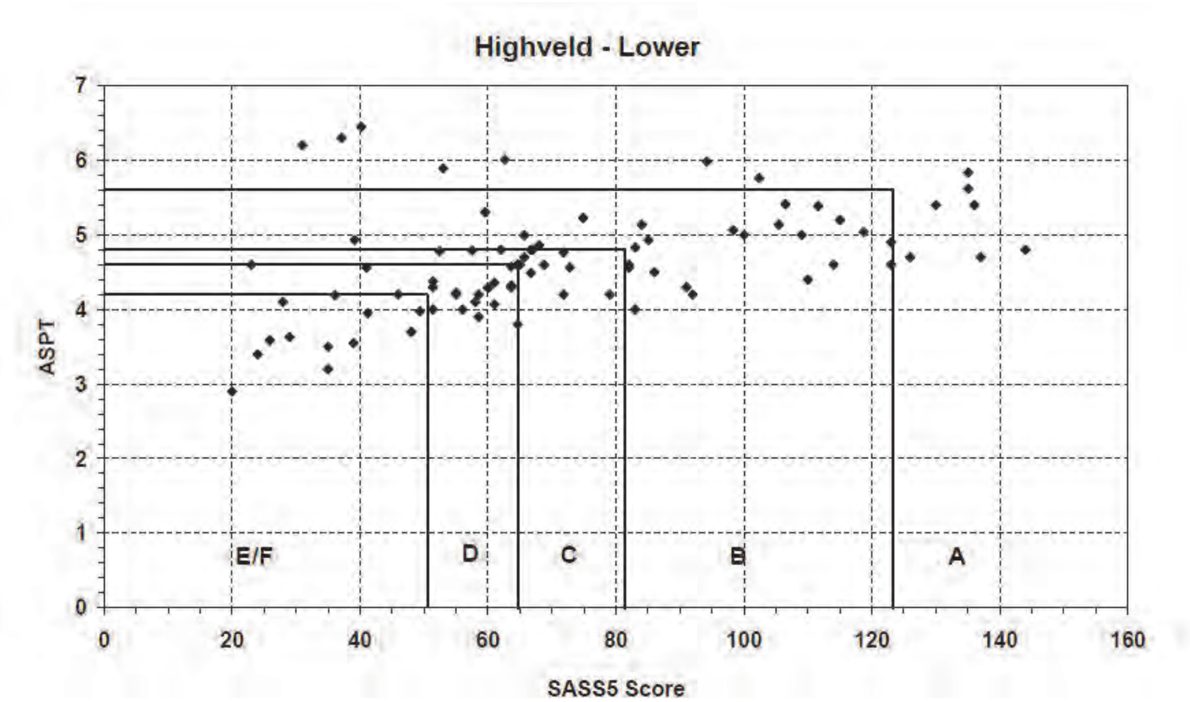


Figure 24: Biological bands for the Highveld Lower Zone (Dallas, 2007).

Table 26: Ecological categories, class key colours and category descriptions presented within the biotic assessment.

| Class | Ecological Category | Description |
|---|---|---|
| A | Natural | Unmodified state – un-impacted state, conditions natural. |
| B | Good | Largely natural – few modifications, mostly natural. |
| C | Fair | Moderately modified – Community modifications, some impairment of river health. |
| D | Poor | Largely modified – Distinct impairment of river health, impacted state. |
| E | Seriously modified | Seriously modified – most community characteristics modified, seriously impacted state. |
| F | Critically modified | Critically modified – extremely low species diversity and abundance, unacceptable modified state. |

## 2.6 Fish Community Assessment

The RHP (Mangold, 2001) and Fish Response Assessment Index (FRAI) (Kleynhans, 2007) sampling methodologies were used to assess the fish populations in the Klip River catchment. The technique used to sample was electro-shocking (Meador et al., 1993;

Barbour et al., 1999). Smith and Root battery-operated electro-shocking equipment was used to sample fish in the available habitat at each site. The electro-shocking technique was implemented for at least 45 minutes depending on the site and habitat availability. All the fish caught were identified and returned to the water. When the fish could not be identified on site it was preserved in 10% formalin for identification in the laboratory.

The current index of choice to determine the fish community integrity for the RHP and Reserve determinations is the FRAI (Kleynhans, 2007) as developed within the EcoClassification methodology (Kleynhans and Louw, 2007). EcoClassification is a rule-based method that aims to integrate the biophysical components of a river to provide a realistic and reproducible result of the EcoStatus of a river (Kleynhans and Louw, 2007). FRAI is based on the responses of fish species and the fish community to various stressors within the ecosystem. These stressors can vary from the lack of natural environmental requirements of the fish, or the effects of driver changes, to the habitat conditions within the ecosystem. The ecological category for the fish community was determined by comparing the environmental requirements and the responses to modified habitat conditions of the observed (in some cases derived) fish communities to a reference fish community (Kleynhans, 2007).

The FRAI index makes uses of the rating, ranking and weighting procedure adopted by the EcoStatus approach (Kleynhans and Louw, 2007). The drivers and responders within an ecosystem do not have the same ecological importance or sensitivity in a specific river. In effect this means that a specific metric may be modified but the effect within the ecosystem will be low if the metric has a low ecological importance or sensitivity. The specific importance and sensitivity of each metric could potentially change from river to river or from ecoregion to ecoregion depending on the importance and sensitivity. Thus, the ranking and weighting approach was incorporated to help deal with this inert variability of rivers (Kleynhans, 2007). The FRAI index has different metrics and sub-metrics to calculate the ecological category. The approach of using various metrics helped to develop a more consistent index of the fish community and helped with the mathematical integration of the metrics.

A reference fish species list, as well as a fish frequency of occurrence within the system, is required for the FRAI index. This information is available in the Reference Fish Frequency of Occurrence (FFROC) (Kleynhans et al., 2007). If no data is available from this source, previous studies and literature should be used to derive the frequency of occurrences and reference species lists for the site and system. The reference list and frequency of occurrences are entered into the FRAI index together with the sampled fish species and their frequency of occurrence. The index then calculates an automated FRAI index value based on the relative intolerances, reference frequency of occurrence and current frequency of occurrence of the reference fish species. This value can be adjusted for each metric within the FRAI index due to changes in habitat conditions observed at the site or expert opinion to provide a more accurate FRAI index value. The FRAI index value is given in terms of an ecological category from A-F according to Table 26.

## 2.7 Fish Health Assessment

Literature review and previous experience indicated that *C. gariepinus* would have the highest probability of occurring at each of the sites selected for the fish health assessments. Therefore, *C. gariepinus* was selected as the test organism for the study. Additionally, it is a common species targeted by local communities as a food source. The fish were collected by means of gill nets (90mm mesh) and sampled fish were then transferred into a keep net. Fish were killed according to the acceptable ethical method of cutting through their spinal cord. Autopsy was performed and the fish were assessed according to the HAI procedures described by McHugh et al. (2011).

Fish collection for the Health Assessment Index (HAI) took place together with the collection of tissues from the *Clarias gariepinus.* The HAI considers the general health of fish using a variety of lines of evidence including parasitic infection, gross tissue structure states and the physiological state of the organism's blood (Heath et al., 2003). Advantages of this method includes: that the life span of fish are generally long and can thus give an indication of long term effects on the system, fish live in close association with their environment therefore the appearance of their internal and external features as well as their blood parameters will indicate if they are in accord with their environment and the HAI can indicate first level health problems in the fish (McHugh et al., 2011).

Fish muscle samples were taken for metal analysis during the survey from the side of each fish. Muscle samples were frozen until analysis will be carried out at the laboratories of the University of Johannesburg. Fish muscle and liver samples were taken for the various biomarker analyses, preserved in Hendrikson's buffer and frozen with liquid nitrogren. Muscle, liver and blood samples were taken, preserved in Hendrikson's buffer and frozen in liquid nitrogen for the DNA analysis to be carried out in the laboratories of the University of Johannesburg. All of the samples were transferred to -80°C once returned to the University of Johannesburg.

## 2.8 Fish Bioaccumulation

Fish muscle samples were taken for metal and organic analysis during the survey from the side of each fish. Muscle samples were frozen until analysis will be carried out at the laboratories of the University of Johannesburg. Pollutant accumulation makes use of the fish muscle tissue as that is the portion of fish that are generally consumed by the human population. The muscle tissue was fillet and samples for organic pollutants were encased in aluminium foil while metal accumulation samples will be placed in a sample tube. Both samples were then frozen until the analysis takes place. The analysis uses a method adapted from Wepener et al. (2012). This method uses a known mass of dried sample for digestion with 7ml nitric acid ($HNO_3$) and 1ml hydrogen peroxide ($H_2O_2$) in an Ethos Microwave digester at 200°C for 20 min. The samples are allowed to cool before being diluted to 50 mℓ. The metal concentrations of selected metals were determined on an Inductively Coupled Plasma – Optical Emission Spectrophotometer (ICP-OES). Any metals that were found to be below the detection limits of the ICP-OES were re-analysed on an Inductively Coupled Plasma – Mass Spectrophotometer (ICP-MS).

## 2.9 Fish Biomarker Assessment

Fish biomarker analysis makes use of liver and muscle tissue which are preserved in Hendrikson's buffer and then frozen in liquid nitrogen until it can be placed at -80°C in the laboratory. The biomarker analysis included biomarkers of both exposure and effect. The biomarker of exposure will be acetylcholinesterase (AChE – pesticide exposure), metallothioniens (MT) and Cytochrome P450 (EROD) activity. The biomarkers of effects will be catalase (CAT) activity, reduced glutathione activity (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), and protein carbonyls (PC). These biomarkers are all indicative of oxidative stress that can be caused by the exposure and effects of organic substances. Additionally cellular energy allocation (CEA) wasere determined to indicate if there were any energetic disturbances in the fish. All the methodologies that were used to determine the biomarker activity have been validated in numerous studies internationally as well as nationally and are presented in Table 27.

Table 27: Biomarkers that form part of the study.

| **Biomarkers of Exposure** | | **Biomarkers of Effect** | | **Others** | |
|---|---|---|---|---|---|
| AChE | Ellman et al. (1961) | CAT | Cohen et al. (1970) | CEA | De Coen and Janssen (1997 |
| CYP450 | Elisa kit | MDA | Ohkawa et al. (1979 | | |
| MT | Viarengo et al. (1997) | PC | Floor and Wetzel (1998) | | |
| | | SOD | Misra (1989) | | |

Approximately 1 g each of tigerfish liver and muscle were placed in cryotubes, mixed with Hendrickson stabilising buffer (Wepener et al., 2005) and placed in liquid nitrogen for biomarker analysis. The remaining portions of the axial muscle were removed and frozen for further analysis. Dissection boards and tools were rinsed with 99.8% ethanol between dissections.

Approximately 0.2 g of collected liver tissue were placed in Eppendorf tubes labeled A and B respectively, and 0.2 g of muscle tissue was placed in an Eppendorf tube labeled as C. The sample in Eppendorf A was homogenized on ice in 200 µL of General Homogenizing Buffer (GHB), centrifuged at 10 000 r.p.m. (Sigma 2-15 centrifuge) for 10 minutes at 4°C and aliquots of the supernatant taken for SOD, CAT, AChE, PC, LP and CYP450 activity analysis. The sample in Eppendorf B was homogenized on ice in 600 µL Tris-sucrose Buffer (Tris) and used solely for MT analysis. The sample in Eppendorf C was homogenized on ice in 200 µL ETS Buffer and used solely for CEA analysis.

### 2.9.1 Acetylcholinesterase

The methodology for AChE analysis was adapted from Ellman et al. (1961). The following chemical solutions were added to 24 of the 96 wells in a microtitre plate:

- 210 µL of Potassium Phosphate Buffer (PPB)
- 10 µL of s-Acetylthiocholine iodide
- 10 µL Ellmans' (2,2'-Dinitro-5,5'dithio-dibenzoic acid) reagent

The sides of the well were lightly tapped to ensure homogeneity, and the plate was covered with the plate lid and allowed to incubate at 37°C for 5 minutes. After incubation, 5 µl GHB was added to the first three wells as a procedure blank. 5 µl of sample was added to the other wells in triplicate so that there were 7 samples being read. The sides of the plate were lightly tapped to ensure mixing and the plate was read immediately at 405 nm, using an automated microplate reader (Elx800-Universal microplate reader; BioTek instruments, USA), in 1 minute intervals over a 6 minute time period. The protein content was determined separately using the method of Bradford (1976), where the absorbance was measured at 630 nm and bovine serum albumin (BSA) used as a standard. Protein content is determined because each biomarker concentration is measured in activity per milligram protein.

### 2.9.2 Cytochrome P450 Activity

Cytochrome P450 activity was determined using a DetectX P450 demethylating fluorescent activity kit (Arbor Assays, K011-F1) where the samples were first diluted with assay buffer in a 1:6 ratio and the samples read using a Multi-Detection microplate reader (Synergy HT; BioTek instruments, USA). Protein content was determined using the method of Bradford (1976).

### 2.9.3 Metallothioneins

The method for MT analysis was adapted from Viarengo et al. (1997; 1999) for analysis on invertebrates using the modification as indicated by Atli & Canli (2008). The samples were homogenised in 3:1 ratio of MT Tris homogenising buffer, and were centrifuged at 72 500 r.p.m (Biofuge stratus, Heraeus instruments) at 4°C for 20 minutes. Five hundred µl of cold (4°C) absolute ethanol and 40 µl of chloroform were added to 500 µl of the supernatant, and vortexed to ensure homogeneity. These samples were then centrifuged at 7 000 r.p.m (Sigma 2-15 centrifuge) (4°C) for 10 minutes. Three further volumes of cold ethanol were added to the mixture, vortexed and incubated at -20°C for 4 hours until a pellet formed. The supernatant was decanted and the pellet washed twice with 1 ml of washing buffer (87% ethanol, 1% chloroform, 12% homogenising buffer), after which it was vortexed and centrifuged at 3000 r.p.m (Sigma 2-15 centrifuge) (4°C) for 20 minutes. The pellet was dried using compressed air, and the pellet resuspended in 300 µL of Tris-Ethylene diamine tetraacetate (EDTA) and vortexed. Ellman's reagent (5,5' dithio-bis (2-nitrobenzoic acid); DTNB; 210 µl) and 15 µl of homogenising buffer were added to the first three wells as a procedure blank in triplicate. Ellman's reagent (210 µl) and 15 µl supernatant were added in triplicate per sample and the samples incubated at room temperature for 15 minutes. The absorbance of samples was read at 412 nm using an automated microplate reader and the protein content determined using the method of Bradford (1976).

### 2.9.4 Cellular Energy Allocation (CEA)

The method for CEA analysis was adapted from De Coen & Janssen (2003), for which protein content, glucose content, lipid content and electron transport system (ETS) activity were determined. 100 µl supernatant (as described previously) was further diluted, using 400 µl ETS buffer and 400 µl ultrapure water, and all analyses carried out on ice.

#### 2.9.4.1 Available Energy Reserves (Ea)

Protein was determined using the method of Bradford (1976). Carbohydrate was determined using a glucose content test kit (GOD-PAP 1 448 668, Roche) and glucose standard (C FAS 759 350, Roche) at 560 nm with an automated microplate reader. Total lipids were extracted following the method of Bligh & Dyer (1959) using tripalmitin as a standard, where 250 µl supernatant was added to 500 µl chloroform and vortexed. Methanol (500 µL) and 250 µl ultrapure water was added to this solution, vortexed and then centrifuged at 4°C for 10 mintues at 7 250 r.p.m (Sigma 2-15 centrifuge). One hundred µl of the organic phase was placed in glass tubes and a blank prepared from 100 µl chloroform. Sulphuric acid ($H_2SO_4$; 500 µl) was added to each tube and the tubes covered with foil and incubated at 200°C for 15 minutes. One ml of ultrapure water was added to each tube and the samples allowed to cool down. Two hundred and forty five µl of each sample and the blank was added in triplicate to polyethylene microtitre plates and the sample absorbancies were read at 360 nm using an automated microplate reader.

#### 2.9.4.2 Energy Consumption ($E_c$)

The cellular respiration rate (energy consumption) was determined by measuring the ETS activity. The samples were centrifuged at 7 250 r.p.m (Sigma 2-15 centrifuge) for 10 minutes at 4°C. Twenty five µl of supernatant of ETS buffer was placed in the first 3 wells in a microplate as a procedure blank. Twenty five µl of supernatant from each sample was placed in triplicate on a microplate with a maximum of 5 samples per plate. Buffered substrate solution (BSS; 0.3% (v/v; 75 µl) Triton X-100, and Tris-HCl), 25 µl NAD(P)H solution and 50 µl p-IodoNitro Tetrazolium violet/chloride (INT) was added to each well and the samples read kinetically at 490 nm at 20°C at 1 minute intervals over a 5 minute period using an automated microplate reader.

### 2.9.4.3 Cellular Energy Allocation (CEA)

The energy reserves were converted into energetic equivalents using the enthalpy of combustion values as indicated by De Coen & Janssen (1997), where these values were 17 500 mJ/mg glycogen, 39 500 mJ/mg lipid and 24 000 mJ/mg protein. The $E_c$ was determined using the theoretical stochiometric relationship that indicates that for each 2 µmol of formazan formed, 1 µmol of oxygen is consumed in the ETS system. The amount of oxygen was transformed into energetic equivalents using an average oxyenthalpic equivalent of 484 kJ/mol $O_2$. The total energy budget was calculated using the following equation:

$CEA= E_a-E_c$
Where: $E_a = E_{glucose}+E_{lipid}+E_{protein}$
$Ec=E_{ETS}$

### 2.9.5 Superoxide Dismutase (SOD)

The methodology for SOD was adapted from Greenwald (1989) where 3 ml Tris Buffer was added to each sample and the reaction initiated by adding 25 µl pyrogallol solution and the samples read on a Multi-Detection microplate reader (Synergy HT; BioTek instruments, USA).

### 2.9.6 Catalase Activity (CAT)

The methodology for CAT was adapted from Cohen et al. (1970). While working on ice, 15 µl of the homogenate from Eppendorf A supernatant was placed in an Eppendorf with 60 µL 0.01 M Catalase Phosphate Buffer (CAT PP buffer; pH 7.0) and centrifuged at 10 000 r.p.m. (Sigma 2-15 centrifuge) for 10 minutes at 4°C. GHB (10 µl) was added in triplicate to the microtitre plate as a procedure blank and 10 µl of each supernatant was added to a microtitre plate in triplicate (maximum of 15 samples per plate). $H_2O_2$ (93 µl) was added to each well, once all of the wells had been filled the plate was tapped gently on the side and allowed to incubate at room temperature for 3 minutes. Sulphuric acid ($H_2SO_4$; 19 µl) was added to each well to stop the reaction, followed immediately by the addition of 130 µl 2 mM potassium permanganate ($KMnO_4$) to measure the amount of unreacted $KMnO_4$ spectrophotometrically at 409 nm using an automated microplate reader. The protein content was measured using Bradford reagent (Bradford 1976). Catalase Activity was expressed as µmol $H_2O_2$/mg protein/minute.

### 2.9.7 Lipid Peroxidation (LP)

The methodology for LP determination was adapted from Ohkawa et al. (1979). Twenty five µl of supernatant from each sample was placed in an acid washed glass tube where 50 µl 8.1% sodium dodecyl sulphate (SDS), 375 µl acetic acid, 375 µl thiobarbituric acid, and 175 µl ultrapure water was added to each tube. The tubes were placed in a hot water bath at 95°C for 30 minutes, thereafter it was allowed to cool down to room temperature. Ultrapure water (250 µl), and 1 250 µl of butanol-pyridine solution (15:1) was added to each sample, vortexed and centrifuged at 4 000 r.p.m (Sigma 2-15 centrifuge) for 10 minutes at room temperature. Two hundred and forty five µl of samples and the blank were added in triplicate to the microtitre plate and read at 540 nm using an automated microplate reader. Protein content was determined following the method of Bradford (1976).

### 2.9.8 Protein Carbonyls (PC)

The methodology for PC was based on the modified Floor and Wetzel (1998) protocol. Supernatant (500 µl) was added to 500 µl 2,4-Dinitrophenylhydrazine (DNPH) and incubated for an hour at room temperature, during which time it was vortexed every 10-15 minutes. Trichloroacetic acid (6%; 500 µl) was added to each sample in order to precipitate the proteins, and was centrifuged at 24 166 r.p.m (Biofuge stratos, Haraeus instruments) for 3 minutes. The supernatant was discarded and the pellet washed three times and resuspended in 1 ml ethanol in order to remove the free reagent. The samples were allowed to stand for 10 minutes before centrifugation and the subsequent removal of supernatant. Guanidine hydrochloride (400 µl) was added to each sample in order to make the proteins soluble and allowed to stand at room temperature for 15 minutes. The samples were centrifuged at 38 666 r.p.m (Biofuge stratos, Haraeus instruments) for 5 minutes in order to remove any trace of insoluble material and the sample read in triplicate at 366 nm using an automated microplate reader and the proteins determined following the method of Bradford (1976).

### 2.9.9 Population genetics of *Clarias gariepinus*

#### 2.9.9.1 Extraction of highly pure deoxyribonucleic acid (DNA) by using a NucleoSpin Tissue DNA extraction kit

The DNA (deoxyribonucleic acid) was extracted from the respective samples according to the standard protocol for human or animal tissue and cultured cells as detailed in the instruction manual of the NucleoSpin Tissue DNA extraction kit (Macherey-Nagel, Düren, Germany). A ground representative 25 mg portion of muscle tissue was placed in a microcentrifuge tube, 180 µl of Buffer T1 and 25 µl of Proteinase K added after which each tube was briefly vortexed. The mixture was incubated at 56°C for 3 h until complete lysis was obtained. Following, 200 µl of Buffer B3 was added and the mixture vortexed and the solution incubated at 70°C for 10 min. After the incubation period expired, 200 µl of 96% ethanol was added and vortexed. Each sample was applied to a NucleoSpin Tissue Column placed in a collection tube and centrifuged at 11 000 g for 1 min. The contents of the collection tube were discarded and the Column re-inserted into the collection tube. This washing step was repeated twice with 500 µl of Buffer BW and 600 µl of Buffer B5, respectively. In order to elute the highly pure DNA, the column containing the DNA was placed into a 1.5 ml microcentrifuge tube, 100 µl of Buffer BE (70°C) added and incubated at room temperature for 1 min. Lastly, the Column was centrifuged at 11 000 g for 1 min. The contents collected in the microcentrifuge tubes were stored at -20°C until PCR amplification could continue.

#### 2.9.9.2 Polymerase chain reaction (PCR) amplification of cytochrome b gene

In order to amplify the *C. gariepinus* cytochrome (cyt) b gene (found in the mitochondrial DNA), the primer pair L15267 and H15891 (Briolay et al., 1998) was used. A solution consisting of 1 µl mM of L15267 primer, 1 µl mM of H15891 primer, 12.5 µl of DreamTaq DNA polymerase (Inqaba Biotec) and 9 µl of nuclease-free water was added to 25 µl microcentrifuge tubes. Lastly, 1 µl of 20 ng/µl pure DNA was added to the microcentrifuge tube. Using a Bio-Rad C1000 Touch$^{TM}$ Thermal Cycler (Bio-Rad, Hemel Hempstead, UK), the PCR sequence runs were performed with the following conditions: Initial denaturing step at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing temperature at 55°C for 30 s and extension at 72°C for 45 s. The final extension step was performed at 72°C for 5 min after which the samples were kept at 4°C until retrieved from the thermo cycler (Roodt-Wilding et al., 2010).

The amplicons were visualized in ultraviolet light using a 1% agarose gel (GelRed) on a ultra-violet Bio-Rad GelDoc Imaging System (Bio-Rad). Successfully amplified PCR products were sent to Inqaba Biotec (South African Genomics Company) for sequencing in both directions.

#### 2.9.9.3 Alignment and analyses of *Clarias gariepinus* cytochrome b sequences

All sequences were edited and aligned using Geneious (v7.1) bioinformatics software package (created by Biomatters. Available at http://www.geneious.com). In order to create high quality sequences, chromatogram based contigs were generated in the above named software package. Following, sequences were manually checked for any undefined base pairs (bp), corrected accordingly and aligned with the MUSCLE (Edgar, 2004) algorithm implemented within Geneious bioinformatics software package. All the specimen samples were identified by using the Basic Local Alignment Search Tool (BLAST) (Available at http://www.ncbi.nlm.nih.gov/blast/Blast). All of the sequences were imported into DnaSP (v5.10.1) software package (Available at http://www.ub.edu/dnasp), which was used to identify and group haplotypes as well as calculate haplotype and nucleotide diversity. The nucleotide data was exported in the relevant file formats and further analysed in Arlequin (v3.5.1.3) (Available at http://cmpg.unibe.ch/software/arlequin35) and Network (v4.612) (Available at http://www.fluxus-engineering.com) software packages. While the former software package was used to analyse the genetic distance [Pairwise $F_{ST}$ and analysis of molecular variance (AMOVA)] between the respective populations, the latter software package was used to create haplotype (median-joining) networks.

## 2.10 Statistical analyses

### 2.10.1 Univariate analyses

The variations in each assessment endpoint were tested by one-way analysis of variance (ANOVA), considering sites as variables. Data were tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests, respectively. When the ANOVA revealed significant differences, post-hoc multiple comparisons between sites were made using the appropriate Scheffé (parametric) or Dunnette-T3 (non-parametric) test to determine which values differed significantly. The significance of results was ascertained at $p<0.05$ (Zar, 1996).

Various univariate diversity indices have been used to assess community structure, as they may emphasize the species richness or equitability components of diversity to varying degrees. Indices that were used were the Shannon-Weiner diversity index (*H*'), which incorporates both species richness and equitability components (Clarke & Warwick, 1994), species richness, which compares the numbers of species present for any given number of individuals, Pielou's evenness index (*J*') and Margalef's index (*d*).

### 2.10.2 Multivariate analyses

The statistical community analysis of data was carried out using Primer Multivariate Software (Clarke & Warwick, 1994). For the analysis of the invertebrate and fish communities, presence/absence data was used. To display the community similarities and groupings, cluster analysis was done to represent community response in the form of a dendrogram. Multidimensional scaling was also carried out to show the correlation and similarity groupings of the sample sites, and from this the sites were grouped together to show their similarities. The analysis of similarities (ANOSIM) test was carried out to show that the results obtained and the groupings displayed via the community response in the cluster and MDS diagrams were statistically significant.

In this study Principle Component Analysis (PCA) (Canoco for Windows Version 4.53) statistical package was used to assess the spatial patterns associated with water and sediment quality, bioaccumulation in fish tissue, biomarker responses and fish community structures (Ter Braak & Smilauer, 2004). The PCA is based on a linear response model relating species and environmental variables (van den Brink et al., 2003). Results of the ordination are a map of the samples being analysed on a 2 dimensional basis, where the placements of the samples reflect the dissimilarities or similarities between the samples; in this case the sampling sites. To determine which factors were responsible for the structure or groupings obtained in the PCA a Redundancy Analysis (RDA) assessment was carried out. A RDA is a derivative of a PCA with one additional feature, which allows for the selection of the driving variables which are intended to be overlaid onto the PCA. The values entered into the RDA analysis are not the original data but the best-fit values estimated from a multiple linear regression between each variable in turn and a second matrix of complementary biological or environmental data. The RDA plots are interpreted through 2-dimentional bi-plots that present the similarities or dissimilarities between the samples analysed (Shaw, 2003).

# 3 WATER AND SEDIMENT QUALITY OF THE KLIPRIVER SYSTEM

## 3.1 Water quality

The in situ water quality results for the June 2013 sampling survey (Table 28 and 29) indicated that the oxygen content and saturation at all of the Klip River sites (KR1-KR5) is within 6-10 mg/l and 70-100% ranges respectively (Table 28). The TWQR for aquatic ecosystems indicate that the dissolved oxygen saturation should be between 80-120% (DWAF, 1996). The Natal Spruit (NS), Riet Spruit (RS) and Vaal River (VR) indicated that the oxygen saturation ranged from 76-104%. The pH for the Klip River and its tributaries indicated a range of 7.5 to 7.9 while the Vaal River sites ranged from 8.1 to 8.8. The TWQR for aquatic ecosystems for pH stipulates that the pH for a specific river should not deviate by more than 5%. The TDS and conductivity of the Klip River increase from site KR1 to KR4. The concentrations at site KR4 and KR5 were similar. The TWQR specifies that the TDS should not vary by more than 15% from the background TDS concentrations. The TDS and conductivity measurements in the Natal Spruit and Riet Spruit were higher than all of the measurements at the Klip River sites with the highest measurements at the Upper Rietspuit. The TDS and conductivity in the Vaal River, VR1, was similar to the upper Klip River site, KR1, while the lower Vaal River, VR2, was similar to the sites KR4 and KR5.

Table 28: In situ water quality parameters measured in the Klip River sites during the low flow survey in June 2013.

| Parameter | Unit | KR1 | KR2 | KR3 | KR4 | KR5 |
|---|---|---|---|---|---|---|
| Oxygen content | mg/l | 7.8 | 9.67 | 9.96 | 6.88 | 8.32 |
| Oxygen saturation | % | 73 | 96.1 | 92.4 | 67.5 | 85.8 |
| pH | - | 7.84 | 7.65 | 7.59 | 7.58 | 7.92 |
| Temperature | °C | 10.3 | 14.7 | 16.3 | 14.8 | 15.1 |
| Conductivity | µS/cm | 292 | 463 | 501 | 589 | 623 |
| TDS | mg/l | 195 | 316 | 346 | 407 | 404 |

Table 29: In situ water quality parameters measured at the Riet Spruit, Natal Spruit and Vaal River sites during the low flow survey in June 2013.

| Parameter | Unit | NS | RS1 | RS2 | VR1 | VR2 |
|---|---|---|---|---|---|---|
| Oxygen content | mg/l | 9.23 | 10.88 | 7.63 | 9.28 | 7.52 |
| Oxygen saturation | % | 91.3 | 104 | 78.4 | 100.2 | 76.2 |
| pH | - | 7.63 | 7.71 | 7.57 | 8.85 | 8.12 |
| Temperature | °C | 17.1 | 13.9 | 15.9 | 18.5 | 17 |
| Conductivity | µS/cm | 766 | 1415 | 794 | 297 | 623 |
| TDS | mg/l | 529 | 981 | 538 | 195 | 424 |

The in situ water quality parameters measured during the January 2014 survey in Table 30 and Table 31 indicated that the majority of parameters were within the TWQR set by DWAF (1996b). The oxygen content was generally slightly lower than the recommended 80% oxygen saturation. This could indicate that some oxygen deficits could occur at certain times. The oxygen content and saturation was also slightly lower at site KR2 that is situated at the Olifantsvlei WWTW. When comparing the two surveys it can be seen that in general the oxygen content and saturation is lower in the January 2014 survey, especially in the Riet Spruit and Natal Spruit. The pH measurements at all of the sites during January 2014 were within the TWQR of 6-9 as set by the DWAF (1996b) guidelines for aquatic ecosystems. The pH measurements were similar between June 2013 and January 2014. The temperature of the various sites all ranged from 18-24°C and the variation that were seen is due to the different times of day that the sites were sampled. Differences between temperature during June 2013 and January 2014 are related to winter and summer seasonality. The EC during January 2014 indicated a general increase in the Klip River from site KR1 to site KR5. The tributary sites indicated a very high EC at site RS1 in the Riet Spruit but at site RS2 it showed a decrease to the same levels of the Klip River. The EC comparison between June 2013 and January 2014 indicated a slightly higher EC during January 2014.

Table 30: In situ water quality parameters measured at the Klip River sites during the high flow survey in January 2014.

| **Parameter** | **Unit** | **KR1** | **KR2** | **KR3** | **KR4** | **KR5** |
|---|---|---|---|---|---|---|
| Oxygen content | mg/l | 7.65 | 5.43 | 7.11 | 4.99 | 6.12 |
| Oxygen saturation | % | 81.7 | 62.5 | 81.5 | 58.7 | 72.5 |
| pH | - | 8.04 | 7.61 | 7.97 | 7.81 | 7.95 |
| Temperature | °C | 18.5 | 22.3 | 23.1 | 23.5 | 24.0 |
| Conductivity | µS/cm | 300 | 480.3 | 508.9 | 593 | 582 |

Table 31: In situ water quality parameters measured in tributaries of the Klip River during the high flow survey in January 2014.

| **Parameter** | **Unit** | **NS** | **RS1** | **RS2** |
|---|---|---|---|---|
| Oxygen content | mg/l | 5.65 | 5.0 | 5.37 |
| Oxygen saturation | % | 64 | 55.4 | 64.7 |
| pH | - | 7.64 | 7.78 | 7.69 |
| Temperature | °C | 21.6 | 20.1 | 22.3 |
| Conductivity | µS/cm | 766 | 1166 | 524.3 |

The results of the nutrient variable analysis are tabulated in Table 32 for the June 2013 survey and Table 32 for January 2014 in the Klip River system. The nutrient variables were compared to the TWQR as set out by the Department of Water Affairs in the South African Water Quality guidelines (DWAF, 1996a).

The June 2013 results indicated that the turbidity increased from site K1 to site K4 while site K5 was similar to site K4. The tributary sites on the Natal Spruit (N1) and Riet Spruit (R1) indicated higher concentrations than in the Klip River; however, at site R2 the turbidity was lower. The Vaal River sites indicate higher concentrations at the reference site V1 than at the site downstream of the confluence with the Klip River. The January 2014 results follow the same trend but the turbidity measurements are higher than seen during June 2013. Turbidity does not have a TWQR for aquatic ecosystems but for domestic use it should be between 0-1 and thus this criteria is exceeded at all sites except at sites K1 and K2.

The COD concentrations indicated the lowest concentration at site K1 while similar concentrations were measured at site K2 to site K5. COD concentrations in the Riet Spruit and Natal Spruit were slightly lower than measured in the Klip River. The Vaal River concentrations were similar to concentrations in the Klip River. No TWQR for aquatic ecosystems or domestic use is specified for COD.

The June 2013 nitrite concentrations ranged from 0.02 to 0.5 mg/l with the highest concentrations measured in the Natal Spruit (N1) and the Riet Spruit (R2). Concentrations were also seen to increase from site K1 to site K5. The January 2014 results indicate all of the nitrite concentrations were below the detection limit. Ammonium concentrations were similar at all sites on the Klip River (K1 to K5) while concentrations in the Natal Spruit (N1) and Riet Spruit (R1) were the highest measurements. These measurements were three and six times higher than concentrations in the Klip River. The ammonium concentration during January 2014 was lower than the June 2013 results with the exception of site R1 in the Riet Spruit that was slightly higher. The nitrate concentrations were the lowest at site K1 and site R1 while the concentrations at sites K2 to K5 were similar. The nitrate concentration at site R2 on the Riet Spruit was similar to the Klip River sites while site N1 on the Natal Spruit showed slightly lower nitrate concentrations. The January 2014 nitrate concentrations indicated that some sites had higher concentrations than in June 2013 while other sites where lower. However, most of the concentrations were similar. There is no TWQR for domestic use for inorganic nitrogen and a few categories exist for the aquatic ecosystem criteria. In general the inorganic nitrogen should not vary by more than 15% from the natural or reference condition. However, nitrogen concentrations can either be less than 0.5 mg/l indicating the system is oligotrophic, between 0.5-2.5 mg/l indicating the system is mesotrophic and more than 2.5 mg/l indicating it is eutrophic. All of the sites are indicating eutrophic conditions are predominant, with the exception of site K1, R1 and V1. Eutrophic conditions generally result in a low biotic diversity as well as nuisance growth of aquatic macrophytes and algal blooms. Algal blooms can contain species that are toxic to humans and livestock (DWAF, 1996b).

Table 32: Nutrient variables for sites sampled during June 2013 in the Klip River system. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

| Site Unit | | Turbidity FAU | COD mg/l | $NO_2$-N mg/l | $NH_4$-N mg/l | $NO_3$-N mg/l | $PO_4$-P mg/l | Cl mg/l |
|---|---|---|---|---|---|---|---|---|
| **K1** | | 0 | 6.8 | < 0.01 | 0.33 | 0.59 | 0.03 | 48 |
| **K2** | | 1 | 11.9 | 0.051 | 0.24 | 3.16 | 0.31 | 15 |
| **K3** | | 5 | 13.3 | 0.124 | 0.38 | 3.61 | 0.42 | 39 |
| **K4** | | 13 | 13.2 | 0.142 | 0.36 | 2.47 | 0.48 | 50 |
| **K5** | | 11 | 12.2 | 0.17 | 0.35 | 3.38 | 0.38 | 53 |
| **N1** | | 19 | 10.5 | 0.58 | 1.96 | 1.77 | 0.1 | 66 |
| **R1** | | 16 | 2.4 | 0.005 | 0.03 | 0.24 | 0.02 | 40 |
| **R2** | | 6 | 9.3 | 0.473 | 0.97 | 3.1 | 0.11 | 77 |
| **V1** | | 16 | 12.8 | 0.021 | 0.06 | 0.21 | 0.03 | 13 |
| **V2** | | 7 | 15 | 0.192 | 0.5 | 2.72 | 0.29 | 62 |

Table 33: Nutrient variables for sites sampled during January 2014 in the Klip River system. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

| Site Unit | | Turbidity FAU | $NO_2$-N mg/l | $NH_4$-N mg/l | $NO_3$-N mg/l | $PO_4$-P mg/l | Cl mg/l |
|---|---|---|---|---|---|---|---|
| **K1** | | 3 | < 0.01 | < 0.03 | 0.9 | < 0.03 | 18.1 |
| **K2** | | 6 | < 0.01 | < 0.03 | 2.4 | 0.28 | 7.8 |
| **K3** | | 22 | < 0.01 | 0.06 | 3.0 | 0.35 | 13.2 |
| **K4** | | 13 | < 0.01 | 0.12 | 3.4 | 0.4 | 18.6 |
| **K5** | | 34 | < 0.01 | 0.11 | 2.8 | 0.42 | 19.5 |
| **N1** | | 13 | < 0.01 | 0.64 | 2.6 | 0.23 | 18.9 |
| **R1** | | 20 | < 0.01 | 0.1 | 1.9 | 0.06 | 15.6 |
| **R2** | | 7 | < 0.01 | 0.09 | 3.8 | 0.26 | 23.4 |

The phosphate concentrations ranged from 0.02 mg/l to 0.48 mg/l during June 2013. The highest concentrations were measured in the Klip River from site K2 to site K5. All these concentrations are indicative of hypertrophic conditions. The Natal Spruit and Riet Spruit tributaries did not indicate high concentrations of phosphates. The concentrations for phosphate in January 2014 were in a similar range to June 2013. The TWQR for domestic use indicates no criteria for phosphates (DWAF, 1996c) but the criteria for aquatic ecosystems are set at 15% change from natural conditions. Phosphate concentrations below 5 µg/l are indicative of oligotrophic conditions while higher concentrations above 25 µg/l are indicative of eutrophic conditions (DWAF, 1996b). The chloride concentrations ranged from 13 mg/l to 77 mg/l with the highest concentrations measured in the Vaal River, Natal Spruit and the Riet Spruit.

The bacteriological analysis results in Figure 25 indicate that most of the sites in the study area contained high counts of colony forming bacteria. The highest counts were measured at sites K1, K4 and K2. The lowest concentrations were measured at site K3 and site V1. TWQR indicates that these counts should be significantly lower if the water is to be used for domestic use.

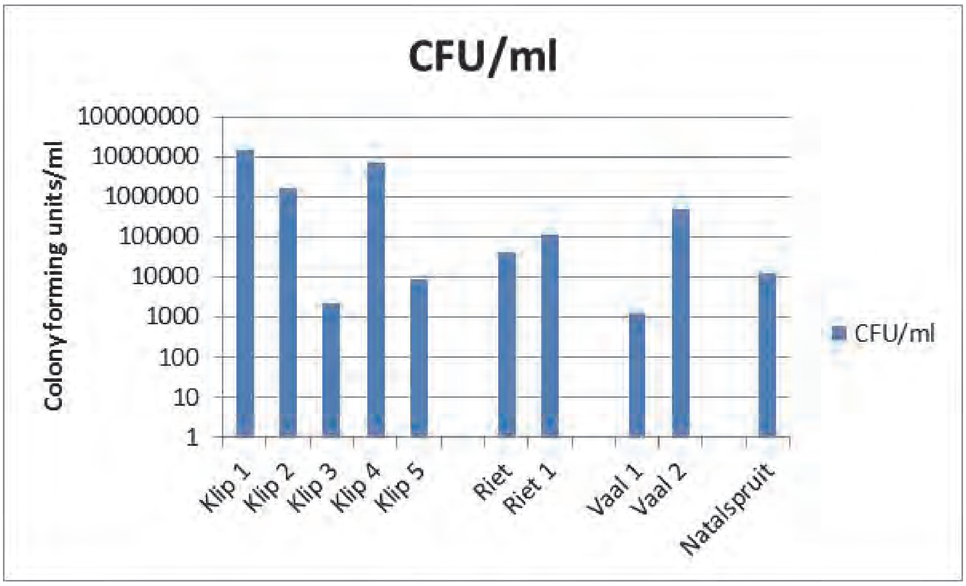


Figure 25: Results of the bacteriological analysis for the sites in the Klip River system for the June 2013 sampling survey.

A follow up survey on the Klip River for bacteriological analysis was initiated in January 2015 to increase the available information in the system. The focus was on the testing of *Clostridium spp*. that is able to cause various waterborne diseases. The *Escherichia coli* results were also determined as comparison. The results of this survey are presented in Table 13 and 14 for the January 2015 survey. The results indicated that *E.coli* was found at sites KR1, KR2 and KR5 in numbers that were too numerous to count. These values were true for both the *E.coli* (Table 34) and *Clostridium spp* (Table 35). Clostridium is an anaerobic bacteria and the genus Clostridium includes many species that produce toxins like *C. botilinum* and *C. perfringes,* which is associated with food poisoning, bactericemia and gangrene.

Table 34: *Escherichia coli* results of the bacteriological analysis for the sites in the Klip River system for the January 2015 sampling survey. TNTC = too numerous to count

| Sample name | CFU/100ml | STDEV |
|---|---|---|
| KR1 | TNTC | |
| KR2 | TNTC | |
| KR3 | 496 | 45.3 |
| KR4 | 338 | 19.8 |
| KR5 | TNTC | |
| NS1 | 7.5 | 10.6 |
| RS1 | 272 | 45.3 |
| RS2 | 355.5 | 27.6 |

Table 35: *Clostridium* spp results of the bacteriological analysis for the sites in the Klip River system for the January 2015 sampling survey. TNTC = too numerous to count

| ***Clostridium* spp. CFU/7 ml** | | | |
|---|---|---|---|
| **Sample ID** | **No.1** | **No.2** | **Avg** |
| KR1 | 700+ | 700+ | 700+ |
| KR2 | 189 | 171 | 180 |
| KR3 | 73 | 65 | 69 |
| KR4 | 87 | 94 | 90.5 |
| KR5 | 248 | 455 | 351.5 |
| NS1 | 114 | 64 | 89 |
| RS1 | 136 | 152 | 144 |
| RS2 | 131 | 192 | 161.5 |

The metal analysis in the water phase is presented below for the Klip River sites for the June 2013 (Figure 26 to 28) and the January 2014 (Figure 29-31) sampling surveys. Aluminium concentrations were similar at all of the sites with the exception of site V1 during the June 2013 survey (Figure 26). The concentration at site V1 exceeds the 0.005 mg/l TWQR set by DWAF (1996a) for aquatic ecosystem. The January 2014 survey indicated higher concentrations at all of the sites except site K2 and N1 (Figure 29). The aluminium concentrations were highest at site R1 while site K1, K3, K4, K5 and R2 were similar. The TWQR was exceeded at all sites except site K2 and N1.

Arsenic in June 2013 showed a similar trend at all of the sites indicating a similar concentration with the exception of site R1, which was higher (Figure 26). The January 2014 survey indicated higher arsenic concentrations at all sites with site K1 and R1 being the highest (Figure 29). The peak arsenic concentrations at the various sites were not higher than the TWQR of 0.01 mg/l (DWAF, 1996a) for aquatic ecosystems and domestic use.

The cobalt concentration at site R1 was also the highest concentration measured during both surveys (Figure 29 and Figure 29), while all the other sites had similar concentrations during both surveys. The cobalt concentration at site R1 was slightly higher in June 2013 than in January 2014. No TWQR has been set for cobalt concentrations for aquatic ecosystems and water for domestic human consumption (Figure 26). The January 2014 concentrations indicated peak concentrations at sites K1, K4 and R1 while the other concentrations were similar. The lowest concentrations were measured at site N1. All of the concentrations measured during 2014 were higher than in June 2013 with the exception of site N1 that was similar. The chromium concentrations during 2013 were much lower than the 0.007 mg/l and 0.05 mg/l TWQR for aquatic ecosystems and domestic use. However, the peak concentrations at sites K1, K4 and R1 were close to the 0.007 mg/l TWQR for aquatic ecosystems.

The June 2013 copper concentration generally increases from site K1 to site K4 with the highest concentration measured at site K4 (Figure 26). The copper concentrations at the Vaal River sites were similar to the concentrations measured at site K4 while the Riet Spruit (R1 and R2) and Natal Spruit (N1) concentrations were lower. The January 2014 copper concentrations were higher at sites K1, R1 and R2 while the concentrations at sites K2 – K5 were similar to June 2013 (Figure 29). The TWQR for aquatic ecosystems and domestic use is set at 0.0003 mg/l and 1 mg/l respectively. All of the concentrations of copper exceeded these TWQR.

The June 2013 iron concentration was the highest at site V1 while all of the other sites had similar concentrations but lower than at V1 (Figure 26). The January 2014 iron concentrations were higher than the June 2013 concentrations (Figure 29). The iron concentrations at site R1 were found to be the highest while all of the other iron concentrations were similar. As iron is a common metal found in the environment no TWQR for aquatic ecosystems have been set. The manganese concentrations varied between all of the sites with the highest concentration measured at site R2 and the lowest concentrations were measured at the Vaal River sites (Figure 27). The majority of sites during January 2014 indicated that the manganese were below the detection limit of the analysis with the exception of sites K1 and R1 (Figure 30). These concentrations were similar to the concentrations measured at the sites during the June 2013 surveys. The TWQR for aquatic ecosystems and domestic use is set at 0.18 mg/l and 0.05 mg/l, respectively. The concentrations measured in this study were all below these concentrations.

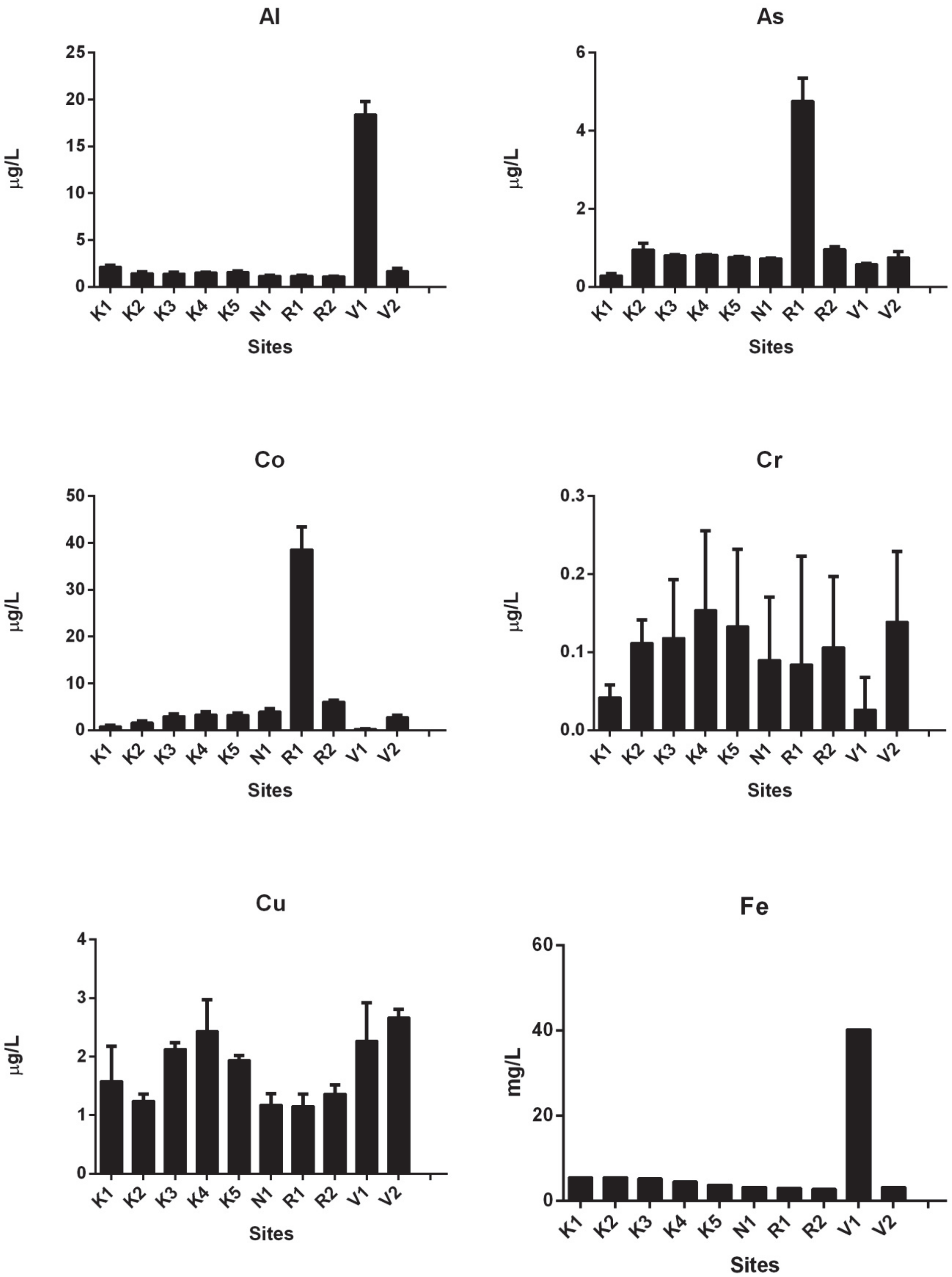


Figure 26: Selected dissolved metal concentrations in the water phase for sites in the Klip River system sampled during June 2013. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

The June 2013 chromium concentration increases from site K1 to site K4 with the highest concentration measured at site K4. The concentration decreases slightly at site K5 while all of the sites on the Riet Spruit, Natal Spruit and Vaal River are lower than in the Klip River

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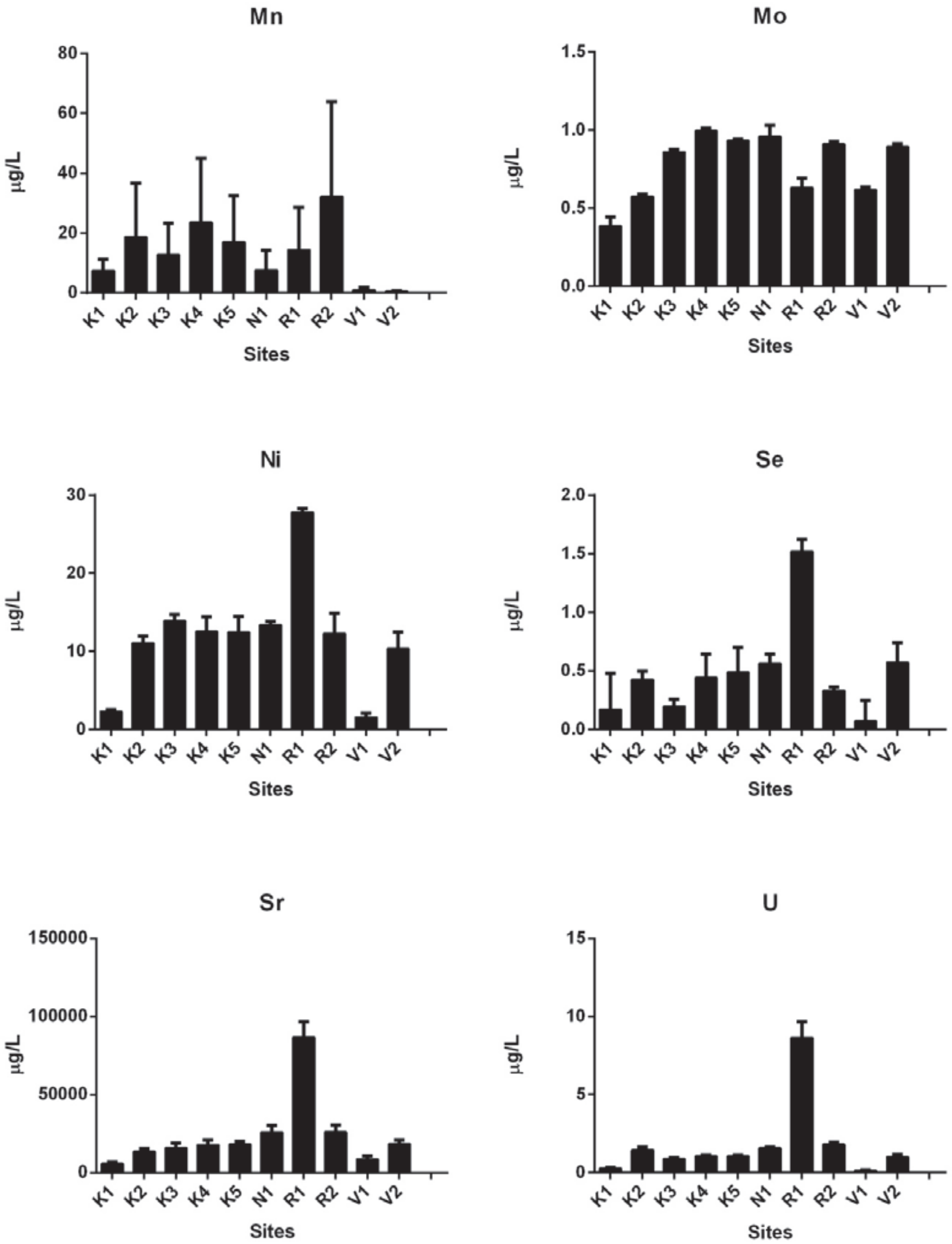


Figure 27: Selected dissolved metal concentrations in the water for sites in the Klip River system sampled during June 2013. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

The June 2013 molybdenum concentrations increase from site K1 to site K4 while all of the other sites have concentrations similar to the concentrations measured at site K4 (Figure 27). The January 2014 concentrations were below the detection limit with the exception of site K1 (Figure 30). The concentration measured at site K1 during January 2014 was higher than measured during June 2013. No TWQR for molybdenum have been set for either aquatic ecosystems of domestic use. The June 2013 nickel, selenium, strontium and uranium concentrations (Figure 27) indicated similar trends with the highest concentrations measured at site R1 while all of the other sites had similar concentrations. The lowest concentrations of these metals where all measured at site K1 on the Klip River. The January 2014 survey indicated that higher concentrations were measured for nickel but the trend remained similar while the strontium concentrations were higher during June 2013. A similar trend between the two surveys was evident. The selenium and uranium concentrations during January 2014 indicated that the highest concentrations were measured at site K1 (Figure 30). Selenium was generally found to be slightly higher in January 2014 than in June 2013 at site K1 while all the other sites were below the detection limit in January 2014. The uranium indicated high concentrations at sites K1 and R1 while all the other concentrations were fairly similar; however all of the concentrations were higher in January 2014 than measured in June 2013. No TWQR have been set for nickel, strontium and uranium for either aquatic ecosystems or domestic use. The TWQR for selenium for aquatic ecosystems is set at 0.002 mg/l and 0.02 mg/l for domestic use. The selenium concentration at site K1 during January 2014 exceeded the TWQR for aquatic ecosystems but none of the other site concentrations measured in this study for selenium exceeded the TWQR.

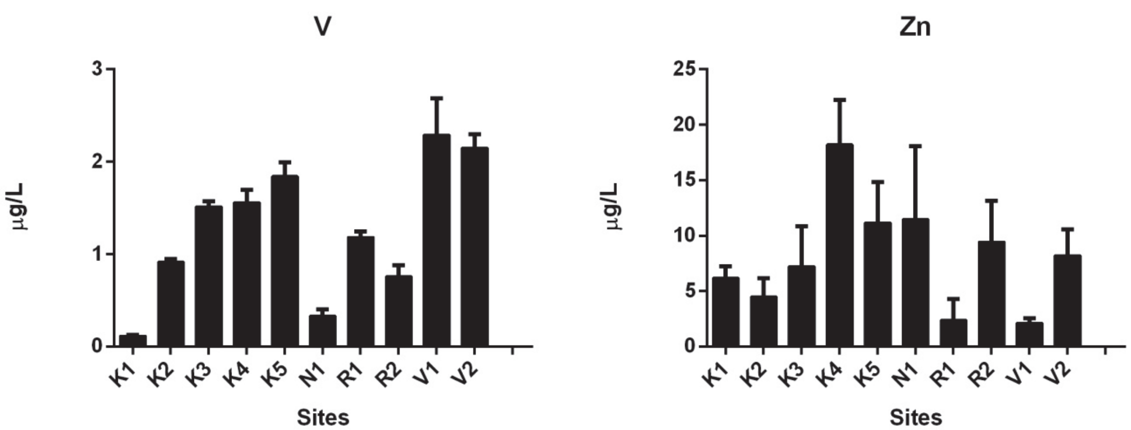


Figure 28: Dissolved vanadium and zinc metal concentrations in the water for sites in the Klip River system sampled during June 2013. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

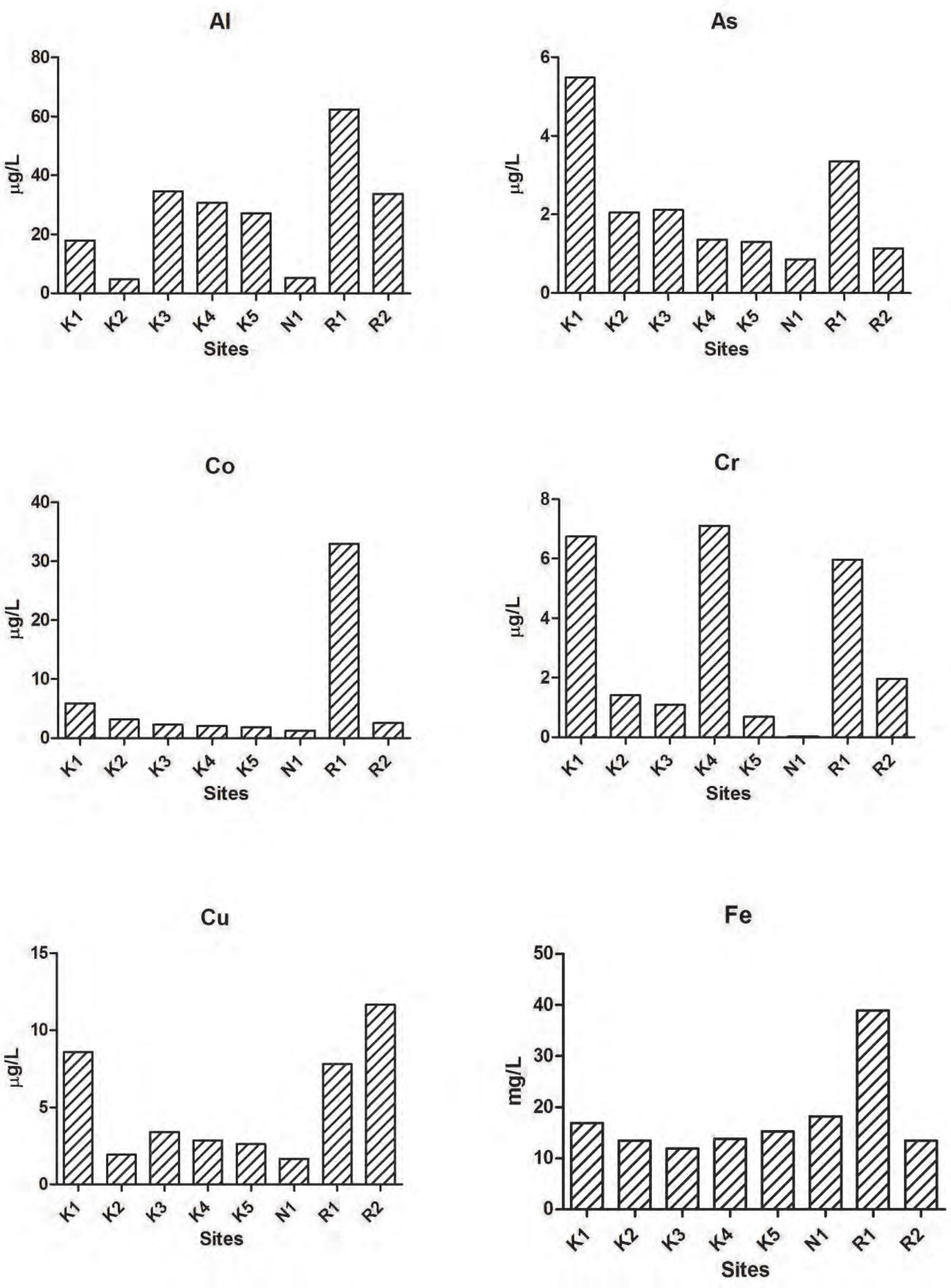


Figure 29: Selected dissolved metal concentrations in the water for sites in the Klip River system sampled during January 2014. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

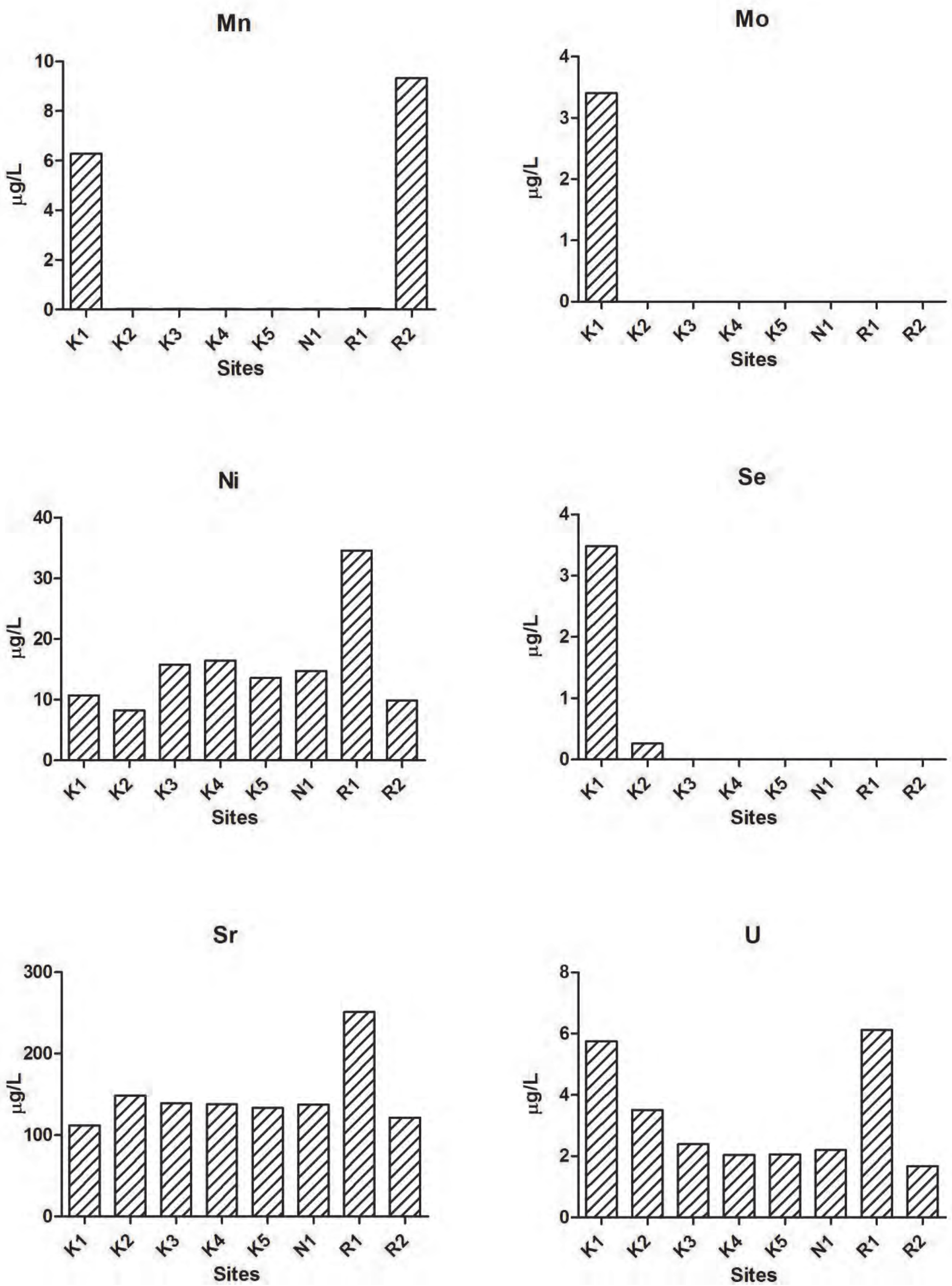


Figure 30: Selected dissolved metal concentrations in the water for sites in the Klip River system sampled during January 2014. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

The June 2013 vanadium concentrations increased from site K1 to site K5 and the highest concentrations were measured at the sites on the Vaal River. The concentrations in the Natal Spruit and Riet Spruit were similar to site K2 on the Klip River system but lower than the Vaal River and site K5 (Figure 28). The January 2014 vanadium concentrations were found to be higher than the June 2013 survey with the exception of site N1 that was similar. The trend also changed with the highest concentration measured at site K1 (Figure 31). The vanadium concentration at site K5 was slightly lower than at site K1 while all the other sites had slightly lower concentrations. Vanadium does not have a TWQR for aquatic ecosystems but a 3 mg/l requirement has been set for domestic use. None of the concentrations measured for vanadium exceeded this value during both surveys.

The zinc concentrations were similar at site K1 to site K3 while site K4 had the highest zinc concentrations. The lowest concentrations were measured at site R1 and site V1 (Figure 28). The January 2014 zinc concentrations were higher than measured during June 2013 for most sites. The trend was also different with the highest concentration measured at site R2. The lowest concentrations were seen at sites K2 and K3 while all the other sites had similar concentrations. The TWQR for aquatic use for zinc is set at 0.002 mg/l and 3 mg/l for domestic use. The aquatic ecosystem requirement for zinc is exceeded at most of the sites with the exception of site R1 and site V1 during June 2013 which was slightly lower.

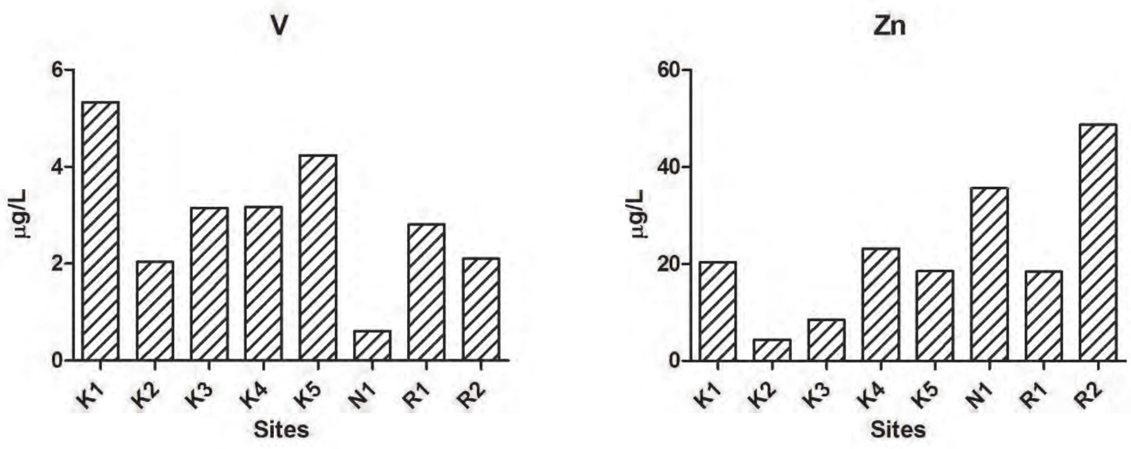


Figure 31: Dissolved vanadium and zinc metal concentrations in the water for sites in the Klip River system sampled during January 2014. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

## 3.2 Sediment

The sediment moisture content in Table 36 for the June 2013 survey indicates similar results for all sites in the Klip River system. The moisture content ranged from 6.5% to 12%. The organic content ranged from 0.38% to 5.29%. The organic content was found to be low at sites K1, R2 and V1 (Table 36). The organic content at site N1 was found to be moderately low during June 2013 while it was found to be very high during January 2014. A medium organic content was found at sites K3, K4, K5, R1 and V2 while a high organic content was found at site K2. This trend was present during both surveys in June 2013 and January 2014.

Table 36: Sediment moisture and organic content from the field survey during June and January 2013 in the Klip River system. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

| **Sample** | **Moisture Content** | **Organic content** June 2013 | **Organic content** January 2014 |
|---|---|---|---|
| **K1** | 6.58 | 0.38 | 1.64 |
| **K2** | 11.18 | 5.29 | 4.46 |
| **K3** | 10.82 | 2.15 | 2.61 |
| **K4** | 8.45 | 3.97 | 3.64 |
| **K5** | 12.85 | 4.33 | 2.83 |
| **N1** | 7.63 | 1.53 | 11.73 |
| **R 1** | 9.25 | 2.02 | 4.61 |
| **R 2** | 9.26 | 0.94 | 1.56 |
| **V1** | 8.40 | 0.81 | - |
| **V2** | 8.71 | 2.20 | - |

The sediment grain size results for June 2013 (Figure 32) and January 2014 (Figure 33) were classified according to Table 23 in the methodology section. During June 2013, sites K1, K3 and K5 had mostly very fine sand and mud grain sizes while at sites K2 and K4 there was a very good spread of each grain size fraction. The grain size results of the tributaries indicated that the majority of grain sizes was either medium sand or coarse sand. The Vaal River grain sizes indicated at site V1 to have mostly coarse and medium sand while at site V2 the medium sand and very fine sand dominated the fractions. The January 2014 results indicated that the majority of the grain sizes are composed of mud, very fine sand and medium sand. The exceptions are sites K4, N1 and R1 which had a higher percentage of coarse sand (Figure 33).

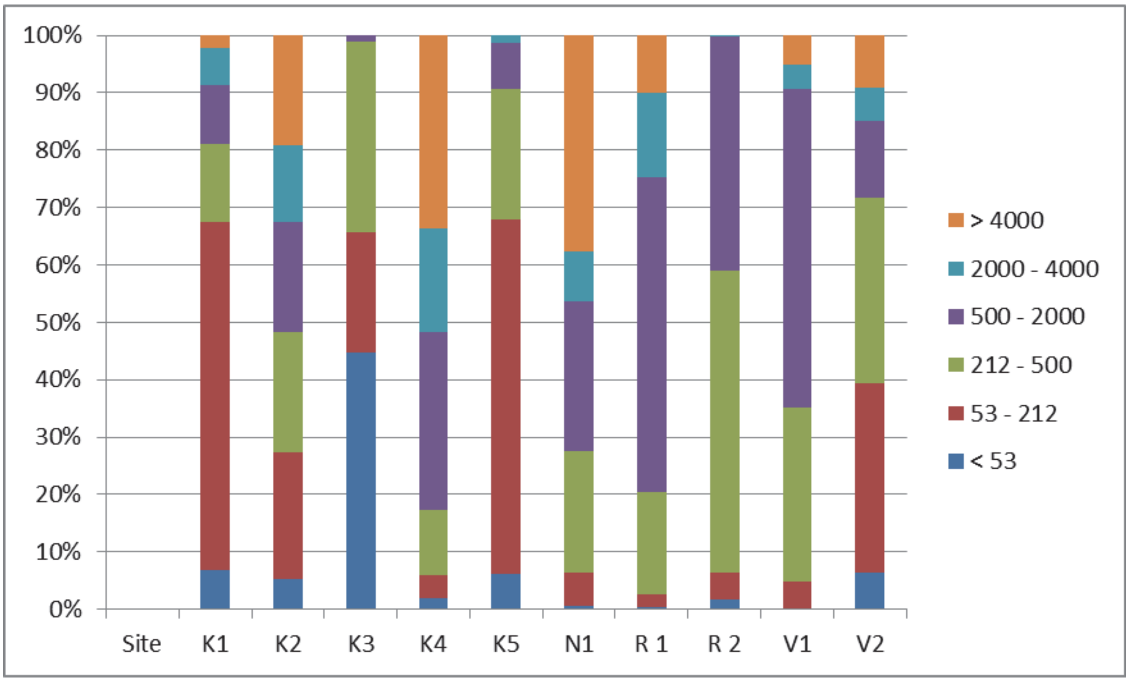


Figure 32: Sediment grain size distribution for the June 2013 sampling survey in the Klip River system. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

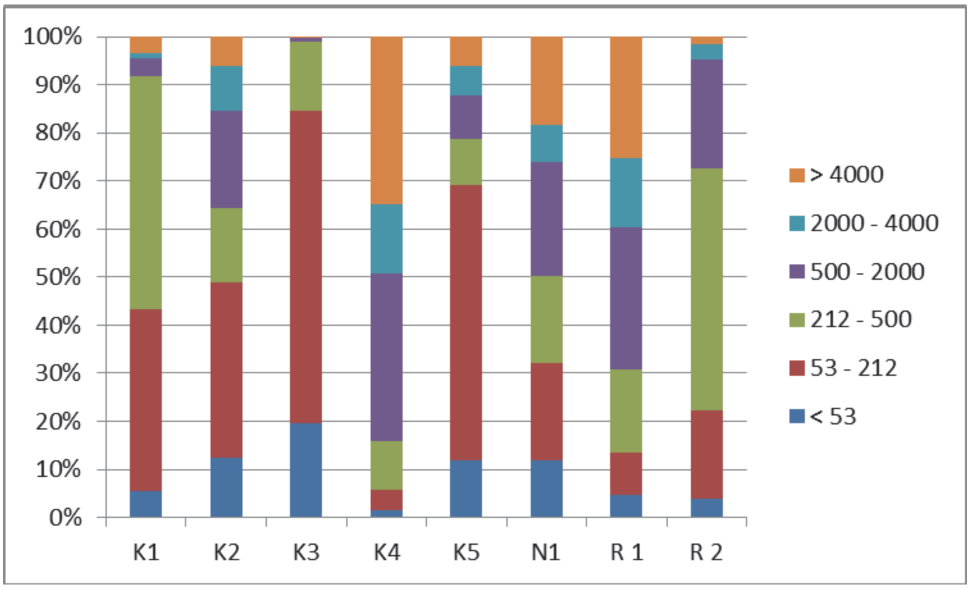


Figure 33: Sediment grain size distribution for the January 2014 sampling survey in the Klip River system. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

The metal concentrations within the sediment was characterised using a total digestion method. The resulting graphs of the various metal concentrations analysed are presented in Figure 34 to Figure 35 for the June 2013 survey and Figure 36 to 37 for the January 2014 survey. The June 2013 aluminium concentrations indicated similar values at site K2 and K5 while concentrations increased from site K3 to site K5 (Figure 34). Aluminium concentrations at site K1 was less than at the other Klip River sites but concentrations where similar than measured in the Vaal River, Riet Spruit and Natal Spruit. The January 2014 survey results indicated that the Klip River concentrations were similar to the June 2013 survey but the tributary sites (Sites N1, R1 and R2) had higher concentrations (Figure 37).

The highest concentrations of arsenic during June 2013 were measured at site K1 where after the concentrations decreased until site K3 (Figure 34). Concentrations at site K4 was slightly higher than at site K3. The sites K5, Vaal River, Natal Spruit (N1) and Riet Spruit (R1 and R2) all indicated similar arsenic concentrations which were less than the concentrations measured at the other Klip River sites. The January 2014 survey indicated that the highest concentration was found at site K4; however, the concentration was similar to the June 2013 survey. Sites K1 to K3 had lower concentrations in January 2014 compared to June 2013, while sites K5, N1, R1 and R2 were similar to the June 2013 concentrations (Figure 37).

The June 2013 cadmium concentrations were generally below the detection limit or very low with the exception of site K2 that indicated a higher concentration than measured at the other sites (Figure 34). The January 2014 concentrations were all within the 0.1 to 0.2 µg/g range which is lower than the concentrations measured during June 2013. The highest concentration during January 2014 was measured at sites K1 and R2 while site K4 was also slightly higher than the other site’s concentrations (Figure 37).

The June 2013 cobalt concentration was the highest at site K2 but then generally showed a decreasing trend towards site K5 (Figure 34). Sites on the Riet Spruit (R1 and R2) indicated concentrations that were similar to site K5 on the Klip River. The Vaal River together with site K1 had the lowest cobalt concentrations in the study area. The January 2014 concentrations indicated that sites K4 and R2 had higher concentrations than the other sites in the study. These concentrations are similar to the highest concentrations at site K2 during June 2013.

Chromium concentrations during June 2013 were found to be low at most of the sites in the study area with the exception of site K2 and site K4 on the Klip River (Figure 34). These sites had similar concentrations but the concentrations were higher than measured in the rest of the system. The chromium concentrations during January 2014 were similar to the June 2013 survey with the exception that only site K4 was seen to give a spiked concentration (Figure 34) while site K2 were similar to the other sites. The concentration at site K4 was, however, similar to the June 2013 concentration.

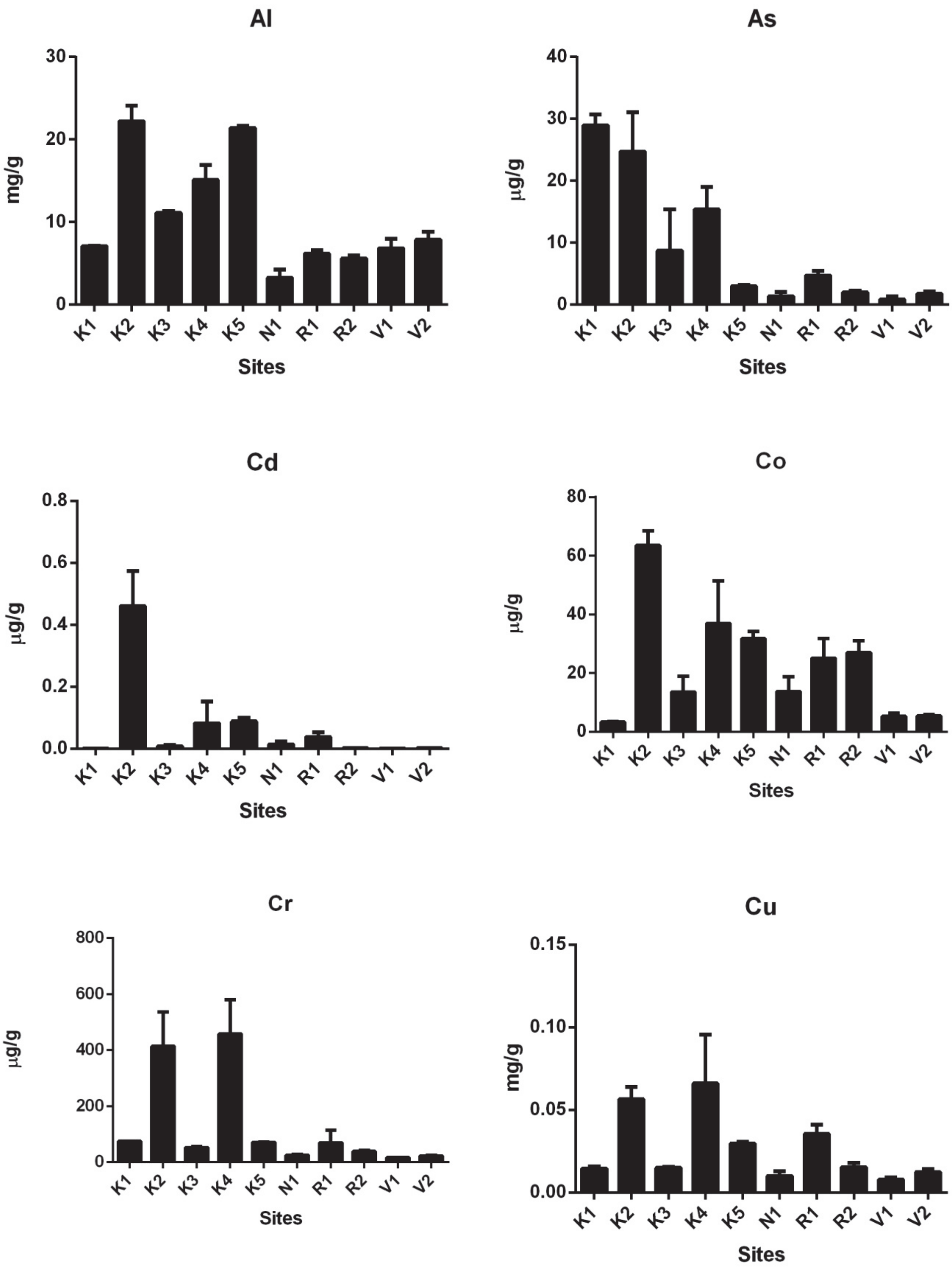


Figure 34: Selected sediment metal concentrations from the Klip River system for the June 2013 sampling survey. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

The June 2013 copper concentrations at the sites indicated the highest concentrations occurred at site K2 and K4 (Figure 34). The concentrations at the other sites were lower when compared to site K2 and K4. Site R1 also indicated a slightly higher concentration than site R2 on the Riet Spruit (Sites R1 and R2). The January 2014 copper concentrations indicated that sites K1, K2, K3 and K5 had similar concentrations that were lower than the concentrations measured at the other sites. However, all of the concentrations measured during January 2014 were higher than the concentrations measured during June 2013. The highest concentration of copper was measured at site K4 while the concentration decreased from site N1 to site R2 (Figure 37).

The June 2013 iron, manganese, molybdenum and lead concentrations indicated similar trends with sites K2 and K4 always indicating the highest sediment metal concentrations while all of the other sites in the study area have similar concentrations (Figure 35). A similar trend was seen with these metals during January 2014 with the exception that site K2 concentrations were similar to all of the sites and only site K4 was higher than the other sites (Figure 38). Additionally, the manganese concentration at site R1 during June 2013 was also found to be slightly higher than all of the other sites but not as high as measured at sites K2 and K4. The concentrations for iron and manganese were generally similar between the June 2014 and January 2014 surveys while the molybdenum and lead concentrations were lower in January 2014 than in June 2013.

The June 2013 nickel concentrations indicated the highest concentrations at sites K2, K4 and K5 in the Klip River (Klip River). The Riet Spruit sites (R1 and R2) had higher concentrations but still lower than the concentrations at sites K2, K4 and K5 (Figure 35). The concentrations at sites K1, K3, N1 and the Vaal River sites (V1 and V2) where all similar but lower than the concentrations at the other sites. The January 2014 results were similar to the June 2013 concentrations but the trends were different. Concentrations in January increased from site K1 to site K4 and then showed a decreased concentration at site K5 that corresponded with site K1. The concentrations at site N1 and R2 were similar to the concentrations at site K3 but lower than at site K5. Site R2 was similar to site K2 (Figure 38).

The highest selenium concentrations during June 2013 were measured at sites K2 and R1 (Figure 35). Concentrations of selenium at sites K3 and K4 where similar during the survey. All of the other sites indicated similar selenium concentrations. The January 2014 survey indicated selenium concentrations higher than measured during June 2013 as well as an increasing trend from site K1 to site K4. Concentrations at site K5 decreased to a similar concentration than at site K1 (Figure 38). The concentrations of selenium in site N1 was the highest throughout the study and concentrations decreased further from site R1 to site R2 but the concentrations were still higher than all of the sites in the Klip River with the exception of site K4.

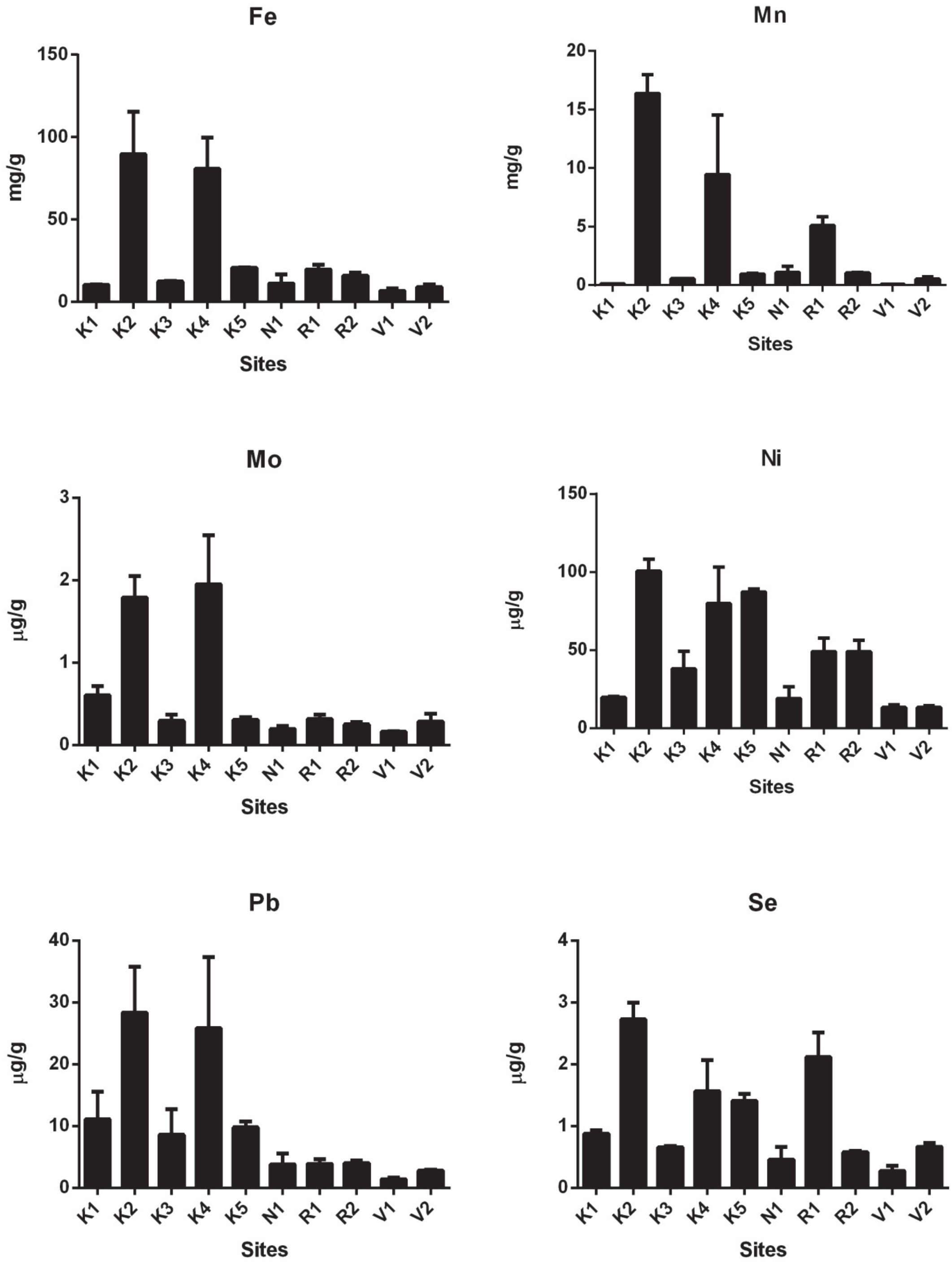


Figure 35: Selected sediment metal concentrations from the Klip River system for the June 2013 sampling survey. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

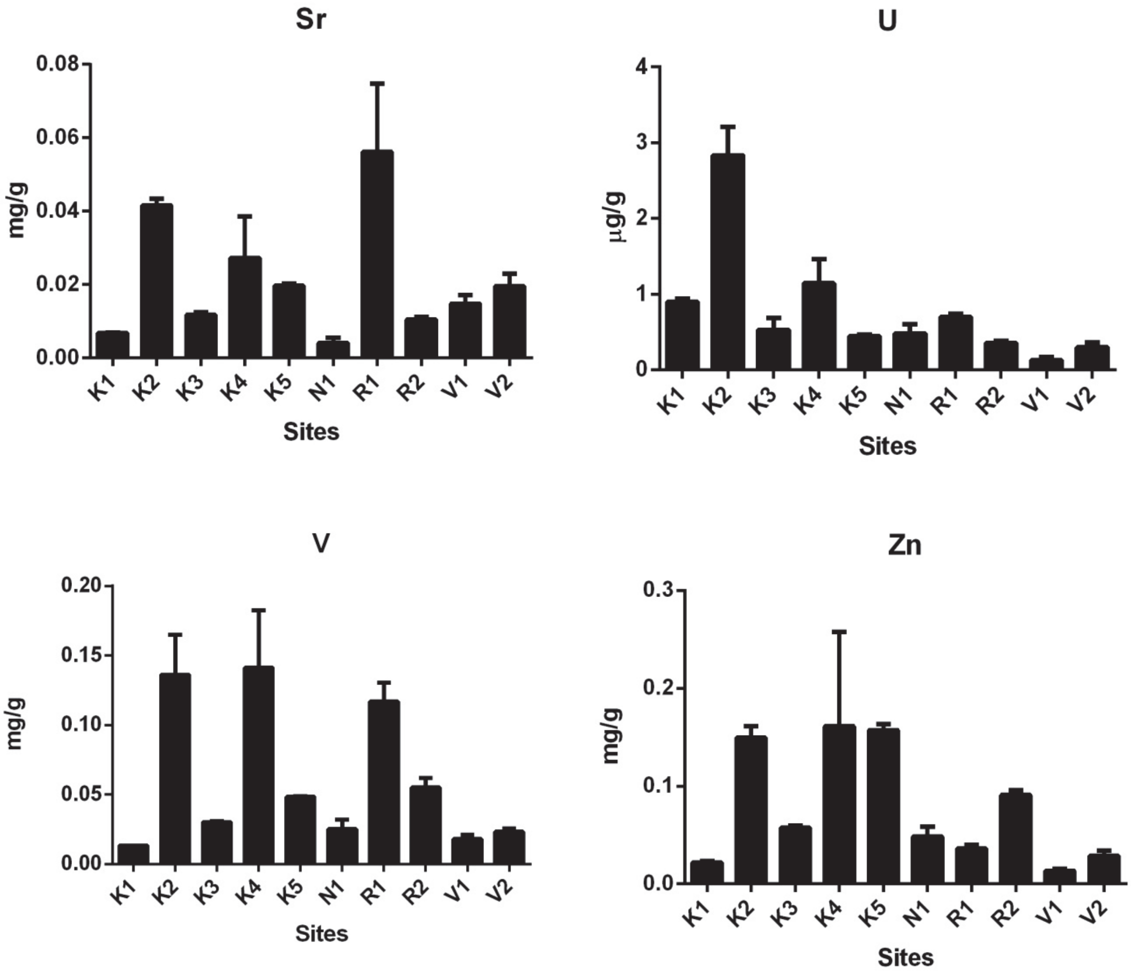


Figure 36: Selected sediment metal concentrations from the Klip River system for the June 2013 sampling survey. (K – Klip River; N – Natal Spruit; R – Riet Spruit; V – Vaal River).

The June 2013 strontium concentrations were the highest at sites R1 and K2 while sites K1 and N1 had the lowest concentrations (Figure 36). The other sites in the study area indicated similar strontium concentrations.

The June 2013 uranium concentrations in the sediment for the study area indicated that all of the sites had similar concentrations with the exception of site K2 (Figure 36). The uranium concentrations at site K2 was higher than measured at any of the other sites. The January 2014 concentrations were similar to the June 2013 concentrations except that site N1 had a high spike concentration instead of site K2 as seen during June 2013.

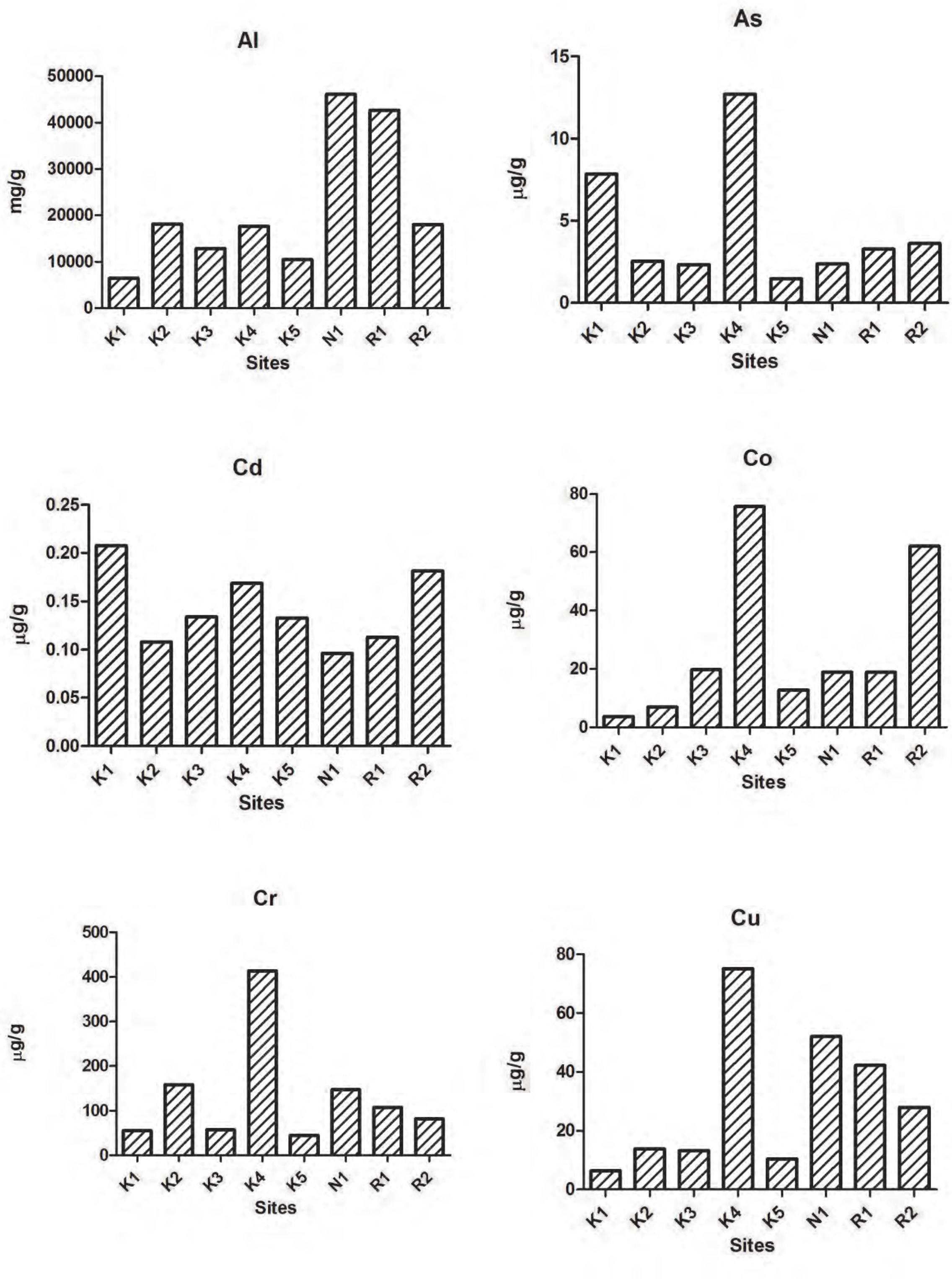


Figure 37: Selected sediment metal concentrations from the Klip River system for the January 2014 sampling survey. (K – Klip River; N – Natal Spruit; R – Riet Spruit).

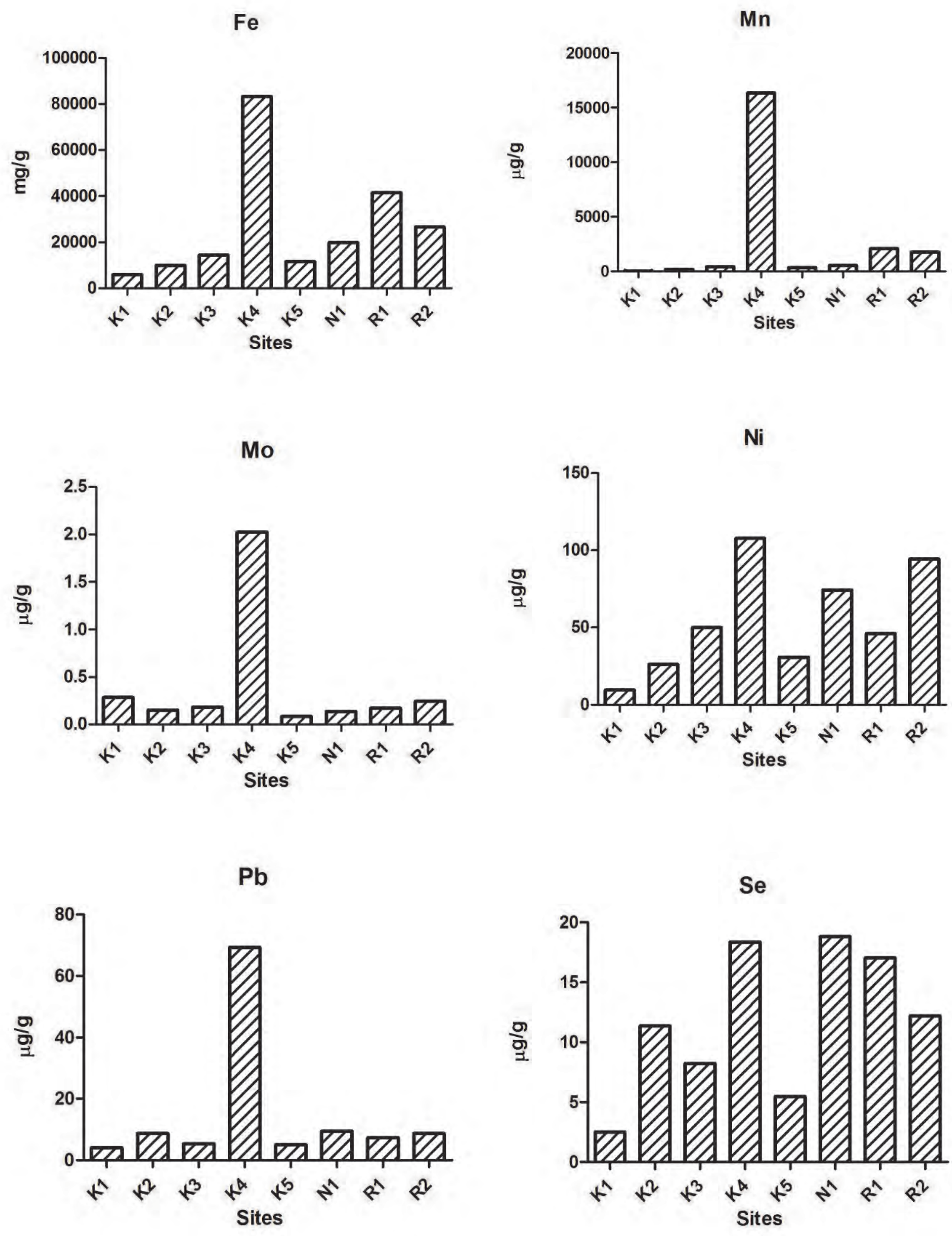


Figure 38: Selected sediment metal concentrations from the Klip River system for the January 2014 sampling survey. (K – Klip River; N – Natal Spruit; R – Riet Spruit).

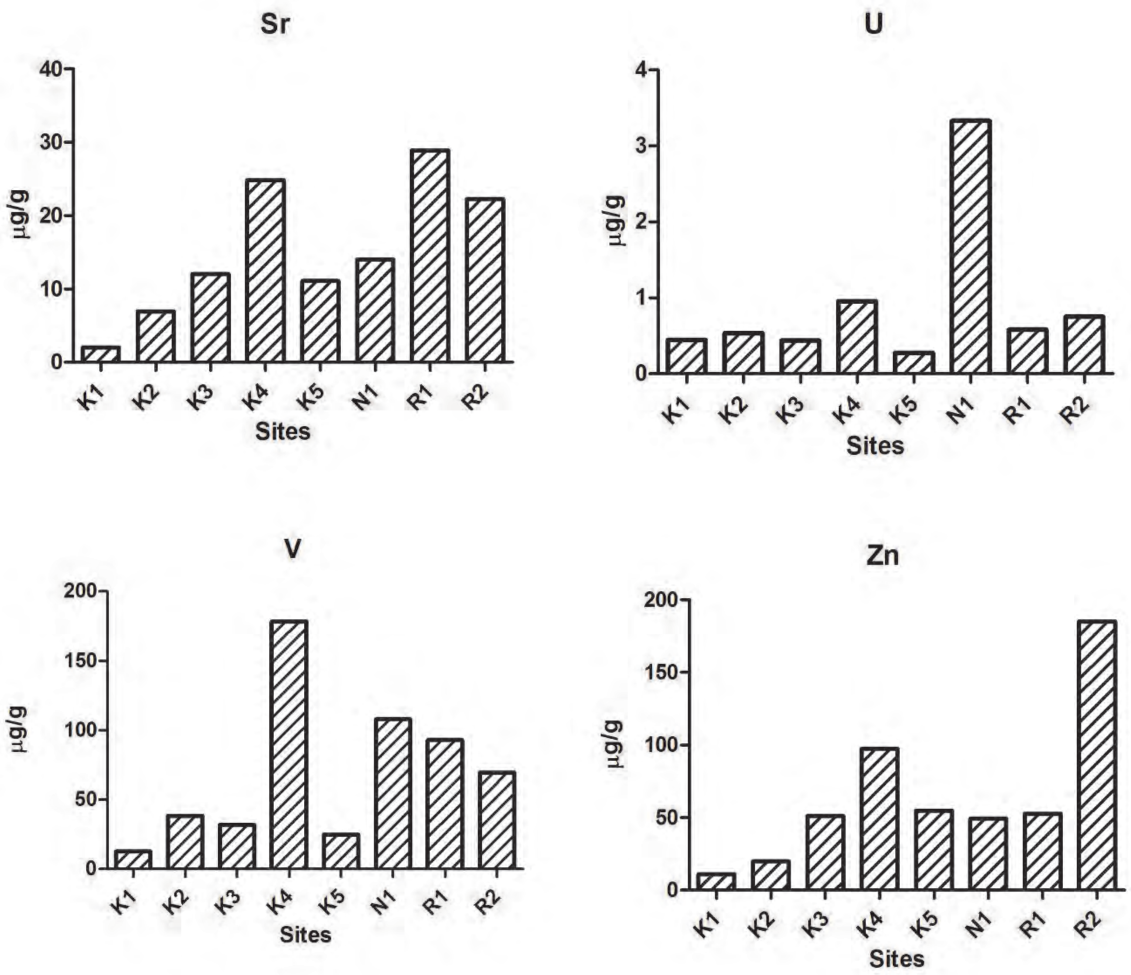


Figure 39: Selected sediment metal concentrations from the Klip River system for the January 2014 sampling survey. (K – Klip River; N – Natal Spruit; R – Riet Spruit).

The June 2013 vanadium concentrations indicated that the highest concentrations were measured at sites K2, K4 and R1 (Figure 36). Site K1 had the lowest vanadium concentration in the study area while all of the other sites indicated similar concentrations. The January 2014 concentrations were similar to the June 2013 concentrations for vanadium. Site K4 showed a high concentration as was seen in June 2013 while all the other Klip River sites were similar (Figure 39). The concentrations at sites N1, R1 and R2 were higher than measured in the Klip River and decreased slightly from site N1 to site R2 (Figure 39).

The June 2013 zinc concentrations at the various sites indicated that sites K2, K4 and K5 had the highest zinc concentrations in the sediment of the selected sites. As seen with the other metal concentrations, site K1 had the lowest concentration. All of the remaining sites indicated similar zinc concentrations in the sediment (Figure 36). The January 2014 concentrations for zinc were higher than the concentrations measured in June 2013. The concentrations increased from site K1 to site K4. The concentrations at sites K5, N1 and R1 were similar while site R2 had the highest concentration measured during January 2014 (Figure 39).

# 4 DIATOM AND MACRO-INVERTEBRATE COMMUNITY STRUCTURES OF THE KLIP RIVER SYSTEM

## 4.1 Diatom Community structures

Algae (including diatoms) and other micro-organisms attached to submerged surfaces occur in most shallow aquatic habitats where there is penetration of sufficient light. In most wetlands, these aggregations of algae known as periphyton grow attached to submerged substrata such as sediment, woody and herbaceous plants and rocky substrata. Because of their high dispersal rates, rapid growth rate and their direct response to environmental changes, algae provide the first indication of changes and are thus one of the most widely used indicators of biological integrity and physico-chemical conditions in aquatic ecosystems.

Diatoms, which constitute approximately 40% of any algal community, are unicellular, occasionally filamentous algae belonging to the group Bacillariophyceae, which are characterized by having a cell wall composed of silica. These microscopic organisms are found throughout most aquatic, sub-aerial and terrestrial habitats. Their communities react rapidly and specifically to changes in environmental conditions such as eutrophication, organic enrichment, salinisation and changes in pH.

Diatom community structures can be used to study current water quality as well as historical conditions. These organisms are very easy to sample and permanent records can be made from each sample collected. They differ from fish and macro-invertebrates in that, in general, they do not need any specialised food, habitat, depth or velocity of water and they occur anywhere where there is water. For these reasons, the use of diatoms for bioassessment in wetlands may provide a valuable tool for inferring water quality. As micro-organisms they lack dispersal barriers and may be transported by wind, aerosols, by wading birds and may even survive passage through insect’s digestive tracts. Many hundreds of thousands of cells may be produced within a few square centimetres of a wetland environment and this adds to the ease with which they are dispersed.

As diatoms cell walls are composed mostly of silica they can remain preserved for thousands of years. These preserved cell walls or frustules when removed in a core from the sediment may be used to trace the history of a wetland. The persistence of diatoms in sediments, even when wetlands are dry, may provide a year round approach for assessing the ecological integrity of wetlands when other organisms are not present. Furthermore, their rapid growth rates enable experimental manipulation of environmental conditions to determine cause-effect relationships between diatomic response and specific environmental stressors. The cumulative response of the diatoms to environmental stressors is reflected as an index score.

Diatom indices function in the following manner: In a sample from a body of water with a particular level or concentration of determinant (e.g. phosphorus), diatom taxa with their optimum close to that level will be most abundant. Therefore an estimate of the level of that determinant in the sample can be made from the average of the pollution sensitivity of all the taxa in that sample, each weighted by its abundance. This means that a taxon that is found frequently in a sample has more influence on the result than one that is rare. A further refinement is the provision of an 'indicator value' which is included to give greater weight to those taxa which are good indicators of particular environmental conditions. In practice, use of diatom indices involves making a list of the taxa present in a sample, along with a measure of their abundance. The index is expressed as the mean of the pollution sensitivity of the taxa in the sample, weighted by the abundance of each taxon. The indicator value acts to further increase the influence of certain species. The Specific Pollution sensitivity Index used in this report was developed and refined over a period of 20 years in France and has been tested in South Africa for 6 years and was found to accurately reflect water quality.

The results of the diatom analysis for the June 2013 and January 2014 surveys are interpreted based on Table 37 which provides a class and index score for the diatoms analysed. The diatom results are summarised into a table with index scores (Table 38) and the taxa found during each survey (Table 39 and Table 40). The indices used in the assessment are known as the Specific Pollution sensitivity Index (SPI). In addition the percentage of pollution tolerant valves (%PTV) is given, if this is above 20% it may indicate the presence of organic pollutants. Deformity in cells of 2% or more may be taken to indicate the presence of a toxicant or pollutant which disturbs cell wall morphogenesis.

Table 37: Guideline for the interpretation of the diatom indices scores.

| **Interpretation of index scores** | |
|---|---|
| **Index score** | **Class** |
| > 17 | high quality |
| 13 to 17 | good quality |
| 9 to 13 | moderate quality |
| 5 to 9 | poor quality |
| 05 | bad quality |

Table 38: Summarised diatom results for the June 2013 and January 2014 surveys on the Klip River indicating the various indices used for the assessment.

| Sample number | Date | NWU coll. Number | Count | No. of species | SPI | %PTV | % deformed cells | Diversity | Evenness | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| NS1 | 2013 | 14-180/1218 | 400 | 6 | 4.30 | 78.00 | 19.25 | 1.06 | 0.41 | |
| RS1 | 2013 | 14-181/1219 | 400 | 36 | 10.40 | 36.60 | 3.75 | 4.45 | 0.86 | |
| RS2 | 2013 | 14-183/1220 | 100 | 23 | 9.40 | 30.50 | 5.00 | 3.85 | 0.85 | Very high sediment load |
| KR2 | 2013 | 14-186/1223 | 400 | 40 | 5.20 | 28.40 | 2.25 | 3.83 | 0.72 | |
| KR3 | 2013 | 14-187/1224 | 400 | 37 | 9.00 | 33.20 | 1.75 | 4.15 | 0.80 | |
| KR4 | 2013 | 14-188/1225 | 400 | 32 | 3.70 | 72.80 | 1.00 | 3.76 | 0.75 | |
| KR5 | 2013 | 14-189/1226 | 400 | 31 | 7.20 | 60.60 | 1.00 | 4.08 | 0.82 | |
| Vaal 1 | 2013 | 14-184/1221 | 400 | 36 | 10.40 | 49.30 | 1.50 | 3.35 | 0.65 | |
| Vaal 2 | 2013 | 14-185/1222 | 400 | 34 | 8.20 | 44.80 | 0.00 | 3.34 | 0.66 | |
| | | | | | | | | | | |
| NS1 | 2014 | 14-333/1233 | 400 | 15 | 3.90 | 88.70 | 1.50 | 2.48 | 0.63 | |
| RS1 | 2014 | 14-327/1227 | 400 | 27 | 8.10 | 65.50 | 4.25 | 2.43 | 0.51 | |
| RS2 | 2014 | 14-328/1228 | 400 | 55 | 7.20 | 41.50 | 1.75 | 4.97 | 0.86 | |
| KR2 | 2014 | 14-329/1229 | 400 | 35 | 5.50 | 64.70 | 4.25 | 3.39 | 0.66 | |
| KR3 | 2014 | 14-330/1230 | 400 | 53 | 5.50 | 51.50 | 1.50 | 4.44 | 0.78 | |
| KR4 | 2014 | 14-331/1231 | 400 | 54 | 6.70 | 35.70 | 2.25 | 4.78 | 0.83 | |
| KR5 | 2014 | 14-332/1232 | 50 | 29 | 7.20 | 38.00 | 0.00 | 4.64 | 0.96 | Very high sediment load |

Table 39: Diatom taxa found at the various sites on the Klip River during the June 2013 sampling survey.

| Taxon | Site – June 2013 Survey | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | NS1 | RS1 | RS2 | KR2 | KR3 | KR4 | KR5 | Vaal 1 | Vaal 2 |
| Abnormal diatom valve or sum of deformities | 77 | 15 | 5 | 9 | 7 | 4 | 4 | 6 | |
| *Achnanthidium exiguum* (Grunow) Czarnecki | | | | | | | 1 | | |
| *Achnanthidium* sp. | | | 3 | | | | | 10 | |
| *Amphora montana* Krasske | | | | 2 | | 4 | 24 | | |
| *Amphora pediculus* (Kützing) Grunow | 23 | 1 | 5 | | | | 3 | | 14 |
| *Aulacoseira granulata* (Ehrenberg) Simonsen | | | | 12 | 1 | | | | |
| *Caloneis bacillum* (Grunow) Cleve | | | | | | | | | 3 |
| *Cocconeis pediculus* Ehrenberg | | 1 | 2 | | | | | 2 | |
| *Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow | | | | | 1 | | 1 | 57 | 3 |
| *Craticula accomoda* (Hustedt) Mann | | | | | | 1 | | | |
| *Craticula molestiformis* (Hustedt) Lange-Bertalot | | | 1 | 1 | | 3 | | 5 | 1 |
| *Cyclostephanos dubius* (Fricke) Round | | | | | | | | 1 | |
| *Cyclostephanos invisitatus* (Hohn & Hellerman) Theriot, Stoermer & Håkansson | | | | | | | | | 1 |
| *Cyclotella meneghiniana* Kützing | | | | 1 | 2 | | | | 1 |
| *Cymbella* sp. | | | | | | | | 1 | |
| *Cymbella turgidula* Grunow | | | | | | | | 3 | |
| *Diatoma vulgaris* Bory | | 1 | | 1 | | | | | |
| *Discostella pseudostelligera* (Hustedt) Houk et Klee | | | | 1 | | | | 2 | |
| *Encyonema minutum* (Hilse) D.G. Mann | | | | | | | | | |
| *Encyonema silesiacum* (Bleisch in Rabh.) D.G. Mann | | | | | | | | | |
| *Eolimna minima* (Grunow) Lange-Bertalot | | 1 | 3 | | | 5 | 4 | 3 | 5 |
| *Eolimna subminuscula* (Manguin) Moser Lange-Bertalot & Metzeltin | | 32 | 10 | 2 | 31 | 33 | 48 | 1 | 2 |
| *Eunotia arcus* Ehrenberg | | | | | | | | 3 | |
| *Fallacia pygmaea* (Kützing) Stickle & Mann | | | | | 1 | | | | 1 |
| *Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot | | 7 | | | 17 | 35 | 2 | | |
| *Fragilaria biceps* (Kützing) Lange-Bertalot | | 2 | | 14 | 11 | | | | 1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Fragilaria capucina* Desmazieres | | | | | | 1 | | | |
| *Fragilaria capucina* var. *vaucheriae* (Kützing) Lange-Bertalot | | | | | | | | 2 | |
| *Frustulia vulgaris* (Thwaites) De Toni | | | | | 1 | | 2 | | |
| *Gomphonema acuminatum* Ehrenberg | | | | 1 | | | | | |
| *Gomphonema parvulum* (Kützing) Kützing | 368 | 24 | 13 | 46 | 41 | 52 | 35 | 1 | 3 |
| *Gomphonema pseudoaugur* Lange-Bertalot | | | | 12 | 4 | 1 | | | 1 |
| *Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot | | | 2 | 1 | 44 | | 6 | 1 | 54 |
| *Gomphonema* sp. | | | | 2 | | | | 1 | |
| *Gyrosigma scalproides* (Rabenhorst) Cleve | | | | | 1 | | | | |
| *Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski | | | | 2 | | | | | |
| *Mayamaea atomus* (Kützing) Lange-Bertalot | | 1 | | | | | | | 1 |
| *Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot | | 18 | 3 | 1 | 8 | 23 | 17 | 2 | 1 |
| *Melosira varians* Agardh | | 8 | | | | | | 1 | |
| *Navicula antonii* Lange-Bertalot | | | | | 19 | | 8 | 2 | |
| *Navicula arvensis* var. *maior* Lange-Bertalot | | | | | 1 | | | 1 | 3 |
| *Navicula cari* var. *linearis* (Ostrup) Cleve-Euler | | 1 | | | | | | | |
| *Navicula cryptocephala* Kützing | | | | 15 | 5 | | | | |
| *Navicula cryptotenella* Lange-Bertalot | | 11 | 3 | | 7 | 1 | 28 | 11 | 1 |
| *Navicula erifuga* Lange-Bertalot | | | | | 5 | | 9 | | 1 |
| *Navicula gregaria* Donkin | | 1 | | 18 | 12 | 1 | 58 | 1 | |
| *Navicula libonensis* Schoeman | | | | | | 3 | | | |
| *Navicula recens* (Lange-Bertalot) Lange-Bertalot | | | | 1 | | | | | 32 |
| *Navicula reichardtiana* Lange-Bertalot | | | | | | | | 6 | |
| *Navicula riediana* Lange-Bertalot & Rumrich | | | | 8 | 3 | | | | |
| *Navicula rostellata* Kützing | | | | 5 | 4 | 1 | | | |
| *Navicula schroeteri* Meister | | | | | | | | | 12 |
| *Navicula* sp. | | 1 | 1 | 1 | 61 | 10 | 8 | 2 | 4 |
| *Navicula symmetrica* Patrick | | 3 | 13 | 4 | 59 | 9 | 5 | 5 | 20 |
| *Navicula tenelloides* Hustedt | | | | | | | 4 | | |
| *Navicula tripunctata* (O.F.Müller) Bory | | 33 | 4 | 1 | | | | 22 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Navicula trivialis* Lange-Bertalot | | | | 1 | | 2 | | | 1 |
| *Navicula veneta* Kützing | 4 | 6 | 3 | 3 | | 5 | 20 | | |
| *Nitzschia acicularis* (Kützing) W.M.Smith | | | | 3 | | | | | |
| *Nitzschia amphibia* Grunow | | 1 | | 32 | 5 | 4 | 3 | | 5 |
| *Nitzschia clausii* Hantzsch | | | | 4 | | | | | |
| *Nitzschia desertorum* Hustedt | | 3 | | 1 | | | | | |
| *Nitzschia dissipata* (Kützing) Grunow | | 42 | 1 | 19 | 1 | | | 26 | 2 |
| *Nitzschia filiformis* (W.M.Smith) Van Heurck | | | | | | | 2 | | 3 |
| *Nitzschia frustulum* (Kützing) Grunow | | 18 | | | | 9 | 58 | | 26 |
| *Nitzschia heufleriana* Grunow | | 23 | | | | | | 1 | |
| *Nitzschia intermedia* Hantzsch | | | | | 1 | | | | |
| *Nitzschia liebetruthii* Rabenhorst | | | | | | | | 164 | |
| *Nitzschia linearis* (Agardh) W.M.Smith | | 7 | | 4 | 2 | 1 | 1 | | |
| *Nitzschia palea* (Kützing) W.Smith | | | | 34 | 19 | 112 | 9 | | 1 |
| *Nitzschia paleacea* (Grunow) Grunow | | 8 | | | | | | | |
| *Nitzschia solita* Hustedt | | 7 | | 1 | | | | | |
| *Nitzschia* sp. | | 19 | 2 | 129 | 13 | 24 | 17 | 10 | 2 |
| *Pinnularia subbrevistriata* Krammer | | 10 | 1 | | 5 | | | | |
| *Planothidium daui* (Foged) Lange-Bertalot | | | | | | | | | 1 |
| *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot | 1 | 27 | 23 | 3 | 1 | 1 | 1 | 1 | 1 |
| *Reimeria uniseriata* Sala, Guerrero & Ferrario | | | | | | | | 1 | |
| *Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot | | 16 | 1 | 3 | 4 | | | 18 | 59 |
| *Sellaphora pupula* (Kützing) Mereschkowksy | | | 1 | 4 | | | | | |
| *Sellaphora seminulum* (Grunow) D.G. Mann | 4 | | | 5 | 3 | 17 | 10 | 26 | 133 |
| *Stephanodiscus agassizensis* Håkansson & Kling | | | | | | | | 1 | |
| *Surirella angusta* Kützing | | 2 | | 2 | 4 | 8 | 3 | | |
| Surirella Brèbissonii Krammer & Lange-Bertalo | | 38 | | | 1 | 6 | 12 | | |
| *Surirella ovalis* Brèbisson | | 23 | 2 | | 2 | 21 | | | |
| *Thalassiosira weissflogii* (Grunow) Fryxell & Hasle | | 2 | | | | 1 | | | |
| *Tryblionella apiculata* Gregory | | 2 | 3 | | | 5 | 1 | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Tryblionella debilis* Arnott ex O'Meara | | | | | | | | | 1 |
| *Tryblionella hungarica* (Grunow) D.G. Mann | | | | | | 1 | | | |

Table 40: Diatom taxa found at the various sites on the Klip River during the January 2014 sampling survey.

| Taxon | Site – January 2014 survey | | | | | | |
|---|---|---|---|---|---|---|---|
| | NS1 | RS1 | RS2 | KR2 | KR3 | KR4 | KR5 |
| Abnormal diatom valve or sum of deformities | 6 | 17 | 7 | 17 | 6 | 9 | |
| *Achnanthes inflata* (Kützing) Grunow | | | 2 | | | | |
| *Achnanthidium exiguum* (Grunow) Czarnecki | | | 1 | 2 | 1 | 1 | 1 |
| *Achnanthidium* sp. | | | 6 | 1 | 1 | 3 | 1 |
| *Amphora montana* Krasske | | | 1 | 17 | | | 2 |
| *Amphora pediculus* (Kützing) Grunow | 16 | 3 | 35 | 1 | 1 | 3 | 1 |
| *Aulacoseira granulata* (Ehrenberg) Simonsen | | | | 1 | 52 | 20 | 2 |
| *Aulacoseira granulata* var. *angustissima* (O.Müler)Simonsen | | | | 31 | 6 | 2 | |
| *Brachysira neoexilis* Lange-Bertalot | | | 1 | | | | |
| *Capartogramma crucicula* (Grunow) Ross | | | | | | | 1 |
| *Cocconeis pediculus* Ehrenberg | | 1 | | | | | |
| *Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow | | | 6 | | 5 | 10 | 1 |
| *Craticula accomoda* (Hustedt) Mann | | | | | 1 | | |
| *Craticula molestiformis* (Hustedt) Lange-Bertalot | 5 | | | 1 | | 1 | |
| *Cyclostephanos invisitatus* (Hohn & Hellerman)  Theriot, Stoermer & Hakans | | | | | 1 | | |
| *Cyclotella meneghiniana* Kützing | | | 1 | 3 | 3 | | |
| *Cymatopleura solea* var. *apiculata* (W.Smith) Ralfs | | | | | | 1 | |
| *Cymbella cuspidata* Kützing | | | | | 1 | 3 | |
| *Cymbella kappii* (Cholnoky) Cholnoky | | | | | | 1 | |
| *Cymbella tumida* (Brèbisson)Van Heurck | | | 1 | | | | |
| *Diadesmis confervacea* Kützing | | 1 | | | 1 | 17 | 1 |
| *Diatoma vulgaris* Bory | | | | | | | 1 |
| *Encyonema minutum* (Hilse in Rabh.) D.G. Mann | | | | | | 1 | |
| *Eolimna minima* (Grunow) Lange-Bertalot | | | 5 | 1 | 2 | 2 | |
| *Eolimna subminuscula* (Manguin) Moser, Lange-Bertalot & Metzeltin | 27 | 4 | 7 | 12 | 11 | 9 | 1 |
| *Fallacia monoculata* (Hustedt) D.G. Mann | | | | | | | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Fallacia pygmaea* ssp. *pygmaea* Lange-Bertalot | | | | | | | 1 |
| *Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot | 17 | | 1 | | 4 | | |
| *Fragilaria biceps* (Kützing) Lange-Bertalot | | | | 2 | | | |
| *Fragilaria ulna* (Nitzsch.) Lange-Bertalot var. ulna | | | | 6 | 5 | | 2 |
| *Frustulia vulgaris* (Thwaites) De Toni | | | 6 | | 1 | | |
| *Gomphonema acuminatum* Ehrenberg | | | | 1 | | | |
| Gomphonema parvulum (Kützing) Kützing | 157 | 1 | 15 | 28 | 51 | 20 | 5 |
| *Gomphonema pseudoaugur* Lange-Bertalot | | | | 2 | | 3 | |
| *Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot | | | | 2 | 6 | 14 | |
| *Gyrosigma acuminatum* (Kützing)Rabenhorst | | 1 | | | | | |
| *Gyrosigma scalproides* (Rabenhorst)Cleve | | | | | 1 | | |
| *Gyrosigma* sp. | | | | | | 1 | |
| *Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski | | | 2 | | | | |
| *Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot | | | 3 | 1 | 1 | 1 | |
| *Melosira varians* Agardh | | 2 | 4 | | 2 | 5 | |
| *Navicula antonii* Lange-Bertalot | | | | | 2 | 1 | |
| *Navicula arvensis* Hustedt var. *maior* Lange-Bertalot | 2 | | 3 | | 5 | 1 | 1 |
| *Navicula cryptocephala* Kützing | 1 | | | 10 | 7 | 3 | |
| *Navicula cryptotenella* Lange-Bertalot | | 4 | 1 | | | | |
| *Navicula cryptotenelloides* Lange-Bertalot | | | | | | 32 | |
| *Navicula erifuga* Lange-Bertalot | | | 5 | 1 | 3 | 5 | 2 |
| *Navicula germainii* Wallace | | | | | | 1 | |
| *Navicula gregaria* Donkin | | | 10 | | | 1 | |
| *Navicula notha* Wallace | | | | | | | |
| *Navicula riediana* Lange-Bertalot & Rumrich | | | | | 3 | 1 | |
| *Navicula rostellata* Kützing | | | 3 | | 3 | 2 | |
| *Navicula schroeteri* Meister | | | 1 | | | 1 | |
| *Navicula* small species | | | 3 | | | | |
| *Navicula* sp. | | 3 | 14 | | 6 | 4 | 1 |
| *Navicula symmetrica* Patrick | | 5 | 19 | | 18 | 52 | 4 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Navicula tripunctata* (O.F.Müller) Bory | | 2 | | | | 1 | |
| *Navicula trivialis* Lange-Bertalot var. trivialis | 1 | | 1 | | | 7 | |
| *Navicula veneta* Kützing | 14 | 3 | 8 | 1 | 5 | 5 | 2 |
| *Nitzschia acicularis* (Kützing) W.M.Smith | | | | 1 | 2 | | |
| *Nitzschia amphibia* Grunow | 1 | 3 | 20 | 18 | 9 | 2 | |
| *Nitzschia capitellata* Hustedt in A.Schmidt & al. | | | 1 | | | | |
| *Nitzschia clausii* Hantzsch | | | | 1 | 6 | 1 | |
| *Nitzschia communis* Rabenhorst | | | | | 1 | | |
| *Nitzschia desertorum* Hustedt | | 2 | | | | 1 | |
| *Nitzschia dissipata* (Kützing) Grunow | | 19 | | | 1 | | |
| *Nitzschia filiformis* (W.M.Smith) Van Heurck | | | 9 | 9 | 11 | 1 | |
| *Nitzschia frustulum* (Kützing) Grunow | | 247 | 13 | 2 | | 17 | 2 |
| *Nitzschia liebetruthii* Rabenhorst | | | | 175 | 27 | 9 | |
| *Nitzschia linearis* (Agardh) W.M.Smith var.linearis | | 11 | 13 | | 1 | 4 | 2 |
| *Nitzschia palea* (Kützing) W.Smith | 38 | 2 | 35 | 31 | 78 | 40 | 3 |
| *Nitzschia paleacea* (Grunow) Grunow in van Heurck | | | 1 | | | | |
| *Nitzschia solita* Hustedt | | 1 | 6 | | | | |
| *Nitzschia* sp. | | 6 | 35 | 15 | 18 | 26 | 4 |
| *Nitzschia umbonata*(Ehrenberg)Lange-Bertalot | | | 1 | | 2 | | |
| *Nupela* species | | | 1 | | 1 | | |
| *Pinnularia* sp. | | | 1 | | | | |
| *Pinnularia subbrevistriata* Krammer | | 3 | 1 | | 5 | | |
| *Pinnularia viridis* (Nitzsch) Ehrenberg | | | 1 | | | | |
| *Placoneis dicephala* (W.Smith) Mereschkowsky | | | | | 1 | | |
| *Placoneis* sp. | | | | 1 | | | |
| *Planothidium frequentissimum*( Lange-Bertalot) Lange-Bertalot | 1 | 9 | 19 | 9 | 7 | 20 | 1 |
| *Planothidium lanceolatum* (Brèbisson) Lange-Bertalot | 1 | | | | | | |
| *Rhoicosphenia abbreviata* (C.Agardh) Lange-Bertalot | | 59 | 3 | | 2 | 8 | |
| *Sellaphora pupula* (Kützing) Mereschkowksy | | | 11 | 2 | 7 | 10 | 1 |
| *Sellaphora seminulum* (Grunow) D.G. Mann | 119 | | 15 | 10 | 8 | 8 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Seminavis strigosa* (Hustedt) Danieledis & Economou-Amilli | | | | | 1 | | |
| *Stauroneis kriegeri* Patrick | | | 6 | 1 | | 1 | |
| *Surirella angusta* Kützing | | | 5 | 1 | | 3 | 2 |
| *Surirella brebissonii* Krammer & Lange-Bertalot | | 1 | 3 | | 1 | | |
| *Surirella ovalis* Brèbisson | | 3 | 3 | | | | |
| *Tryblionella apiculata* Gregory | | 4 | 27 | | | 12 | |
| *Tryblionella calida* (Grunow) D.G. Mann | | | 1 | | | | 1 |
| *Tryblionella hungarica* (Grunow) D.G. Mann | | | | | | 1 | 2 |
| *Tryblionella levidensis* W.M.Smith | | | 3 | | 1 | | |
| *Tryblionella littoralis* (Grunow) D.G. Mann | | | | | | 1 | 1 |
| Tryblionella sp. | | | 1 | | | | |

The diatom results indicate that the SPi index was generally lower than 11 which indicate a moderate to poor water quality. The worst affected site was site NS1 that indicated a bad water quality during both surveys. The %PTV of most sites was also above the 20% limit that indicates the presence of organic pollutants. The percentage of deformed cells was the highest at site NS1 during June 2013 while all the other sites were only slightly higher than the 2% range as mentioned previously. Lists of all the diatom taxa found during both surveys are presented in Table 39 and Table 40.

## 4.2 Macro-Invertebrates

The macro-invertebrate results during the June 2013 survey for the Klip River and the various tributaries yielded taxa from 22 different families across all of the sites sampled (Table 41). Site KR2 had the highest number of taxa at 15 while site KR1 had the lowest at 1. The majority of the taxa sampled where relatively hardy with the most sensitive taxa being more than two species each of the Hydropsychidae and Baetidae families sampled at site KR3. The SASS scores for the various sites range from 1 to 70 while the ASPT ranged from 3 to 5.4. Low SASS scores are often a reflection of alterations to habitat, while the ASPT is a good reflection of water quality (Dickens & Graham, 2001). The taxa also reflected that very little Ephemeroptera, Trichoptera and Odonata, which are regarded as good water quality indicators, were present at the sites. A study by Ferreira et al. (2010) on the Klip River completed SASS5 sampling at the same sites on the Klip River as used in this survey. Their results were similar at sites KR2 to KR3 while the SASS scores during this survey were lower at sites KR4 and KR5. However, when looking at the ASPT scores for sites KR4 and KR5 the scores were similar. The one major difference from the Ferreira et al. (2010) study was the condition of site KR1. Since 2010 this site has been critical modified and all instream habitats have been destroyed due to the sand mining taking place at the site as well as upstream of the site.

Table 41: Macro-invertebrate taxa collected during the June 2013 sampling survey on the Klip River and tributaries (KR – Klip River; NS – Natal Spruit; RS – Riet Spruit).

| **Taxa** | **Sensitivity** | **KR1** | **KR2** | **KR3** | **KR4** | **KR5** | **NS** | **RS1** | **RS2** |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| Aeshnidae | 8 | | | 1 | | | | | |
| Ancylidae | 6 | | 1 | | | | | | 1 |
| Atyidae | 8 | | | | | | | | 1 |
| Baetidae 1sp | 4 | | B | | | | A | | |
| Baetidae 2sp | 6 | | | | A | A | | B | A |
| Baetidae > 2sp | 12 | | | B | | | | | |
| Belastomatidae | 3 | | | 1 | | | | | |
| Caenidae | 6 | | 1 | A | | | | | |
| Ceratopogonidae | 5 | | A | | | | | 1 | |
| Chironomidae | 2 | 1 | A | B | C | A | B | B | B |
| Coenagrionidae | 4 | | | B | 1 | B | | A | B |
| Corixidae | 3 | | D | B | | A | | C | A |
| Culicidae | 1 | | 1 | | | | | | |
| Gomphidae | 6 | | | 1 | | | | | |
| Gyrinidae | 5 | | | A | | | | | 1 |
| Hirudinea | 3 | | 1 | | 1 | | | | A |
| Hydrophilidae | 5 | | | | | | | A | |
| Hydropsychidae 1sp | 4 | | B | | | | | B | |
| Hydropsychidae 2sp | 6 | | | | A | A | | | B |
| Hydropsychidae > 2sp | 12 | | | B | | | | | |
| Notonectidae | 3 | | A | | | | | | |
| Oligochaeta | 1 | | A | B | B | A | A | A | A |
| Potamonautidae | 3 | | A | A | | | | A | 1 |
| Simuliidae | 5 | | A | B | C | B | A | A | B |
| Turbellaria | 3 | | A | | B | | | | |
| Veliidae | 5 | | 1 | | | A | | | |
| | | | | | | | | | |
| **SASS score** | | **1** | **54** | **70** | **30** | **32** | **12** | **38** | **52** |
| **Number of Taxa** | | **1** | **15** | **13** | **8** | **8** | **4** | **10** | **12** |
| **ASPT** | | **1** | **3.6** | **5.4** | **3.75** | **4** | **3** | **3.8** | **4.3** |
| **Ecological Category** | | **E/F** | **E/F** | **C** | **E/F** | **E/F** | **E/F** | **E/F** | **D** |

The macro-invertebrate results from the January 2014 survey for the Klip River and the various tributaries yielded taxa from 20 different families across all of the sites sampled

(Table 42). The low flow survey on the Klip River resulted in the sampling of 22 different families. Site RS2 had the highest number of taxa at 15 while site KR1 had the lowest at 5; this was similar to the low flow survey with the major difference being that more taxa were sampled at site KR1. The majority of the taxa sampled where relatively hardy with the most sensitive taxa being more than two species each of the Hydropsychidae and Baetidae families sampled at site KR3. The SASS scores for the various sites range from 16 to 63 while the ASPT ranged from 3.1 to 5.2. The taxa also reflected that very little Ephemeroptera, Trichoptera and Odonata, which are regarded as good water quality indicators, were present at the sites. However, when looking at the ASPT scores for sites KR4 and KR5 the scores were similar. The one major difference from the Ferreira et al. (2010) study was the condition of site KR1. Since 2010 this site has been critical modified and all instream habitats have been destroyed due to the sand mining taking place at the site as well as upstream of the site.

The SASS5 results of the Riet Spruit (RS) and Natal Spruit (NS) indicate that the macro-invertebrate community is severely degraded in especially the Natal Spruit. The taxa found in the Natal Spruit are very hardy and literature indicates that taxa like Chironomidae, Simuliidae and Oligochaeta will dominate when organic pollution is present (Day et al., 2002). The Lower Riet Spruit (RS2) did indicate a recovery when compared to the Upper Riet Spruit site in terms of the SASS score but the ASPT scores were similar. Overall, the macro-invertebrate community is comprised of hardy taxa with a distinct absence of many of the sensitive macro-invertebrate taxa. This reflects that the community is modified, possibly due to the numerous impacts that the river do receive. The SASS5 results did indicate that at site KR3 a good ecological category (EC of B) was calculated. This result is an indication of the very good habitat at the site rather than a reflection of good water quality.

Table 42: Macro-invertebrate taxa collected during the January 2014 sampling survey on the Klip River and tributaries. SASS5 indices and ecological categories are also indicated.

| **Taxa** | **Sensitivity** | **KR1** | **KR2** | **KR3** | **KR4** | **KR5** | **NS** | **RS1** | **RS2** |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| Baetidae 1sp | 4 | 1 | | | | 1 | B | | A |
| Baetidae 2sp | 6 | | B | | A | | | A | |
| Baetidae > 2sp | 12 | | | B | | | | | |
| Belostomatidae | 3 | | 1 | | A | A | | 1 | |
| Caenidae | 6 | | | 1 | | | | | |
| Ceratopogonidae | 5 | | | 1 | | | | | |
| Chironomidae | 2 | A | A | B | B | A | C | B | A |
| Coenagrionidae | 4 | | 1 | A | A | 1 | | A | |
| Corixidae | 3 | A | B | A | A | A | A | B | 1 |
| Culicidae | 1 | | 1 | | | | | | |
| Gyrinidae | 5 | | 1 | A | | A | | 1 | |
| Hirudinea | 3 | | 1 | | 1 | | 1 | | |
| Hydropsychidae 1 | 4 | | | | | | 1 | | A |
| Hydropsychidae 2 | 6 | | A | | B | | | | |
| Hydropsychidae > 2 | 12 | | | B | | | | B | |
| Leptoceridae | 6 | 1 | | | | | | | |
| Lymnaeidae | 3 | | | | | | | 1 | |
| Notonectidae | 3 | | | | | | | A | A |
| Oligochaeta | 1 | A | 1 | A | B | A | B | A | A |
| Planorbinae | 3 | | | | | | | 1 | |
| Potamonautidae | 3 | | A | A | A | | A | A | A |
| Simuliidae | 5 | | D | D | B | | B | A | A |
| Turbellaria | 3 | | A | | | | | | |
| Veliidae | 5 | | | A | | A | | A | |
| | | | | | | | | | |
| **SASS score** | | **16** | **45** | **63** | **34** | **27** | **25** | **58** | **25** |
| **Number of Taxa** | | **5** | **13** | **12** | **10** | **8** | **8** | **15** | **8** |
| **ASPT** | | **3.2** | **3.46** | **5.25** | **3.4** | **3.38** | **3.13** | **3.87** | **3.13** |
| **Ecological Category** | | **E/F** | **E/F** | **B** | **E/F** | **E/F** | **E/F** | **E/F** | **E/F** |

# 5 FISH COMMUNITY STRUCTURE AND HEALTH IN THE KLIP RIVER SYSTEM

## 5.1 Fish Community Assessment

The fish community assessment was completed at selected sites, i.e. KR2, KR3, KR4, KR5 and RS2. The other sites were deemed not important for the fish community due to the lack of sufficient habitat to support larger fish individuals for the pollutant accumulation and biomarker studies. The FROC database (Kleynhans, 2007) and a study by Kotze (2002) were used to determine the expected species list for the fish community. Table 43 provides the FROC database sites and associated fish species expected to be present at sites on the Klip River. The RHP site codes C2KLIP-ZWART and C2KLIP-SLANG correspond to the sites KR3 and KR4 in the current study. Kotze (2002) used the same fish species list as given in the Table 43 below. The species list comprises of 14 species that includes three alien fish species. The table also indicates that two species *Labeobarbus kimberleyensis* (BKIM) and *Austroglanis sclateri* (ASCL) are Code 3 species that relate to no records of their presence exist but they were expected to occur historically. However, Kotze (2002) does indicate that *A. sclateri* were sampled at sites in the Lower Klip River that would correspond to the present sites KR4 and KR5. Both of these fish species are sensitive to changes in water quality and quantity and therefore these species would probably not occur in the Klip River at present, due to all the impacts on water quality in the river. The other nine indigenous species on the reference list are all fairly tolerant to changes in water quality and it is expected that these fish should occur within the Klip River at present.

The fish species that were sampled during the June 2013 sampling survey on the Klip River is provided in Table 44 and comprised of eleven species of the possible twelve on the expected list. This included all three of the alien fish species, i.e. *Gambusia affinis*, *Micropterus salmoides* and *Cyprinus carpio*. Fish sampling was extremely difficult due to the cold water temperatures resulting in fish movement being minimal. The most fish activity was seen at site KR5 where *C. carpio* was observed surfacing but none of these individuals were sampled in the nets or with electroshocking. The most fish species were sampled at site KR2 and site KR3 with five and four species being sampled respectively. In general, the abundance of fish species was also low at all of the sites. It is expected that fish species would be more active during spring and summer and a higher species diversity and abundance is expected to be found at all of the sites during these times. One dead *Labeobarbus aeneus* were seen at site RS2 and anecdotal evidence suggests this species occurs in abundance in the Riet Spruit.

Table 43: Fish reference list for the Klip River based on the FROC database (Kleynhans et al., 2007)

| RHP Site Code | SPP | CODE 3 SP | FFROC | CONFIDENCE | RELATIVE ABUNDANCE |
|---|---|---|---|---|---|
| C2KLIP-ZWART | BANO | | 4 | 5 | 2 |
| C2KLIP-ZWART | BNEE | | 3 | 3 | 1 |
| C2KLIP-ZWART | BPAU | | 3 | 3 | 2 |
| C2KLIP-ZWART | BAEN | | 4 | 5 | 2 |
| C2KLIP-ZWART | | BKIM | 3 | 2 | 1 |
| C2KLIP-ZWART | LCAP | | 4 | 5 | 2 |
| C2KLIP-ZWART | LUMB | | 3 | 5 | 1 |
| C2KLIP-ZWART | CGAR | | 4 | 5 | 1 |
| C2KLIP-ZWART | | ASCL | 3 | 3 | 1 |
| C2KLIP-ZWART | PPHI | | 4 | 5 | 1 |
| C2KLIP-ZWART | TSPA | | 4 | 5 | 1 |
| C2KLIP-ZWART | CCAR | | | | |
| C2KLIP-ZWART | MSAL | | Alien | | |
| C2KLIP-ZWART | GAFF | | | | |
| C2KLIP-SLANG | BANO | | 4 | 5 | 2 |
| C2KLIP-SLANG | | BNEE | 3 | 3 | 1 |
| C2KLIP-SLANG | BPAU | | 3 | 3 | 1 |
| C2KLIP-SLANG | BAEN | | 5 | 4 | 2 |
| C2KLIP-SLANG | | BKIM | 3 | 3 | 1 |
| C2KLIP-SLANG | LCAP | | 5 | 4 | 2 |
| C2KLIP-SLANG | LUMB | | 4 | 3 | 1 |
| C2KLIP-SLANG | CGAR | | 4 | 3 | 1 |
| C2KLIP-SLANG | ASCL | | 3 | 3 | 1 |
| C2KLIP-SLANG | PPHI | | 4 | 3 | 1 |
| C2KLIP-SLANG | TSPA | | 4 | 3 | 1 |
| C2KLIP-SLANG | | GAFF | | | |
| C2KLIP-SLANG | | MSAL | Alien | | |
| C2KLIP-SLANG | CCAR | | | | |

The fish species that were sampled during the June 2013 sampling survey on the Klip River is provided in Table 45 and comprised of ten species of the possible twelve on the expected list. This included all three of the alien fish species, i.e. *Gambusia affinis*, *Micropterus salmoides* and *Cyprinus carpio*. The most fish activity was seen at site KR5 where *C. carpio* was observed surfacing but none of these individuals were sampled in the nets or with electroshocking. The most fish species were sampled at site KR3 and site KR4 with five and six species being sampled respectively. In general, the abundance of fish species was low at all of the sites. When comparing these results with the June 2013 survey it is evident that the same amount of species were sampled but that the abundances of especially the *Labeobarbus aeneus* were higher during the current survey.

Table 44: Fish sampled during the June 2013 survey on the Klip River and Riet Spruit

| | KR2 | KR3 | KR4 | KR5 | RS2 |
|---|---|---|---|---|---|
| *Gambusia affinis* | 2 | | | > 100 | |
| *Clarias gariepinus* | 1 | | 22 | | |
| *Barbus paludinosus* | 1 | | | | |
| *Barbus anoplus* | 12 | 1 | | | |
| *Pseudocrenilabrus philander* | 3 | | | | 4. |
| *Labeo capensis* | | 2 | | | |
| *Labeobarbus aeneus* | | 2 | | | |
| *Tilapia sparrmanii* | | 8 | | | |
| *Labeo umbratus* | | | 2 | | |
| *Cyprinus carpio** | | | | 5 | |
| *Micopterus salmoides* | | | | | 1 |

* Visual identification

Table 45: Fish sampled during the January 2014 survey on the Klip River and Riet Spruit

| | KR2 | KR3 | KR4 | KR5 | RS2 |
|---|---|---|---|---|---|
| *Gambusia affinis* | 4 | - | - | > 50 | 2 |
| *Clarias gariepinus* | 2 | 2 | 4 | - | 10 |
| *Barbus paludinosus* | - | - | 11 | - | - |
| *Barbus anoplus* | - | - | - | - | 14 |
| *Pseudocrenilabrus philander* | 9 | 3 | - | - | 4 |
| *Labeo capensis* | - | 2 | 1 | 1 | - |
| *Labeobarbus aeneus* | - | 2 | 26 | - | 5 |
| *Tilapia sparrmanii* | - | 8 | 1 | - | 1 |
| *Labeo umbratus* | - | - | - | 1 | - |
| *Cyprinus carpio** | - | - | - | 5 | - |
| *Micopterus salmoides* | 5 | - | - | - | 1 |

* Visual identification

When the fish diversity and abundance is compared to the Kotze (2002) study it is evident that the diversity and abundance have dramatically decreased from the work completed by Kotze (2002). This decrease in abundance could possibly be due to the impacts experienced on the Klip River. As the reference fish species are generally hardy the indication of impacts are more than likely to be reflected in the abundance rather than the diversity.

The FRAI assessment results (Table 46) indicated that the majority of the sampled reaches in the Klip River and Riet Spruit are largely modified (Category D). The exception is the upper reach in the Klip River where the ecological category was found to be in a seriously modified condition (Category E). These results are mainly due to the absence and less frequent occurrence of especially the *Labeo spp*. and yellowfish species (*Labeobarbus aeneus*) in the upper reaches of the Klip River. However, during the sampling surveys at all of the sites the only species that were not sampled was *Austroglanis sclateri* and *Labeobarbus kimberleyensis (*Largemouth Yellowfish). It is expected that flow modifications and water quality conditions have possibly resulted in these species not being present in the Klip River anymore.

Table 46: Fish Response Assessment Index (FRAI) for the various sites in the study area for the combined June 2013 and January 2014 fish surveys.

| Sites / Reaches | **FRAI score (%)** | **Ecological category** | **PESEIS Category** |
|---|---|---|---|
| Sites KR1 / KR2 | 36.5 | **E** | **E** |
| Site KR3 | 43.7 | **D** | **D** |
| Sites KR4 / KR5 | 48.2 | **D** | **D** |
| Riet Spruit | 48.1 | **D** | **D** |

### 5.2 Fish Bioaccumulation

The results of the fish bioaccumulation results in the muscle tissue of *Clarias gariepinus* are presented in Figure 40 and Figure 41 for the June 2013 survey. The results for site KR2 are only based on one individual while site KR4 is based on 20 individuals of *Clarias gariepinus*. The results in Figure 40 indicate that the aluminium, cobalt, copper and iron concentrations between KR2 and KR4 were similar for the June 2013 survey. The arsenic concentrations indicated that there was an increase from site KR2 to site KR4. The chromium concentrations indicated that the concentration decreased from site KR2 to site KR4.

The cobalt concentrations were also measured in the *C. gariepinus* tissue in the Ferreira et al. (2010) survey on the Klip River. Those results indicated that the current survey had slightly higher cobalt concentrations at both sites sampled during the survey. The chromium concentration at site KR4 was lower during the current survey when compared to the concentrations measured during the Ferreira et al. (2010) survey. These concentrations are lower than concentrations reported in the Olifants River by Coetzee et al. (2002), where the chromium concentrations ranged from 11-56 µg/g in *C. gariepinus* and *Labeo umbratus*.

The copper concentrations measured during the current study were higher than the concentrations measured during the 2010 survey by Ferreira et al. (2010). The concentrations at site KR4 was however lower than the copper concentrations reported by Kotze (2001) at the same site on the Klip River. Coetzee et al. (2002) found average concentrations of copper in muscle tissue to range between 2-19 μg/g for *C. gariepinus* and *L. umbratus*.

The uranium, vanadium and molybdenum concentrations in the muscle tissue in Figure 41 were generally similar between the two sites on the Klip River. No data from previous surveys on the Klip River were available for comparison. The selenium concentration showed an increase from site KR2 to site KR4 for the current survey. No previous surveys on the Klip River had measured selenium concentrations in fish muscle tissue. Internationally, selenium has been known to cause adverse effects in aquatic ecosystems as well as in human consumption when the concentrations are high. This has especially occurred in areas of gold mining as is the case in the Klip River system.

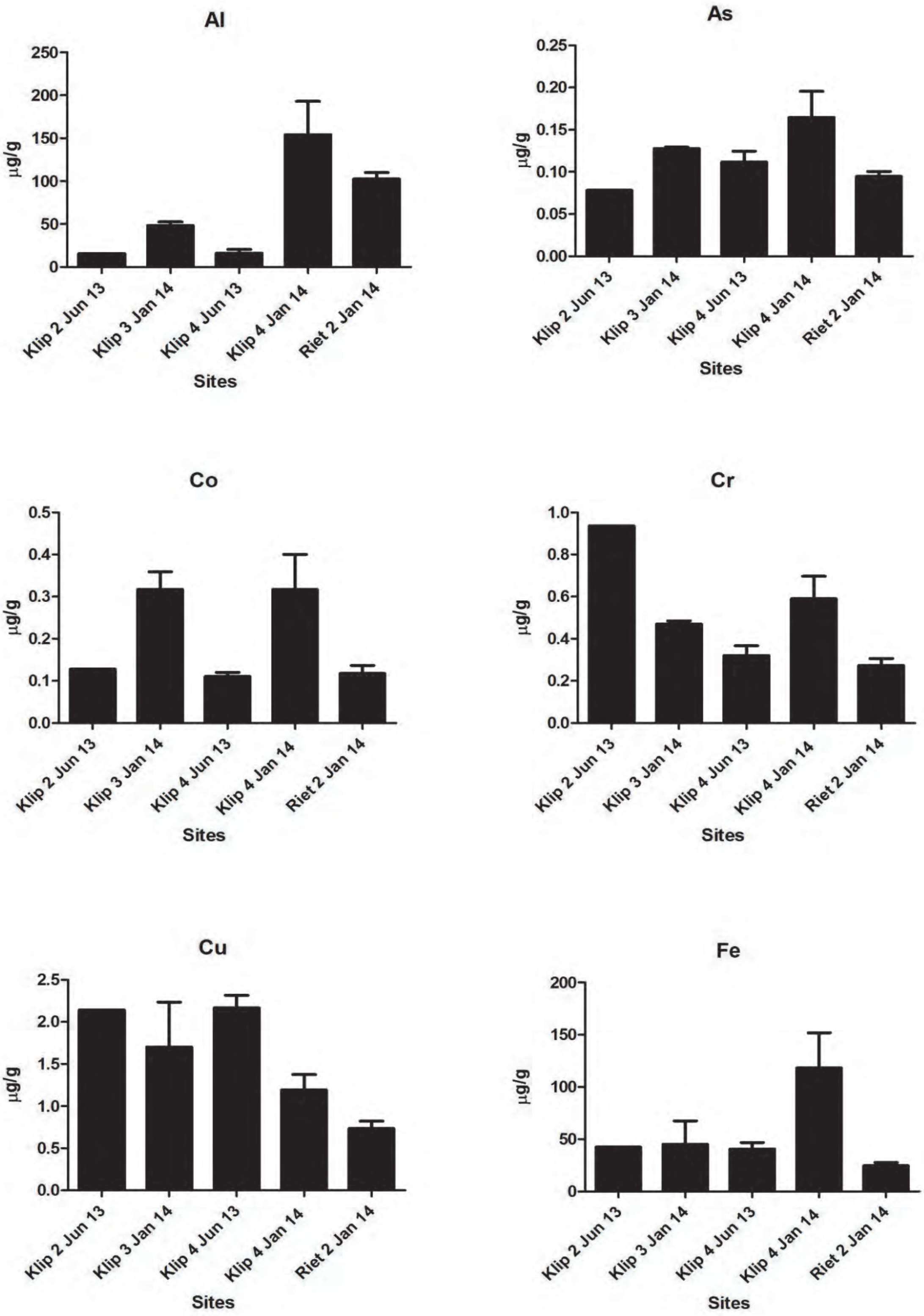


Figure 40: Selected metal concentrations in *Clarias gariepinus* muscle tissue for site KR2, KR4 on the Klip River fand RS2 on the Riet Spruit during the June 2013 and January 2014 sampling surveys.

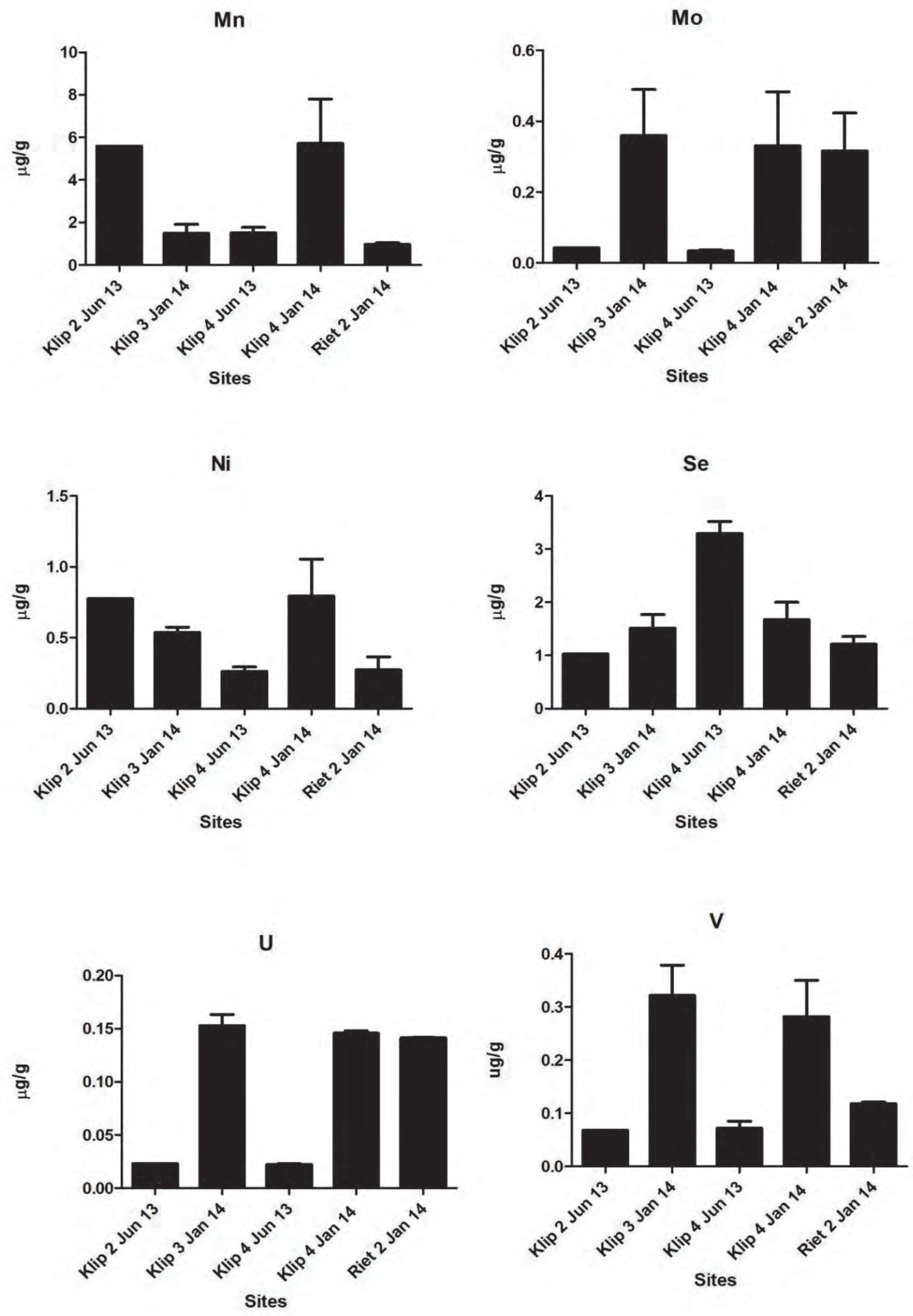


Figure 41: Selected metal concentrations in *Clarias gariepinus* muscle tissue for site KR2, KR4 on the Klip River fand RS2 on the Riet Spruit during the June 2013 and January 2014 sampling surveys.

The nickel and manganese concentrations measured in the fish muscle tissue indicated decreased concentrations from site KR2 to site KR4 for the current survey. The Ferreira et al. (2010) and the Kotze (2001) studies indicated lower concentrations for manganese in the fish tissue of *C. gariepinus* than measured during the current survey. The previous studies indicated around 1 µg/g while the current survey was between 2 and 6 µg/g. The manganese concentrations from Coetzee et al. (2002) ranged from 2-9 µg/g for the fish tissue from the Olifants River. No nickel concentrations were determined in the previous surveys on the Klip River for comparison.

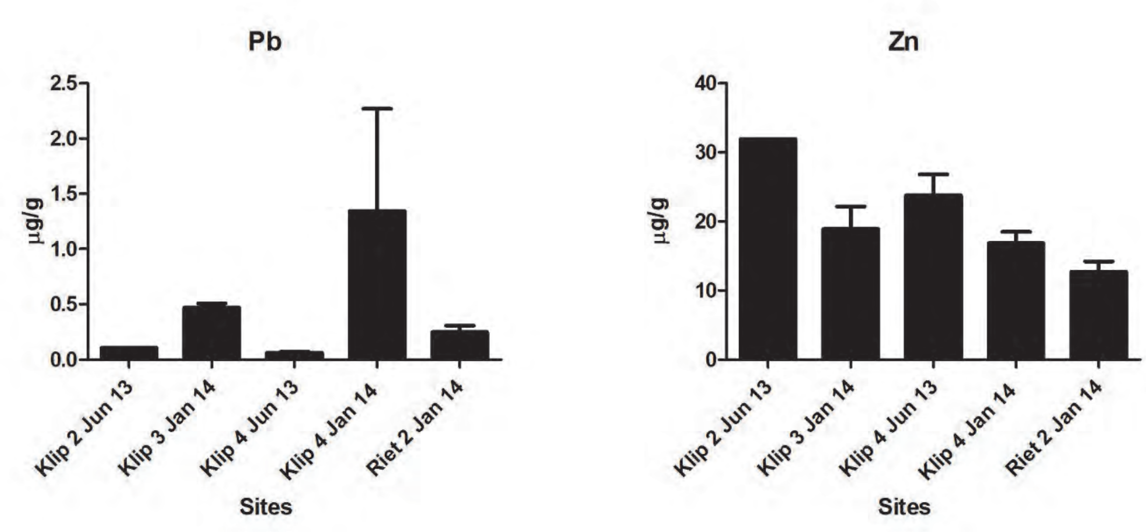


Figure 42: Selected metal concentrations in *Clarias gariepinus* muscle tissue for site KR2, KR4 on the Klip River fand RS2 on the Riet Spruit during the June 2013 and January 2014 sampling surveys.

The lead and zinc concentrations in Figure 42 indicated that these concentrations decreased from site KR2 to site KR4 for the June 2013 survey. No lead concentrations were available for comparison. The zinc concentrations indicated that higher concentrations were measured during the current survey when compared to the Ferreira et al. (2010) survey. The current survey results were similar to the Kotze (2001) survey, which reported zinc concentrations of 20 µg/g and 40 µg/g at site KR2 and site KR4. The current results are similar to the 29-73 µg/g reported in the muscle tissue of *C. gariepinus* in the Olifants River (Coetzee et al., 2002).

Organochlorine contaminants were analysed in the muscle tissue of *C. gariepinus* for selected sites on the Klip River (Table 47). Overall the levels of hexachlorobenzenes (HCBs) and chlordanes were higher in fish from the Klip River, whereas the other OCPs were higher in the Riet Spruit. Dieldrin levels in the Riet Spruit were similar to those recorded in tigerfish from the Luvuvhu River (Smit et al., 2014) but higher than the tigerfish levels from Lake Pongolapoort (Wepener et al., 2012). All OCP levels with the exception of hexachlorocyclohexanes (HCHs) were lower than those measured in *Labeo capensis* from the Vaal River (Wepener et al., 2011). No *o,p'*-DDT levels were measured in any of the fish samples indicating only historic levels remaining in the environment. However of concern are the high levels of γ-HCH (lindane) that were measured in the Riet Spruit.

Table 47: Mean ± standard error of organochlorine pesticides (ng/g lipid) in the fish muscle tissue of *Clarias gariepinus* from selected sites in the Klip River for June 2013 and January 2013. Level of detection and below detection levels are represented by LOD and ND respectively.

| Compound | LOD (ng/g) | Riet Spruit Jan 13 (n=6) | Klip River Jan 13 (n=3) | Klip River Jun 13 (n=10) |
|---|---|---|---|---|
| | 4 | ND | 8.4 ± 7.9 | 11.6 ± 2.4 |
| HCB | | | | |
| | 4 | 20.5 ± 13.6 | 19.9 ± 12.2 | 6.5 ± 1.2 |
| *o,p'*-DDD | 4 | 44.3 ± 18.7 | 9.5 ± 9.4 | 14.5 ± 3.2 |
| *p,p'*-DDD | 4 | ND | ND | ND |
| *o,p'*-DDE | 4 | 409.9 ± 115.2 | 120.2 ± 24.3 | 68.2 ± 11.7 |
| *p,p'*-DDE | 4 | 7.9 ± 7.1 | ND | ND |
| *o,p'*-DDT | 4 | ND | ND | ND |
| *p,p'*-DDT | 4 | 482.5 ± 154.5 | 149.6 ± 45.9 | 89.2 ± 16.1 |
| **ΣDDTs** | | | | |
| | | | | |
| OxC | 2 | 29.5 ± 26.1 | ND | ND |
| TN | 2 | 51.3 ± 31.3 | ND | 9.5 ± 2.4 |
| Trans CHL | 2 | ND | 68.3 ± 42.6 | 38.2 ± 4.6 |
| ΣCHLs | 4 | 3.49 ± 1.2 | 68.3 ± 42.6 | 47.7 ± 7 |
| | | | | |
| α-HCH | 2 | ND | 4.2 ± 1.9 | 3.4 ± 0.8 |
| β-HCH | 2 | ND | ND | 9.3 ± 3.7 |
| γ-HCH | 2 | 542.6 ± 271.3 | 55.9 ± 34.2 | 5.5 ± 1.9 |
| ΣHCHs | 4 | 542.6 ± 271.3 | 60.1 ± 36.1 | 18.2 ± 6.4 |
| | | | | |
| Dieldrin | 2 | 247.6 ± 72.8 | 102.0 ± 24.3 | 16.6 ± 2.1 |
| Endrin | 2 | 0.4 ± 0.26 | ND | ND |

## 5.3 Fish Health Assessment

The January 2014 sampling for the fish health assessment resulted in *Clarias gariepinus* at four sites, i.e. KR2, KR3, KR4 and RS2. The abundance of fish at sites KR2 and KR3 were low with only two individuals sampled. The sampling at site KR4 resulted in six individuals and at site RS2 resulted in ten individuals. The June 2013 sampling survey results were based on one individual at site KR2 and 20 individuals at site KR4. The fish health assessment results for the surveys are provided in Tables 47 to Table 55. A study by Ferreira et al. (2010) also completed the Fish Health Assessment on *C. gariepinus* at three sites on the Klip River of which two sites corresponds to the sites in this study. At site KR2 the condition factor (CF = 1.09) during the previous survey in June 2013 was similar to the average condition factor (CF = 0.96) from the previous study (Ferreira et al., 2010). However, previously some alterations in the skin and liver were seen that was not present during this time. Unfortunately, no large individuals of *C. gariepinus* were caught at the site. There is some anecdotal evidence of illegal netting at the site which could have resulted in the decreased population at the site compared to the Ferreira et al. (2010) study. The fish from site KR3 that were sampled during the January 2014 survey indicated a condition factor of 0.8 which is lower than measured at site KR2.

The fish sampling at site KR4 resulted in six *C. gariepinus* available to complete the fish health assessment on. The January 2014 sex ratio of the fish was 66% female and 34% male while the average condition factor was calculated at 0.98 with a standard deviation of 0.24. The June 2013 sex ratio of the fish was 55% female and 45% male while the average condition factor was calculated at 1.07 with a standard deviation of 0.12. This studies results are lower than the average condition factor of 1.12 (SD = 0.18) from Ferreira et al. (2010) while the sex ratio was 70% females during that study. Externally, the eyes, skin, fins, opercula and gills were mostly normal with some frayed gills. Internally, the hindgut, kidney and spleen were mostly normal while 90% of the individuals had 50% or less mesenteric fat present. The June 2013 liver results indicated that 50% was normal, 40% showed discolouration, 5% showed focal discolouration and one individual had a nodular liver. Five individuals showed signs that trematode cysts were present. The January 2014 liver results indicated mostly normal results with some focal discolouration present. The liver results were slightly different to the Ferreira et al. (2010) study as their 40% of the individuals had alterations to the liver tissue. Overall, the fish health at site KR4 indicated that the fish are in a good condition with minimal alterations in the organs.

Table 48: Health Assessment Index (HAI) values of site KR3 from the January 2014 survey.

| Health Assessment Index (HAI) Variables | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish # | Sex | Eyes | Skin | Fins | Opercula | Gills | Bile | Messenteric Fat | Liver | Spleen | Hindgut | Kidney | Parasites | Comments |
| CGAR 1 | Female | Normal | Normal | Normal | Normal | Normal | D Straw | 50% | Normal | Normal | Normal | Normal | No | - |
| CGAR 2 | Femail | Normal | Normal | Normal | Normal | Normal | L Straw | none | Normal | Normal | Normal | Normal | No | - |

Table 49: Body mass, body length and organ masses from *Clarias gariepinus* at site KR3 from the January 2014 survey.

| | | | Body Mass | | Body lengths | | Liver Mass | Spleen Mass | Gonad mass | Condition Factor |
|---|---|---|---|---|---|---|---|---|---|---|
| Date | Fish # | Sex | Total (g) | Gutted (g) | Total (mm) | STD (mm) | g | g | Total | |
| 2014/02/30 | CGAR 1 | Male | 700 | 480 | 460 | 428 | 2 | 1 | < 1 | 0.89 |
| 2014/02/30 | CGAR 2 | Female | 34 | 300 | 190 | 165 | - | - | - | 0.76 |
| | | **AVERAGE** | 367 | 390 | 325 | 296.5 | 2 | 1 | - | 0.82 |
| | | **STDEVA** | 470.93 | 127.28 | 190.92 | 185.97 | - | - | - | 0.096 |

Table 50: Health Assessment Index (HAI) values of site KR4 from the January 2014 survey.

| Health Assessment Index (HAI) Variables | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish # | Sex | Eyes | Skin | Fins | Opercula | Gills | Bile | Messenteric Fat | Liver | Spleen | Hindgut | Kidney | Parasites | Comments |
| CGAR 1 | Female | Normal | Normal | Normal | Normal | Normal | L Straw | < 50% | Normal | Normal | Inflamed | Normal | No | - |
| CGAR 2 | Male | Normal | Normal | Normal | Normal | Pale | L Straw | > 50% | Fatty | Normal | Normal | Normal | No | - |
| CGAR 3 | Female | Normal | Normal | Normal | Normal | Normal | L Straw | < 50% | Normal | Normal | Normal | Normal | No | - |
| CGAR 4 | Male | Normal | Normal | Normal | Normal | Normal | D Straw | < 50% | Discoloured | Normal | Normal | Normal | No | - |
| CGAR 5 | Female | Normal | Normal | Normal | Normal | Normal | L Straw | < 50% | Normal | Normal | Normal | Normal | No | - |
| CGAR 6 | Female | Normal | Normal | Normal | Normal | Normal | L Straw | < 50% | Normal | Normal | Normal | Normal | No | - |

Table 51: Body mass, body length and organ masses from *Clarias gariepinus* at site KR4 from the January 2014 survey.

| Date | Fish # | Sex | Body Mass | | Body lengths | | Liver Mass | Spleen Mass | Gonad mass (g) | Condition Factor |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total (g) | Gutted (g) | Total (mm) | STD (mm) | g | g | Total | |
| 2014/01/27 | CGAR 1 | Female | 3060 | 2520 | 670 | 610 | 30 | 2 | 234 | 1.35 |
| 2014/01/27 | CGAR 2 | Male | 3240 | 2800 | 730 | 660 | 24 | 4 | < 1 | 1.13 |
| 2014/01/27 | CGAR 3 | Female | 4760 | 4000 | 830 | 760 | 32 | 6 | 366 | 1.08 |
| 2014/01/27 | CGAR 4 | Male | 760 | 680 | 535 | 470 | 4 | < 1 | 2 | 0.73 |
| 2014/01/27 | CGAR 5 | Female | 1440 | 1320 | 605 | 560 | 22 | 1 | 94 | 0.82 |
| 2014/01/27 | CGAR 6 | Female | 460 | 400 | 425 | 390 | 4 | < 1 | 13 | 0.78 |
| | | **AVERAGE** | 2286.67 | 1953.33 | 632.50 | 575.00 | 19.33 | 3.25 | 141.80 | 0.98 |
| | | **STDEVA** | 1673.83 | 1390.12 | 143.69 | 132.78 | 12.44 | 2.22 | 155.87 | 0.24 |

Table 52: Health Assessment Index (HAI) values of site RS2 from the January 2014 survey.

| Health Assessment Index (HAI) Variables | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish # | Sex | Eyes | Skin | Fins | Opercula | Gills | Bile | Messenteric Fat | Liver | Spleen | Hindgut | Kidney | Parasites | Comments |
| CGAR 1 | Female | Normal | Normal | Normal | Normal | Discoloured | D Straw | < 50% | Discoloured | Normal | Normal | Normal | No | - |
| CGAR 2 | Male | Normal | Normal | Normal | Normal | Normal | D Straw | < 50% | Normal | Normal | Normal | Normal | No | - |
| CGAR 3 | Male | Normal | Normal | Normal | Normal | Normal | L Straw | < 50% | Nodular | Normal | Normal | Normal | No | - |
| CGAR 4 | Female | Normal | Normal | Normal | Normal | Normal | D Straw | < 50% | Normal | Normal | Normal | Normal | No | - |
| CGAR 5 | Female | Normal | Normal | Normal | Normal | Normal | D Straw | 50% | Normal | Normal | Normal | Normal | No | - |
| CGAR 6 | Male | Normal | Normal | Normal | Normal | Normal | D Straw | < 50% | Normal | Normal | Normal | Normal | No | - |
| CGAR 7 | Female | Normal | Normal | Normal | Normal | Normal | D Straw | < 50% | Discoloured | Normal | Normal | Normal | No | - |
| CGAR 8 | Male | Normal | Normal | Normal | Normal | Normal | D Straw | < 50% | Normal | Normal | Normal | Normal | No | - |
| CGAR 9 | Female | Normal | Normal | Normal | Normal | Normal | L Straw | < 50% | Normal | Normal | Normal | Normal | No | - |
| CGAR 10 | Male | Normal | Normal | Normal | Normal | Normal | L Straw | < 50% | Normal | Normal | Normal | Normal | No | - |

Table 53: Body mass, body length and organ masses from *Clarias gariepinus* at site RS2 from the January 2014 survey.

| Date | Fish # | Sex | Body Mass | | Body lengths | | Liver Mass | Spleen Mass | Gonad mass (g) | Condition Factor |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total (g) | Gutted (g) | Total (mm) | STD (mm) | g | g | Total | |
| 2014/01/31 | CGAR 1 | Female | 1580 | 1400 | 625 | 560 | 14 | 2 | 114 | 0.90 |
| 2014/01/31 | CGAR 2 | Male | 920 | 860 | 520 | 460 | 8 | 4 | 1 | 0.95 |
| 2014/01/31 | CGAR 3 | Male | 1880 | 1760 | 685 | 625 | 10 | 2 | 2 | 0.77 |
| 2014/01/31 | CGAR 4 | Female | 1500 | 1380 | 620 | 560 | 8 | 2 | 116 | 0.85 |
| 2014/01/31 | CGAR 5 | Female | 1520 | 1420 | 610 | 555 | 14 | 2 | 2 | 0.89 |
| 2014/01/31 | CGAR 6 | Male | 3200 | 2780 | 795 | 720 | 16 | 4 | 6 | 0.86 |
| 2014/01/31 | CGAR 7 | Female | 1320 | 1140 | 625 | 570 | 6 | 1 | 104 | 0.71 |
| 2014/01/31 | CGAR 8 | Male | 1720 | 1360 | 640 | 575 | 8 | 2 | 8 | 0.90 |
| 2014/01/31 | CGAR 9 | Female | 980 | 880 | 555 | 550 | 8 | 2 | 46 | 0.59 |
| 2014/01/31 | CGAR 10 | Male | 420 | 380 | 440 | 405 | 2 | < 1 | < 1 | 0.63 |
| | | **AVERAGE** | 1504.00 | 1336.00 | 611.50 | 558.00 | 9.40 | 2.33 | 44.33 | 0.81 |
| | | **STDEVA** | 737.49 | 638.18 | 95.05 | 84.50 | 4.22 | 1.00 | 52.21 | 0.12 |

Table 54: Body mass, body length and organ masses from *Clarias gariepinus* at site KR2 from the June 2013 survey.

| | | | Body Mass | | Body lengths | | Liver Mass | Spleen Mass | Gonad mass | Condition Factor |
|---|---|---|---|---|---|---|---|---|---|---|
| Date | Fish # | Sex | Total (g) | Gutted (g) | Total (mm) | STD (mm) | g | g | Total | |
| 2013/06/03 | CGAR 1 | Female | 1360 | 1320 | 550 | 500 | 21 | 3 | 15 | 1.09 |

Table 55: Body mass, body length and organ masses from *Clarias gariepinus* at site KR4 from the June 2013 survey.

| Date | Fish # | Sex | Body Mass | | Body lengths | | Liver Mass | Spleen Mass | Gonad mass (g) | Condition Factor |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total (g) | Gutted (g) | Total (mm) | STD (mm) | g | g | Total | |
| 2013/06/06 | CGAR 1 | Female | 2260 | 2200 | 685 | 601 | 27 | 5.000 | 18 | 1.04 |
| 2013/06/06 | CGAR 2 | Male | 2100 | 1980 | 665 | 600 | 43 | 4.000 | 5 | 0.97 |
| 2013/06/06 | CGAR 3 | Male | 1980 | 1820 | 681 | 598 | 22 | 4.000 | 4 | 0.93 |
| 2013/06/06 | CGAR 4 | Male | 2240 | 1683 | 611 | 558 | 21 | 4.000 | 3 | 1.29 |
| 2013/06/06 | CGAR 5 | Female | 1920 | 1760 | 626 | 571 | 27 | 2.000 | 15 | 1.03 |
| 2013/06/06 | CGAR 6 | Female | 1440 | 1340 | 571 | 505 | 14 | 3.000 | 19 | 1.12 |
| 2013/06/06 | CGAR 7 | Female | 2880 | 2620 | 716 | 630 | 38 | 6.000 | 26 | 1.15 |
| 2013/06/06 | CGAR 8 | Male | 1440 | 1280 | 539 | 485 | 28 | 1.000 | < 1 | 1.26 |
| 2013/06/06 | CGAR 9 | Male | 1460 | 1320 | 591 | 535 | 16 | 2.000 | 2 | 0.95 |
| 2013/06/06 | CGAR 10 | Female | 2040 | 1840 | 641 | 575 | 25 | 5.000 | 13 | 1.07 |
| 2013/06/06 | CGAR 11 | Male | 2340 | 2180 | 685 | 605 | 27 | 6.000 | 4 | 1.06 |
| 2013/06/06 | CGAR 12 | Male | 2240 | 2040 | 675 | 621 | 38 | 3.000 | 7 | 0.94 |
| 2013/06/06 | CGAR 13 | Male | 2660 | 2420 | 725 | 659 | 32 | 4.000 | 5 | 0.93 |
| 2013/06/06 | CGAR 14 | Female | 2300 | 2020 | 656 | 595 | 35 | 4.000 | 28 | 1.09 |
| 2013/06/06 | CGAR 15 | Female | 1700 | 1580 | 607 | 549 | 18 | 2.000 | 19 | 1.03 |
| 2013/06/06 | CGAR 16 | Female | 2040 | 1920 | 641 | 587 | 24 | 2.000 | < 1 | 1.01 |
| 2013/06/06 | CGAR 17 | Female | 1520 | 1420 | 585 | 532 | 16 | 3.000 | 7 | 1.01 |
| 2013/06/06 | CGAR 18 | Female | 1700 | 1520 | 595 | 531 | 30 | 3.000 | 16 | 1.14 |
| 2013/06/06 | CGAR 19 | Female | 1540 | 1420 | 541 | 482 | 28 | 3.000 | 7 | 1.38 |
| 2013/06/06 | CGAR 20 | Male | 1820 | 1660 | 625 | 558 | 32 | 4.000 | 3 | 1.05 |
| | | **AVERAGE** | **1981** | **1801.15** | **633.05** | **568.85** | **27.05** | **3.5** | **11.166667** | 1.07 |
| | | **STDEVA** | **409.3885** | **375.490034** | **53.52763674** | **47.582919** | **7.910519312** | **1.357241785** | **8.5438497** | 0.12 |

A different fish health assessment was used in the study by Kotze (2002) with the aim to determine external fish health. This indicate a range of health related impacts on fish sampled during the study that included abnormal opercula, gill damage, severe active fin erosion, skin damage, cysts and skin necrosis on *C. gariepinus* and *L. capensis*. However, no serious fish health issues as mentioned by Kotze (2002) were seen in the present study.

The results of site RS2 in Table 53 indicated that a 50:50 ratio of females to males were caught during January 2014. The average condition factor was measured at 0.81 (SD = 0.12) which is again lower than values measured during Ferreira et al. (2010) in the Klip River. The external results at site RS2 indicated that the eyes, skin, fins, opercula and gills were mostly normal. The internal examination indicated that the liver was predominantly normal with some discolouration present. The hindgut, kidney and spleen were also normal. The mesenteric fat percentage for these individuals was mostly less than 50%. No parasites were visible either externally or internally. Overall, the fish health at site RS2 indicated that the fish are in a good condition with minimal alterations in the organs. Unfortunately, no data from the Riet Spruit were available for comparison. However, if compared to the Ferreira et al. (2010) survey on the Klip River, the fish health scores appear to be similar to the current survey on the Riet Spruit.

## 5.4 Fish Biomarkers

The results of the selected biomarker analyses on the *C. gariepinus* are presented in Figures 43 and 44 for the June 2013 and January 2014 sampling survey. Each of these biomarkers responds differently when exposure of pollutants has occurred. Therefore, Table 56 provides a short summary of the expected response of the various biomarkers that were used in this study. Not very many biomarker studies have been completed in the Klip River and therefore comparison is difficult. The study on the Klip River by Ferreira et al. (2010) also completed biomarker analysis on *C. gariepinus* but all of those results were found to be significantly higher than the present study making comparison problematic.

Table 56: Summary of the diagnostic nature of the biomarker responses and their interpretation [modified from van der Oost et al. (2003) and Wepener et al. (2011)].

| **Biomarker** | **Increase / Decrease** | **Exposure or effect interpretation** |
|---|---|---|
| Acetylcholinesterase | Decrease | Inhibition due to pesticide exposure |
| Metallothionein | Increase | Stimulation in response to metal exposure |
| GSH | Increase | Detoxification enzyme |
| SOD | Increase | Produced in response to ROS formation |
| Catalase | Increase | Produced in response to ROS formation |
| Malondialdehyde | Increase | Indicative of liver peroxidation due to ROS |
| Cytochrome P450 | Increase | Stimulation in the presence of organochlorine compounds |
| Protein carbonyls | Increase | Damage to proteins due to ROS |
| Cellular energy allocation | Increase / Decrease | Decrease due to stress compensation requiring additional energy sources. Increases associated with additional energy sources. |

The biomarker results for site KR2 is a representation of the one individual sampled during the June 2013 survey. The biomarker results for acetylcholinesterase (AChE) in Figure 43 indicate similar concentrations at all the sites except for site KR3 during January 2014. Unfortunately, the results at site KR3 are based on only two individuals of *C. gariepinus* which could explain the increased concentrations. The catalase activity indicated the highest concentrations at site KR3 as was seen for the AChE. The catalase activity at site KR4 was also measured higher in January 2014 than compared to June 2013.

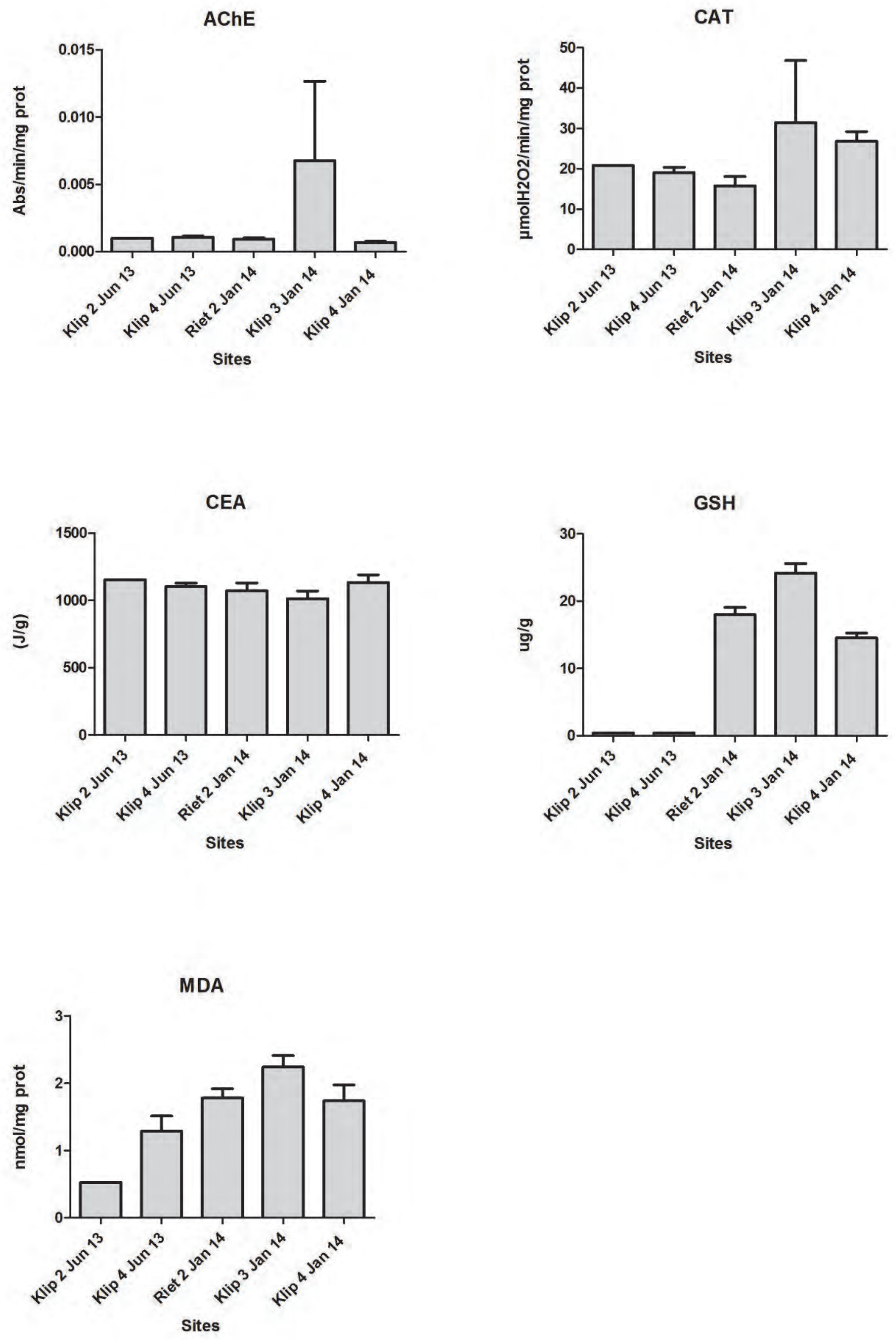


Figure 43: Biomarker results from *Clarias gariepinus* at sampled sites on the Klip River and Riet Spruit for the June 2013 and January 2014 sampling survey.

The cellular energy allocation (CEA) biomarker looks at how energy is used within an organism. It is calculated by determining the energy consumption and energy available for consumption. Similar concentrations were seen for all of the sampled sites during the two surveys in 2013 and 2014 (Figure 43). The glutathione (GSH) biomarker responds to pollutant exposure by increasing as it is an enzyme that is responsible for detoxification in the liver. The GSH results in Figure 43 indicated significantly higher concentrations were measured during the January 2014 survey as compared to the June 2014 survey. The concentration at site KR3 during January 2014 was the highest. The concentrations measured at site RS2 and KR4 were similar to concentrations of the biomarker at site KR3 while the concentrations at site RS2 and site KR4 were similar during January 2014 while the June 2013 results were slightly lower. However, the results seem to indicate that MDA increased at site KR4 from June 2013 to January 2014. Increases in MDA are generally as a result of ROS species present within the system or site. ROS results in lipid peroxidation in the fish that results in increased MDA concentrations.

The protein carbonyls indicated lower concentrations at all of the sites during January 2014 (Figure 44). The concentrations measured during each of the surveys appeared similar across the various sites. Increases in catalase can point to response due to the formation of reactive oxygen species (ROS) in the tissue. Another biomarker that responds to oxidative stress and the formation of ROS in tissue is superoxide dismutase (SOD), which generally increases in concentration when ROS is present. In Figure 44 the results of the SOD indicates that the highest concentration were measured at site RS2 while the concentrations at all of the other sites were similar. Concentrations at site KR4 during June 2013 and January 2014 were similar although a larger variation in concentration was noted during January 2014.

Another biomarker that was used in this study is metallothioniens which responds in the presence of metal pollution by increasing in concentration. In Figure 44 it is evident that the concentration of metallothioniens was the highest at site KR3 during 2014 and KR4 during 2013. The concentration at site KR4 during January 2014 was slightly lower than during the June 2013 survey. Metal pollution is potentially a major pollutant in the system due to all of the mining and industries present in the catchment. A range of metal concentrations are expected to be present at higher than background levels in the system. The metal analysis in the water, sediment and fish tissue will be able to indicate whether metals are higher at site KR3 than at the other sites.

The cytochrome P450 enzyme generally increases in the presence of organochlorine pollutants. The results for this biomarker in Figure 44 indicated an increased concentrations during the January 2014 survey as compared to the June 2013 survey while concentrations were similar at all sites during the specific sampling survey. The oxidative stress biomarker MDA in Figure 43 also indicated higher levels during the 2014 surveys.

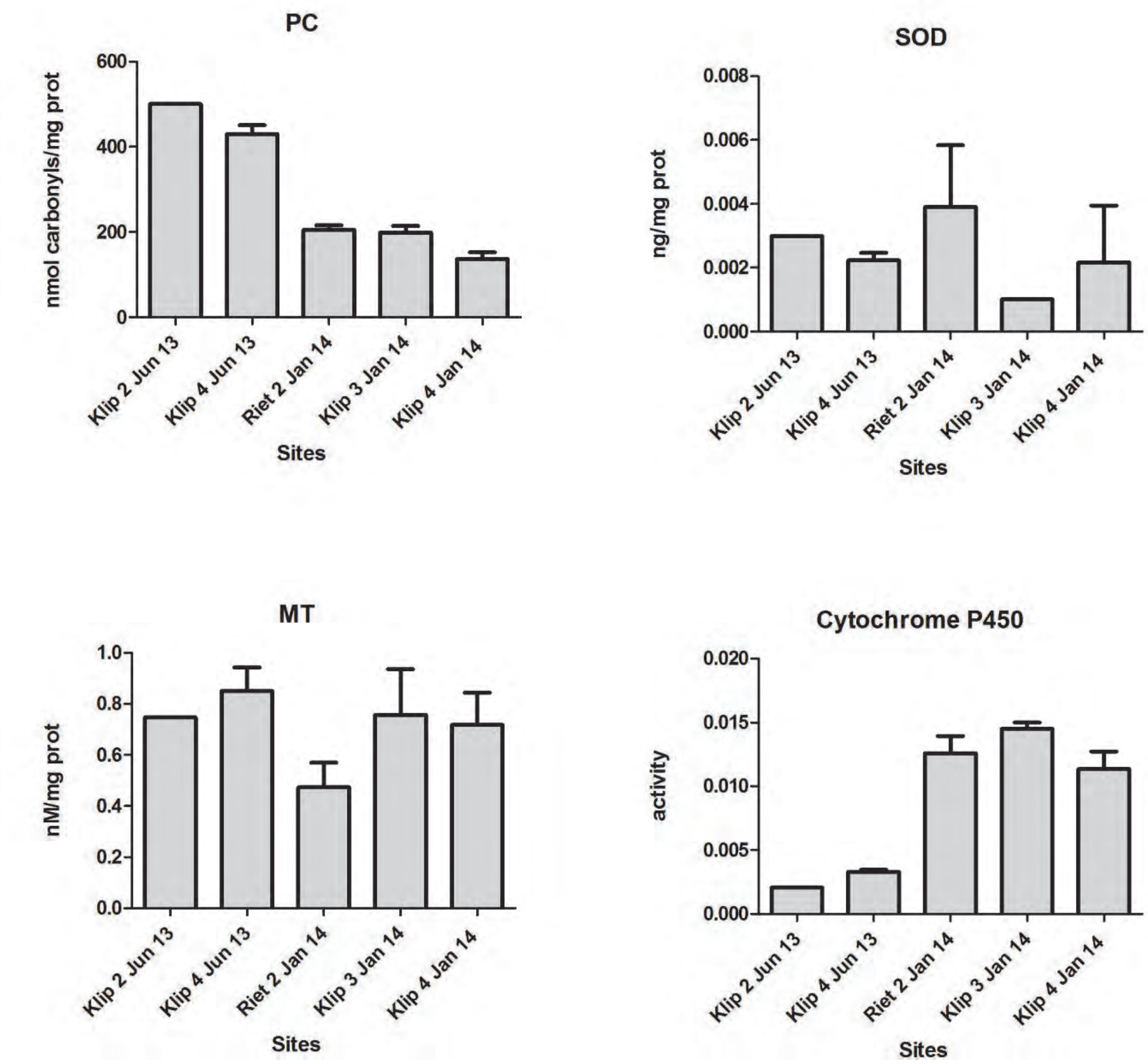


Figure 44: Selected biomarker results from *Clarias gariepinus* at two sites on the Klip River and Riet Spruit for the June 2013 sampling survey.

## 5.5 Population genetics in *Clarias gariepinus*

The genetic analysis of the cyt b gene [mitochondrial (mt) DNA] was performed in order to determine whether the fish from the Klip River have undergone genetic changes due to prolonged exposure to pollutants in the system. The *C. gariepinus* sequences of the specimens sampled from the Wonderfontein Spruit (n = 9) and Klip River/Riet Spruit (n = 10) were approximately 600 bp long. Also, cyt b sequences available on GenBank for North [Accession No. HQ70170(0-4).1] and East [Accession No. HQ7016(88-91). One, HQ7016(94-99).1] African *C. gariepinus* clades were used for comparative purposes. The Klip River and Riet Spruit had lower nucleotide diversity (Table 57) as illustrated by a higher degree of polymorphism when compared to the Wonderfontein Spruit and other African populations. There were no obvious differences in haplotype diversity observed in the populations.

Table 57: Measures of genetic variance in the *Clarias gariepinus* populations from the Klip River, Riet Spruit and Wonderfontein Spruit compared to the North and East African populations.

| | ***n*** | **Number of haplotypes** | **Haplotype diversity (*h*)** | **Nucleotide diversity ($\pi$)** |
|---|---|---|---|---|
| Klip River | 10 | 5 | 0.76 | 0.006 |
| Riet Spruit | 10 | 4 | 0.78 | 0.005 |
| Wonderfontein Spruit | 9 | 5 | 0.81 | 0.0035 |
| North Africa | 5 | 3 | 0.80 | 0.0077 |
| East Africa | 10 | 4 | 0.78 | 0.0035 |

The pairwise $F_{ST}$ fixation index (Table 58) was used to determine the genetic distances between the respective *C. gariepinus* populations. The significance of each comparison was calculated by following 16 000 permutations with a 95% confidence interval. The $F_{ST}$ analysis produced significant ($p < 0.05$) high values between the North and East African populations, as well as between the latter populations and South African populations, respectively. This indicated that most of the observed molecular variance was attributed to differentiation between and not within the populations. However, zero ($p > 0.05$) genetic distance was observed between the Riet Spruit, Wonderfontein Spruit and Klip River populations. Therefore, 100% of the observed molecular variance was within the three populations.

A haplotype (median-joining) network (Figure 45) was created to illustrate the genetic divergence (structure) between the different *C. gariepinus* populations. The network clearly illustrates that the North and East African populations (clades) were genetically diverged from each other, as well as from the South African systems' populations. In contrast, it was evident that most of the haplotypes from the Klip River clade occurred in both the Wonderfontein Spruit and Riet Spruit populations; only one haplotype was unique to the Wonderfontein Cave population.

Table 58**:** Pairwise $F_{ST}$ values indicating the genetic distances between the Wonderfontein Cave, Stoffels Dam, North and East African *Clarias gariepinus* populations. Significant ($p < 0.05$) $F_{ST}$ values are indicated with an asterisk (*).

| $F_{ST}$ | | (1) | (2) | (3) | (4) | (5) |
|---|---|---|---|---|---|---|
| Wonderfontein Spruit | (1) | - | | | | |
| Klip River | (2) | -0.039 | - | | | |
| Riet Spruit | (3) | 0.042 | 0.07 | - | | |
| East Africa | (4) | 0.96* | 0.97* | 0.96* | - | |
| North Africa | (5) | 0.95* | 0.95* | 0.83* | 0.097* | - |

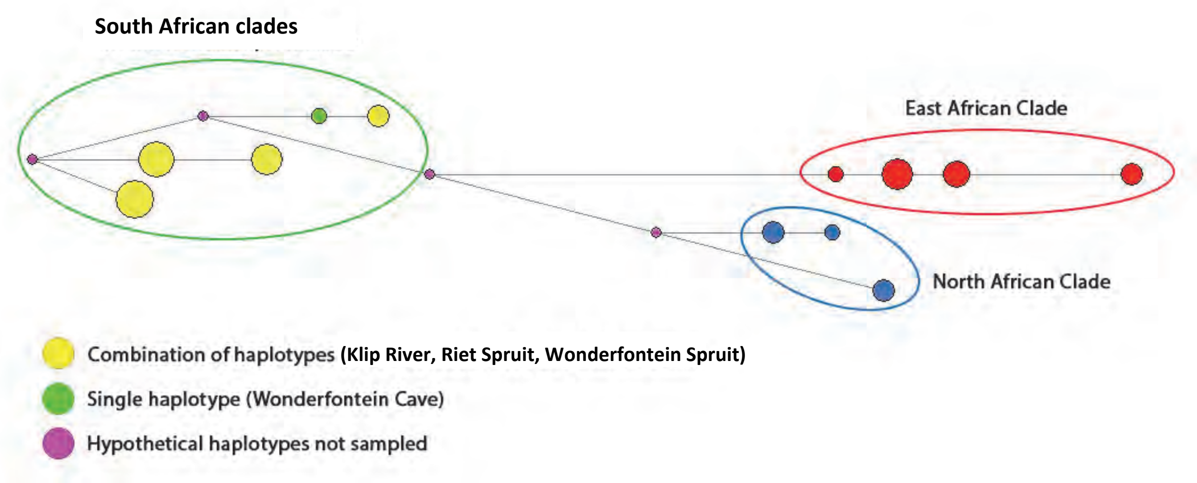


Figure 45: A haplotype (median-joining) network illustrating the genetic divergence (structure) between the North African, East African and Wonderfontein Spruit *Clarias gariepinus* clades. The size of the circles is proportional to the haplotype frequency.

An analysis of molecular variance (AMOVA; Table 59) was performed based on the genetic structure illustrated by Figure 45. For this analysis the South African populations were grouped together, while each of the North and East African populations were representative of its own group. For each comparison, a fixation index ($F_{CT}$, $F_{SC}$ and $F_{ST}$) was calculated to test the significance of the analysis. The AMOVA analysis revealed that no significant variance was observed among populations within groups ($F_{SC}$).

Table 59: Analysis of molecular variance (AMOVA) between the Wonderfontein Spruit (Wonderfontein Cave and Stoffels Dam populations), North African and East African *Clarias gariepinus* clades. Significant ($p < 0.05$) fixation index values are indicated with an asterisk (*).

| **Source of variation** | **Df** | **Sum of squares** | **Variance components** | **Percentage of variation** | **Fixation Index** |
|---|---|---|---|---|---|
| Among groups | 2 | 976.04 | 50.16 | **96** | $F_{CT} = 0.96$ |
| Among populations within groups | 1 | 1.44 | -0.07 | **0** | $F_{SC} = 0.0$ |
| Within populations | 29 | 61.75 | 2.12 | **4** | $F_{ST} = 0.96$* |
| Total | 32 | 1039.24 | 52.22 | | |

The study of population genetics has been widely applied to various fish species, including *C. gariepinus* (So et al., 2006; Nwafili and Gao, 2007; Roodt-Wilding et al., 2010; Sousa-Santos et al., 2014). The genetic distance ($F_{ST}$ and AMOVA) analyses revealed that there existed zero to minimal genetic divergence between the *C. gariepinus* populations associated with the Klip River, Riet Spruit and Wonderfontein Spruit. Also, the haplotype network analysis revealed that most of the identified *C. gariepinus* haplotypes from the Klip River clade were present within both the Riet Spruit and Wonderfontein Spruit populations. It is thus evident that the South African populations have not diverged and that gene flow is likely still possible.

# 6 INTEGRATION OF THE BIO-PHYSICAL DATA USING THE REGIONAL SCALE RISK ASSESSMENT PROCEDURE

The excessive use of water resources in South Africa is resulting in a continued deterioration in the wellbeing of aquatic ecosystems and a decline in the provision of key ecosystem services upon which the social and economic development of the country depends (Driver et al., 2005; MEA, 2005; Ashton, 2007). The National Water Act (NWA) (No. 36 of 1998) aims to ensure the sustainable use of water resources for the benefit of all South Africans. To achieve sustainability sufficient protection measures must be afforded to water resources to ensure that use is not excessive so that the wellbeing and availability of key ecosystem can be maintained. To achieve this, the NWA prescribes a number of protection measures for water resources including; the establishment of a societal vision to direct the level use and or protection of resources, the classification of water resources by establishing a Management Class to represent the vision, establish the Ecological Reserve that provides for the ecological requirements and then determine Resource Quality Objectives (RQO) for water resources, which gives effect to the Management Classes (DWA, 2011). The narrative and or numeric RQOs that relate to the quantity, quality, habitat and biota of water resources, establish clear goals for the desired quality of the resources to achieve a balance between the need to use water resources and protect them (DWA, 2011). Following the determination of RQOs for all relevant water resources, usually include rivers, wetlands, groundwater, dams (man-made lakes) and or estuaries, RQOs are gazetted and as a result become binding on all authorities and institutions (DWA, 2011). The Department of Water and Sanitation (DWS) is the custodian of the water resources in South Africa and it is their responsibility to implement established RQOs through Source Directed Control measures as prescribed by the NWA. To implement RQOs the DWS initially needs to characterise the risks to the achieving RQOs on multiple spatial scales, within the context of existing socio-ecological systems, and use a robust validated measure/s to evaluate the socio-economic and ecological consequences of alternative management options which can provide the information required to achieve RQOs using Source Directed Control measures, such as Water Licences.

The RQO determination procedures for the Upper Vaal Water Management Area (WMA) have recently been completed (DWS, 2014). The Upper Vaal RQOs includes numerous quantity, quality, habitat and biota subcomponent objectives for regional and prioritised river, groundwater, wetland and dam ecosystems. The Klip River which forms a part of the greater Vaal River WMA is one of South Africa's most economically valuable aquatic ecosystems, and with the Vaal River, one of South Africa's hardest working rivers (Braune and Rodgers, 1987). Although the ecological importance of the Klip River is noteworthy, a wide range of socio-economic services are provided by the ecosystem to various local and regional stakeholders, including the provision of water for basic human needs and natural products to local communities, and the removal of waterborne wastes from Gauteng (DWA, 2012). As a result, the use of ecological services of the Klip River is currently excessive and unsustainable (Wepener et al., 2011). To address this, the Water Resource Classification procedure for the Upper Vaal WMA established a Heavily Used (Class III) class for the Upper Vaal IUA U1 which includes the Klip River (DWA, 2012). While the Class III considers the current significantly altered state of the local water resources, a minimum, largely modified, but sustainable ecological integrity state must be achieved for the local water resources. This vision for IUA U1 has been used to establish regional RQOs and RQOs for priority river, groundwater and wetland resources (Figure 46). This study aims to demonstrate the suitable use of the Regional Scale Relative Risk Assessment approach incorporating the Relative Risk Model (RRM), as a suitable measure to evaluate the risks of achieving riverine Resource Quality Objectives for water resources in the Klip River (RUs 63-65) portion of the Vaal River Catchment in South Africa. In addition the study aims to demonstrate the use of the RRM to evaluate the socio-economic and ecological consequences of alternative management options to provide the information for Source Directed Control measures, to achieve RQOs.

A Regional Scale Risk assessment using the RRM is a form of ecological risk assessment that is carried out on a spatial scale where considerations of multiple sources of multiple stressors affecting multiple endpoints or objectives are allowed (Landis, 2005 O'Brien & Wepener, 2012). This transparent, adaptable, scientifically validated approach is being continually developed both locally and internationally and has been used extensively as a robust and reliable technique that importantly allows for the uncertainty associated with the outcomes to be carefully evaluated (Landis, 2005; Ayre & Landis, 2012; O'Brien & Wepener, 2012). The RRM has been shown to contribute towards the management of surface aquatic ecosystems in South Africa to achieve a balance in the protection of biodiversity, while allowing for the social and economic needs of society (O'Brien & Wepener, 2012). This study demonstrates the use of the latest RRM approach to contribute to the implementation of RQOs for the Klip River in South Africa.

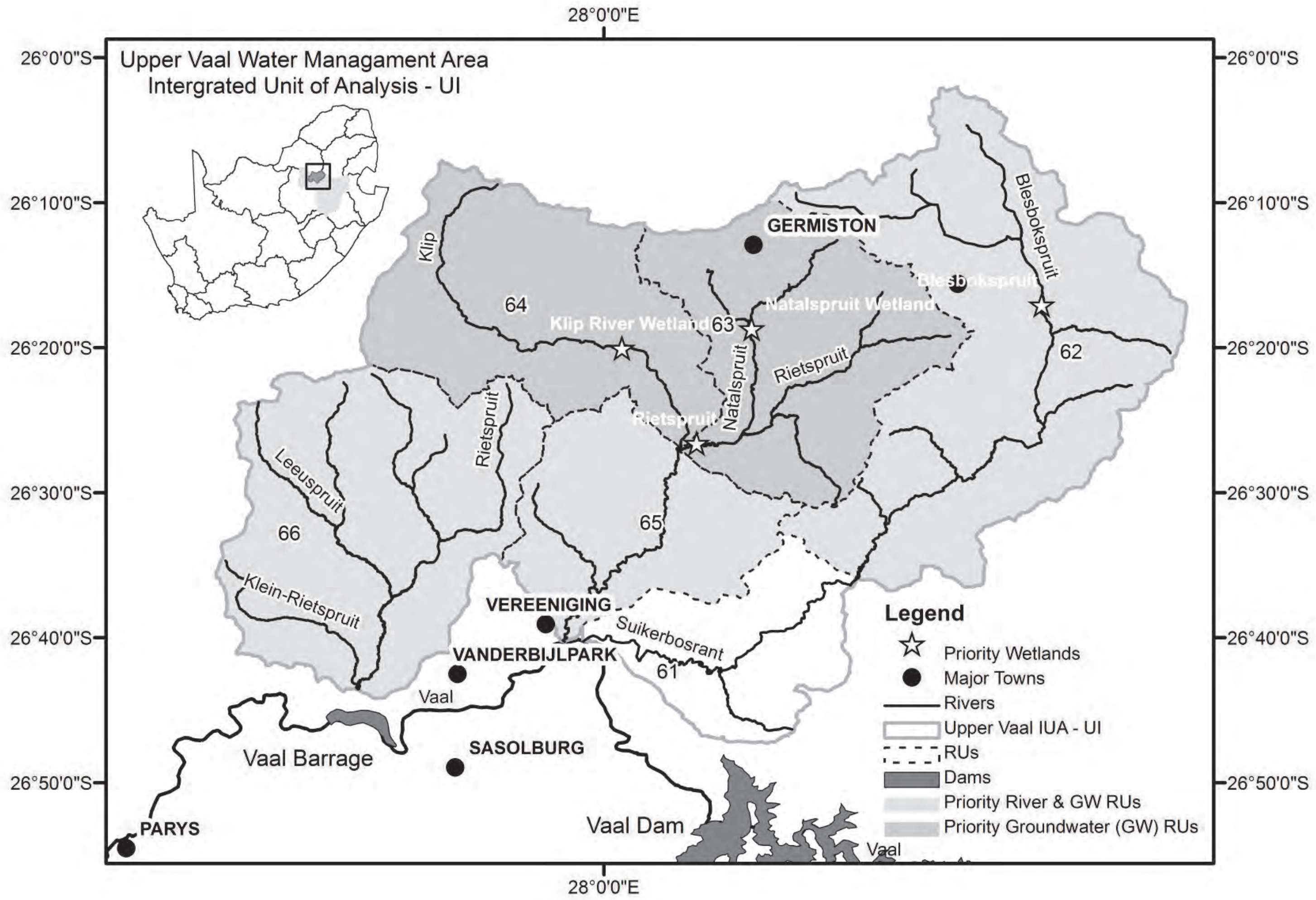


Figure 46: The Integrated Unit of Analyses (IUA) U1 from the Upper Vaal Water Management Area (WMA), with priority groundwater and river Resource Units (RUs) and priority wetland resources obtained from the Resource Quality Objective determination procedure for the WMA (DWS, 2014).

## 6.1 Methodology

The RRM implemented in this study is based on the ten procedural steps of the RRM including:

1. List the important management goals for the region.
2. Generate a map on which the potential sources and habitats relevant to the established management goals are indicated.
3. Demarcate the map into regions based on a combination of the management goals, sources and habitats.
4. Construct a conceptual model that links the sources of stressors to receptors and to the assessment endpoints.
5. Decide on a ranking scheme to calculate the relative risk to the assessment endpoints.
6. Calculate the relative risks.
7. Evaluate uncertainty and sensitivity analysis of the relative rankings.
8. Generate testable hypotheses for future field and laboratory investigations to reduce uncertainties and to confirm the risk rankings.
9. Test the hypotheses that were generated in Step 8.
10. Communicate the results in a fashion that effectively portrays the relative risk and uncertainty in response to the management goals.

**Step 1: List the important management goals for the region.**

The management goals considered for this study include the RQOs established for the river ecosystems in study area (DWS, 2014). The RQO determination procedure for the Upper Vaal WMA adopted a requisite simplicity approach, whereas few RQOs as necessary to ensure that the visions (Management Class) and associated site specific recommended ecological categories of water resources, for the WMA, were established (DWA, 2012; DWS, 2014). Resource Quality Objectives for rivers on a regional (whole IUA U1) and Resource Unit (RU) scale have been established (DWS, 2014). The regional scale narrative RQOs with context information for IUA U1 includes:

- Many of the rivers in this IUA are heavily impacted and it is important that the ecosystem be maintained in an acceptable quality (D or better ecological category) so that there can be a continued supply of ecosystem services.
- Altered low flows conditions are of particular importance in this IUA. Elevated low flows need to be managed to be sympathetic to the ecosystem. In addition, there are

numerous water quality issues that need to be managed so that wellbeing of the ecosystem does not deteriorate to unacceptable conditions, below a D category.

- The consumption of fish harvested from rivers in the IUA must not pose a threat to human health.
- The recommended ecological category (REC) of any river reach as described in the Water Resource Classification for the Upper Vaal (DWA, 2012) and must be adhered to, unless superseded by the detailed Resource Quality Objectives for the RUs (Table 60).

The RU scale RQOs for the Klip River include objectives for quantity, quality, habitat and biota sub-components for RU 65 alone (Table 60, DWS, 2014).

Table 60: Summary of the riverine Resource Quality Objectives for the Klip River catchment (adapted from DWS, 2014).

| Component | Sub-component | Narrative Resource Quality Objective | Indicator/ measure | Numerical Limits | Threshold of potential concern |
|---|---|---|---|---|---|
| Quantity | Low Flow | Low flows should be capped to protect low flow ecosystem processes. | Base flows in rivers (consider wetland RESERVE) | ≥ Largely modified state (equivalent to EcoClassification score > 40). This data is not presently available. | NA |
| Quality | Salts | Salts need to be improved to levels that do not threaten the ecosystem and to provide for users. | Electrical conductivity | ≤ 111 mS/m | 98 |
| | Toxicants | The river water should not be toxic to aquatic organisms or be a threat to human health. | F | ≤ 3.0 mg/L | 2.8 mg/L |
| | | | Al | ≤ 150 µg/L | 128 µg/L |
| | | | As | ≤ 130 µg/L | 113 µg/L |
| | | | Cd (Hard) | ≤ 5.0 µg/L | 4.0 µg/L |
| | | | Cr(VI) | ≤ 200 µg/L | 161 µg/L |
| | | | Cu hard | ≤ 8.0 µg/L | 7.0 µg/L |
| | | | Hg | ≤ 1.70 µg/L | 1.34 µg/L |
| | | | Mn | ≤ 1300 µg/L | 1145 µg/L |
| | | | Pb hard | ≤ 13.00 µg/L | 11.25 µg/L |
| | | | Se | ≤ 30 µg/L | 26 µg/L |
| | | | Zn | ≤ 36 µg/L | 31 µg/L |
| | | | Chlorine | ≤ 5.0 µg/L free Cl | 4.1 µg/L free Cl |
| | | | Endosulfan | ≤ 0.200 µg/L | 0.165 µg/L |
| | | | Atrazine | ≤ 100 µg/L | 89 µg/L |
| | Pathogens | Pathogens should be maintained at levels safe for human use (excluding for direct consumption). | *E.coli* | ≤ 130 counts/100 ml | NA |
| Habitat | Instream Habitat | The instream habitat should be maintained to a level that sustains this ecosystem. | State of instream habitat according to Rapid Habitat Assessment Method (RHAM) | EcoStatus (RHAM) ≥C category (≥62), and or maintenance of habitat for indicator species in a ≥C ecological category. | EcoStatus (RHAM) ≥B/C category (≥78) |
| Biota | Fish | The fish community needs to be maintained to sustainable levels. | State of fish populations according to Fish Response Assessment Index (FRAI) Score | FRAI Score ≥40 (≥D category (equivalent to EcoClassification score > 40)) | FRAI Score C/D |
| | Aquatic invertebrates | Invertebrates should be maintained/improved to a sustainable condition to support biodiversity. | State of aquatic invertebrates according to Macroinvertebrate Response Assessment Index (MIRAI) Score, using the SASS5 sampling method and maintenance of critical habitat according to Rapid Habitat Assessment Method (RHAM). | MIRAI Score ≥D category (equivalent to EcoClassification score > 40) and maintenance of critical habitat for invertebrates in a in a state equivalent to ≥D ecological category. | MIRAI Score C/D |

**Step 2: Generate a map and include potential sources and habitats relevant to the established management goals**

As outlined in the RRM process (O'Brien and Wepener, 2012), in the next step considerations of the spatial extent of the activity associated with the management of the research are made. These considerations were made by experts and stakeholders of the Klip River Catchment in a formal stakeholder engagement process included in the RQO determination process (DWS, 2014).

**Step 3: Demarcate map into regions based on a combination of the management goals, sources and habitats**

In the RQO determination process the RU delineation and then prioritisation phase result in the prioritisation of RU 65 for RQO establishment in the Klip River catchment alone (DWS, 2014). The RQO approach purposefully prioritised RU 65 which includes the lower portion of the Klip River upstream of the confluence of the Klip and Vaal Rivers (Figure 46). These RQOs are influenced by all sources and stressors located upstream of and includes RU65, i.e. the whole Klip River catchment (RU63 and RU64). As such, although the risk assessment for the Klip River considered here is based on RQOs located in RU 65 alone, all sources and stressors in the catchment upstream of RU 65 were considered to be represented.

**Step 4: Construct a conceptual model that links the sources of stressors to receptors and to the assessment endpoints**

The conceptual model delineates the potential relationships between sources, stressors, habitats and endpoints that will be used in the assessment of each risk region (Landis, 2005). The information used to establish the maps for the RRM in Step 2 and through generating resource-use scenarios, based on information gathered from stakeholders through the RQO determination process, was achieved (DWS, 2014). The conceptual model for the study is graphically presented in Figure 47. It has been used as the basis to convert the model into a Bayesian Network belief model, which is presented in Figure 48. This process has allowed for the establishment of complex theoretical relationships between stressors and sources that will be tested in the assessment. This also allows for the modification of relationships following the evaluation of uncertainty and the sensitivity assessments (Step 7) to establish a scenario of relationships between stressors and sources.

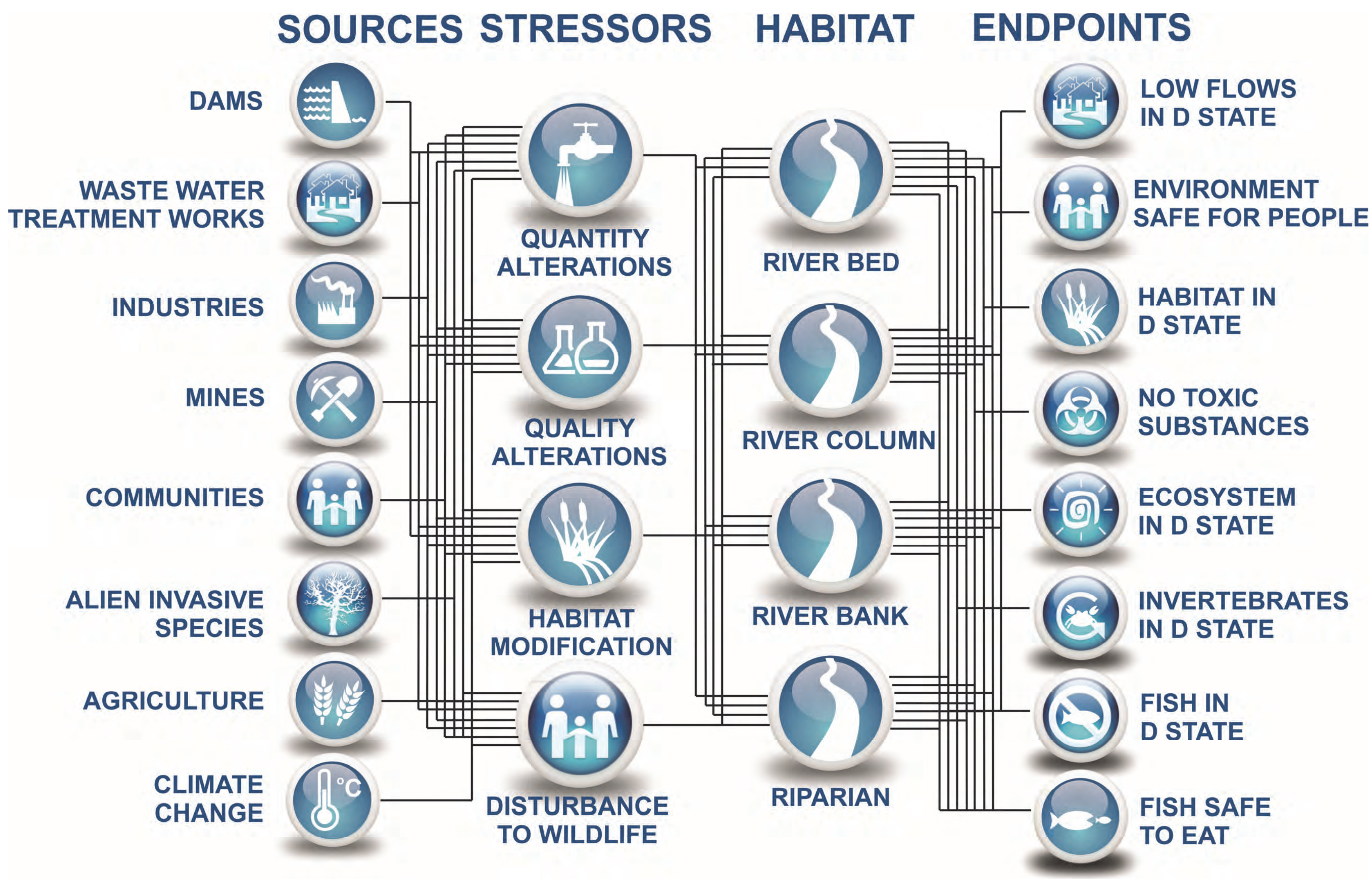


Figure 47: Conceptual model linking Source and Stressor combinations to habitats and receptors considered in the study.

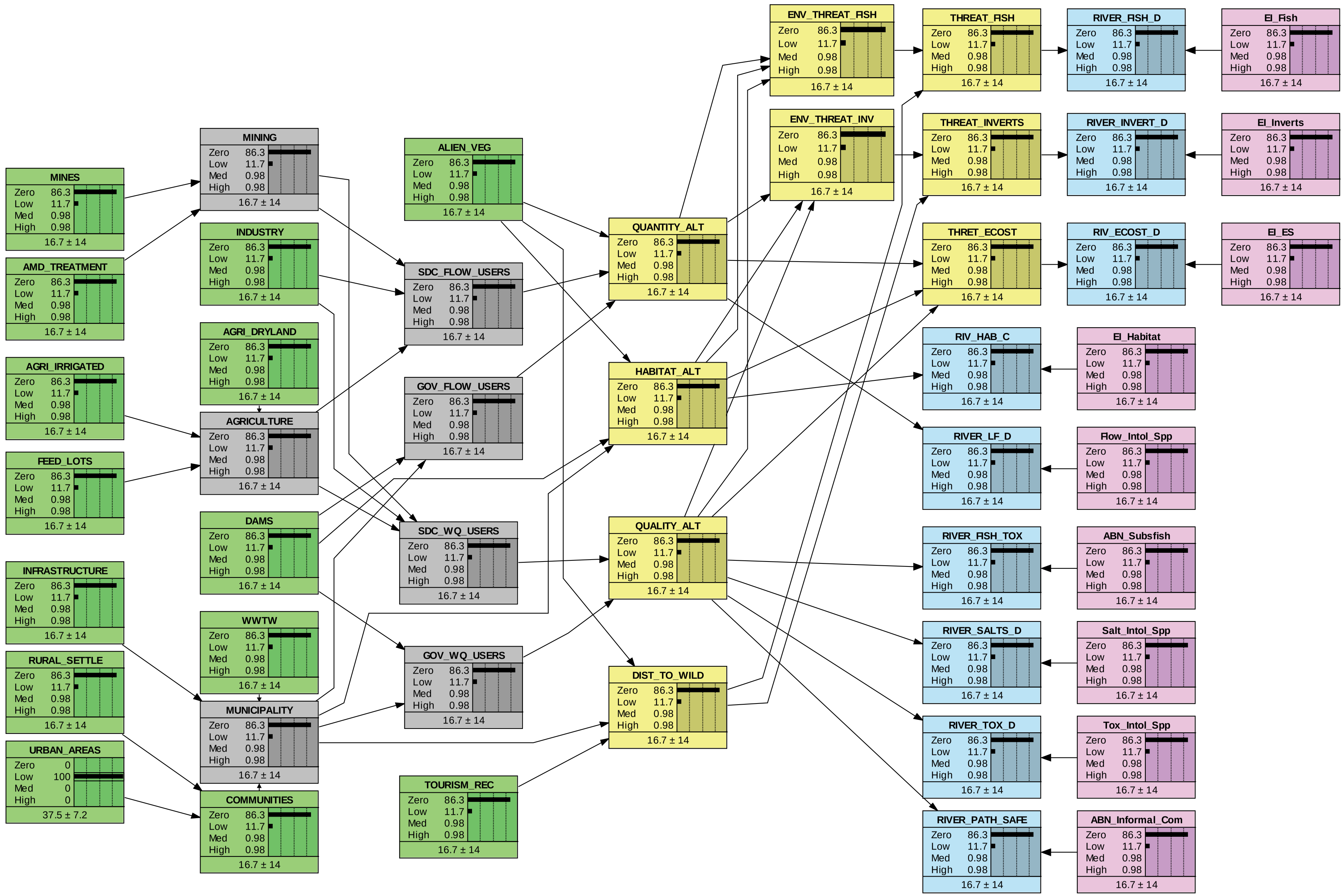


Figure 48: Bayesian Network belief network established in the study to evaluate the risk of source/stressors to endpoints in the study.

For the RRM assessment of the Klip River the Bayesian Belief network (Figure 48) comprises of 48 Nodes, including 62 causal pathways or Links between nodes and a total of 3672 Conditional probability relationships. Each daughter node considered is limited to be considered a function of a maximum of three variables to reduce uncertainty in the 34 Conditional Probability tables established for the assessment. The descriptions of the nodes considered in the Bayesian Belief network (Figure 48) are based on outcomes of the RQO determination procedures by DWS (2014) and include:

**Source nodes (Name and descriptions):**

- ALIEN_VEG: Alien vegetation in the study area is considered to be an important source although very little quantitative evidence is available that describes the extent of the infestation and impact of this source (consider PESEIS, 2011).
- MINES: The mining sector in the study area is extensive, particularly, in RU63 and RU 64. The industry in IUA U1 contributes approximately R 7056.6M annually to the GDP (DWS, 2014). Water quality problems relating to upstream activities are particularly concerning in RU 65, which are located downstream of RU 63 and RU 64. The IUA is characterised by water quality related problems due to pollution from gold mining slimes dams and mine dewatering. Within IUA U1 about 50 million $m^3$/annum of treated urban wastewater and mine water discharges from Grootvlei Mine (now referred to as Petrex) is discharged into the Suikerbosrand and Blesbokspruit rivers. The Far West Basin Mines release approximately 18 million $m^3$/annum into the Riet Spruit primarily.
- AMD_TREATMENT: Acid Mine Drainage (AMD) Neutralisation Plan Phase I is being established in the catchment. This includes objectives to reach acceptable dilution of AMD from IBTs and water treatment by 2017 (DWS, 2014).
- AGRI_IRRIGATED: Irrigated agriculture sector in the study area is limited with a relatively small contribution to GDP (approximately R 232.4M, DWS, 2014). However, there is evidence of extensive irrigation especially along the Blesbokspruit (RU64) and Klip River (RU63) according to satellite imagery (DWS, 2014).
- FEED_LOTS: Concentrated animal feed lots are uncommon but are considered by stakeholders to occur in the study area. These intensive agricultural activities have been documented to result in water quality stressors in particular.
- AGRI_DRYLAND: Large areas of agriculture in RU 63 and 65 in particular occur in the study area. Evidence of dryland agriculture especially along the Riet Spruit and lower Klip River is considered to pose a threat to the wellbeing of the riverine ecosystems in the study area.

- COMMUNITIES: The IUA is highly urbanised, particularly along the Riet Spruit and Klip River in Gauteng. Urban centres that influence the study area include Johannesburg, Soweto, Boksburg, Brakpan, Benoni, Springs and Sebokeng (with any associated informal peri-urban settlements). The IUA is characterised by water quality related issues in particular, associated with pollution through surface runoff from urban centres, leaking sewers and effluent from Waste Water Treatment Works (DWS, 2014). There are a number of poor urban and informal communities that make use of the fish and other natural products from the riparian zone of the rivers, in the study area. Despite the well documented poor water quality from rivers in the study area, and suspected unsafe fish quality for human consumption, the local subsistence fisheries activities in and along the Klip River are considered to be very important. Although some species of vegetation from the riparian zone along the rivers in the Klip River catchment were considered to be important, the actual utilisation is low and possibly associated with restrictions on access to the river and associated riparian areas.
- DAMS: Numerous small impoundments occur throughout the study area. These impoundments are considered to affect the water quality and quantity in the study area; and affect the movement of fishes in particular, in the rivers between the Vaal River and the upper reaches of the Klip River, Riet Spruit and Natal Spruit. These dams also harbour many alien, invasive fishes that indirectly threaten the wellbeing of the river ecosystems.
- INFRASTRUCTURE: This includes all infrastructure associated with agriculture and irrigation, water supply and sanitation, urban and peri-urban centres, roads, power infrastructure and manufacturing. In the study area, the paved/sealed surfaces of the urban areas affect the water quantity and quality.
- RURAL_SETTLE: Although small holdings (PESEIS; 2011) are common throughout the study area and there are a broad range of communities present on these farms, most are urbanised and the dependence on the goods and services is likely to be limited.
- URBAN_AREAS: The IUA is highly urbanised, particularly along the Riet Spruit and Klip River in Gauteng. Urban centres that influence the study area include Johannesburg, Soweto, Boksburg, Brakpan, Benoni, Springs and Sebokeng (with any associated informal peri-urban settlements). The IUA is characterised by water quality related issues in particular, associated with pollution from surface runoff from urban centres, leaking sewers and effluent from Waste Water Treatment Works (DWS, 2014).

- WWTW: The Klip River within the study area receives partially treated water from the Goudkoppies and Olifantsvlei Waste Water Treatment Works which receives waste water from Johannesburg, Soweto, Boksburg, Brakpan, Benoni, Springs and Sebokeng (with associated informal urban settlements). The IUA is characterised by water quality related problems.
- TOURISM_REC: Tourism and Recreation activities in the study area are relatively limited, picnicking and angling activities for recreation do occur.
- INDUSTRY: The industrial sector within IUA UI contributes approximately R 46,559.7M to the GDP of South Africa. This very important user in the study area includes the industrial centres and densely populated area of Johannesburg, Soweto, Boksburg, Brakpan, Benoni, Springs and Sebokeng. The industrial sector is the main contributor to GDP, employment opportunities and household income in the area, involving approximately 204,252 employment opportunities and contribution to household incomes of R 37,793.6M/annum.

**User category nodes (Name and descriptions):**

The sources were categorised into sectors to facilitate the risk descriptions in the study as follows:

- GOV_WQ_USERS: Represents sources that contribute to the water quality stressors that are managed directly by national and provincial governments in the study area.
- MUNICIPALITY: Represents sources that contribute to stressors that are managed directly by local municipal governments in the study area.
- SDC_WQ_USERS: Represents sources that contribute to the water quality stressors that are regulated through Source Directed Control Measures by issuing water licences to private formal ecosystem users.
- GOV_FLOW_USERS: Represents sources that contribute to the quality stressors that are managed directly by national and provincial governments in the study area.
- SDC_FLOW_USERS: Represents sources that contribute to the quality stressors that are regulated through Source Directed Control Measures by issuing water licences to private formal ecosystem users.

**Stressor nodes (Name and descriptions):**

- QUANTITY_ALT: Refers to water quantity alteration stressors associated with alterations to the volume, timing and duration of flows through abstraction and excessive releases of flows.

- DIST_TO_WILD: Refers to disturbance to wildlife stressors associated with community and other source activities that cause a response from ecosystems components due to the mere presence/activity without any impacts to the habitat or water quantity/quality.
- QUALITY_ALT: Refers to water quality alteration stressors primarily due to the releases of effluents into the ecosystem.
- HABITAT_ALT: Refers to habitat alteration stressors associated with instream and riparian habitat modifications and or removals.

**Step 5: Decide on a ranking scheme to allow the calculations of the relative risk to the assessment endpoints**

In this step, ranking schemes are defined, conditional probability tables (CPTs) constructed, and all relevant data collected for input into the model. Within ELOHA, this step considers acceptable ecological conditions and societal values to determine the ranking scheme while flow-ecology, hydrologic foundation, river classification, and flow alteration are considered to construct the CPTs for the RRM model. Ecological data from local surveys, historical surveys within the study area and or similar areas and specialist opinion provides input data/evidence. A plan for use of probabilistic results should be incorporated into the construction of the ranking schemes which represent the state of and or risk to variables (Figures 48 and 49). For the RRM approach to be transparent and adaptable, all decisions and assumptions for each node and causal relationship need to be described based on existing knowledge available at the time of the creation of the model. In this example (Figure 47), we have selected a four rank risk rankings scheme that is comparable with regional ecosystem wellbeing and sustainability classification schemes to facilitate with the establishment of the rank thresholds including:

- *Zero risk rank* which refers to the state of each component considered in the study that is comparable to natural (pre-anthropogenic influence) conditions.
- *Low risk rank* refers to an ideal state for each component including anthropogenic activities. This condition can also be considered to represent the best attainable conditions for the endpoints considered in the study.
- *Moderate risk rank* refers to the state of each component considered in the study in a modified state which is still sustainable but includes an acceptable loss in ecological services, processed and biodiversity.  This condition is usually only maintained in highly utilised ecosystems and is indicative of the change in the wellbeing of the component considered from an ideal state towards an unacceptably impaired state (high risk), where mitigation measures should be implemented. This rank can also be

considered to represent the threshold of potential concern for the wellbeing of the component considered.

- *High risk rank* refers to the state of each component considered in the study in a severely impaired, unsustainable condition, where a significant change in the wellbeing has occurred/or is likely to occur.

In this step evidence is required to:

- Select sources, indicators and receptors as input variables for the assessment and define the relationships between variables, and use evidence to construct conditional probability tables that will govern the relationships (Figure 50).
- Generate sources (such as hydrological statistics from a dam), indicators (such as the flow-dependent habitat requirements of fishes) and receptor ranks that conform to the zero, low, moderate and high ranking scheme thresholds (Table 60). Evaluate available data and define scenario modelling data requirements for the assessment to calculate the risk.

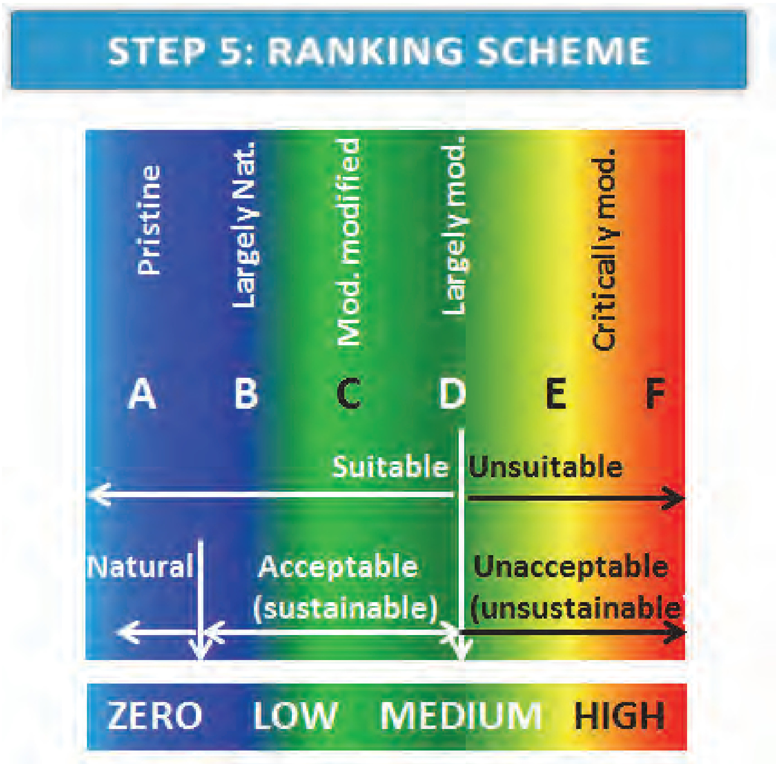


Figure 49: Graphical presentation of the relationship between ecoclassification (A-F) scale and descriptions, suitability/acceptability thresholds and risk rank scales.

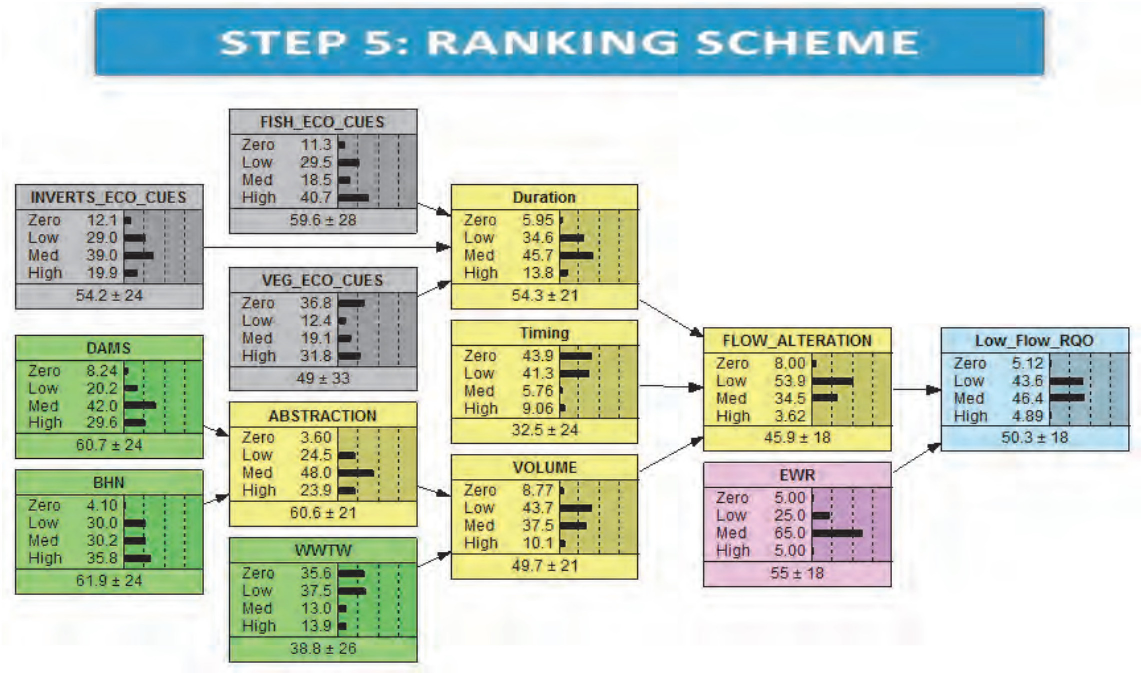


Figure 50: Bayseian Network model for a RRM assessment to assess the risk of sources to low flow Resource Quality Objectives in The model includes Sources (green) known to increase/decrease flows, the environmental requirements of selected ecological cues in the assessment (gray) and a receptor variable against which the threat of flow alterations can be made (Pink) and the overall endpoint (Blue).

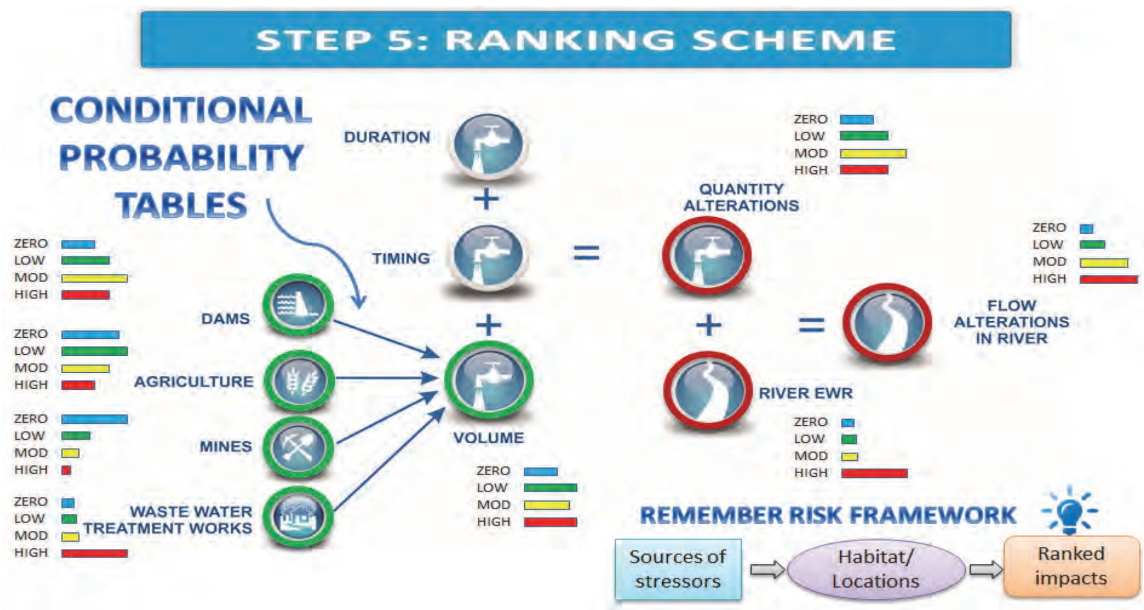


Figure 51: Schematic relationship between sources and endpoints used to model the risk of altered flows in a river, and the requirement for a conceptual probability table to govern the relationship between sources considered (Green). Addition and equal symbols used to demonstrate that the risk is a function of quantity alterations and the Ecological Water Requirement variables (Red). Zero, Low, Moderate and High graphs represent hypothetical state of each variable considered.

Table 61: Example of a ranking scheme with ranks and measure ranges associated with the ranks, for a RRM assessment to define the state of, and evaluate the suitability of substrates within an ecological endpoint model.

| DESCRIPTIONS | RANKS | | MEASURE RANGES FOR RANKS | JUSTIFICATION | DATA/ REFERENCES |
|---|---|---|---|---|---|
| EXPOSURE VARIABLE: Substrate suitability for fish (Substrate_Threat) | Zero | 0-25 | Maximum shear range state ensures that habitat is maintained in ideal/near natural state. No threat to wellbeing of fishes. | Similarly substrate availability is an important component of, and contributes to the wellbeing of the habitat which the fish have been observed to depend on. Substrate requirements of the fishes observed in the study area were available from historical data and from evidence collected in this study. The measure selected to represent substrate suitability variable is discharge (m3/s). Ranks based on shear stress values of various substrate types (Rowntree and Mzobe, in preparation) assessed by geomorphology specialists with available hydrology modelled through channel considering slope and velocity-depth profiles in relation to available substrate types. Zero rank assigned to high sheer stress range which would maintain existing rocky substrate types which are required to maintain specialised fishes. Low rank assigned to moderate sheer stress range which would maintain similar diversities of existing substrate types. Moderate rank assigned to sheer stress range which would result in a reduction of rocky substrate types but not in the total removal of rocky substrate types due to sedimentation. High rank assigned to sheer stress range which would result in total sedimentation of substrate types the removal of rocky substrates. Also consider turbidity associated with movement of water. At P1 because of the dam there will be very little sediment and even in high discharges there will be limited cover and increased predation. From P2 to P6 increased velocities (low/zero states) will facilitate indigenous fish. | Kleynhans, 2007; Kleynhans and Louw, 2007; King et al., 2011; Skelton, 2001, Lucas, 2003 O'Brien et al., 2013; |
| | Low | 25-50 | Moderate shear range state where habitat is maintained in a suitable state. Minimal threat to wellbeing of fishes. | | |
| | Moderate | 50-75 | Low shear range state which ensures that habitat are moderately impaired. Worst acceptable state, moderate threat to wellbeing of fishes. Management action required. | | |
| | High | 75-100 | Lowest shear range state results in highly modified habitats with extreme threat to wellbeing of fishes. | | |

### Step 6: Calculate the relative risks

In this step the posterior probability distributions in the BNs are initially calculated (sources, indicators and receptors), and then the BN outputs are integrated using a Monte Carlo analysis (Figures 52 and 53). This step correlates with the ELOHA flow alteration-ecological response relationship for each river type node. Risk calculations in Bayesian networks – The posterior probability distributions will calculate the probability of risk to the endpoints. The risk calculated may be compared between individual endpoints by risk region/site or by management scenario, but in order to compare the cumulative risk of the social, ecological and all endpoints within a risk region or management scenario, a Monte Carlo analysis (or alternatively Latin Hypercube assessment) must be conducted.

The outcomes of the integration include a graphical description of the relative risk distributions (relative scale) of the endpoints considered, with the peak of each curve representing the highest probability and the width representing the variability of the profile. These curves can be compared in a relative manner and present the relative risk of the scenario/risk region considered to the endpoint/s considered. In this hypothetical example we have presented total risk profiles to all endpoints considered (Figure 54) and then social and ecological endpoints considered separately (Figures 55 and 56) for clarification.

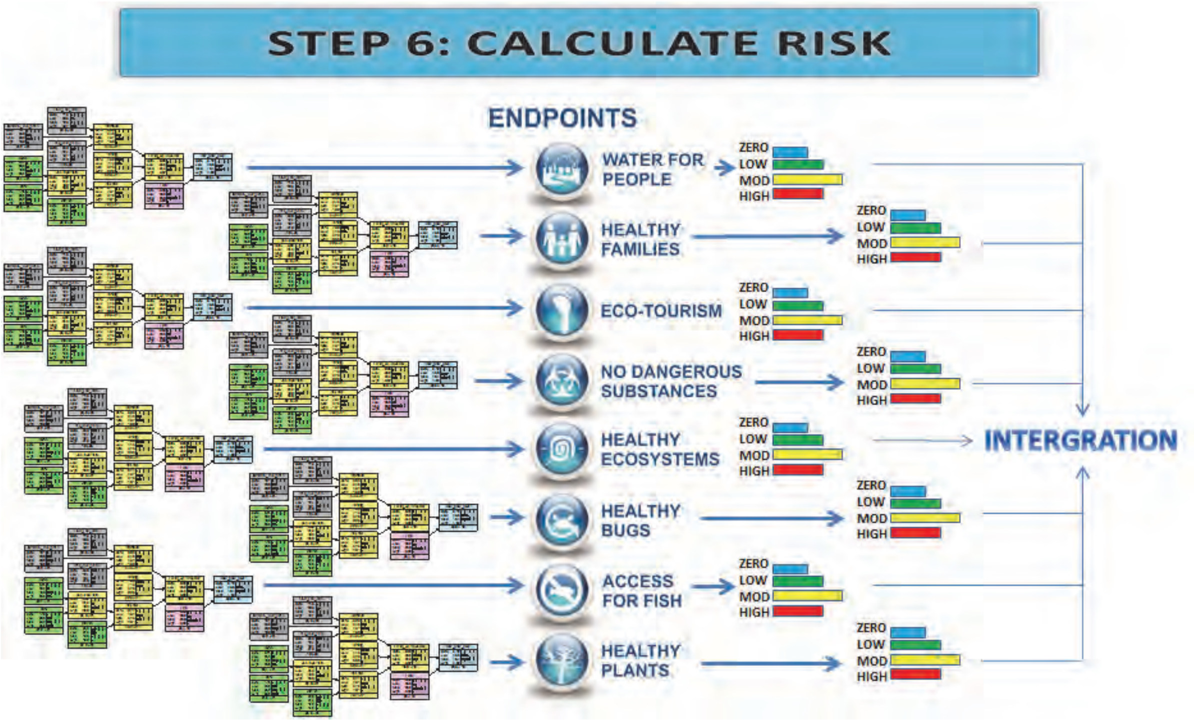


Figure 52: Schematic demonstration of the risk calculation phase of a RRM assessment including the use of the risk outputs for numerous socio-ecological endpoints and their integration.

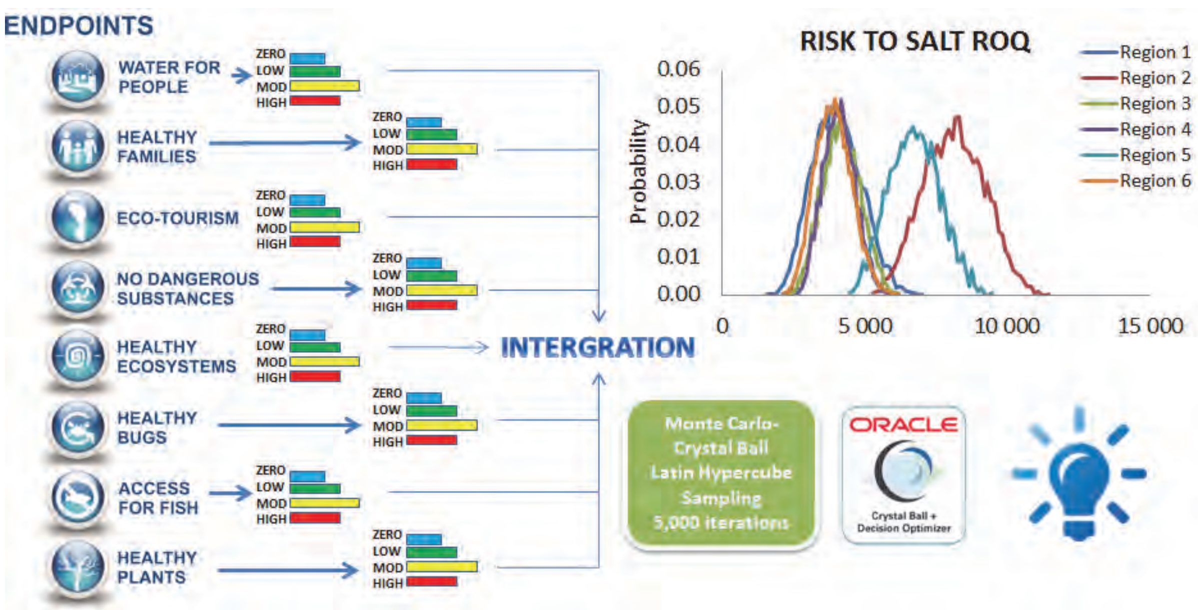


Figure 53: Continued Schematic demonstration of the risk calculation phase of a RRM assessment including the use of the risk outputs for numerous socio-ecological endpoints and their integration using Monte-Carlo permutations with Oracle ® Crystal Ball software.

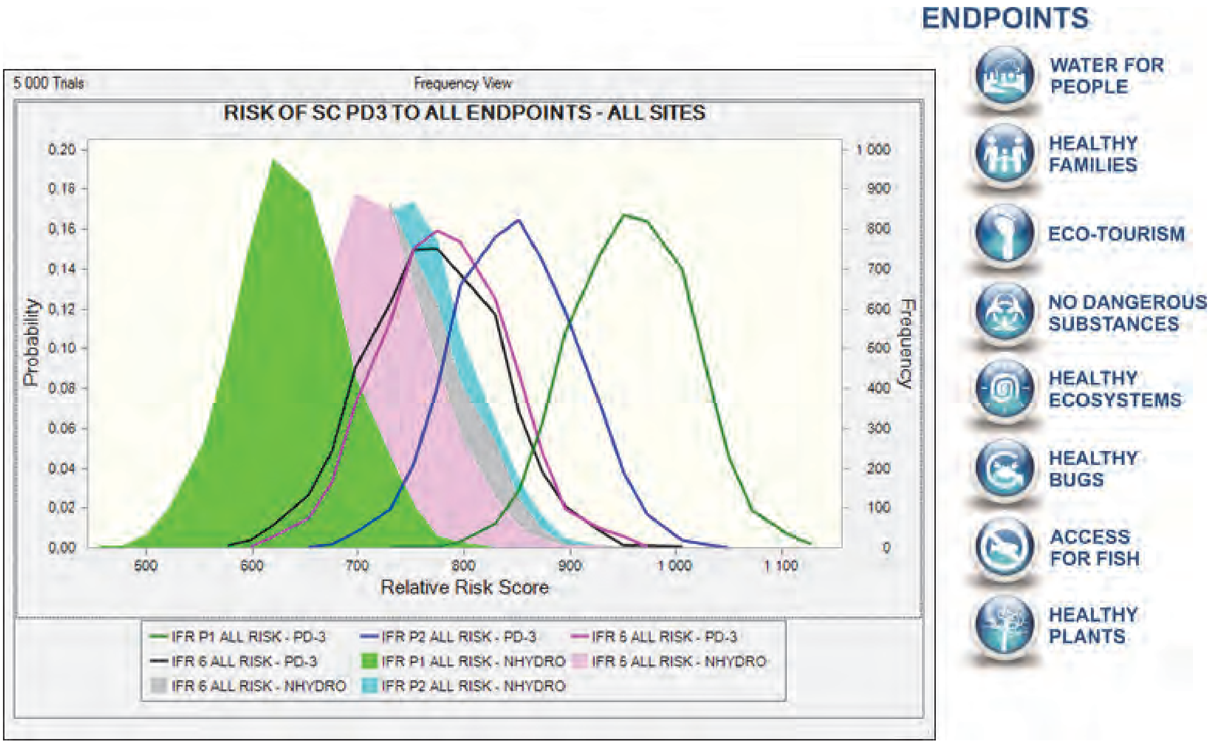


Figure 54: Risk profile distributions to all of the endpoints considered in an assessment within one risk region/site. The relative position, height and width of each curve represent the risk score, highest point of probability and variability respectively.

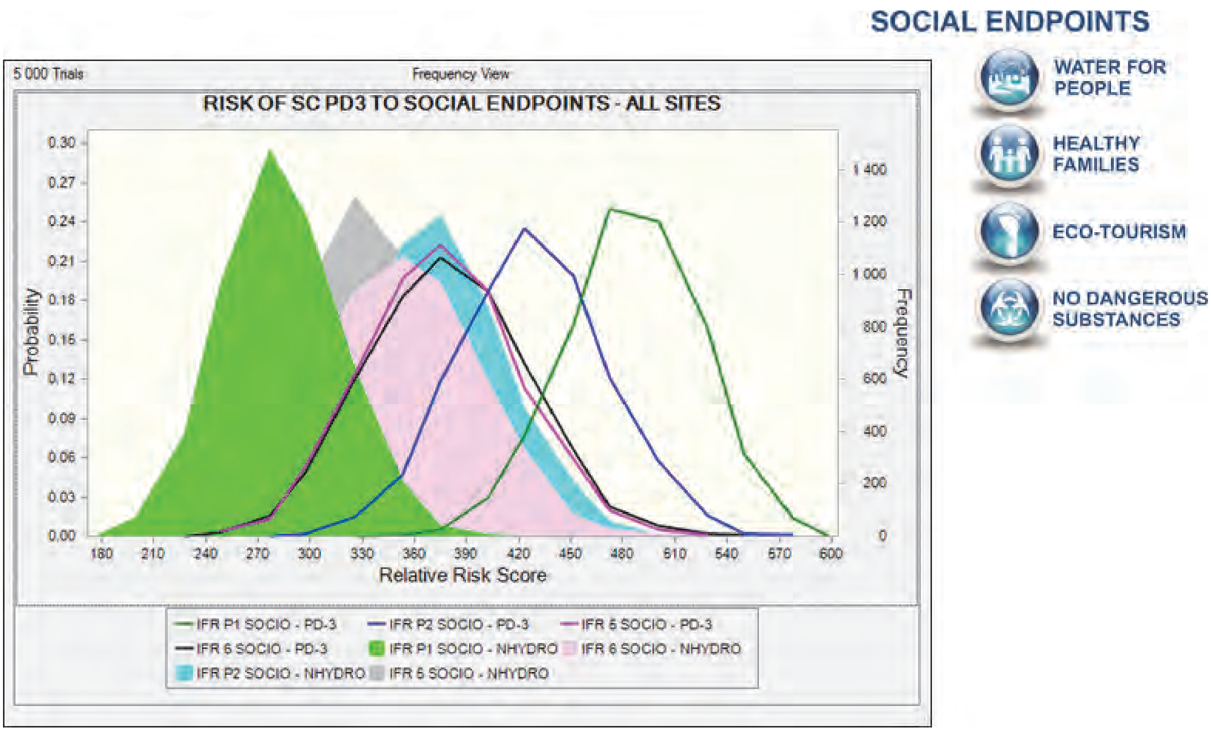


Figure 55: Risk profile distributions to social endpoints considered in an assessment within one risk region/site. The relative position, height and width of each curve represent the risk score, highest point of probability and variability respectively.

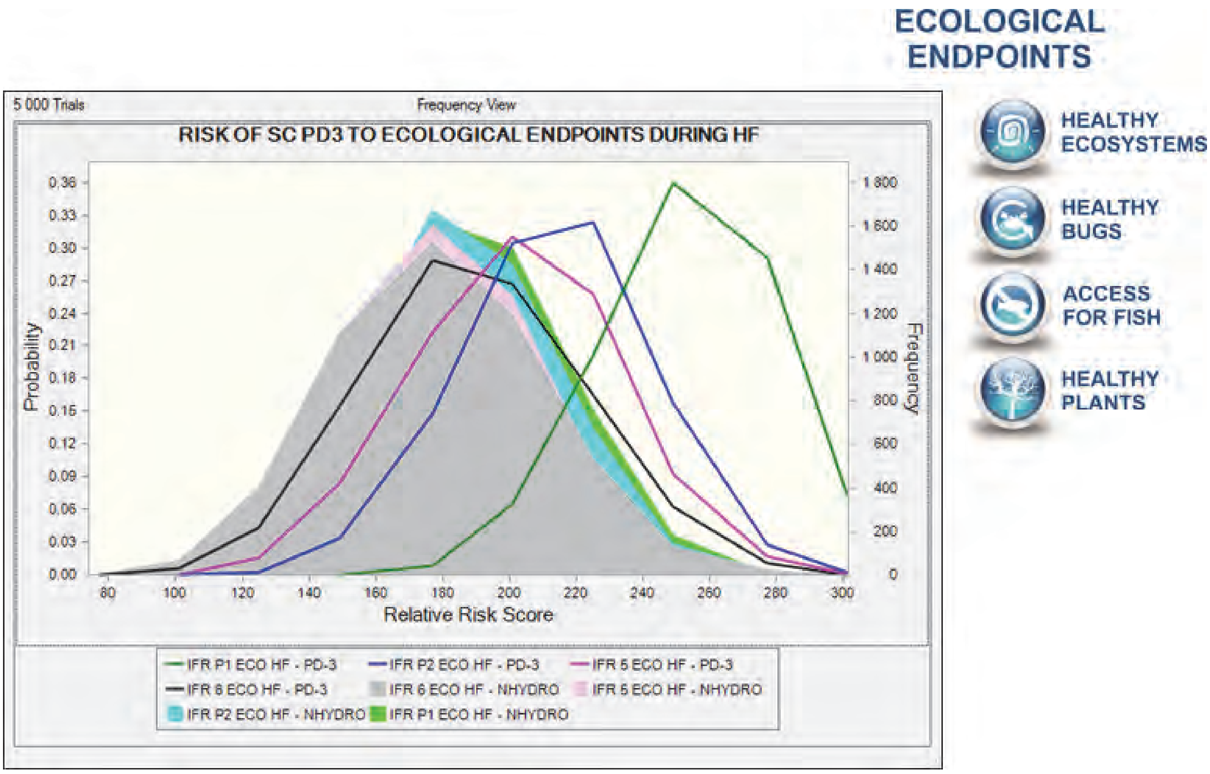


Figure 56: Risk profile distributions to ecological endpoints considered in an assessment within one risk region/site. The relative position, height and width of each curve represent the risk score, highest point of probability and variability respectively.

**Step 7: Evaluate uncertainty and sensitivity of the relative rankings**

In a RRM assessment it is necessary to conduct a sensitivity and uncertainty analysis. In this step any uncertainty associated with the data used (or lack thereof), modelling processes and integration processes are defined and presented. This allows managers to consider the amount of uncertainty associated with a risk profile to facilitate decision making processes (Figure 57). This step allows examination of what management decisions could be made to optimize riverine ecosystem services by identifying the key drivers which are the inputs that most influence the model output. By evaluating uncertainty, data gaps may be identified to direct future research and refine the model to reduce uncertainty where possible. This step can fit well within the adaptive management framework.

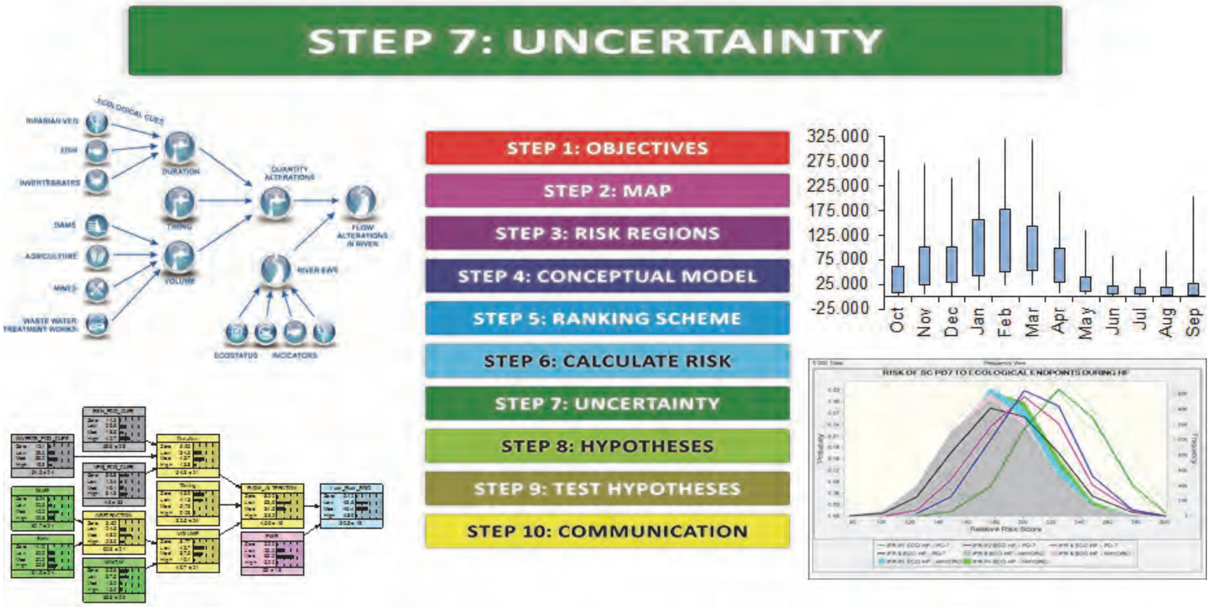


Figure 57: All components of a RRM assessment may cause uncertainty to be generated which must be evaluated.

**Step 8: Generate testable hypotheses for future field and laboratory investigation to reduce uncertainties and to confirm the risk rankings**

RRM assessments result in the establishment of Instream Flow Requirements (or Ecological Water Requirements) and are used to evaluate the socio-ecological consequences of altered flows in aquatic ecosystems. Managers use these outcomes to make resource use and or protection decisions. There will always be a level of uncertainty associated with the outcomes of a RRM assessment. The RRM includes two strategies to address this uncertainty; initially the process includes explicit descriptions of the uncertainty and possible implications to the outcomes and then the approach incorporates hypotheses generation steps to identify and test aspects of uncertainty in the process (Figures 58 to 60). In this process indicators of the models are identified that can be used to test the relationships established (Figure 58). This may include for example from a hypothetical model to evaluate the effects of flow alterations by sources (Figure 58). This process is used to:

- Generate data to reduce uncertainty pertaining to the state of input components,
- Generate evidence to reduce uncertainty associated with the use of conditional probability tables to define the relationships between variables,
- Generate evidence to reduce uncertainty associated with the outcomes of the RRM assessment.

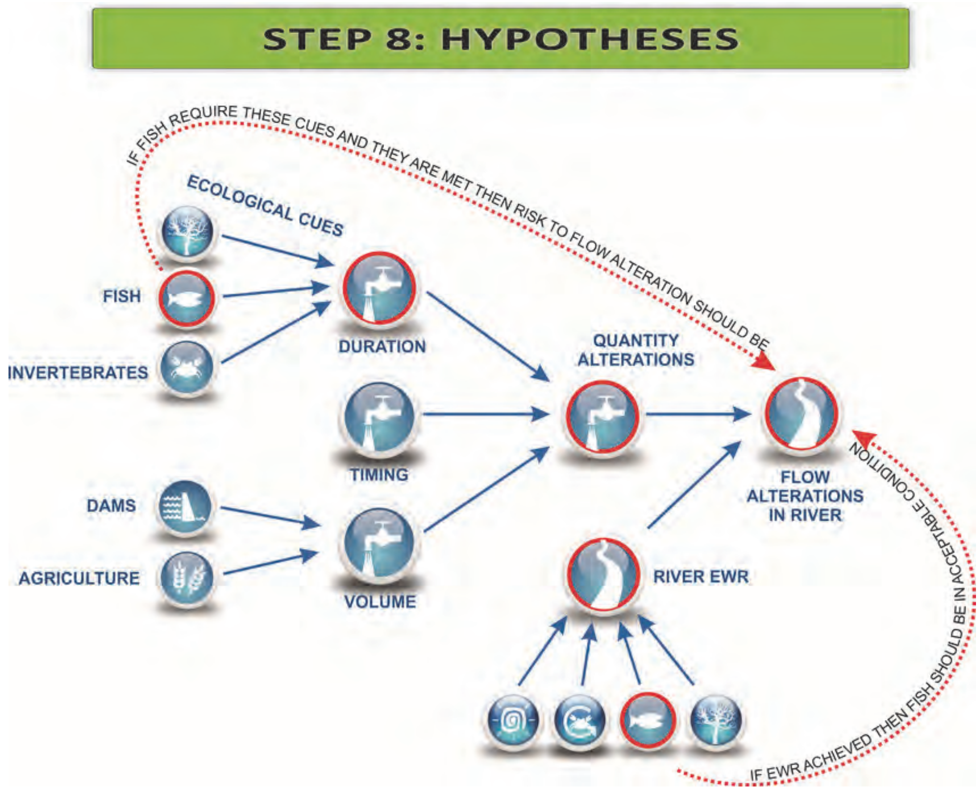


Figure 58: Graphical representation of the selection of indicators identified in a RRM assessment which can be used to establish hypotheses and test them to reduce uncertainty.

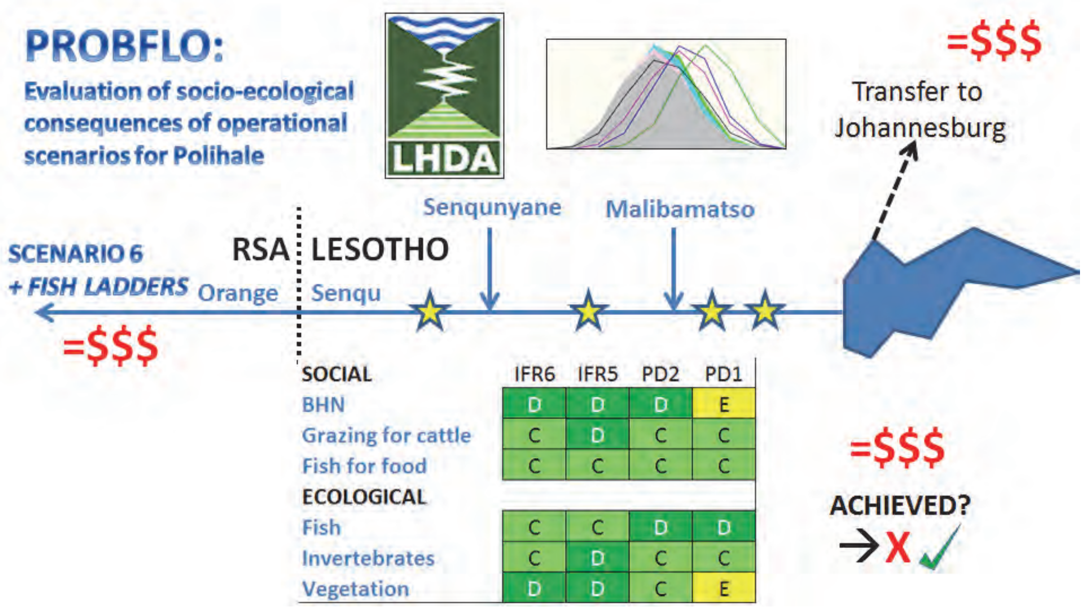


Figure 59: Schematic demonstration of the economic, social and Ecological consequences of implementing a management scenario for the Lesotho Highland Water Transfer Scheme (Phase II), and the ultimate goal of the implementation of an assessment, to monitor the successes or failures of the PROBLFO process and the socio-ecological consequences of released flows.

**Step 9: Test hypothesis established in Step 8**

The implementation process requires the establishment of a RRM implementation data management system to receive and interpret data, update existing RRM assessments and produce outcomes to compare historical and current RRM assessment results. Although this process can be automated, it is recommended that a risk assessor review the outcomes of an implementation process to ensure that they are representative of the new information. To implement the RRM process the following procedural steps are followed (Figure ):

- Indicators of the model that can be used to test the uncertainty and or the outcomes of a RRM assessment are identified,
- A monitoring plan is designed to collect data that describes the state of selected indicator components and or describes the relationships between variables. In this example a range of ecosystem driver components (water quality, discharge and habitat states) and response components (fish, riparian vegetation and invertebrate data) were selected for a monitoring plan with multiple levels of details for surveys (annual rapid surveys and comprehensive three yearly surveys for example).
- The monitoring plan is implemented and the results are captured into a data management system which then:
  - Updates available evidence and immediately provides descriptive analyses of the new data,
  - Converts the information into a format which the RRM process can use/query,
  - Populates the RRM models and integrates the outcomes.
- The automated outputs of the data management system include:
  - descriptive analyses of the new sampling data,
  - outcomes of the RRM assessment with comparisons to the original assessment,
  - a description of the results of the hypotheses testing to reduce uncertainty, and
  - information on RRM uncertainty mitigation measures, and model refinement recommendations which can be agreed to for automatic amendments or refused for testing, etc.
  - RRM outcomes can be compared with original modelling outcomes to update the socio-ecological consequence assessment of reduced flows based on measured data, and provide scenario amendment information to evaluate alternative management implications.

These procedural steps will reduce the uncertainty associated with the original RRM assessment, and allow the approach to be used in an adaptive management framework as advocated as best scientific practice. This will allow managers to constantly update the assessment with new information and consider the refined socio-ecological implications of water resource use decisions. The approach also allows for later add-on components which can be used in the future to evaluate the cumulative impacts of additional stressors to the endpoints considered, etc.

**Step 10: Communicate the results in a fashion that portrays the relative risk and uncertainty in response to the management goals**

Throughout the RRM process, communication needs to occur so that relative risk and uncertainty in response to management goals are effectively portrayed using a range of tools (reports, presentations, etc.). The graphical display outputs by BNs and Monte Carlo clearly portray the risk given in probability distributions which can serve as useful communication tools to managers and stakeholders. In this step the reporting phase for the whole study.

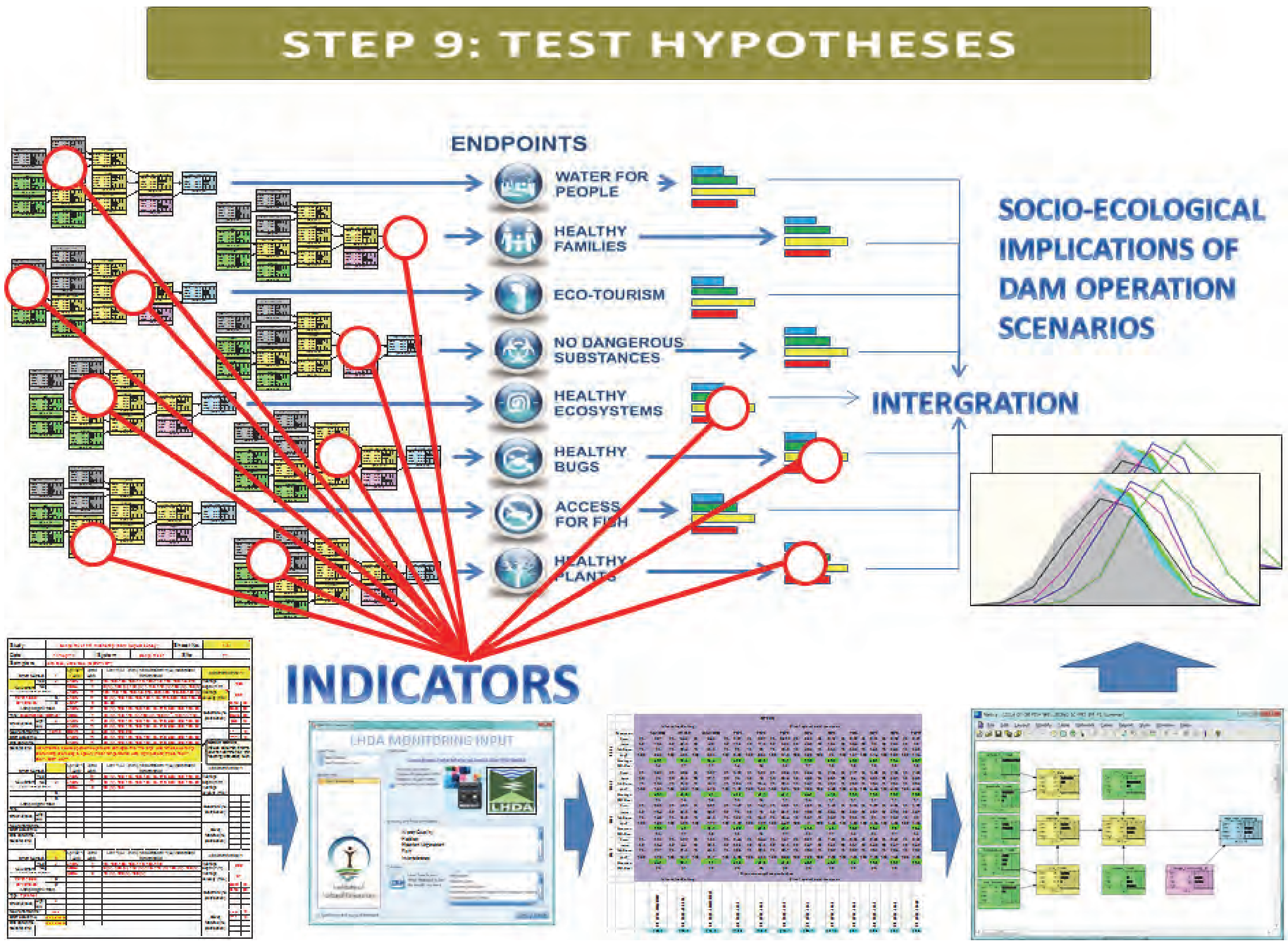


Figure 60: Demonstration of the RRM implementation process to continually evaluate the risk of altered flows, and reduce uncertainty associated with the assessment. The process includes the selection of indicators for a monitoring plan, collection of data, uploading the data into a data management system, the automatic evaluation of the data and incorporation into the RRM system and generation of outcomes based on monitoring for comparison to proposed outcomes from the RRM assessment.

# 7 CONCLUSIONS: LINKING THE RELATIVE RISK METHODOLOGY TO THE WATER QUALITY OBJECTIVES FOR THE KLIP RIVER SYSTEM

The Bayesian Network (BN) approach has successfully been applied to evaluate different impacts to forested landscapes in Oregon, USA (Ayer & Landis, 2012), low impact developments in relation to catchment management goals (Hines & Landis, 2013) and assessment of risk posed by parasitic infections caused by whirling disease on natural fish populations (Ayre et al., 2014). During this study (Chapter 6) we demonstrated how the causal structure of a risk assessment tool such as the relative risk methodology (RRM) can be translated into a graphical BN models. The tiered nodal structure of the BN allowed for the causal linking of sources of stressors, habitats and endpoints of the Klip River.

For the construction of the BN models multiple data types were applied a priori. Data types included published literature, new data generated during the field surveys and expert knowledge. According to Ayre & Landis (2012) the BN methods are particularly useful to reduce uncertainty because of these different data types through the use of conditional probability tables. As new data become available the BN is easily updated, thereby reducing uncertainty in risk predictions (Howes et al., 2010). The continuous updating of the BN model and the ensuing risk profiles provides a “learning by doing” environment, which is the foundation of the adaptive management process (Nyberg et al., 2006). One application of the BN model is to investigate alternative management scenarios. By altering the source/stressor input variables it is possible to evaluate changes in risk. Furthermore, the application of an RRM-based BN is ideal for situations where management strategies are implemented on a spatial scale since the evaluation of these options can then be undertaken by taking regional risk differences into account.

## 7.1 Management scenarios

To demonstrate the adaptive management application value of the BN that was developed during this study we selected eight scenarios which are presented in Table 61. For the purposes of meeting the requirements of the RQO process for the Vaal River system, the whole Klip River catchment was regarded as a single risk region (and not the five risk regions described in Chapter 2). The reason for this is that the lower portion of the Klip River just before the confluence with the Vaal River (RU 65) integrates all the risks posed by all activities within the catchment and therefore any risk mitigation measures that are implemented within the catchment will be reflected in the risk profile before the confluence with the Vaal River.

Table 62: Summary of the additional scenarios used to demonstrate the adaptive management application value of the RRM-BN.

| Scenario name | Description |
|---|---|
| **Scenario 1** (Present state) | This represents the present condition of the Klip River based on the data that were generated during this project. |
| **Scenario 2** (Natural state) | This scenario represents the conditions that would present in the Klip River at the turn of the 1900s. |
| **Scenario 3** (Desired condition) | This scenario relates to changing most of the risk projections from the current high to medium and low risks |
| **Scenario 4** (Mitigate AMD) | Risk of mines and AMD treatments were decreased by allocation zero to low risks |
| **Scenario 5** (Mitigate WWTW) | Risk posed by WWTW were mines and AMD treatments were decreased by allocation zero to low risks |
| **Scenario 6** (Reduce DTW impacts) | Risk posed by human disturbances to wildlife were decreased with by allocation zero to low risks |
| **Scenario 7** (Increase DTW impacts) | Risk posed by human disturbances to wildlife were increased with by allocation medium to low risks |
| **Scenario 8** (Reduce EI/ES) | Discount the contribution of the ecological importance/sensitivity to maintaining the ecological status. |

To evaluate the sensitivity of the RR-BN we exaggerated the conditions represented by the different scenarios. In most instances they could be regarded as worst-case scenarios. The different scenarios can also be related to aspects covered under the aims that were set for the study. The condition probability table (CPT) that allows for the structuring of the risks under the different scenarios is presented in Table 62. In Scenario 1 the current conditions as determined during this study was presented. The natural condition (Scenario 2) involved the changing in the risk profiles associated with the sources of stressors (e.g. mining and human habitation / encroachment on the river) to those that would have been expected in the early 1900's. Since mining and human activities were already present the risks could not be entirely zero and therefore some of the risk was in the low to medium risk categories. For Scenario 3 a desired condition was constructed, where the majority of risk would be low to medium. Scenarios 4 and 5 involved mitigation of the mining (and associated AMD) and WWTW risks by changing the risk profiles from high to a medium/low. The reduction of disturbance to wildlife involves the removal or reduction in the physical proximity of human habitation to the system. Scenario 6 therefore has a risk profile associated with the human stressors ranging from low to medium. The increased disturbance to wildlife impacts involves the changing for the risk profile to a predominantly high risk. The importance of allocating an ecological sensitivity rating to deriving ecological categories was tested in Scenario 8. Here the contribution of the ecological sensitivity / importance was removed completely.

## 7.2 Scenario assessment

The changes in risk profiles brought about by the different scenarios are risks presented in Figures 61 to 69. Figures 61 and 62 represent the risk profiles associated with changes to aquatic biodiversity. For both macro-invertebrates and fish the profiles are similar with only the desired (and historical conditions) occurring within low risk regions. Interestingly the removal of mining and WWTW stressors does not result in a decrease in the risks posed to biodiversity. Even the sensitivity and importance rating does not make a significant contribution to decreasing the risks.

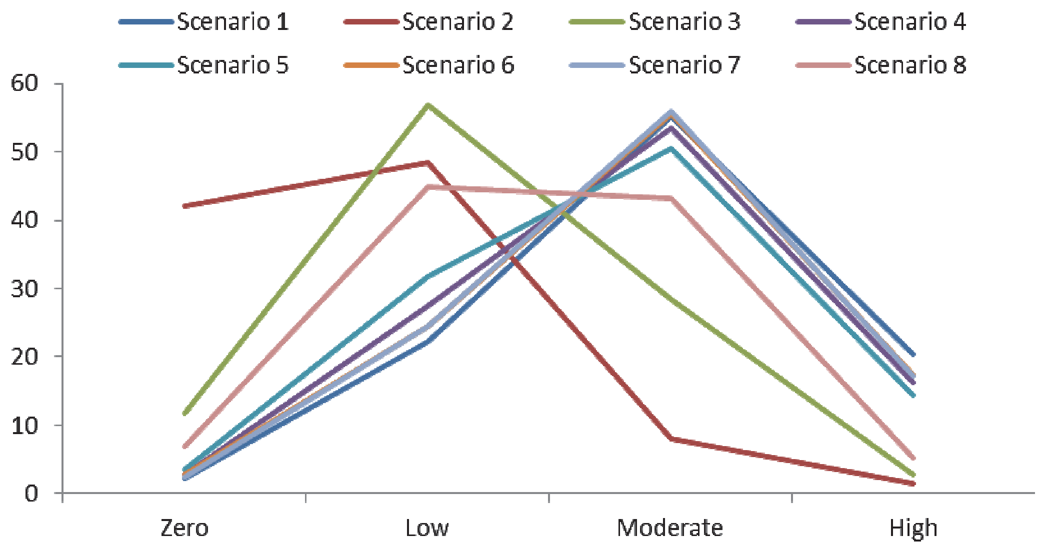


Figure 61: Risk posed to aquatic macro-invertebrate biodiversity under the eight scenarios.

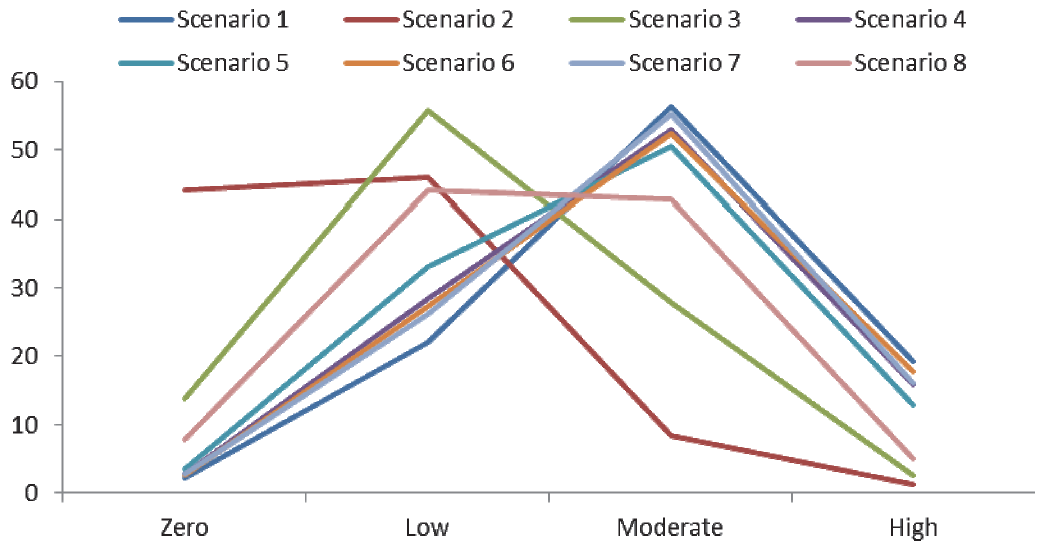


Figure 62: Risk posed to fish biodiversity under the eight scenarios.

In Figure 63 the changes in risk profiles of the ecological status related to the eight scenarios are presented. Surprisingly the EI/ES does not “protect” the overall ecological status of the system and by removing it the risk actually decreases, indicating a low ecological importance of the system. The risks posed to habitat structure of the Klip River are presented in Figure 64. The most notable change in profile is related to reducing the influence of humans, which does result in a decrease in risks but the largest portion of risk remains in the medium range. By taking away the EI/ES rating the risks are also decreased.

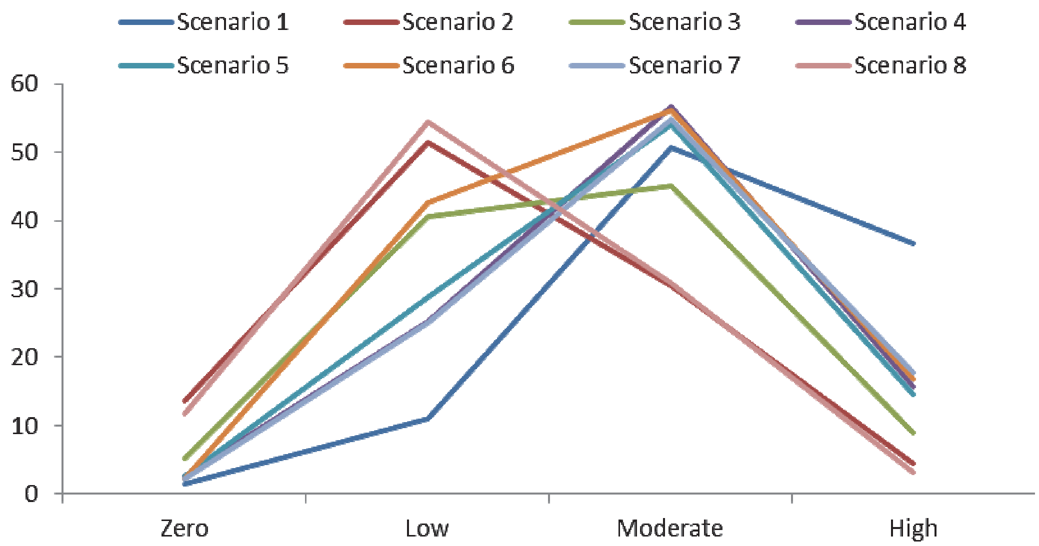


Figure 63: Risk posed to ecological status of the Klip River under the eight scenarios.

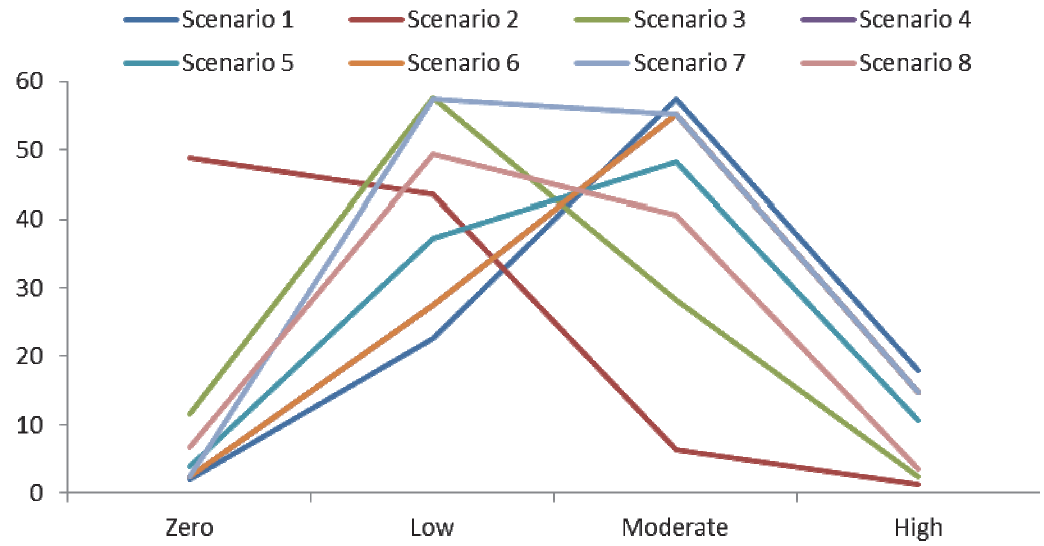


Figure 64: Risk posed to habitat structure of the Klip River under the eight scenarios.

The risks posed to humans by toxicant levels in the water and water-borne pathogens are presented in Figures 65 and 66 respectively. In both instances it is clear that mitigation of mining and WWTW activities under present day conditions will not reduce the risk to the natural or desired states.

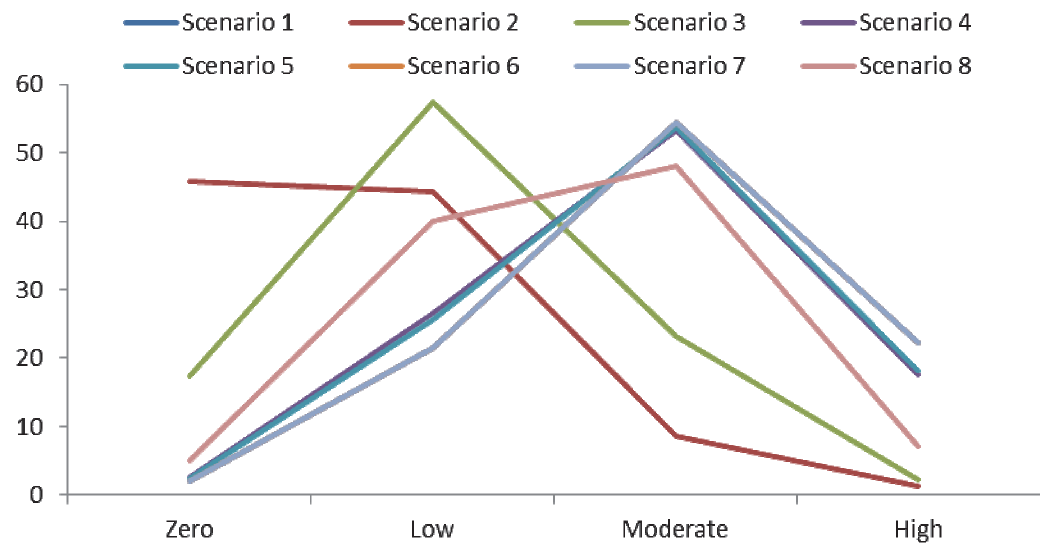


Figure 65: Risk posed to human health by toxicants in water of the Klip River under the eight scenarios

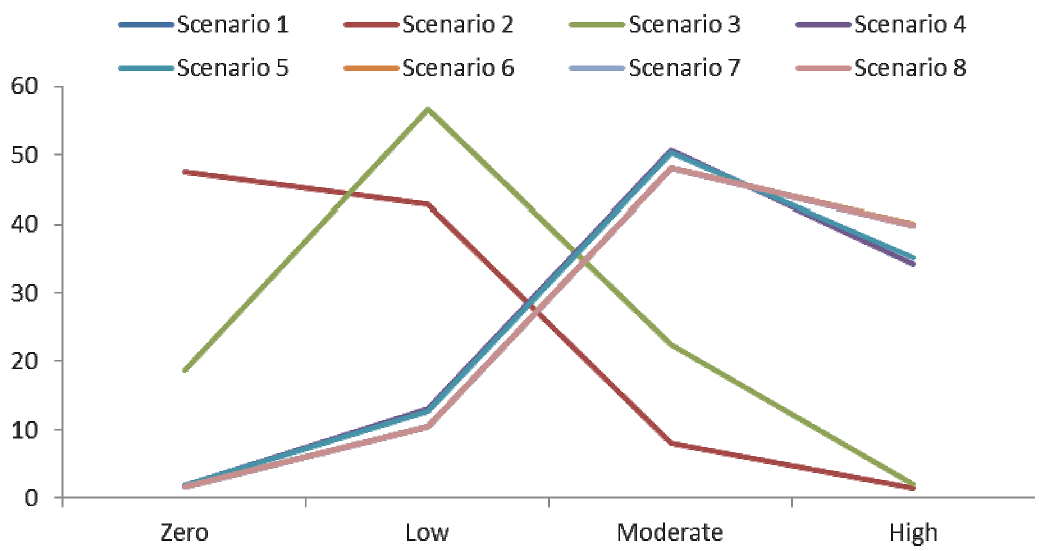


Figure 66: Risk posed to human health by water-borne pathogens of the Klip River under the eight scenarios

According to Ayre et al. (2014) BNs can be used to “back-calculate” what the initial conditions would need to be to reach the desired outcome. In this instance the desired outcome is to reduce all risks to obtain an ecological category D.

Figure 67 represents the integrated risk posed to all ecological endpoints under the different scenarios. The changes in risk profiles were related relative to the natural (scenario 2) conditions. To reduce overall risk to “D” category protective measures (Scenario 3) were modelled and the ecological importance and sensitivity of local ecosystem was reduced (Scenario 8). Although considerable portion of SC4, SC5, SC6 and SC7 was still in an “unacceptable state” the highest risk probability is in sustainable “D” category. Therefore, the present state remains in the worst condition allowed for the Klip River system.

Figure 68 represents the risk posed to social or human related endpoints under the different scenarios that were considered. To reduce overall risk to “D” category the desired conditions scenario was implemented. Not surprisingly, the reduction of the ecological importance and sensitivity does not reduce risk to humans. Notwithstanding the mitigation measures that were considered, the risk cannot be reduced to the “acceptable” D state.

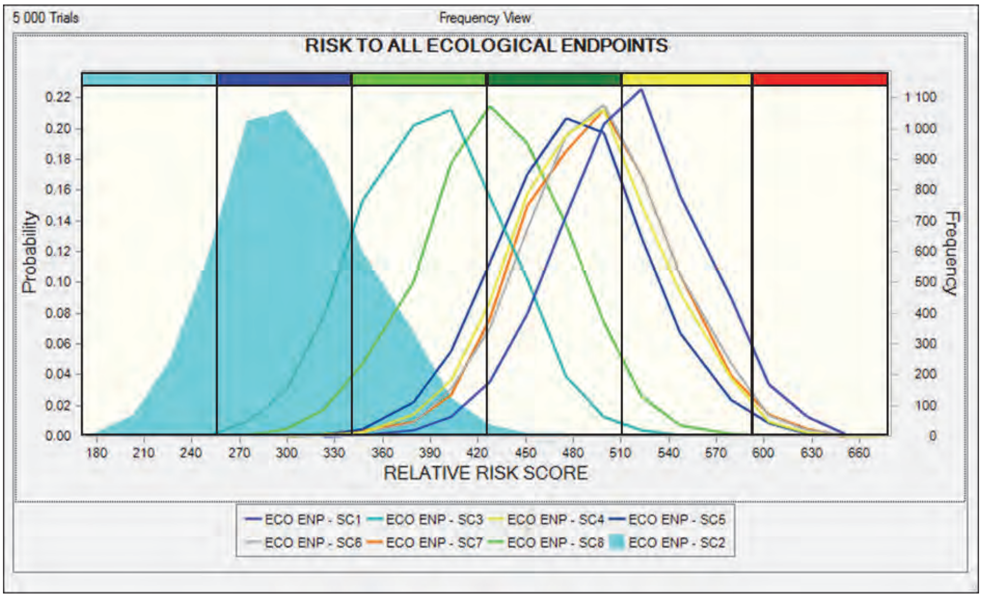


Figure 67: Integrated relative risk distributions of all ecological endpoints considered for seven of the scenarios with Natural conditions as the benchmark (light blue). Six category risk classes from no risk on left (pristine equivalent, Ecostatus class "A") to extreme risk on right (critical modification equivalent, Ecostatus class "F") is included.

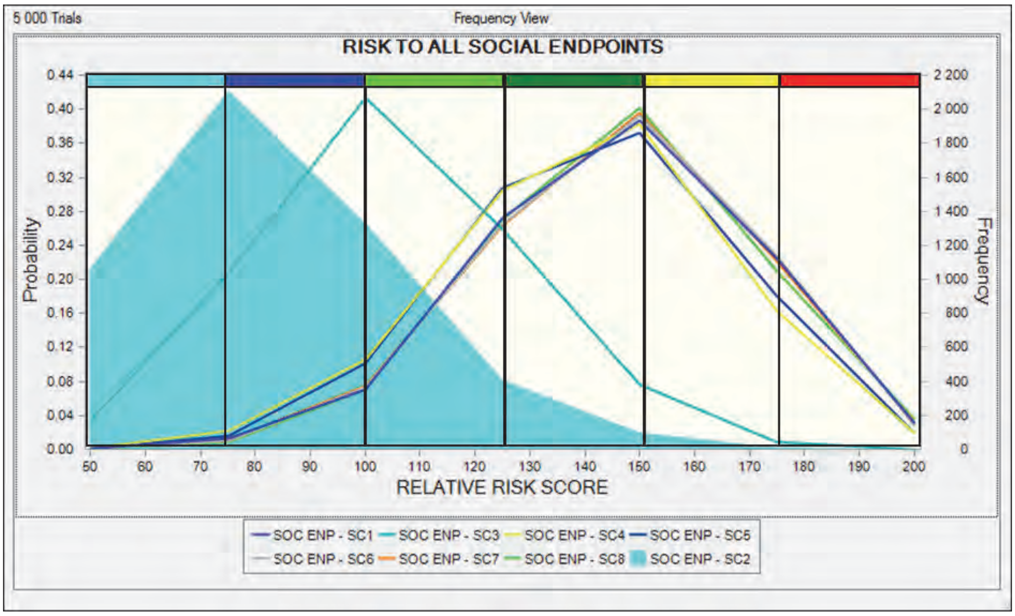


Figure 68: Integrated relative risk distributions of all social/human endpoints considered for seven of the scenarios with Natural conditions as the benchmark (light blue). Six category risk classes from no risk on left (pristine equivalent, Ecostatus class "A") to extreme risk on right (critical modification equivalent, Ecostatus class "F") is included.

Figure 69 presents the integrated risk posed to all ecological and social endpoints. The protective measures that were implemented to reduce overall risk to "D" category (Scenario

3), as well as the reduction in the role of the ecological importance and sensitivity of local ecosystem (Scenario 8), resulted in a considerable portion of SC4 and SC5 (mitigate AMD and WWTW) remaining in an "unacceptable state". However, as with the ecological endpoints, the highest probability of the integrated risk still remained in the sustainable "D" category. This indicates that the present state of the system is in the most degraded condition that is allowable.

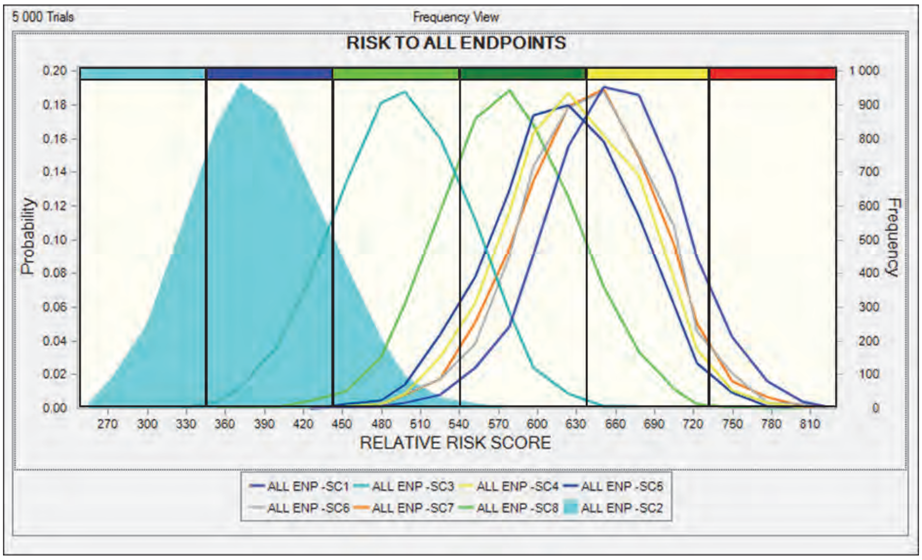


Figure 69: Integrated relative risk distributions of all the endpoints considered, for seven of the scenarios with Natural conditions as the benchmark (light blue). Six category risk classes from no risk on the left (pristine equivalent, Ecostatus class "A") to extreme risk on the right (critical modification equivalent, Ecostatus class "F"), is included.

The conceptual models with the BN nodes for each of the different scenarios are presented in Figures 69 to 76.

### 7.3 General conclusions

- In this study we demonstrated that the BN approach could effectively be used as a tool for water resource and conservation managers.
- We were able to demonstrate that the water resource management goals can be assessed against the backdrop of different scenarios. The trade-offs of cost and benefits can be evaluated in this way, e.g. it was demonstrated that even with mitigation of AMD in the Klip River, there would still not be any change to the macro-invertebrate status.
- The graphic nature of the interface and outputs coupled to the ability of the BN models to generate and evaluate alternative scenarios makes it a useful tool for resource management.

- The form of the risk distribution curves serves as a sensitivity analysis of the BN model outputs. It therefore provides an indication of where additional information will be required to reduce uncertainty.
- The generation of information to reduce the uncertainty in the risk predictions will in essence drive the structuring of future monitoring programmes, i.e. the monitoring becomes hypothesis driven.
- The new information generated by the monitoring can be used to update the input node descriptions and if necessary the rank scores making the BN model ideal for adaptive management application.
- The application of RRM-BN models can contribute to greater application of adaptive management practices in water resource and conservation management of the Klip River and Upper Vaal WMA.
- According to Ayre and Landis (2012) the application value within an adaptive management framework is due to the RRM-BN model communicating uncertainty in a quantifiable manner. The interactions of dispariate ecological values are visually observed through the graphical interface, and once the model has been developed it can easily be updated and refined by the resource manager. Thereby increasing ownership in the adaptive management process.

### 7.4 Recommendations

- The broad base risk distribution patterns are indicative of the degree of uncertainty related to the data used for scoring the input parent nodes, as well as the input distributions used to set up condition probabilities.
- These uncertainties can only be reduced by filling the knowledge gaps through hypothesis-driven fundamental research projects.
- Further reduction in uncertainty in particularly the Klip River catchment can be decreased through focused monitoring and field surveys.
- The focus of this study was primarily on ecological endpoints and since the RRM framework was based on both ecological as well as human health aspects it was not surprising that there was still overall high risk even when the factor contributing to the ecological risk were mediated. It is therefore essential that future studies should focus on the aspects that relate to both human health risk as well as economical risks. For example, what is the health risk associated with the consumption of fish from the Klip River system or consumption of products irrigated from surface and ground water from the system. Further what financial risks are associated when irrigation from Klip River water resources is stopped?  The RRM would allow for the evaluation of trade-offs be

between reducing human health risks by stopping irrigation and the loss of income through irrigation based agriculture.

Table 63: Condition probability table summarising the exposure and effect variable (input node) input distributions for all scenarios considered in the study.

| | EI_ES | | | | ALIEN_VEG | | | | MINES | | | | AMD_TREATMENT | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zero | Low | Med | High | Zero | Low | Med | High | Zero | Low | Med | High | Zero | Low | Med | High |
| Scenario 1 (Present state) | 1 | 9 | 25 | 65 | 1 | 9 | 25 | 65 | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 |
| Scenario 2 (Natural < 1900) | 1 | 20 | 59 | 20 | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 |
| Scenario 3 (Desired condition) | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 |
| Scenario 4 (Mitigate AMD) | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 |
| Scenario 5 (Mitigate WWTW) | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 |
| Scenario 6 (Reduce DTW Impacts) | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 |
| Scenario 7 (Increase DTW Impacts) | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 |
| Scenario 8 (Reduce EI/ES) | 65 | 25 | 9 | 1 | 1 | 20 | 59 | 20 | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 |

Table 63 Continued

| | INDUSTRY | | | | AGRI_DRYLAND | | | | AGRI_IRRIGATED | | | | FEED_LOTS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zero | Low | Med | High | Zero | Low | Med | High | Zero | Low | Med | High | Zero | Low | Med | High |
| Scenario 1 (Present state) | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 2 (Natural < 1900) | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 |
| Scenario 3 (Desired condition) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 |
| Scenario 4 (Mitigate AMD) | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 5 (Mitigate WWTW) | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 6 (Reduce DTW Impacts) | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 7 (Increase DTW Impacts) | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 8 (Reduce EI/ES) | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |

| | DAMS | | | | WWT | | | | INFRASTRUCTURE | | | | RURAL_SETTLE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zero | Low | Med | High | Zero | Low | Med | High | Zero | Low | Med | High | Zero | Low | Med | High |
| Scenario 1 (Present state) | 20 | 59 | 20 | 1 | 1 | 9 | 25 | 65 | 20 | 30 | 49 | 1 | 1 | 20 | 59 | 20 |
| Scenario 2 (Natural < 1900) | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 |
| Scenario 3 (Desired condition) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 |
| Scenario 4 (Mitigate AMD) | 20 | 59 | 20 | 1 | 1 | 9 | 25 | 65 | 20 | 30 | 49 | 1 | 1 | 20 | 59 | 20 |
| Scenario 5 (Mitigate WWTW) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 30 | 49 | 1 | 1 | 20 | 59 | 20 |
| Scenario 6 (Reduce DTW Impacts) | 20 | 59 | 20 | 1 | 1 | 9 | 25 | 65 | 20 | 30 | 49 | 1 | 20 | 59 | 20 | 1 |
| Scenario 7 (Increase DTW Impacts) | 20 | 59 | 20 | 1 | 1 | 9 | 25 | 65 | 20 | 30 | 49 | 1 | 1 | 9 | 25 | 65 |
| Scenario 8 (Reduce EI/ES) | 20 | 59 | 20 | 1 | 1 | 9 | 25 | 65 | 20 | 30 | 49 | 1 | 1 | 20 | 59 | 20 |

Table 63 Continued

| | URBAN_AREAS | | | | Flow_Intol_Spp | | | | EI_Habitat | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zero | Low | Med | High | Zero | Low | Med | High | Zero | Low | Med | High |
| Scenario 1 (Present state) | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 2 (Natural < 1900) | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 |
| Scenario 3 (Desired condition) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 |
| Scenario 4 (Mitigate AMD) | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 5 (Mitigate WWTW) | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 6 (Reduce DTW Impacts) | 20 | 59 | 20 | 1 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 7 (Increase DTW Impacts) | 1 | 9 | 25 | 65 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 8 (Reduce EI/ES) | 1 | 20 | 59 | 20 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 |

Table 63 Continued

| | ABN_Subsfish | | | | Salt_Intol_Spp | | | | Tox_Intol_Spp | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zero | Low | Med | High | Zero | Low | Med | High | Zero | Low | Med | High |
| Scenario 1 (Present state) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 1 | 20 | 59 | 20 |
| Scenario 2 (Natural < 1900) | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 |
| Scenario 3 (Desired condition) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 |
| Scenario 4 (Mitigate AMD) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 1 | 20 | 59 | 20 |
| Scenario 5 (Mitigate WWTW) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 1 | 20 | 59 | 20 |
| Scenario 6 (Reduce DTW Impacts) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 1 | 20 | 59 | 20 |
| Scenario 7 (Increase DTW Impacts) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 1 | 20 | 59 | 20 |
| Scenario 8 (Reduce EI/ES) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 |

| | TOURISM_REC | | | | EI_Fish | | | | EI_Inverts | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zero | Low | Med | High | Zero | Low | Med | High | Zero | Low | Med | High |
| Scenario 1 (Present state) | 20 | 59 | 20 | 1 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 2 (Natural < 1900) | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 | 65 | 25 | 9 | 1 |
| Scenario 3 (Desired condition) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 |
| Scenario 4 (Mitigate AMD) | 20 | 59 | 20 | 1 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 5 (Mitigate WWTW) | 20 | 59 | 20 | 1 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 6 (Reduce DTW Impacts) | 20 | 59 | 20 | 1 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 7 (Increase DTW Impacts) | 20 | 59 | 20 | 1 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 |
| Scenario 8 (Reduce EI/ES) | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 | 20 | 59 | 20 | 1 |

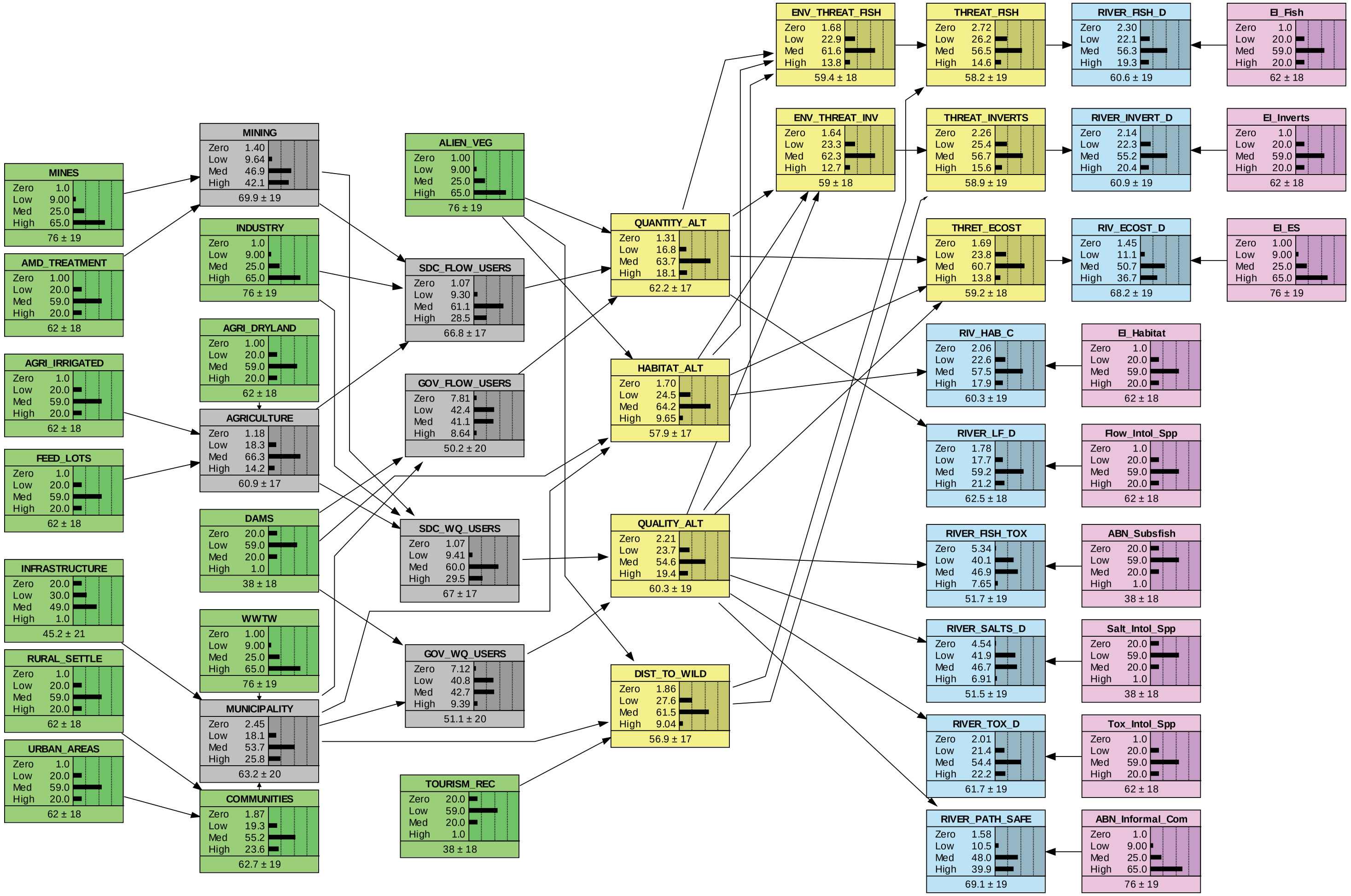


Figure 70: Bayesian Network belief network established in the study to evaluate the risk of source/stressors to endpoints under Scenario 1 – current conditions.

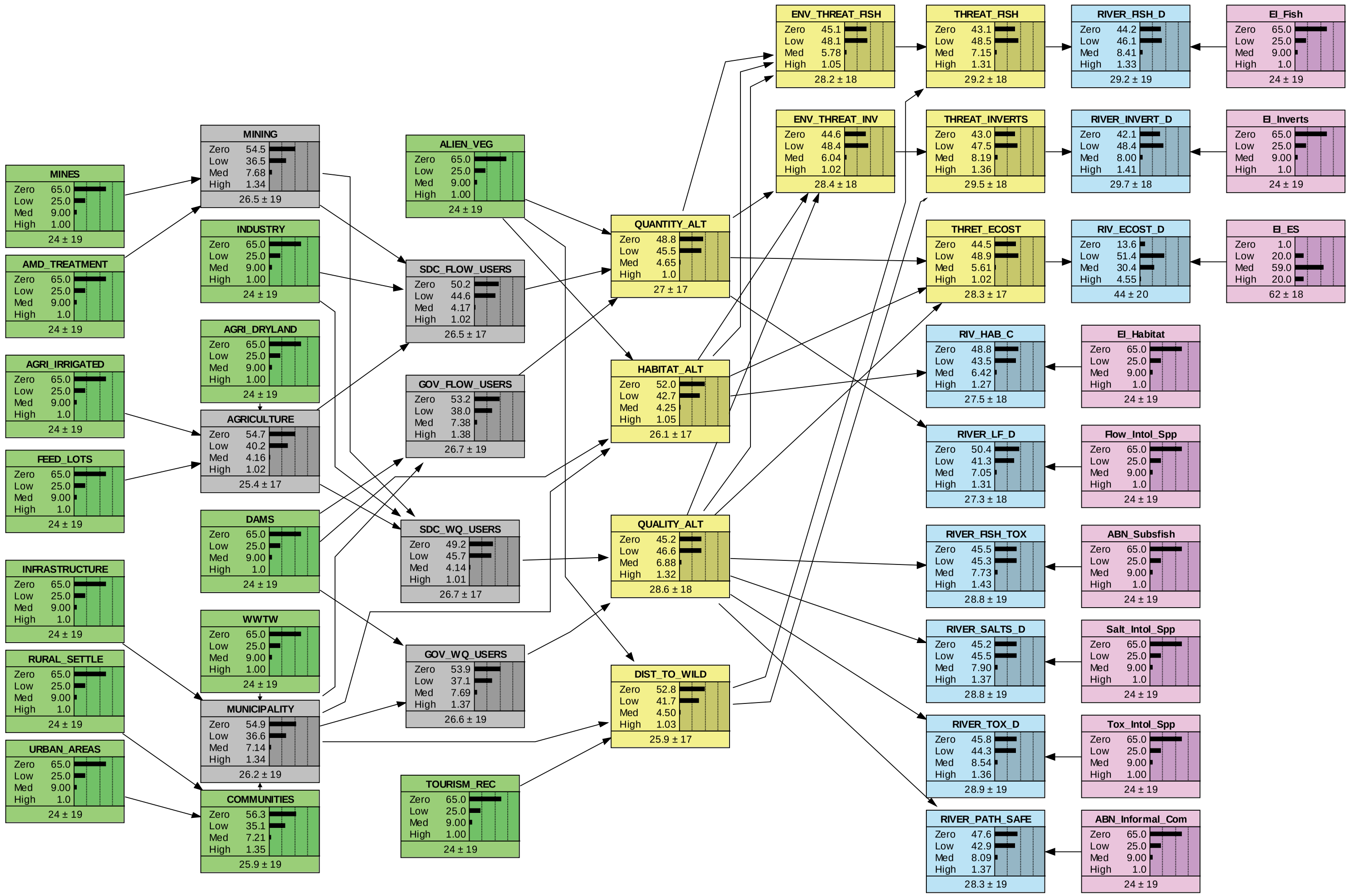


Figure 71: Bayesian Network belief network established in the study to evaluate the risk of source/stressors to endpoints under Scenario 2 – natural conditions.

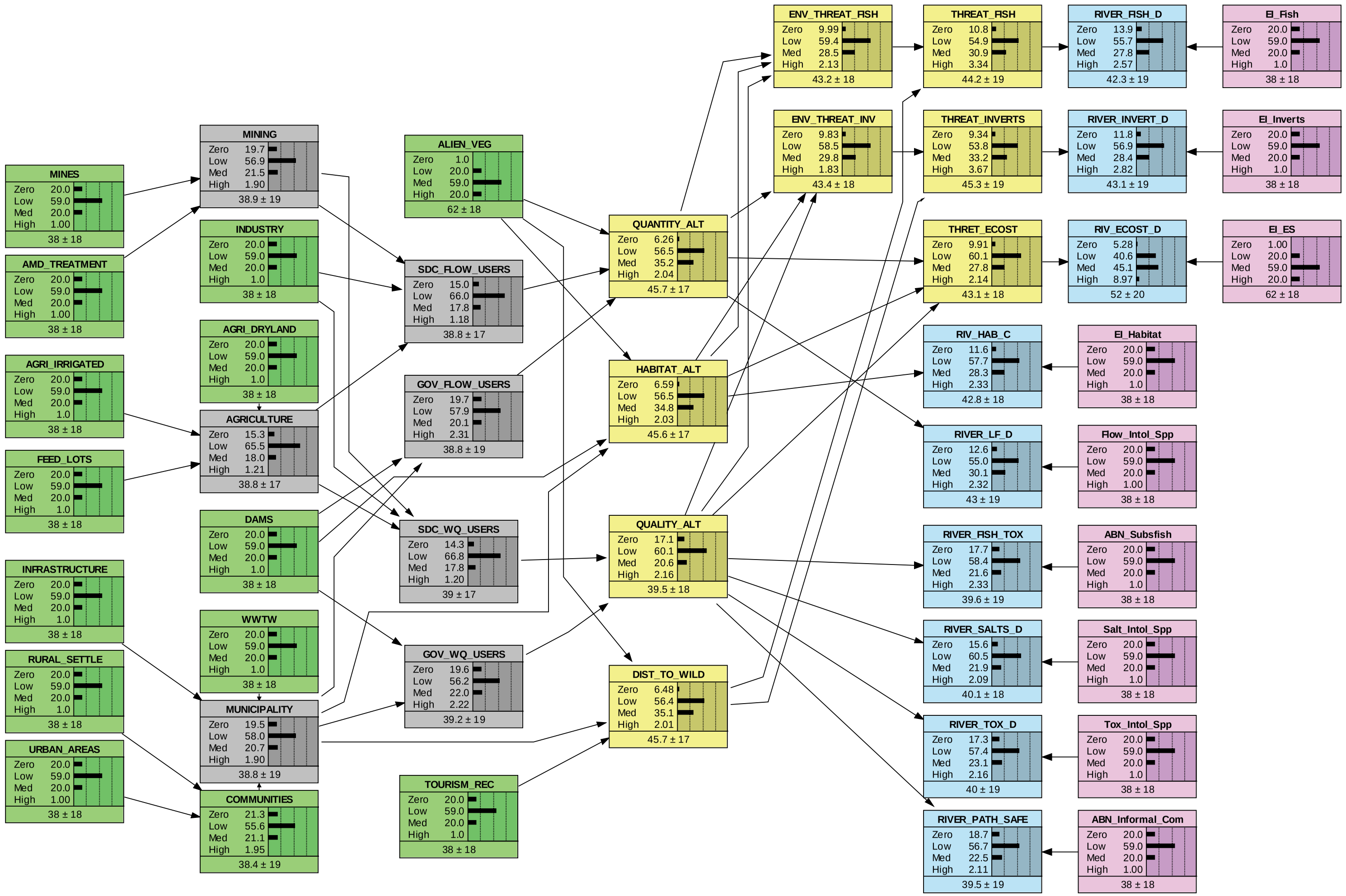


Figure 72: Bayesian Network belief network established in the study to evaluate the risk of source/stressors to endpoints under Scenario 3 – desired conditions.

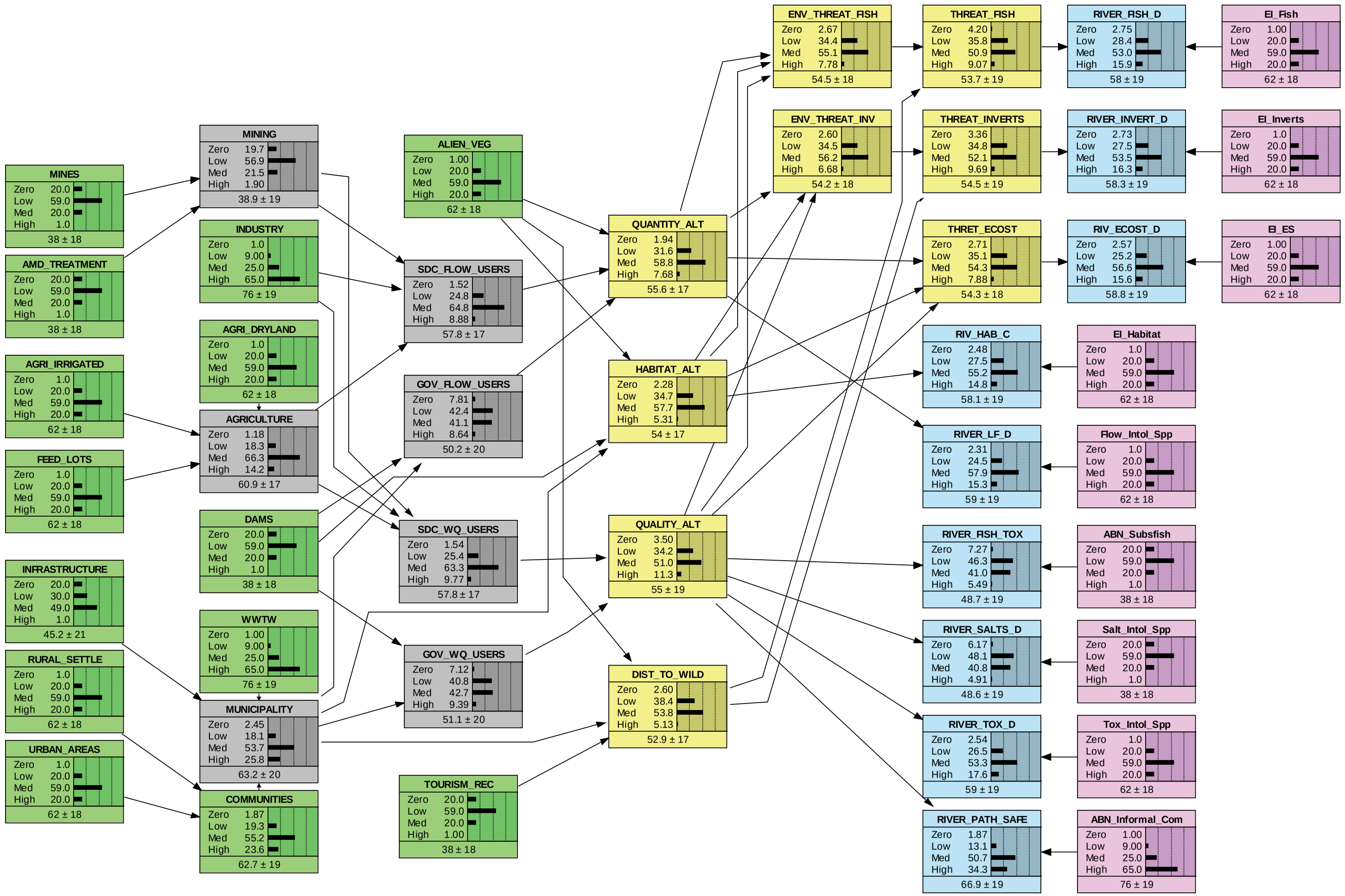


Figure 73: Bayesian Network belief network established in the study to evaluate the risk of source/stressors to endpoints under Scenario 4 – mitigation of mines.

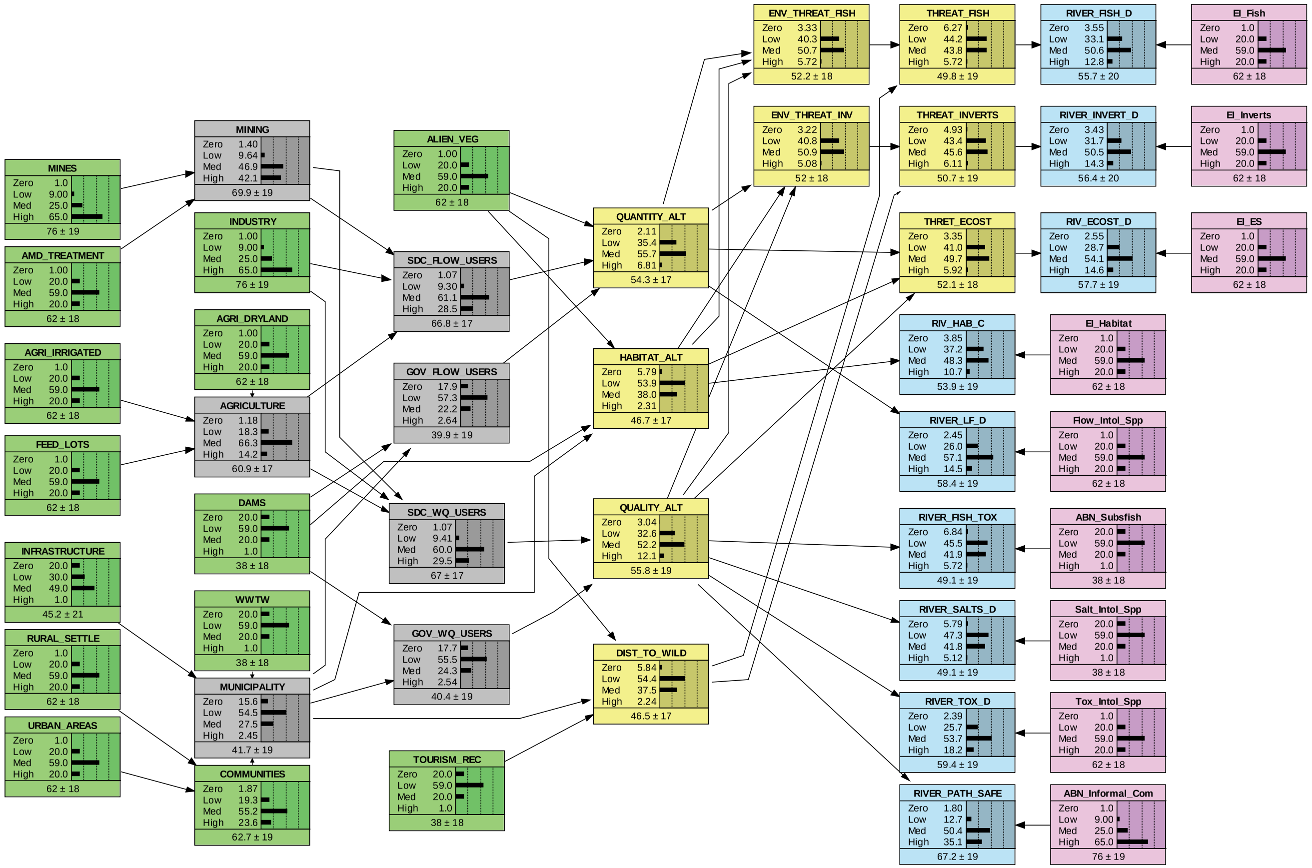


Figure 74: Bayesian Network belief network established in the study to evaluate the risk of source/stressors to endpoints under Scenario 5 – mitigation of WWTW.

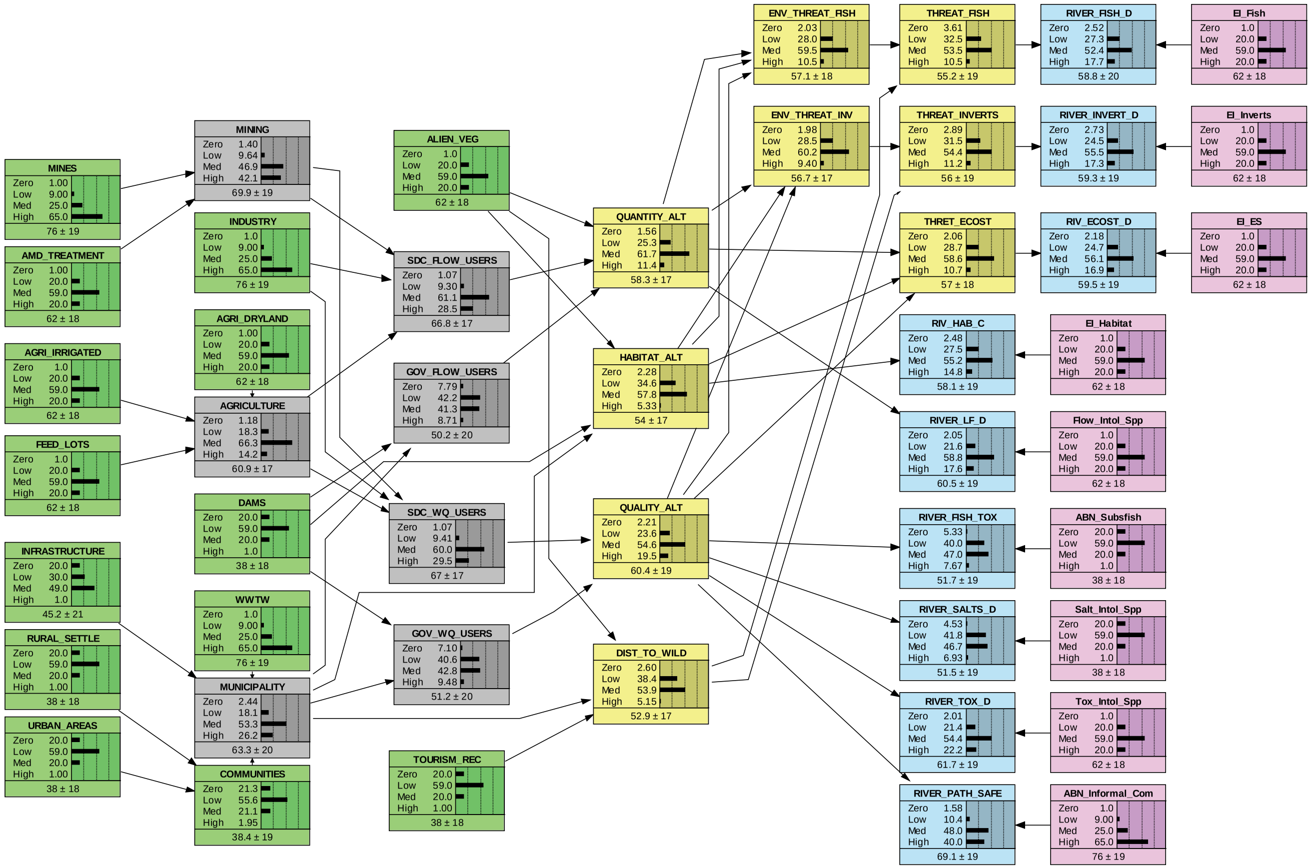


Figure 75: Bayesian Network belief network established in the study to evaluate the risk of source/stressors to endpoints under Scenario 6 – decrease the disturbance to wildlife.

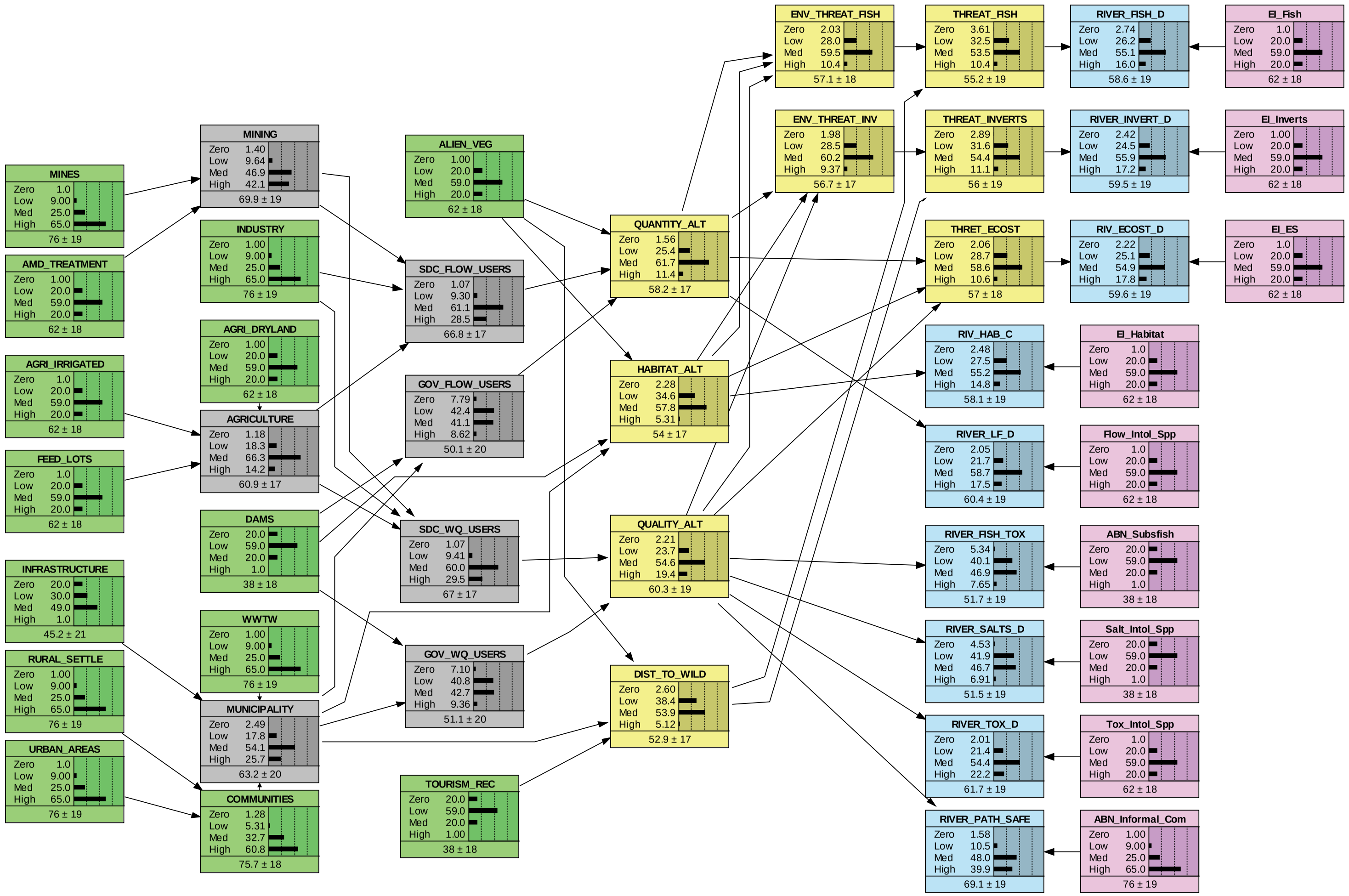


Figure 76: Bayesian Network belief network established in the study to evaluate the risk of source/stressors to endpoints under Scenario 7 – increase the disturbance to wildlife.

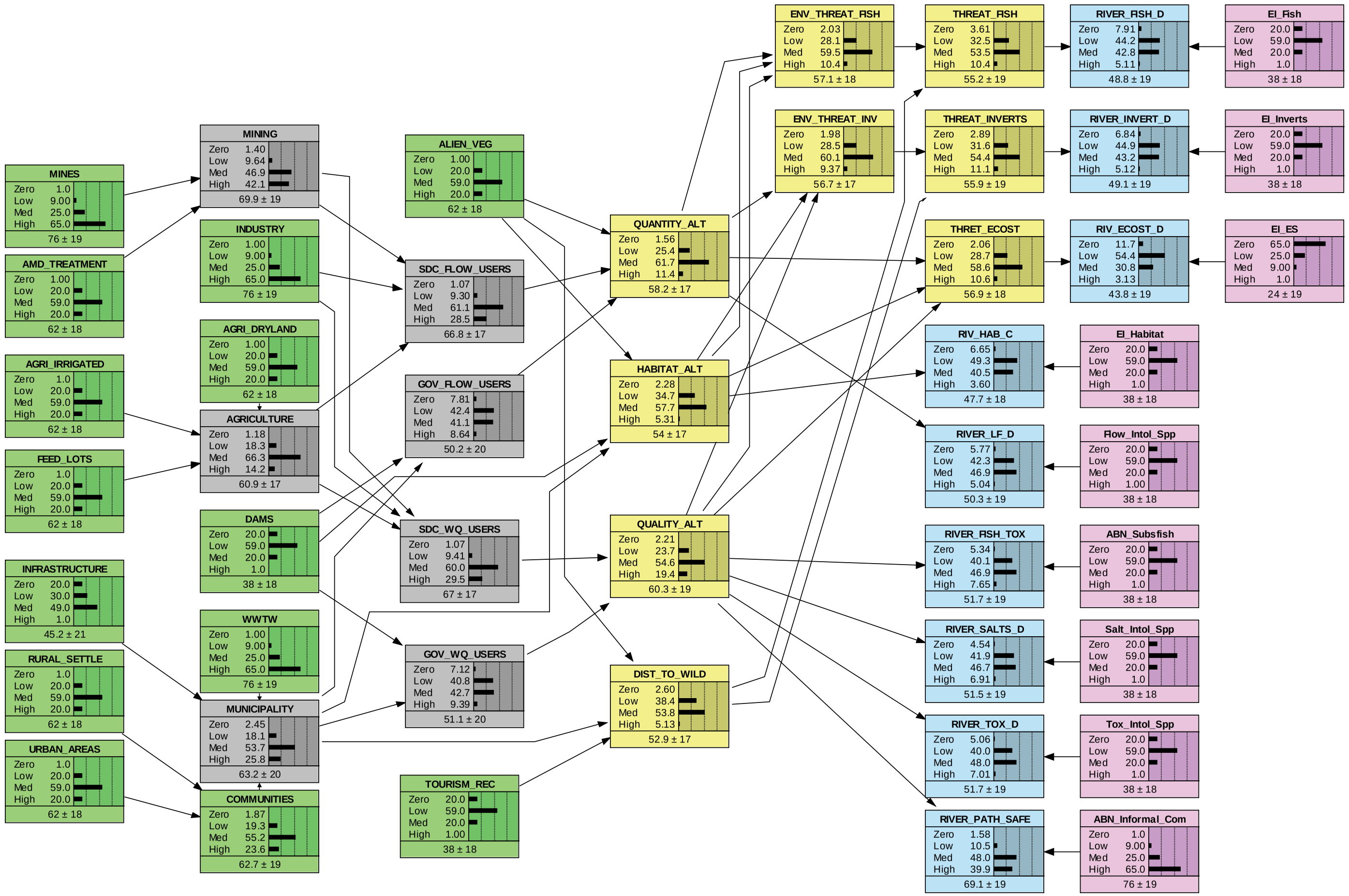


Figure 77: Bayesian Network belief network established in the study to evaluate the risk of source/stressors to endpoints under Scenario 8 – reduce the contribution of the ecological importance and sensitivity scores to the overall ecological status.

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