The macroinvertebrate diversity, chemical and physical factors in the Loop Spruit and Mooi River, North-West Province

JH Erasmus
22119809

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Supervisor: Prof CT Wolmarans
Co-supervisor: Prof V Wepener
Assistant supervisor: Prof KN de Kock

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It all starts here ™

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Abstract

The Loop Spruit, originating near Fochville, forms part of the Mooi River catchment in the North-West Province and ultimately forms part of the Vaal River catchment. This is a relatively small river with an average rainfall of 683 mm per annum, but serves as the main natural water source for many activities in the area including, agriculture. It is also a sink for mining effluent from several mines in the area. These circumstances may have a detrimental effect on the water quality of this river caused by metals originating from mining activities and nutrients produced by agricultural activities. These phenomena may also influence the ecological health, as well as the quality of potable water downstream. There is no State of River Report on the ecosystem health of the Mooi River or Loop Spruit catchment. Furthermore, little research has been done on the Loop Spruit, especially with regard to metal concentrations and macroinvertebrate diversity. The ten preselected sites were selected based on a variety of biotopes present in the rivers, availability of water and accessibility to the rivers. It included sites mostly located in natural areas, but also in areas mostly impacted by anthropogenic activities such as mining and sewage treatment plants.

The aim of this study was to determine the environmental quality and influence on the macroinvertebrates of the Loop Spruit, as well as that part of the Mooi River after the confluence of the Loop Spruit.

In order to determine the metal concentrations in the water and sediment at selected sites within the Loop Spruit catchment, three surveys were conducted during the dry seasons in July 2014, September 2014 and May 2015. Selected abiotic factors, including electrical conductivity, pH, temperature, turbidity, and flow-rate were measured *in situ* at each site. Metal concentrations in the sediment and water samples were determined using inductively coupled plasma mass spectrometry and were analysed for twelve metals considered potentially toxic to aquatic biota. Particle size determination was also done in order to determine the percentage composition of the total sediment sample. This was done using an Endecott dry sieving system with different mesh sized sieves. The clay particles (< 53 µm) were transferred for X-ray diffraction and scanning electron microscopy analyses. The results showed that quartz was the most abundant mineral with illite, muscovite and kaolinite, to name a few, occurring at different sites in smaller percentages. High percentages of silicon and oxygen were further found at all the sites

which could have been due to the high percentage of quartz located at every site, since quartz is composed of SiO_2. The same composition is also found in some of the other minerals. The results indicated that mining activities within the Loop Spruit catchment, could contribute to higher concentrations of selected metals in surface water at Site 2 and 4 (in close proximity to mines) while sediment concentrations of some metals were significant at these two sites. A decrease in concentration of these metals occurred downstream from the sites nearest to the mines, but the results also indicate that not only mining, but other anthropogenic activities such as agriculture and sewage treatment plant effluent can contribute to metal concentrations in the Loop Spruit.

To establish the diversity of aquatic macroinvertebrates and its association with selected abiotic factors and biotopes within the Loop Spruit, biota was collected during the three surveys at the ten sites using standard sampling methods in all available biotopes. Abiotic factors, biotope descriptions and vegetation types were noted at each site. The organisms were identified up to species level, whenever possible, otherwise up to genus or family level, using the aid of the guides to Freshwater Invertebrates of Southern Africa. All the specimens were counted and grouped into relevant orders. Sensitivity values were allocated to the families and classified into three classes: tolerant, moderately sensitive and highly sensitive towards organic pollution. Species Richness, Shannon-Wiener and Pielou's evenness indices were used to describe the community structure of the organisms. A total of 137 taxa within 72 families were collected during this study and the family assemblages were relatively consistent. The results indicated that 16 families occurred most commonly, while the majority of these preferred low to very low water quality regarding organic enrichment. Exceptions to this were Baetidae and Hydropsychidae, which indicate good water quality when represented by two or more species at a specific site. Dytiscidae, Tubificidae and Chironomidae, to name only a few, occurred at a majority of the sites during all three surveys. In contrast to this, 19 of the 72 families only occurred during one survey at less than five of the ten sites. This could be ascribed to several reasons including their preference for high water quality and sensitivity towards organic enrichment. A further temporal variation was noted at some of the sites and also a clear spatial variation. Highly sensitive taxa were represented at only two sites, while moderately sensitive taxa were present, to a lesser extent and tolerant taxa occurring in abundance at several sites. These results indicate that the Loop Spruit is largely organically enriched, enabling the tolerant taxa to thrive, but the impact was not to such an extent as to prohibit the occurrence of moderately sensitive taxa.

Possible associations between metal concentrations in functional feeding groups and habitat preference (benthic or pelagic) in selected macroinvertebrate families were also investigated. This was achieved by using inductively coupled plasma mass spectrometry analysis. Caenidae differed significantly ($p < 0.05$) from all the other families and high concentrations were further found in Simuliidae and Chironomidae, all categorised as benthic organisms. Low concentrations of the majority of metals were found in macroinvertebrates classified as predators. From the results obtained during this study a significant variation ($p < 0.05$) in metal concentrations were evident between functional feeding groups, as well as between benthic and pelagic macroinvertebrate families. The fact that some metals do not biomagnify within the food chain can be ascribed to the lower concentrations found in the predator families. The lower trophic levels (scraper/grazer, shredder, collector-gatherer and collector-filterer FFGs) had significantly higher ($p < 0.05$) metal concentrations. The benthic families also had significantly higher ($p < 0.05$) metal concentrations than the pelagic families in the majority of the metals. Although these metals are all considered as potentially toxic to aquatic biota, these high concentrations may not have had a detrimental effect possibly due to strategies such as elimination, detoxification, as well as metabolisation.

The main aim of the study was successfully achieved through 1) determining the primary lithology and secondary minerals of the area surrounding the study area, in order to establish the metals which originate from mining activities or from natural weathering; 2) determining *in situ* water quality and metal concentrations in water at each site; 3) determining the physical characteristics and metal concentrations in sediments from the selected sampling sites; 4) determining the aquatic macroinvertebrate diversity within the study area; 5) determining metal bioaccumulation in selected macroinvertebrates from an impacted site; and 6) finally to establish a relationship between measured environmental factors and the aquatic macroinvertebrate community structure. These results can serve as a baseline for future studies in this respect.

Keywords: aquatic macroinvertebrates, Loop Spruit, Mooi River catchment, North-West Province, mining effluent, anthropogenic activities, metal bioaccumulation.

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Chapter 1: Introduction

1.1 Background

Freshwater is a precious commodity for humans and every other type of living organism. Without water life could not be sustained, therefore it is essential to manage water to ensure a healthy society, as well as a healthy ecosystem. Although freshwater comprise only about 2.5 % of the Earth's water (Gleick, 1993; Griffiths *et al*., 2015), merely 0.3 % is surface water found in wetlands, lakes and rivers (Griffiths *et al*., 2015). The demand for water increases every year due to population increases, an increase in food production and a growing economy that goes hand in hand with more intense industrial activities. These water bodies are not just under severe threat of over exploitation, but also by polluting it with industrial and sewage effluents which include, amongst others, chemicals, high concentrations of nutrients, as well as agricultural runoff (Griffiths *et al*., 2015). Habitat destruction, altering of flow and impoundments in river systems also pose a threat to the ecosystem health of a river, which will affect the water quality, impacts not only found in developed countries, but also in developing countries.

In South Africa, a developing country, it is even more challenging to address the problem since it is classified as a semi-arid country (DWAF, 1999). According to DWAF (1999), South Africa is the 30th driest country in the world, with an average rainfall of 497 mm per year in contrast to the global average rainfall of 860 mm per year. According to the River Health Program (2013) and DWA (2013), 53 rivers exhibit a critically endangered ecosystem status, 25 rivers are endangered and 20 are vulnerable, while only 20 rivers have a status considered as least threatened. It is therefore of critical importance that the over exploitation of all surface waters must be prevented and the usage of water be optimally managed to ensure the maintenance of healthy biological assemblages.

The Loop Spruit, originating near Fochville, is situated in the Gauteng and North-West Provinces and is part of the Mooi River catchment area which again forms part of the Vaal River catchment area (van der Walt *et al*., 2002; Merafong City Local Municipality, 2014; Tlokwe City Council, 2014). Although this stream is relatively small due to the fact that the average evaporation potential (1 650 mm) exceeds that of the average rainfall (683 mm) (van der Walt *et al*., 2002), it serves as the main water source for nearby agricultural activities, while it also acts as a sink for the effluent of several mines in the area. These circumstances may have a detrimental effect on the water quality of this stream caused by metals originating from mining activities and nutrients produced by agricultural activities. These phenomena may also influence the ecological health, as well as the quality of potable water downstream. Although DWAF (1999) developed a programme (River Health Programme) in 1994 to determine and monitor the ecosystem health of South African river systems, no State of Rivers report was compiled on the Mooi River catchment area, or on the Loop Spruit (River Health Programme, 2013).

Although extensive research has been done on the Wonderfontein Spruit (van Veelen, 2009; Hamman, 2012; DWS, 2014; Tlokwe City Council, 2014), also a tributary of the Mooi River, little research has been done on the Loop Spruit, with regard to metal concentrations and macroinvertebrate diversity.

1.2 Hypotheses

The hypothesis for Chapter 3 states that mining activities in the upper catchment results in an increase in metals in water and sediment of the Loop Spruit. The aim of this chapter was thus to determine the metal concentrations in the water and sediment at selected sites within the Loop Spruit.

For Chapter 4 the hypothesis states that, the macroinvertebrate community structure will be altered by mining activities from the upper reaches of the Loop Spruit. The aim of this chapter was then to establish the diversity of aquatic macroinvertebrates and its association with selected abiotic factors and biotopes within the Loop Spruit.

The hypotheses stated for Chapter 5 were the following: 1) Feeding groups differ in their ability to accumulate metals. 2) Benthic macroinvertebrates will accumulate higher metal concentrations than pelagic macroinvertebrates.

1.3 Aims and objectives

The aim of this study was therefore to determine the environmental quality and influence on the macroinvertebrates of the Loop Spruit, as well as that part of the Mooi River after the confluence of the Loop Spruit. This will be achieved through the following objectives:

- Determining the primary lithology and secondary minerals of the area surrounding the study area in order to establish the metals which originate from mining activities or from natural weathering.
- Determining *in situ* water quality and metal concentrations in water at each site.

- Determining the physical characteristics and metal concentrations in sediments from the selected sampling sites.
- Determining the aquatic macroinvertebrate diversity within the study area.
- Determining metal bioaccumulation in selected macroinvertebrates from an impacted site.
- Establishing a relationship between measured environmental factors and the aquatic macroinvertebrate community structure.

Chapter 2: Study area and site description

2.1 Study area

The Mooi River catchment area is situated in the western part of the Gauteng Province and in the North-West Province (van der Walt *et al*., 2002), with a relatively flat topography, which have an elevation range of 1 520 m in the north and 1 300 m in the southwest (van Veelen, 2009; Tlokwe City Council, 2014). This catchment area is situated in the Upper Vaal Water Management Area (Merafong City Local Municipality, 2014; Tlokwe City Council, 2014) and has an annual rainfall of 683 mm, but an average evaporation potential of 1 650 mm (van der Walt *et al*., 2002). According to Cilliers and Bredenkamp (2000), the mean temperatures of the catchment area ranges from > 32 °C in the summer months to -1 °C in the winter months and frost occurs frequently in the winter. The Mooi River catchment area consists of the Mooi River, with two main tributaries, the Wonderfontein Spruit in the northeast and the Loop Spruit in the southeast (van Veelen, 2009; Merafong City Local Municipality, 2014; Tlokwe City Council, 2014).

These two main tributaries are separated by the Gatsrand geological ridge, - a steep, rocky ridge, which contains some of the richest gold reserves in South Africa (Gauteng Department of Agriculture and Rural Development, 2011; Tlokwe City Council, 2014). Several goldmines are situated on this ridge, including Tau Tona, Savuka, Deelkraal, Elandsrand and Blyvooruitsig mines on the northern side and Mponeng mine on the southern side of the Gatsrand ridge.

The Loop Spruit originates from various springs approximately 8 km northeast of the town Fochville in the southwestern part of the Gauteng Province. The Kraalkop Spruit, a tributary of the Loop Spruit, originates about 4 km north of Fochville and flows east of the town until it joins the Loop Spruit in the Piet Viljoen Dam, south of the town. A second, ephemeral tributary, the Leeu Spruit, joins the Loop Spruit before the informal settlement of Kokosi, west of Fochville and meanders through the settlement. Another stream that feeds the Loop Spruit, originates on the Kraalkop nature reserve and flows south of Mponeng and joins the Loop Spruit on a farm approximately 2.4 km west of Fochville's sewage treatment plant. The Loop Spruit and its main tributary, the Ensel Spruit, provide water to the Klipdrift Dam, where the water is used for irrigation purposes in the surrounding area (Tlokwe City Council, 2014). Further downstream of the Klipdrift Dam, the Loop Spruit provides water to the Modder Dam, which is also used for irrigation purposes before its confluence with the Mooi River, approximately 500 m upstream of the Potchefstroom's sewage treatment plant (Tlokwe City Council, 2014). The Mooi River flows into the Vaal River 20 km south of Potchefstroom (Tlokwe City Council, 2014).

The study was conducted at ten preselected sites (Figure 2.1), of which eight sites are located within the Loop Spruit and its tributaries and the rest in the lower reaches of the Mooi River. Site selection was based on the availability of water, accessibility to the rivers, and includes sites mostly located in natural areas, but also in areas mostly impacted by anthropogenic activities such as mining and sewage treatment plants. The availability of different biotopes was also taken into consideration in the process of site selection, to ensure that all the different biotopes represented throughout the river system were sampled. One of the sites (i.e. Site 8) is located below an impoundment in order to evaluate the possible effects of the impoundment on the river and the downstream habitat. Detailed site descriptions that include coordinates, available biotopes, lithology and dominant marginal and aquatic vegetation are provided in Tables 2.1 to 2.10.

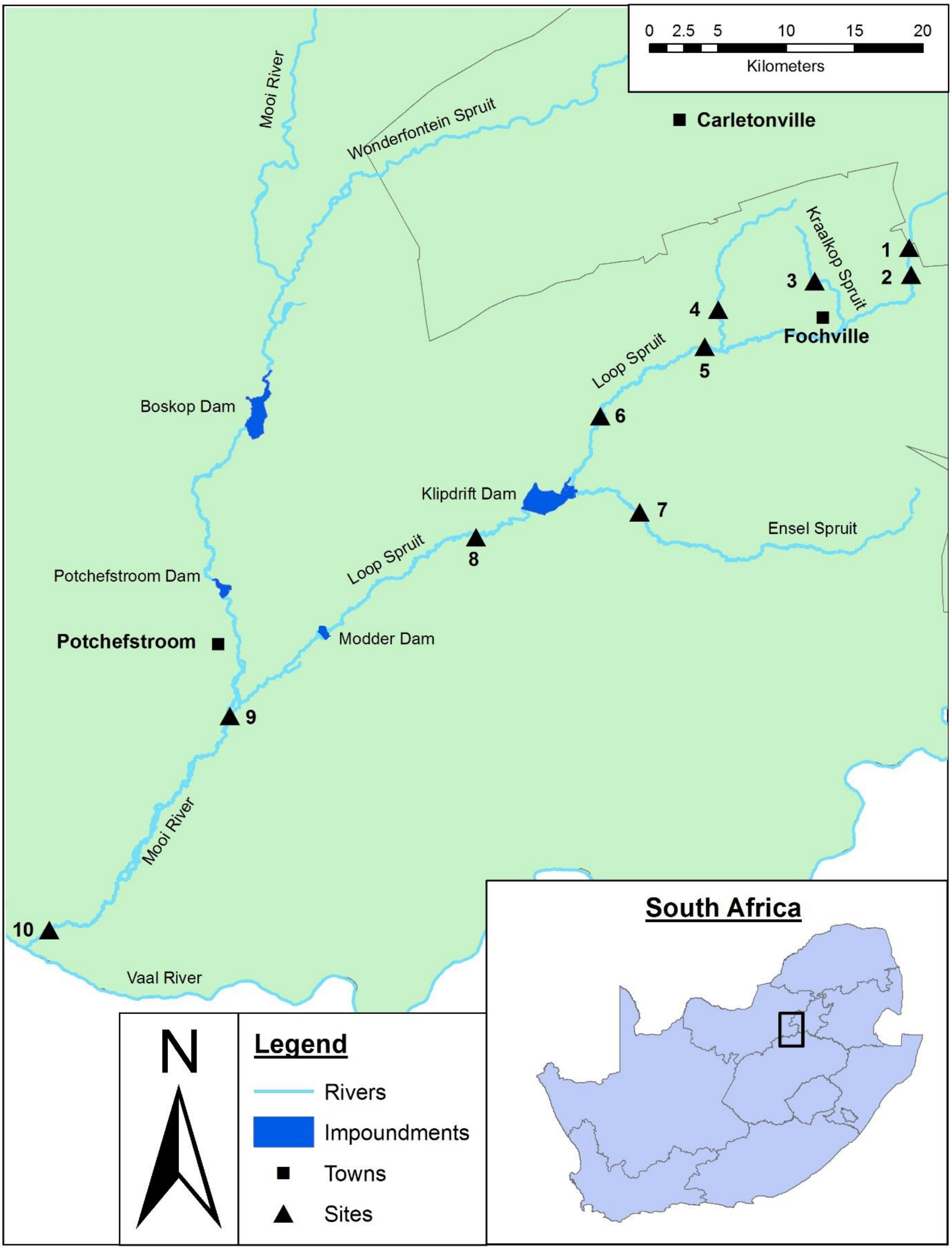


Figure 2.1: Map of the study area and the sampling sites.

2.2 Site description

<u>Table 2.1:</u> Physical characteristics of Site 1.

Site 1 Loop Spruit near the spring, Fochville area.	
	
Coordinates	S26˚25’49.3” E27˚33’09.4”
Height above sea level	1 555 m
Site description	This site is located on a farm approximately 8 km northeast of Fochville. Several of the springs that feed the Loop Spruit are located on this farm. Few anthropogenic impacts are present.
Biotope description	Headwater zone, sandy/muddy substratum, marginal and aquatic vegetation with algae in the water and a run biotope.
Primary lithology	Ferruginous shale, Hornfels (Keyser, 1986).
Dominant vegetation	*Ludwigia adscendens diffusa, Myriophyllum aquaticum, Rumex conglomeratus, Spirodela polyrhiza, Spirogyra* sp., *Typha capensis* and *Veronica annagallis-aquatica*.

Table 2.2: Physical characteristics of Site 2.

Site 2 Loop Spruit where mine effluent enters the river.	
	
Coordinates	S26˚25'54.5" E27˚33'08.7"
Height above sea level	1 553 m
Site description	This site is located about 300 m downstream of Site 1 and was selected to investigate the influence of gold mine discharge from an underground gulley, on the Loop Spruit.
Biotope description	Headwater zone, sandy streambed, marginal and aquatic vegetation with algae in the water and a run biotope.
Primary lithology	Andesite, agglomerate and tuff (Keyser, 1986).
Dominant vegetation	*Lagarosiphon major, Marsilea* sp., *Myriophyllum aquaticum, Nasturtium officinale, Rumex conglomeratus, Spirodela polyrhiza, Spirogyra* sp., *Typha capensis* and *Veronica annagallis-aquatica.*

Table 2.3: Physical characteristics of Site 3.

Site 3 Kraalkop Spruit just north of Fochville.	
	
Coordinates	S26˚27'53.7" E27˚29'30.5"
Height above sea level	1 505 m
Site description	This site is located in the Kraalkop Spruit just north of Fochville before flowing into an impoundment. This site was selected to gain information on the possible influence of the Kraalkop Spruit on the Loop Spruit. Surrounding land uses consist of livestock farming.
Biotope description	Middle water zone, overhanging tree canopy, marginal and aquatic vegetation with algae, sandy substratum and a pool biotope.
Primary lithology	Ferruginous shale, quartzite (Keyser, 1986).
Dominant vegetation	*Juncus lomatophyllus, Rumex conglomeratus, Spirogyra* sp., *Typha capensis* and *Veronica annagallis-aquatica.*

Table 2.4: Physical characteristics of Site 4.

Site 4 Tributary of the Loop Spruit.	
	
Coordinates	S26˚28'41.7" E27˚25'44.4"
Height above sea level	1 460 m
Site description	This site is located in a tributary that meanders past the Anglo Ashanti goldmine, Mponeng. Various anthropogenic activities are present in the surrounding area, including a scrapyard, as well as agricultural activities. The stream flows underneath the busy N12 route between Potchefstroom and Johannesburg.
Biotope description	Middle water zone, sandy substratum, overhanging tree canopy, marginal and aquatic vegetation with algae, sand, run and pool biotopes.
Primary lithology	Soil cover, alluvium (Keyser, 1986).
Dominant vegetation	*Nasturtium officinale, Potamogeton pusillus, Rumex conglomeratus, Spirodela polyrhiza, Veronica annagallis-aquatica* and *Zygnema* sp.

Table 2.5: Physical characteristics of Site 5.

Site 5 Loop Spruit on the farm Lepat.	
	
Coordinates	S26˚30'44.9" E27˚25'38.9"
Height above sea level	1 422 m
Site description	This site is located on a farm west of Fochville after the confluence of the tributary in which Site 4 was selected. Site 5 is downstream of Fochville, as well as Kokosi's sewage treatment plants. Water is abstracted for irrigational purposes.
Biotope description	Middle water zone, sandy substratum, stones in and out of current, marginal and aquatic vegetation, riffle and run biotopes.
Primary lithology	Soil cover, alluvium (Keyser, 1986).
Dominant vegetation	*Arundo donax, Nasturtium officinale, Phragmites australis, Rumex conglomeratus, Spirodela polyrhiza* and *Veronica annagallis-aquatica.*

Table 2.6: Physical characteristics of Site 6.

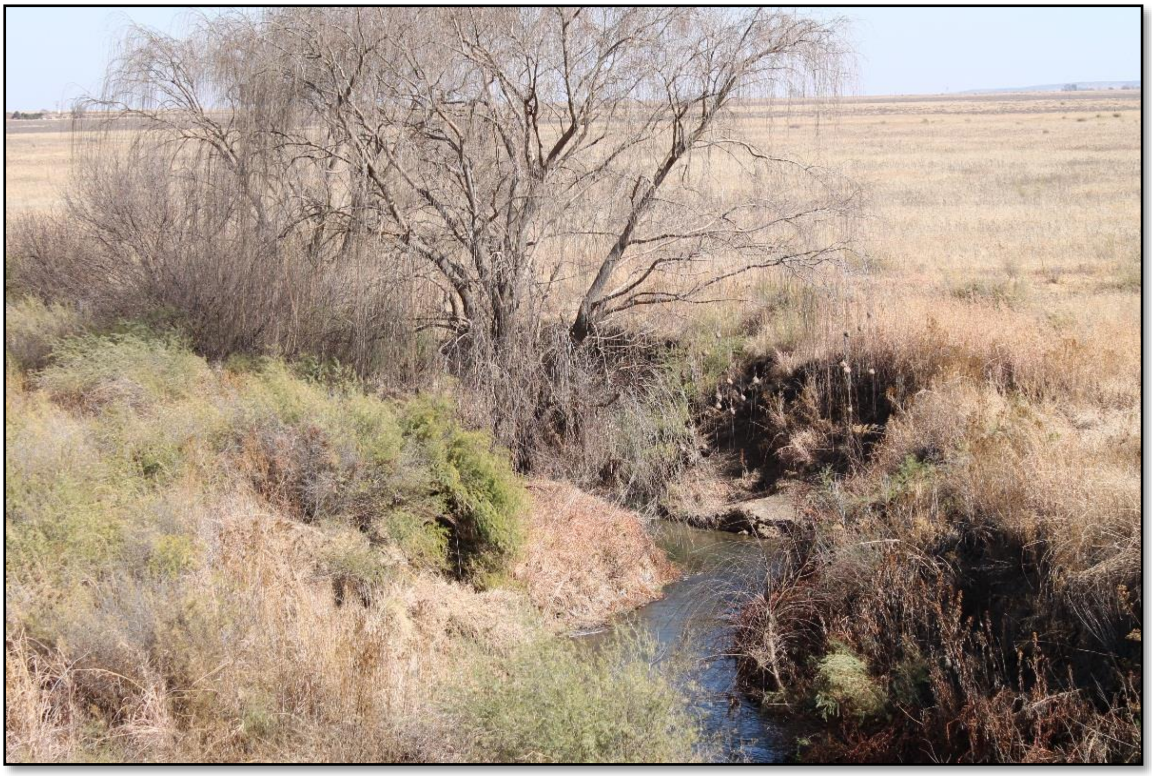
Site 6 Loop Spruit before Klipdrift Dam.	
	
Coordinates	S26˚33'22.6" E27˚20'31.5"
Height above sea level	1 388 m
Site description	This site in the Loop Spruit is located a few kilometres upstream from Klipdrift Dam. The land surrounding the river at this site is mainly used for agricultural purposes, which include crops and livestock farming. Several poultry farms are located approximately 4 km upstream of this site.
Biotope description	Middle water zone, overhanging tree canopy and marginal vegetation with algae, sandy substratum, stones in current, riffle and run biotopes.
Primary lithology	Soil cover, alluvium (Keyser, 1986).
Dominant vegetation	*Nasturtium officinale, Spirodela polyrhiza, Spirogyra* sp. and *Veronica annagallis-aquatica.*

Table 2.7: Physical characteristics of Site 7.

Site 7 Ensel Spruit before Klipdrift Dam.	
	
Coordinates	S26˚37’08.3” E27˚22’16.2”
Height above sea level	1 390 m
Site description	The Ensel Spruit, which is the main tributary of the Loop Spruit, was surveyed at this site which is located on the R54 between Potchefstroom and Vereeniging, approximately 28 km northeast from Potchefstroom. The reason for selection of this site was to determine the possible influence of the Ensel Spruit on water conditions in the Loop Spruit. The surrounding land use includes crop farming and other agricultural activities. Both the Ensel Spruit and the Loop Spruit supply water to the Klipdrift Dam.
Biotope description	Lower water zone, sandy/muddy substratum, overhanging tree canopy, marginal vegetation, algae in water and a pool biotope.
Primary lithology	Soil cover, alluvium (Keyser, 1986).
Dominant vegetation	*Cyperus dives, Myriophyllum aquaticum, Phragmites mauritianus, Spirogyra* sp., *Typha capensis* and *Veronica annagallis-aquatica.*

Table 2.8: Physical characteristics of Site 8.

Site 8 Loop Spruit below Klipdrift Dam.	
	
Coordinates	S26˚37'57.3" E27˚15'15.1"
Height above sea level	1 356 m
Site description	This site located 4.7 km downstream of the embankment of Klipdrift Dam was selected to study the possible influence of this dam on the river. This dam is mainly used for irrigation purposes, while the surrounding area is used for intensive agricultural purposes.
Biotope description	Middle water zone, muddy substratum, marginal vegetation and run biotope.
Primary lithology	Soil cover, alluvium (Keyser, 1986).
Dominant vegetation	*Cyperus dives, Persicaria lapathifolia, Phragmites australis* and *Typha capensis.*

Table 2.9: Physical characteristics of Site 9.

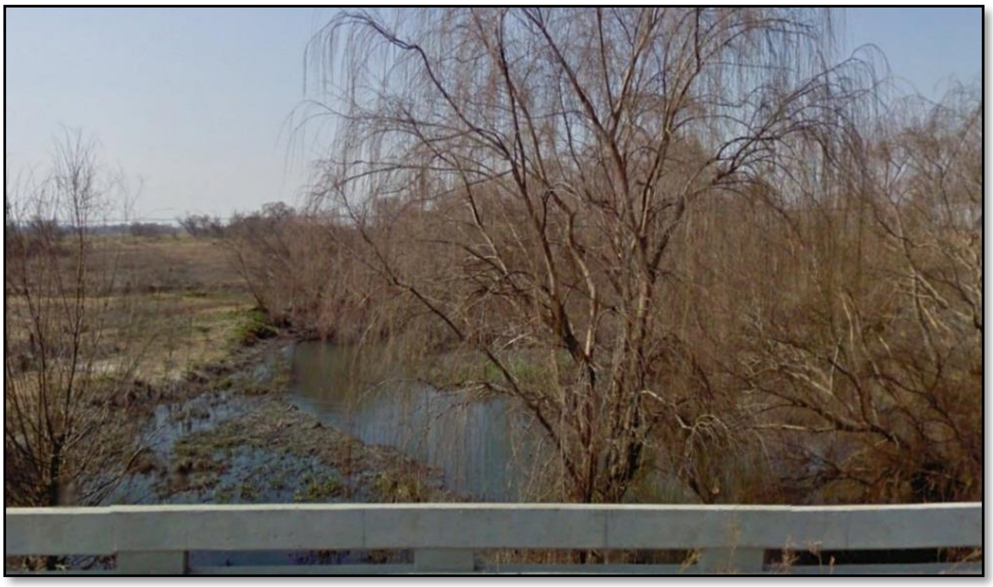
Site 9 Mooi River, after its confluence of the Loop Spruit.	
	
Coordinates	S26˚45'08.6" E27˚06'01.2"
Height above sea level	1 324 m
Site description	This site is located in the Mooi River, just outside Potchefstroom on the R501 to Viljoenskroon. The town's sewage treatment plant is located approximately 500 m west from this site. The Loop Spruit confluences with the Mooi River approximately 1.2 km upstream from this site. The Wasgoed Spruit, which receives industrial effluent, enters the Mooi River 5.7 km upstream from this site and its influence can be assessed at Site 9. Effluent from the town's sewage treatment plant enters the Mooi River downstream from this site.
Biotope description	Lower water zone, clay substratum, overhanging tree canopy with marginal vegetation and algae in water, run and pool biotopes.
Primary lithology	Soil cover, alluvium (Keyser, 1986).
Dominant vegetation	*Cyperus dives, Phragmites mauritianus, Rumex conglomeratus, Spirogyra* sp. and *Typha capensis.*

Table 2.10: Physical characteristics of Site 10.

Site 10 Mooi River before its confluence with the Vaal River.	
	
Coordinates	S26˚52'50.2" E26˚57'51.0"
Height above sea level	1 302 m
Site description	This site is located at Kromdraai, 1.3 km before the Mooi River enters the Vaal River and was chosen in order to assess the water quality of the Mooi River before its confluence with the Vaal River. Farms located between Sites 9 and 10, extracts water from canals provided by Boskop, as well as Potchefstroom Dam.
Biotope description	Lower water zone, sandy substratum, overhanging tree canopy, marginal and aquatic vegetation, algae in the water and riffle and run biotopes.
Primary lithology	Soil cover, alluvium (Keyser, 1986).
Dominant vegetation	*Cyperus dives, Phragmites australis, Phragmites mauritianus, Potamogeton pusillus, Spirogyra* sp.*, Typha capensis* and *Veronica annagallis-aquatica.*

Chapter 3: Geochemical assessment of water and sediment in the Loop Spruit.

3.1 Introduction

To support life within an aquatic environment and enable it to be suitable for different uses, water needs to contain various trace elements (Chapman, 1998). Metals such as copper (Cu), manganese (Mn) and zinc (Zn) are important to perform physiological functions and regulate various biochemical processes in living organisms, when they are present within water sources in trace concentrations (Chapman, 1998). When these metals enter natural waters from various anthropogenic sources like industrial and mining effluent or sewage discharge, in excessive concentrations, it can have detrimental effects on the aquatic environment, as well as on humans (Chapman, 1998; Hoffman *et al*., 2002; Griffiths *et al*., 2015). According to literature (Runnells *et al*., 1992; Stumm & Morgan, 1996; Chapman, 1998; Griffiths *et al*., 2015) water pollution, as a result of metals from anthropogenic sources, is increasing and have serious ecological effects on aquatic environments worldwide. This is aggravated due to the fact that there is no natural elimination process for metals (Chapman, 1998).

Several metals that occur within freshwater systems in trace amounts, are however present due to the natural weathering of rocks, soils and minerals (Runnells *et al*., 1992; Stumm & Morgan, 1996; Chapman, 1998; Tchounwou *et al*., 2012; Griffiths *et al*., 2015). Metals in the aquatic environment can be influenced by various factors, which include pH, alkalinity, temperature, mineralogy, redox potential, suspended particulates, total organic content, water velocity, volume of water, as well as the duration of water availability (John & Leventhal, 1995; DWAF, 1996; Stumm & Morgan, 1996; Hoffman *et al*., 2002). Literature (John & Leventhal, 1995; DWAF, 1996; Chapman, 1998; Harper *et al*., 1998; Hoffman *et al*., 2002; Karbassi *et al*., 2008) states that metals can be accumulated and during a process of adsorption, can result in their precipitation. This may result in much higher metal concentrations in the sediment than in the water column. For the purpose of this study the focus will only be on those regarded as toxic to aquatic biota, which include aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), Cu, iron (Fe), lead (Pb), Mn, nickel (Ni), titanium (Ti) and Zn (McKinney & Rogers, 1992; John & Leventhal, 1995; DWAF, 1996; Chapman, 1998; Hoffman *et al*., 2002; USEPA, 2007).

The hypothesis for this chapter states that mining activities in the upper catchment results in an increase in metal concentrations in water and sediment of the Loop Spruit. The aim of this chapter was thus to determine the metal concentrations in the water and sediment at selected sites within the Loop Spruit.

3.2 Materials and Methods

3.2.1 Fieldwork

Three surveys (July 2014, September 2014 and May 2015) were conducted during the dry season at the ten preselected sites (Figure 2.1). Water and sediment samples were collected in triplicate during each survey at all the sites. Both the water and sediment samples were collected in pre-cleaned 250 ml polyethylene bottles. The cleaning method consisted of washing the bottles with a phosphate-free detergent, rinsed in distilled water, after which it was washed in 1 % nitric acid and finally rinsed in double distilled water. For the sediment samples only the upper 7 cm of the substratum were collected and transferred into the bottles. The samples were transported back to the laboratory and stored in a refrigerator at 4 °C until analyses were conducted. Selected abiotic factors, including electrical conductivity (EC) (DIST 3, MI98303, Hanna Instruments), pH (MI98128, Hanna Instruments), temperature (Checktemp, Hanna Instruments), turbidity (GroundTruth Clarity Tube) and flow-rate (Global Water Flow Probe, model FP111) were measured *in situ* at each site.

3.2.2 Laboratory methods

All laboratory methods were adapted from Wolmarans *et al*. (2017).

3.2.2.1 Metal concentration in water samples

From the 250 ml polyethylene bottle, 9.85 ml water was extracted by means of a clean 60 ml syringe and was filtered through a 0.22 µm Whatmann® filter into a 15 ml plastic inductively coupled plasma mass spectrometry (ICP-MS) test tube. The water sample in the ICP-MS test tube was acidified with 0.15 ml 65 % nitric acid to an acid concentration of 1 %. The test tubes were sealed with Parafilm®, labelled with relevant information and sent for ICP-MS analyses to determine the metal concentrations. All of the metal concentrations in the water during all three surveys, were measured in µg/L.

3.2.2.2 Metal concentration in sediment samples

In the laboratory a representative sediment sample was transferred from the 250 ml polyethylene bottle into a weigh boat and was dried at 60 °C in a Labcon 5016U oven for 24 hours. This was done for all of the samples from each of the sites. After the sediment samples were dried, 0.5 g of sediment was used for digestion. Analyses of metals in the total sediment, regarded as bioavailable to aquatic macroinvertebrates (John & Leventhal, 1995), were done using an ICP-MS. Triplicate samples were digested using aqua regia (HCL: HNO3 = 3:1) and a microwave digester (Advanced Microwave Digestion System, Ethos Easy Maxi 44). After digestion, 9.7 ml Milli-Q® water was added to a 15 ml ICP-MS test tube, after which 300 µl supernatant was added to the test tube with a 1 000 µl pipette. The test tubes were labelled with the relevant information, sealed with Parafilm® and sent for ICP-MS analyses to determine the metal concentrations in the sediment. For each sample run, the appropriate quality assurance and -control were applied. Certified Reference Material ((CNS392-050) Trace Elements on Freshwater Sediments from Resource Technology Corporation and CN Schmidt BV) was used and the standard calibration protocol was performed before each run. A < 10 % deviation range was found during the certified reference material analyses, as well as in the percentage recoveries for the standards (Table 3.1). The metal concentrations in the sediment of all three surveys, were measured in mg/kg.

Table 3.1: Metal concentrations recovered from Certified Reference Material. All the concentrations was measured in mg/kg.

	Sediment		
	Reference	**Measured**	**% Recovery**
Ti	1510.0	1490.4	98.7
Cr	35.0	36.6	104.6
Mn	1400.0	1529.6	109.3
Co	8.8	9.3	105.7
Ni	12.8	12.2	95.3
Cu	1230.0	1330.2	108.1
Zn	498.0	468.2	94.0
As	115.0	108.3	94.2
Cd	4.0	4.2	105.0
Pb	285.0	304.5	106.8

3.2.2.3 Particle size determination

An Endecott dry sieving system was used to collect fractions from a 30 g sub-sample of dried sediment of each survey. The sub-sample was sieved to determine the composition of the sediment at the pre-selected sites. The sediment was sieved for 20 minutes using a King Test VB200/300 shaker and sieves with mesh sizes of 4000 µm, 2000 µm, 500 µm, 212 µm and 53 µm. After the 20 minutes the sediment which remained in each sieve was weighed and recorded, then divided by the 30 g to determine the percentage composition of each particle size of the total sediment sample. The clay (particles < 53 µm) were transferred to a 60 ml polyethylene bottle for X-ray diffraction (XRD) and scanning electron microscopy (SEM) analyses.

3.2.2.4 X-ray diffraction analyses

Characterization of the crystalline materials present in the sample were used to identify minerals. A back loading technique was used for preparation after which a Cu X-ray tube was used to scan the samples with X-rays generating a unique diffractogram per sample. Different phases and phase concentrations of present crystalline materials in every sample are represented by this diffractogram. The International Centre for Diffraction Data (ICDD) were used to identify the different phases in an X'Pert Highscore plus program. For mineral identification, the ICDD database PDF 4+ was again used with the ICSD-PANanalytical program to interpret the results. Rietveld quantification is used to determine the weight percentage of each mineral in a sample. In this study, this was done by using the different phases of the diffractograms of each of the samples.

3.2.2.5 Scanning electron microscopy analyses

Elemental composition in the clay particles (< 53 µm) was determined by means of an FEI Quanta 250 FEG ESEM microscope. This microscope is equipped with an integrated Oxford Inca X- MAX 20 EDS-system and incorporated internal standards. The clay particles were added to microscope stubs with double sided tape. After all the elements of each site were identified, a percentage abundance for each element was calculated.

3.2.3 Statistics

Univariate and multivariate statistical analyses were conducted using SPSS (v23) and Canoco (v5). One-way analysis of variance (ANOVA) was used to test the significant variations of metals in water and sediment between sites and surveys. Normality and homogeneity of the data were tested using Levene's test. *Post-hoc* comparisons were applied using Tukey's post-test for homogeneous data and Dunnett's-T3 test for non-homogeneous data. Results obtained from the two tests indicated whether significant differences ($p < 0.05$) between the data sets occurred.

A Principal Component Analysis (PCA) for the water and sediment, were applied which uses multivariate data and creates a scatterplot, which makes the interpretation of the data easier. The PCAs were created using Canoco (v5).

3.3 Results and Discussion

3.3.1 Mineral composition

The most abundant mineral that occurred at every site in high percentages was quartz, with illite, muscovite, kaolinite, magnesioferrite, chrysotile, calcite, vermiculite, montmorillonite and albite occurring at different sites in smaller percentages (Table 3.2). Sites 1, 2 and 3 had lower percentages of quartz (Table 3.2) possibly due to the fact that they are situated on andesite, quartzite and shale, classified as hard rocks and a rock with medium hardness, respectively (Klein & Dutrow, 2007; Wenk & Bulakh, 2008) (Tables 2.1, 2.2 and 2.3). In contrast Sites 4 – 9, all situated in soil cover, had higher percentages of quartz (Tables 2.4 – 2.9), however Site 10, which is also situated in soil cover (Table 2.10), had a lower percentage of quartz for no obvious explanation. The sediment of this site also consisted of 33.5 % albite (Table 3.2).

Table 3.2: The percentage mineral composition of clay particles found during XRD analysis at each site.

Mineral Identification	Sites									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Quartz	72.9	76.7	69.5	96.6	98.7	93.1	99.3	99.7	98.8	64.8
Illite	12.5	0	0	0	0	0	0	0	0	0
Muscovite	1.0	15.3	0	0	0	4.6	0	0	0	0
Kaolinite	13.7	7.9	28.6	0	0	0	0	0	0	0
Magnesioferrite	0	0.1	1.8	3.1	1.3	0	0	0	0	0
Chrysotile	0	0	0.1	0.3	0	0	0	0	0	0
Calcite	0	0	0	0	0	2.0	0	0	0	0
Vermiculite	0	0	0	0	0	0.4	0	0.3	0.8	1.7
Montmorillonite	0	0	0	0	0	0	0.7	0	0.4	0
Albite	0	0	0	0	0	0	0	0	0	33.5

3.3.2 Element composition

The percentage element composition as analysed in the clay by SEM at each site, is shown in Table 3.3. High percentages of silicon (Si) and oxygen (O) were found at all sites and can be explained by the high percentage quartz found at every site (Table 3.2). According to Anthony *et al*. (2001) the chemical composition of quartz consists of SiO_2, while minerals such as illite, muscovite, kaolinite, chrysotile, vermiculite, montmorillite and albite also consist of amongst others, SiO_2. Although most of the remaining minerals were present in very small percentages these could in the long term contribute to metals like Fe, Al and Ti. The Fe percentages can be due to illite, magnesioferrite, vermiculite and montmorillonite (Table 3.2). Aluminium is found in illite, muscovite, kaolinite, magnesioferrite, vermiculite, montmorillonite and albite in the form Al_2O_3 (Anthony *et al*., 2001). Titanium is found in clay minerals (Neal *et al*., 2011) and illite, kaolinite and montmorillionite are all clay minerals, which occurred at several sites (Table 3.2). Although magnesioferrite is not classified as a clay, it consists of 23.4 % TiO_2 (Anthony *et al*., 2001). The presence of carbon, which varied between the sites, could be ascribed to organic content in the sediment samples.

Table 3.3: The percentage element composition of the clay particles found by the SEM analysis at each site.

Elements	Sites									
	1	2	3	4	5	6	7	8	9	10
C	11.98	10.44	10.82	9.24	13.24	10.32	12.16	13.80	17.10	15.40
Al	6.13	5.22	6.89	5.47	3.50	4.01	4.17	3.44	3.13	3.51
Si	17.13	20.43	16.63	19.51	17.76	21.91	18.93	16.55	12.56	13.46
Ti	0.42	0.32	0.49	0.35	0.29	0.48	0.33	0.30	0.26	0.45
Fe	5.56	5.94	8.49	10.47	4.97	5.39	5.05	5.45	3.21	5.75
O	58.77	57.65	56.67	54.95	60.24	57.89	59.35	60.45	63.74	61.43
Total	100	100	100	100	100	100	100	100	100	100

3.3.3 Metal concentrations in water

A total of 34 elements were detected in water by the ICP-MS at each of the sites during this study, which presents a baseline for metals within the Loop Spruit (Appendix A, Tables A1, A2 and A3). Twelve metals based on their potential toxicity towards aquatic biota will be discussed in this chapter.

Table 3.4: The mean metal concentration and standard deviation (µg/L) in the water, at ten preselected sites during all three surveys, as well as selected abiotic factors and target water quality range (TWQR), chronic effect value (CEV) and acute effect value (AEV). A colour was allocated where concentrations exceeded the above mentioned values (TWQR – blue, CEV – green, and AEV – red). Within columns means with common alphabetical superscripts indicate significant temporal ($p < 0.05$) differences at each sites. Spatial significant differences ($p < 0.05$) during surveys are not indicated in the table but are pointed out in the text.

Sites	Surveys	Al pH <6.5	Al pH >6.5	Ti	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb	pH	EC (µS/cm)	Temp (˚C)	Turbidity (NTU)	Flow-rate (m/s)
TWQR		5	10		7	180				1.2	2	10	0.35	1					
CEV		10	20		14	370				2.4	3.6	20	0.7	2					
AEV		100	150		200	1 300				7.5	36	130	10	13					
1	1	295.5 ± 1.7^{a}		2.1 ± 0.2	1.8 ± 0.2^{a}	0.3 ± 0.2^{a}	200.9 ± 2.1^{a}	0.1 ± 0.03^{a}	2.0 ± 0.4	2.2 ± 0.3^{a}	18.7 ± 0.4^{a}	0.1 ± 0.06^{a}	0.03 ± 0.01	0.4 ± 0.1^{a}	7.2	65	9.2	7	0.2
	2	13.5 ± 0.3^{ab}		1.5 ± 0.3	1.4 ± 0.3	2.1 ± 0.2^{ab}	318.2 ± 1.9^{a}	0.3 ± 0.05	2.1 ± 0.2	2.1 ± 0.1^{b}	42.6 ± 1.6^{ab}	1.0 ± 0.2	0.1 ± 0.04	0.3 ± 0.2^{b}	6.0	80	12.6	6	0.2
	3	47.5 ± 2.1^{ab}		3. ± 0.08	0.03 ± 0.01^{a}	5.8 ± 0.2^{ab}	258.6 ± 18.0	0.4 ± 0.02^{a}	1.9 ± 0.07	12.2 ± 1.2^{ab}	121.5 ± 0.6^{ab}	0.9 ± 0.09^{a}	0.03 ± 0.01	27.4 ± 0.4^{ab}	7.9	70	10.1	19	0.1
2	1	35.6 ± 0.02^{a}		1.3 ± 0.3	1.1 ± 0.1^{a}	3.7 ± 0.8^{a}	194.0 ± 0.4^{a}	0.1 ± 0.02^{a}	4.2 ± 0.02^{a}	3.2 ± 0.7^{a}	60.6 ± 1.5^{a}	0.1 ± 0.02^{a}	0.03 ± 0.01^{a}	0.4 ± 0.2^{a}	6.7	901	12.3	5	1.2
	2	7.4 ± 0.6^{ab}		2.4 ± 0.3	2.2 ± 0.2^{ab}	3.2 ± 0.2^{b}	787.4 ± 11.5^{ab}	2.4 ± 0.2^{ab}	117.2 ± 8.2^{ab}	3.2 ± 0.2^{b}	37.5 ± 2.8^{ab}	4.3 ± 0.3^{a}	0.1 ± 0.02	0.1 ± 0.02^{b}	6.4	783	14.7	5	0.5
	3	64.5 ± 0.7^{ab}		1.2 ± 0.03	0.03 ± 0.01^{ab}	100.6 ± 1.0^{ab}	190.5 ± 4.4^{b}	40.7 ± 0.9^{ab}	199.1 ± 2.3^{ab}	11.5 ± 0.3^{ab}	120.3 ± 1.6^{ab}	4.8 ± 0.06^{a}	0.1 ± 0.003^{a}	25.4 ± 1.9^{ab}	7.0	1076	15.6	5	0.6
3	1	33.3 ± 0.2^{a}		1.3 ± 0.2^{a}	0.9 ± 0.1	2.5 ± 0.6^{a}	105.0 ± 0.4^{a}	0.2 ± 0.09^{a}	3.4 ± 0.4	3.3 ± 0.5^{a}	56.9 ± 0.3^{a}	0.02 ± 0.009^{a}	0.03 ± 0.01	0.7 ± 0.09^{a}	7.0	198	13.2	5	0.1
	2	22.6 ± 1.2^{ab}		2.0 ± 0.1^{b}	1.3 ± 0.2	1.9 ± 0.1^{b}	365.2 ± 3.2^{ab}	0.3 ± 0.04^{b}	1.7 ± 0.2^{a}	1.4 ± 0.2^{b}	37.3 ± 2^{ab}	0.9 ± 0.1	0.1 ± 0.009	0.3 ± 0.03^{b}	6.3	297	15.5	5	0.1
	3	77.1 ± 1.8^{ab}		3.1 ± 0.02^{ab}	0.03 ± 0.01	3444.3 ± 23.6^{ab}	271.4 ± 2.1^{ab}	1.7 ± 0.1^{ab}	5.0 ± 0.02^{a}	13.4 ± 0.3^{ab}	175.4 ± 2.0^{ab}	0.7 ± 0.04^{a}	0.03 ± 0.01	27.2 ± 0.7^{ab}	7.2	340	10.6	84	0.1
4	1	23.7 ± 0.2^{a}		0.7 ± 0.2	2.8 ± 0.2^{a}	1.2 ± 0.1^{a}	89.5 ± 0.4^{a}	5.2 ± 0.2^{a}	10.5 ± 0.6^{a}	6.4 ± 0.4^{a}	49.6 ± 0.2^{a}	0.6 ± 0.2	0.03 ± 0.01^{a}	0.1 ± 0.01^{a}	6.8	1207	11.7	5	0.3
	2	5.9 ± 0.1^{ab}		1.5 ± 0.1	6.0 ± 0.05^{ab}	3.5 ± 0.2^{a}	1084.0 ± 8.9^{ab}	6.3 ± 0.1^{b}	9.0 ± 0.02^{b}	6.0 ± 0.04^{b}	37.5 ± 0.7^{ab}	1.9 ± 0.1^{a}	0.1 ± 0.001^{ab}	0.3 ± 0.02^{ab}	6.2	1362	15.7	84	0.3
	3	45.0 ± 1.2^{ab}		1 ± 0.02	3.2 ± 0.03^{b}	4.7 ± 0.06^{a}	318.3 ± 5.8^{ab}	2.8 ± 0.09^{ab}	5.5 ± 0.09^{ab}	17.6 ± 0.6^{ab}	218.4 ± 1.8^{ab}	0.4 ± 0.01^{a}	0.03 ± 0.01^{b}	31.6 ± 1.5^{ab}	7.1	1341	12.0	7	0.2

Table 3.4 (continued)

5	**1**	24.2 ± 0.2^{a}	1.3 ± 0.1^{a}	1.7 ± 0.2^{a}	1.9 ± 0.1^{a}	87.4 ± 0.2^{a}	1.9 ± 0.07^{a}	9.1 ± 0.2^{a}	4.7 ± 0.2^{a}	48.4 ± 0.1^{a}	0.7 ± 0.08^{a}	0.03 ± 0.01^{a}	0.4 ± 0.03^{a}	6.9	906	10.9	6	0.4
	2	13.5 ± 0.3^{ab}	3.2 ± 0.04^{a}	2.6 ± 0.09^{b}	2.2 ± 0.02^{b}	341.9 ± 3.4^{ab}	1.5 ± 0.03^{ab}	7.6 ± 0.06^{ab}	2.9 ± 0.1^{ab}	41.8 ± 1.4^{b}	2.3 ± 0.05^{ab}	0.1 ± 0.002^{ab}	0.2 ± 0.002^{b}	6.7	823	16.5	10	0.2
	3	62.3 ± 2.1^{ab}	3.1 ± 0.04^{a}	0.01 ± 0.001^{ab}	7.4 ± 0.2^{ab}	631.6 ± 1.8^{ab}	3.4 ± 0.03^{ab}	11.0 ± 0.4^{b}	25.2 ± 0.3^{ab}	535.8 ± 6.0^{ab}	1.6 ± 0.05^{ab}	0.03 ± 0.01^{b}	23.2 ± 2.0^{ab}	7.1	906	15.6	6	0.2
6	**1**	24.1 ± 0.06^{a}	0.7 ± 0.05^{a}	1.5 ± 0.2^{a}	1.8 ± 0.1	83.0 ± 0.07^{a}	1.6 ± 0.1^{a}	9.6 ± 0.5	5.0 ± 0.1^{a}	56.2 ± 0.04^{a}	0.6 ± 0.1^{a}	0.03 ± 0.01^{a}	0.2 ± 0.04^{a}	7.0	806	10.5	10	0.5
	2	9.3 ± 0.07^{ab}	2.9 ± 0.08^{a}	2.5 ± 0.04^{b}	2.4 ± 0.03^{a}	499.4 ± 6.9^{ab}	1.4 ± 0.07^{b}	7.1 ± 0.09^{a}	4.0 ± 0.03^{ab}	28.7 ± 0.3^{ab}	3.4 ± 0.1^{ab}	0.1 ± 0.002^{ab}	0.6 ± 0.03^{ab}	6.7	772	16.6	6	0.5
	3	118.3 ± 2.0^{ab}	3.3 ± 0.05^{a}	0.03 ± 0.01^{ab}	2.1 ± 0.04^{a}	377.4 ± 9.0^{ab}	2.6 ± 0.2^{ab}	10.3 ± 0.1^{a}	12.0 ± 0.4^{ab}	164.5 ± 2.2^{ab}	1.7 ± 0.1^{ab}	0.03 ± 0.01^{b}	22.0 ± 1.2^{ab}	7.0	797	12.3	10	0.4
7	**1**	27.0 ± 1.3^{a}	1.1 ± 0.2^{a}	0.9 ± 0.1	1.9 ± 0.1	71.3 ± 2.7^{a}	0.2 ± 0.02^{a}	5.9 ± 0.2^{a}	4.2 ± 0.2^{a}	59.0 ± 2.5^{a}	0.3 ± 0.04^{a}	0.03 ± 0.01^{a}	0.3 ± 0.04^{a}	8.3	480	10.7	35	0.1
	2	21.9 ± 1.3^{b}	2.4 ± 0.2^{ab}	1.5 ± 0.02^{a}	2.7 ± 0.07	463.1 ± 5.4^{ab}	0.5 ± 0.01^{ab}	2.4 ± 0.02^{ab}	2.9 ± 0.05^{b}	29.7 ± 0.3^{ab}	1.8 ± 0.03^{a}	0.1 ± 0.001^{ab}	0.1 ± 0.0004^{b}	7.2	558	21.3	7	0.1
	3	44.8 ± 2.0^{ab}	0.8 ± 0.05^{b}	0.03 ± 0.01^{a}	2.5 ± 0.1	304.8 ± 2.2^{ab}	0.7 ± 0.02^{ab}	5.4 ± 0.2^{b}	13.6 ± 0.5^{ab}	204.6 ± 2.3^{ab}	1.4 ± 0.1^{a}	0.03 ± 0.01^{b}	21.2 ± 1.5^{ab}	7.5	766	13.6	7	0.01
8	**1**	54.2 ± 1.8^{a}	0.9 ± 0.2^{a}	1.8 ± 0.2^{a}	6.3 ± 0.3^{a}	112.4 ± 0.5^{a}	1.0 ± 0.04	5.7 ± 0.3	3.9 ± 0.2^{a}	54.5 ± 0.6^{a}	0.8 ± 0.07^{a}	0.03 ± 0.01^{a}	0.3 ± 0.05^{a}	7.4	781	7.3	6	0.3
	2	31.0 ± 1.5^{ab}	1.8 ± 0.02^{b}	3.3 ± 0.05^{ab}	3.8 ± 0.04^{ab}	512.7 ± 3.6^{ab}	0.9 ± 0.01	4.5 ± 0.2	3.6 ± 0.05^{b}	36.7 ± 2.0^{ab}	3.3 ± 0.06^{ab}	0.1 ± 0.002^{ab}	0.5 ± 0.01^{b}	7.6	1303	20.4	5	0.1
	3	57.3 ± 1.6^{b}	6 ± 0.09^{ab}	2.0 ± 0.05^{b}	211.7 ± 1.8^{ab}	362.4 ± 1.8^{ab}	1.3 ± 0.05	4.5 ± 0.2	17.2 ± 0.3^{ab}	253.2 ± 2.4^{ab}	1.8 ± 0.2^{b}	0.03 ± 0.01^{b}	28.9 ± 0.7^{ab}	7.4	1120	13.5	6	0.1
9	**1**	37.6 ± 2.6	1.6 ± 0.2	1.2 ± 0.2	3.5 ± 0.2^{a}	134.1 ± 0.6^{a}	1.6 ± 0.2	5.1 ± 0.2^{a}	2.9 ± 0.2^{a}	41.0 ± 0.4^{a}	0.3 ± 0.1^{a}	0.03 ± 0.01^{a}	0.1 ± 0.02^{a}	7.7	695	12.3	6	0.3
	2	24.5 ± 1.6^{a}	2.5 ± 0.07	1.9 ± 0.05^{a}	1.7 ± 0.1^{a}	265.5 ± 2.8^{ab}	1.7 ± 0.07	2.2 ± 0.05^{ab}	2.7 ± 0.03^{b}	44.1 ± 1.7^{b}	1.2 ± 0.07^{ab}	0.1 ± 0.005^{ab}	0.3 ± 0.01^{ab}	7.1	789	19.4	11	0.5
	3	41.4 ± 1.6^{a}	2.2 ± 0.06	0.03 ± 0.01^{a}	1.7 ± 0.06^{a}	211.3 ± 1.5^{ab}	1.6 ± 0.05	1.8 ± 0.02^{ab}	8.9 ± 0.09^{ab}	109.5 ± 1.0^{ab}	0.5 ± 0.008^{ab}	0.03 ± 0.01^{b}	21.2 ± 0.8^{ab}	7.5	661	15.5	7	0.3
10	**1**	152.7 ± 1.7^{a}	1.2 ± 0.07^{a}	1.2 ± 0.1^{a}	14.4 ± 0.4^{a}	113.7 ± 0.2^{a}	1.5 ± 0.2	3.6 ± 0.3	18.3 ± 0.4^{a}	55.6 ± 0.2^{a}	0.4 ± 0.1^{a}	0.03 ± 0.01^{a}	2.9 ± 0.3^{a}	8.3	737	11.7	5	0.2
	2	10.8 ± 0.2^{ab}	1.7 ± 0.1^{b}	2.4 ± 0.2^{ab}	3.8 ± 0.09^{ab}	513.0 ± 4.5^{ab}	2.4 ± 0.2	3.4 ± 0.2	3.1 ± 0.1^{ab}	45.4 ± 1.6^{b}	1.6 ± 0.2^{a}	0.1 ± 0.005^{ab}	0.2 ± 0.03^{ab}	7.1	788	17.4	6	0.1
	3	246.4 ± 0.6^{ab}	4.9 ± 0.03^{ab}	0.03 ± 0.01^{ab}	2.5 ± 0.02^{ab}	447.2 ± 1.7^{ab}	1.7 ± 0.03	2.7 ± 0.03	11.7 ± 0.1^{ab}	191.3 ± 0.7^{ab}	0.8 ± 0.006	0.03 ± 0.01^{b}	22.3 ± 1.0^{ab}	7.7	754	16.2	6	0.4

Aluminium, Ti and Fe were present in the highest concentrations at Site 1, during the first survey (Table 3.4), where Al differed significantly ($p < 0.05$) with all other sites, Ti differed significantly ($p < 0.05$) between Sites 1 and 6 with Sites 4 and 10, respectively, while Fe at Site 1 differed significantly ($p < 0.05$) with all the sites, except with Site 4. Chromium, Co and Ni were the highest at Site 4, where Cr and Co differed significantly ($p < 0.05$) with all the sites, while Ni also differed significantly ($p < 0.05$) from all the sites, except with Sites 5 and 6. Site 10 had the highest concentrations of Mn, Cu and Pb (Table 3.4) and differed significantly ($p < 0.05$) with all the sites. The highest concentration of Zn were measured at Site 2 and only differed significantly ($p < 0.05$) with Sites 1, 5 and 9, whereas Site 8 had the highest concentration of As (Table 3.4) and differed significantly ($p < 0.05$) with Sites 1, 2, 3 and 7.

With regard to Al, it exceeded the target water quality range (TWQR), as well as the chronic effect value (CEV) at all ten sites, while at Sites 1 and 10, it only exceeded the acute effect value (AEV) as set by DWAF (1996) (Table 3.4). Copper exceeded the TWQR at all ten sites, but the CEV at Sites 2 to 9, while the AEV was only exceeded at Site 10 (Table 3.4). Zinc also exceeded the TWQR at all of the sites, but the AEV at Sites 2 to 10 and only the CEV at Site 1 (Table 3.4). Lead exceeded the TWQR and CEV at Site 10 (Table 3.4).

The highest Al and Mn concentrations during the second survey, were recorded at Site 8 (Table 3.4). Aluminium differed significantly ($p < 0.05$) with all the sites except with Sites 3 and 9, while Mn also differed significantly ($p < 0.05$) with all the sites, except with Sites 2, 4 and 10. The highest concentrations of Ni, As and Cd were recorded at Site 2, where Ni differed significantly ($p < 0.05$) with all the sites, while As differed significantly ($p < 0.05$) with all the sites, except Sites 5, 6 and 8. Cadmium had no significant differences. High concentrations of Cr, Fe, Co and Cu were present at Site 4 (Table 3.4) and differed significantly ($p < 0.05$) with all the other sites. The highest concentration of Zn were at Site 10 and only differed significantly ($p < 0.05$) with Sites 6 and 7, while the highest Pb concentration was recorded at Site 6 (Table 3.4) and differed significantly ($p < 0.05$) with all the sites except with Sites 1 and 8. Titanium was recorded in the highest concentrations at Site 5 (Table 3.4) and differed significantly ($p < 0.05$) with Sites 3, 4, 8, 9 and 10. Aluminium exceeded the TWQR again during this survey at all of the sites except at Site 6 and exceeded the CEV at Sites 1, 3, 7, 8 and 9 (Table 3.4). Copper exceeded the TWQR again at all of the sites, while it also exceeded the CEV at Sites 2 and 4 to 10 (Table 3.4). Zinc exceeded the TWQR at every site and the CEV at Sites 6 and 7, while exceeding the AEV at Sites 1 to 5 and 8 to 10 (Table 3.4).

During the third survey the highest Co, Ni, As and Cd concentrations were recorded at Site 2 and differed significantly ($p < 0.05$) with all the sites, while Site 3 had the highest Mn concentration (Tables 3.4) and also differed significantly ($p < 0.05$) with all the sites. At Site 4 the highest concentrations of Cr and Pb were recorded, where Cr differed significantly ($p < 0.05$) with all the sites, while Pb only differed significantly ($p < 0.05$) with Sites 6, 7, 9 and 10. High concentrations of Fe, Cu and Zn were found at Site 5 (Table 3.4), which differed significantly ($p < 0.05$) with all the sites. Site 8 had the highest recorded Ti concentration, while the highest Al concentration was found at Site 10 (Table 3.4) and both differed significantly ($p < 0.05$) with all the sites. The Al concentrations at all the sites exceeded the TWQR as well, and were higher than the CEV at all the sites, except for Site 10 where it exceeded the AEV (Table 3.4). The Mn concentrations were higher than the TWQR at Sites 3 and 8, while the concentration at Site 3 was higher than the AEV (Table 3.4). Copper and Zn concentrations exceeded the TWQR again at all the sites in this survey, while also exceeding the AEV at all of the sites (Table 3.4).

The high concentrations found in the case of Al can mainly be ascribed to the fact that the earth’s crust contains 8 % of this metal and it is considered as the most abundant metal in the natural environment (WHO, 2010). Aluminium enters the environment through natural processes where numerous factors can influence its mobility (DWAF, 1996; WHO, 2010; Lenntech, 2016a). These factors, among others, include the hydrological flow paths, chemical speciation, the geological composition and soil-water interactions (WHO, 2010). Aluminium containing minerals (Anthony *et al*., 2001) found during this study include illite, muscovite, kaolinite, vermiculite, montmorillonite and albite (Table 3.2). Anthropogenic sources including the addition of Al salts for water purification purposes, various industries and the combustion of coal, are also sources of Al in surface water (DWAF, 1996; WHO, 2010; Lenntech, 2016a). Although the mean Al concentrations in water was found to vary between 5.9 µg/L and 295.5 µg/L (Table 3.4) during this study and exceeded the TWQR of South Africa, these values still remain within tolerable ranges stated for natural surface waters (WHO, 2010). Soluble Al species are mainly found in acid mine drainage waters and are of great concern due to the impacts on the environment (DWAF, 1996). However, in this study, the high Al concentrations can rather be ascribed to the weathering of minerals than to acid mine drainage, since it was recorded throughout the river system. The toxicity of Al is influenced by pH and the calcium (Ca) concentration in the water (DWAF, 1996), where the neutral pH (Table 3.4) and high Ca concentrations (Appendix A, Tables A1, A2 and A3) in this river system are possible reasons why the Al seems to have no toxic effects.

Copper also occurs naturally within most aquatic environments and is considered as a common element in minerals and rocks within the earth's crust (DWAF, 1996; WHO, 2004a). Copper can enter the aquatic environment in a natural manner, due to weathering of rocks or by means of dissolution of native copper and copper minerals (DWAF, 1996; Chapman, 1998). Although Cu can occur naturally, 33 – 60 % of Cu that enters the aquatic environment in South Africa annually, is probably due to anthropogenic sources (DWAF, 1996). These anthropogenic sources include, amongst others, Cu salts in algaecides, insecticides and fertilizers, as well as in sewage treatment plant effluents, while elemental Cu can originate from corrosion of copper and brass pipes and from industrial and mining sources (DWAF, 1996; Walker *et al*., 1999; WHO, 2004a). The factors that can decrease the toxicity of Cu include the occurrence of chelating agents, the presence of other elements (i.e. Ca, Zn, magnesium (Mg) and sulphate (SO_4^{2-})), organic matter, as well as a rise in alkalinity (DWAF, 1996). During the current study it was found that Cu was present in significantly higher ($p < 0.05$) concentrations at Site 10 (first survey), Site 4 (second survey) and Site 5 (third survey) (Table 3.4). The possible reasons for the high concentrations at Site 10 and Site 5 could be that these sites are located downstream of the Potchefstroom and Fochville's sewage treatment plants and are situated in agricultural intensive areas, where fertilizers and pesticides are used. The origin of the high concentration recorded at Site 4 (Table 3.4), can be from mining effluent that enters this tributary of the Loop Spruit (Figure 2.1), or can be due to the runoff from a scrapyard in the vicinity of the stream. Comparing the first two surveys with the third survey (Table 3.4) it is evident that the concentrations found, during the last survey were significantly higher ($p < 0.05$) and was probably due to a concentration effect caused by the severe drought, resulting in lower water levels. This conclusion is also supported by Chen *et al*. (2008), who found higher concentrations during the dry season in the Seine River, France. Although the Cu concentrations generally exceeded the CEV and AEV there was no indication that it was toxic to the macroinvertebrates. This is possibly due to the interaction of Cu with Ca, Zn and Mg (DWAF, 1996, WHO, 2004a) (Appendix A, Tables A1, A2 and A3).

Zinc occurs naturally in aquatic environments (Lenntech, 2016b), as ores, rocks and sulphides, by which it can enter rivers by means of natural weathering or erosion (DWAF, 1996; WHO, 2003a; Lenntech, 2016b). Zinc can occur in rivers through anthropogenic influences from a variety of industries, fertilizers, insecticides and growth stimulant in animal feed, rubber and tires, as well as automotive exhausts (DWAF, 1996; Walker *et al*., 1999; WHO, 2003a; Chen *et al*., 2008; Lenntech, 2016b). The solubility of Zn is dependent on temperature and pH within the aquatic environment (Lenntech, 2016b), whereas the speciation is influenced by the alkalinity and pH (DWAF, 1996). During the current investigation the highest Zn concentrations fluctuated between sites and during surveys, where the highest concentration for the first survey was recorded at Site 2 (Table 3.4) and could possibly be ascribed to mining effluent at this site. The highest concentration during the second and last survey was recorded at Site 10 and Site 5 (Table 3.4), respectively, and can possibly be ascribed to anthropogenic impacts originating from Potchefstroom and Fochville, as well as from the surrounding farms that use fertilizers and insecticides. The Zn concentrations were significantly higher ($p < 0.05$) during the third survey at all the sites than the first two surveys (Table 3.4) and can be ascribed to a concentration effect, due to lower water levels as supported by Chen *et al*. (2008) during studies conducted in the Seine River, France. Although the Zn concentrations exceeded the TWQR and AEV at several sites during this study, it seems that it did not have a toxic effect on the organisms. This could possibly be ascribed to the alkaline waters within the Loop Spruit, which reduces its toxicity.

Although Pb mainly enters the aquatic environment by the weathering and dissolution of sulphide ores (DWAF, 1996; WHO, 2011a), anthropogenic activities can also contribute to the Pb concentration in surface waters (John & Leventhal, 1995; DWAF, 1996; Walker *et al*., 1999; WHO, 2011a; Lenntech, 2016c). Lead toxicity is dependent on the degree of the alkalinity of water, and its uptake is thus also influenced by the Ca concentration (DWAF, 1996). The high concentrations of Pb during the third survey at Site 4 (Table 3.4) could possibly be due to mining effluent, as well as Pb contamination originating from the nearby scrapyard. It must however, be emphasized that the concentrations of Pb measured at all sites during the third survey exceeded, in contrast to the first two surveys, the TWQR, as well as the AEV (Table 3.4). This significant increase ($p < 0.05$) in concentration could possibly be ascribed to the effect of lower water levels within the river due to the drought. Although Pb is considered toxic to aquatic biota, the alkalinity of the water could have ameliorated the toxicity.

Manganese is one of the most abundant metals in sedimentary and metamorphic rocks and it is therefore expected to be present in surface waters (DWAF, 1996; WHO, 2004b). This phenomenon was also observed during the current study with the highest concentration present at Site 3 during the third survey (Table 3.4). The drought experienced during this survey resulting in the concentration of water in isolated pools, was most probably responsible for this. The fact that such habitats were characterized by decomposed biota, creating an anaerobic environment that lead to a significant increase ($p < 0.05$) in bioavailability at this site (DWAF, 1996). Although mining activities and mineral processing also contribute to Mn in surface waters (DWAF, 1996; WHO, 2004b), this was not evident during the current investigation. Other factors contributing to Mn in water include redox potential, organic matter and pH and natural weathering of magnesioferrite (DWAF, 1996; Anthony *et al*., 2001; WHO, 2004b).

Although Ti is regarded as an abundant element which occurs in various rocks, including anatase, titanite, rutile, ilmenite, silicates, brookite and clay minerals and can enter aquatic environments through natural weathering (Neal *et al*., 2011; Lenntech, 2016d), it can originate from various anthropogenic sources as well and enter surface waters (Neal *et al*., 2011; Lenntech, 2016d). Concentrations found during this study never exceeded 6 µg/L which was measured at Site 8 (Table 3.4) and can be assumed that it originated from natural weathering.

Chromium, a widely distributed metal in the earth's crust (WHO, 2003b), is most commonly found in the mineral chromite (DWAF, 1996) and can also enter the aquatic environment through natural sources like the weathering of rock constituents, as well as runoff from terrestrial surroundings (DWAF, 1996). Various anthropogenic activities can act as sources of Cr in surface waters (DWAF, 1996; Kotaś & Stasicka, 2000; WHO, 2003b; Lenntech, 2016e). The fact that this metal only occurs in very low concentrations in natural water (DWAF, 1996), is supported by the finding that the highest concentration recorded during this study at Site 4 (Table 3.4) was just 6 µg/L, a value lower than the TWQR of 7 µg/L. Even though Cr occurred in low concentrations, a significant increase ($p < 0.05$) at Site 4 can not only be due to natural weathering but also be ascribed to mining effluent and possible Cr compounds used in cooling waters (Kotaś & Stasicka, 2000).

The significantly higher ($p < 0.05$) concentrations of Fe found in the surface water during this study at a number of sites (Sites 2, 4, 5, 8 and 10) (Table 3.4) was expected as this element is the fourth most abundant metal in the environment (DWAF, 1996). The significant variation ($p < 0.05$) in concentrations recorded at the different sites, as well as the surveys (Table 3.4), are due to the chemical properties of the water and the minerals in which the water body is situated (DWAF, 1996; WHO, 2003c; Lenntech, 2016f). Iron entering the aquatic environment can also be of anthropogenic origin, including mining activities (DWAF, 1996; WHO, 2003c; Lenntech, 2016f). The elevated concentrations at Sites 2 and 4 which were influenced by mining effluent (Table 3.4), serve as proof for this. The significant variation ($p < 0.05$) in Fe concentrations found during this study can be ascribed to factors (pH, oxidation reduction reactions, organic complexing agents and the presence of coexisting inorganic compounds), which influence the behaviour of Fe within the aquatic environment (DWAF, 1996; WHO, 2003c).

It is apparent that the highest concentrations of Co were recorded at Sites 2 and 4 (Table 3.4). According to DWAF (1996) no TWQR exists for Co in South Africa. The significant decrease ($p < 0.05$) in the Co concentration downstream from Site 4 is possibly due to an increase in pH resulting in the precipitation of this metal. This is supported by WHO (2006) that several factors including redox conditions, pH and ionic strength, as well as the total dissolved organic matter influences the concentration and distribution of Co in water.

Although it may also originate from the weathering of sandstone, slate, basalt and clay (Lenntech, 2016g), the significant increase ($p < 0.05$) in the Ni concentration directly downstream from Site 1 (Table 3.4) is most probably due to the mining effluent from the nearby mine. Due to the tendency of Ni to form complexes with ligands within the water, it is not bioavailable for biota (WHO, 2005).

It is established that As is a widely distributed metalloid element in the earth's crust (DWAF, 1996; WHO, 2011b) and is introduced into the aquatic environment by the weathering and dissolution of As containing ores, rocks and minerals (DWAF, 1996; WHO, 2011b; Lenntech, 2016h). According to literature (DWAF, 1996; Smedley &

Kinniburgh, 2002; WHO, 2011b; Lenntech, 2016h), As can also originate from various anthropogenic sources. The significantly high ($p < 0.05$) concentration of As recorded during this study was found at Site 2 (Table 3.4). However, this concentration (4.8 µg/L) did not exceed the TWQR. A significant variation ($p < 0.05$) in the concentrations recorded during the three surveys can be observed with a minimum concentration of 0.02 µg/L and a maximum of 4.8 µg/L (Table 3.4). High concentrations of As were found at Site 8 and Site 6 (Table 3.4). This could possibly be ascribed to nearby chicken farms (Smedley & Kinniburgh, 2002) at Site 6 and the use of pesticides, fertilizers and herbicides (DWAF, 1996) at both sites, since this is an intensive agricultural area (Tables 2.6 and 2.8, Figure 2.1).

Cadmium is generally considered to be highly toxic to biota in various environments, especially in the aquatic environment (DWAF, 1996; WHO, 2011c; USEPA, 2016). It is generally found in association with other metals (i.e. Cu, Pb and Zn) or in sulphide ores and can enter natural water through natural weathering (DWAF, 1996). Anthropogenic activities, including mining and industrial activities can also contribute to Cd in natural surface waters (DWAF, 1996; WHO, 2011c). During the current investigation mining influence was evident, at Site 2 during the second and third survey. No plausible explanation could be found for the significantly higher ($p < 0.05$) concentrations during the second survey at most of the sites (Table 3.4). The Cd concentrations never exceeded the TWQR during this study.

The concentrations of Fe, Cr, Co, Ni, As and Cd showed a significant increase ($p < 0.05$) at Sites 2 and 4, which are the sites where mining effluent enters surface water of the Loop Spruit. Mining effluent can, amongst others, be possible reasons for the increase in metal concentrations, and aligns with the hypothesis stated for this chapter.

3.3.4 Spatial and temporal variation of metals in water

In Figure 3.1 the associations between toxic metal concentrations and selected abiotic factors in the water, as found at the ten preselected sites during all three surveys, are displayed in a PCA biplot. From Figure 3.1, Pb, Cu, Zn and Mn described 44.59 % of the variation on the first axis (x-axis), while Ni, As, Co and Al described 17.95 % of the variation on the second axis (y-axis). The first axis indicated that the water quality from the sites surveyed during the third survey, was different than the water quality from the sites surveyed during the first two surveys. The third survey was characterized by a severe drought and showed an increase in dissolved metal concentrations and pH, compared to sites surveyed during the first two surveys with a more natural flow. In the PCA biplot, it can be seen that As, Ni and Co associated with EC, while Cd and Cr grouped together. All of these metals, as well as EC associated with Sites 2 and 4, especially in the second and third surveys, where these metals and the EC were the highest (Table 3.4). This phenomenon indicates that the mining effluent entering the Loop Spruit at these sites had an influence on the metal concentrations in the water. Manganese associated with Site 3 during the third survey due to the high concentration found at this site. The high concentration of Cu and Zn measured at Site 5 is possibly responsible for the association with this site during the third survey. The association between Al and pH is also supported by literature (DWAF, 1996; WHO, 2010; Lenntech, 2016a) which all stated that pH strongly influences the solubility, toxicity and speciation of Al. The association between temperature and flow-rate may be ascribed to a decrease in temperature with an increase in flow-rate.

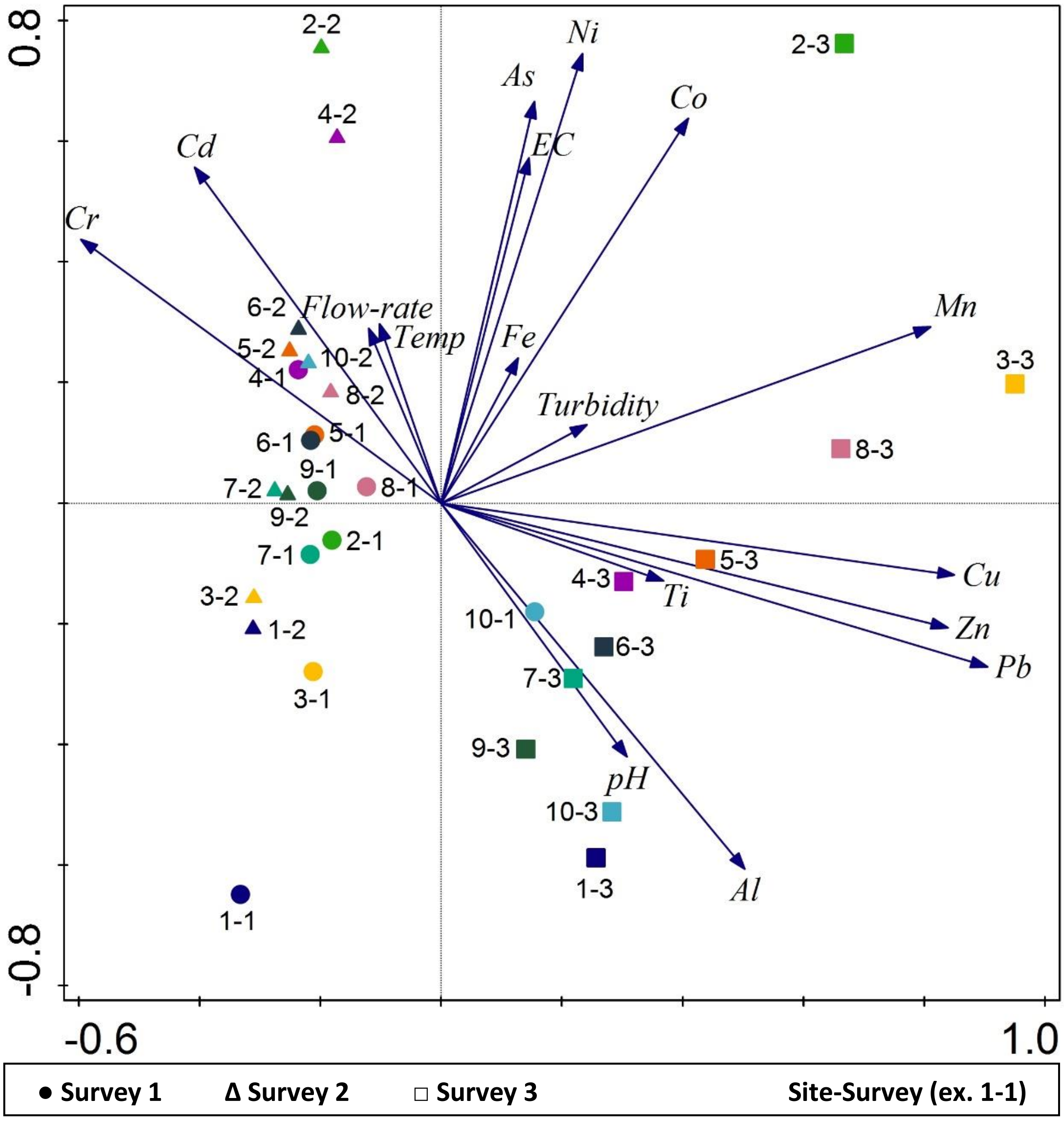


Figure 3.1: PCA biplot illustrating associations between metal concentrations and selected abiotic factors (pH, EC, temperature, turbidity and flow-rate) in the water found at the sampling sites during all three surveys. The biplot describes 62.55 % of the variation with 44.59 % on the first axis and 17.95 % on the second axis.

3.3.5 Metal concentrations in sediment

A total of 31 elements were above instrument detection limits in sediments at each of the sites (Appendix A, Tables A4, A5 and A6). The same 12 metals discussed for water will be addressed here (Table 3.5).

The concentrations of all the metals in the sediment (Table 3.5) are considerably higher than the concentrations in the water (Table 3.4). This is mainly due to several processes that can remove metals from the liquid phase (dissolved) and transfer it to the solid phase. These include surface precipitation, absorption and adsorption, or sorption overall (Honeyman & Santschi, 1988; John & Leventhal, 1995; DWAF, 1996; Harper *et al*., 1998). Suspended particulate materials and sediments perform an intricate role in the sorption of metals, and can form a possible reservoir for these metals (Hoffman *et al*., 2002; Karbassi *et al*., 2007; Karbassi *et al*., 2008). It is also important to note that precipitated metals are, to a large extent, no longer bioavailable and pose little threat to biota (John & Leventhal, 1995; Hoffman *et al*., 2002; Karbassi *et al*., 2007; Karbassi *et al*., 2008). These bound metals can still, due to processes including desorption, be released back into the water column when there is a change in the physiochemical conditions of the water (John & Leventhal, 1995; Karbassi *et al*., 2007; Karbassi *et al*., 2008).

The significantly higher ($p < 0.05$) concentrations found for the majority of metals at most of the sites (Sites 1, 3, 4, 5, 7 and 10) during the third survey, can possibly be ascribed to a concentration effect caused by the drought conditions that are experienced (Table 3.5). The accompanying increase in the clay fraction (Table 3.5) due to a decrease in flow-rate (Table 3.4) during this survey could also contribute to the increase in the metal concentrations (Semlali *et al*., 2001; Cai *et al*., 2002; Ljung *et al*., 2006; Yao *et al*., 2015).

Table 3.5: The mean metal concentration and standard deviation (mg/kg dry weight) in the sediment, at ten preselected sites during all three surveys, as well as the percentage particle size composition. Within columns means with common alphabetical superscripts indicate significant temporal ($p < 0.05$) differences at each site. Spatial significant differences ($p < 0.05$) during surveys are not indicated in the table but are pointed out in the text.

Site	Survey	Al	Ti	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb	% Gravel	% Sand	% Silt	% Clay
1	1	205548.7 ± 203.1^{a}	992.3 ± 1.8^{a}	389.4 ± 1.3^{a}	278.7 ± 1.8^{a}	96320.5 ± 114.5^{a}	28.9 ± 1.4^{a}	166.7 ± 1.3^{a}	116.7 ± 0.8^{a}	153.0 ± 1.6^{a}	24.8 ± 0.9^{a}	0.2 ± 0.003^{a}	80.5 ± 0.9^{a}	10.0	15.5	69.4	5.1
	2	54566.4 ± 5.4^{ab}	344.4 ± 0.5^{ab}	227.1 ± 0.2^{ab}	385.5 ± 0.4^{ab}	54911.7 ± 11.5^{ab}	24.1 ± 0.1^{b}	69.4 ± 0.2^{ab}	44.0 ± 0.1^{ab}	104.5 ± 0.2^{ab}	17.8 ± 0.2^{ab}	0.1 ± 0.002^{ab}	24.5 ± 0.8^{ab}	10.4	16.3	66.6	6.7
	3	130759.4 ± 5.8^{ab}	131.7 ± 1.2^{ab}	853.9 ± 1.0^{ab}	1510.3 ± 2.0^{ab}	279315.0 ± 7.5^{ab}	123.6 ± 0.8^{ab}	183.5 ± 1.3^{ab}	193.7 ± 0.5^{ab}	351.8 ± 0.9^{ab}	136.6 ± 0.3^{ab}	0.5 ± 0.03^{ab}	1426.5 ± 2.0^{ab}	3.0	12.9	77.0	7.1
2	1	68460.6 ± 104.2^{a}	540.4 ± 0.8^{a}	364.3 ± 1.4	537.3 ± 0.6^{a}	85421.3 ± 143.4^{a}	37.3 ± 0.3^{a}	74.8 ± 0.6^{a}	62.8 ± 0.2^{a}	115.1 ± 0.3^{a}	36.4 ± 0.2^{a}	0.2 ± 0.001^{a}	35.3 ± 0.7^{a}	18.6	40.3	39.3	1.7
	2	37316.2 ± 2.5^{ab}	188.1 ± 0.08^{a}	366.0 ± 0.9	905.5 ± 0.2^{ab}	176747.7 ± 4.5^{ab}	206.9 ± 0.1^{ab}	438.4 ± 0.3^{ab}	93.4 ± 0.6^{ab}	329.6 ± 0.4^{ab}	116.3 ± 0.3^{ab}	0.6 ± 0.01^{ab}	77.3 ± 0.9^{ab}	37.0	22.7	40.0	0.4
	3	42617.1 ± 17.6^{ab}	196.4 ± 2.0^{a}	362.8 ± 0.2	756.4 ± 0.9^{ab}	97513.6 ± 1.9^{ab}	42.6 ± 0.3^{ab}	97.6 ± 0.3^{ab}	72.8 ± 0.3^{ab}	237.9 ± 0.2^{ab}	58.3 ± 0.2^{ab}	0.4 ± 0.03^{b}	68.4 ± 0.3^{ab}	34.8	13.7	49.3	2.1
3	1	32163.7 ± 190.4^{a}	518.4 ± 0.9^{a}	362.4 ± 0.5^{a}	662.8 ± 0.9^{a}	88048.7 ± 59.0^{a}	36.7 ± 0.3^{a}	83.6 ± 0.4^{a}	79.6 ± 0.3^{a}	174.1 ± 0.2^{a}	16.0 ± 0.08^{a}	0.2 ± 0.03^{a}	55.3 ± 1.7^{a}	29.1	20.0	49.1	1.8
	2	123523.5 ± 120.2^{ab}	737.1 ± 0.4^{ab}	612.8 ± 1.1^{ab}	1886.4 ± 1.8^{ab}	176226.7 ± 30.6^{ab}	66.1 ± 0.4^{ab}	246.2 ± 0.4^{ab}	132.3 ± 0.9^{ab}	328.3 ± 0.1^{ab}	30.2 ± 0.1^{ab}	0.5 ± 0.02^{a}	91.2 ± 1.3^{ab}	54.2	18.3	24.7	2.9
	3	77021.7 ± 3.5^{ab}	67.3 ± 0.2^{ab}	732.7 ± 0.4^{ab}	5316.7 ± 4.3^{ab}	190872.1 ± 4.7^{ab}	138.3 ± 0.3^{ab}	197.3 ± 0.2^{ab}	185.2 ± 0.2^{ab}	195.5 ± 0.3^{ab}	17.7 ± 0.2^{ab}	0.3 ± 0.2	268.1 ± 0.5^{ab}	25.7	16.9	47.1	10.3
4	1	38358.7 ± 104.3^{a}	693.4 ± 0.6^{a}	1486.6 ± 7.6^{a}	2559.3 ± 1.2^{a}	172176.0 ± 8.3^{a}	101.2 ± 0.4^{a}	252.9 ± 0.3^{a}	134.3 ± 0.4^{a}	131.6 ± 0.4^{a}	26.2 ± 0.2^{a}	0.2 ± 0.005^{a}	28.7 ± 0.2^{a}	37.5	37.2	25.1	0.1
	2	38663.9 ± 4.6^{b}	555.1 ± 1.7^{ab}	519.2 ± 1.2^{ab}	1234.0 ± 3.8^{ab}	88676.0 ± 5.3^{ab}	61.7 ± 0.3^{ab}	250.5 ± 0.9^{b}	76.7 ± 0.3^{ab}	109.0 ± 1.5^{ab}	17.6 ± 0.3^{ab}	0.2 ± 0.05	21.2 ± 0.9^{ab}	0.8	8.3	90.3	0.6
	3	39824.6 ± 4.3^{ab}	998.3 ± 0.2^{ab}	1772.6 ± 1.1^{ab}	4593.7 ± 0.8^{ab}	191927.5 ± 5.2^{ab}	157.3 ± 0.2^{ab}	327.7 ± 0.3^{ab}	177.1 ± 0.3^{ab}	155.7 ± 0.4^{ab}	20.7 ± 0.3^{ab}	0.2 ± 0.002^{a}	226.6 ± 2.0^{ab}	39.0	30.6	29.6	0.8

Table 3.5 (continued)

5	1	33971.0 ± 66.6^{a}	264.5 ± 0.6^{a}	293.9 ± 0.5^{a}	2061.9 ± 1.9^{a}	63363.7 ± 67.6^{a}	52.5 ± 0.2^{a}	127.8 ± 0.2^{a}	75.7 ± 0.07^{a}	158.4 ± 0.5^{a}	9.5 ± 0.1^{a}	0.2 ± 0.005^{a}	23.6 ± 0.2^{a}	19.9	22.5	55.2	2.4
	2	14226.5 ± 7.5^{ab}	166.8 ± 0.7^{ab}	201.8 ± 1.3^{ab}	1034.7 ± 2.1^{ab}	39784.1 ± 4.8^{ab}	35.6 ± 0.4^{ab}	107.5 ± 0.7^{ab}	26.3 ± 0.2^{ab}	58.3 ± 0.3^{ab}	8.4 ± 0.1^{ab}	0.1 ± 0.02^{b}	13.7 ± 0.2^{ab}	22.6	28.1	48.6	0.8
	3	87951.3 ± 4.3^{ab}	43.9 ± 0.3^{ab}	618.9 ± 0.5^{ab}	2735.1 ± 0.6^{ab}	141768.5 ± 4.8^{ab}	129.3 ± 0.5^{ab}	372.9 ± 0.7^{ab}	320.6 ± 0.3^{ab}	826.3 ± 0.4^{ab}	22.4 ± 0.4^{ab}	0.9 ± 0.03^{ab}	170.5 ± 0.6^{ab}	8.5	41.6	44.2	5.7
6	1	30728.6 ± 15.1^{a}	214.8 ± 0.2^{a}	325.2 ± 0.2^{a}	5752.4 ± 2.3^{a}	101019.8 ± 13.7^{a}	142.3 ± 0.5^{a}	352.1 ± 0.1^{a}	71.5 ± 0.2^{a}	100.7 ± 0.7^{a}	18.2 ± 0.2	0.2 ± 0.002	47.6 ± 0.5^{a}	33.6	48.9	17.3	0.3
	2	25225.0 ± 2.7^{ab}	206.6 ± 0.6^{ab}	525.7 ± 2.0^{a}	2372.8 ± 3.09^{ab}	60153.3 ± 3.5^{ab}	46.6 ± 0.3^{ab}	165.2 ± 0.2^{ab}	36.4 ± 0.2^{ab}	47.2 ± 0.2^{ab}	19.2 ± 0.2^{a}	0.1 ± 0.02	23.3 ± 0.4^{ab}	49.4	26.5	23.3	0.8
	3	24248.4 ± 1.2^{ab}	13.3 ± 0.3^{ab}	534.4 ± 0.5^{a}	9969.0 ± 1.0^{ab}	75913.3 ± 1.7^{ab}	102.7 ± 0.2^{ab}	211.4 ± 0.6^{ab}	59.7 ± 0.4^{ab}	78.7 ± 0.1^{ab}	17.2 ± 0.2^{a}	0.1 ± 0.02	291.2 ± 0.3^{ab}	27.8	25.4	44.5	2.3
7	1	39316.9 ± 93.7^{a}	236.2 ± 0.3^{a}	221.4 ± 1.0^{a}	1877.6 ± 2.2^{a}	47514.7 ± 239.4^{a}	44.3 ± 0.6^{a}	99.4 ± 0.2^{a}	55.7 ± 0.1^{a}	95.2 ± 0.2^{a}	5.9 ± 0.06^{a}	0.2 ± 0.002^{a}	27.3 ± 0.3^{a}	16.0	18.8	64.1	1.1
	2	20486.8 ± 6.5^{ab}	252.5 ± 0.6^{ab}	88.5 ± 0.5^{ab}	1102.8 ± 3.2^{ab}	20750.1 ± 3.7^{ab}	19.6 ± 0.2^{ab}	59.5 ± 0.7^{ab}	20.3 ± 0.5^{ab}	34.6 ± 0.9^{ab}	3.7 ± 0.2^{ab}	0.1 ± 0.005^{ab}	9.5 ± 0.02^{ab}	24.4	18.3	54.3	3.0
	3	132924.9 ± 3.4^{ab}	19.3 ± 0.4^{ab}	684.1 ± 0.5^{ab}	4956.5 ± 2.9^{ab}	164077.8 ± 7.7^{ab}	147.7 ± 0.2^{ab}	354.8 ± 0.2^{ab}	278.7 ± 0.3^{ab}	861.6 ± 0.7^{ab}	12.2 ± 0.3^{ab}	1.8 ± 0.2^{ab}	345.4 ± 0.5^{ab}	10.1	35.2	48.6	6.1
8	1	104069.8 ± 86.8^{a}	6625.1 ± 1.3^{a}	358.7± 0.2^{a}	1441.1 ± 1.9^{a}	247371.0 ± 13.8^{a}	141.5 ± 1.0^{a}	322.7 ± 1.2^{a}	378.4 ± 1.0^{a}	287.4 ± 1.4^{a}	10.3 ± 0.02^{a}	0.4 ± 0.005^{a}	20.7 ± 0.1^{a}	45.3	28.1	26.1	0.5
	2	9690.1 ± 1.2^{ab}	228.3 ± 0.5^{ab}	165.1 ± 0.3^{ab}	664.6 ± 0.5^{ab}	19126.3 ± 2.05^{ab}	17.4 ± 0.03^{ab}	48.4 ± 0.05^{ab}	18.9 ± 0.2^{ab}	17.3 ± 0.3^{ab}	4.4 ± 0.2^{ab}	0.1 ± 0.0004^{a}	4.8 ± 0.3^{ab}	11.0	18.7	68.7	1.6
	3	59466.4 ± 3.4^{ab}	205.8 ± 0.3^{ab}	698.6 ± 0.4^{ab}	5833.5 ± 2.1^{ab}	127709.0 ± 10.5^{ab}	163.3 ± 0.4^{ab}	281.4 ± 0.4^{ab}	150.7 ± 0.4^{ab}	159.9 ± 0.7^{ab}	17.6 ± 0.7^{ab}	0.2 ± 0.02^{a}	397.2 ± 0.2^{ab}	34.0	29.6	35.3	1.1
9	1	158861.3 ± 184.3^{a}	617.4 ± 0.9^{a}	692.1 ± 1.3^{a}	2717.6 ± 1.2^{a}	140190.3 ± 71.7^{a}	92.8 ± 0.3^{a}	247.1 ± 1.1^{a}	328.4 ± 0.8^{a}	936.2 ± 1.8^{a}	10.9 ± 0.06^{a}	0.7 ± 0.01^{a}	90.4 ± 0.9^{a}	35.5	23.2	37.1	4.2
	2	87014.9 ± 4.6^{ab}	314.6 ± 0.5^{ab}	302.7 ± 0.3^{ab}	2255.6 ± 1.9^{ab}	76032.4 ± 3.3^{ab}	38.7 ± 0.3^{ab}	167.7 ± 0.6^{ab}	74.7 ± 0.3^{ab}	212.9 ± 0.3^{ab}	6.6 ± 0.2^{ab}	0.2 ± 0.06^{a}	28.3 ± 0.3^{ab}	1.8	17.4	79.0	1.9
	3	56337.6 ± 2.8^{ab}	14.2 ± 0.3^{ab}	415.3 ± 0.5^{ab}	1585.7 ± 2.1^{ab}	72375.4 ± 5.3^{ab}	56.3 ± 0.3^{ab}	122.7 ± 0.4^{ab}	126.7 ± 0.3^{ab}	432.5 ± 0.4^{ab}	4.5 ± 0.2^{ab}	0.4 ± 0.06	257.8 ± 2.0^{ab}	13.2	16.3	68.7	1.7
10	1	68660.1 ± 25.6^{a}	3622.8 ± 2.6^{a}	240.6 ± 0.4^{a}	3292.3 ± 0.7^{a}	148743.0 ± 71.8^{a}	141.7 ± 0.4^{a}	270.4 ± 0.6^{a}	252.0 ± 0.07^{a}	211.9 ± 0.02^{a}	10.9 ± 0.01^{a}	0.7 ± 0.01^{a}	44.3 ± 0.7^{a}	70.4	12.9	16.0	0.6
	2	63333.5 ± 1.1^{ab}	1193.5 ± 0.4^{ab}	172.0 ± 1.7^{ab}	1514.9 ± 1.8^{ab}	82633.5 ± 4.3^{ab}	50.1 ± 0.03^{ab}	154.7 ± 0.2^{ab}	99.3 ± 0.3^{ab}	160.5 ± 1.0^{ab}	6.5 ± 0.04^{ab}	0.2 ± 0.02^{ab}	14.9 ± 0.3^{ab}	55.9	21.2	21.8	1.1
	3	143881.0 ± 5.4^{ab}	4761.3 ± 0.3^{ab}	426.2 ± 0.4^{ab}	3347.2 ± 4.3^{ab}	207504.0 ± 5.4^{ab}	193.6 ± 0.3^{ab}	341.1 ± 0.6^{ab}	623.8 ± 0.4^{ab}	424.3 ± 0.5^{ab}	20.1 ± 0.5^{ab}	0.7 ± 0.05^{b}	528.2 ± 2.4^{ab}	49.1	26.6	22.4	1.9

The significantly higher ($p < 0.05$) concentrations of Al, Fe, As and Pb at Site 1 (Table 3.5), characterized by a number of springs are most probably due to the weathering effect of minerals caused by these springs. In spite of the fact that significant variation ($p < 0.05$) in metal concentrations were found at the different sites during the surveys, the concentration of Cr, Co, Ni and As were, in most of the cases, significantly higher ($p < 0.05$) at Sites 2 and 4 (Table 3.5), where mining effluent enters the Loop Spruit. The elevated concentrations of these four metals were no longer evident downstream from these sites (Table 3.5).

With regard to the remaining metals no apparent deviations could be found downstream from Site 1 and it can be concluded that the concentrations measured for these metals are mainly due to a combination of natural and various anthropogenic influences.

3.3.6 Spatial and temporal variation of metals in sediment

The PCA (Figure 3.2) represents the associations between the metal concentrations and supplementary variables (particle size and minerals), at the sites found during the three surveys. This PCA triplot only describes 29.50 % of the total variation with Sites 6, 7, 8 and 9 separating from Sites 1, 2, and 10 on the first axis. This separation between these sites can be explained by the metals Ti, Cu and Fe, describing 16.95 % of the variation on the first axis (x-axis). On the second axis (y-axis) a slight temporal influence is evident during the third survey at Sites 1, 2, 3, 5, 6 and 7, which separated in the upper right quadrant. This separation can be explained by the metals Pb, Cd, Al and Zn describing 12.55 % of the variation on the second axis (y-axis). These metal concentrations are rather due to a combination of factors and not solely due to the influence of mines. These groupings based on the metal concentrations can further be explained by the dominant lithology (Table 3.2) and substrate characteristics of the respective sites (Table 3.5). The clay particles associated with higher metal concentrations at all of the sites. The silt particles associated with Sites 4 and 8, during the second survey and Site 9, during the second and last surveys since it was the dominant particle size at these sites. Calcite associated with Site 6, during all the surveys, since this was the only site where calcite occurred.

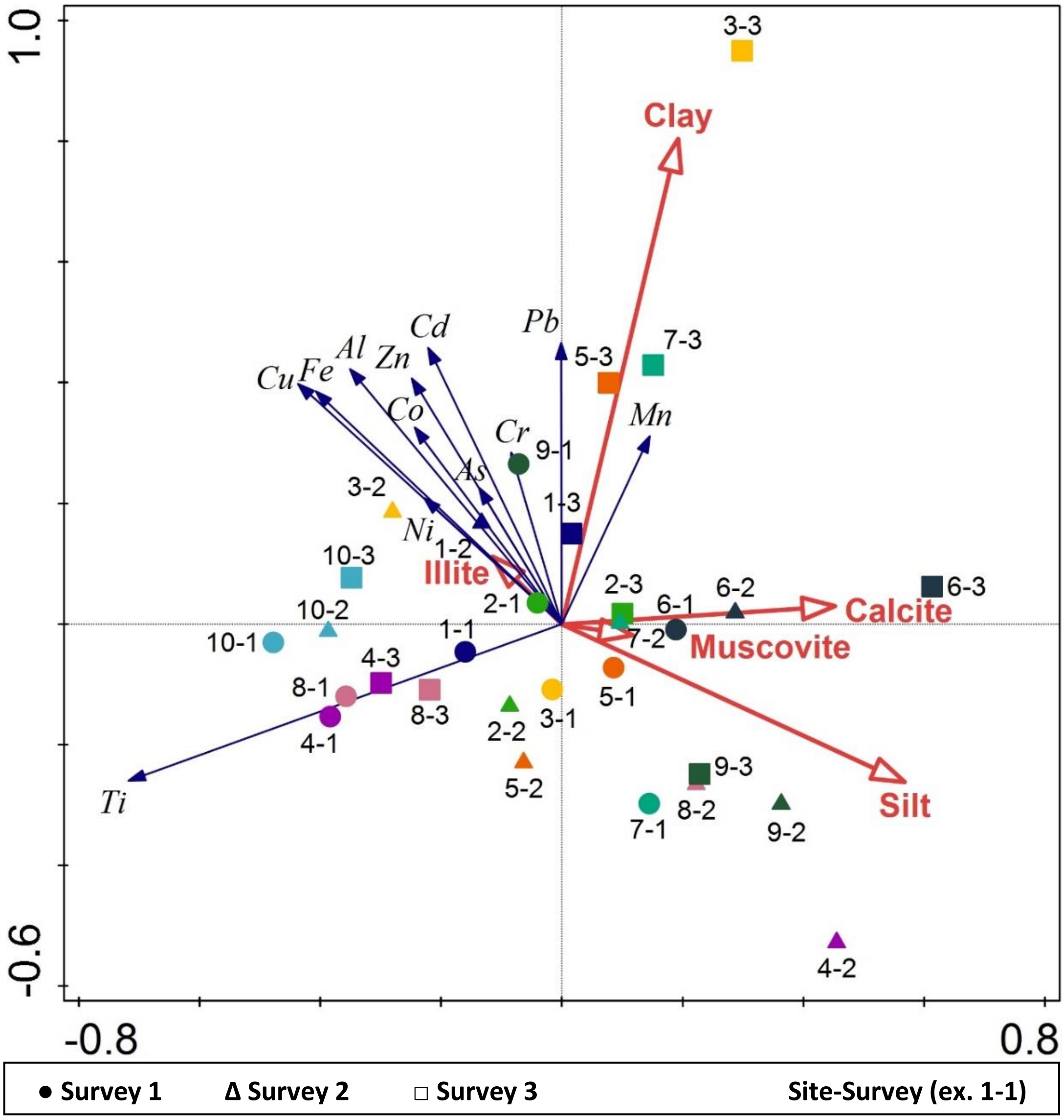


Figure 3.2: PCA triplot illustrating associations between metal concentrations and supplementary variables (sediment particle size (gravel > 2 000 µm, sand > 500 µm, silt 53 µm - 500 µm and clay < 53 µm) and minerals (quartz, illite, muscovite, kaolinite, magnesioferrite, chrysotile, calcite, vermiculite, montmorillonite and albite)) in the sediment found at the sampling sites during all three surveys. Only the supplementary variables with significant ($p < 0.05$) influence are indicated in the graph. The triplot describes 29.50 % of the variation with 16.95 % on the first axis and 12.55 % on the second axis.

3.4 Conclusion

From the results obtained during this study, it seems that mining activities within the Loop Spruit catchment, could contribute to the higher concentrations of Cr, Fe, Co, Ni, As, Cd and Pb in the surface water at Sites 2 and 4 (Table 3.4), while Cr, Co, Ni and As were significant ($p < 0.05$) in the sediment of these two sites. Even though the Cr concentration for the surface water was low, high concentrations were detected in the sediment, which can possibly be ascribed to a past mining event no longer evident in the water column. The fact that a decrease in concentration of these metals occurred downstream from the sites nearest to the mines, accepts the hypothesis stated that mining activities in the upper catchment results in an increase in metal concentrations in water and sediment of the Loop Spruit. From this investigation it was evident that not only mining but other anthropogenic activities, like agricultural activities and sewage treatment plant effluent, also influenced the quality of the water and concentrations of metals found in the sediment of the lower Loop Spruit catchment.

Chapter 4: Macroinvertebrate diversity and its association with selected physico-chemical factors, as well as biotopes.

4.1 Introduction

Biological assessment in rivers is internationally recognised as a method for determining the ecosystem health (Chapman, 1998; Davies & Day, 1998; Karr & Chu, 1999; Thirion, 2007; Malherbe *et al.*, 2010; Griffiths *et al.*, 2015). Aquatic macroinvertebrates are regarded as valuable bio-indicators of ecosystem health due to their different sensitivities to habitat alteration and pollution (Dickens & Graham, 2002; Dallas, 2007; Thirion, 2007; Griffiths *et al*., 2015). Excessive organic, as well as chemical pollution can alter community structures resulting in a reduction in species diversity (Thirion, 2007). The use of macroinvertebrates as indicators have various advantages including their ability to populate a variation of aquatic biotopes, low mobility, small size, as well as the fact that they are easy to sample (Rosenberg & Resh, 1993; Dickens & Graham, 2002; Thirion, 2007; Malherbe *et al*., 2010). Macroinvertebrates further perform various important functions within a freshwater ecosystem, such as the retention and breakdown of organic material, the recycling of nutrients and minerals, while they also contribute to energy processing at different trophic levels (Rosenberg & Resh, 1993; Allan, 1995; DWAF, 1999; Malherbe *et al.*, 2010).

Although water of suitable quality is essential to sustain a healthy population, different taxa exhibit different tolerances to specific water quality variables (Thirion, 2007). Some of the major physical and chemical properties of water that can affect the abundance and distribution of aquatic organisms, include amongst others temperature, oxygen, salinity, EC and dissolved solids, turbidity, light and acidity (Chapman, 1998; Davies & Day, 1998; Griffiths *et al.*, 2015). A rise in temperature will result in an increase in the rate of metabolism (Griffiths *et al.*, 2015), while EC may disturb, in case of extreme fluctuations, the salt balance in the organism's body (Griffiths *et al.,* 2015). Turbidity on the other hand, can lead to a decrease in the oxygen concentration of the water, leading to an increase in anaerobic respiration, also influencing the community structure (Griffiths *et al.,* 2015). The pH of the water is of paramount importance to most aquatic organisms, which cannot survive at pH levels lower than 6.5 and higher than 8.5, partly due to the fact that metabolic processes are slowed down (Petrin *et al*., 2007; Peters *et al*., 2013; Griffiths *et al*., 2015). More significantly is the increase in the metal concentration caused by a decrease in pH, which makes the metals more toxic (Peters *et al*., 2013; Griffiths *et al.,* 2015).

With regard to sediment, it is well known that suspended particulates may have a detrimental effect on the organisms by inhibiting respiration through the clogging of gills (Jones *et al*., 2012). It also reduces the visibility of predators and prey to detect each other while some particles can absorb or release nutrients or toxicants from their surfaces (Jones *et al*., 2012; Griffiths *et al.*, 2015).

Besides the above mentioned factors the availability of specific biotopes such as pool, riffle, run, gravel, sand and mud (GSM), marginal and aquatic vegetation, as well as stones in and out of current have a determining influence on the community structure. Various studies in the past were performed to establish the association between macroinvertebrate biodiversity and biotopes (Dallas, 2007; Thirion, 2007; Demars *et al*., 2012).

For this chapter the hypothesis states that, the macroinvertebrate community structure will be altered by mining activities from the upper reaches of the Loop Spruit. The aim of this chapter was then to establish the diversity of aquatic macroinvertebrates and its association with selected abiotic factors and biotopes within the Loop Spruit.

4.2 Material and Methods

4.2.1 Fieldwork

Three surveys (July 2014, September 2014 and May 2015) were conducted during the dry seasons for two successive years at ten preselected sites (Figure 2.1). Aquatic macroinvertebrates were collected during each of the surveys at all the sites. Macroinvertebrates were collected based on the same methodology used by Kemp *et al*. (2014) and Wolmarans *et al*. (2014). Macroinvertebrates were sampled using a standard South African Scoring System Version 5 (SASS 5) net (30 cm square frame with a sturdy handle and a Perlon® gauze net with mesh size of 1 mm) (Dickens & Graham, 2002) in three biotopes - stones (in and out of current), GSM, as well as the vegetation (marginal and aquatic). Each of the biotopes was sampled for approximately 15 minutes at each site. The stones biotopes were sampled by disturbing the stones with the feet while continuously sweeping the net downstream over the disturbed area. Macroinvertebrates on the GSM substrata were sampled as described for the stones. Marginal and aquatic vegetation were sampled by pushing the net vigorously into the vegetation and moving it backwards and forwards through the same area. The contents of the net, after sampling each biotope, were transferred to a white SASS 5 tray (360 x 470 x 80 mm) (Dickens & Graham, 2002) which was filled up to half with site water. Most of the coarse plant material was carefully removed by hand without discarding any organisms with it. The contents of the SASS 5 tray were decanted into a cone-shaped net (0.25 mm mesh) suspended on a stand to eliminate any excessive water. The decanted material was transferred from the net into a plastic container with a tight-fitting lid after which an adequate amount of 70 % ethanol was added to fix and preserve the samples. Each plastic container was labelled with relevant site information and the samples were transported back to the laboratory for identification.

Selected abiotic factors including EC (DIST 3, MI98303, Hanna Instruments), pH (MI98128, Hanna Instruments), temperature (Checktemp, Hanna Instruments), turbidity (GroundTruth Clarity Tube) and flow-rate (Global Water Flow Probe, model FP111) were measured *in situ* at each site. Biotope descriptions of each site were noted and the dominant marginal and aquatic vegetation were identified with the aid of a field guide (Gerber *et al*., 2004) and noted at each site.

4.2.2 Laboratory methods

4.2.2.1 Macroinvertebrate identification

The macroinvertebrate samples of each site were transferred to a Perspex® sorting tray (300 x 200 x 25 mm). The organisms were sorted into morpho-types using a stereo microscope with a light source from top and bottom. Each of the morpho-types was transferred to a 30 ml bottle equipped with a tight fitting cap in which 70% ethanol was added as preservative. Each bottle was labelled with the relevant information, which included site identification, GPS reference and sampling date. Morpho-types were identified up to species level, whenever possible, otherwise identification was done up to genus or family level, with the aid of the Guides to Freshwater Invertebrates of Southern Africa (Hamer, 1999; Seaman *et al*., 1999; Griffiths & Stewart, 2001; Hart *et al*., 2001; Martens, 2001; Rayner, 2001; van As & van As, 2001; Appleton, 2002a; Appleton, 2002b; Dippenaar-Schoeman, 2002; Jansen van Rensburg & Day, 2002; Oosthuizen & Siddall, 2002; Rayner *et al*., 2002; van Hoven & Day, 2002; Barber-James & Lugo-Ortiz, 2003; Coetzee, 2003; de Meillon & Wirth, 2003; de Moor, 2003; de Moor & Scott, 2003; Harrison, 2003; Harrison *et al*., 2003a; Harrison *et al*., 2003b; Henning, 2003; Reavell, 2003; Samways & Wilmot, 2003; Stevens & Picker, 2003;

Biström, 2007; Endrödy-Younga, 2007a; Endrödy-Younga, 2007b; Endrödy-Younga & Stals, 2007a; Endrödy-Younga & Stals, 2007b; Endrödy-Younga & Stals, 2007c; Endrödy-Younga & Stals, 2007d; Grobbelaar, 2007; Nelson, 2007a; Nelson, 2007b; Perkins, 2007; Stals, 2007a; Stals, 2007b; Stals & Endrödy-Younga, 2007; Villet & Endrödy-Younga, 2007) compiled for the Water Research Commission in Pretoria, as well as additional literature (Davies & Day, 1998; Gerber & Gabriel, 2002a; Gerber & Gabriel, 2002b). All of the specimens were counted and grouped into the relevant orders.

4.2.2.2 Sensitivity values

After the macroinvertebrates were identified, counted and recorded in Excel sheets, the SASS 5 sensitivity values (Dickens & Graham, 2002) were allocated to each family to classify the macroinvertebrates into three classes (Table 4.1). These classes were tolerant (values 1-5), moderately sensitive (values 6-10) and highly sensitive (values 11-15) taxa towards organic pollution. No sensitivity value was allocated to those families collected that do not have SASS 5 sensitivity values.

Table 4.1: SASS 5 sensitivity values.

Sensitivity values	Sensitivity category
1-5	Tolerant taxa
6-10	Moderately sensitive taxa
11-15	Highly sensitive taxa

4.2.3 Statistics

Statistical analyses, which included the relevant biodiversity indices were applied. Species Richness (SR) was used to determine the total number of species present in a community (Heip *et al*., 1998), while the Shannon-Wiener-index (H') is used to characterize species diversity in a community (Heip *et al*., 1998; Beals *et al*., 2000). The higher the value of H', the greater the biodiversity is in a given locality. This value is calculated by the following equation:

$$H' = \sum_{i=1}^{s} p_i \ln p_i$$

Pielou's evenness index (J') describes the even distribution between species at a given sample point and is defined as the Shannon-Wiener-indices (H') divided by the natural logarithm of the species richness (SR). In a healthy ecosystem it is accepted that an even distribution between species in a given community will occur. Subsequently community stability can be related (Heip *et al*., 1998). Values of J’ can vary between 0 and 1, thus 1 indicates an even distribution and 0 an uneven distribution. To calculate J’ the following equation is used:

$$J' = -\frac{H'}{\ln S}$$

Statistical significance of the spatial and temporal variation of the selected abiotic factors and biodiversity indices were determined at $p < 0.05$. Normality and homogeneity of variance were tested using D’Agostino and Pearson omnibus normality test and Kolmogorov-Smirnov test (with Dallal-Wilkinson-Lilliefor P value) respectively. When data was parametric, ANOVA and Tukey’s multiple comparison tests were performed. In the case of non-parametric data, Kruskal-Wallis tests with Dunn’s Multiple Comparison Tests were performed to test for significant differences between sites and surveys.

A Redundancy Analysis (RDA) is used to study the relationship between two tables of variables (families, biotopes and selected abiotic factors) and is non-symmetric (Kent, 2012). The RDA was created using Canoco (v5).

4.3 Results and Discussion

4.3.1 Abiotic factors and biotopes

The mean of the three surveys and the standard error of the temperature (A), pH (B), EC (C), turbidity (D) and flow-rate (E), between the sites are depicted in Figure 4.1, respectively. During this study the mean temperatures ranged between 10.6 ˚C at Site 1 and 15.7 ˚C at Site 9 (Tables 4.2, 4.3 and 4.4 and Figure 4.1A). The mean temperature between sites did not differ significantly (Figure 4.1A).

Table 4.2: Selected abiotic factors and biotopes at each of the sites, obtained during the first survey.

Abiotic factors and Biotopes	Sites									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
pH	7.2	6.7	7.0	6.8	6.9	7.0	8.3	7.4	7.7	8.3
EC (µS/cm)	65	901	198	1 207	906	806	480	781	695	737
Temperature (°C)	9.2	12.3	13.2	11.7	10.9	10.5	10.7	7.3	12.3	11.7
Turbidity (NTU)	7	5	5	5	6	10	35	6	6	5
Flow-rate (m/s)	0.2	1.2	0.1	0.3	0.4	0.5	0.1	0.3	0.3	0.2
Marginal Vegetation	X	X	X	X	X	X	X	X	X	X
Aquatic Vegetation		X		X						X
Algae		X	X			X	X			X
SiC		X		X	X	X		X		X
SoC					X			X		
Riffle		X		X	X	X				X
Run	X	X	X	X	X	X		X	X	X
Pool				X			X			
GSM	X	X	X	X	X	X	X	X	X	X

X indicates the biotopes that were present at the preselected sites.

The mean pH fluctuated between 6.7 and 7.7, at Sites 2 and 10 (Tables 4.2, 4.3 and 4.4 and Figure 4.1B). Although the pH did fluctuate between the sites, differences were only minimal and did not differ significantly between the sites (Figure 4.1B). The lowest mean pH values recorded during the three surveys, were measured at Sites 2 and 4 (Tables 4.2, 4.3 and 4.4 and Figure 4.1B), which were the only two sites where mining effluent enters the river system and this could possibly account for the low pH values.

Table 4.3: Selected abiotic factors and biotopes at each site, recorded during the second survey.

Abiotic factors and Biotopes	Sites									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
pH	6.0	6.4	6.3	6.2	6.7	6.7	7.2	7.6	7.1	7.1
EC (µS/cm)	80	783	297	1362	823	772	558	1303	789	788
Temp (°C)	12.6	14.7	15.5	15.7	16.5	16.6	21.3	20.4	19.4	17.4
Turbidity (NTU)	6	5	5	84	10	6	7	5	11	6
Flow-rate (m/s)	0.2	0.5	0.1	0.3	0.2	0.5	0.1	0.1	0.5	0.1
Marginal Vegetation	X	X	X	X	X	X	X	X	X	X
Aquatic Vegetation	X	X	X	X	X					X
Algae	X	X					X		X	X
SiC		X		X	X	X		X		X
SoC					X			X		
Riffle				X	X	X				X
Run	X	X		X	X	X		X	X	X
Pool			X				X			
GSM	X	X	X	X	X	X	X	X	X	X

X indicates the biotopes that were present at the preselected sites.

The mean EC varied from 71.7 µS/cm, at Site 1 to 1 303.3 µS/cm, at Site 4, while Sites 2, 5, 6 and 8 were above the mean (Tables 4.2, 4.3 and 4.4 and Figure 4.1C). Electrical conductivity differed significantly ($p < 0.05$) between Site 1 and all the sites except Site 3, between Site 2 and Sites 3 and 4, between Site 3 and Sites 4, 5, 6, 8, 9 and 10, between Site 4 and Sites 5, 6, 7, 9 and 10, between Site 7 and Site 8, as well as between Site 8 and 9 (Figure 4.1C). The low EC value at Site 1 indicates that it is clean groundwater at the origin of the Loop Spruit (Tables 4.2, 4.3 and 4.4 and Figure 4.1C). Site 4 had the highest EC value and is located in an area with agricultural and mining activities, as well as a scrapyard located on the banks of the stream. Site 2 also receives mining effluent, while Site 8 is located within an intensive agricultural area. The high EC values could be ascribed to the use of fertilizers and other products which will increase the conductivity because of the surplus chloride, nitrate and phosphate ions (Rose *et al*., 2014).

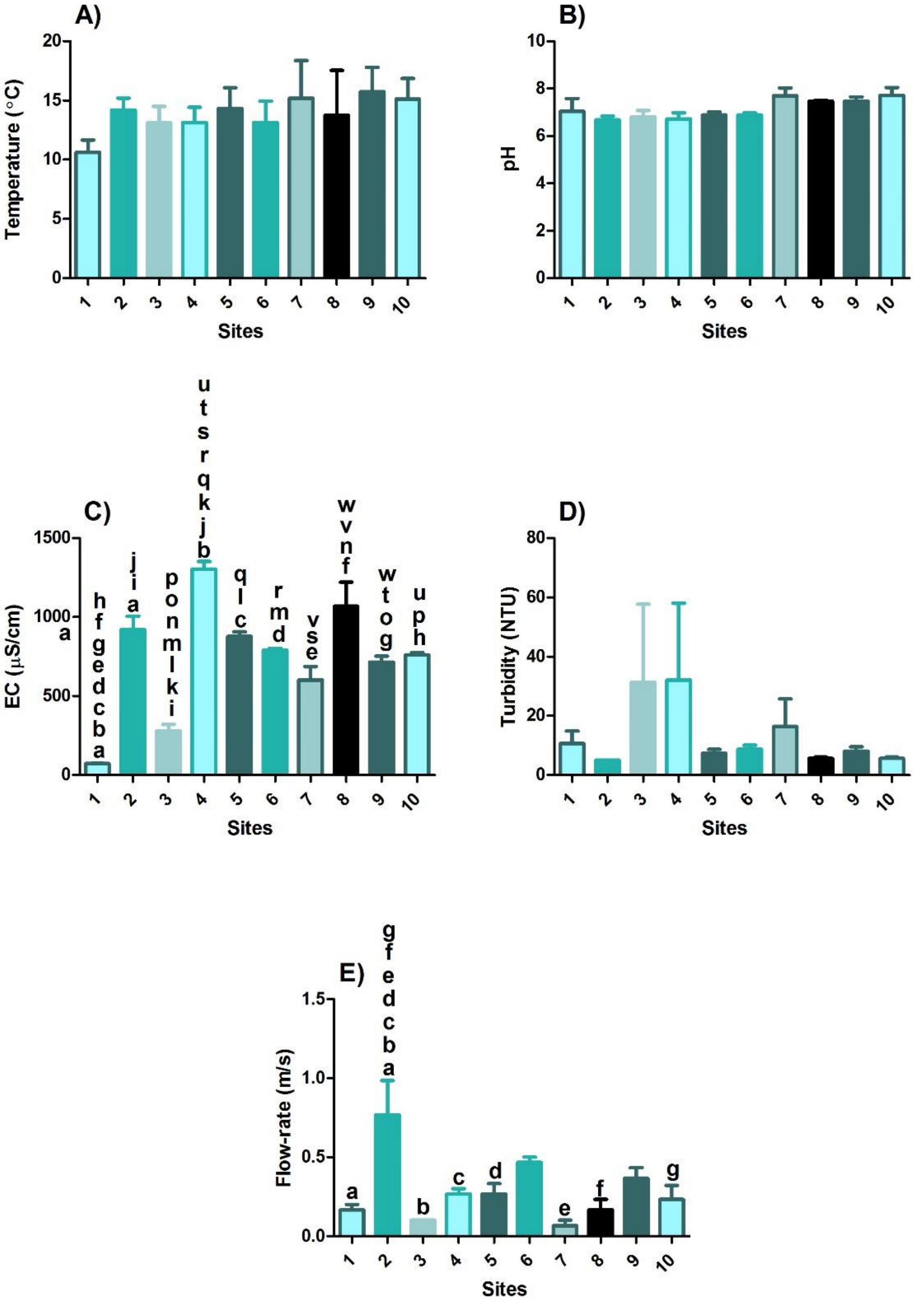
<u>Table 4.4:</u> Selected abiotic factors and biotopes at each site, during the third survey.

Abiotic factors and Biotopes	Sites									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
pH	7.9	7.0	7.2	7.1	7.1	7.0	7.5	7.4	7.5	7.7
EC (µS/cm)	70	1076	340	1341	906	797	766	1120	661	754
Temp (°C)	10.1	15.6	10.6	12	15.6	12.3	13.6	13.5	15.5	16.2
Turbidity (NTU)	19	5	84	7	6	10	7	6	7	6
Flow-rate (m/s)	0.1	0.6	0.1	0.2	0.2	0.4	0.01	0.1	0.3	0.4
Marginal Vegetation	X	X	X	X	X	X	X	X	X	X
Aquatic Vegetation	X	X		X					X	X
Algae		X	X	X	X	X		X	X	X
SiC		X		X	X	X		X		X
SoC					X			X		
Riffle					X	X		X		X
Run		X		X	X	X		X	X	X
Pool	X		X				X	X		
GSM	X	X	X	X	X	X	X	X	X	X

X indicates the biotopes that were present at the preselected sites.

The turbidity was the highest at Site 4 with a mean value of 32 NTU and the lowest at Site 2 with a mean value of 5 NTU, although Sites 3, 4 and 7 had a high mean there was no significant difference between the sites (Tables 4.2, 4.3 and 4.4 and Figure 4.1D). As for flow-rate, the means varied between 0.07 m/s at Site 7 and 0.77 m/s at Site 2 (Tables 4.2, 4.3 and 4.4 and Figure 4.1E). There was a significant difference ($p < 0.05$) between Site 2 and Sites 1, 3, 4, 5, 7, 8 and 10 (Figure 4.1E). The consistent high flow-rate at Site 2 can be ascribed to the fact that mining effluent enters the Loop Spruit at this site. Although the flow-rate decreased at all of the other sites during the last survey due to the drought, a higher flow-rate was recorded at Site 2 and 10 (Table 4.4). The reason for the higher flow-rate at Site 10 can be attributed to the fact that Site 10 is located below Potchefstroom's sewage treatment plant, thus receiving effluent from Potchefstroom's residential area.

The marginal vegetation and GSM biotopes were present at all of the sites during all of the surveys (Tables 4.2, 4.3 and 4.4). The run biotope was present at all of the sites during all the surveys, except for Site 7 during all three surveys, Site 3 during the last two surveys and Site 1 during the last survey. Aquatic vegetation occurred at Sites 2, 4 and 10 during all three surveys, at Site 1 during the last two surveys, Sites 3 and 5 during the second survey and at Site 9 in the last survey (Tables 4.2, 4.3 and 4.4). The stones in current (SiC) were present at Sites 2, 4, 5, 6, 8 and 10 during all three surveys, while stones out of current (SoC) occurred at Sites 5 and 8 during this study. Algae varied between the sites and surveys (Tables 4.2, 4.3 and 4.4), but were more dominant in the last survey and could be due to the low water levels and concentrated nutrient concentrations.



<u>Figure 4.1:</u> The mean of the three surveys and standard error of selected abiotic factors between the sites. Common alphabetical superscripts indicate significant differences ($p < 0.05$). The selected abiotic factors include: Temperature (A), pH (B), EC (C), turbidity (D) and flow-rate (E).

4.3.2 Biodiversity

Throughout this study a total of 72 families with 137 taxa were collected and identified. During the first survey 57 families (Table 4.5) and 105 taxa were collected, whereas the second survey had 55 families (Table 4.5) with 98 taxa present, and during the third survey 57 families (Table 4.5) with 98 taxa were collected. The family assemblages collected throughout the surveys were relatively consistent. Of the 57, 55 and 57 families collected during the three surveys, 44 families were present during all three surveys, while 17 families varied between sites and surveys. From the 44 families that were present throughout the three surveys, 16 families occurred most commonly and was found at five or more sites during all three surveys. Nineteen families were found at less than five of the sites and furthermore, only during one survey.

The majority of the 16 families that occurred most commonly, have a preference for poor or very poor water quality, with regards to organic enrichment (Brown, 1994; Seaman *et al*., 1999; Appleton, 2002b; Dickens & Graham, 2002; Oosthuizen & Siddall, 2002; van Hoven & Day, 2002; Barber-James & Lugo-Ortiz, 2003; de Meillon & Wirth, 2003; de Moor, 2003; de Moor & Scott, 2003; Harrison, 2003; Reavell, 2003; Samways & Wilmot, 2003; Biström, 2007; Perkins, 2007; Thirion, 2007; Griffths *et al*., 2015). The only exceptions were the Baetidae and Hydropsychidae, which are indicators of good water quality, when represented by more than two species at a specific site (Dickens & Graham, 2002; Barber-James & Lugo-Ortiz, 2003; de Moor & Scott, 2003; Thirion, 2007; Griffiths *et al*., 2015). This phenomenon occurred at Site 4 (Tables 4.7 and 4.8) during the second and third surveys, Site 6 (Tables 4.6, 4.7 and 4.8), during all three surveys and at Site 10 (Tables 4.7 and 4.8), during the last two surveys. With regard to the families that occurred frequently, at most of the sites during the three surveys, were Chironomidae, Tubificidae, Baetidae, Coenagrionidae and Dytiscidae (Table 4.5). Chironomidae occurred at all the sites during all the surveys, while Tubificidae occurred at nine of the ten sites in the first survey and at all the sites during the second and third surveys (Table 4.5). Baetidae were present at nine of the sites during all three surveys, whereas Coenagrionidae occurred at nine sites during the first two surveys and at eight sites in the last survey, while Dytiscidae were present at nine sites in the first survey, ten sites during the second survey and seven sites throughout the last survey (Table 4.5).

Table 4.5: Distribution of families over three surveys, at the ten preselected sites. (The values listed in the table below represent the number of sites, out of the ten preselected sites, where the families occurred).

Families	Survey		
	1	**2**	**3**
Branchipodidae	2	1	0
Daphniidae	7	8	5
Isotomidae	2	0	1
Cypridoidea	2	2	2
Atyidae	3	4	2
Potamonautidae	1	5	5
Hydroida	1	4	4
Rhabdocoela	5	2	6
Planariidae	1	2	0
Tubificidae	9	10	10
Glossiphoniidae	8	8	8
Salifidae	1	1	1
Hydrachnidae	1	1	0
Hygrobatidae	1	0	0
Limnesiidae	0	0	3
Teratothyasidae	0	2	0
Lymnaeidae	3	3	3
Ancylidae	7	8	9
Planorbidae	8	7	7
Physidae	5	4	4
Corbiculidae	2	2	3
Sphaeriidae	8	8	7
Baetidae	9	9	9
Caenidae	7	8	6
Lestidae	0	1	0
Protoneuridae	1	2	2
Chlorocyphidae	1	1	1
Coenagrionidae	9	9	8
Gomphidae	5	5	2
Aeshnidae	3	4	3
Libellulidae	3	5	1
Perlidae	1	0	0
Mesoveliidae	0	0	2
Veliidae	1	2	1
Gerridae	1	0	1
Corixidae	6	7	10
Notonectidae	4	4	4
Pleidae	1	2	6
Nepidae	0	2	0
Belostomatidae	2	5	3
Hydropsychidae	6	5	6
Hydroptilidae	2	1	3
Ecnomidae	0	1	3
Crambidae	1	0	0
Tipulidae	5	3	1
Blephariceridae	0	0	1
Psychodidae	0	1	2
Dixidae	0	1	0
Ceratopogonidae	7	5	5
Culicidae	3	3	5
Simuliidae	8	7	9
Chironomidae	10	10	10
Tabanidae	2	3	3
Athericidae	1	2	1
Stratiomyidae	4	0	0
Empididae	1	0	0
Sciomyzidae	1	0	2
Dolichopodidae	0	2	0
Ephydridae	2	2	1
Muscidae	3	7	1
Sphaeriusidae	0	0	1
Gyrinidae	5	6	3
Haliplidae	0	2	3
Dytiscidae	9	10	7
Spercheidae	1	0	0
Hydrophilidae	4	3	2
Hydraenidae	5	6	6
Scirtidae	0	0	1
Elmidae	2	1	1
Limnichidae	2	0	0
Chrysomelidae	0	0	4
Curculionidae	0	0	1
Total families per survey	**57**	**55**	**57**

According to Harrison (2003), Chironomidae can comprise up to 10 – 50 % of the biomass of aquatic invertebrates, thus forming an important link in the food chain and serving as a food source for various aquatic organisms. They also have haemoglobin, which enables them to enhance oxygen uptake in organically enriched environments, making it possible to survive and thrive in severely polluted water (Harrison, 2003; Griffiths *et al*., 2015). They feed on algae and detritus, while they can inhabit a wide variety of environments, from standing to running freshwater habitats, aquatic vegetation, GSM and stones in and out of current (Harrison, 2003; Thirion, 2007; Griffiths *et al*., 2015). Although the chironomids were present at all of the sites during the three surveys (Table 4.5), their abundance varied between sites and surveys from 11 to 951 specimens (Tables 4.6, 4.7 and 4.8). The sites where chironomids were collected in high abundance were Sites 3 and 10, during the first survey (Table 4.6), Site 2 in the second survey (Table 4.7) and Sites 4 and 5, during the third survey (Table 4.8), while Site 8 had a consistently high abundance throughout the study (Tables 4.6, 4.7 and 4.8). All of these sites had high EC values which varied from 737 to 1 362 µS/cm, except for Site 3 where a value of 198 µS/cm was recorded during the first survey (Tables 4.2, 4.3 and 4.4). The presence of algae was noted at five of the eight sites during sampling (Tables 4.2, 4.3 and 4.4). As seen from Tables 4.2, 4.3 and 4.4 the flow-rate varied between 0.1 and 0.5 m/s with the GSM biotope present at all of the sites.

<u>Table 4.6:</u> Biodiversity list of the macroinvertebrates collected during the first survey at all the sites and their sensitivity towards organic pollution.

Taxa		Sites										Sen[$]
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Anostraca												
Branchipodidae	*Branchipodopsis* sp.			8							3	
Cladocera												
Daphniidae	*Daphnia magna*		20					70	71	7	5	
	Simocephalus sp.	2		59				18	22		3	
Collembola												
Isotomidae	*Isotomides* sp.								2	1		
Ostracoda												
Cypridoidea	*Parastenocypris junodi*	2		1								
Decapoda												
Atyidae	*Caridina nilotica*				3			11			44	**
Potamonautidae	*Potamonautes warreni*									1		*
Cnidaria												
Hydroida	*Hydra* sp.		6									*
Turbellaria												
Rhabdocoela	*Mesostoma* sp.		2	3			2			11	8	*
Planariidae	*Planaria* sp.						6					*
Oligochaeta												
Tubificidae	*Branchiura sowerbyi*		5			4		4		3	3	*

Table 4.6 (continued)

	Tubifex sp.	41	20	287	4	154		57	183	291	25	*
Hirudinea												
Glossiphoniidae	*Alboglossiphonia* sp.		1	146		31			2	3		*
	Batracobdelloides tricarinata									1		*
	Helobdella stagnalis		2		4			2	17			*
	Marsupiobdella africana					24					2	*
	Placobdelloides jaegerskioeldi			24		217			1	12	6	*
Salifidae	*Salifa perspicax*										1	*
Trombidiformes												
Hydrachnidae											1	**
Hygrobatidae	*Atractides* sp.							3				**
Mollusca												
Lymnaeidae	*Lymnaea collumella*	1		3								*
	Lymnaea natalensis			1				1				*
Ancylidae	*Burnupia mooiensis*				11		7	1	4	5	3	**
	Ferrissia cawstoni	1								1	2	**
Planorbidae	*Gyraulus connollyi*	2			2			11			4	*
	Bulinus tropicus	5	162	20					19	2		*
Physidae	*Physa acuta*				2	2			402	3	51	*
Corbiculidae	*Corbicula fluminalis africana*						6				5	*
Sphaeriidae	*Pisidium costulosum*		7	2	2			1		1	1	*
	Pisidium langleyanum		44				1	10		13		*
	Pisidium viridarium	5	112	1	3							*
Ephemeroptera												
Baetidae	*Acanthiops erepens*				76		23		48			**
	Acanthiops sp.	56			2	1	462	6	22	4		**
	Baetidae sp.			15			50				31	**
	Cloeon & Procloeon sp.							56				**
	Ophelmatostoma sp.						16					**
Caenidae	*Caenis* sp.			2	5		190	13	55	4	23	**
Zygoptera												
Protoneuridae	*Ellattoneura glauca*		1									**
Chlorocyphidae	*Platycypha caligata*						1					**
Coenagrionidae	*Pseudagrion* sp.	4	20	3	23		2	25	4	20	20	*
	Enallagma glaucum							8				*
Anisoptera												
Gomphidae	*Notogomphus* sp.		1									**
	Ceratogomphus sp.										1	**
	Paragomphus sp.		1		13							**
	Onychogomphus sp.	3				1						**
Aeshnidae	*Aeshna* sp.		1									**
	Anax sp.			1					1			**
Libellulidae	*Notiothemis* sp.	1										*
	Orthetrum sp.		3									*
	Trithemis sp.										2	*
Plecoptera												
Perlidae		1										***
Hemiptera												
Veliidae	*Rhagovelia* sp.				1							*
Gerridae	*Gerris* sp.			1								*
Corixidae	*Micronecta* sp.					1						*
	Sigara sp.					7		1		10	6	*
	Agraptocorixa sp.			36				7	74	221	34	*
Notonectidae	*Anisops* sp.	1						4				*

Table 4.6 (continued)

	Notonecta sp.										1	*
	Enithares sp.			1								*
Pleidae	*Plea pullula*		4									*
Belostomatidae	*Appasus* sp.							1	2			*
Trichoptera												
Hydropsychidae	*Cheumatopsyche* sp.	1										*
	Cheumatopsyche afra						47					*
	Cheumatopsyche thomasseti		116	1	5		8	1				*
	Diplectronella medialis						2					*
Hydroptilidae	*Orthotrichia* sp.		84							1		**
Lepidoptera												
Crambidae	Schoenobiinae							1				***
Diptera												
Tipulidae	*Tipula* sp.	1			1	1	1					*
	Erioptera sp.								1			*
Ceratopogonidae	*Bezzia* sp.	2	3				8		1	2	3	*
	Culicoides sp.			1								*
Culicidae	Anophelinae	1						1	1			*
Simuliidae	*Simulium adersi*	29	186	1	17				39			*
	Simulium hargreavesi		247	3						4	2	*
	Simulium nigritarse			6			15					*
	Simulium ruficorne		228									*
Chironomidae	Tanypodinae	53	93	52	58		3	121	388	140	19	*
	Chironominae	12	56	899	9	35			18	6	266	*
	Orthocladiinae		7		4	1	97	12	109	7	10	*
	Rheotanytarsus sp.		6									*
Tabanidae		2			2							*
Athericidae			2									**
Stratiomyidae		1		1				1		3		
Empididae								1				**
Sciomyzidae									2			
Ephydridae									1		3	*
Muscidae			3		1						2	*
Coleoptera												
Gyrinidae	*Orectogyrus* sp.		2	2						7		*
	Dineutus sp.		1	1	19		3					*
Dytiscidae	*Dytiscidae sp.*			1								*
	Derovatellus sp.			1					2		4	*
	Laccophilus sp.	1	2		7		1	80	2	3	54	*
	Philodytes sp.				2			1	5		2	*
	Hydrovatus sp.			2							12	*
	Hydaticus sp.			1								*
Spercheidae	*Spercheus* sp.						1					
Hydrophilidae	*Laccobius* sp.			5					1			*
	Hydrophilus sp.								1			*
	Enochrus sp.		1					2				*
Hydraenidae	Hydraenidae sp.						86					**
	Ochthebius sp.						1				4	**
	Coelometopon sp.				2				1			**
	Hydreana sp.			1			1					**
Elmidae	Elmidae sp.			8				29				**
Limnichidae			1					2				**
	Number of Organisms	228	1450	1600	278	479	1040	562	1501	787	666	
	Species Richness (SR)	24	35	36	26	13	26	33	32	29	37	
	Shannon-Wiener (H')	2.15	2.51	1.57	2.45	1.43	1.88	2.57	2.20	1.83	2.43	
	Pielou's Evenness (J')	0.68	0.71	0.44	0.75	0.56	0.58	0.74	0.63	0.54	0.67	

$ Sensitivity, * tolerant taxa, ** moderately sensitive taxa, *** highly sensitive taxa.

The family Tubificidae also possesses haemoglobin, an attribute which enables this family to survive in anoxic environments, as well as under grossly organic enriched conditions (Dickens & Graham, 2002; van Hoven & Day, 2002; Griffiths *et al*., 2015). Tubificidae is therefore used as indicators of poor water quality and highly organic polluted sediments, where they mainly feed on organic material and prefers the GSM biotope (Dickens & Graham, 2002; van Hoven & Day, 2002; Thirion, 2007; Griffiths *et al*., 2015). This family occurred at all the sites during the three surveys, except for Site 6 during the first survey (Tables 4.6, 4.7 and 4.8). The number of specimens collected of this family ranged from 4 to 294 throughout this study, with high abundance occurring at Sites 3, 5 and 9 during all three surveys, at Site 1 during only the last two surveys and at Site 8 throughout the first and last survey (Tables 4.6, 4.7 and 4.8). The EC values at these sites varied between 70 µS/cm to 1 120 µS/cm and the presence of algae was noted at seven of the 13 above mentioned surveys (Tables 4.2, 4.3 and 4.4).

Baetidae can be an indicator of both poor and good water quality. They can occur in a variety of habitats, from lentic to lotic ecosystems and are benthic organisms, which occupy both stones and vegetation (Barber-James & Lugo-Ortiz, 2003; Thirion, 2007; Griffiths *et al*., 2015). Baetidae can be collector-gatherers, deposit feeders or scrapers, while some are predacious, which feed on midge larvae (Barber-James & Lugo-Ortiz, 2003; Griffiths *et al*., 2015). According to Barber-James and Lugo-Ortiz (2003), Baetidae have the ability to reproduce asexually, in which the young develops from unfertilized eggs, when experiencing extreme conditions and the development of baetids accelerates with a rise in temperature. The number of specimens collected throughout this study ranged between 1 and 551 (Tables 4.6, 4.7 and 4.8). As mentioned earlier, sites where more than two species of baetids were present are an indication of good water quality, and this was recorded for Sites 4 and 10, during the second and third surveys (Tables 4.7 and 4.8), as well as for Site 6, during all three surveys (Tables 4.6, 4.7 and 4.8). The EC values varied between 754 µS/cm and 1 362 µS/cm at these sites (Tables 4.2, 4.3 and 4.4), which does not necessarily indicate good water quality, but the presence of more than two baetid species could be ascribed to their habitat preferences. The baetid species that were the most abundant at these sites were *Acanthiops spp*., which according to Griffiths *et al*. (2015) are primarily found on small to medium size stones in fast to moderate flowing streams. All of these sites had SiC, riffles and a flow-rate between 0.1 m/s to 0.5 m/s (Tables 4.2, 4.3 and 4.4).

Table 4.7: Biodiversity list of the macroinvertebrates collected at each site, during the second survey and their sensitivity towards organic pollution.

Taxa		Sites										Sen[$]
		1	2	3	4	5	6	7	8	9	10	
Anostraca												
Branchipodidae	*Branchipodopsis* sp.							1				
Cladocera												
Daphniidae	*Daphnia magna*	20	19	16				112	221		16	
	Simocephalus sp.	14		58			2	62	53	2	9	
Ostracoda												
Cypridoidea	*Parastenocypris junodi*	119						15				
Decapoda												
Atyidae	*Caridina nilotica*				5			14	6		31	**
Potamonautidae	*Potamonautes warreni*		1				1	2	4	1		*
Cnidaria												
Hydroida	*Hydra* sp.		25						7	45	1	*
Turbellaria												
Rhabdocoela	*Mesostoma* sp.								5	2		*
Planariidae	*Planaria* sp.						1		2			*
Oligochaeta												
Tubificidae	*Branchiura sowerbyi*	3				5	6	3	2	12	4	*
	Tubifex sp.	122	56	98	10	218	8	71	32	186	58	*
Hirudinea												
Glossiphoniidae	*Alboglossiphonia* sp.			42		63	4	1	26	5		*
	Marsupiobdella africana		1			35			4	3	3	*
	Placobdelloides jaegerskioeldi		9	16		256		3	39	18	4	*
Salifidae	*Salifa perspicax*										4	*
Trombidiformes												
Hydrachnidae								1				**
Teratothyasidae							1		2			**
Mollusca												
Lymnaeidae	*Lymnaea collumella*	14				1					1	*
	Lymnaea natalensis	3				1						*
	Lymnaea truncatula	1										*
Ancylidae	*Burnupia mooiensis*				43	66	1	1	73	9	1	**
	Ferrissia cawstoni	2					1	1		1	1	**
Planorbidae	*Gyraulus connollyi*	1			20			6		1	3	*
	Bulinus tropicus	8	153	1	2			1		3	2	*
Physidae	*Physa acuta*				17				1	1	20	*
Corbiculidae	*Corbicula fluminalis africana*									41	6	*
Sphaeriidae	*Pisidium costulosum*	12	32	7	8			47	3	3		*
	Pisidium langleyanum	3	51					6	3	1		*
	Pisidium viridarium	7	24			1			3	2		*
Ephemeroptera												
Baetidae	*Acanthiops erepens*				10		12				18	**
	Acanthiops sp.			2	12		138	5		4	43	**
	Baetidae sp.	19	3		6		66			11	20	**
	Cloeon & Procloeon sp.	10		1				15	54			**
Caenidae	*Caenis* sp.		2	1	37		176	12	8	3	89	**
Zygoptera												
Lestidae	*Lestes plagiatus*	1										**
Protoneuridae	*Ellattoneura glauca*		1	3								**
Chlorocyphidae	*Platycypha caligata*				5							**
Coenagrionidae	*Pseudagrion* sp.	10	6	14	45		2	32	16	2	75	*
	Enallagma glaucum		2	2	21			5				*

Table 4.7 (continued)

Anisoptera												
Gomphidae	*Notogomphus* sp.			3								**
	Paragomphus sp.		1		13		1					**
	Onychogomphus sp.	3		1								**
Aeshnidae	*Aeshna* sp.	2	2	3	3							**
Libellulidae	*Tetrathemis* sp.			1								*
	Orthetrum sp.	1		1	3							*
	Bradinopyga sp.	1			4				2			*
	Trithemis sp.			3	1			1				*
Hemiptera												
Veliidae	*Rhagovelia* sp.				1		1					*
Corixidae	*Micronecta* sp.	3		8				25	94	2	30	*
	Sigara sp.			1					30		13	*
	Agraptocorixa sp.		29						38			*
Notonectidae	*Anisops* sp.							72	5	1	1	*
Pleidae	*Plea pullula*	2	5									*
Nepidae	*Laccotrephes* sp.		1	2								*
Belostomatidae	*Appasus* sp.	2		1	3			1			1	*
Trichoptera												
Hydropsychidae	*Cheumatopsyche afra*				3		19					*
	Cheumatopsyche thomasseti		1				20			1	1	*
	Diplectronella medialis		1									*
Hydroptilidae	*Hydroptila cruciata*		11									**
Ecnomidae	*Ecnomus thomasseti*						1					**
Diptera												
Tipulidae	*Tipula* sp.				1	1						*
	Erioptera sp.		1									*
	Limonia sp.		2									*
Psychodidae		2										*
Dixidae	Dixidae sp.		2									**
Ceratopogonidae	*Bezzia* sp.	7			1		12		2		19	*
	Culicoides sp.								10			*
Culicidae	Culicinae							2	5		50	*
	Anophelinae								6			*
Simuliidae	*Simulium adersi*	6		4			6	1				*
	Simulium chutteri		8									*
	Simulium hargreavesi	3	15		8		11	1				*
	Simulium nigritarse	47	64	1	90		137	1	7			*
	Simulium ruficorne		10				1					*
Chironomidae	Tanypodinae	55	79	25	21	5	15	29	22	4	15	*
	Chironominae	78	416	99	22	3	90	23	180	7	95	*
	Orthocladiinae	13	40	6	3		13		2		13	*
Tabanidae		2			1	1						*
Athericidae		1	1									**
Dolichopodidae		3			6							
Ephydridae			4			2						*
Muscidae		1	56			1	5	1	13		3	*
Coleoptera												
Gyrinidae	*Orectogyrus* sp.	2		4						17		*
	Dineutus sp.						1				7	*
	Aulonogyrus sp.				32						37	*
Haliplidae	*Haliplus sp.*			1				3				*
Dytiscidae	*Dytiscidae sp.*		3		1				5			*
	Derovatellus sp.	1		1		2	1	2	2		24	*
	Laccophilus sp.	17		5	2			27	49	3	31	*
	Philodytes sp.	3			6			1				*

Table 4.7 (continued)

	Africophilus sp.	1		1								*
	Hydrovatus sp.							15			17	*
	Hydaticus sp.		1	1	1							*
Hydrophilidae	*Enochrus* sp.	1							5		1	*
Hydraenidae	Hydraenidae sp.						73					**
	Ochthebius sp.				1			1	5		4	**
	Hydreana sp.	23					41				1	**
Elmidae	Elmidae sp.										2	**
	Number of Organisms	649	1138	433	468	661	867	622	1046	391	774	
	Species Richness (SR)	44	38	34	37	16	32	39	40	29	41	
	Shannon-Wiener (H')	2.77	2.40	2.42	2.94	1.54	2.45	2.79	2.77	2.06	3.06	
	Pielou's Evenness (J')	0.73	0.66	0.69	0.82	0.55	0.71	0.76	0.75	0.61	0.82	

[$] Sensitivity, * tolerant taxa, ** moderately sensitive taxa, *** highly sensitive taxa.

According to literature (Dickens & Graham, 2002; Samways & Wilmot, 2003; Thirion, 2007; Griffiths *et al*., 2015), Coenagrionidae have a preference for moderately fast flowing water (0.3 – 0.6 m/s), vegetation habitats and poor water quality. This family occurred in numbers ranging from 2 to 75 specimens throughout this study (Tables 4.6, 4.7 and 4.8). As for *Pseudagrion* spp., which were the dominant species of this family during the study, they can inhabit a vast array of freshwater habitats including fast flowing streams, ponds and lakes, while they prefer habitats with shaded overhanging vegetation, as well as aquatic vegetation (Samways & Wilmot, 2003; Thirion, 2007; Griffiths *et al*., 2015). *Pseudagrion* spp. were present at all of the sites except for Site 5, during the first two surveys (Tables 4.6 and 4.7) and Sites 3 and 7, during the last survey (Table 4.8). These species were present in abundance at Site 7 in the first survey (Table 4.6), Site 10 in the second survey (Table 4.7), Site 2 in the third survey (Table 4.8) and Site 4 in the last two surveys (Tables 4.7 and 4.8). All of these sites had an overhanging tree canopy at the sampling points and also had aquatic vegetation and marginal biotopes, except for Site 7 which only had marginal vegetation (Tables 4.2, 4.3 and 4.4). The flow-rate at these sites varied from 0.01 m/s to 0.6 m/s (Tables 4.2, 4.3 and 4.4).

The family Dytiscidae is regarded as true water beetles and are the largest of all the water beetle families (Biström, 2007). They are considered a keystone species within the freshwater food web, due to the fact that they can act as prey, as well as predators (Biström, 2007; Griffiths *et al*., 2015). They can occur in both fresh and brackish waters, also in standing to slow flowing waters (< 0.1 m/s), as well as in temporary waters due to the ability of flight in the adult beetles (Biström, 2007; Thirion, 2007). They have a preference for vegetation and poor water quality (Dickens & Graham, 2002; Thirion, 2007). The reason why this family was collected at several sites during the three surveys could most probably be ascribed to the fact that they are an important component of the aquatic macroinvertebrate assemblage (Biström, 2007; Griffiths *et al*., 2015). The sites where Dytiscidae were found in abundance were Sites 1 and 8 during the second survey (Table 4.7), Site 10 during the first two surveys (Tables 4.6 and 4.7) and Site 7 during all three surveys (Tables 4.6, 4.7 and 4.8). All of these sites had a slow flow-rate (0.01 – 0.5 m/s) and marginal vegetation in common, as well as algae, except for Site 7 during the last survey and Site 8 during the second survey (Tables 4.2, 4.3 and 4.4). The EC were also relatively high at all of these sites with values ranging from 480 to 1 303 µS/cm (Tables 4.2, 4.3 and 4.4), which can be taken as an indication of, amongst others, organic enrichment, considered as a preferred water quality for this family. Although this family was present at several sites throughout the three surveys, the number of specimens per sample ranged from only 1 to 81 organisms (Tables 4.6, 4.7 and 4.8).

Table 4.8: Biodiversity list of macroinvertebrates collected at each site, during the third survey and their sensitivity towards organic pollution.

Taxa		**Sites**										**Sen$^{\$}$**
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Cladocera												
Daphniidae	*Daphnia magna*			63		6		4				
	Simocephalus sp.			205		192	3		16			
Collembola												
Isotomidae	*Isotomides* sp.									1		
Ostracoda												
Cypridoidea	*Parastenocypris junodi*	22						3				
Decapoda												
Atyidae	*Caridina nilotica*				19						38	**
Potamonautidae	*Potamonautes warreni*	1			1		3		2	6		*
Cnidaria												
Hydroida	*Hydra* sp.							5	3	1	1	*
Turbellaria												
Rhabdocoela	*Mesostoma* sp.	1	1			1	1	1	2			*
Oligochaeta												
Tubificidae	*Branchiura sowerbyi*	3	2	1			3		7	6		*
	Tubifex sp.	110	20	123	6	186	36	96	114	140	36	*
Hirudinea												
Glossiphoniidae	*Alboglossiphonia* sp.							14	34	4		*
	Helobdella stagnalis							36	67	7	4	*
	Marsupiobdella africana						1					*
	Placobdelloides jaegerskioeldi			19	1	14	1		6			*
Salifidae	*Salifa perspicax*										4	*
Trombidiformes												
Limnesiidae				8				4	1			**
Mollusca												
Lymnaeidae	*Lymnaea collumella*	3										*
	Lymnaea natalensis					5		1				*
Ancylidae	*Burnupia mooiensis*		1		13	1	2	6	19	4	5	**
	Ferrissia cawstoni	9										**

Table 4.8 (continued)

Planorbidae	*Gyraulus connollyi*	1			1		1			3		*
	Bulinus tropicus	3	31					3	1			*
Physidae	*Physa acuta*		1		5	140					1	*
Corbiculidae	*Corbicula fluminalis africana*					1	1				1	*
Sphaeriidae	*Pisidium costulosum*		1	2				3		1	1	*
	Pisidium langleyanum	1	9					2				*
	Pisidium viridarium	5	9					2	1		1	*
Ephemeroptera												
Baetidae	*Acanthiops erepens*				15		10				52	**
	Acanthiops sp.		2		9	4	115	24		6	136	**
	Baetidae sp.	9			7		41				64	**
	Cloeon & Procloeon sp.							8	12			**
	Ophelmatostoma sp.						60					**
Caenidae	*Caenis* sp.	3	3		10		12		3		18	**
Zygoptera												
Protoneuridae	*Ellattoneura glauca*		6				1					**
Chlorocyphidae	*Platycypha caligata*				5							**
Coenagrionidae	*Pseudagrion* sp.	7	40		43	2	11		9	35	7	*
	Enallagma glaucum	1	15		18					2		*
	Agriocnemis pinheyi		1									*
Anisoptera												
Gomphidae	*Notogomphus* sp.						1					**
	Paragomphus sp.		13									**
Aeshnidae	*Aeshna* sp.	4			1							**
	Anax sp.		2		1							**
Libellulidae	*Tetrathemis* sp.	3										*
	Notiothemis sp.	3										*
	Bradinopyga sp.	9										*
	Trithemis sp.	1										*
Hemiptera												
Mesoveliidae	*Mesovelia* sp.	2			3							*
Veliidae	*Rhagovelia* sp.				7							*
Gerridae	*Neogerris* sp.				22							*
Corixidae	*Micronecta* sp.	10	7	294	35	15	9	292	193	11	45	*
	Sigara sp.							14	2		1	*
Notonectidae	*Anisops* sp.			3	7			5			26	*
Pleidae	*Plea pullula*	2	6	2	2		1		3			*
Belostomatidae	*Appasus* sp.	1	2							4		*
Trichoptera												
Hydropsychidae	*Cheumatopsyche* sp.		3									*
	Cheumatopsyche thomasseti		53		3		46	6	1		1	*
Hydroptilidae	*Hydroptila cruciata*				9					1	8	**
Ecnomidae	*Ecnomus thomasseti*	3			3		1					**
Diptera												
Tipulidae	*Tipula* sp.	4										*
	Limnophila sp.	1										*
Blephariceridae	*Clogmia* sp.	2										***
Psychodidae	Psychodidae sp.								2			*
	Pericoma sp.	1										*
Ceratopogonidae	*Bezzia* sp.				4							*
	Culicoides sp.			1				19	1	1		*
Culicidae	Culicinae				1	5		3	4			*
	Anophelinae	3			9			2				*
Simuliidae	*Simulium adersi*	1	28		73	4	205	7	36	2	8	*
	Simulium hargreavesi	1	3			2			10		2	*
	Simulium nigritarse		5		27	3	15					*

Table 4.8 (continued)

	Simulium ruficorne		5						12			*
Chironomidae	Tanypodinae	19	19	170	22	19	5	175	5	5	28	*
	Chironominae	63	67		189	566	25	63	180	37	63	*
	Orthocladiinae	1	4	8	16	4			26	5	8	*
Tabanidae		1			4					1		*
Athericidae					1							**
Sciomyzidae		3							3			
Ephydridae								34				*
Muscidae			12									*
Coleoptera												
Sphaeriusidae		1										
Gyrinidae	*Aulonogyrus* sp.				122		7				79	*
Haliplidae	*Haliplus sp.*			1	3			3				*
Dytiscidae	*Derovatellus* sp.	1		1								*
	Laccophilus sp.	5	1	1								*
	Philodytes sp.	1		3	4			2			7	*
	Philaccolus sp.								2			*
	Hyphydrus sp.			2								*
	Hydrovatus sp.	1			6			20			3	*
Hydrophilidae	*Berosus* sp.							2				*
	Laccobius sp.				1							*
Hydraenidae	*Ochthebius* sp.		1		1			1	5			**
	Limnebius sp.		1									**
	Hydreana sp.	6							2			**
	Parasthetops sp.				19		1	1				**
Scirtidae	*Cyphon* sp.							2				
Elmidae	Elmidae sp.				1							**
Chrysomelidae	*Donacia* sp.	24	2	1		1						
Curculionidae	*Bagous* sp.						1					
	Number of Organisms	356	376	908	749	1171	619	863	784	283	648	
	Species Richness (SR)	44	34	19	43	20	29	34	33	22	28	
	Shannon-Wiener (H')	2.66	2.81	1.76	2.78	1.56	2.24	2.27	2.36	1.90	2.59	
	Pielou's Evenness (J')	0.70	0.80	0.60	0.74	0.52	0.66	0.64	0.67	0.61	0.78	

$^{\$}$ Sensitivity, * tolerant taxa, ** moderately sensitive taxa, *** highly sensitive taxa.

As for the 19 families that occurred during only one survey at less than five of the ten sites (Table 4.5), there are no distinct characteristic common to all of them, according to literature, but nine of these families have a preference for moderate to good water quality, which include Hygrobatidae, Limnesiidae, Teratothyasidae, Lestidae, Perlidae, Crambidae, Blephariceridae, Dixidae and Limnechidae (Dickens & Graham, 2002; Harrison *et al*., 2003a; Harrison *et al*., 2003b; Henning, 2003; Stevens & Picker, 2003; Endrödy-Younga, 2007b; Thirion, 2007; Griffiths *et al*., 2015). The family Empididae are moderately sensitive towards organic pollution according to Dickens and Graham (2002) but according to Thirion (2007) has a preference for poor water quality. However, results obtained during this study showed that Empididae occurred at Site 7 (Table 4.6), which support the view of Dickens and Graham (2002). Three of the 19 families collected during this study can occur in very poor water quality, which include Mesoveliidae, Nepidae and Stratiomyidae (Dickens & Graham, 2002; Thirion, 2007), while the remaining six families (Dolichopodidae, Spercheidae, Scirtidae, Chrysomelidae and Curculionidae) have no information available regarding their water quality preferences.

The nine families with a preference for moderate to good water quality occurred five times at Site 7, with four of the five times during the first survey (Table 4.6) and the remaining one in the last survey (Table 4.8). This can be ascribed to the fact that Site 7 is a tributary of the Loop Spruit, which is subjected to only few anthropogenic influences. These families also occurred three times at Site 1, during all three surveys (Tables 4.6, 4.7 and 4.8) indicating that this site is largely a natural habitat within the Loop Spruit with only a few anthropogenic influences. These two sites seem to be the least impacted by organic pollution and can therefore accommodate families highly sensitive towards organic pollution.

The biodiversity indices, of the lower taxonomic data, with regards to the species richness (SR), Shannon-Wiener diversity index (H'), Pielou's Evenness Index (J') and number of organisms are displayed in Figure 4.2. From the figure it is clear that Site 1 had the highest SR, with a mean of 37 species, while Site 5 had the lowest SR, with a mean of 16 species (Figure 4.2A). Site 1 is located in a relatively natural area within the Loop Spruit and Site 5 is located in the Loop Spruit where various anthropogenic activities (agricultural, urban, industrial and mining activities) could possibly have had an influence. There was a significant difference ($p < 0.05$) in the SR and H' between Site 5 and Sites 1, 2, 4, 7, 8 and 10 (Figure 4.2 A and B). Site 5 (downstream of Fochville's waste water treatment plant, on the farm Lepat) also had the lowest Pielou's evenness value, with a mean of 0.54 (Figure 4.2C). The low J' value can be ascribed to the high abundance of *Tubifex* sp. during all three surveys, *Placobdelloides jaegerskioeldi* during the first two surveys and *Simochephalus* sp., *Physa acuta* and Chironominae during the last survey (Tables 4.6, 4.7 and 4.8). All of these taxa have a preference for very poor water quality, whereas *P. acuta*, also known as the sewage snail, is known to live in organically enriched freshwater habitats near human settlements (Brown, 1994; Appleton, 2002b; de Kock & Wolmarans, 2007; Griffiths *et al*., 2015). This snail not only tolerates organic pollution, but is an invasive species which is widespread within South Africa (Brown, 1994; Appleton, 2002b; Griffiths *et al*., 2015). According to de Kock and Wolmarans (2007), and Griffiths *et al*. (2015) this snail was initially from North America or the north west of Europe, but is now considered the most widespread freshwater snail in the world. The highest H' and J' values were found at Site 4 (Figure 4.2 B and C), which indicates the highest diversity and an even distribution between the species. The significant difference ($p < 0.05$) in the J' values between the sites, were between Site 3 and 4 and 4 and 5, as well as between Site 5 and 10 (Figure 4.2C). The number of organisms differed between the sites with the lowest mean of 411 organisms at Site 1 and the highest mean of 1 110 organisms at Site 8 (Figure 4.2D). It is also evident that the number of organisms varied between the surveys at each site.

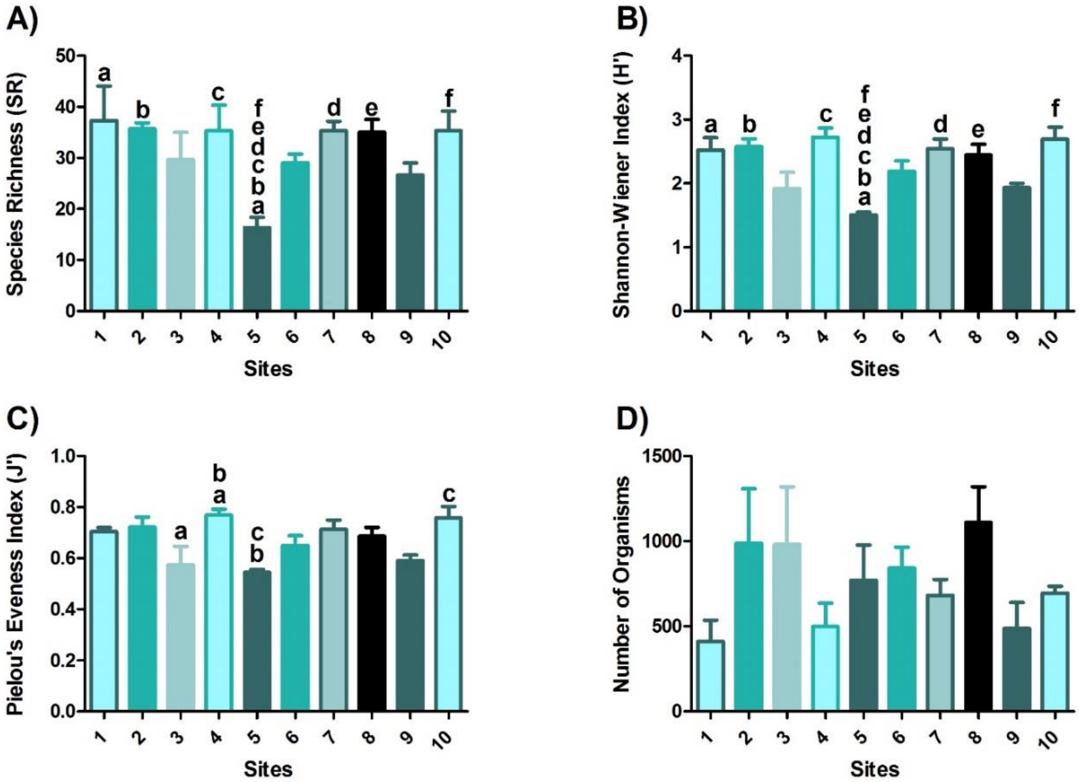


Figure 4.2: The mean of the three surveys and the standard error of calculated biodiversity indices between the sites. Common alphabetical superscripts indicate significant differences ($p < 0.05$). The indices include: A) Species Richness (SR), B) Shannon-Wiener diversity index (H'), C) Pielou's Evenness Index (J') and D) the number of organisms.

Figure 4.3 illustrates the percentage composition between highly sensitive, moderately sensitive and tolerant taxa towards organic pollution at each site, during the first survey. It is clearly visible that tolerant taxa are predominantly present at all the sites, and Site 5 showcased the highest percentage composition of tolerant taxa with 84.6 %. Site 6 had the lowest percentage of tolerant taxa (60 %) and the highest percentage of moderately sensitive taxa present (40 %). The only two sites with highly sensitive taxa present, were Sites 1 and 7, the origin of the Loop Spruit and the Ensel Spruit, respectively. These highly sensitive taxa included Perlidae and Crambidae at Site 1 and 7, respectively (Tables 4.6).

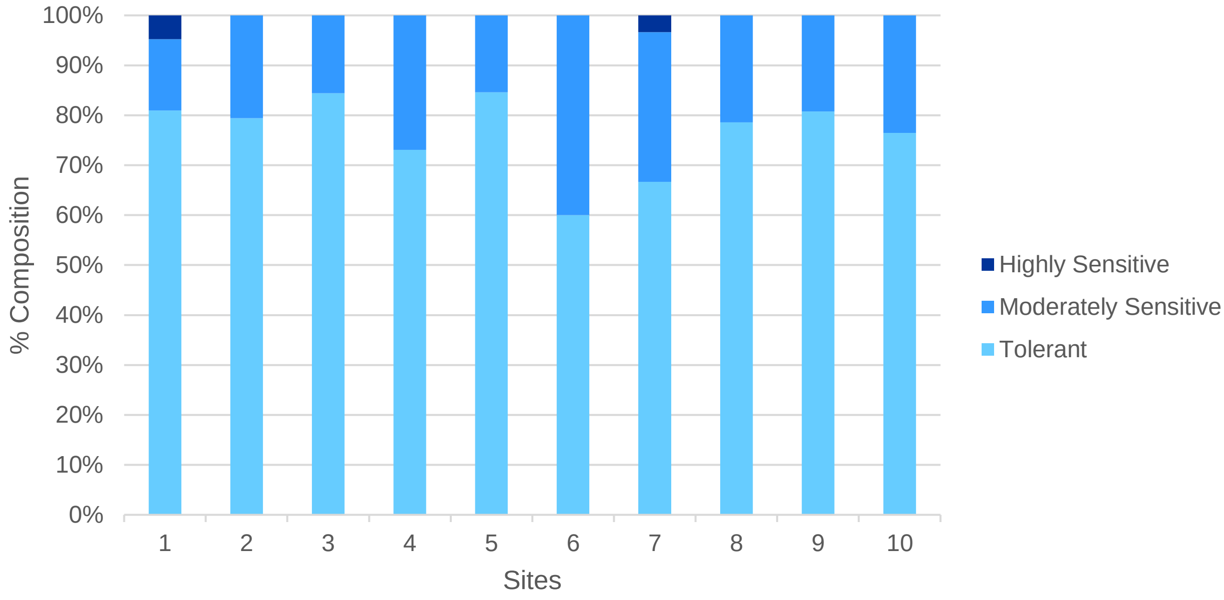
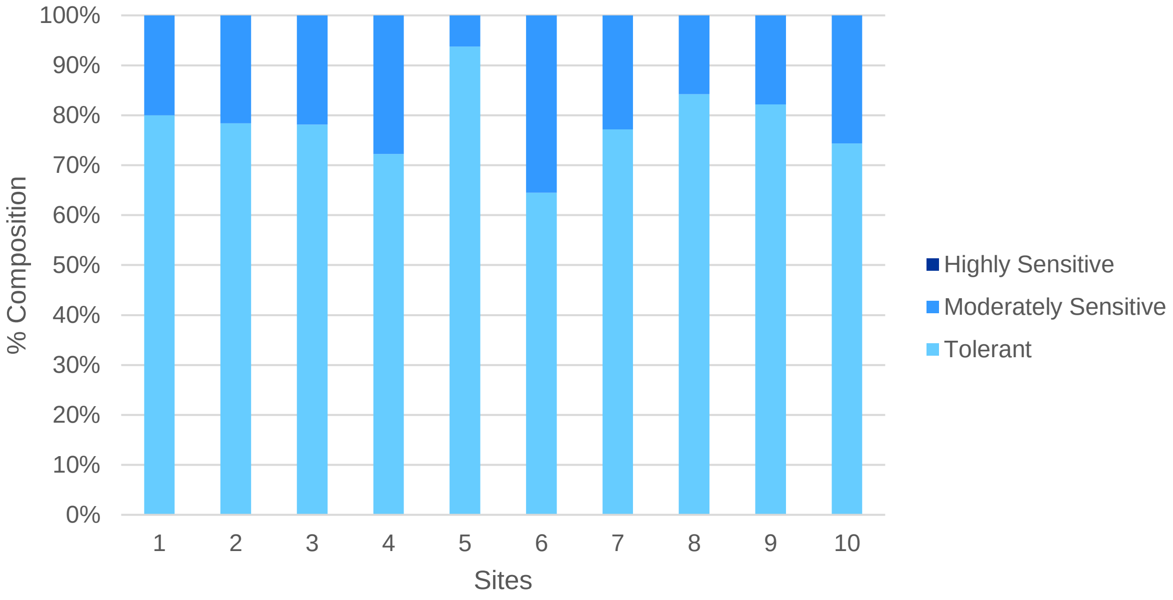


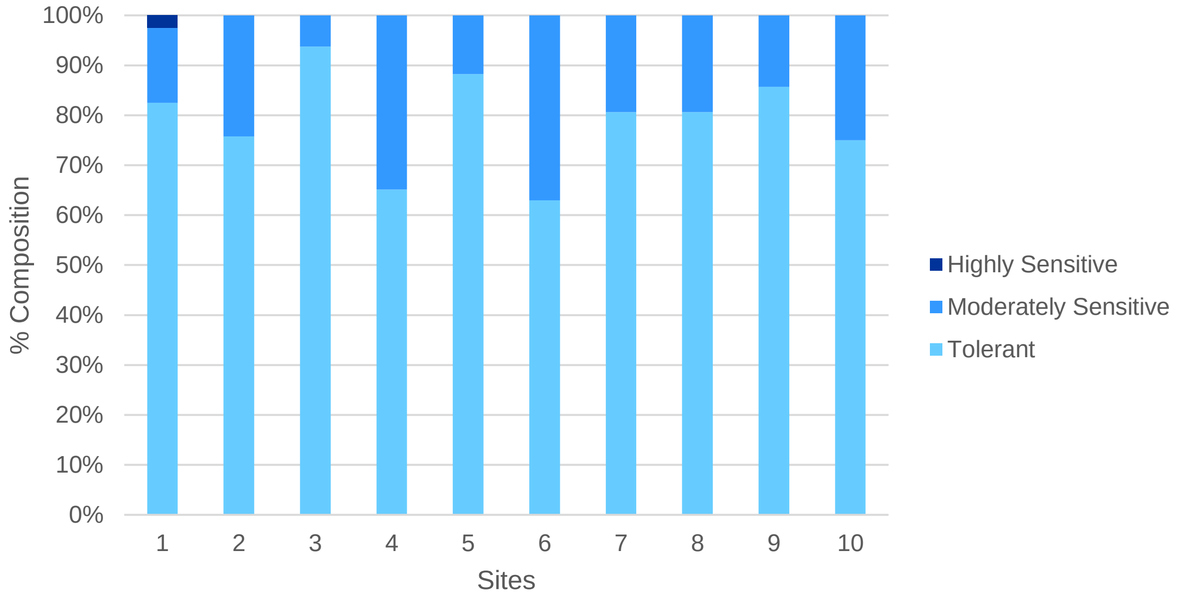
Figure 4.3: Percentage composition of highly sensitive, moderately sensitive and tolerant taxa per site, collected during the first survey.

The percentage composition of highly sensitive, moderately sensitive and tolerant taxa, towards organic pollution at each site, during the second survey is depicted in Figure 4.4. During this survey tolerant taxa were collected at each of the sites. The highest percentage tolerant taxa (93.8 %) and the lowest percentage moderately sensitive taxa (6.2 %) were collected at Site 5. Site 6 recorded the lowest percentage tolerant taxa (64.5 %) and the highest percentage moderately sensitive taxa (35.5 %). The fact that more than two Baetidae species were collected at Site 6, accounted for the presence of the moderately sensitive taxa (Table 4.7). Throughout the second survey no highly sensitive taxa were collected.



<u>Figure 4.4:</u> Percentage composition of highly sensitive, moderately sensitive and tolerant taxa per site, collected during the second survey.

Figure 4.5 shows the percentage composition of highly sensitive, moderately sensitive and tolerant taxa at each site, during the third survey. From this figure it is evident that tolerant taxa were primarily present at all of the sites. Site 3 and Site 5 had the highest percentage of tolerant taxa (93.8 %) and second highest percentage of tolerant taxa (88.2 %), respectively. Site 6 and Site 3 recorded the highest percentage of moderately sensitive (37.0 %) and the lowest percentage of moderately sensitive taxa (6.2 %), respectively. Although Site 1 was the only site where highly sensitive taxa were collected during this survey, it only represented 2.5 % of the taxa collected. Blephariceridae were the only highly sensitive family collected during this survey (Table 4.8).



<u>Figure 4.5:</u> Percentage composition of highly sensitive, moderately sensitive and tolerant taxa per site, collected during the third survey.

4.3.3 Spatial and temporal changes of macroinvertebrate community structure in relation to selected abiotic factors and biotopes.

The RDA triplot (Figure 4.6) illustrates the associations between the macroinvertebrate families, selected abiotic factors and biotopes of all of the sites during all three surveys. The major factors responsible for 75 % of the variation were EC and flow-rate which had a significant influence ($p < 0.05$) on the distribution of the macroinvertebrate families. From the RDA it is evident that flow-rate, riffle, run, SiC and EC together with the families Athericidae, Hydroptilidae, Hydropsychidae and Simuliidae together with Sites 1, 2, 4 and 6, all associated with the right halve. The pH, temperature, turbidity, pool and SoC in conjunction with Sites 3, 5, 7, 8, 9 and 10, plus the families Atyidae, Ancylidae, Corixidae, Culicidae, Cypridoidea, Dytiscidae, Lymnaeidae and Tubificidae all associated with the left halve. It is also evident that there was temporal variation at Sites 1, 2, 4 and 8, while a clear spatial variation is visible, as the different surveys of each site (Sites 3, 4, 6, 7, 9 and 10) group together but sites differ from each other. Athericidae, Hydropsychidae, Hydroptilidae and Simuliidae associated with flow-rate, which is in correspondence with literature regarding these families' habitat preferences (de Moor, 2003; de Moor & Scott, 2003; Harrison *et al*., 2003b; Thirion, 2007; Griffiths *et al*., 2015), except for Hydroptilidae which prefers slow flowing water or backwaters (de Moor & Scott, 2003; Thirion, 2007; Griffiths *et al*., 2015). Cypridoidea, Lymnaeidae, and Tubificidae all associated with the pool biotope, which is in accordance with literature that they prefer very slow moving waters or standing waters (Brown, 1994; Appleton, 2002b; van Hoven & Day, 2002; Thirion, 2007; Griffiths *et al*., 2015), while Cypridoidea can also occur in temporary waters (Martens, 2001). Dytiscidae associated with SoC, which is in accordance with literature which mentions their preference for very slow moving streams (Biström, 2007; Thirion, 2007). Corixidae associated with pH as seen in the RDA triplot and according to Griffiths *et al*. (2015), this family, especially *Sigara* sp. is an indicator of acidification, as well as eutrophication, but the pH values were not as low during this study. Ancylidae, Atyidae and Culicidae associated with Sites 4, 8 and 10 where this family occurred in abundance (Tables 4.6, 4.7 and 4.8). Ancylidae mostly associated with Site 8 during the last two surveys, while Atyidae associated with Sites 4 (third survey), 8 (second survey) and 10 (all three surveys) (Tables 4.6, 4.7 and 4.8). Culicidae associated with Site 4, during third survey, Site 8, during the last two surveys and Site 10, during the second survey (Tables 4.6, 4.7 and 4.8).

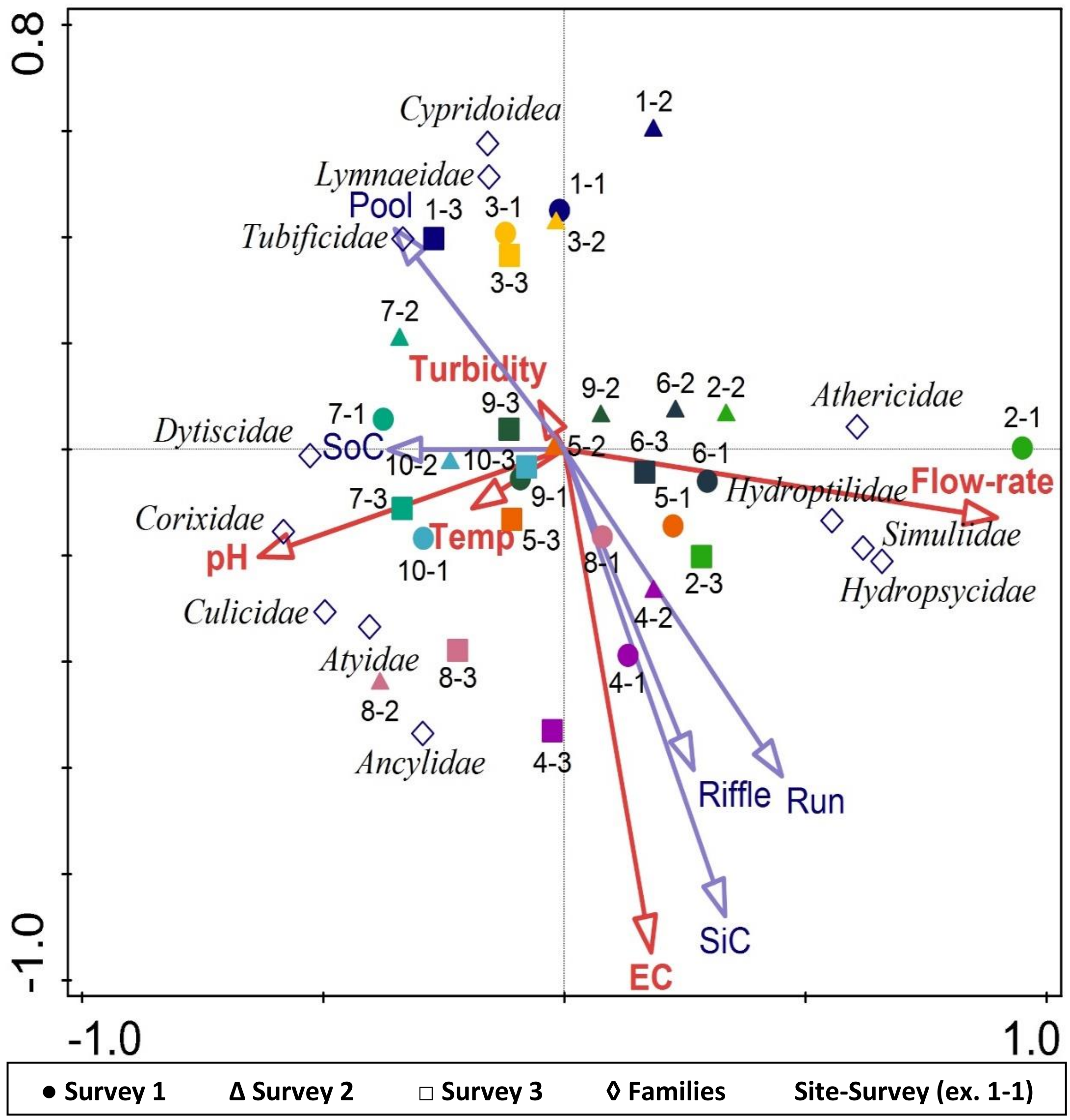


Figure 4.6: RDA triplot (with supplementary variables) illustrating associations between macroinvertebrate families, selected abiotic factors (pH, EC, temperature, turbidity, flow-rate) and biotopes (riffle, run, pool, stones in current (SiC) and stones out of current (SoC)) found at the sampling sites during all three surveys. The triplot describes 18.65 % of the variation with 12.38 % on the first axis and 6.27 % on the second axis.

4.4 Conclusion

In this study a variety of families were collected, consisting of several taxa. These families varied between the sites and surveys as seen in the results above. Although the majority of these families, that were present at the sites, are tolerant towards organic enrichment, there were two sites where highly sensitive taxa occurred. Moderately sensitive taxa also occurred, to a lesser extent, and differed between sites and surveys. The tolerant taxa occurred in abundance at several sites, while the moderately sensitive taxa were present in lower numbers and the highly sensitive taxa were only represented by a single specimen. These results indicate that the Loop Spruit is largely organically enriched, from sources mentioned above, which enables the tolerant taxa to thrive, but not to such an extent as to prohibit the occurrence of moderately sensitive taxa. The origin of the Loop Spruit and the Ensel Spruit, a tributary of the Loop Spruit, are the only two sites where highly sensitive taxa were found. This indicates that these sites were in a relatively natural state with few anthropogenic impacts. Although the sensitivity towards organic enrichment should be taken into account, several other factors can also influence the macroinvertebrate community structure. These factors include, amongst others, biotope preferences of each family, habitat availability, as well as abiotic factors. From the results obtained it is evident that the sites (Sites 2 and 4) where mining effluent enters the Loop Spruit, a high species richness, diversity and even distribution between species were found (Figure 4.2). At these sites significantly higher ($p < 0.05$) EC values were recorded, which indicates, amongst others, organic enrichment and act as a food source for the mostly tolerant families that occurred at these sites. The hypothesis stated for this chapter, that the macroinvertebrate community structure will be altered by mining activities from the upper reaches of the Loop Spruit, is thus rejected.

Chapter 5: Metal accumulation by selected aquatic macroinvertebrate families collected in a highly impacted river.

5.1 Introduction

Aquatic macroinvertebrates are generally regarded as good indicators of metal contamination due to their abundance and presence in a wide variety of freshwater habitats (Goodyear & McNeill, 1999; Hoffman *et al*., 2002; Chapman *et al*., 2003; Santoro *et al*., 2009; Cid *et al*., 2010; Schmidt *et al*., 2011; Griffiths *et al*., 2015). According to Goodyear and McNeill (1999), they are further relatively sedentary and can reflect the conditions of a specific site. Due to their life-cycles they may also be exposed to the aquatic environment for extended periods during which time metals can be accumulated (Goodyear & McNeill, 1999). Metal accumulation in macroinvertebrates can differ due to factors such as type of species, specific life stage, habitat preference (benthic or pelagic) and functional feeding groups (FFGs) (Goodyear & McNeill, 1999; Hoffman *et al*., 2002; Santoro *et al*., 2009). These FFGs include scraper/grazers (S-G), shredders (Sh), collector-gatherers (C-G), collector-filterers (C-F) and predators (Pr). Other factors that may also influence accumulation include specific morphological features and physiological processes.

Bioconcentration in aquatic organisms are the accumulation of bioavailable metals in the water column by means of non-dietary uptake routes (Vieth *et al*., 1979; Gray, 2002; Hoffman *et al*., 2002; Corbi *et al*., 2010; Schmidt *et al*., 2011). These routes may include respiration, adsorption onto the exoskeleton of macroinvertebrates, absorption through the skin, as well as binding sites in the digestive tract of organisms (Dallinger & Rainbow, 1993; Hoffman *et al*., 2002, Cid *et al*., 2010; Corbi *et al*., 2010). There are several factors that can have an influence on the bioavailability of metals in the water column, which include, among others, the presence of dissolved organic matter (DOM) and particles, stearic hindrance and the exposure concentration (Knezovich *et al*., 1987; Barron, 1990; John & Leventhal, 1995; DWAF, 1996; Hoffman *et al*., 2002).

Pathways by which aquatic organisms can get exposed to sediment-associated metals can include, *inter alia*, the ingestion of DOM and sediment particles, sediment pore water exposure, body surfaces that are in direct contact with sediment, as well as contact with the periphery layer of water covering the sediment (Knezovich *et al*., 1987;

Gray, 2002; Hoffman *et al*., 2002; Corbi *et al*., 2010). Benthic organisms generally utilize sediments as a food source (Watling, 1991; Corbi *et al*., 2010) and especially ingest particles of the sediment, by means of filter feeding from the water column or direct ingestion and thus could be an important route of exposure (Landrum & Faust, 1991; Hoffman *et al*., 2002; Corbi *et al*., 2010). Studies done by Corbi *et al*. (2010) in the Jacaré-Guaçu River basin (Brazil), found that collector-gatherer and collector-filterer FFGs, as well as benthic families, accumulated higher metal (Al, Cd, Cr, Cu, Fe, Mn and Zn) concentrations than other aquatic macroinvertebrates, due to their feeding habits and close association with sediments.

The hypotheses stated for this chapter were: 1) Feeding groups differ in their ability to accumulate metals. 2) Benthic macroinvertebrates will accumulate higher metal concentrations than pelagic macroinvertebrates.

5.2 Materials and methods

5.2.1 Field surveys

Macroinvertebrates were collected during the second survey (September 2014) at Site 4 as described in Chapter 4, Section 4.2.1. This site was selected on account of the nearby mining effluent, as well as other anthropogenic influences including a scrapyard in the vicinity of a tributary of the Loop Spruit (Figure 2.1). Organisms collected during this survey represented a large number of families and were also present in abundance (Table 4.7), providing sufficient biomass for replicate metal analyses.

5.2.2 Laboratory methods

5.2.2.1 Macroinvertebrate identification

Macroinvertebrates were identified using the same methods described in Chapter 4, Section 4.2.2.1.

5.2.2.2 Macroinvertebrate FFGs and habitat preference

After the macroinvertebrates were identified, they were grouped into FFGs as determined by Goodyear and McNeill (1999), Rawer-Jost *et al*. (2000), Naiman and Bilby (2001), Cummins *et al*. (2005) and Tomanova *et al*. (2006). They were hereafter separated into benthic or pelagic organisms according to habitat preferences as described by Dallas (2007), Thirion (2007) and Griffiths *et al*. (2015) (Table 5.1).

Table 5.1: Families grouped according to FFGs and habitat preference.

	Scraper/ grazers (S-G)	Shredders (Sh)	Collector-gatherers (C-G)	Collector-filterers (C-F)	Predators (Pr)
Benthic					
Ancylidae	X				
Caenidae	X		X		
Physidae	X				
Chironomidae		X	X	X	
Baetidae			X		
Simuliidae				X	
Aeshnidae					X
Chlorocyphidae					X
Gomphidae					X
Libellulidae					X
Pelagic					
Atyidae	X		X		
Belostomatidae					X
Coenagrionidae					X
Dytiscidae					X
Gyrinidae					X

5.2.2.3 Metal concentrations in macroinvertebrates

In the laboratory, organisms were prepared for metal analysis using methods described by Wolmarans and van Aardt (1985). The wet mass of three representative samples of each of the families were determined and then transferred to a pre-cleaned multi-cell Teflon® digestion block equipped with tight fitting lids (Wolmarans & van Aardt, 1985). One ml 65 % nitric acid was added to each sample. The cells were then sealed in order to prevent sample loss during digestion, which was done under high pressure at a temperature of 70 ˚C for 24 hours. The supernatant from the digested samples were then transferred to 15 ml plastic ICP-MS test tubes and diluted up to 14 ml with MilliQ® water. It was hereafter analysed by making use of ICP-MS and the concentrations expressed in µg/g wet weight. Certified Reference Material (CRM) for mussel tissue (ERM-CE278k) exposed to the same digestion procedure was used. A < 10 % deviation range was found during the CRM analyses, as well as in the percentage recoveries for the standards (Table 5.2).

Table 5.2: Metal concentrations recovered from Certified Reference Material (CRM). All the concentrations were measured in µg/g.

	Macroinvertebrates		
	Reference	**Measured**	**% Recovery**
Cr	0.73	0.8	105.5
Mn	4.88	5.0	102.7
Fe	161.0	156.0	96.9
Ni	0.69	0.7	94.2
Cu	5.98	6.2	103.0
Zn	71.0	68.0	95.8
As	6.7	6.2	92.5
Cd	0.336	0.4	107.1
Pb	2.18	2.2	102.8

5.2.3 Statistics

Statistical significance ($p < 0.05$) between the metal concentrations and selected families classified into FFGs and according to habitat preference were determined. Normality and homogeneity of variance were tested using D'Agostino and Pearson omnibus normality test and Kolmogorov-Smirnov test (with Dallal-Wilkinson-Lilliefor P value) respectively. Where data were parametric, ANOVA and Tukey's multiple comparison tests were performed. In the case of non-parametric data, Kruskal-Wallis tests with Dunn's Multiple Comparison Tests were performed to test for significant differences ($p < 0.05$) between macroinvertebrate families.

A PCA for the metal concentrations in the families and supplementary variables (FFG and habitat preference (benthic or pelagic)) were constructed, using Canoco (v5). This analysis uses multivariate data and creates a scatterplot, which assists with the interpretation of the data.

5.3 Results and discussion

5.3.1 Metal concentrations in macroinvertebrates

The mean Al concentrations ranged from 66.8 µg/g in Aeshnidae to 3 048.3 µg/g in Caenidae (Figure 5.1A). Caenidae differed significantly ($p < 0.05$) from all the other families, while high concentrations of Al were also found in the Simuliidae (1 484.3 µg/g) and Chironomidae (1 136.5 µg/g), of which all are benthic families. Caenidae belongs to the scraper/grazer and collector-gatherer FFGs, while Simuliidae and Chironomidae belong to the collector-filterer FFG (Table 5.1). In contrast to this Aeshnidae, also a family associating with the benthos, displayed the lowest concentration of Al (Figure 5.1A). Although high concentrations of Al were only found in families associating with the benthos, this conclusion was not valid for families categorized as predators also living in the benthos. Low concentrations of Al were present in all the pelagic families (Figure 5.1A). The mean Ti concentrations (Figure 5.1B) were low in all the families regardless of feeding groups or habitat preference and varied between 1.1 µg/g in Aeshnidae and 12.3 µg/g in Simuliidae, where the families Chironomidae, Physidae, Ancylidae and Caenidae all have mean Ti concentrations that exceed 5 µg/g (Figure 5.1B). All of the benthic families differed significantly ($p < 0.05$), except for the predator families, from the pelagic families (Figure 5.1B). It is further evident that Aeshnidae had significantly lower ($p < 0.05$) metal concentrations than all of the other families (Figures 5.1, 5.2 and 5.3). With regard to Cr, the highest concentrations were again measured in the benthic families except for those categorized as predators (Figure 5.1C). The highest concentration of Cr was measured in Caenidae (30.4 µg/g), which differed significantly ($p < 0.05$) from all the other families (Table 5.1C). This is in contrast to findings by Eisler (1986), which states that benthic macroinvertebrates seem to have a limited ability to accumulate high Cr concentrations from clays and sediments. In the case of Mn it is evident that Simuliidae (521.0 µg/g) had a significantly higher ($p < 0.05$) concentration than most of the families (Figure 5.1D). The benthic families, except the predator families, differed significantly ($p < 0.05$) from the pelagic families (Figure 5.1D). Caruso *et al*. (2012) have found that aquatic biota occurring in lower trophic levels tend to bioconcentrate Mn more readily than organisms of a higher trophic level. It was also the case in this study, where families of lower trophic levels (Ancylidae, Caenidae, Physidae, Chironomidae, Baetidae and Simuliidae) had significantly higher ($p < 0.05$) concentrations than the predators Aeshnidae, Chlorocyphidae, Gomphidae, Libellulidae, Belostomatidae, Coenagrionidae, Dytiscidae and Gyrinidae (Figure 5.1D).

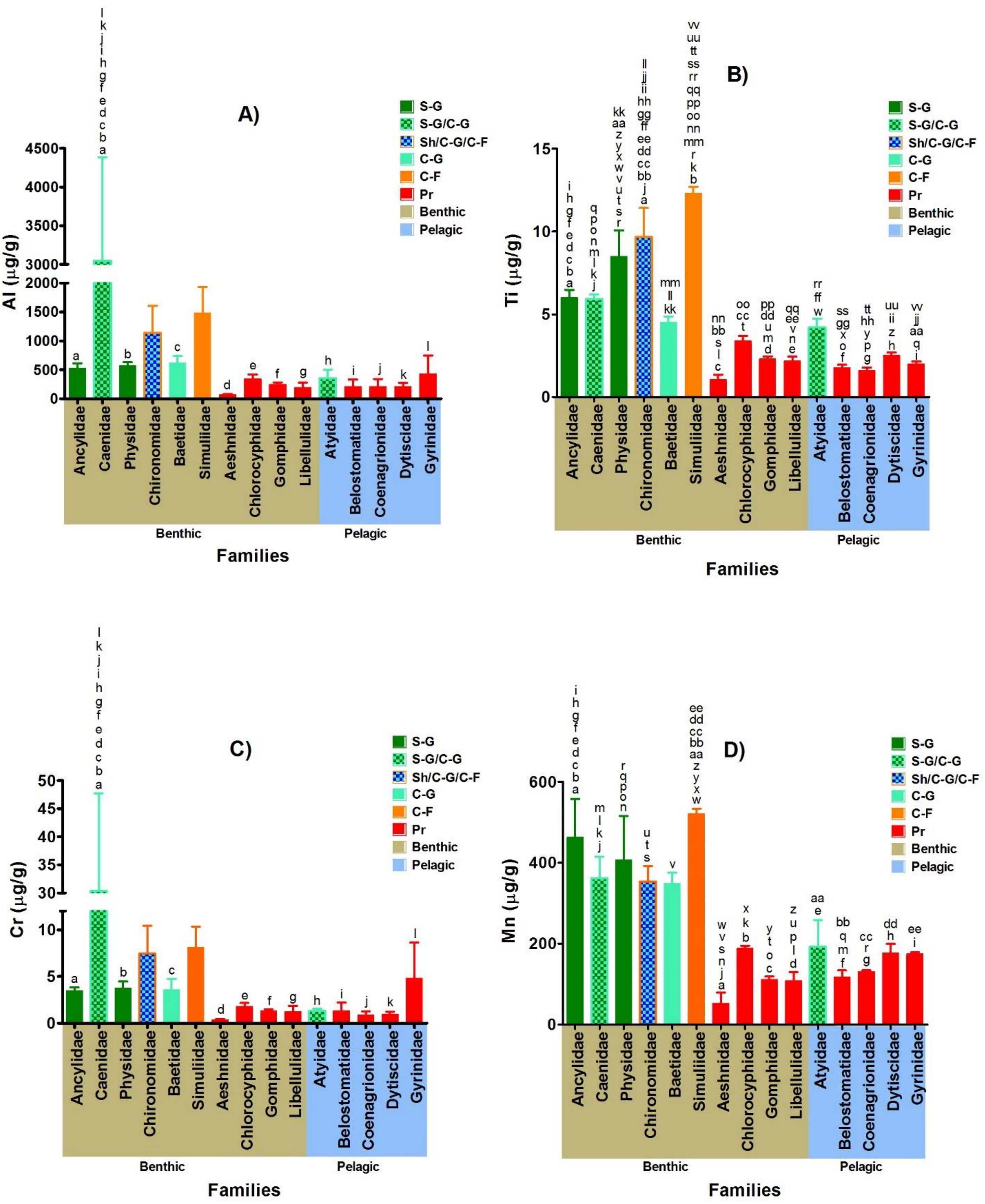


Figure 5.1: The mean and standard error of metal concentrations (µg/g wet weight) of Al (A), Ti (B), Cr (C) and Mn (D), measured in selected families. Means with common alphabetical superscripts indicate significant differences ($p < 0.05$). Selected families are indicated according to FFGs (S-G – scraper/grazers; Sh – shredders; C-G – collector-gatherers; C-F – collector-filterers and Pr – predators).

Variations occurred with regards to the Fe concentrations measured in the different families (Figure 5.2A). Although the lowest concentrations were still present in the predators, high concentrations were measured in both benthic, as well as pelagic families (Figure 5.2A). According to Vouri (1995), the uptake of Fe by aquatic organisms can only occur from two sources, either from the water itself, or from the food they consume, while the uptake of Fe is much higher in rivers rich in humic compounds, than in rivers with clear water. The high turbidity measured at this site during the second survey could thus have contributed to the high Fe concentrations in the mentioned families (Table 3.3). The fact that Caenidae had significantly higher ($p < 0.05$) concentrations of Fe, Al, Cr, Zn, As and Cd, can be ascribed to a number of characteristics. These include their affinity for the benthos, feeding on metal containing organic materials, a body covered with fine setae and frilly gills enlarging the adsorption surface, as well as having 15 to 30 nymphal instars, implying an extended exposure to the environment (Barber-James & Lugo-Ortiz, 2003; Griffiths *et al*., 2015). Although low concentrations of Co and Ni were found in all the families (Figures 5.2B and 5.2C) the benthic families, except for the predators differed significantly ($p < 0.05$) from the pelagic families, while the scraper/grazer, shredder, collector-gatherer and collector-filterer FFGs differed significantly ($p < 0.05$) from the predator FFG (Figures 5.2B and 5.2C). Chironomidae had significantly higher ($p < 0.05$) Cu concentration than the other families (Figure 5.2D), while the benthic families, except for the predator families differed significantly ($p < 0.05$) from the pelagic families. According to literature (Eisler, 1998a; Goodyear & McNeill, 1999; Gray, 2002; Cardwell *et al*., 2013) Cu concentrations were the highest in detritivore organisms, and do not biomagnify within freshwater food chains. In this study it was found that all of the families that had a significantly higher ($p < 0.05$) Cu concentrations feed on detritus (Appleton, 2002b; Barber-James & Lugo-Ortiz, 2003; de Moor, 2003; Harrison, 2003; Griffiths *et al*., 2015), while all the predators had significantly low ($p < 0.05$) concentrations of Cu (Figure 5.2D).

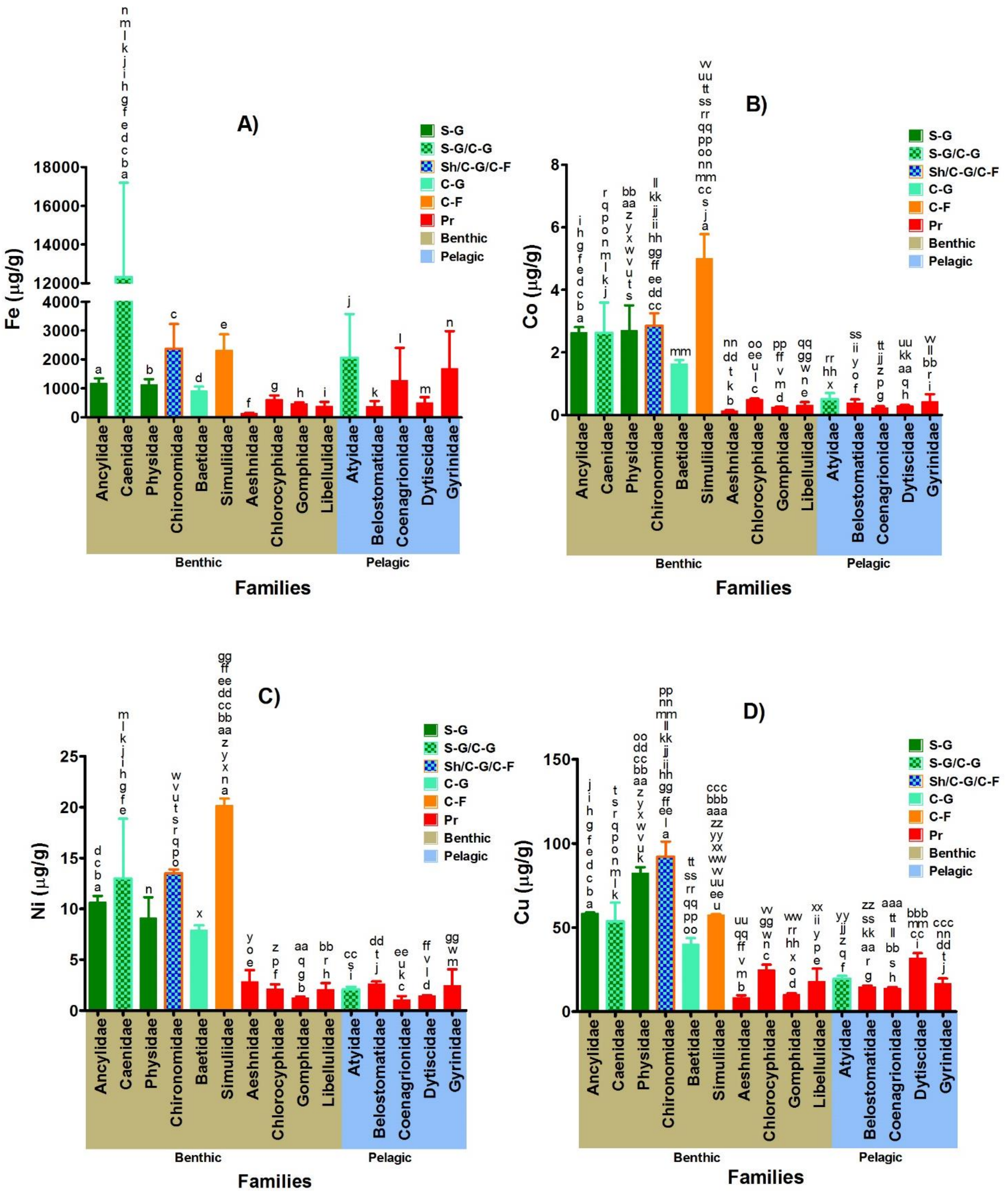


Figure 5.2: The mean and standard error of metal concentrations (µg/g wet weight) of Fe (A), Co (B), Ni (C) and Cu (D), measured in selected families. Means with common alphabetical superscripts indicate significant differences ($p < 0.05$). Selected families are indicated according to FFGs (S-G – scraper/grazers; Sh – shredders; C-G – collector-gatherers; C-F – collector-filterers and Pr – predators).

The highest concentrations of Zn were present in Chironomidae, Caenidae and Atyidae (Figure 5.3A), where Caenidae differed significantly ($p < 0.05$) from the majority of the other families, of which the first two were categorized as benthic families and Atyidae as pelagic (Table 5.1). According to Eisler (1993) Zn concentrations in the tissue generally exceed that necessary for normal metabolism needed by aquatic macroinvertebrates. These concentrations, however, have no toxic effects, since it occurs as insoluble metal inclusions within the tissue, or is bound to macromolecules. High Zn concentrations in freshwater crustaceans (Atyidae) are typically associated with industrial pollution (Eisler, 1993), a phenomenon also present at Site 4 as substantiated by the presence of anthropogenic activities. Although large variation in the concentrations of As were found when all the families are compared, only Caenidae differed significantly ($p < 0.05$) from all the other families and no distinct accumulation pattern was evident, these concentrations were relatively low (Figure 5.3B). It is interesting to note that in Cd, although present in low concentrations, higher concentrations were found in the pelagic families than in the benthic families but were not significantly higher (Figure 5.3C). Caenidae was the only family that differed significantly ($p < 0.05$) from the majority of the other families (Tables 5.3C). Cadmium can be bioaccumulated by organisms but there is no evidence that it can biomagnify via the aquatic food chain (DWAF, 1996; Goodyear & McNeill, 1999; Gray, 2002; Cardwell *et al*., 2013). The highest concentrations of Pb was found in Chironomidae and Caenidae and differed significantly ($p < 0.05$) from the majority of the other families (Figure 5.3D). Higher concentrations of Pb were found in the benthic families than in the pelagic families, while lower trophic level organisms had significantly higher ($p < 0.05$) concentrations than the predator families, thus biomagnification also does not take place within the aquatic food chain (Dallinger & Rainbow, 1993; DWAF, 1996; Gray, 2002; Cardwell *et al*., 2013). The high abundance observed for Chironomidae (Table 4.7) during the course of the entire study, is an indication of its tolerance towards Pb, as supported by studies conducted by Grosell *et al*. (2006).

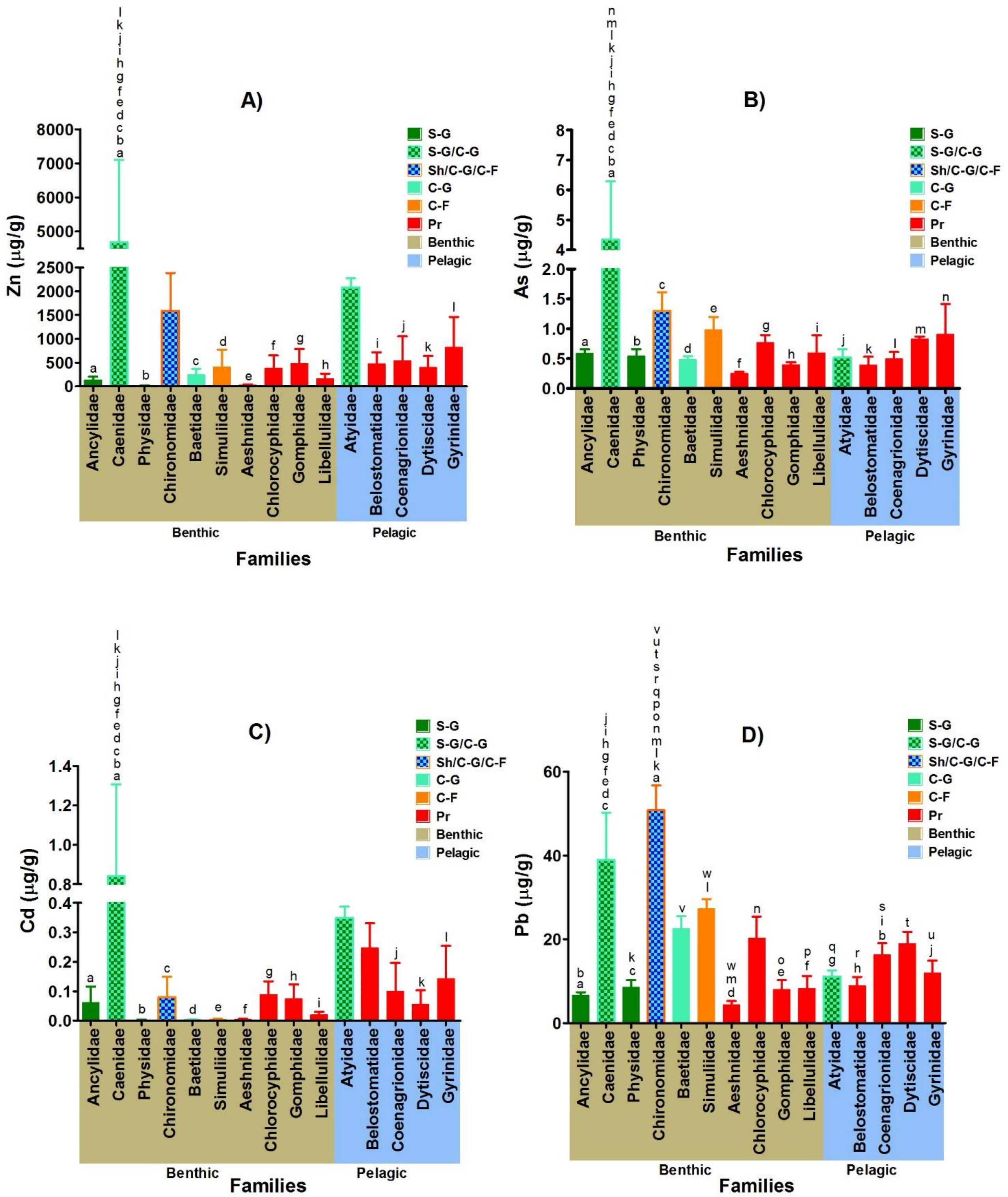


Figure 5.3: The mean and standard error of metal concentrations (µg/g wet weight) of Zn (A), As (B), Cd (C) and Pb (D), measured in selected families. Means with common alphabetical superscripts indicate significant differences ($p < 0.05$). Selected families are indicated according to FFGs (S-G – scraper/grazers; Sh – shredders; C-G – collector-gatherers; C-F – collector-filterers and Pr – predators).

From the results in Figures 5.1, 5.2 and 5.3 it is evident that families with different FFGs accumulated metal concentrations differently, where the predators had significantly lower ($p < 0.05$) concentrations of all the metals than the other FFGs, which can be explained by the fact that these metals do not biomagnify within the food chain that consists of primary producers and macroinvertebrates (Dallinger & Rainbow, 1993; DWAF, 1996; Eisler, 1998a; Eisler, 1998b; Goodyear & McNeill, 1999; Gray, 2002; Cardwell *et al*., 2013). Cardwell *et al*. (2013) found that Cd, Ni, Cu, Zn and Pb usually do not biomagnify in macroinvertebrate consumers in laboratory studies, as well as field studies conducted in the Augraben and Leiferer Rivers (Italy), Illinois River (USA), River Wandle (England), as well as in the Upper Sacramento River (USA). Studies done by Gray (2002) also found that these metals do not biomagnify in the aquatic food chain, except for Zn, where it was found that predators had the highest Zn concentrations. Results obtained from the current study were in contrast with the findings of Gray (2002) as the concentrations in the predators were significantly lower ($p < 0.05$) than Caenidae (Figure 5.3A), which is classified as a scraper/grazer and collector-gatherer. It is also evident from these results that the majority of benthic macroinvertebrate families had significantly higher ($p < 0.05$) metal concentrations than the pelagic families. The families Caenidae, Simuliidae and Chironomidae had significantly higher ($p < 0.05$) metal concentrations throughout the study (Figures 5.1, 5.2 and 5.3).

The family Simuliidae is classified as a collector-filterer and is regarded as an exceptionally effective biological filterer by de Moor (2003) and Griffiths *et al*. (2015), and use cephalic fans to filter the water for suspended detritus. Its lifestyle consists of an exclusively sedentary existence and they secure themselves on rocks or on the substratum as larvae and have seven instars before becoming a pupa, which also occurs on rocks or sediment (de Moor, 2003; Griffiths *et al*., 2015). The high metal concentrations can be accumulated through the suspended detritus that they feed on or it can be adsorbed from their cephalic fans or on their exoskeleton, as well as through close contact with sediments or by respiration through their anal gills (de Moor, 2003; Griffiths *et al*., 2015). According to Harrison (2003) and Griffiths *et al*. (2015) Chironomidae have only four larval instars but the larval phase can occur over several months while living in tubes constructed of debris and salivary secretions, which is attached to the sediment. This family also consumes detritus and algae and breathe through gills, while anal and procercal setae are present (Harrison, 2003; Griffiths *et al*., 2015; Bervoets *et al*., 2016). The possible reasons for high metal concentrations could be due to close contact with sediments, adsorption of metals to setae and gills or on the detritus that they feed on. According to Corbi *et al*. (2010) and Bervoets *et al*. (2016) high concentrations of Cr, Cu, Zn, Fe, Mn and Pb were found in Chironomidae in studies conducted in the Jacaré-Guaçu River basin (Brazil) and the Meuse River basin (Netherlands), respectively, and indicated that Chironomidae can be classified as a family resistant towards metal pollution. In this study significantly higher ($p < 0.05$) concentrations of Cu and Pb were found in Chironomidae, while Zn concentration was also high in this family (Figures 5.2D, 5.3A and 5.3D).

5.3.4 Associations between metal concentrations and aquatic macroinvertebrate families

The associations between metal concentrations and selected macroinvertebrate families, as well as supplementary variables (FFGs and habitat preference (benthic or pelagic)) are shown in a PCA triplot (Figure 5.4). Simuliidae associated with Ni, Co, Cu, Ti and Mn. This grouping can be explained by the fact that the Ti, Ni, Co, Mn and Cu concentrations were the highest in Simuliidae, where the Cu concentration was the fourth highest in Simuliidae. Chironomidae associated with Pb, As, Fe, Al and Cr since high concentrations of these metals were measured in this family. This is also true for Caenidae which associated with Pb, As and Fe, and Atyidae with which an association was found with Zn and Cd. The clear grouping of all the predators (Libellulidae, Chlorocyphidae, Dytiscidae, Belostomatidae, Gomphidae and Coenagrionidae), except for Aeshnidae and Gyrinidae in the left quadrants can be ascribed to the significantly low ($p < 0.05$) concentrations of metals measured in these families. The family Aeshnidae had the lowest concentrations of all the metals, except for Ni and Cd, which can be the reason for its solitary appearance in the PCA, while the family Gyrinidae associated with Zn and Cd, highlighting the fact that the fourth highest Zn and Cd concentrations were found in this family. The scraper/grazer FFG, grouped together (Physidae and Ancylidae), while the collector-gatherer families also grouped together (Chironomidae and Caenidae).

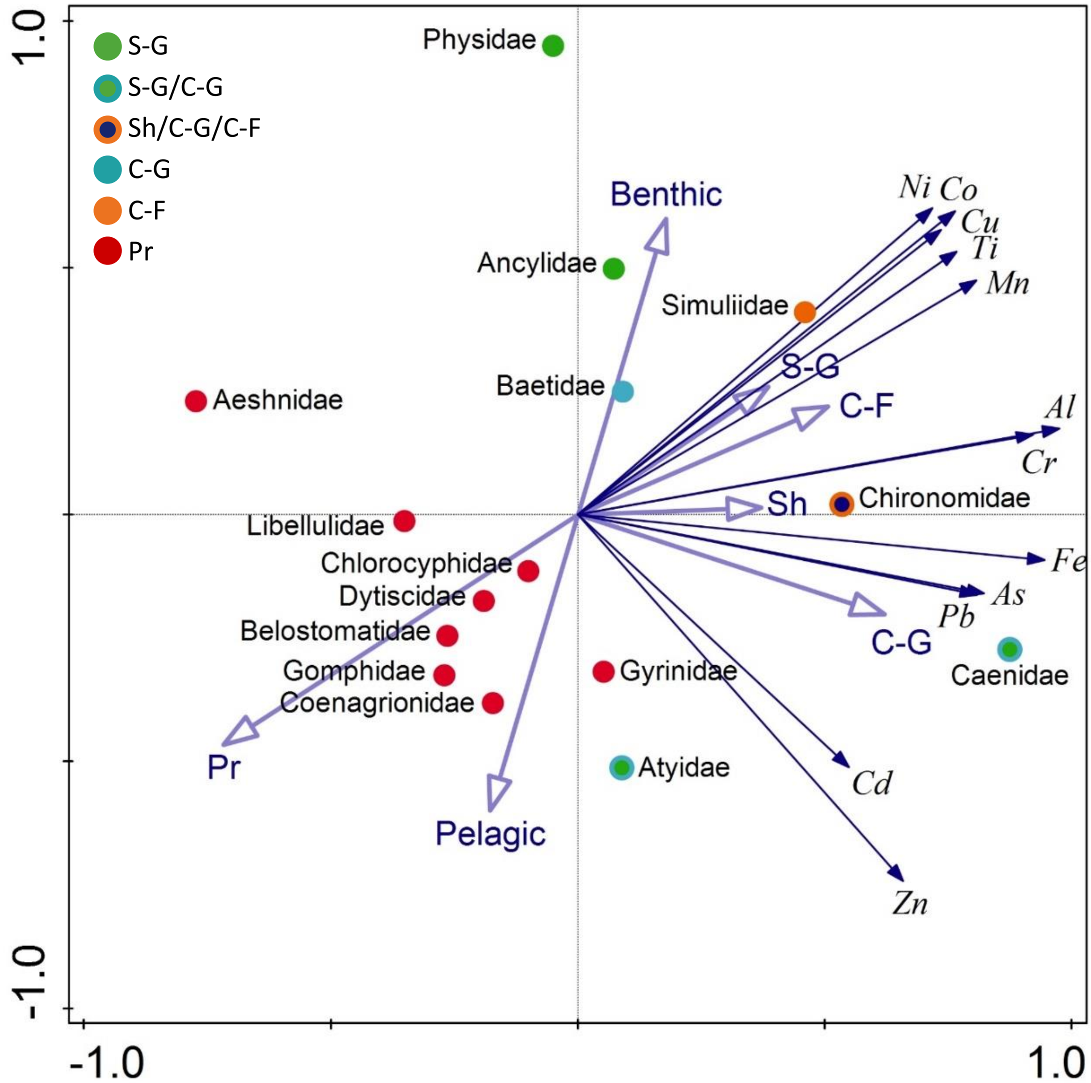


Figure 5.4: PCA triplot (with supplementary variables) illustrating associations between metal concentrations and selected macroinvertebrate families, with FFG (scraper/grazer (S-G), shredder (Sh), collector-gatherer (C-G), collector-filterer (C-F) and predator (Pr)) and habitat preference (benthic or pelagic) found at the sampling site. The triplot describes 91.34 % of the variation with 65.45 % on the first axis and 25.89 % on the second axis.

5.4 Conclusion

From the results obtained during this study a significant variation ($p < 0.05$) in metal concentrations are evident between FFGs, as well as between benthic and pelagic macroinvertebrate families (Figures 5.1, 5.2 and 5.3). The predator families had significantly lower ($p < 0.05$) metal concentrations, which can be ascribed to the fact these metals do not biomagnify within the food chain (Dallinger & Rainbow, 1993; DWAF, 1996; Eisler, 1998a; Eisler, 1998b; Goodyear & McNeill, 1999; Gray, 2002; Cardwell *et al*., 2013). In contrast to this, the lower trophic levels (scraper/grazer, shredder, collector-gatherer and collector-filterer FFGs) had significantly higher ($p < 0.05$) metal concentrations. The benthic families also had significantly higher ($p < 0.05$) metal concentrations than the pelagic families in the case of most of the metals. Although these metals are all considered as potentially toxic to aquatic biota, these high concentrations may not have had a detrimental effect possibly due to strategies such as elimination (excretion and biotransformation processes), detoxification (metallothionein) and sequestration, as well as metabolisation (Phillips & Rainbow, 1989; Hoffman *et al*., 2002; Ahearn *et al*., 2004; Cardwell *et al*., 2013; Mugwar & Harbottle, 2016). The hypotheses state that feeding groups differ in their ability to accumulate metals and that benthic macroinvertebrates will accumulate higher metal concentrations than pelagic macroinvertebrates. From the results both of these hypotheses can thus be accepted.

Chapter 6: Conclusions and Recommendations.

6.1 Conclusions

Throughout the last decade extensive research has been dedicated to the monitoring of rivers and natural water sources due to an increase in mining, industrial, agricultural and urbanisation activities and increasing human populations. Even though monitoring plans have been designed and in some cases implemented, the critical importance of these water systems and the management thereof is essential.

The aim of this study was to determine the environmental quality and influence on the macroinvertebrates of the Loop Spruit, as well as that part of the Mooi River after the confluence of the Loop Spruit.

The hypothesis, that mining activities in the upper catchment results in an increase in metal concentrations in water and sediment of the Loop Spruit, was stated for Chapter 3. The aim of this chapter was thus to determine the metal concentrations in the water and sediment at selected sites within the Loop Spruit. From the results obtained it can be concluded that mining activities could have been responsible for higher concentrations of Cr, Fe, Co, Ni, As, Cd and Pb in the upper reaches of the Loop Spruit, but other anthropogenic activities (i.e. agricultural, urban and sewage treatment plant effluent) also contributed to metal concentrations in the Loop Spruit. The first hypothesis of this study was thus accepted.

The second hypothesis of this study was stated in Chapter 4, namely, that the macroinvertebrate community structure will be altered by mining activities from the upper reaches of the Loop Spruit. The aim of this chapter was therefore to establish the diversity of aquatic macroinvertebrates and their association with selected abiotic factors and biotopes within the Loop Spruit. From the results obtained it can be concluded that although a variety of families were collected during the study, the majority of these are classified as tolerant towards organic enrichment, whereas moderately sensitive families also occurred but to a lesser extent and highly sensitive families were only found at Sites 1 and 7. High species richness, diversity and even distribution between taxa were found at the sites where mining effluent enters the Loop Spruit, which indicate that the mining effluent did not have a negative effect on the community structure of the macroinvertebrates present at these sites. The hypothesis stated for this chapter was thus rejected.

The hypotheses stated for Chapter 5 were: 1) Feeding groups differ in their ability to accumulate metals, and 2) Benthic macroinvertebrates will accumulate higher metal concentrations than pelagic macroinvertebrates. The third and fourth hypotheses of the study were also accepted due to the fact that families with different FFGs had different metal concentrations, while the benthic families had higher metal concentrations than the pelagic families. The predator families had the lowest metal concentrations of the FFGs, while families of lower trophic levels (scraper/grazers, shredders, collector-gatherers and collector-filterers) had significantly higher ($p < 0.05$) metal concentrations. Although the metals discussed in this study are considered as potentially toxic to aquatic biota, it seemed to have no detrimental effect on the macroinvertebrates that were present at this site, which can possibly be due to several strategies to eliminate, detoxify and metabolise the metals.

The main aim of the study was successfully achieved through 1) determining the primary lithology and secondary minerals of the area surrounding the study area in order to establish the metals which originate from mining activities or from natural weathering; 2) determining *in situ* water quality and metal concentrations in water at each site; 3) determining the physical characteristics and metal concentrations in sediments from the selected sampling sites; 4) determining the aquatic macroinvertebrate diversity within the study area; 5) determining metal bioaccumulation in selected macroinvertebrates from an impacted site; and 6) finally to establishing a relationship between measured environmental factors and the aquatic macroinvertebrate community structure.

6.2 Recommendations

For future studies it is recommended that the following aspects should be considered to supplement this study:

- Conducting surveys during dry seasons, which are not affected by a drought and compare the results.
- To conduct wet season surveys in which metal concentrations can be determined to supply a holistic view of metal distribution and concentrations in the Loop Spruit.
- To conduct an in depth study to determine the origin of metals in the Loop Spruit.
- More physico-chemical parameters should be incorporated into the study, including, amongst others, nutrients and dissolved oxygen from each sampling site during each survey. This would give more detailed results and can be linked to the biodiversity.
- Surveys can be conducted after extreme events such as rainfall to identify succession patterns (e.g. pioneer species or family changes).
- Metal analysis in more macroinvertebrate families can be conducted, as well as in an unimpacted site to compare the data with the results obtained during this study.

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Appendix A

<u>Table A1:</u> Mean metal concentrations (µg/L), standard deviation and selected abiotic factors in water samples collected during the first survey.

	1	2	3	4	5	6	7	8	9	10
Be	0.2 ± 0.02	0.1 ± 0.03	0.1 ± 0.04	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.005	0.1 ± 0.01	0.1 ± 0.02	0.2 ± 0.07	0.1 ± 0.03
B	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	25.4 ± 0.2	35.5 ± 0.3	29.5 ± 0.1	15.6 ± 0.2	27.4 ± 0.3	2.7 ± 0.2	3.9 ± 0.3
Na	1704.7 ± 8.9	3082.3 ± 3.3	10329.9 ± 5.9	34500.0 ± 3.8	34505.1 ± 12.3	34500.9 ± 5.1	26550.7 ± 2.5	34499.8 ± 7.7	28915.0 ± 121.2	33564.7 ± 69.3
Mg	525.6 ± 3.0	3317.2 ± 2.6	6785.3 ± 2.9	20380.4 ± 1.0	25320.3 ± 1.9	22410.5 ± 1.2	30920.8 ± 2.5	24556.8 ± 68.9	41730.5 ± 81.0	42776.8 ± 88.1
Al	295.5 ± 1.7	35.6 ± 0.02	33.3 ± 0.2	23.7 ± 0.2	24.2 ± 0.2	24.1 ± 0.06	27.0 ± 1.3	54.2 ± 1.8	37.6 ± 2.6	152.7 ± 1.7
P	47.2 ± 1.7	54.8 ± 1.1	51.8 ± 0.2	121.5 ± 0.2	379.5 ± 0.3	236.6 ± 0.3	58.5 ± 2.7	71.2 ± 1.4	83.7 ± 3.1	240.7 ± 1.9
K	2193.5 ± 5.3	2419.2 ± 3.9	1832.4 ± 4.5	5874.2 ± 1.1	6327.1 ± 1.2	6737.1 ± 1.8	2375.2 ± 5.2	7759.7 ± 82.8	2997.9 ± 6.4	4302.7 ± 32.6
Ca	609.9 ± 2.1	4171.8 ± 3.3	17558.5 ± 4.9	60990.6 ± 4.7	62530.3 ± 1.8	55019.9 ± 1.8	32336.7 ± 341.0	41958.3 ± 91.7	57367.1 ± 88.8	57541.3 ± 46.0
Ti	2.1 ± 0.2	1.3 ± 0.3	1.3 ± 0.2	0.7 ± 0.2	1.3 ± 0.1	0.7 ± 0.05	1.1 ± 0.2	0.9 ± 0.2	1.6 ± 0.2	1.2 ± 0.07
V	1.3 ± 0.5	0.3 ± 0.2	0.3 ± 0.2	1.1 ± 0.3	1.7 ± 0.2	2.1 ± 0.1	3.6 ± 0.3	2.2 ± 0.2	1.9 ± 0.2	2.6 ± 0.2
Cr	1.8 ± 0.2	1.1 ± 0.1	0.9 ± 0.1	2.8 ± 0.1	1.7 ± 0.2	1.5 ± 0.2	0.9 ± 0.1	1.8 ± 0.2	1.2 ± 0.2	1.2 ± 0.1
Mn	0.3 ± 0.2	3.7 ± 0.8	2.5 ± 0.6	1.2 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	6.3 ± 0.3	3.5 ± 0.2	14.4 ± 0.4
Fe	200.9 ± 2.1	194.0 ± 0.4	105.0 ± 0.3	89.5 ± 0.4	87.4 ± 0.2	83.0 ± 0.07	71.3 ± 2.7	112.4 ± 0.5	134.1 ± 0.6	113.7 ± 0.2
Co	0.1 ± 0.02	0.1 ± 0.02	0.2 ± 0.09	5.2 ± 0.2	1.9 ± 0.07	1.6 ± 0.1	0.2 ± 0.02	1.0 ± 0.04	1.6 ± 0.2	1.5 ± 0.2
Ni	2.0 ± 0.4	4.2 ± 0.02	3.4 ± 0.4	10.5 ± 0.6	9.1 ± 0.2	9.6 ± 0.5	5.9 ± 0.2	5.7 ± 0.3	5.1 ± 0.2	3.6 ± 0.3
Cu	2.2 ± 0.3	3.2 ± 0.7	3.3 ± 0.5	6.4 ± 0.4	4.7 ± 0.2	5.0 ± 0.1	4.2 ± 0.2	3.9 ± 0.2	2.9 ± 0.2	18.3 ± 0.3
Zn	18.7 ± 0.4	60.6 ± 1.5	56.9 ± 0.3	49.6 ± 0.2	48.4 ± 0.1	56.2 ± 0.04	59.0 ± 2.5	54.5 ± 0.6	41.0 ± 0.4	55.6 ± 0.2
As	0.1 ± 0.1	0.1 ± 0.02	0.01 ± 0.01	0.6 ± 0.2	0.7 ± 0.08	0.6 ± 0.1	0.3 ± 0.04	0.8 ± 0.07	0.3 ± 0.1	0.4 ± 0.1

Table A1 (continued)

Se	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	1.6 ± 0.2	0.4 ± 0.05	0.2 ± 0.1	0.03 ± 0.01	0.3 ± 0.02	0.03 ± 0.01	0.03 ± 0.01
Rb	1.6 ± 0.2	1.3 ± 0.2	1.7 ± 0.3	8.0 ± 0.3	7.0 ± 0.2	7.5 ± 0.5	1.2 ± 0.2	5.4 ± 0.2	3.5 ± 0.3	4.9 ± 0.4
Sr	4.6 ± 0.6	21.5 ± 0.7	59.5 ± 0.4	443.4 ± 1.5	348.5 ± 0.6	300.4 ± 0.6	142.0 ± 1.4	264.3 ± 1.1	88.2 ± 0.7	80.6 ± 1.1
Mo	0.1 ± 0.03	0.3 ± 0.2	0.3 ± 0.07	1.2 ± 0.1	1.8 ± 0.1	1.7 ± 0.05	0.3 ± 0.09	1.6 ± 0.1	0.4 ± 0.2	0.4 ± 0.2
Pd	1.0 ± 0.07	1.1 ± 0.1	1.1 ± 0.1	1.4 ± 0.2	1.4 ± 0.08	1.4 ± 0.1	1.2 ± 0.2	1.3 ± 0.2	1.1 ± 0.1	1.1 ± 0.2
Ag	0.03 ± 0.01	0.01 ± 0.02	0.2 ± 0.09	0.03 ± 0.01	0.02 ± 0.001	0.04 ± 0.004	0.2 ± 0.07	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Cd	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Sb	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.08	0.7 ± 0.07	0.7 ± 0.07	0.6 ± 0.06	0.4 ± 0.08	0.6 ± 0.05	0.6 ± 0.03	1.2 ± 0.2
Ba	16.3 ± 0.2	10.4 ± 0.1	37.5 ± 0.5	29.7 ± 0.07	28.4 ± 0.3	32.1 ± 0.07	34.7 ± 0.9	50.9 ± 0.2	22.5 ± 0.4	21.8 ± 0.8
Pt	0.03 ± 0.01	0.01 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.2 ± 0.02	0.03 ± 0.01	0.02 ± 0.002
Au	1.7 ± 0.2	1.6 ± 0.2	1.6 ± 0.3	1.8 ± 0.2	1.7 ± 0.04	1.6 ± 0.2	1.7 ± 0.2	1.7 ± 0.1	1.7 ± 0.2	1.7 ± 0.2
Tl	0.01 ± 0.02	0.01 ± 0.02	0.01 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.006	0.01 ± 0.01	0.01 ± 0.008	0.01 ± 0.006	0.01 ± 0.02
Pb	0.4 ± 0.1	0.4 ± 0.2	0.7 ± 0.09	0.1 ± 0.02	0.4 ± 0.03	0.2 ± 0.04	0.3 ± 0.04	0.3 ± 0.05	0.1 ± 0.02	2.9 ± 0.3
Bi	0.1 ± 0.02	0.1 ± 0.02	0.3 ± 0.09	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.02	4.3 ± 0.3
Th	0.6 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.05	0.5 ± 0.02	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.2
U	0.2 ± 0.1	0.1 ± 0.03	0.5 ± 0.2	16.2 ± 0.2	21.8 ± 0.1	18.0 ± 0.05	1.2 ± 0.1	7.3 ± 0.3	3.4 ± 0.4	3.0 ± 0.3
pH	7.2	6.7	7.0	6.8	6.9	7.0	8.3	7.4	7.7	8.3
EC (µS/cm)	65	901	198	1207	906	806	480	781	695	737
Temperature (˚C)	9.2	12.3	13.2	11.7	10.9	10.5	10.7	7.3	12.3	11.7
Turbidity (NTU)	7	5	5	5	6	10	35	6	6	5
Flow-rate (m/s)	0.2	1.2	0.1	0.3	0.4	0.5	0.1	0.3	0.3	0.2

Table A2: Mean metal concentrations (µg/L), standard deviation and selected abiotic factors in water samples collected during the second survey.

	1	2	3	4	5	6	7	8	9	10
Be	0.1 ± 0.04	0.1 ± 0.06	0.1 ± 0.02	0.1 ± 0.006	0.1 ± 0.01	0.1 ± 0.006	0.1 ± 0.005	0.1 ± 0.002	0.1 ± 0.009	0.1 ± 0.03
B	0.03 ± 0.01	57.6 ± 4.1	0.03 ± 0.01	40.9 ± 1.3	31.4 ± 0.5	34.1 ± 0.4	22.7 ± 0.9	41.9 ± 1.8	0.03 ± 0.01	13.3 ± 0.5
Na	3524.1 ± 4.2	62913.3 ± 137.1	14483.1 ± 91.3	68914.5 ± 43.6	63517.7 ± 159.5	67196.3 ± 95.6	32625.7 ± 13.4	68891.7 ± 36.2	29565.3 ± 89.0	67936.7 ± 52.0
Mg	4494.0 ± 6.1	23957.0 ± 96.0	10672.4 ± 43.2	27167.4 ± 81.6	20924.0 ± 79.2	23349.3 ± 144.2	36513.3 ± 26.2	55939.3 ± 44.0	49821.0 ± 42.6	78484.7 ± 34.2
Al	13.5 ± 0.3	7.4 ± 0.6	22.6 ± 1.2	5.9 ± 0.1	13.5 ± 0.3	9.3 ± 0.07	21.9 ± 1.3	31.0 ± 1.5	24.5 ± 1.6	10.8 ± 0.2
P	0.03 ± 0.01	820.1 ± 3.6	0.03 ± 0.01	103.3 ± 2.0	1250.2 ± 6.2	1052.7 ± 4.7	12.5 ± 0.5	282.9 ± 2.9	75.5 ± 1.5	466.0 ± 3.0
K	6420.0 ± 34.5	9821.7 ± 82.1	6963.5 ± 6.4	18642.3 ± 52.9	17083.0 ± 26.1	15659.0 ± 38.5	9562.0 ± 10.1	9539.7 ± 40.5	6761.3 ± 25.1	16382.7 ± 14.1
Ca	6104.9 ± 27.2	62250.7 ± 106.5	28747.5 ± 23.4	86677.0 ± 151.2	52168.3 ± 158.6	56711.3 ± 194.1	38425.7 ± 32.9	85833.3 ± 35.1	70945.7 ± 14.4	21961.0 ± 8.5
Ti	1.5 ± 0.3	2.4 ± 0.3	2.0 ± 0.1	1.5 ± 0.1	3.2 ± 0.04	2.9 ± 0.08	2.4 ± 0.2	1.8 ± 0.02	2.5 ± 0.07	1.7 ± 0.1
V	0.7 ± 0.2	1.3 ± 0.2	0.8 ± 0.03	2.4 ± 0.2	2.5 ± 0.2	5.9 ± 0.07	4.1 ± 0.1	5.9 ± 0.03	4.0 ± 0.09	6.5 ± 0.3
Cr	1.4 ± 0.3	2.2 ± 0.2	1.3 ± 0.2	6.0 ± 0.1	2.6 ± 0.09	2.5 ± 0.04	1.5 ± 0.02	3.3 ± 0.05	1.9 ± 0.05	2.4 ± 0.2
Mn	2.1 ± 0.2	3.2 ± 0.2	1.9 ± 0.1	3.5 ± 0.2	2.2 ± 0.02	2.4 ± 0.03	2.7 ± 0.07	3.8 ± 0.04	1.7 ± 0.1	3.8 ± 0.1
Fe	318.2 ± 1.9	787.4 ± 11.5	365.2 ± 3.2	1084.0 ± 8.9	341.9 ± 3.4	499.4 ± 6.9	463.1 ± 5.4	512.7 ± 3.6	265.5 ± 2.8	513.0 ± 4.5
Co	0.3 ± 0.05	2.4 ± 0.2	0.3 ± 0.04	6.3 ± 0.1	1.5 ± 0.03	1.4 ± 0.07	0.5 ± 0.01	0.9 ± 0.01	1.7 ± 0.07	2.4 ± 0.2
Ni	2.1 ± 0.2	117.2 ± 8.2	1.7 ± 0.2	9.0 ± 0.02	7.6 ± 0.06	7.1 ± 0.09	2.4 ± 0.02	4.5 ± 0.2	2.2 ± 0.05	3.4 ± 0.2
Cu	2.1 ± 0.1	3.2 ± 0.2	1.4 ± 0.2	6.0 ± 0.04	2.9 ± 0.1	4.0 ± 0.03	2.9 ± 0.05	3.6 ± 0.05	2.7 ± 0.03	3.1 ± 0.1
Zn	42.6 ± 1.6	37.5 ± 2.8	37.3 ± 2.0	37.5 ± 0.7	41.8 ± 1.4	28.7 ± 0.3	29.7 ± 0.3	36.7 ± 2.0	44.1 ± 1.7	45.4 ± 1.6
As	1.0 ± 0.2	4.3 ± 0.3	0.9 ± 0.1	1.9 ± 0.1	2.3 ± 0.05	3.4 ± 0.09	1.8 ± 0.03	3.3 ± 0.06	1.2 ± 0.07	1.6 ± 0.2

Table A2 (continued)

Se	0.6 ± 0.2	1.4 ± 0.2	0.8 ± 0.1	2.5 ± 0.1	1.4 ± 0.03	1.3 ± 0.05	1.4 ± 0.03	2.7 ± 0.2	1.2 ± 0.02	1.5 ± 0.09
Rb	2.2 ± 0.2	11.5 ± 1.9	2.8 ± 0.2	13.3 ± 0.3	10.0 ± 0.08	10.6 ± 0.2	2.1 ± 0.03	5.8 ± 0.1	4.4 ± 0.2	12.5 ± 0.2
Sr	28.3 ± 2.7	364.6 ± 8.3	86.3 ± 1.9	574.5 ± 4.7	243.1 ± 3.0	274.4 ± 1.5	160.4 ± 1.9	401.3 ± 3.1	89.8 ± 0.6	50.3 ± 0.4
Mo	0.5 ± 0.05	5.6 ± 0.4	0.5 ± 0.007	2.9 ± 0.08	2.3 ± 0.03	2.7 ± 0.1	0.5 ± 0.02	1.5 ± 0.05	0.6 ± 0.006	0.8 ± 0.03
Pd	1.4 ± 0.2	1.7 ± 0.3	1.5 ± 0.1	2.0 ± 0.08	1.6 ± 0.02	1.7 ± 0.1	1.5 ± 0.02	1.9 ± 0.05	1.5 ± 0.1	1.4 ± 0.06
Ag	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Cd	0.1 ± 0.04	0.1 ± 0.02	0.1 ± 0.009	0.1 ± 0.001	0.1 ± 0.002	0.1 ± 0.002	0.1 ± 0.001	0.1 ± 0.002	0.1 ± 0.005	0.1 ± 0.005
Sb	0.4 ± 0.2	1.1 ± 0.09	0.4 ± 0.04	0.9 ± 0.01	0.6 ± 0.02	0.6 ± 0.006	0.4 ± 0.04	0.6 ± 0.006	0.4 ± 0.02	0.5 ± 0.01
Ba	11.8 ± 2.0	24.1 ± 3.2	57.3 ± 1.3	26.7 ± 0.6	23.2 ± 1.7	16.2 ± 0.2	35.1 ± 0.3	26.2 ± 1.3	22.6 ± 1.3	20.7 ± 0.3
Pt	0.1 ± 0.02	0.01 ± 0.02	0.02 ± 0.004	0.01± 0.003	0.1 ± 0.002	0.02 ± 0.002	0.03 ± 0.001	0.1 ± 0.001	0.1 ± 0.004	0.04 ± 0.003
Au	1.9 ± 0.3	1.9 ± 0.2	1.9 ± 0.07	2.0 ± 0.02	1.9 ± 0.09	2.0 ± 0.05	1.9 ± 0.07	2.9 ± 0.05	2.0 ± 0.1	2.1 ± 0.09
Tl	0.03 ± 0.01	0.01 ± 0.001	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Pb	0.3 ± 0.2	0.1 ± 0.02	0.3 ± 0.03	0.3 ± 0.02	0.2 ± 0.002	0.6 ± 0.03	0.1 ± 0.001	0.5 ± 0.01	0.3 ± 0.01	0.2 ± 0.03
Bi	1.3 ± 0.3	1.3 ± 0.2	1.3 ± 0.09	1.3 ± 0.05	1.3 ± 0.05	1.3 ± 0.03	1.4 ± 0.02	1.3 ± 0.2	1.4 ± 0.2	1.4 ± 0.06
Th	0.5 ± 0.3	0.5 ± 0.2	0.5 ± 0.03	0.5 ± 0.02	0.5 ± 0.005	0.5 ± 0.01	0.5 ± 0.006	0.5 ± 0.007	0.5 ± 0.02	0.5 ± 0.01
U	0.2 ± 0.08	64.3 1.4	0.4 0.01	29.6 ± 1.6	15.8 ± 0.1	24.8 ± 0.4	1.4 ± 0.02	6.5 ± 0.2	3.9 ± 0.2	4.3 ± 0.2
pH	6.0	6.4	6.3	6.2	6.7	6.7	7.2	7.6	7.1	7.1
EC (µS/cm)	80	783	297	1362	823	772	558	1303	789	788
Temperature (˚C)	12.6	14.7	15.5	15.7	16.5	16.6	21.3	20.4	19.4	17.4
Turbidity (NTU)	6	5	5	84	10	6	7	5	11	6
Flow-rate (m/s)	0.2	0.5	0.1	0.3	0.2	0.5	0.1	0.1	0.5	0.1

Table A3: Mean metal concentrations (µg/L), standard deviation and selected abiotic factors in water samples collected during the third survey.

	1	2	3	4	5	6	7	8	9	10
Be	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
B	12.5 ± 0.2	105.6 ± 1.3	10.7 ± 0.4	39.3 ± 0.6	76.4 ± 2.4	61.3 ± 1.4	48.5 ± 1.5	18.2 ± 0.2	8.9 ± 0.2	16.3 ± 0.6
Na	5909.7 ± 10.5	100333.7 ± 65.5	22225.3 ± 37.4	101419.0 ± 103.8	94915.0 ± 89.6	89986.3 ± 49.6	79929.3 ± 102.0	101414.3 ± 66.7	33636.3 ± 81.7	50271.3 ± 25.0
Mg	7939.7 ± 43.4	38672.7 ± 91.0	22443.7 ± 45.8	16819.3 ± 38.9	38284.3 ± 50.6	33808.0 ± 75.7	74934.3 ± 72.7	67237.7 ± 44.0	54861.3 ± 56.4	60264.0 ± 23.3
Al	47.5 ± 2.1	64.5 ± 0.7	77.1 ± 1.8	45.0 ± 1.2	62.3 ± 2.1	118.3 ± 2.0	44.8 ± 2.0	57.3 ± 1.6	41.4 ± 1.6	246.4 ± 0.6
P	587.9 ± 10.8	265.1 ± 1.2	111.4 ± 2.0	115.4 ± 2.1	724.6 ± 2.5	678.1 ± 1.8	181.7 ± 2.0	389.2 ± 5.1	119.4 ± 1.6	822.6 ± 1.2
K	6263.0 ± 74.5	8107.3 ± 16.3	3286.0 ± 40.7	4816.0 ± 23.5	9219.3 ± 38.8	9467.0 ± 41.2	8245.7 ± 32.9	11391.0 ± 19.3	2377.3 ± 15.0	5270.0 ± 16.5
Ca	10926.0 ± 73.5	30261.7 ± 157.7	47935.3 ± 72.1	60123.0 ± 55.2	95723.3 ± 74.7	84208.3 ± 173.5	59027.3 ± 55.4	62638.3 ± 51.5	46485.7 ± 97.6	70634.3 ± 74.7
Ti	3.0 ± 0.08	1.2 ± 0.03	3.1 ± 0.02	1.0 ± 0.02	3.1 ± 0.04	3.3 ± 0.05	0.8 ± 0.05	6.0 ± 0.09	2.2 ± 0.06	4.9 ± 0.03
V	0.3 ± 0.02	0.7 ± 0.02	0.8 ± 0.01	1.0 ± 0.005	3.8 ± 0.2	4.5 ± 0.1	2.6 ± 0.1	2.8 ± 0.07	2.7 ± 0.04	4.5 ± 0.03
Cr	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	3.2 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	2.0 ± 0.05	0.03 ± 0.01	0.03 ± 0.01
Mn	5.8 ± 0.2	100.6 ± 1.0	3444.3 ± 23.6	4.7 ± 0.06	7.4 ± 0.2	2.1 ± 0.04	2.5 ± 0.1	211.7 ± 1.8	1.7 ± 0.06	2.5 ± 0.02
Fe	258.6 ± 18.0	190.5 ± 4.4	271.4 ± 2.1	318.3 ± 5.8	631.6 ± 1.8	377.4 ± 9.0	304.8 ± 2.2	362.4 ± 1.8	211.3 ± 1.5	447.2 ± 1.7
Co	0.4 ± 0.02	40.7 ± 0.9	1.7 ± 0.1	2.8 ± 0.09	3.4 ± 0.03	2.6 ± 0.1	0.7 ± 0.02	1.3 ± 0.05	1.6 ± 0.05	1.7 ± 0.03
Ni	1.9 ± 0.07	199.1 ± 2.3	5.0 ± 0.02	5.5 ± 0.09	11.0 ± 0.4	10.3 ± 0.1	5.4 ± 0.2	4.5 ± 0.2	1.8 ± 0.02	2.7 ± 0.03
Cu	12.2 ± 1.2	11.5 ± 0.3	13.4 ± 0.3	17.6 ± 0.6	25.2 ± 0.3	12.0 ± 0.4	13.6 ± 0.5	17.2 ± 0.3	8.9 ± 0.09	11.7 ± 0.1
Zn	121.5 ± 0.6	120.3 ± 1.6	175.4 ± 2.0	218.4 ± 1.8	535.8 ± 6.0	164.5 ± 2.2	204.6 ± 2.3	253.2 ± 2.4	109.5 ± 1.0	191.3 ± 0.7
As	0.9 ± 0.09	4.8 ± 0.06	0.7 ± 0.04	0.4 ± 0.01	1.6 ± 0.05	1.7 ± 0.1	1.4 ± 0.1	1.8 ± 0.2	0.5 ± 0.008	0.8 ± 0.006

Table A3 (continued)

Se	0.03 ± 0.01	1.6 ± 0.03	1.4 ± 0.03	2.4 ± 0.01	1.9 ± 0.06	1.4 0.1	1.3 ± 0.1	4.4 ± 0.2	0.5 ± 0.01	0.5 ± 0.002
Rb	3.6 ± 0.06	17.1 ± 0.1	5.7 ± 0.03	7.7 ± 0.06	12.3 ± 0.3	12.0 ± 0.2	4.5 ± 0.09	7.8 ± 0.2	3.6 ± 0.06	6.9 ± 0.02
Sr	46.8 ± 0.9	251.3 ± 1.5	131.8 ± 1.3	469.2 ± 9.7	442.2 ± 1.9	381.2 ± 2.2	252.6 ± 1.8	300.6 ± 1.9	54.2 ± 1.0	81.7 ± 0.9
Mo	0.1 ± 0.004	3.1 ± 0.02	0.7 ± 0.006	1.8 ± 0.2	2.9 ± 0.1	2.3 ± 0.07	0.5 ± 0.01	0.3 ± 0.04	0.2 ± 0.006	0.4 ± 0.008
Pd	0.2 ± 0.01	0.5 ± 0.01	0.4 ± 0.006	1.1 ± 0.05	0.9 ± 0.02	0.7 ± 0.02	0.5 ± 0.01	0.7 ± 0.03	0.2 ± 0.01	0.2 ± 0.01
Ag	0.03 ± 0.01	0.1 ± 0.003	0.03 ± 0.01	1.0 ± 0.09	0.03 ± 0.01	0.03 ± 0.01	2.9 ± 0.1	0.03 ± 0.01	0.03 ± 0.01	0.4 ± 0.01
Cd	0.03 ± 0.01	0.1 ± 0.003	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Sb	0.6 ± 0.005	1.0 ± 0.01	0.6 ± 0.003	0.7 ± 0.07	0.8 ± 0.03	0.8 ± 0.01	0.6 ± 0.06	0.9 ± 0.02	0.7 ± 0.01	0.7 ± 0.01
Ba	15.2 ± 0.7	10.5 ± 0.7	184.4 ± 0.9	19.4 ± 1.2	42.3 ± 1.9	47.5 ± 1.6	81.3 ± 1.5	80.7 ± 1.2	11.6 ± 0.4	24.6 ± 0.7
Pt	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.2 ± 0.004	0.01 ± 0.004	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.1 ± 0.002
Au	0.2 ± 0.02	0.2 ± 0.006	0.4 ± 0.002	0.4 ± 0.05	0.2 ± 0.02	0.3 ± 0.02	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.003	0.2 ± 0.007
Tl	0.1 ± 0.007	0.1 ± 0.005	0.1 ± 0.005	0.1 ± 0.004	0.1 ± 0.006	0.1 ± 0.003	0.1 ± 0.005	0.1 ± 0.01	0.1 ± 0.002	0.1 ± 0.005
Pb	27.4 ± 0.4	25.4 ± 1.9	27.2 ± 0.7	31.6 ± 1.5	23.2 ± 2.0	22.0 ± 1.2	21.2 ± 1.5	28.9 ± 0.6	21.2 ± 0.8	22.3 ± 1.0
Bi	0.1 ± 0.007	0.1 ± 0.002	0.1 ± 0.004	0.1 ± 0.003	0.1 ± 0.01	0.1 ± 0.003	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.002	0.1 ± 0.005
Th	0.01 ± 0.001	0.1 ± 0.001	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.002	0.01 ± 0.001	0.03 ± 0.002	0.1 ± 0.001	0.03 ± 0.01	0.01 ± 0.001
U	0.02 ± 0.001	58.5 ± 1.4	1.3 ± 0.02	25.4 ± 1.2	37.0 ± 1.9	27.6 ± 2.0	2.6 ± 0.2	2.0 ± 0.05	1.7 ± 0.05	2.1 ± 0.02
pH	7.9	7.0	7.2	7.1	7.1	7.0	7.5	7.4	7.5	7.7
EC (µS/cm)	70	1076	340	1341	906	797	766	1120	661	754
Temperature (˚C)	10.1	15.6	10.6	12.0	15.6	12.3	13.6	13.5	15.5	16.2
Turbidity (NTU)	19	5	84	7	6	10	7	6	7	6
Flow-rate (m/s)	0.1	0.6	0.1	0.2	0.2	0.4	0.01	0.1	0.3	0.4

Table A4: Mean metal concentrations (mg/kg), standard deviation and particle size composition in sediment samples collected during the first survey.

	1	2	3	4	5	6	7	8	9	10
Be	4.1 ± 0.04	2.2 ± 0.02	1.3 ± 0.02	2.3 ± 0.04	1.2 ± 0.01	1.7 ± 0.01	1.1 ± 0.01	1.9 ± 0.01	3.4 ± 0.07	1.8 ± 0.01
B	3.2 ± 0.02	2.3 ± 0.03	0.01 ± 0.002	0.01 ± 0.002	8.1 ± 0.1	0.01 ± 0.001	7.2 ± 0.2	5.0 ± 0.02	1.8 ± 0.01	0.01 ± 0.001
Na	878.6 ± 1.3	490.9 ± 1.1	256.2 ± 1.1	579.3 ± 1.3	745.3 ± 0.3	493.5 ± 0.7	754.4 ± 1.5	2020.7 ± 1.7	1243.9 ± 1.3	865.5 ± 1.6
Mg	2761.2 ± 55.7	1960.4 ± 1.8	4293.4 ± 89.5	8592.1 ± 11.4	5932.7 ± 3.2	8203.6 ± 3.5	13937.8 ± 61.2	47298.8 ± 12.1	28727.9 ± 93.5	39562.3 ± 54.3
Al	205548.7 ± 203.1	68460.6 ± 104.2	32163.7 ± 190.4	38358.7 ± 104.3	33971.0 ± 66.6	30728.6 ± 15.1	39316.9 ± 93.7	104069.8 ± 86.8	158861.3 ± 184.3	68660.1 ± 25.6
P	817.4 ± 1.0	771.0 ± 1.1	882.7 ± 1.1	992.4 ± 0.6	3977.1 ± 10.2	1018.3 ± 0.4	561.5 ± 0.8	1731.8 ± 1.9	2514.5 ± 1.0	1613.1 ± 1.8
K	12855.9 ± 78.8	3841.1 ± 4.2	1768.8 ± 1.5	1331.7 ± 1.4	2605.3 ± 1.7	1680.4 ± 0.6	3681.1 ± 1.3	11726.9 ± 4.9	10403.8 ± 4.5	3095.4 ± 0.8
Ca	3118.1 ± 15.6	2744.5 ± 1.9	4848.2 ± 2.1	10138.0 ± 45.8	10779.5 ± 23.8	12693.1 ± 8.6	29777.8 ± 87.9	48158.4 ± 17.6	66541.7 ± 57.0	255290.0 ± 95.0
Ti	992.3 ± 1.8	540.4 ± 0.8	518.4 ± 0.9	693.4 ± 0.6	264.5 ± 0.6	214.8 ± 0.2	236.2 ± 0.3	6625.1 ± 1.3	617.4 ± 0.9	3622.8 ± 2.5
V	287.4 ± 0.9	170.6 ± 0.8	176.6 ± 0.5	423.8 ± 0.2	145.0 ± 0.7	256.2 ± 0.2	130.2 ± 0.02	721.6 ± 0.9	249.9 ± 0.4	472.3 ± 0.6
Cr	389.4 ± 1.3	364.3 ± 1.4	362.4 ± 0.5	1486.6 ± 7.6	293.9 ± 0.5	325.2 ± 0.2	221.4 ± 1.0	358.7± 0.2	692.1 ± 1.3	240.6 ± 0.4
Mn	278.7 ± 1.8	537.3 ± 0.6	662.8 ± 0.9	2559.3 ± 1.2	2061.9 ± 1.9	5752.4 ± 2.3	1877.6 ± 2.1	1441.1 ± 1.9	2717.6 ± 1.2	3292.3 ± 0.6
Fe	96320.5 ± 114.5	85421.3 ± 143.4	88048.7 ± 59.0	172176.0 ± 80.3	63363.7 ± 67.6	101019.8 ± 13.7	47514.7 ± 239.4	247371.0 ± 13.7	140190.3 ± 71.7	148743.0 ± 71.8
Co	28.9 ± 1.4	37.3 ± 0.3	36.7 ± 0.3	101.2 ± 0.4	52.5 ± 0.2	142.3 ± 0.5	44.3 ± 0.6	141.5 ± 1.0	92.8 ± 0.3	141.7 ± 0.4
Ni	166.7 ± 1.3	74.8 ± 0.6	83.6 ± 0.4	252.9 ± 0.3	127.8 ± 0.2	352.1 ± 0.1	99.4 ± 0.2	322.7 ± 1.2	247.1 ± 1.1	270.4 ± 0.6
Cu	116.7 ± 0.8	62.8 ± 0.2	79.6 ± 0.3	134.3 ± 0.4	75.7 ± 0.07	71.5 ± 0.2	55.7 ± 0.1	378.4 ± 1.0	328.4 ± 0.8	252.0 ± 0.07
Zn	153.0 ± 1.6	115.1 ± 0.2	174.1 ± 0.2	131.6 ± 0.4	158.4 ± 0.5	100.7 ± 0.7	95.2 ± 0.2	287.4 ± 1.4	936.2 ± 1.8	211.9 ± 0.02
As	24.8 ± 0.9	36.4 ± 0.2	16.0 ± 0.08	26.2 ± 0.2	9.5 ± 0.1	18.2 ± 0.2	5.9 ± 0.06	10.3 ± 0.02	10.9 ± 0.06	10.9 ± 0.01

Table A4 (continued)

Se	5.0 ± 0.03	2.1 ± 0.008	1.2 ± 0.02	1.6 ± 0.02	1.5 ± 0.01	2.3 ± 0.01	1.6 ± 0.1	4.7 ± 0.02	5.0 ± 0.05	5.3 ± 0.01
Sr	74.4 ± 0.6	38.3 ± 0.5	26.7 ± 0.2	44.7 ± 0.7	57.5 ± 0.2	46.1 ± 0.07	86.9 ± 0.5	126.6 ± 0.7	87.3 ± 1.1	79.1 ± 0.2
Mo	1.4 ± 0.005	2.2 ± 0.02	1.5 ± 0.02	1.6 ± 0.04	0.9 ± 0.002	0.8 ± 0.02	0.4 ± 0.01	1.2 ± 0.02	0.8 ± 0.008	0.6 ± 0.009
Pd	3.6 ± 0.02	2.3 ± 0.06	1.7 ± 0.02	2.0 ± 0.03	2.6 ± 0.02	2.4 ± 0.05	2.0 ± 0.02	3.7 ± 0.02	2.8 ± 0.1	4.8 ± 0.02
Ag	0.6 ± 0.002	0.2 ± 0.003	0.1 ± 0.002	0.1 ± 0.003	0.5 ± 0.002	0.1 ± 0.001	0.1 ± 0.002	0.8 ± 0.03	1.1 ± 0.01	0.5 ± 0.01
Cd	0.2 ± 0.003	0.2 ± 0.001	0.2 ± 0.03	0.2 ± 0.005	0.2 ± 0.005	0.2 ± 0.002	0.2 ± 0.002	0.4 ± 0.005	0.7 ± 0.01	0.7 ± 0.01
Sb	0.6 ± 0.004	1.3 ± 0.001	0.6 ± 0.02	0.6 ± 0.02	0.6 ± 0.006	0.6 ± 0.01	0.6 ± 0.01	0.6 ± 0.03	0.6 ± 0.01	0.7 ± 0.006
Ba	469.5 ± 1.0	234.3 ± 1.4	112.8 ± 1.1	104.3 ± 0.6	159.4 ± 0.5	136.4 ± 0.6	208.5 ± 0.4	519.1 ± 1.8	360.1 ± 1.1	357.7 ± 0.6
Pt	0.1 ± 0.001	0.1 ± 0.001	0.1 ± 0.002	0.1 ± 0.001	0.1 ± 0.001	0.1 ± 0.002	1.1 ± 0.003	0.1 ± 0.002	0.1 ± 0.006	0.5 ± 0.002
Au	2.1 ± 0.1	2.7 ± 0.01	2.3 ± 0.08	2.2 ± 0.02	2.9 ± 0.007	2.3 ± 0.02	2.1 ± 0.007	2.6 ± 0.03	2.2 ± 0.05	2.2 ± 0.03
Tl	1.6 ± 0.03	0.6 ± 0.009	0.4 ± 0.02	0.4 ± 0.02	0.4 ± 0.02	0.3 ± 0.007	0.4 ± 0.01	0.5 ± 0.01	1.1 ± 0.003	0.5 ± 0.002
Pb	80.5 ± 0.9	35.3 ± 0.7	55.3 ± 1.7	28.7 ± 0.2	23.6 ± 0.2	47.6 ± 0.5	27.3 ± 0.3	20.7 ± 0.1	90.4 ± 0.9	44.3 ± 0.7
U	10.6 ± 0.1	4.1 ± 0.01	4.3 ± 0.02	18.1 ± 0.2	14.9 ± 0.02	7.2 ± 0.1	1.3 ± 0.007	27.2 ± 0.2	9.6 ± 0.02	4.3 ± 0.02
% Gravel	10.0	18.6	29.1	37.5	19.9	33.6	16.0	45.3	35.5	70.4
% Sand	15.5	40.3	20.0	37.2	22.5	48.9	18.8	28.1	23.2	12.9
% Silt	69.4	39.3	49.1	25.1	55.2	17.3	64.1	26.1	37.1	16.0
% Clay	5.1	1.7	1.8	0.1	2.4	0.3	1.1	0.5	4.2	0.6

<u>Table A5:</u> Mean metal concentrations (mg/kg), standard deviation and particle size composition in sediment samples collected during the second survey.

	1	2	3	4	5	6	7	8	9	10
Be	1.6 ± 0.005	1.9 ± 0.01	2.6 ± 0.2	1.3 ± 0.1	0.6 ± 0.05	1.0 ± 0.04	0.4 ± 0.1	0.3 ± 0.02	1.8 ± 0.03	1.2 ± 0.03
B	0.01 ± 0.001	0.01 ± 0.002	0.01 ± 0.001	0.01 ± 0.02	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.004	0.01 ± 0.003	0.01 ± 0.002
Na	275.4 ± 0.3	240.7 ± 0.9	531.8 ± 1.7	474.3 ± 2.5	215.4 ± 1.7	243.1 ± 1.8	186.5 ± 1.7	182.5 ± 0.8	490.5 ± 0.9	556.1 ± 1.6
Mg	1499.5 ± 1.2	3200.5 ± 2.7	6299.6 ± 2.6	5257.0 ± 3.3	3586.0 ± 3.2	2669.3 ± 5.9	4476.3 ± 3.7	1755.7 ± 0.8	12004.6 ± 4.2	16131.0 ± 3.4
Al	54566.4 ± 5.4	37316.2 ± 2.5	123523.5 ± 120.2	38663.9 ± 4.6	14226.5 ± 7.5	25225.0 ± 2.7	20486.8 ± 6.5	9690.1 ± 1.2	87014.9 ± 4.6	63333.5 ± 1.1
P	355.3 ± 0.5	1589.4 ± 0.6	922.5 ± 0.6	691.9 ± 1.1	753.4 ± 0.7	500.4 ± 0.7	0.01 ± 0.001	45.7 ± 0.2	739.6 ± 0.4	596.5 ± 2.0
K	3672.3 ± 0.6	2851.4 ± 0.5	9790.4 ± 2.8	2905.6 ± 5.8	1177.3 ± 1.8	2065.2 ± 1.8	1806.8 ± 3.5	885.4 ± 0.4	6211.8 ± 0.7	4704.4 ± 3.8
Ca	1327.8 ± 0.5	2225.4 ± 0.3	7600.7 ± 2.4	7876.9 ± 6.2	17465.4 ± 6.4	3406.4 ± 2.5	11706.7 ± 5.9	9276.8 ± 0.7	32868.1 ± 2.2	34888.0 ± 1.4
Ti	344.4 ± 0.4	188.1 ± 0.08	737.1 ± 0.4	555.1 ± 1.7	166.8 ± 0.7	206.6 ± 0.6	252.5 ± 0.6	228.3 ± 0.5	314.6 ± 0.5	1193.5 ± 0.4
V	148.7 ± 0.1	227.5 ± 0.2	445.5 ± 1.8	282.2 ± 0.5	132.2 ± 1.2	168.3 ± 0.5	81.8 ± 1.5	88.7 ± 0.3	166.9 ± 0.5	258.6 ± 0.8
Cr	227.1 ± 0.2	366.0 ± 0.9	612.8 ± 1.1	519.2 ± 1.2	201.8 ± 1.3	525.7 ± 2.0	88.5 ± 0.5	165.1 ± 0.3	302.7 ± 0.3	172.0 ± 1.7
Mn	385.5 ± 0.3	905.5 ± 0.2	1886.4 ± 1.8	1234.0 ± 3.8	1034.7 ± 2.1	2372.8 ± 3.1	1102.8 ± 3.2	664.6 ± 0.5	2255.6 ± 1.9	1514.9 ± 1.8
Fe	54911.7 ± 11.5	176747.7 ± 4.5	176226.7 ± 30.6	88676.0 ± 5.3	39784.1 ± 4.8	60153.3 ± 3.5	20750.1 ± 3.7	19126.3 ± 2.1	76032.4 ± 3.3	82633.5 ± 4.3
Co	24.1 ± 0.1	206.9 ± 0.1	66.1 ± 0.4	61.7 ± 0.3	35.6 ± 0.4	46.6 ± 0.3	19.6 ± 0.2	17.4 ± 0.03	38.7 ± 0.3	50.1 ± 0.03
Ni	69.4 ± 0.2	438.4 ± 0.3	246.2 ± 0.4	250.5 ± 0.9	107.5 ± 0.7	165.2 ± 0.2	59.5 ± 0.7	48.4 ± 0.05	167.7 ± 0.6	154.7 ± 0.2
Cu	44.0 ± 0.1	93.4 ± 0.6	132.3 ± 0.9	76.7 ± 0.3	26.3 ± 0.2	36.4 ± 0.2	20.3 ± 0.6	18.9 ± 0.2	74.7 ± 0.3	99.3 ± 0.3
Zn	104.5 ± 0.2	329.6 ± 0.4	328.3 ± 0.1	109.0 ± 1.5	58.3 ± 0.3	47.2 ± 0.2	34.6 ± 0.9	17.3 ± 0.3	212.9 ± 0.3	160.5 ± 1.0
As	17.8 ± 0.2	116.3 ± 0.3	30.2 ± 0.1	17.6 ± 0.3	8.4 ± 0.1	19.2 ± 0.2	3.7 ± 0.2	4.4 ± 0.2	6.6 ± 0.2	6.5 ± 0.04

Table A5 (continued)

Se	1.9 ± 0.01	2.8 ± 0.01	2.8 ± 0.05	1.9 ± 0.02	1.5 ± 0.01	1.6 ± 0.04	1.3 ± 0.02	1.4 ± 0.05	2.3 ± 0.3	2.4 ± 0.04
Sr	27.5 ± 0.1	63.6 ± 0.2	44.7 ± 0.6	33.7 ± 0.5	36.2 ± 0.4	24.3 ± 0.4	26.6 ± 0.5	14.3 ± 0.3	34.7 ± 0.3	30.6 ± 2.1
Mo	1.1 ± 0.001	5.0 ± 0.05	1.7 ± 0.02	0.5 ± 0.006	0.1 ± 0.001	0.1 ± 0.005	0.01 ± 0.002	0.01 ± 0.003	0.01 ± 0.002	0.01 ± 0.004
Pd	0.5 ± 0.005	0.6 ± 0.02	0.7 ± 0.02	0.4 ± 0.05	0.4 ± 0.01	2.2 ± 0.04	0.3 ± 0.01	0.3 ± 0.04	0.7 ± 0.02	0.7 ± 0.03
Ag	0.01 ± 0.002	0.1 ± 0.1	0.1 ± 0.01	0.01 ± 0.003	0.01 ± 0.002	0.01 ± 0.002	0.01 ± 0.002	0.01 ± 0.003	0.01 ± 0.004	0.01 ± 0.003
Cd	0.1 ± 0.002	0.6 ± 0.01	0.5 ± 0.02	0.2 ± 0.05	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.003	0.2 ± 0.06	0.2 ± 0.02
Sb	0.2 ± 0.01	0.3 ± 0.03	0.2 ± 0.02	0.2 ± 0.03	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.04	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.01
Ba	151.4 ± 1.2	149.2 ± 1.2	292.7 ± 1.9	97.5 ± 1.2	77.3 ± 0.9	125.3 ± 0.7	71.8 ± 1.8	35.8 ± 0.4	190.5 ± 0.7	149.4 ± 0.5
Pt	0.2 ± 0.01	0.4 ± 0.004	0.2 ± 0.005	0.2 ± 0.04	0.2 ± 0.003	0.2 ± 0.05	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02
Au	0.4 ± 0.1	0.5 ± 0.01	0.5 ± 0.04	0.7 ± 0.02	0.5 ± 0.01	0.7 ± 0.03	0.5 ± 0.05	0.5 ± 0.03	0.5 ± 0.04	0.5 ± 0.03
Tl	0.2 ± 0.005	0.1 ± 0.02	0.5 ± 0.05	0.1 ± 0.02	0.01 ± 0.001	0.01 ± 0.002	0.01 ± 0.003	0.01 ± 0.002	0.2 ± 0.03	0.1 ± 0.007
Pb	24.5 ± 0.8	77.3 ± 0.9	91.2 ± 1.3	21.2 ± 0.9	13.7 ± 0.2	23.3 ± 0.3	9.5 ± 0.02	4.8 ± 0.3	28.3 ± 0.3	14.9 ± 0.3
U	2.7 ± 0.07	52.2 ± 0.8	6.6 ± 0.2	14.6 ± 0.3	6.5 ± 0.2	5.8 ± 0.1	0.3 ± 0.03	1.5 ± 0.3	1.5 ± 0.03	2.4 ± 0.3
% Gravel	10.4	37.0	54.2	0.8	22.6	49.4	24.4	11.0	1.8	55.9
% Sand	16.3	22.7	18.3	8.3	28.1	26.5	18.3	18.7	17.4	21.2
% Silt	66.6	40.0	24.7	90.3	48.6	23.3	54.3	68.7	79.0	21.8
% Clay	6.7	0.4	2.9	0.6	0.8	0.8	3.0	1.6	1.9	1.1

<u>Table A6:</u> Mean metal concentrations (mg/kg), standard deviation and particle size composition in sediment samples collected during the third survey.

	1	2	3	4	5	6	7	8	9	10
Be	6.9 ± 0.2	3.5 ± 0.3	4.2 ± 0.2	3.2 ± 0.02	3.7 ± 0.05	2.0 ± 0.3	5.5 ± 0.3	2.6 ± 0.3	2.6 ± 0.3	3.9 ± 0.1
B	1.2 ± 0.2	1.7 ± 0.2	0.01 ± 0.002	1.4 ± 0.05	2.8 ± 0.2	0.01 ± 0.003	1.2 ± 0.2	4.8 ± 0.2	0.06 ± 0.01	22.4 ± 0.4
Na	604.4 ± 2.0	387.4 ± 1.1	653.3 ± 0.9	1071.6 ± 0.8	2504.5 ± 2.2	587.3 ± 0.6	2926.4 ± 1.5	1587.9 ± 0.6	738.4 ± 2.2	1989.4 ± 1.1
Mg	4667.4 ± 4.3	2913.7 ± 3.5	8709.3 ± 1.7	11480.6 ± 1.5	19575.5 ± 5.9	6089.3 ± 0.7	44457.3 ± 2.9	20417.5 ± 3.5	15906.2 ± 3.5	62224.9 ± 4.6
Al	130759.4 ± 5.8	42617.1 ± 17.6	77021.7 ± 3.5	39824.6 ± 4.3	87951.3 ± 4.3	24248.4 ± 1.2	132924.9 ± 3.4	59466.4 ± 3.4	56337.6 ± 2.8	143881.0 ± 5.4
P	3505.2 ± 3.2	1873.9 ± 1.7	872.2 ± 0.2	1514.8 ± 2.7	8908.3 ± 1.8	1469.4 ± 1.1	1874.5 ± 0.4	2106.6 ± 0.6	1322.6 ± 0.5	2346.8 ± 0.8
K	4686.3 ± 2.8	4249.6 ± 2.8	3496.1 ± 1.3	2064.5 ± 0.2	7809.2 ± 0.3	2133.5 ± 1.2	11443.0 ± 2.7	5812.0 ± 1.7	4005.0 ± 2.2	5345.6 ± 3.4
Ca	5664.0 ± 4.4	3641.4 ± 1.2	11346.4 ± 2.7	13569.6 ± 2.0	39678.7 ± 4.2	10153.7 ± 2.2	82751.1 ± 7.7	35547.4 ± 5.4	40101.8 ± 3.3	92440.1 ± 5.5
Ti	131.7 ± 1.2	196.4 ± 2.0	67.3 ± 0.2	998.3 ± 0.2	43.9 ± 0.3	13.3 ± 0.3	19.3 ± 0.4	205.8 ± 0.2	14.2 ± 0.3	4761.3 ± 0.3
V	451.8 ± 0.8	193.5 ± 0.7	433.8 ± 0.2	455.3 ± 0.2	288.2 ± 0.2	181.0 ± 0.7	362.7 ± 0.3	358.6 ± 0.3	103.4 ± 0.3	715.7 ± 0.3
Cr	853.9 ± 1.0	362.8 ± 0.2	732.7 ± 0.4	1772.6 ± 1.1	618.9 ± 0.4	534.4 ± 0.5	684.1 ± 0.5	698.6 ± 0.4	415.3 ± 0.5	426.2 ± 0.4
Mn	1510.3 ± 2.0	756.4 ± 0.9	5316.7 ± 4.3	4593.7 ± 0.8	2735.1 ± 0.6	9969.0 ± 1.0	4956.5 ± 2.9	5833.5 ± 2.1	1585.7 ± 2.1	3347.2 ± 4.3
Fe	279315.0 ± 7.5	97513.6 ± 1.9	190872.1 ± 4.7	191927.5 ± 5.2	141768.5 ± 4.8	75913.3 ± 1.6	164077.8 ± 7.7	127709.0 ± 10.5	72375.4 ± 5.3	207504.0 ± 5.4
Co	123.6 ± 0.7	42.6 ± 0.2	138.3 ± 0.3	157.3 ± 0.2	129.3 ± 0.5	102.7 ± 0.2	147.7 ± 0.2	163.3 ± 0.4	56.3 ± 0.3	193.6 ± 0.3
Ni	183.5 ± 1.3	97.6 ± 0.3	197.3 ± 0.2	327.7 ± 0.3	372.9 ± 0.7	211.4 ± 0.5	354.8 ± 0.2	281.4 ± 0.4	122.7 ± 0.4	341.1 ± 0.6
Cu	193.7 ± 0.5	72.8 ± 0.3	185.2 ± 0.2	177.1 ± 0.3	320.6 ± 0.3	59.7 ± 0.3	278.7 ± 0.3	150.7 ± 0.3	126.7 ± 0.3	623.8 ± 0.4
Zn	351.8 ± 0.9	237.9 ± 0.2	195.5 ± 0.3	155.7 ± 0.3	826.3 ± 0.4	78.7 ± 0.1	861.6 ± 0.7	159.9 ± 0.7	432.5 ± 0.4	424.3 ± 0.5
As	136.6 ± 0.3	58.3 ± 0.1	17.7 ± 0.2	20.7 ± 0.3	22.4 ± 0.4	17.2 ± 0.2	12.2 ± 0.3	17.6 ± 0.7	4.5 ± 0.2	20.1 ± 0.5

Table A6 (continued)

Se	7.5 ± 0.08	1.9 ± 0.02	3.6 ± 0.2	1.8 ± 0.02	5.9 ± 0.3	2.4 ± 0.08	6.1 ± 0.2	4.4 ± 0.05	2.9 ± 0.1	7.9 ± 0.04
Sr	53.3 ± 0.3	51.4 ± 0.08	30.7 ± 1.0	49.4 ± 0.3	223.6 ± 0.3	56.9 ± 0.3	232.8 ± 0.4	114.1 ± 1.1	41.0 ± 0.8	79.5 ± 0.4
Mo	1.5 ± 0.02	4.8 ± 0.2	0.3 ± 0.02	1.0 ± 0.004	0.8 ± 0.01	0.6 ± 0.03	0.4 ± 0.03	0.4 ± 0.02	0.2 ± 0.02	0.6 ± 0.08
Pd	4.2 ± 0.2	2.0 ± 0.02	3.3 ± 0.2	2.2 ± 0.2	3.4 ± 0.02	2.5 ± 0.02	4.6 ± 0.03	3.5 ± 0.05	2.4 ± 0.3	5.5 ± 0.03
Ag	6.9 ± 0.1	0.1 ± 0.02	1.5 ± 0.2	0.01 ± 0.003	5.1 ± 0.2	0.01 ± 0.004	0.8 ± 0.03	0.01 ± 0.004	0.1 ± 0.004	2.6 ± 0.2
Cd	0.5 ± 0.03	0.4 ± 0.03	0.3 ± 0.2	0.2 ± 0.002	0.9 ± 0.03	0.1 ± 0.02	1.8 ± 0.2	0.2 ± 0.02	0.4 ± 0.06	0.7 ± 0.05
Sb	0.4 ± 0.03	0.6 ± 0.01	0.3 ± 0.07	0.4 ± 0.02	0.3 ± 0.01	0.4 ± 0.02	1.9 ± 0.02	0.4 ± 0.03	0.4 ± 0.03	0.5 ± 0.05
Ba	597.1 ± 1.2	213.4 ± 0.8	688.7 ± 0.7	225.7 ± 1.2	783.5 ± 0.3	484.5 ± 0.5	964.3 ± 0.7	681.1 ± 9.6	223.7 ± 1.3	665.5 ± 2.3
Pt	0.4 ± 0.03	0.2 ± 0.02	0.1 ± 0.02	0.1 ± 0.004	0.01 ± 0.006	1.3 ± 0.03	0.1 ± 0.01	0.1 ± 0.004	0.2 ± 0.02	0.1 ± 0.04
Au	1.1 ± 0.1	1.2 ± 0.09	0.5 ± 0.02	0.9 ± 0.03	4.1 ± 0.02	0.6 ± 0.02	0.6 ± 0.03	1.1 ± 0.1	0.6 ± 0.1	0.6 ± 0.02
Tl	1.3 ± 0.2	0.2 ± 0.02	0.9 ± 0.2	0.7 ± 0.02	1.1 ± 0.02	0.4 ± 0.01	1.3 ± 0.03	0.7 ± 0.04	0.7 ± 0.1	0.8 ± 0.03
Pb	1426.5 ± 2.0	68.4 ± 0.3	268.1 ± 0.5	226.6 ± 2.0	170.5 ± 0.6	291.2 ± 0.3	345.4 ± 0.5	397.2 ± 0.2	257.8 ± 2.0	528.2 ± 2.4
U	11.8 ± 0.2	29.0 ± 0.03	5.6 ± 0.2	21.8 ± 0.2	123.9 ± 0.3	11.1 ± 0.2	17.8 ± 0.3	11.0 ± 0.4	3.7 ± 0.1	10.1 ± 0.2
% Gravel	3.0	34.8	25.7	39.0	8.5	27.8	10.1	34.0	13.2	49.1
% Sand	12.9	13.7	16.9	30.6	41.6	25.4	35.2	29.6	16.3	26.6
% Silt	77.0	49.3	47.1	29.6	44.2	44.5	48.6	35.3	68.7	22.4
% Clay	7.1	2.1	10.3	0.8	5.7	2.3	6.1	1.1	1.7	1.9

Appendix B

Table B1: The mean metal concentrations (µg/g wet weight) and standard deviation in selected macroinvertebrate families.

	Al	Ti	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb
Benthic												
Ancylidae	529.9 ± 145.5	6026.0 ± 0.8	3493.0 ± 0.6	462.6 ± 164.6	1185.0 ± 288.0	2631.0 ± 0.3	10.7 ± 1040.0	58.5 ± 0.7	138.5 ± 109.7	0.6 ± 0.1	0.06 ± 0.1	6694.0 ± 1153.0
Caenidae	3048.0 ± 2318.0	5938.0 ± 0.5	30.4 ± 30.1	362.5 ± 91.2	12314.0 ± 8459.0	2624.0 ± 1678.0	13.0 ± 10.2	53.7 ± 19.1	4684.0 ± 4181.0	4333.0 ± 3383.0	0.8 ± 0.8	39.0 ± 19.6
Physidae	570.0 ± 105.5	8500.0 ± 2723.0	3779.0 ± 1161.0	406.4 ± 189.9	1125.0 ± 333.4	2704.0 ± 1383.0	9099.0 ± 3548.0	82.3 ± 5995.0	19.2 ± 6651.0	0.5 ± 0.2	0.003 ± 0.002	8541.0 ± 2943.0
Chironomidae	1136.0 ± 813.3	9668.0 ± 3058.0	7477.0 ± 5116.0	353.9 ± 65.4	2370.0 ± 1485.0	2857.0 ± 0.7	13.5 ± 0.6	92.0 ± 15.7	1590.0 ± 1383.0	1294.0 ± 0.5	0.1 ± 0.1	50.8 ± 10.3
Baetidae	613.1 ± 212.0	4513.0 ± 0.6	3554.0 ± 2093.0	348.8 ± 45.3	908.3 ± 264.8	1636.0 ± 0.2	7941.0 ± 0.8	40.2 ± 6212.0	243.6 ± 222.6	0.5 ± 0.1	0.004 ± 0.001	22.6 ± 5119.0
Simuliidae	1484.0 ± 771.3	12.3 ± 0.7	8102.0 ± 3858.0	521.0 ± 23.0	2317.0 ± 948.8	5002.0 ± 1345.0	20.2 ± 1173.0	57.3 ± 1.0	412.3 ± 624.4	1.0 ± 0.4	0.005 ± 0.002	27.3 ± 3934.0
Aeshnidae	66.8 ± 26.9	1076.0 ± 0.5	0.4 ± 0.1	52.5 ± 46.7	140.5 ± 18.9	0.1 ± 0.1	2833.0 ± 1988.0	8401.0 ± 2315.0	28.0 ± 21.1	0.2 ± 0.05	0.004 ± 0.005	4423.0 ± 1513.0
Chlorocyphidae	340.2 ± 138.8	3387.0 ± 0.5	1824.0 ± 0.6	188.7 ± 10.5	616.6 ± 239.9	0.5 ± 0.1	2136.0 ± 0.8	24.8 ± 5315.0	383.1 ± 457.5	0.8 ± 0.2	0.1 ± 0.1	20.3 ± 8971.0
Gomphidae	247.0 ± 61.1	2313.0 ± 0.3	1360.0 ± 0.2	111.0 ± 13.5	473.4 ± 80.7	0.2 ± 0.02	1253.0 ± 0.2	10.1 ± 1460.0	486.8 ± 523.0	0.4 ± 0.1	0.1 ± 0.1	7982.0 ± 3943.0
Libellulidae	199.4 ± 141.0	2173.0 ± 0.5	1249.0 ± 1059.0	108.3 ± 37.1	378.1 ± 271.6	0.3 ± 0.2	2092.0 ± 1070.0	17.9 ± 13.5	161.4 ± 183.7	0.6 ± 0.5	0.02 ± 0.02	8290.0 ± 5082.0
Pelagic												
Atyidae	355.4 ± 258.9	4209.0 ± 0.9	1297.0 ± 0.4	192.3 ± 114.4	2057.0 ± 2629.0	0.5 ± 0.3	2073.0 ± 0.5	19.5 ± 2978.0	2083.0 ± 327.2	0.5 ± 0.2	0.3 ± 0.1	11.2 ± 2474.0
Belostomatidae	211.2 ± 206.3	1765.0 ± 0.3	1320.0 ± 1511.0	118.1 ± 27.1	364.7 ± 343.7	0.4 ± 0.2	2651.0 ± 0.4	14.8 ± 1137.0	471.7 ± 428.8	0.4 ± 0.2	0.2 ± 0.1	8976.0 ± 3531.0
Coenagrionidae	208.4 ± 230.8	1611.0 ± 0.3	0.9 ± 0.7	130.7 ± 5851.0	1272.0 ± 1962.0	0.2 ± 0.1	1096.0 ± 0.6	14.0 ± 0.9	540.6 ± 890.7	0.5 ± 0.2	0.1 ± 0.2	16.3 ± 4908.0
Dytiscidae	210.1 ± 110.8	2512.0 ± 0.3	1.0 ± 0.4	177.4 ± 38.3	493.7 ± 355.5	0.3 ± 0.05	1441.0 ± 0.1	31.9 ± 4981.0	400.0 ± 419.9	0.8 ± 0.1	0.1 ± 0.1	18.9 ± 4933.0
Gyrinidae	431.7 ± 545.1	2010.0 ± 0.2	4836.0 ± 6557.0	175.0 ± 6844.0	1681.0 ± 2248.0	0.4 ± 0.4	2465.0 ± 2755.0	16.8 ± 5098.0	819.5 ± 1107.0	0.9 ± 0.9	0.1 ± 0.2	12.0 ± 5059.0