

The macroinvertebrate diversity and selected physical and chemical factors of the Mooi River and the Wonderfontein Spruit

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Abstract

The Mooi River catchment area has been the sole water source for Potchefstroom (North-West Province) since 1842. The Mooi River and the Wonderfontein Spruit are subjected to a substantial number of detrimental impacts which result in, amongst others, the influx of metals that are deposited and adsorbed on the sediment. Anthropogenic activities, such as agricultural and mining activities in the catchment area, not only reduce the diversity of macroinvertebrate assemblages, but also the community structure. According to the literature, aquatic macroinvertebrates are well known for their sensitivity towards extensive organic pollution, habitat transformation and selected physico-chemical factors and therefore utilising them for the assessment of the ecological health of rivers is a widely recognised method.

The aim of this study was to determine the aquatic macroinvertebrate diversity and the possible influences that selected physico-chemical factors may have on the macroinvertebrates. This will contribute towards a meaningful assessment of the current state of environmental (water and sediment) and ecological (macroinvertebrate) quality of the Mooi River catchment.

Three surveys were conducted at 11 preselected sites in the Mooi River, Gerhard Minnebron and Wonderfontein Spruit. Macroinvertebrates were collected from the vegetation, as well as the substratum by using standard sampling procedures. Sediment and water samples were also collected at each of the sites, while electrical conductivity (EC), pH, temperature, turbidity and flow-rate were measured *in situ* at all sites. Whenever possible, macroinvertebrates were identified up to species level in the laboratory, otherwise identification was done up to genus or family level. Subsequently, all specimens were counted and classified. Sediment samples were digested, sieved and sent for Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analyses, X-Ray Diffraction (XRD), as well as Scanning Electron Microscopy (SEM)-analyses to determine the metal concentrations, mineral composition and elemental composition, respectively. Water samples were filtered and acidified, where after the samples were sent for ICP-MS analyses to determine the metal concentrations. In addition, selected macroinvertebrate taxa were digested and sent for ICP-MS analysis to determine the metal concentrations.

Thirty four (34) and 31 metals were detected in the water and sediment samples, respectively. Only those metals that may be, according to literature, potentially toxic to macroinvertebrates were included. The results indicated that, although some metal concentrations measured in the water and sediment samples may have been affected by the mining effluent originating from Site 1B, the presence of the majority was probably as a result of natural weathering of the minerals and rocks present in the catchment area, as well as soil erosion due to the agricultural activities around the rivers. The concentrations of the metals in the water and sediment could thus not be attributed to the adverse effects of the mining effluent.

Regarding the macroinvertebrate taxa, a total of 142 taxa belonging to 66 families were collected at the 11 selected sites during the three surveys. The majority of these families were classified as highly tolerant. Only one highly sensitive family was collected during this study, suggesting that organic enrichment is present throughout the catchment area. Although the levels of organic enrichment observed at some sites during this study could be advantageous for those families known to be tolerant to, or have a preference for such environments or conditions, these levels were probably not to such an extent as to exclude the less tolerant families from these sites. Different biotope types and selected parameters (specifically flow-rate and EC) played a decisive role in the distribution of the families in the Mooi River catchment area.

The results obtained from the metals measured in the macroinvertebrates taxa revealed that, in contrast to the limited variations in metal concentrations measured in the pelagic organisms, of which the majority were classified as predators, a substantial variation in the metal concentrations measured in the benthic macroinvertebrates was evident. The majority of macroinvertebrates that associated with the benthic environment had the highest metal concentrations, which may be ascribed to the ingestion of sediment and organic particles, direct contact with sediments or contact with interstitial water.

KEY WORDS: Mooi River, Wonderfontein Spruit, metals, anthropogenic activities, macroinvertebrate assemblages.

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Chapter 1: General Introduction

1.1 Background

The Department of Water and Sanitation (DWS) (2016) describes South Africa as the 30th driest country in the world, with an average rainfall of 450 mm per annum, which is almost half the global average of 860 mm per annum (DWS, 2016). This situation is exacerbated by the relatively high temperatures, the growing human population, increasing demand for freshwater, pollution, climate change, over-abstraction and unsustainable water usage (Dallas & Day, 2004; DWA, 2011), which all contribute to a scarcity of freshwater (Dallas & Day, 2004). According to Oberholster and Ashton (2008), even with a zero increase in population in South Africa, pollutants will continue to accumulate in freshwater systems and further deteriorate the water quality, which in turn may affect the health of aquatic ecosystems. These factors threaten the integrity of natural ecosystems and aquatic life, and therefore, the use of freshwater needs to be thoroughly and accurately managed.

It was further established that approximately 71% of rivers in South Africa are considered as either endangered or critically endangered, due to anthropogenic activities that include mining and agricultural activities (Nel *et al.*, 2004). The State of the Rivers assessment found that all of the rivers in the North-West Province, have been impacted by human activities and therefore the habitat integrity and overall health of these ecosystems are in a state of deterioration. The majority of the rivers that were assessed, are considered to be in a moderately to largely modified state (category C to D) (RHP, 2007a). With regard to the habitat integrity of the Mooi River catchment, which includes the Wonderfontein Spruit, also situated in the North-West Province, the same conclusions were made (DWS, 2014; RHP, 2007b).

The Mooi River catchment area has been the sole water source for Potchefstroom since 1842 (Van der Walt *et al.*, 2002). Regarding the surrounding geology, the catchment area is divided between the Wonderfontein Spruit and the Loop Spruit (both tributaries of the Mooi River) by the Gatsrand geological ridge, where the upper part of the catchment mainly lies in a dolomite-rich area (Barnard *et al.*, 2013; DWA, 2009; DWS, 2014; Tlokwe City Council, 2014). The Mooi River and the Wonderfontein Spruit are subjected to a substantial number of detrimental impacts. These impacts result in, amongst others, the influx of metals that are deposited and adsorbed onto the sediment (Tchounwou *et al.*, 2012). Informal settlements, agricultural activities and sewage treatment plants are also some of the pollution sources and are partly responsible for the increased nitrogen and phosphate levels (DWS, 2014). According to Batty *et al.* (2010), anthropogenic activities, such as those mentioned above, not only reduce the diversity of macroinvertebrate

assemblages, but also the community structure. This is due to their sensitivity towards organic pollution and habitat transformation and therefore utilising them for the assessment of the ecological health of rivers is a widely recognized method (Dickens & Graham, 2002).

1.2 Hypotheses

The following four hypotheses will be tested during this study:

1. Mining effluent from the Wonderfontein Spruit is responsible for an increase in metal contamination of the water and sediment of the Mooi River (Chapter 3).
2. The anthropogenic activities (mining and agriculture) in the upper reaches of the Wonderfontein Spruit has a detrimental effect on the macroinvertebrate assemblages downstream (Chapter 4).
3. Trophic transfer of metals will be reflected in the FFGs, with higher concentrations in the predators compared to collector-gatherers and scraper-grazers (Chapter 5).
4. Macroinvertebrates associating with the benthic environment, will display higher metal concentrations than those associated with the water column (Chapter 5).

1.3 Aims and objectives

The aim of this study was to determine the aquatic macroinvertebrate diversity and the possible influences that selected physico-chemical factors may have on the macroinvertebrates. This will contribute towards a meaningful assessment of the current state of environmental (water and sediment) and ecological (macroinvertebrate) quality of the Mooi River catchment. To this end, the following aspects will be addressed:

- Determine the physico-chemical characteristics in water and sediment.
- Determine the aquatic macroinvertebrate diversity of the Mooi River and Wonderfontein Spruit.
- Determine the interaction between the physico-chemical parameters and the macroinvertebrates.

Chapter 2: Description of the study area and site selection

2.1 Study area

The Mooi River catchment area, along with its two main tributaries, the Wonderfontein Spruit and the Loop Spruit, extends over two provinces, with the upper section located in Gauteng and the lower part of the catchment in the North- West Province (DWS, 2014). The catchment area has a relatively flat topography with altitudes ranging from 1 520 m in the north to 1300 m in the southwest (DWA, 2009; Tlokwe City Council, 2014) and covers a total area of 1 800 km², of which only 55.8% of the total runoff within the catchment area contributes to the surface water of the Mooi River (Van der Walt *et al.*, 2002). The mean rainfall per year is approximately 683 mm, with a mean evaporation potential of 1 650 mm (Van der Walt *et al.*, 2002) and temperatures ranging from > 32 °C during October to January and -1 °C during May to September (Cilliers & Bredenkamp, 2000).

The Mooi River originates in the Boons area, where it flows through three relatively large impoundments, namely the Klerkskraal Dam, Boskop Dam and Potchefstroom Dam, before its confluence with the Vaal River, 20 km downstream from Potchefstroom (Tlokwe City Council, 2014). The Mooi River and its tributaries flow through the districts of Potchefstroom, Westonaria, Oberholzer, Fochville and Carletonville, where a number of informal settlements, including Khutsong, Kokosi and Green Park, are also located (DWS, 2014). The Wonderfontein Spruit originates in the area surrounded by several gold mines that are not in production, on the southern side of Krugersdorp on the Witwatersrand Ridge (DWS, 2014; Tlokwe City Council, 2014). After the Donaldson Dam, the river is channelled into a one meter diameter pipeline for approximately 32 km, which transports water over three dewatered dolomitic compartments (Oberholzer, Venterspos, and Bank) and finally discharges near Carletonville (DWS, 2014; Hamman, 2012; Tlokwe City Council, 2014). The pipeline was constructed to prevent the recirculation of water that was pumped to the surface from underground mining activities (Hamman, 2012). The Wonderfontein Spruit, together with the Gerhard Minnebron confluences with the Mooi River, approximately 10 km above the inflow of Boskop Dam (Tlokwe City Council, 2014). Several springs feed the Mooi River and Wonderfontein Spruit, of which the Gerhard Minnebron, Bovenste Oog and Turffontein Spring are the largest (Tlokwe City Council, 2014).

Land use around the Mooi River mainly include agricultural activities, where water is drawn from the river for livestock, as well as irrigation and crop farming (Barnard *et al.*, 2013; DWS, 2014). Industrial and general recreational uses of water from this river are

mainly concentrated in and around the town of Potchefstroom and Boskop Dam area (DWS, 2014). In the case of the Wonderfontein Spruit, land use mainly include mining activities in the upper reaches and agricultural activities along the banks of the river (Barnard *et al.*, 2013; DWA, 2009; Tlokwe City Council, 2014).

The study was conducted at five preselected sites (Figure 2.1) in the Mooi River (Sites 1A to 5A), as well as the Wonderfontein Spruit (Sites 1B to 5B) and one site located at the Gerhard Minnebron (Site 1C). Sites were selected based on a number of criteria, including the availability of water, accessibility to the river, possible impact areas such as mine and agricultural runoff, the availability of a variety of suitable biotopes, such as stones in current, stones out of current, marginal, and aquatic vegetation, gravel, sand and mud (GSM), runs, riffles and pool biotopes. The location of impoundments and sewage treatment plants, as well as the presence of springs, were also taken into consideration. The locations of these sites are depicted in Figure 2.1, while site, habitat and biotope descriptions are displayed in Tables 2.1 to 2.11.

2.2 Map of the study area

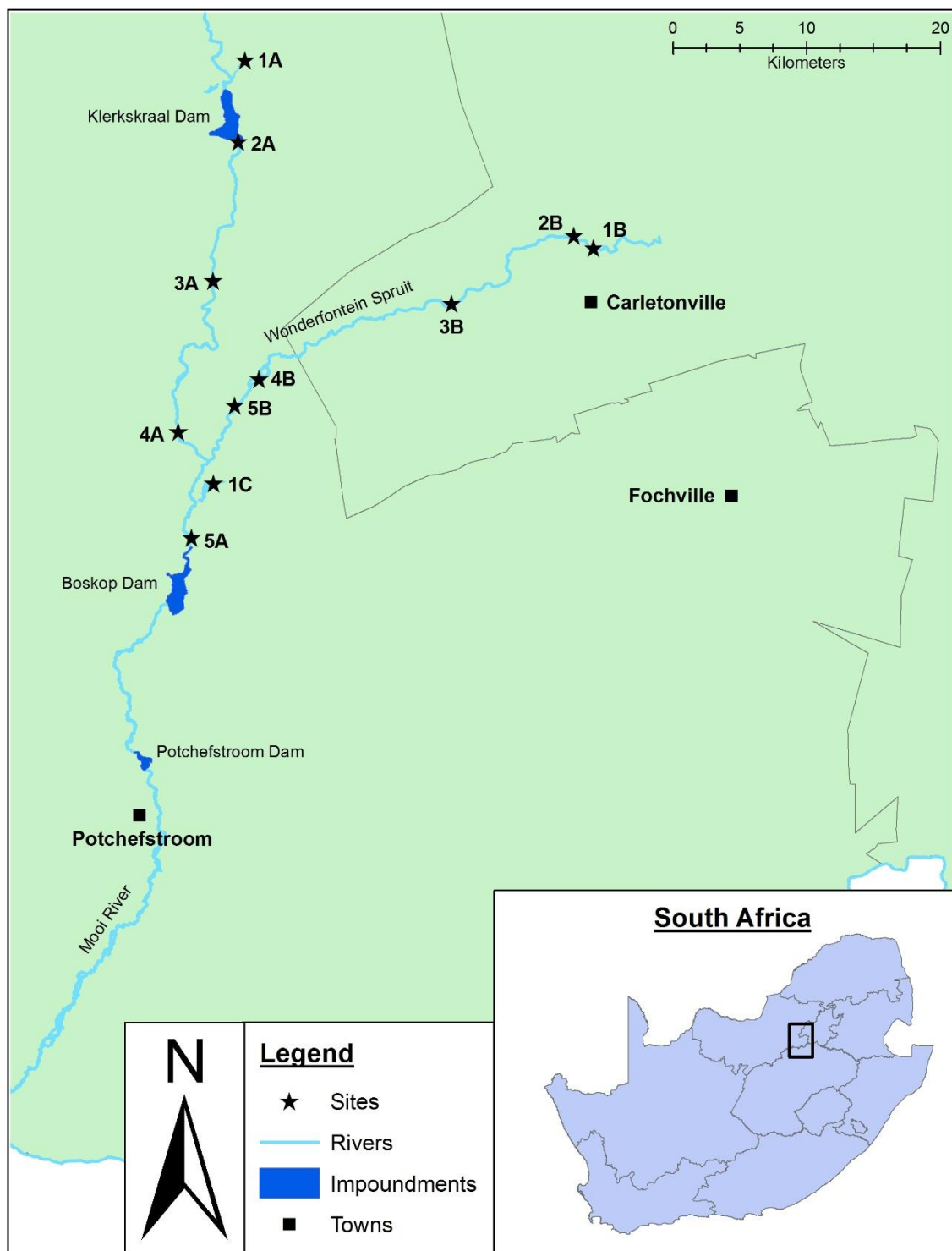


Figure 2.1: Sampling sites within the Mooi River and Wonderfontein Spruit, North-West Province, South Africa.

2.3 Site description

Table 2.1: Physical characteristics of Site 1A.

Site 1A Bovenste Oog	
	
Coordinates	S 26°11'52.4" E 27°09'52.5"
Altitude	1 472 m
Site description and land use	Site 1A is considered as one of the main springs that feed the Mooi River and is located approximately 6 km upstream of the Klerkskraal Dam. Little to no impacts occur here and land uses include limited agricultural activities.
Habitat and biotope description	Headwater zone, stones in streambed, abundant marginal, aquatic and overhanging riparian vegetation, clear, shallow water, with a pool biotope.
Primary lithology	Alluvium, chert-rich dolomite, interbedded oolitic chert and gravel, diamondiferous in places (Keyser, 1986).
Dominant vegetation	<i>Berula erecta</i> , <i>Nymphaea nouchali</i> var. <i>caerulea</i> , <i>Typha capensis</i> , <i>Veronica annagallis aquatica</i> , <i>Schoenoplectus corymbosus</i> , <i>Nasturtium officinale</i> and <i>Juncus lomatophyllus</i> .

Table 2.2: Physical characteristics of Site 2A.

Site 2A Mooi River below the Klerkskraal Dam	
	
Coordinates	S 26°15'09.9" E 27°09'35.1"
Altitude	1 462 m
Site description and land use	This site is located directly below the Klerkskraal Dam wall. Land uses around the dam include agricultural, as well as various recreational activities.
Habitat and biotope description	Stones in streambed, marginal and abundant aquatic vegetation, stones in current, stones out of current, riffle and run biotopes, directly below impoundment.
Primary lithology	Chert-rich dolomite, interbedded oolitic chert, quartzite, conglomerate, shale and gravel, diamondiferous in places (Keyser, 1986).
Dominant vegetation	<i>Berula erecta</i> , <i>Juncus lomatophyllus</i> , <i>Phragmites mauritianus</i> , <i>Typha capensis</i> , <i>Veronica annagallis aquatica</i> , <i>Rumex conglomeratus</i> , <i>Potomageton pusillus</i> , <i>Potomageton schweinfurthii</i> , <i>Nitella</i> sp., <i>Ludwigia natans</i> and <i>Persicaria decipiens</i> .

Table 2.3: Physical characteristics of Site 3A.

Site 3A Mooi River (Rysmierbult)	
	
Coordinates	S 26°21'35.5" E 27°08'20.1"
Altitude	1 439 m
Site description and land use	Site 3A is located in the Mooi River, in the Rysmierbult area. This site is situated approximately 12 km downstream from the Klerkskraal Dam. Land uses include agricultural activities, mainly crop farming and informal cattle farming, as well as limited industrial activities. During the last survey, this site was completely dry due to the drought experienced throughout South Africa.
Habitat and biotope description	Middle water zone, stones in streambed, marginal, aquatic and overhanging riparian vegetation, stones in current, sandy and muddy substratum, run and riffle biotopes.
Primary lithology	Gravel, diamondiferous in places and dolomite, chert and remnants of chert breccia of the Rooihogte Formation (Keyser, 1986).
Dominant vegetation	Filamentous algae, <i>Veronica annagallis aquatica</i> , <i>Ranunculus rionii</i> , <i>Ludwigia natans</i> , <i>Plantago major</i> , <i>Rumex conglomeratus</i> , <i>Schoenoplectus corymbosus</i> and <i>Cyperus dives</i> .

Table 2.4: Physical characteristics of Site 4A.

Site 4A Mooi River (Muiskraal)	
	
Coordinates	S 26°26'43.1" E 27°07'06.5"
Altitude	1 412 m
Site description and land use	This site is also located in the Mooi River, approximately 13 km downstream of Site 3A, in the Muiskraal area. Agricultural activities include mainly cattle farming, as well as small-scale cultivation.
Habitat and biotope description	Middle water zone, stones in streambed, marginal and abundant aquatic vegetation, overhanging riparian vegetation, sandy substratum, stones in current, riffle and run biotopes.
Primary lithology	Gravel, diamondiferous in places and dolomite, chert and remnants of chert breccia of the Rooihogte Formation (Keyser, 1986).
Dominant vegetation	<i>Typha capensis</i> , <i>Veronica annagallis aquatica</i> , <i>Schoenoplectus corymbosus</i> , <i>Ludwigia natans</i> , Filamentous algae, <i>Berula erecta</i> , <i>Cyperus dives</i> , <i>Juncus lomatophyllus</i> , <i>Persicaria decipiens</i> and <i>Fissidens</i> sp.

Table 2.5: Physical characteristics of Site 5A.

Site 5A Mooi River above Boskop Dam	
	
Coordinates	S 26°30'53.2" E 27°07'28.4"
Altitude	1 399 m
Site description and land use	Site 5A is located at the inlet of the Boskop Dam, about 9.5 km downstream of Site 4A. The confluence of the Wonderfontein Spruit and the Gerhard Minnebron (Site 1C) with the Mooi River occurs approximately 2.7 km upstream of this site. Limited agricultural activities, including the planting of crops, occur here.
Habitat and biotope description	Middle water zone, limited marginal and overhanging riparian vegetation, muddy substratum, deep, murky water, run and pool biotopes.
Primary lithology	Dolomite, chert and remnants of chert breccia of the Rooihogte Formation (Keyser, 1986).
Dominant vegetation	<i>Phragmites mauritanus</i> , <i>Veronica annagallis aquatica</i> , <i>Nasturtium officinale</i> , <i>Persicaria decipiens</i> , <i>Phragmites australis</i> and <i>Typha capensis</i> .

Table 2.6: Physical characteristics of Site 1B.

Site 1B Wonderfontein Spruit (discharge from pipeline)	
	
Coordinates	S 26°19'04.9" E 27°23'33.7"
Altitude	1 476 m
Site description and land use	This site is located in the Wonderfontein Spruit, where the one meter diameter pipeline discharges water from the mines surrounding the headwaters of the Wonderfontein Spruit. Land uses around this site mainly include informal cattle farming and mining activities in the upper reaches.
Habitat and biotope description	Headwater zone, stones in streambed, marginal and aquatic vegetation, algae present in water, muddy and sandy substratum and riffle and run biotopes.
Primary lithology	Alluvium, dolomite, chert and remnants of chert breccia of the Rooihoogte Formation (Keyser, 1986).
Dominant vegetation	<i>Berula erecta</i> , <i>Cyperus</i> sp., <i>Spirodela</i> sp., <i>Veronica annagallis aquatica</i> , <i>Juncus</i> sp., <i>Rumex conglomeratus</i> , <i>Potamogeton pusillus</i> and <i>Persicaria lapathifolia</i> .

Table 2.7: Physical characteristics of Site 2B.

Site 2B Wonderfontein Spruit	
	
Coordinates	S 26°18'57.4" E 27°22'58.4"
Altitude	1 473 m
Site description and land use	This site is located approximately 650 m downstream of Site 1B, on the R500 between Carletonville and Holfontein. Land uses include mainly informal cattle farming.
Habitat and biotope description	Middle water zone, muddy substratum, marginal and aquatic vegetation, deep water, run and pool biotopes.
Primary lithology	Alluvium, gravel, diamondiferous in places and dolomite, chert and remnants of chert breccia of the Rooihoogte Formation (Keyser, 1986).
Dominant vegetation	<i>Azolla pinnata</i> , <i>Berula erecta</i> , <i>Cyperus eragrostis</i> , <i>Plantago longissima</i> , <i>Spirodela</i> sp., <i>Potamogeton pusillus</i> and <i>Rumex conglomeratus</i> .

Table 2.8: Physical characteristics of Site 3B.

Site 3B Wonderfontein Spruit below the Khutsong sewage treatment plant	
	
Coordinates	S 26°21'15.1" E 27°18'05.8"
Altitude	1 465 m
Site description and land use	Site 3B is located approximately 9 km downstream from Site 2B. This site is also located downstream from the Abe Bailey Nature Reserve where the river forms a wetland. Land uses at this site mainly include informal agricultural activities, informal settlements, as well as the Khutsong sewage treatment plant, which is situated 600 m upstream of this site.
Habitat and biotope description	Middle water zone, stones in streambed, marginal, aquatic and limited overhanging riparian vegetation, algae present, stones in current, stones out of current, run, riffle and pool biotopes.
Primary lithology	Alluvium, gravel, diamondiferous in places and dolomite, chert and remnants of chert breccia of the Rooihoogte Formation (Keyser, 1986).
Dominant vegetation	<i>Azolla pinnata</i> , <i>Berula erecta</i> , <i>Spirodela</i> sp., <i>Typha capensis</i> , <i>Rumex conglomeratus</i> , <i>Ludwigia stolonifera</i> , <i>Ludwigia natans</i> , <i>Potamogeton pusillus</i> , <i>Lemna gibba</i> , <i>Persicaria decipiens</i> , <i>Veronica annagallis aquatic</i> and <i>Schoenoplectus brachyceras</i> .

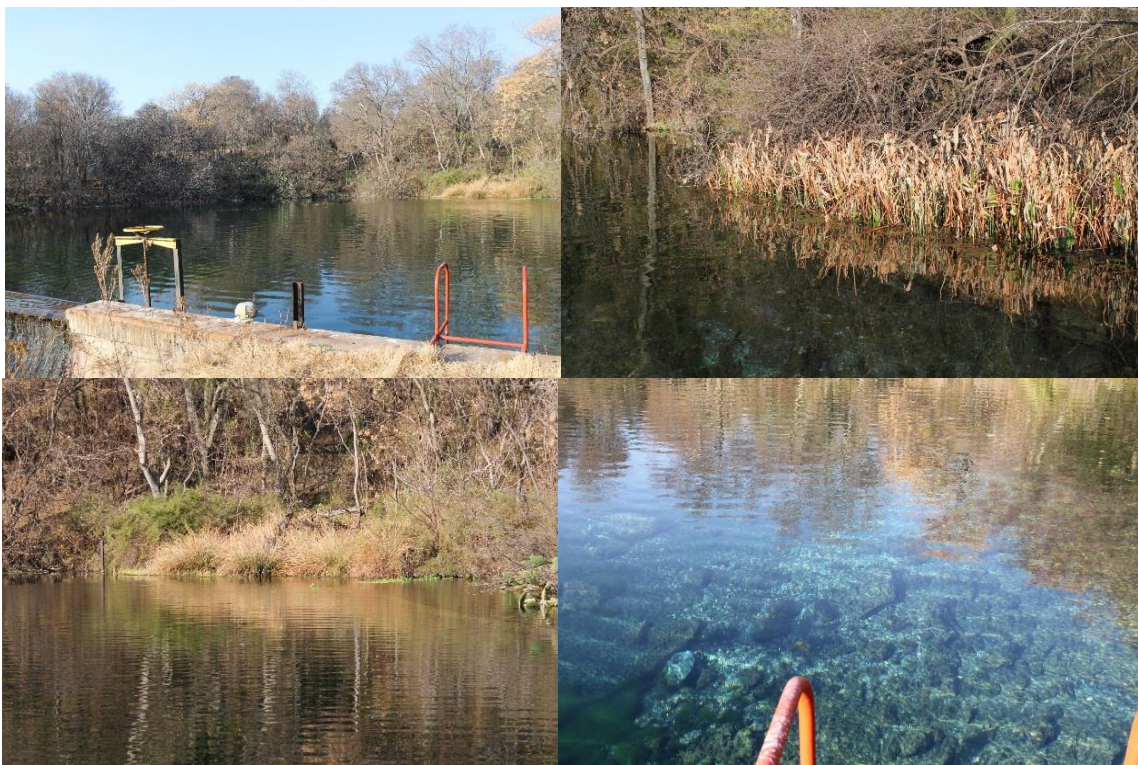
Table 2.9: Physical characteristics of Site 4B.

Site 4B Wonderfontein Spruit above the Turffontein Spring	
	
Coordinates	S 26°23'46.2" E 27°11'28.3"
Altitude	1 427 m
Site description and land use	This site is also situated in the Wonderfontein Spruit on the Turffontein farm, which is located between Carletonville and Potchefstroom. Land uses include mainly large-scale cattle farming, where these waters serve as drinking water for cattle.
Habitat and biotope description	Middle water zone, marginal and aquatic vegetation, mostly a muddy substratum and a pool biotope.
Primary lithology	Dolomite, chert and remnants of chert breccia of the Rooihooft Formation (Keyser, 1986).
Dominant vegetation	<i>Azolla pinnata</i> , <i>Spirodela</i> sp., <i>Potamogeton pusillus</i> , <i>Veronica annagallis</i> aquatic, <i>Lemna gibba</i> , <i>Nasturtium officinale</i> , <i>Phragmites australis</i> and <i>Rumex conglomeratus</i> .

Table 2.10: Physical characteristics of Site 5B.

Site 5B Turffontein Spring	
	
Coordinates	S 26°24'31.3" E 27°10'39.6"
Altitude	1 426 m
Site description and land use	Site 5B, the Turffontein Spring, is located approximately 2 km downstream of Site 4B and is also situated on the Turffontein farm. Land uses include mainly large-scale cattle farming, where these waters serve as drinking water for the cattle.
Habitat and biotope description	Headwater zone, stones in streambed, abundant marginal, aquatic and limited overhanging riparian vegetation, sandy substratum, stones out of current and a pool biotope.
Primary lithology	Gravel, diamondiferous in places and dolomite, chert and remnants of chert breccia of the Rooihogte Formation (Keyser, 1986).
Dominant vegetation	<i>Azolla pinnata</i> , <i>Berula erecta</i> , <i>Phragmites mauritianus</i> , <i>Spirodela</i> sp., <i>Typha capensis</i> , <i>Veronica annagallis aquatica</i> , <i>Rumex conglomeratus</i> , <i>Nasturtium officinale</i> , <i>Hydrocotyle verticillata</i> , <i>Juncus lomatophyllus</i> , <i>Persicaria decipiens</i> , <i>Riccia fluitans</i> and <i>Schoenoplectus corymbosus</i> .

Table 2.11: Physical characteristics of Site 1C.

Site 1C Gerhard Minnebron	
	
Coordinates	S 26°28'44.8" E 27°09'03.4"
Altitude	1 415 m
Site description and land use	The Gerhard Minnebron is one of the largest water sources in the Mooi River catchment area and is situated approximately 12 km from Site 5B. At this location, land uses mainly include limited agricultural activities.
Habitat and biotope description	Headwater zone, stones in streambed, abundant marginal, aquatic and overhanging riparian vegetation, mostly a sandy and muddy substratum, stones out of current and pool and run biotopes.
Primary lithology	Gravel, diamondiferous in places and dolomite, chert and remnants of chert breccia of the Rooihoogte Formation (Keyser, 1986).
Dominant vegetation	<i>Arundo donax</i> , <i>Berula erecta</i> , <i>Typha capensis</i> , <i>Veronica annagallis aquatica</i> , <i>Ludwigia natans</i> , <i>Pontaderia cordata</i> , <i>Juncus lomatophyllus</i> , <i>Nasturtium officinale</i> and <i>Phragmites australis</i>

Chapter 3: Analysis of metals in water and sediment, as well as selected sediment characteristics in the Mooi River and Wonderfontein Spruit.

3.1 Introduction

Metals present in aquatic systems are usually naturally occurring elements, which are widespread throughout the environment (Etesin & Benson, 2007; Tchounwou *et al.*, 2012; Vuković *et al.*, 2011). However, in recent years, there has been a growing concern over the increase in metal contamination in freshwater (Etesin & Benson, 2007; Tam & Wong, 2000; Tchounwou *et al.*, 2012). As a result of their toxicity, persistence and bioaccumulation potential, metals are considered as some of the more severe pollutants in the aquatic environment (Tam & Wong, 2000).

Metals in the aquatic environment may originate either from human mediated activities, which include industrial and domestic effluent, agricultural runoff, as well as mining activities, or natural sources, such as geological weathering, atmospheric deposition, leaching, sediment re-suspension and soil erosion (Etesin & Benson, 2007; Lau *et al.*, 1998; Newman & Watling, 2007; Shine *et al.*, 1995; Smolders *et al.*, 2003; Tchounwou *et al.*, 2012; Wolmarans *et al.*, 2016). Metals introduced into the aquatic environment readily adsorb onto suspended sediments, organic material, hydroxides, sulphides and clay minerals, which ultimately become integrated into bottom sediments (Binning & Baird, 2001; Brown *et al.*, 1999; Inengite *et al.*, 2010; Lin & Chen, 1998; Madkour *et al.*, 2011; Newman & Watling, 2007; Vuković *et al.*, 2011). As a result of this phenomenon, concentrations of metals in sediments usually surpass those of the overlying water column by several orders of magnitude (Lin & Chen, 1998; Newman & Watling, 2007). The sorption of metals from the overlying water column to the bottom sediments are dependent on numerous factors, which include, amongst others, the pH, redox potential, as well as the available surface area (Binning & Baird, 2001; Davies *et al.*, 1991; Inengite *et al.*, 2010). It is further evident from the literature that grain size is another important factor in controlling metal concentrations in sediments (Huang & Lin, 2003; Yao *et al.*, 2015), as fine-grained sediments have a tendency to accumulate metals due to their sorptive potential (Eggleton & Thomas, 2004) and larger surface area to volume ratio (Eggleton & Thomas, 2004; Parizanganeh, 2008; Smith, 1999; Yao *et al.*, 2015).

The Mooi River and Wonderfontein Spruit are subjected to a substantial number of human activities (DWA, 2009; DWS, 2014; Tlokwe City Council, 2014), which include agricultural, industrial and mining activities (see Chapter 2). These may result in, amongst others, the influx of metals into surface waters, which in turn may be adsorbed

onto the sediments (Lau *et al.*, 1998; Smolders *et al.*, 2003; Tchounwou *et al.*, 2012). Although numerous metals are considered as essential nutrients, they can wield toxic effects at concentrations often found in environments polluted by anthropogenic activities (Etesin & Benson, 2007). In addition to this, metals are well known to biopersist and can become, in some cases, bioaccumulated in the food chain (Etesin & Benson, 2007; Radojevic & Baskin, 1999).

The following hypothesis will be tested in this chapter:

1. Mining effluent from the Wonderfontein Spruit is responsible for an increase in metal contamination of the water and sediment of the Mooi River.

The purpose of this chapter is to determine the sediment characteristics, metal concentrations in water and sediment samples, pH, electrical conductivity (EC), turbidity and flow-rate in the Mooi River, Wonderfontein Spruit and Gerhard Minnebron, and the influence that mining effluent from the Wonderfontein Spruit catchment has on the water and sediment quality of the Mooi River catchment.

3.2 Material and methods

3.2.1 Fieldwork

Water and sediment samples were collected at each of the selected sites during three low-flow seasons (July 2014 (Survey 1), September 2014 (Survey 2) and May 2015 (Survey 3)). Water samples were collected separately in 250 ml polyethylene bottles, which were washed with detergent, rinsed with distilled water, followed by 1 % nitric acid solution and then rinsed again with double distilled water. Sediment samples were collected with the same container as described above, from the upper 7 cm of the substratum (Wolmarans *et al.*, 2016). Both the water and sediment samples were transported to the laboratory and stored in a refrigerator at 4 °C, until used for analyses. The coordinates and altitude were determined by means of a Garmin Nuvi 500 GPS, for each sampling site. Electrical conductivity (EC), pH and temperature were measured *in situ* at all sites during each survey using a portable digital conductivity meter (DIST 3, HI 98303, Hanna Instruments), a portable digital pH probe (HI 98128, Hanna Instruments) and a portable digital thermometer (Checktemp, Hanna Instruments). The flow-rate, measured by a Global Water Flow probe model FP111, as well as the turbidity, measured with a Groundtruth Clarity Tube, was also recorded at each of the sites.

3.2.2 Laboratory methods

3.2.2.1 Sediment digestion

Laboratory methods are based on the techniques described in the study of Wolmarans *et al.* (2016). In the laboratory, a representative sample of the sediment, collected with the 250 ml polyethylene bottles in the field, was transferred to a weigh boat, which was provided with the relevant information of the site. Thereafter the samples were air dried at 70 °C for 36 hours. After the samples were dried, 0.5 g of each of the samples were weighed off with a Zeiss Sartorius digital scale and together with 10 ml of aqua regia ($\text{HCl}:\text{HNO}_3 = 3:1$), added in a Microwave digester Teflon tube. The samples were then digested at 200 °C and 20 bar pressure in an Ethos Easy Microwave Digester Maxi 44 for 20 minutes and then cooled for 15 minutes. For each batch of samples, quality control and quality assurance protocols were applied. Certified Reference Materials (CRM) (Resource Technology Corporation and CN Schmidt BV: Trace Elements on Fresh Water Sediments (CNS392-050)) were used and standard calibration runs were performed. The percentage recoveries for the standards and certified reference materials were within an acceptable range (< 10 % deviation). Calculations are depicted in Table 3.1. After the digestion, 300 µl of the supernatant was drawn up, using a 1000 µl pipette and transferred to a 15 ml test tube. The samples were diluted with 9.7 ml MiliQ Water. The tubes were marked with relevant site information and sent for Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analyses using standard recognised methods, to determine the metal concentrations in the sediment.

Table 3.1: Metal concentrations (mg/kg) recovered from Certified Reference Material (CRM).

	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

3.2.2.2 Particle size determination, X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM) analyses

→ Particle size determination

A sub-sample of 30 g (or the remainder of sediment) from each site was weighed with the digital scale mentioned above and sieved for 20 minutes each by making use of a King Test VB 200300 dry sieving system, to determine the particle sizes of the sediment sample (Wolmarans *et al.*, 2016). The mesh sizes of the sieves that were used, are as follows: 4000 μm , 2000 μm , 500 μm , 212 μm , and 53 μm . After the samples were sieved, the content of each sieve was weighed using the digital scale and recorded. The sediment with a particle size smaller than 53 μm , were transferred to a 60 ml plastic container and provided with a label that contained the relevant information. After this, the samples were sent for X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM) analyses for determining the mineral composition and the elements present, respectively.

→ X-Ray Diffraction (XRD)

The clay samples (fraction < 53 μm) were used to classify the crystalline materials present in the sample, in order to identify the minerals. Preparation of the samples was done by making use of a back loading technique (Wolmarans *et al.*, 2016). After preparations, the samples were scanned using copper (Cu) X-ray tube-generated X-rays, which produced an individual diffractogram for each sample. Each diffractogram represents the different phases and phase concentrations of all the crystalline materials present in the sample. Identification of phases was done by using X'Pert Highscore Plus and International Centre for Diffraction Data (ICDD). ICDD database PDF 4+ (2014) and the ICSD-PANanalytical program were used to identify the minerals present in the samples (Wolmarans *et al.*, 2016). A Rietveld quantification was prepared with the phases, to determine the weight percentage of each mineral in the sample.

→ Scanning Electron Microscopy (SEM)

An FEI Quanta 250 FEG ESEM microscope equipped with an integrated Oxford Inca X-MAX 20 EDS-system with incorporated internal standards were used to determine the element composition in the clay samples (fraction < 53 μm) (Wolmarans *et al.*, 2016). Samples were prepared by adding the clay fraction to microscope stubs, which were

provided with double sided tape (Wolmarans *et al.*, 2016). The abundance of each element was expressed as a percentage of the total elements identified per site.

3.2.2.3 Water samples

After the water samples were transported to the laboratory, 9.85 ml of each sample was drawn up, using a 60 ml syringe and filtered through a 0.22 μm Millipore® filter paper. The filtered samples were transferred to ICP-MS test tubes. The samples were labelled with the relevant information and acidified using 0.15 ml 65% nitric acid. After this, the samples were sent for ICP-MS analysis, to determine the metal concentrations.

3.2.3 Statistics

3.2.3.1 Univariate and multivariate statistical analyses

Statistical analyses were conducted using IBM SPSS Statistics 23. Statistical significance of the spatial and temporal variation of the selected metals were determined at $p < 0.05$. Normality and homogeneity of variance were tested using Levene's tests. When the one-way analysis of variance (ANOVA) revealed significant differences, *post hoc* multiple comparisons between sites and surveys, were made using the appropriate Tukey (parametric) or Dunnett-T3 (non-parametric) test to determine which values differed significantly. A Principal Component Analysis (PCA), a multivariate analysis, was performed using Canoco (version 5) to determine the spatial and temporal distributions based on the metal concentrations and physico-chemical parameters.

3.3 Results and discussion

Although 34 and 31 metals were above instrument detection limits in water and sediment samples at each of the sites, respectively (Tables A1 to A6, Appendix A), only those metals that may be potentially toxic to macroinvertebrates, including aluminium (Al), titanium (Ti), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn) arsenic (As), cadmium (Cd) and lead (Pb) (DWAF, 1996; Jonh & Leventhal, 1995; Tchounwou *et al.*, 1995), will be reported on and discussed. Furthermore, attention will also be given to the minerals associated with these sites, their metal and element composition, as well as the selected physical characteristics.

3.3.1 Mineral composition

The mineral composition of each site (expressed as a percentage of all the minerals present at a specific site) is displayed in Table 3.2. Quartz was the most abundant mineral found at all of the sampling sites, contributing to the silicone (Si) and oxygen (O) element composition (Table 3.2). Calcite was the second most abundant mineral and found at Sites 2A, 3A, 5A, 1B, 2B and 4B and contributes to the calcium (Ca) concentration, an essential trace metal to macroinvertebrates, in the sediment. It is further evident that kaolinite, vermiculite and dolomite only occurred at Sites 2B, 3B and 4B; Sites 5A and 1B; and Sites 1A and 1C, respectively. Minerals which were only present at one of the 11 preselected sites, included goethite, muscovite and pyrite.

Table 3.2: The mean mineral composition at each site expressed as a percentage of all the minerals present at a specific site during the three surveys.

Minerals	Sites and percentage composition										
	1A	2A	3A	4A	5A	1B	2B	3B	4B	5B	1C
Quartz	98.3	29.4	96.9	100	93.8	87.3	60.4	89	75.5	100	94.4
Goethite	0	0	0	0	0	0	0	1.9	0	0	0
Muscovite	0	0	0	0	0	0	19.9	0	0	0	0
Kaolinite	0	0	0	0	0	0	15.8	9.1	17.5	0	0
Calcite	0	70.6	3.1	0	6.1	12.5	3.8	0	6.2	0	0
Vermiculite	0	0	0	0	0.1	0.2	0	0	0	0	0
Montmorillonite	0	0	0	0	0	0	0.1	0	0	0	0
Dolomite	1.7	0	0	0	0	0	0	0	0	0	5.6
Pyrite	0	0	0	0	0	0	0	0	0.8	0	0
Total %	100	100	100	100	100	100	100	100	100	100	100

3.3.2 Element composition

Regarding the elements identified in the sediment samples, it is evident from the results that these elements are present in some of the identified minerals as shown in Tables 3.2 and 3.3. Aluminium may be present in muscovite, kaolinite, vermiculite and montmorillonite; Si and O mainly in quartz, muscovite, kaolinite, vermiculite and montmorillonite; Ca in calcite and dolomite and Fe in goethite and pyrite (Tables 3.2 and 3.3). The presence of carbon in the sediment samples may possibly be ascribed to the organic content in the samples.

Table 3.3: The mean element composition representing the identified minerals as measured using Scanning Electron Microscopy (SEM). The abundance of each element is expressed as a percentage of the abundance of all the elements in total for each site.

	Sites										
	1A	2A	3A	4A	5A	1B	2B	3B	4B	5B	1C
C	14.9	15.9	15.7	16.2	19.4	13.7	12.2	9.4	18.1	17.9	10.5
Al	1.5	2.1	2.6	2.5	1.7	4.3	6.5	5.5	3.5	0.8	4.3
Si	17.7	10.1	15.5	15.4	10.9	15.2	15.7	19.7	11.4	15.2	21.6
Ca	0.0	8.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ti	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.3	0.3	0.0	0.4
Fe	3.6	3.2	3.2	2.2	1.6	6.9	7.1	9.8	1.8	0.3	5.1
O	62.2	60.2	62.8	63.6	66.2	59.7	58.3	55.3	64.9	65.8	58.1
Total %	100	100	100	100	100	100	100	100	100	100	100

3.3.3 Metal concentrations in water

The large variation in metal concentration found during the different surveys, as well as sites is evident from Table 3.4. This phenomenon is especially true for Al, Fe, Ni, Cu, Zn and Pb identified for Site 1B, located in the Wonderfontein Spruit. It is, however, unknown whether these results can be ascribed to mining activities. In contrast to this, less variation was observable in the case of Ti, Cr, Mn, Co, As, and Cd. Although the variation in the Al concentrations was evident from Site 1B to Site 5B, these concentrations were, except for Site 5B, the lowest during Survey 2. The significant increase in the Al concentration at Site 5B (the Turffontein Spring) during the third survey may possibly be ascribed to the weathering activities present at this site and the contribution of anthropogenic activities upstream from this site. Regarding the concentrations of Al in the Mooi River (Sites 1A to 5A), it is evident that these concentrations, although it exceeded the Target Water Quality Range (TWQR) values for aquatic ecosystems (DWAf, 1996) at all the sites, did not differ greatly from the concentrations in the Wonderfontein Spruit (Sites 1B to 5B), as well as the Gerhard

Minnebron (Site 1C). The decrease in the Al concentration during the second survey at the majority of the sites cannot be explained, as all the surveys were conducted during the low-flow season. Although an elevated Al concentration was found at Site 5B (151.5 µg/L) during Survey 3, the concentration of this metal was significantly lower at Site 5A (34.7 µg/L), most probably due to the confluence of the Wonderfontein Spruit and the Gerhard Minnebron with the Mooi River. From this it can be deduced that the mining effluent originating at Site 1B did not have an adverse effect on the Al concentrations, as the origin of this metal in water may be due to the weathering of various minerals including muscovite, kaolinite, vermiculite and montmorillonite (DWAF, 1996; Klein & Dutrow, 2007), all identified during this study. Aluminium is further one of the most abundant elements present in the environment (DWAF, 1996).

Regarding Ti, limited variation occurred between all the sites surveyed in the Wonderfontein Spruit, the Mooi River, as well as the Gerhard Minnebron, with a minimum of 0.6 µg/L and a maximum of 5.4 µg/L (Table 3.4), indicating that this metal probably did not originate from mining effluent. Titanium is a relatively abundant metal in the environment and dissolves in stream water through the weathering of ferromagnesian minerals and rocks including goethite, vermiculite, montmorillonite, pyrite and dolomite (Table 3.2) (Bhuvana *et al.*, 2014; Klein & Dutrow, 2007; Venugopal *et al.*, 2009), which was probably the source of Ti during this study. Furthermore, it seems that the mining effluent was, as in the case of Ti, not the source of Cr or Mn in this catchment. Table 3.3 shows that the concentrations of Cr, as well as Mn did not vary considerably between the two rivers, however some spatial significant differences were found at both metals. Chromium is considered as a relatively scarce metal and quantities thereof in natural waters are generally low (DWAF, 1996). Minerals containing Cr do occur, but are relatively scarce and elevated concentrations of Cr in the aquatic ecosystem are usually as a result of industrial and agricultural activities (Eisler, 1986; DWAF, 1996; Ling *et al.*, 2012; Mattuck & Nikolaidis, 1996; Oliveira, 2012; Tchounwou *et al.*, 1995). No TWQR values (Table 3.5) were exceeded by either of these metals at any of the sites or surveys (Table 3.4). The concentrations of Mn may possibly be ascribed to the natural weathering of minerals, as well as agricultural activities in the surrounding area, as Mn is used in various fertilizers (Nádaská *et al.*, 2012).

Regarding Fe, it is evident that the concentrations of this metal were significantly elevated at the sites located in the Mooi River, before the confluence of the Wonderfontein Spruit and Gerhard Minnebron, indicating that this metal did not originate

from the mining effluent present at Site 1B. The substantial variation in the concentrations of Fe may be attributed to the fact that this metal is the fourth most abundant metalloid in the environment and is present in various quantities depending on the weathering of various minerals identified during this study, which include, amongst others, goethite and pyrite (Table 3.2) (DWAF, 1996; Klein & Dutrow, 2007; Yao *et al.*, 2015). The metals identified in these minerals are depicted in Table 3.2.

It is further evident that the concentrations of Co and Ni measured in the Mooi River and Gerhard Minnebron were generally lower, when compared to the concentration found in the Wonderfontein Spruit (Table 3.4). The elevated concentrations of both these metals at the majority of sites located in the Wonderfontein Spruit, may be ascribed to anthropogenic activities, such as mining effluent and the use of fertilizers in agriculture (Cemple & Nickel, 2006; Kim *et al.*, 2004; Smith & Carson, 1981). The elevated concentrations of Co probably give rise to a limited increase in the concentrations found in the Mooi River, after the confluence of the Wonderfontein Spruit (Site 5A). Whether this increase influences the composition of metals further downstream in the Mooi River, is not known. Nickel, on the other hand, did not show an increase in concentrations at Site 5A (Table 3.4).

Regarding Cu, considerable higher concentrations were found during the third survey at most of the sites, a phenomenon also true for the concentrations of Zn. In contrast to Cu, where only Sites 2A, 2B and 5B showed significant differences between Surveys 1 and 2, and Survey 3, the concentrations of Zn were significantly higher in the third survey at the majority of sites. The increase in concentrations of both Cu and Zn during the last survey, may possibly be ascribed to the severe drought, which may have led to a concentration effect of metals in the water, an effect also demonstrated in Figure 3.1. The origin of both Cu and Zn may possibly be attributed to the weathering and erosion of rocks and ores, as well as the agricultural activities in the catchment area, which include the use of fungicides, pesticides and fertilizers (DWAF, 1996). According to DWAF (1996), anthropogenic sources account for 33 to 60 % of the total concentration of Cu found in natural waters. Although the concentrations of Cu and Zn exceeded the TWQR values at all the sampling sites during all three surveys, the toxicity of both these metals may possibly have been reduced due to high concentrations of Ca and magnesium (Mg) (Tables A1 to A3, Appendix A), resulting in alkaline water, thus rendering these metals non-toxic to macroinvertebrates (DWAF, 1996).

The concentrations of As, a non-metallic element, were considerably higher at most of the sites in the Wonderfontein Spruit than in the Mooi River and Gerhard Minnebron and generally exceeded the TWQR (Tables 3.4 and 3.5). From this it can be concluded that the mining effluent originating at Site 1B may have contributed towards these elevated concentrations in the Wonderfontein Spruit. The lower concentrations measured at the Mooi River and Gerhard Minnebron may be ascribed to the weathering of minerals such as As-containing pyrite, as well as agricultural activities (DWAF, 1996). From this it can be deduced that the mining effluent originating at Site 1B did not have an adverse effect on the As concentrations downstream of the origin.

The extremely low Cd concentrations, which ranged between 0.01 µg/L and 0.1 µg/L, render it unlikely that this metal could influence the chemical integrity of the water in the Mooi River in an adverse way.

In contrast to the limited variation found in the Pb concentrations when the sites are compared, considerable variation was found between different surveys, with a significant increase at the majority of the sites during the third survey, indicating that this phenomenon cannot be ascribed to the mining effluent. The elevated levels of Pb during the third survey, which exceed the TWQR, may rather be ascribed to a concentration effect caused by the drought, natural weathering of sulphide ores and anthropogenic sources including street runoff and waste water discharge (DWAF, 1996; Jain & Ram, 1997).

Table 3.4: Mean and standard deviation of selected metal concentrations ($\mu\text{g/L}$) and selected abiotic factors measured in water samples during this study. Common alphabetical superscripts (a, b) indicate significant difference between surveys at a specific site ($p < 0.05$) (spatial significant differences during the surveys are not indicated in Table 3.4, but are pointed out in the text).

Site	Survey	Al	Ti	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb	Temperature ($^{\circ}\text{C}$)	EC ($\mu\text{S/cm}$)	pH	Turbidity (NTU)	Flow-rate (m/s)
1A	1	23.8 $\pm$ 5.5 ^a	1.5 $\pm$ 0.5	4.2 $\pm$ 1.0	0.9 $\pm$ 0.1	65.8 $\pm$ 10.7 ^a	0.2 $\pm$ 0.1	2.3 $\pm$ 0.1 ^a	2.0 $\pm$ 0.1	50.9 $\pm$ 3.4 ^a	0.04 $\pm$ 0.01 ^a	0.03 $\pm$ 0.01	0.1 $\pm$ 0.01 ^{ab}	19.2	457.0	6.8	5.0	0.1
	2	20.9 $\pm$ 2.5 ^b	2.0 $\pm$ 0.1	5.0 $\pm$ 0.1 ^a	3.3 $\pm$ 1.0	889.6 $\pm$ 14.6 ^{ab}	0.3 $\pm$ 0.1	1.8 $\pm$ 0.4	2.4 $\pm$ 0.6	60.0 $\pm$ 4.8 ^b	0.8 $\pm$ 0.04 ^{ab}	0.1 $\pm$ 0.01	0.6 $\pm$ 0.1 ^a	20.3	443.0	7.2	5.0	0.1
	3	36.6 $\pm$ 2.2 ^{ab}	2.6 $\pm$ 0.6	1.9 $\pm$ 0.1 ^a	1.6 $\pm$ 0.3	307.3 $\pm$ 3.1 ^{ab}	0.4 $\pm$ 0.1	1.3 $\pm$ 0.1 ^a	8.7 $\pm$ 1.6	127.2 $\pm$ 7.4 ^{ab}	0.4 $\pm$ 0.04 ^{ab}	0.03 $\pm$ 0.0	20.1 $\pm$ 2.4 ^b	19.5	453.0	6.7	5.0	0.1
2A	1	42.7 $\pm$ 5.7 ^a	1.4 $\pm$ 0.1	0.6 $\pm$ 0.1 ^a	1.1 $\pm$ 0.1	65.5 $\pm$ 6.9 ^a	0.2 $\pm$ 0.1	2.1 $\pm$ 0.1	2.4 $\pm$ 0.6	37.6 $\pm$ 4.5 ^a	0.2 $\pm$ 0.1 ^a	0.03 $\pm$ 0.0 ^a	0.2 $\pm$ 0.04 ^a	12.0	438.0	7.7	5.0	1.2
	2	5.6 $\pm$ 0.1 ^{ab}	1.6 $\pm$ 0.1	1.2 $\pm$ 0.03 ^b	3.2 $\pm$ 0.8	1 015.1 $\pm$ 19.4 ^{ab}	0.4 $\pm$ 0.1	19. $\pm$ 0.1	2.1 $\pm$ 0.04 ^a	30.3 $\pm$ 2.8 ^b	0.9 $\pm$ 0.1 ^{ab}	0.1 $\pm$ 0.01 ^{ab}	0.2 $\pm$ 0.1 ^b	14.8	430.0	7.8	5.0	0.2
	3	49.7 $\pm$ 2.4 ^b	2.6 $\pm$ 0.5	0.03 $\pm$ 0.01 ^{ab}	2.8 $\pm$ 0.7	323.6 $\pm$ 5.0 ^{ab}	0.4 $\pm$ 0.1	2.1 $\pm$ 1.0	14.1 $\pm$ 1.1 ^a	232.4 $\pm$ 4.0 ^{ab}	0.4 $\pm$ 0.03 ^b	0.03 $\pm$ 0.0 ^b	26.5 $\pm$ 1.1 ^{ab}	14.0	422.0	7.1	5.0	0.1
3A*	1	31.5 $\pm$ 4.2	1.6 $\pm$ 0.1	0.8 $\pm$ 0.1	1.5 $\pm$ 0.1	88.7 $\pm$ 7.4 ^a	0.2 $\pm$ 0.04	2.6 $\pm$ 0.1 ^a	2.5 $\pm$ 0.1	43.0 $\pm$ 4.5	0.1 $\pm$ 0.03 ^a	0.03 $\pm$ 0.01	0.2 $\pm$ 0.01	9.6	441.0	7.8	5.0	0.3
	2	22.5 $\pm$ 5.0	1.4 $\pm$ 0.1	1.1 $\pm$ 0.01	1.8 $\pm$ 0.1	197.9 $\pm$ 10.1 ^a	0.4 $\pm$ 0.1	1.6 $\pm$ 0.04 ^a	2.1 $\pm$ 0.04	34.3 $\pm$ 9.9	1.1 $\pm$ 0.04 ^a	0.1 $\pm$ 0.01	0.5 $\pm$ 0.1	17.4	448.0	7.4	6.0	0.1
	3																	
4A	1	27.8 $\pm$ 3.4	1.1 $\pm$ 0.1	0.9 $\pm$ 0.1	1.1 $\pm$ 0.1 ^a	97.7 $\pm$ 6.0 ^{ab}	0.2 $\pm$ 0.1	7.0 $\pm$ 0.1 ^{ab}	2.3 $\pm$ 0.1	39.3 $\pm$ 7.0 ^a	0.1 $\pm$ 0.01 ^a	0.03 $\pm$ 0.0	0.1 $\pm$ 0.0 ^a	10.2	456.0	7.8	5.0	0.8
	2	22.2 $\pm$ 3.7	1.4 $\pm$ 0.1	1.1 $\pm$ 0.04 ^a	2.2 $\pm$ 0.03 ^a	274.9 $\pm$ 7.0 ^a	0.4 $\pm$ 0.03	1.6 $\pm$ 0.1 ^a	2.4 $\pm$ 0.1	52.7 $\pm$ 6.8 ^b	0.9 $\pm$ 0.1 ^{ab}	0.1 $\pm$ 0.03	0.5 $\pm$ 0.04 ^{ab}	19.7	476.0	7.5	6.0	0.5
	3	30.5 $\pm$ 2.1	2.7 $\pm$ 0.6	0.03 $\pm$ 0.0 ^a	2.6 $\pm$ 0.6	253.5 $\pm$ 6.2 ^b	0.4 $\pm$ 0.1	1.7 $\pm$ 0.04 ^b	9.8 $\pm$ 1.2	136.5 $\pm$ 2.2 ^{ab}	0.2 $\pm$ 0.1 ^b	0.03 $\pm$ 0.01	23.5 $\pm$ 2.0 ^{ab}	13.3	444.0	7.7	5.0	0.3
5A	1	14.3 $\pm$ 2.0 ^a	1.3 $\pm$ 0.1	2.4 $\pm$ 0.1 ^a	0.7 $\pm$ 0.1 ^a	96.8 $\pm$ 8.1 ^a	2.9 $\pm$ 0.2	4.4 $\pm$ 0.1 ^a	2.4 $\pm$ 0.1	36.9 $\pm$ 5.5 ^a	0.1 $\pm$ 0.01	0.03 $\pm$ 0.0	0.04 $\pm$ 0.0 ^a	11.3	697.0	7.4	5.0	1.1
	2	12.8 $\pm$ 2.5 ^b	2.0 $\pm$ 0.1	3.3 $\pm$ 0.3 ^b	7.0 $\pm$ 0.1 ^{ab}	2 723.3 $\pm$ 10.4 ^{ab}	3.5 $\pm$ 0.2	3.1 $\pm$ 0.1 ^a	2.8 $\pm$ 0.2	40.8 $\pm$ 4.5 ^b	0.9 $\pm$ 0.1	0.1 $\pm$ 0.02	0.7 $\pm$ 0.1 ^{ab}	18.0	704.0	7.3	5.0	0.8
	3	34.7 $\pm$ 2.8 ^{ab}	3.1 $\pm$ 0.9	0.03 $\pm$ 0.0 ^{ab}	1.8 $\pm$ 0.04 ^{ab}	388.9 $\pm$ 4.3 ^{ab}	3.7 $\pm$ 0.5	3.9 $\pm$ 0.4	11.5 $\pm$ 2.0	214.7 $\pm$ 8.0 ^{ab}	0.4 $\pm$ 0.1	0.03 $\pm$ 0.0	18.1 $\pm$ 2.2 ^{ab}	16.4	704.0	7.4	5.0	0.5

Table 3.4 continued

1B	1	61.7 ± 7.5 ^a	2.7 ± 0.1	1.5 ± 0.5	1.4 ± 0.1 ^a	141.0 ± 5.8 ^a	13.4 ± 0.8	57.9 ± 4.5	5.2 ± 0.1 ^a	63.9 ± 4.0 ^a	12.6 ± 0.7	0.03 ± 0.0 ^a	0.2 ± 0.02	13.9	1 001.0	7.5	7.0	0.1
	2	36.3 ± 3.8 ^{ab}	2.8 ± 0.1	2.1 ± 0.1 ^a	2.9 ± 0.1 ^a	401.3 ± 5.6 ^{ab}	12.5 ± 0.8	32.9 ± 4.6	4.2 ± 0.1 ^a	42.3 ± 5.8 ^{ab}	18.9 ± 2.4	0.1 ± 0.0 ^{ab}	0.5 ± 0.1	17.7	1 027.0	7.2	8.0	0.5
	3	64.2 ± 3.6 ^b	3.2 ± 1.0	0.9 ± 0.1 ^a	2.5 ± 0.7	441.7 ± 4.4 ^{ab}	15.2 ± 2.8	61.2 ± 2.3	14.7 ± 1.3	252.0 ± 5.5 ^{ab}	21.3 ± 3.2	0.01 ± 0.0 ^b	22.9 ± 3.3	19.1	1 038.0	7.9	10.0	0.4
2B	1	46.4 ± 5.0 ^a	2.3 ± 0.1	1.6 ± 0.1 ^a	1.8 ± 0.1 ^a	152.3 ± 4.8 ^a	10.4 ± 0.8	52.5 ± 5.2	4.5 ± 0.6 ^a	51.9 ± 6.0 ^a	13.2 ± 0.3	0.03 ± 0.0 ^a	0.2 ± 0.02 ^a	12.5	1 055.0	7.1	15.0	0.2
	2	11.6 ± 1.9 ^{ab}	1.8 ± 0.2	18. ± 0.1 ^b	2.5 ± 0.1 ^a	359.1 ± 6.6 ^{ab}	12.6 ± 0.8	29.9 ± 4.0 ^a	4.8 ± 0.5	42.3 ± 2.2 ^b	23.3 ± 3.4	0.1 ± 0.03	0.4 ± 0.1 ^b	17.3	1 027.0	7.5	12.0	0.7
	3	66.4 ± 3.1 ^{ab}	3.0 ± 0.6	0.03 ± 0.0 ^{ab}	2.2 ± 1.0	413.5 ± 8.4 ^{ab}	15.8 ± 1.6	65.0 ± 3.4 ^a	12.3 ± 1.2 ^a	162.3 ± 5.0 ^{ab}	23.5 ± 1.7	0.01 ± 0.0 ^a	19.6 ± 1.6 ^{ab}	18.5	1 046.0	7.7	7.0	0.9
3B	1	28.9 ± 3.8 ^a	1.2 ± 0.1 ^a	1.3 ± 0.1 ^a	1.5 ± 0.03 ^a	107.7 ± 4.4 ^{ab}	7.8 ± 0.6	17.8 ± 1.0	4.5 ± 0.7	52.1 ± 2.8	14.0 ± 1.6	0.03 ± 0.0	0.1 ± 0.03	6.7	974.0	7.0	5.0	0.1
	2	5.8 ± 0.3 ^{ab}	1.9 ± 0.1 ^a	2.4 ± 0.1 ^{ab}	3.9 ± 0.1 ^a	355.3 ± 8.5 ^a	12.3 ± 1.2	24.8 ± 2.8	3.5 ± 1.1	33.3 ± 3.5 ^a	18.4 ± 1.6	0.1 ± 0.03	0.1 ± 0.02	14.7	1 033.0	7.2	6.0	0.2
	3	50.8 ± 2.7 ^{ab}	3.7 ± 0.6	0.03 ± 0.01 ^{ab}	2.3 ± 0.9	405.3 ± 18.3 ^b	7.7 ± 2.0	12.8 ± 2.5	12.8 ± 2.8	178.7 ± 7.1 ^a	16.5 ± 1.7	0.03 ± 0.01	22.8 ± 4.9	14.7	975.0	7.2	5.0	0.2
4B	1	32.1 ± 4.8 ^a	0.6 ± 0.1	1.2 ± 0.02 ^a	0.1 ± 0.1	93.8 ± 1.8 ^{ab}	6.6 ± 0.3	28.8 ± 3.7	4.4 ± 1.2	50.2 ± 4.0	3.5 ± 0.6	0.03 ± 0.0 ^a	0.1 ± 0.01	15.6	996.0	8.0	27.0	0.1
	2	17.9 ± 1.8 ^a	0.7 ± 0.04	1.6 ± 0.1	1.3 ± 0.1	300.3 ± 4.0 ^a	5.7 ± 1.1	36.2 ± 3.1	3.6 ± 1.0	41.2 ± 4.1	7.2 ± 1.0	0.1 ± 0.01 ^a	0.2 ± 0.04	22.9	1 017.0	8.4	27.0	0.1
	3	17.9 ± 2.9 ^a	0.7 ± 0.04	1.7 ± 0.1 ^a	1.3 ± 0.04	303.0 ± 18.1 ^b	5.6 ± 0.9	36.9 ± 3.1	3.6 ± 0.4	40.8 ± 5.0	7.3 ± 0.8	0.1 ± 0.02	21.2 ± 0.04	18.9	1 096.0	8.0	27.0	0.1
5B	1	19.9 ± 1.4 ^a	1.6 ± 0.1	2.5 ± 0.3	0.8 ± 0.3	82.3 ± 7.2 ^{ab}	1.2 ± 0.03 ^a	2.7 ± 0.5	2.2 ± 0.3 ^a	47.9 ± 5.9 ^a	0.02 ± 0.0 ^a	0.03 ± 0.0	0.1 ± 0.01 ^a	19.8	798.0	7.2	5.0	0.1
	2	47.7 ± 3.4 ^{ab}	2.1 ± 0.03	4.5 ± 1.0	1.6 ± 0.1	319.1 ± 2.0 ^a	1.9 ± 0.1 ^a	1.4 ± 0.1	2.5 ± 0.5 ^b	46.9 ± 2.7 ^b	0.7 ± 0.03 ^{ab}	0.1 ± 0.02	1.0 ± 0.0 ^{ab}	21.5	764.0	7.1	5.0	0.1
	3	151.5 ± 7.3 ^{ab}	5.4 ± 1.1	2.4 ± 1.2	2.4 ± 1.2	578.4 ± 4.5 ^b	2.1 ± 1.0	2.0 ± 0.1	10.5 ± 0.8 ^{ab}	173.1 ± 3.7 ^{ab}	0.3 ± 0.1 ^b	0.03 ± 0.0	21.7 ± 1.5 ^{ab}	19.7	786.0	7.5	5.0	0.1
1C	1	29.8 ± 4.4 ^a	1.6 ± 0.4	4.7 ± 0.3	1.1 ± 0.04 ^a	87.0 ± 5.1 ^a	6.3 ± 1.1	3.5 ± 0.5	2.1 ± 0.6	38.7 ± 4.0 ^a	0.03 ± 0.0 ^a	0.03 ± 0.01	0.1 ± 0.01 ^a	19.7	785.0	7.1	5.0	0.1
	2	71.5 ± 1.8 ^{ab}	1.9 ± 0.1 ^a	6.2 ± 1.0	5.5 ± 0.6	1 305.7 ± 19.3 ^{ab}	7.5 ± 0.9	18. ± 0.04 ^a	2.7 ± 0.5	51.6 ± 4.7 ^b	0.7 ± 0.04 ^{ab}	0.1 ± 0.03	1.8 ± 0.2 ^a	21.9	766.0	7.2	5.0	0.1
	3	50.8 ± 3.3 ^{ab}	0.9 ± 0.04 ^a	2.2 ± 1.0	1.4 ± 0.1 ^a	151.8 ± 4.2 ^{ab}	7.3 ± 1.0	1.1 ± 0.1 ^a	7.3 ± 1.1	112.5 ± 3.0 ^{ab}	0.1 ± 0.01 ^{ab}	0.03 ± 0.0	17.6 ± 2.7	19.8	796.0	7.4	5.0	0.1

*Site 3A dry.

Table 3.5: Target Water Quality Range (TWQR) values, Chronic Effect Values (CEV) and Acute Effect Values (AEV) of the metals toxic to aquatic ecosystems (DWAF, 1996).

	TWQR	CEV	AEV
Al	10.0	20.0	150.0
Ti	-	-	-
Cr	7.0	14.0	200.0
Mn	180.0	370.0	1 300.0
Fe	-	-	-
Co	-	-	-
Ni	-	-	-
Cu	1.4	2.8	12.0
Zn	2.0	3.6	36.0
As	10.0	20.0	130.0
Cd	0.4	0.8	13.0
Pb	1.2	2.4	16.0

*- No values available in DWAF (1996).

*Concentrations in µg/L.

3.3.4 Spatial and temporal metal concentrations in water

The PCA bi-plot (Figure. 3.1) illustrates possible interactions between metal concentrations measured, the turbidity, pH, flow-rate, temperature and EC, during Surveys 1, 2 and 3. Figure 3.1 shows associations between metals and the third survey (upper left and right quadrants); turbidity, EC and sites in the Wonderfontein Spruit (bottom right quadrant); and between the different sites in the Mooi River (bottom left quadrant). A clear spatial variation is evident on the first axis (related to EC, Co, As and Ni) between sites located in the Mooi River (Sites 1A to 5A) and the Gerhard Minnebron (Site 1C), and those located in the Wonderfontein Spruit (1B to 4B), with the exception of Site 5B, the Turffontein Spring. It is further evident that a clear temporal variation between the different surveys occurred (second axis), i.e. Survey 3 displaying different water quality patterns to the other two surveys. The association found between metals and Survey 3 confirms the conclusion that the severe drought may have led to an increase in the majority of the metal concentrations. These are similar to findings by Sakalauskiene & Ignatavicius (2003) and Vliet & Zwolsman (2008), who also found increases in metal concentrations during periods of drought in the Lithuanian River system and the Meuse River, respectively. The associations found between EC, turbidity and sites in the Wonderfontein Spruit, is in accordance with the results showing a marked increase in turbidity, as well as EC at the sites located in the Wonderfontein Spruit. Regarding the abiotic factors, temperature, flow-rate and pH appeared to have

the least effect on the metals concentrations, whereas the EC and turbidity showed to have a greater influence during this study.

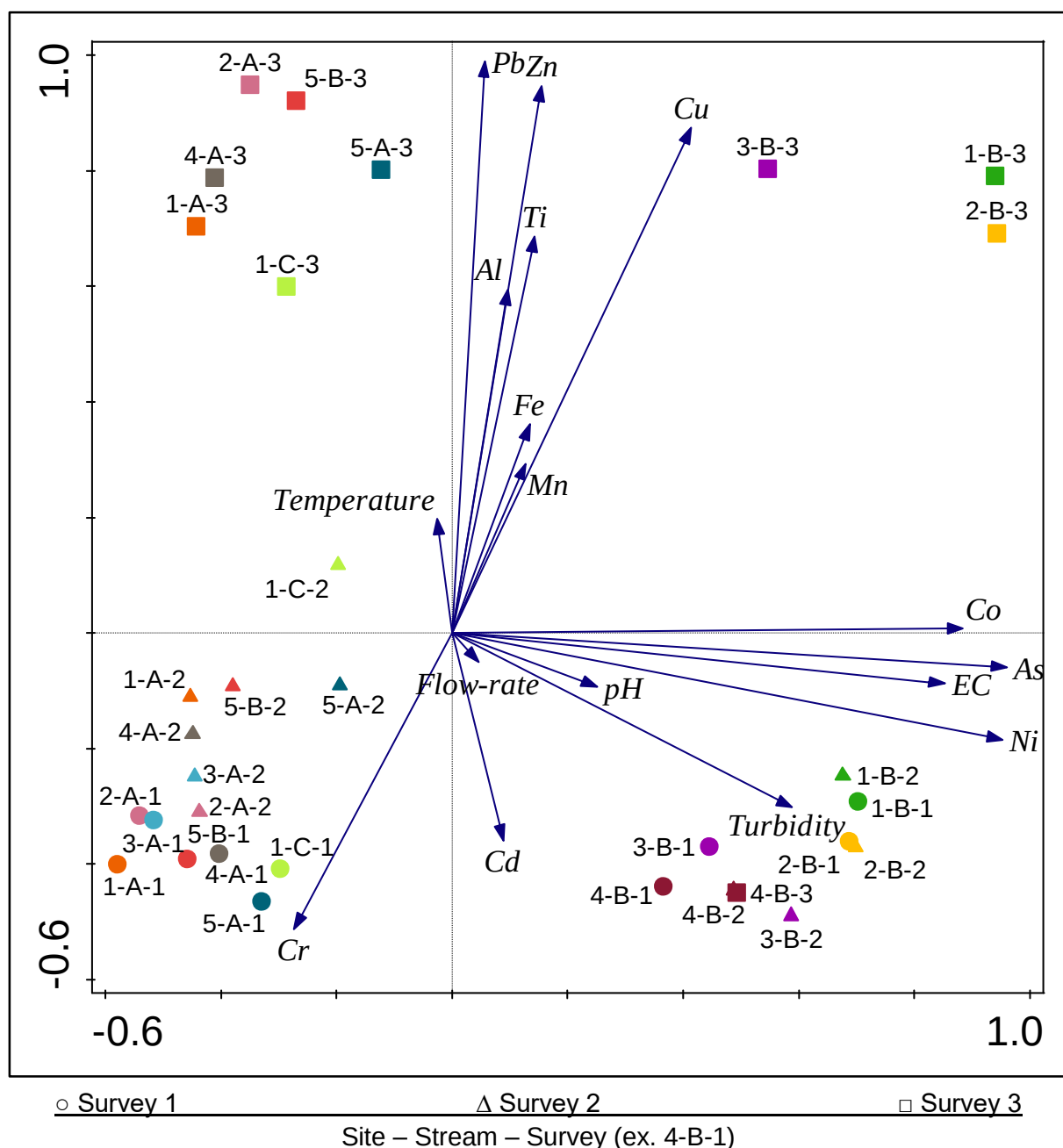


Figure 3.1: PCA bi-plot illustrating associations between metal concentrations of Al, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd and Pb measured in the water samples, the turbidity, pH, flow-rate, temperature and EC, during Surveys 1, 2 and 3. The bi-plot describes 75.5 % of the variation, with 43.3 % on the first axis and 32.2 % on the second axis.

3.3.5 Metal concentrations in sediments

Metals are well known to concentrate in the sediment of aquatic systems and readily adsorb onto clay minerals, hydrous metals oxides (especially Fe and Mn oxides/hydroxides), carbonates and organic materials (Eggletton & Thomas, 2004; Jain & Ram,

1997; Lin & Chen, 1998; Smith, 1999; Westrup *et al.*, 2005; Yao *et al.*, 2015). According to DWAF (1996) and Smith (1999), increased metal concentrations in sediments are the result of both natural weathering and anthropogenic sources such as mining and agricultural activities. It is likely that the sources of metals in sediments recorded during this study are probably as a result of both natural weathering and anthropogenic activities, such as those mentioned above. The concentrations measured in the sediment samples were also considerably higher than those of the water samples, which is supported by literature (Lin & Chen, 1998). A significant increase in the majority of metal concentrations occurred at Site 2B, during all three surveys which decreased further downstream (Table 3.6). These high concentrations at Site 2B were possibly due to the large percentage of clay particles varying from 16.5 % to 39.9% during the third and second surveys, respectively (Table 3.6). It is well known from the literature that clay particles are also capable of adsorbing metals to a great extent due to their adsorptive nature and larger surface area to volume ratio (Eggleton & Thomas, 2004; Huang & Lin, 2003; Jain & Ram, 1997; Parizanganeh, 2008; Singh *et al.*, 1999; Smith, 1999; Yao *et al.*, 2015), a phenomenon also demonstrated in Figure 3.2. The higher concentrations of the majority of metals at most of the sites during the last survey, were also the result of the drought experienced (Table 3.6).

Aluminium, Mn, Fe and Zn were present in markedly high concentrations at all the sites in the Mooi River, Wonderfontein Spruit and Gerhard Minnebron. This was expected as these metals are of the most abundant in nature (DWAF, 1996). Although mining and agricultural activities do occur in this part of the catchment, the elevated levels of these metals may rather be attributed to natural weathering of the minerals found in the study area (Table 3.2), than to anthropogenic activities. The concentrations of Co, Ni, Cu, As and Pb were considerably higher at sites located in the Wonderfontein Spruit associated with mining effluent (Sites 1B, 2B and 3B). These elevated concentrations were, however, no longer evident downstream at the confluence of the Mooi River, Wonderfontein Spruit and Gerhard Minnebron (Site 5A). The remaining metals (Ti, Cr and Cd) showed no drastic variations in concentrations downstream from the origin of the mining effluent (Table 3.6), indicating limited influence from anthropogenic activities.

Table 3.6: Mean and standard deviation of selected metal concentrations (mg/kg) measured in the sediment samples, as well as the percentage composition of gravel (> 2000 µm), sand (> 500 µm), silt (53 – 212 µm) and clay (< 53 µm) of each selected site. Common alphabetical superscripts (a, b) indicate significant difference between surveys at a specific site (p < 0.05) (spatial significant differences during the surveys are not indicated in Table 3.6, but are pointed out in the text).

Sites	Surveys	Al	Ti	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb	% Gravel	% Sand	% Silt	% Clay
1A	1	7 073.9 ± 70.6 ^a	136.6 ± 5.7	376.6 ± 32.7	6 501.3 ± 282.6 ^a	55 948.3 ± 891.5 ^a	46.2 ± 3.7	104.1 ± 77.7	50.9 ± 4.6	61.7 ± 7.1	20.0 ± 2.6	0.2 ± 0.03	38.3 ± 2.9	14.7	34.8	50.3	0.2
	2	14 568.3 ± 104.9 ^{ab}	177.4 ± 22	269.1 ± 17.3 ^a	20 008.4 ± 104.5 ^{ab}	36 837.7 ± 105.3 ^{ab}	47.1 ± 16.2	269.8 ± 16.9	63.4 ± 10.4	55.8 ± 7.4	20.0 ± 8.1	0.2 ± 0.02	40.3 ± 8.4	24.2	43.3	32.4	0.1
	3	9 417.8 ± 66.8 ^{ab}	160.3 ± 17.9	535.1 ± 18.2 ^a	25 824.3 ± 72.9 ^{ab}	65 429.7 ± 111.6 ^{ab}	62.5 ± 7.8	203.5 ± 11.7	56.7 ± 11.1	74.2 ± 6.2	23.4 ± 5.5	0.1 ± 0.03	92.4 ± 8.0	20.6	27.6	51.5	0.3
2A	1	17 797.1 ± 13.9 ^a	177.3 ± 6.9 ^a	722.3 ± 7.9 ^a	7 658.9 ± 34.6 ^a	105 122.8 ± 60.0 ^a	77.0 ± 7.6	307.6 ± 12.2 ^a	97.3 ± 8.2	270.7 ± 17.9 ^a	63.4 ± 5.1 ^a	0.4 ± 0.3	213.0 ± 12.3 ^a	51.1	33.4	14.5	1.0
	2	36 782.0 ± 77.7 ^{ab}	244.0 ± 38.1	826.0 ± 52.1	24 759.8 ± 73.8 ^{ab}	194 526.8 ± 196.4 ^{ab}	98.1 ± 15.7	638.2 ± 33.8 ^{ab}	103.8 ± 13.8	197.7 ± 26.5 ^a	74.5 ± 10.2	0.6 ± 0.1	79.4 ± 8.8 ^{ab}	43.7	40.5	14.9	0.9
	3	25 068.5 ± 62.3 ^{ab}	329.1 ± 7.6 ^a	935.7 ± 27.7 ^a	20 843.9 ± 41.9 ^{ab}	111 538.8 ± 72.1 ^{ab}	73.6 ± 8.5	314.5 ± 9.0 ^b	96.7 ± 8.7	174.2 ± 5.7 ^a	28.7 ± 4.7 ^a	0.4 ± 0.1	231.2 ± 6.9 ^b	30.6	23.6	45.7	0.1
3A*	1	58 678.0 ± 18.9 ^a	415.7 ± 8.9	745.5 ± 35.4 ^a	18 807.9 ± 15.8 ^a	116 814.5 ± 14.3 ^a	97.2 ± 3.9 ^a	198.6 ± 3.3	147.6 ± 4.6 ^a	159.1 ± 7.1	27.1 ± 2.3	0.4 ± 0.1	78.4 ± 3.9	24.4	15.4	54.2	6
	2	131 601.5 ± 95.0 ^a	547.8 ± 26.6	452.9 ± 18.3 ^a	2 790.8 ± 50.3 ^a	90 910.2 ± 45.9 ^a	25.2 ± 8.8 ^a	167.8 ± 17.0	66.3 ± 10.9 ^a	183.8 ± 8.8	13.9 ± 4.5	0.2 ± 0.1	33.9 ± 7.7	7.0	20.4	65	7.6
	3																
4A	1	40 965.8 ± 5.8 ^a	243.1 ± 12.3 ^a	454.3 ± 18.2 ^a	3 440.4 ± 40.7 ^a	33 351.7 ± 24.0 ^a	27.7 ± 7.4 ^a	96.4 ± 6.0 ^a	58.6 ± 6.4 ^a	58.1 ± 3.8 ^a	4.9 ± 0.8	0.1 ± 0.01	36.0 ± 2.8 ^a	30	31.3	37.7	1.0
	2	26 271.6 ± 72.1 ^{ab}	228.0 ± 15.8 ^b	133.7 ± 13.4 ^{ab}	6 099.2 ± 17.9 ^{ab}	47 324.4 ± 102.1 ^{ab}	27.2 ± 6.5 ^b	106.6 ± 10.4	34.3 ± 10.8 ^b	62.7 ± 9.1 ^b	10.1 ± 5.1	0.2 ± 0.1	22.1 ± 2.8 ^b	46.4	34.4	19	0.2
	3	23 549.3 ± 47.5 ^{ab}	123.9 ± 12.3 ^{ab}	294.0 ± 6.5 ^{ab}	87 756.2 ± 55.1 ^{ab}	143 990.0 ± 79.0 ^{ab}	145.6 ± 9.4 ^{ab}	378.7 ± 7.7 ^a	179.1 ± 7.9 ^{ab}	142.5 ± 7.5 ^{ab}	15.8 ± 3.9	0.3 ± 0.03	147.1 ± 5.0 ^{ab}	38.4	38	23.5	0.1
5A	1	104 199.7 ± 14.2 ^a	761.3 ± 19.1 ^a	715.6 ± 8.3 ^a	4 883.3 ± 6.2 ^a	89 698.1 ± 15.4 ^a	61.0 ± 7.4	159.7 ± 4.0 ^a	110.4 ± 5.6 ^a	169.2 ± 6.2 ^a	9.7 ± 3.3	0.3 ± 0.1	49.6 ± 4.5 ^a	6.3	22.1	62.9	8.7
	2	70 868.4 ± 66.0 ^{ab}	460.8 ± 10.7 ^{ab}	337.3 ± 12.2 ^{ab}	4 297.2 ± 14.1 ^{ab}	56 391.0 ± 64.1 ^{ab}	32.7 ± 6.1	119.7 ± 6.6 ^{ab}	53.1 ± 6.3 ^{ab}	103.7 ± 7.7 ^a	8.6 ± 0.5	0.2 ± 0.01	19.8 ± 2.5 ^{ab}	16.4	9.0	72.1	2.5
	3	96 018.2 ± 56.7 ^{ab}	130.6 ± 6.6 ^{ab}	838.0 ± 7.0 ^{ab}	4 884.1 ± 24.7 ^b	90 620.7 ± 52.1 ^{ab}	64.0 ± 6.1	162.7 ± 6.3 ^b	129.2 ± 8.4 ^b	139.4 ± 5.4	9.5 ± 1.7	0.2 ± 0.1	58.0 ± 3.1 ^b	5.3	9.6	76.1	9.0

Table 3.6 continued

1B	1	12 964.1 ± 10.6 ^a	331.0 ± 12.3	380.0 ± 7.1 ^a	4 200.3 ± 13.4 ^a	34 181.8 ± 6.7 ^a	266.7 ± 7.8 ^a	576.6 ± 8.4 ^a	61.6 ± 10.1 ^a	194.2 ± 9.6 ^a	66.4 ± 6.0 ^a	0.7 ± 0.2	17.2 ± 6.4 ^a	3.0	8.2	87.3	1.5
	2	21 326.4 ± 52.5 ^{ab}	339.4 ± 16.1	294.9 ± 11.8 ^{ab}	3 068.2 ± 10.2 ^{ab}	36 210.8 ± 23.3 ^{ab}	245.1 ± 12.9 ^b	633.5 ± 11.4 ^b	169.9 ± 8.2 ^{ab}	318.1 ± 8.2 ^{ab}	68.5 ± 7.2 ^b	0.9 ± 0.1	31.4 ± 6.1 ^b	0.6	5.9	91.3	2.2
	3	45 583.8 ± 38.1 ^{ab}	407.5 ± 23.4	1 564.7 ± 10.2 ^b	23 150.9 ± 48.7 ^{ab}	248 294.1 ± 41.0 ^{ab}	560.3 ± 18.1 ^{ab}	1 619.0 ± 17.1 ^{ab}	293.7 ± 9.8 ^{ab}	566.1 ± 10.0 ^{ab}	183.8 ± 8.0 ^{ab}	1.1 ± 0.1	118.9 ± 5.1 ^{ab}	31.1	34.8	33.6	0.5
2B	1	149 825.5 ± 20.5 ^a	1 015.5 ± 71.9	768.2 ± 16.9 ^a	18 835.7 ± 50.8 ^a	233 225.8 ± 131.1 ^a	1 770.9 ± 15.0 ^a	5 012.2 ± 38.7 ^a	1 311.8 ± 29.0 ^a	1 964.8 ± 23.9 ^a	810.6 ± 40.7 ^a	5.4 ± 1.7	174.5 ± 21.1	5.0	30.5	45.3	19.2
	2	182 692.6 ± 93.5 ^{ab}	1 267.4 ± 18.1 ^a	790.7 ± 12.4 ^b	16 714.9 ± 41.6 ^{ab}	232 190.8 ± 111.2 ^{ab}	1 883.5 ± 21.7 ^{ab}	5 515.8 ± 28.2 ^{ab}	1 817.6 ± 35.3 ^{ab}	2 728.3 ± 26.4 ^{ab}	649.1 ± 18.1 ^{ab}	5.7 ± 1.3	232.5 ± 12.1 ^a	1.4	11.7	47	39.9
	3	27 356.1 ± 51.3 ^{ab}	697.7 ± 12.8 ^a	1 473.7 ± 12.6 ^{ab}	30 204.4 ± 30.3 ^{ab}	302 169.3 ± 87.0 ^{ab}	598.0 ± 12.0 ^{ab}	1 852.7 ± 21.4 ^{ab}	355.2 ± 12.8 ^{ab}	982.0 ± 18.2 ^{ab}	304.7 ± 7.8 ^{ab}	1.8 ± 0.1	143.9 ± 5.4 ^a	15.2	28.8	39.5	16.5
3B	1	84 079.6 ± 30.9 ^a	990.9 ± 24.3 ^a	701.5 ± 14.0 ^a	15 867.9 ± 56.6 ^a	257 629.3 ± 139.4 ^a	151.6 ± 16.5 ^a	286.9 ± 20.4	227.3 ± 26.1	479.0 ± 15.5 ^a	81.1 ± 9.6	0.7 ± 0.2	183.9 ± 22.1	31.8	22.9	41.8	3.5
	2	46 626.3 ± 43.1 ^{ab}	650.1 ± 11.2 ^{ab}	1 645.5 ± 14.9 ^{ab}	35 078.1 ± 73.5 ^{ab}	281 141.2 ± 110.7 ^{ab}	187.9 ± 13.2 ^{ab}	299.9 ± 15.6	185.9 ± 10.5 ^a	338.4 ± 13.5 ^{ab}	83.4 ± 8.7	0.6 ± 0.04 ^a	143.0 ± 9.4 ^a	19.9	26.2	52.3	1.6
	3	35 392.0 ± 51.9 ^{ab}	764.9 ± 12.6 ^{ab}	1 054.5 ± 9.4 ^{ab}	56 212.5 ± 70.6 ^{ab}	352 708.8 ± 55.9 ^{ab}	219.0 ± 6.1 ^{ab}	386.6 ± 10.4	352.3 ± 8.3 ^a	868.0 ± 17.1 ^{ab}	128.7 ± 6.7	1.1 ± 0.1 ^a	285.9 ± 10.9 ^a	25.1	19.8	52.5	2.6
4B	1	84 962.7 ± 101.8 ^a	797.6 ± 25.6 ^a	616.4 ± 22.5	406.2 ± 27.1 ^a	67 623.6 ± 39.7 ^a	88.8 ± 6.9 ^a	593.7 ± 10.6 ^a	128.5 ± 15.2	288.4 ± 12.0 ^a	31.7 ± 6.5	0.4 ± 0.1	50.5 ± 3.7	13.4	32.5	48.1	6.0
	2	225 734.6 ± 56.0 ^{ab}	782.8 ± 15.6 ^b	516.0 ± 13.2 ^a	1 025.9 ± 27.3 ^{ab}	78 411.6 ± 78.2 ^{ab}	155.5 ± 13.1 ^{ab}	1 448.3 ± 27.4 ^{ab}	187.0 ± 10.6 ^a	1 045.9 ± 27.8 ^{ab}	66.0 ± 12.0	0.7 ± 0.1	79.3 ± 8.2	13.6	39.8	36.7	9.9
	3	11 154.1 ± 43.5 ^{ab}	157.9 ± 11.7 ^{ab}	698.8 ± 14.0 ^a	523.1 ± 12.5 ^b	50 132.7 ± 46.6 ^{ab}	30.3 ± 9.2 ^{ab}	185.8 ± 10.1 ^{ab}	41.1 ± 8.2 ^a	175.8 ± 10.6 ^{ab}	16.7 ± 1.1	0.04 ± 0.01	66.8 ± 5.6	10.0	18.7	69.6	1.7
5B	1	9 904.8 ± 29.2 ^a	225.7 ± 27.9	256.2 ± 13.8 ^a	396.5 ± 14.1 ^a	9 442.0 ± 41.5 ^a	7.0 ± 2.8	27.5 ± 9.8	14.9 ± 5.0	22.5 ± 6.4 ^a	5.5 ± 1.6	0.03 ± 0.01 ^a	12.6 ± 2.7 ^a	26.4	18.1	51.2	4.3
	2	14 159.2 ± 51.3 ^{ab}	221.1 ± 10.4 ^a	285.0 ± 8.8 ^b	398.5 ± 11.7 ^b	11 795.9 ± 59.9 ^{ab}	6.2 ± 0.9	39.8 ± 7.5	14.6 ± 2.3	14.1 ± 1.6 ^b	6.4 ± 0.5	0.1 ± 0.0 ^a	11.5 ± 0.9 ^b	22.9	21.8	52.3	3.0
	3	6 606.0 ± 19.4 ^{ab}	126.0 ± 6.2 ^a	515.8 ± 10.3 ^{ab}	779.2 ± 6.5 ^{ab}	14 618.6 ± 35.4 ^{ab}	7.3 ± 0.7	28.4 ± 7.2	22.5 ± 5.7	119.1 ± 7.6 ^{ab}	5.4 ± 0.5	0.02 ± 0.01	63.9 ± 6.3 ^{ab}	10.3	16.9	69.0	3.8
1C	1	18 306.1 ± 83.5 ^a	329.3 ± 25.7 ^a	426.2 ± 26.3	6 002.3 ± 96.6 ^a	86 142.3 ± 138.7 ^a	33.8 ± 11.5 ^a	66.6 ± 13.2	46.5 ± 9.5	144.5 ± 20.6	17.0 ± 7.1	0.1 ± 0.02	58.9 ± 16.9	23.6	15.9	57.3	3.2
	2	25 225.4 ± 20.3 ^{ab}	241.9 ± 20.4 ^b	280.4 ± 15.2 ^a	5 763.3 ± 24.4 ^b	99 955.0 ± 49.1 ^{ab}	22.4 ± 7.5 ^b	82.2 ± 12.2	41.7 ± 6.1	165.6 ± 13.0	24.1 ± 5.1	0.2 ± 0.01	93.9 ± 8.0 ^a	60.5	15.8	23.3	0.4
	3	30 161.7 ± 37.4 ^{ab}	579.9 ± 15.6 ^{ab}	519.7 ± 12.0 ^a	26 865.9 ± 55.6 ^{ab}	79 098.2 ± 92.6 ^{ab}	80.6 ± 8.5 ^{ab}	124.1 ± 12.0	83.0 ± 7.5	162.1 ± 9.6	20.2 ± 5.2	0.2 ± 0.0	146.4 ± 6.5 ^a	35.3	29.6	30.2	4.9

*Site 3A dry.

3.3.6 Spatial and temporal metal concentrations in sediment

Figure 3.2 displays a PCA tri-plot illustrating associations between selected metal concentrations measured in the sediment samples, the percentage of sediment particle sizes, as well as the mineral composition, during Surveys 1, 2 and 3. Only those factors that had a significant influence ($p < 0.05$) on the metal concentrations were included. These factors explain 93.0 % of the total variation. Figure 3.2 shows a clear spatial difference between the sites located in the Mooi River (Sites 1A to 5A) and Gerhard Minnebron (Site 1C), and those located in the Wonderfontein Spruit. This variation on the first axis may be explained by the dominance of quartz and is related to the lithology and associated metals. The association of the majority of the sites located in the Mooi River and quartz is also supported by the results obtained (Table 3.2). The low percentage variation on the second axis is likely related to Site 4B separating from all the other sites. This may be ascribed to the fact that the metals in the sediments, as well as the lithology at Site 4B differ from the other sites (all three surveys cluster together).

The association between the percentage clay, vermiculite, muscovite and the majority of metals was also expected as these three minerals were also categorized as clays. In accordance with the literature, the majority of the metals associated with the percentage of clay particles, as these particles are well known to accumulate metals due to their adsorptive nature and larger surface area to volume ratio (Eggleton & Thomas, 2004; Huang & Lin, 2003; Jain & Ram, 1997; Parizanganeh, 2008; Singh *et al.*, 1999; Smith, 1999; Yao *et al.*, 2015).

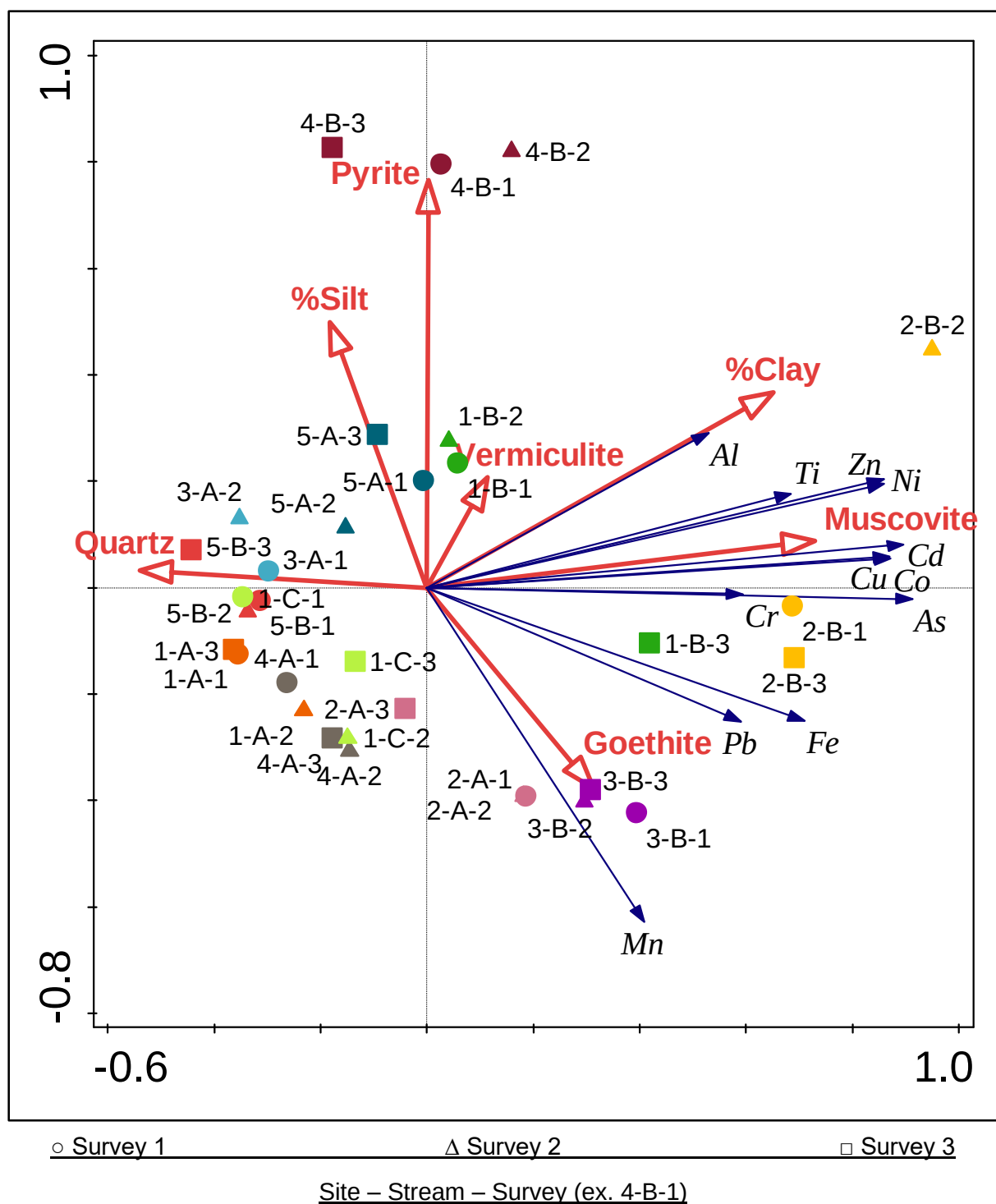


Figure 3.2: PCA tri-plot illustrating associations between metal concentrations of Al, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd and Pb measured in the sediment samples, the percentage of silt and clay, as well as the mineral composition, during Surveys 1, 2 and 3. The tri-plot describes 66.3 % of the variation, with 57.3 % on the first axis and 9.0 % on the second axis.

3.4 Conclusion

Although some metal concentrations may have been affected by the mining effluent originating from Site 1B, the presence of the majority was probably as a result of natural weathering of the minerals and rocks present in the catchment area, as well as soil erosion due to the agricultural activities around the rivers. The concentrations of the metals in the water and sediment could thus not be attributed to the adverse effects of the mining effluent and therefore in this instance, the hypothesis of this chapter is rejected.

**Chapter 4: The aquatic macroinvertebrate
diversity and selected physico-chemical
parameters of the Mooi River and Wonderfontein
Spruit.**

4.1 Introduction

Aquatic macroinvertebrates are the most common organisms found in riverine habitats. Most of these macroinvertebrates commence their lifecycle as larvae of different insect species, of which some will become terrestrial as they develop into adults, while others will remain in the water as adults throughout their complete life cycle (Thirion, 2007). Those macroinvertebrates which spend their entire lifetime in water include mussels, clams, snails, as well as aquatic worms.

Macroinvertebrates occupy various biotopes in riverine habitats where they perform essential ecological functions, which include the following: 1) the decomposition of organic matter and purification of water as illustrated by, amongst others, Tubificidae, Trichoptera, Baetidae, Caenidae, some Mollusca species and Chironomidae, (Anderson & Sedell, 1979; Appleton, 2002b; Barber-James & Lugo-Ortiz, 2003; De Moor & Scott, 2003; Harrison, 2003; Van Hoven & Day, 2002); 2) act as a vital food source for larger invertebrates, fish and birds, such as the families Atyidae and Corixidae (Griffiths *et al.*, 2015; Hart *et al.*, 2001) and 3) become involved in the maintenance of aquatic ecosystems (Farrell, 2014; Salvarrey *et al.*, 2014; Thirion, 2007; Thirion, 2016).

According to Dallas and Day (2004), aquatic macroinvertebrates are well known for their sensitivity towards extensive organic pollution, habitat transformation and selected physico-chemical factors, which include pH, EC, temperature, flow-rate and turbidity. It is thus important that these abiotic factors must be suitable for the survival and reproduction of macroinvertebrates (Blanco & Bécares, 2010; Wolmarans *et al.*, 2014). Most organisms prefer pH values within the neutral range, which falls between a pH of 6 and 8 (Dallas & Day, 2004; Griffiths *et al.*, 2015). A deviation of this pH range may interfere with normal metabolic processes, affecting the efficiency of cells in the body (Griffiths *et al.*, 2015), as well as interfering with the ion exchange and osmotic balance of the organism (Dallas & Day, 2004). A decrease in the pH can also result in the desorption of metals, which may have a detrimental effect on the survival of macroinvertebrates (Griffiths *et al.*, 2015).

Limited information is available with regard to the conductivity tolerances of freshwater organisms (Dallas & Day, 2004). Macroinvertebrates are rarely able to tolerate a large change in the EC, as it affects the movement of water and salts across cell membranes (Dallas & Day, 2004; Griffiths *et al.*, 2015). With regard to the temperature, its influence

on the rate of respiration, metabolic rate, growth, reproduction and egg hatchability, toxicity of many substances and the solubility of dissolved oxygen in water, is well known (Dallas & Day, 2004; Griffiths *et al.*, 2015). Flow-rate exerts a direct physical force on macroinvertebrates, while also influencing them by having an impact on the substrate composition, water chemistry, availability of nutrients and organic particles, as well as the availability of suitable habitats (Dewson *et al.*, 2007). It was further found that an increase in turbidity and the deposition of fine sediments may affect macroinvertebrates in several ways. On the one hand, a reduction in light penetration gives rise to a decrease in photosynthesis, a reduction of the availability of food, as well as the concentration of oxygen in the water (Dallas & Day, 2004). On the other hand, the deposition of fine particles on respiratory structures (i.e. gills) may inhibit proper respiration and feeding activities such as filter feeding (Dallas & Day, 2004; Griffiths *et al.*, 2015).

The availability and diversity of biotopes are also a major factor that determine the presence of these organisms in a river and may contribute to the variation in community composition (Dallas, 2007; RHP, 2007a). The different biotopes include vegetation, stones, in and out of current, riffles, runs, pools, and gravel, sand and mud (GSM). The vegetation biotope which includes marginal and aquatic vegetation, comprises all vegetation that can provide habitat for macroinvertebrates (Thirion, 2007). Macroinvertebrates that are associated with this biotope include families such as Nepidae, Belostomatidae, Coenagrionidae, Dytiscidae, Lymnaeidae and Planorbidae. The stones biotope include all hard surfaces, in and out of current. The riffle biotope is characterized by shallow water, usually with a rapid current and a broken flow, which occurs over substrates such as cobbles or stones (Palmer & O’Keeffe, 1991; Wadeson, & Rowntree, 1998). Taxa associated with this and similar biotopes include Simuliidae, Aeshnidae, Chlorocyphidae, Elmidae, Potamonautidae and Hirudinea, amongst others (Thirion, 2007). The pool biotope is regarded as water of a reasonable depth, with little to no current (Wadeson, & Rowntree, 1998) and families associated with this type of biotope include, amongst others, Veliidae, Caenidae and Physidae (Thirion, 2007). The run biotope can be defined as water of a moderate to rapid current, with rippled flow and typically occurs between riffles and the downstream pool (Wadeson, & Rowntree, 1998). The GSM biotope includes a combination of small grains of gravel, sand and mud (Thirion, 2007). Macroinvertebrates associated with this biotope include, amongst

others, Oligochaeta, Corbiculidae, Tipulidae and Chironomidae (Harrison, 2003; Thirion, 2007).

Assessing biodiversity in rivers, amongst others, has become a widely recognized method for determining the condition or health of a river (Dickens & Graham, 2002). Macroinvertebrates have been used, more often than any other biological group, to assess the integrity of aquatic ecosystems as these organisms reflect the physico-chemical conditions in a river (Clements, 1994; Thirion, 2016; Wolmarans *et al.*, 2014). Regarding the total macroinvertebrate diversity of the Mooi River catchment, limited research has been done and therefore, the amount of available data is insufficient.

The following hypotheses will be tested during this chapter:

1. The anthropogenic activities (mining and agriculture) in the upper reaches of the Wonderfontein Spruit has a detrimental effect on the macroinvertebrate assemblages downstream.

The aim of this chapter was therefore to investigate the relationship between macroinvertebrate assemblages and selected abiotic factors, including water temperature, EC, pH, TDS, turbidity, flow-rate and the availability of suitable biotopes in the Mooi River and the Wonderfontein Spruit.

4.2 Material and methods

4.2.1 Fieldwork

During this study three surveys (July 2014 (Survey 1), September 2014 (Survey 2) and May 2015 (Survey 3)) were conducted in the low-flow season. Surveys were conducted in the Mooi River, Wonderfontein Spruit and Gerhard Minnebron at 11 preselected sites (Figure 2.1) based on methods and techniques described in the study of Kemp *et al.* (2014) and Wolmarans *et al.* (2014). Macroinvertebrates were collected using a standard net consisting of a 30 cm square steel frame with a sturdy handle, to which a Perlon® gauze net with a mesh size of 1 mm is attached (Kemp *et al.*, 2014; Wolmarans *et al.*, 2014). Surveys were conducted by sampling the vegetation, as well as the substratum with the net at each preselected site for approximately 15 minutes (Kemp *et al.*, 2014; Wolmarans *et al.*, 2014). Marginal and aquatic vegetation were sampled by pushing the net vigorously into the vegetation and moving it backwards and forwards through the same area (Dickens & Graham, 2002). Biotopes consisting mainly

of gravel, sand, mud or stones were sampled by dragging the feet through the substratum whilst continuously sweeping the net downstream over the disturbed area (Dickens & Graham, 2002). The content of the net was then transferred to a plastic tray (360 X 470 X 80 mm), filled with site water. Most of the debris and coarse material were carefully removed by hand, where after the contents of the container were decanted into a cone-shaped Perlon® gauze net (0.25 mm mesh), suspended on a stand, to drain the excessive water (Kemp *et al.*, 2014; Wolmarans *et al.*, 2014). The content was then transferred to a plastic container with a tight-fitting lid and 70% ethanol was added to preserve and fixate the samples. The containers were then labelled with relevant site information. Samples collected from the marginal and aquatic vegetation biotopes were dealt with in a similar way.

The coordinates and altitude were determined by means of a Garmin Nuvi 500 GPS, for each sampling site. The species of dominant marginal and aquatic vegetation was identified with the aid of a guide (Gerber *et al.*, 2004) and noted for each site. Electrical conductivity, pH and temperature were measured *in situ* at all sites during each survey using a portable digital conductivity meter (DIST 3, HI 98303, Hanna Instruments), a portable digital pH probe (HI 98128, Hanna Instruments) and a portable digital thermometer (Checktemp, Hanna Instruments). The flow-rate, measured by a Global Water Flow probe model FP111, as well as the turbidity, measured with a Groundtruth Clarity Tube, were also recorded for all of the sites. All the samples collected were taken to the laboratory for further analysis.

4.2.2 Laboratory methods

4.2.2.1 Macroinvertebrate identification

In the laboratory, the macroinvertebrate samples were transferred into a rectangular Perspex® sorting tray (300 x 200 x 25 mm). All macroinvertebrates were sorted using a stereomicroscope, with lighting from above and below, and preliminary sorted into groups. Further identification was done by making use of the microscope mentioned above, as well as the Guides to the Freshwater Invertebrates of Southern Africa (Appelton, 2002a; Appelton, 2002b; Barber-James & Lugo-Ortiz, 2003; Biström, 2007; Coetzee, 2003; De Meillon & Wirth, 2003; De Moor, 2003; De Moor & Scott, 2003; Dippenaar-Schoeman, 2002; Endrödy-Younga, 2007a; Endrödy-Younga, 2007b; Endrödy-Younga & Stals, 2007a; Endrödy-Younga & Stals, 2007b; Endrödy-Younga & Stals, 2007c; Endrödy-Younga & Stals, 2007d; Griffiths & Stewart, 2001; Grobbelaar,

2007; Hamer, 1999; Harrison, 2003; Harrison *et al.*, 2003a; Harrison *et al.*, 2003b; Hart *et al.*, 2001; Henning, 2003; Jansen van Rensburg & Day, 2002; Kensley, 2001; Mansell, 2003; Martens, 2001; Nelson, 2007a; Nelson, 2007b; Oosthuizen & Siddall, 2002; Perkins, 2007; Rayner, 2001; Rayner *et al.*, 2002; Reavell, 2003; Samways & Wilmot, 2003; Seaman *et al.*, 1999; Stals, 2007a; Stals, 2007b; Stals & Endrödy-Younga, 2007; Van As & Van As, 2001; Van Hoven & Day, 2002), as well as additional literature (Davies & Day, 1998; Gerber & Gabriel, 2002a; Gerber & Gabriel, 2002b). Where possible, macroinvertebrates were identified up to species level, otherwise identification was done up to genus or family level. Subsequently, all specimens were counted and classified according to the South African Scoring System, Version 5 (SASS 5) (Dickens & Graham, 2002). All macroinvertebrate samples were preserved with 70% ethanol in 30 ml glass vials.

4.2.2.2 Sensitivity values

After the macroinvertebrates were collected, identified and the data compiled in tables, a sensitivity score were assigned to each family. The SASS 5 values (Dickens & Graham, 2002) were adapted for this study to classify the collected macroinvertebrate families into three groups, namely highly tolerant (scores 1 to 5), moderately sensitive (scores 6 to 10) and highly sensitivity (scores 11 to 15) (Table 4.1). Where families collected during this study were not included in the SASS 5 values, no scores were allocated.

Table 4.1: The sensitivity scores adapted from the SASS 5 values (Dickens & Graham, 2002).

Sensitivity Category Scores		
1-5	1	Highly tolerant
6-10	2	Moderately sensitive
11-15	3	Highly sensitive

4.2.3 Statistics

To further analyse the macroinvertebrate community structure of the Mooi River catchment area, a combination of biological indices and exploratory statistical analyses were conducted.

4.2.3.1 Univariate indices

Macroinvertebrate assemblages were assessed using species richness (SR), the Shannon–Wiener index (H') and Pielou's evenness index (J').

- *Species richness* (SR): The total number of taxa recorded.
- *Shannon-Wiener index* (H'): Accounts for both abundance and proportion contribution to the community. This index is used to indicate community diversity. The H' value generally varies between 1.5 and 3.5, the higher the value, the greater the biodiversity (Khan, 2003).

$$H' = - \sum (p_i \cdot \ln p_i)$$

- *Pielou's evenness* (J'): This index can be described as the distribution of multiplicity between species at a given sampling point and can also be defined as a diversity index or a measure of biodiversity, which quantifies how equal the community is numerically. Values can range between 0 and 1, where 0 indicates an uneven distribution and 1 indicates a uniform distribution of multiplicity.

$$J' = \frac{H'}{\ln SR}$$

4.2.3.2 Univariate and multivariate statistical analyses

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-Lillie for P value), respectively. When data were parametric, analysis of variance (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. Graphical presentations of the data were performed using GraphPad Prism (mean and standard error). A Redundancy analysis (RDA), a multivariate analysis, was performed using Canoco (version 5) to determine the spatial and temporal distributions based on species diversity and abundance.

4.3 Results and discussion

4.3.1 Physico-chemical and biotope characteristics

Figure 4.1 displays the mean and standard error of temperature (A), EC (B), pH (C), turbidity (D) and flow-rate (E) measured at each site during the three surveys, while Tables 4.2, 4.3 and 4.4 shows these physico-chemical parameter measured at each site, during each survey. During this study, the mean temperatures (Figure 4.1A) ranged between 12.0 °C at Site 3B and 20.4 °C at Site 1C, but did not differ significantly between sites. The mean EC values varied between 430.0 µS/cm at Site 2A and 1 042.6 µS/cm at Site 2B. Sites 1A, 2A, 3A and 4A differed significantly from Sites 5A, 1B, 2B, 3B, 4B, 5B and 1C (Figure 4.1B). It is further evident that Site 1B, 2B, 3B and 4B all differed significantly with regard to this parameter from Sites 5B and 1C (Figure 4.1B). In this regard, Site 5A is the only site which differed significantly from all other sites during this study. The elevated EC values at Sites 1B to 4B may possibly be ascribed to the fact that the Wonderfontein Spruit is highly enriched due to various anthropogenic activities, which is also documented in the literature (DWS, 2014; Tlokwe City Council, 2014; Van de Walt *et al.*, 2002). The phenomenon that Site 5A differed significantly from all of the sites, may possibly be ascribed to the confluence of the Mooi River (497.1 µS/cm) (Sites 1A to 5A), Wonderfontein Spruit (975.5 µS/cm) (Sites 1B to 5B) and Gerhard Minnebron (782.3 µS/cm) (Site 1C) at this site. Regarding the pH (Figure 4.1C), the highest mean value was measured for Site 4B (pH of 8.1), while the lowest was measured for Site 1A (pH of 6.9). Site 4B showed a significant difference between Sites 1A, 5A, 3B, 5B and 1C. High rates of photosynthesis due to the abundance of *Azolla pinnata*, *Spirodela* sp. and *Lemna gibba* (Table 2.9, Chapter 2), whether natural or as a result of eutrophication, may possibly result in elevated pH values in standing waters (DWAF, 1996), which was recorded at this site (Tables 4.2 to 4.4; Figure 4.1E). From Figure 4.1C it is further evident that Site 1A differed significantly from Site 4A. The mean turbidity ranged between 5.0 NTU at Sites 1A, 2A, 5A, 5B and 1C, and 27.0 NTU at Site 4B. Site 2B differed significantly between all of the sites, except for Site 1B, while Site 4B showed a significant difference from all other sites. The mean flow-rate varied between 0.8 m/s at Site 5A and 0.1 m/s at Sites 1A, 4B, 5B and 1C (Figure 4.1E). No significant differences were found between the sites in this respect.

Regarding the biotopes found during the study, marginal vegetation and GSM were present at all the sites during the three surveys (Tables 4.2, 4.3 and 4.4). Aquatic

vegetation and pool biotopes were present at the majority of the sites during the three surveys, except for Sites 5A and 3B, and Sites 2A, 3A and 4A, respectively. The run biotope was present at Sites 2A, 3A, 4A, 5A, 1B, 2B, and 3B during all three surveys, with the exception of Site 3A, Survey 3, as it was completely dry. Stones in current and riffle biotopes were found at Sites 2A, 4A, 1B and 3B at Surveys 1, 2 and 3, while these two biotopes were also present at Site 3A only during the first two surveys. It is further evident from Tables 4.2, 4.3 and 4.4 that the stones out of current biotope were present at Sites 1A, 2A, 5B and 1C, and at Site 3B at Surveys 1 and 2 only.

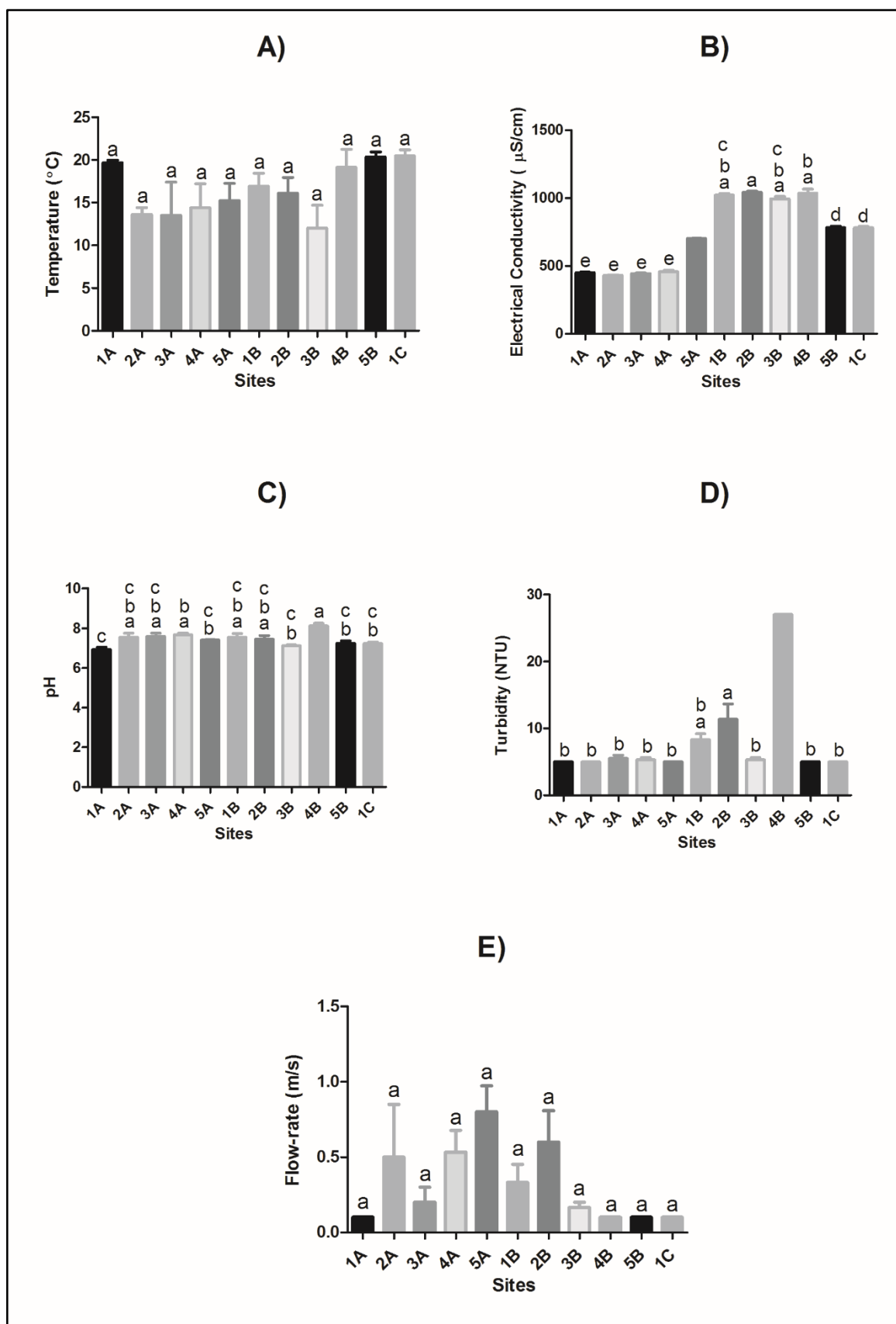


Figure 4.1: The mean with standard error of selected abiotic factors, including A) temperature, B) EC, C) pH, D) turbidity and E) flow-rate, measured at each site. Common superscripts indicate no significant difference. Bars with different superscripts differ significantly ($p < 0.05$).

Table 4.2: Selected abiotic factors and biotopes of each site, during Survey 1.

Selected abiotic factors and biotopes	Sites										
	1A	2A	3A	4A	5A	1B	2B	3B	4B	5B	1C
Temperature (°C)	19.2	12.0	9.6	10.2	11.3	13.9	12.5	6.7	15.0	19.8	19.7
Electrical conductivity (µS/cm)	457.0	438.0	441.0	456.0	697.0	1001.0	1055.0	974.0	996.0	798.0	785.0
pH	6.8	7.7	7.8	7.8	7.4	7.5	7.1	7.0	7.9	7.2	7.1
Total Dissolved Solids (mg/L)	306.2	293.5	295.5	305.5	467.0	670.7	706.9	652.6	667.3	534.7	526.0
Turbidity (NTU)	5.0	5.0	5.0	5.0	5.0	7.0	15.0	5.0	27.0	5.0	5.0
Flow-rate (m/s)	0.1	1.2	0.3	0.8	1.1	0.1	0.2	0.1	0.1	0.1	0.1
Marginal vegetation	X	X	X	X	X	X	X	X	X	X	X
Aquatic vegetation	X	X	X	X		X	X		X	X	X
Stones in current		X	X	X		X		X			
Stones out of current	X	X						X		X	X
Riffle		X	X	X		X		X			
Run		X	X	X	X	X	X	X			
Pool	X				X	X	X	X	X	X	X
Gravel/ Sand/ Mud	X	X	X	X	X	X	X	X	X	X	X

Table 4.3: Selected abiotic factors and biotopes of each site, during Survey 2.

Selected abiotic factors and biotopes	Sites										
	1A	2A	3A	4A	5A	1B	2B	3B	4B	5B	1C
Temperature (°C)	20.3	14.8	17.4	19.7	18.0	17.7	17.3	14.7	22.9	21.5	21.9
Electrical conductivity (µS/cm)	443.0	430.0	448.0	476.0	704.0	1027.0	1027.0	1033.0	1017.0	764.0	766.0
pH	7.2	7.8	7.4	7.5	7.3	7.2	7.5	7.2	8.4	7.1	7.2
Total Dissolved Solids (mg/L)	296.8	288.1	300.2	318.9	471.7	688.1	688.1	692.1	681.4	511.9	513.2
Turbidity (NTU)	5.0	5.0	6.0	6.0	5.0	8.0	12.0	6.0	27.0	5.0	5.0
Flow-rate (m/s)	0.1	0.2	0.1	0.5	0.8	0.5	0.7	0.2	0.1	0.1	0.1
Marginal vegetation	X	X	X	X	X	X	X	X		X	X
Aquatic vegetation	X	X		X		X	X		X	X	X
Stones in current		X	X	X		X		X			
Stones out of current	X	X						X		X	X
Riffle		X	X	X		X		X			
Run		X	X	X	X	X	X	X			
Pool	X				X		X	X	X	X	X
Gravel/ Sand/ Mud	X	X	X	X	X	X	X	X	X	X	X

Table 4.4: Selected abiotic and biotic factors of each site, during Survey 3.

Selected abiotic factors and biotopes	Sites										
	1A	2A	3A*	4A	5A	1B	2B	3B	4B	5B	1C
Temperature (°C)	19.5	14.0		13.3	16.4	19.1	18.5	14.7	18.9	19.7	19.8
Electrical conductivity (µs/cm)	453.0	422.0		444.0	704.0	1038.0	1046.0	975.0	1096.0	786.0	796.0
pH	6.9	7.1		7.7	7.4	7.9	7.7	7.2	8.0	7.5	7.4
Total Dissolved Solids (mg/L)	303.5	282.7		297.5	471.7	695.5	700.8	653.3	734.3	526.6	533.3
Turbidity (NTU)	5.0	5.0		5.0	5.0	10.0	7.0	5.0	27.0	5.0	5.0
Flow-rate (m/s)	0.1	0.1		0.3	0.5	0.4	0.9	0.2	0.1	0.1	0.1
Marginal vegetation	X	X		X	X	X	X	X	X	X	X
Aquatic vegetation	X	X		X			X		X	X	X
Stones in current		X		X		X		X			
Stones out of current	X	X						X		X	X
Riffle		X		X		X		X			
Run		X		X	X	X	X	X			
Pool	X				X		X	X	X	X	X
Gravel/ Sand/ Mud	X	X		X	X	X	X	X	X	X	X

*Site 3A dry.

4.3.2 Biodiversity

The different taxa, as well as the number of specimens per taxa collected during the three low-flow seasons over the course of this study, are presented in Tables 4.6 to 4.8. A total of 142 taxa belonging to 66 families were collected at the 11 preselected sites. A total number of 57, 50 and 53 families were collected during Survey 1, Survey 2 and Survey 3, respectively (Table 4.5). Forty one (41) of these families coincided during the three surveys. Of the 41 families, 12 were present at five or more sites during all three surveys. This can possibly be attributed to the fact that the majority of these families prefer low to very low water quality and are tolerant to organically enriched waters (Appleton, 2002b; Brown, 1994; Dickens & Graham, 2002; Griffiths *et al.*, 2015; Harrison, 2003; Reavell, 2003; Thirion, 2007; Van Hooven & Day, 2002), except for the families Baetidae and Hydropsychidae, which indicate water of high quality when two or more species are present (Dickens & Graham, 2002; Thirion, 2007). It is further evident from Table 4.5 that Tubificidae, Lymnaeidae, Planorbidae, Baetidae, Coenagrionidae, Hydropsychidae, Simuliidae, Chironomidae and Dytiscidae were present at almost all the sites during each of the surveys and will be discussed later in this chapter. In contrast to this, most of the remaining families were generally present at less than 50 % of the sites during the three surveys. Twelve (12) taxa occurred only during one survey at less than five sites and also in very low numbers (the highest being 22 specimens and the lowest one). This may possibly be attributed to various factors, which include

insufficient macrophyte habitats (Rumes, 2010), mass emergences associated with various environmental factors (Griffiths *et al.*, 2015), intolerance to saline conditions (Samways & Wilmot, 2003) and the ability to disperse by flight (Endrödy-Younga & Stals, 2007d). Another factor that must be taken into consideration is the reproductive cycle, particularly generation time, as well as growth and egg hatchability (Dallas & Day, 2004; Kemp *et al.*, 2014).

The presence of Tubificidae at most of the sites can be ascribed to their tolerance for moderate to highly organic enriched habitats, characterised by anaerobic conditions (Griffiths *et al.*, 2015; Thirion, 2007; Van Hooven & Day, 2002). The survival of this family under these conditions is most probably enhanced by the fact that Tubificidae possesses haemoglobin as respiration pigment (Griffiths *et al.*, 2015; Van Hooven & Day, 2002), an attribute not generally found in most of the macroinvertebrate families. Limited spatial variation was found with regard to the sites populated by this family. The tolerance of this family for a large variation in EC (Gillett *et al.*, 2007; Salvarrey *et al.*, 2014) is demonstrated in Figure 4.1B and Tables 4.2 to 4.4, which show that EC varied from approximately 500 $\mu\text{S}/\text{cm}$ (Sites 1A to 4A) to $> 1000 \mu\text{S}/\text{cm}$ (Sites 1B to 4B). The elevated EC value at Site 5A can be attributed to the confluence of the highly enriched Wonderfontein Spruit and the Gerhard Minnebron Spring (Site 1C) with the Mooi River, originating from Site 1A.

Three species of the family Lymnaeidae, namely *Lymnaea columella*, *L. natalensis* and *L. truncatula*, were collected during this study. *Lymnaea natalensis* is the most widespread of the three species, while *L. truncatula* shows the most limited and sporadic distribution patterns (De Kock & Wolmarans, 2008), findings which are both supported by the numbers of individuals collected during this study (Tables 4.2 to 4.4). *Lymnaea columella* is well known to populate a variety of water bodies and is regarded as the most widespread exotic invader species in the country (De Kock *et al.*, 1989; De Kock & Wolmarans, 2008). *Lymnaea columella* and *L. truncatula* are also amphibious, enabling them to crawl out on vegetation and other surfaces (Brown, 1994; Griffiths *et al.*, 2015). The occurrence of the family Lymnaeidae at most of the sites over the course of this study can most probably be ascribed to the factors mentioned above, as well as their habitat preferences, as they are frequently found amongst the marginal and aquatic vegetation, as well as stones and GSM (Brown, 1994; Griffiths *et al.*, 2015; Thirion, 2007). The family Planorbidae, also a mollusc family, was present at almost

every site during Surveys 1, 2 and 3 (Tables 4.5 to 4.8). According to the literature (Appleton, 2002b; Brown, 1994; Griffiths *et al.*, 2015; Thirion, 2007), this family is found in a diversity of aquatic habitats, ranging from standing to flowing freshwater, aquatic vegetation and GSM, and this versatility may possibly be one of the reasons for the presence of this family at the variety of sites. Planorbidae are also classified as highly tolerant (Dickens & Graham, 2002; Thirion, 2007) and possess haemoglobin as blood pigment (Appleton, 2002b), which allows them to survive at a wide range of organic enrichment levels and in a wide variety of habitats.

Baetidae were present at ten, seven and ten out of the possible 11 sites during Survey 1, 2 and 3, respectively (Tables 4.5). From this it is evident that this family can occur in a variety of water bodies and can also occupy a variety of biotopes, from rocks, riffle areas and vegetation to GSM, which is supported by the literature (Barber-James & Lugo-Ortiz, 2003; Griffiths *et al.*, 2015; Thirion, 2007). According to Dickens and Graham (2002) and Thirion (2007), more than two species of Baetidae collected at a specific site, indicates water of a higher quality. More than two species were collected only at Site 3B, during Survey 1 and Site 4A, Survey 2. The occurrence of Baetidae at these sites are probably due to the availability of nutrients and suitable biotopes, rather than the quality of the water. The occurrence of Baetidae at the variety of sites and wide range of parameters could also be attributed to the fact that the female beatids can reproduce asexually in unfavourable environmental conditions, in which the offspring develop from unfertilized eggs (Barber-James & Lugo-Ortiz, 2003).

The presence of the family Hydropsychidae at a variety of sites, can most probably be ascribed to the habitat preferences of this family, which is normally found in very fast flowing waters (> 0.6 m/s) (De Moor & Scott, 2003; Thirion, 2007). This is also demonstrated by the RDA tri-plot (Figure 4.6), which shows an association between this family and a high flow-rate, which will be discussed later in this chapter. In contrast to the literature, the family Hydropsychidae still occurred at one site (Site 1A) with slow-flowing water during the first survey, however in very low numbers (Table 4.6). Simuliidae were present at more than 50 % of the sites during all three surveys (Table 4.5). This may be attributed to the fact that this family is best adapted to life in flowing waters and are commonly found on stones, cobbles and submerged or floating vegetation (De Moor, 2003; Thirion, 2007). From Tables 4.2 to 4.4 it is evident that these biotopes and flow regime were found at the majority of the sites.

The family Coenagrionidae was present at seven, nine and ten out of the possible 11 sites during the three surveys, respectively (Table 4.5). At Site 2A, the numbers of the family increased from zero in the first survey to 610 specimens in the second survey, and then decreased again to 31 specimens during the third survey. This phenomenon can possibly be ascribed to the biotope preferences of Coenagrionidae (Griffiths *et al.*, 2015; Samways & Wilmot, 2003; Thirion, 2007), rather than to water quality preferences. The increase in the number of specimens may be attributed to the upsurge of aquatic vegetation during Survey 2 at this site. Although in low numbers, this family occurred at the majority of sites during the last two surveys.

Chironomidae were collected at almost every site during this study, except for Site 4B during Survey 2 and Site 3A during the last survey (Table 4.7 and 4.8). According to Harrison (2003), Chironomidae are some of the most abundant aquatic macroinvertebrates and comprise up to 10 to 50 % of the biomass. This family is found in nearly every aquatic habitat, from stony torrents to waterfalls, standing to running freshwater and aquatic vegetation to GSM and rocks (Griffiths *et al.*, 2015; Harrison, 2003). They also possess haemoglobin as blood pigment to enhance oxygen uptake, a feature that allows larvae to survive in organically enriched environments (Griffiths *et al.*, 2015; Harrison, 2003), which enables this family to survive in a variety of environmental conditions and also to exploit such a large variety of sites.

The family Dytiscidae is one of the largest of all beetle families and are also a keystone species in the freshwater food web (Biström, 2007; Griffiths *et al.*, 2015). Dytiscidae were collected at eight, 11 and six of the possible 11 sites during Surveys 1, 2 and 3, respectively. In the study of Lundkvist *et al.* (2001) it was concluded that the degree of shading was the most important factor in determining the presence of Dytiscidae. Although aquatic, marginal and overhanging riparian vegetation, the preferred biotopes of Dytiscidae (Thirion, 2007), were present at the majority of the sites during all three surveys, the variation in the occurrence of this particular family could not be attributed to any specific factor.

Table 4.5: The total number of sites where each of the families/ taxa were collected, during each of the three surveys.

Taxa	Surveys		
	1	2	3*
Daphniidae	4	4	2
Cypridoidea	0	0	1
Cyclopoida	1	0	0
Atyidae	4	8	6
Potamonautidae	3	4	7
Hydroida	3	2	0
Rhabdocoela	3	4	3
Nematoda	0	0	1
Tubificidae	8	8	9
Glossiphoniidae	5	5	3
Hydrachnidae	3	1	0
Hygrobatidae	1	0	0
Eylaidae	0	0	1
Lymnaeidae	7	5	7
Ancylidae	7	4	8
Planorbidae	10	11	10
Physidae	5	6	5
Corbiculidae	2	2	4
Sphaeriidae	4	3	8
Baetidae	10	7	10
Caenidae	9	7	7
Polymitarcyidae	0	0	1
Chlorocyphidae	0	1	0
Coenagrionidae	7	9	10
Protoneuridae	0	0	1
Gomphidae	1	4	4
Aeshnidae	3	2	5
Libellulidae	4	5	4
Mesoveliidae	1	0	4
Veliidae	0	2	3
Gerridae	2	1	3
Paraphrynoveliidae	1	1	0
Corixidae	7	6	7
Notonectidae	3	1	1

Taxa	Surveys		
	1	2	3*
Pleidae	3	4	2
Naucoridae	5	3	6
Nepidae	1	0	3
Belostomatidae	5	4	6
Hydropsychidae	7	5	5
Hydroptilidae	3	2	4
Ecnomidae	4	1	3
Crambidae	3	2	1
Psychodidae	0	0	1
Tipulidae	1	2	0
Dixidae	2	2	1
Chaoboridae	2	2	0
Ceratopogonidae	3	5	2
Culicidae	2	2	4
Simuliidae	8	8	7
Chironomidae	11	10	10
Tabanidae	0	3	0
Stratiomyidae	3	0	1
Empididae	1	0	1
Dolichopodidae	1	1	0
Ephydriidae	1	2	4
Muscidae	4	4	6
Gyrinidae	5	5	4
Haliplidae	1	0	2
Dytiscidae	8	11	6
Hydrophilidae	3	3	6
Hydraenidae	5	3	6
Scirtidae	1	0	0
Elmidae	3	1	4
Psephenidae	1	0	0
Chrysomelidae	1	0	1
Curculionidae	1	3	2
Total number of families collected per survey	57	50	53

*During Survey 3, Site 3A was dry.

Table 4.6: Biodiversity list of all macroinvertebrates collected at each site during Survey 1, the number of organisms (n), the species richness (SR), Shannon-Wiener index (H'), Pielou's evenness index (J'), as well as the sensitivity scores.

Taxa		Site											Sensitivity
		1A	2A	3A	4A	5A	1B	2B	3B	4B	5B	1C	
Cladocera													
Daphniidae	<i>Daphnia magna</i>		61				73	932	89				
	<i>Simocephalus</i> sp.						8	419	12				
Copepoda													
Cyclopoida	<i>Thermocyclops oblongatus</i>								22				
Decapoda													
Atyidae	<i>Caridina nilotica</i>		1		1						6	246	**
Potamonautidae	<i>Potamonautes warreni</i>					2					1	2	*
Cnidaria													
Hydridae	<i>Hydra</i> sp.		63				97	196					*
Turbellaria													
Rhabdocoela	<i>Mesostoma</i> sp.		46		5		18						*
Oligochaeta													
Tubificidae	<i>Tubifex</i> sp.		8	24		4	152	133	28		7	1	*
	<i>Branchiura</i> sp.							13					*
Hirudinea													
Glossiphoniidae	<i>Alboglossiphonia</i> sp.		1				270	28	1				*
	<i>Marsupiobdella africana</i>							5	2				*
	<i>Placobdelloides jaegerskioeldi</i>							72	14	6			*
Trombidiformes													
Hydrachnidae			4	2	1								**
Hygrobatidae	<i>Atractides</i> sp.				1								**
Mollusca													
Lymnaeidae	<i>Lymnaea columella</i>									1			*
	<i>Lymnaea natalensis</i>	3	5			1				1	1	2	*

Table 4.6 continued

	<i>Lymnaea truncatula</i>			5									*
Ancylidae	<i>Burnupia mooiensis</i>		1			4	1		1				**
	<i>Ferrissia cawstoni</i>			1	2	1						2	**
Planorbidae	<i>Ceratophallus natalensis</i>			87					7	4	1		*
	<i>Gyraulus connollyi</i>		5		4	1					3	12	*
	<i>Gyraulus costulatus</i>	1	1		1								*
	<i>Bulinus africanus</i>	2											*
	<i>Bulinus tropicus</i>		4	8				58	12				*
Physidae	<i>Physa acuta</i>			112				8	6	37	1		*
Corbiculidae	<i>Corbicula fluminalis africana</i>		5		7								*
Sphaeriidae	<i>Pisidium langleyanum</i>	1			1	15					1		*
	<i>Pisidium viridarium</i>					29					3		*
Ephemeroptera													
Baetidae	Baetidae sp.		149	80	1				26	1			**
	<i>Acanthiops erepens</i>	66					122		6				**
	<i>Acanthiops</i> sp.	13			218	289	7		20		27	29	**
	<i>Cloeon</i> & <i>Procloeon</i>			7									**
Caenidae	<i>Caenis</i> sp.	3	91	1	150	34	1			1	3	1	**
Odonata													
Zygoptera													
Coenagrionidae	<i>Pseudagrion</i> sp.	63				8	11	15	5		1	6	*
	<i>Enallagma</i> sp.							5					*
Anisoptera													
Gomphidae	<i>Nepogomphoides</i> sp.					2							**
Aeshnidae	<i>Anax</i> sp.	1					1					1	**
Libellulidae	<i>Notiothemis</i> sp.										1		*
	<i>Trithemis</i> sp.				1		1					2	*

Table 4.6 continued

[illegible]

Table 4.6 continued

[illegible]

Table 4.6 continued

	<i>Hydrovatus</i> sp.	2		2					1	1			*
	<i>Herophydrus</i> sp.	6											*
	<i>Nebrioporus</i> sp.						1						*
	<i>Hydaticus</i> sp.						2						*
Hydrophilidae	<i>Regimbartia</i> sp.	1											*
	<i>Enochrus</i> sp.					1						2	*
Hydraenidae	<i>Ochthebius</i> sp.		12	1	11				1	2			**
	<i>Hydraena</i> sp.								2				**
Scirtidae							3						
Elmidae	<i>Elminae</i> sp.					1							**
	<i>Potamodytes</i> sp.				18				1				**
Psephenidae	<i>Afrobrianax</i> sp.						1						**
Chrysomelidae	<i>Donicia</i> sp.					1							
Curculionidae	<i>Bagous</i> sp.									7			
	Number of organisms (n)	265	1233	528	552	481	1059	1932	331	99	81	356	
	Species richness (SR)	24	30	28	25	26	33	21	32	18	19	23	
	Shannon-Wiener index (H')	2.1	2.4	2.4	1.9	1.7	2.5	1.6	2.7	2.0	2.3	1.3	
	Pielou's Evenness index (J')	0.7	0.7	0.7	0.6	0.5	0.7	0.5	0.8	0.7	0.8	0.4	

*Highly tolerant, **Moderately sensitive, ***Highly sensitive.

Table 4.7: Biodiversity list of all macroinvertebrates collected at each site during Survey 2, the number of organisms (n), the species richness (SR), Shannon-Wiener index (H'), Pielou's evenness index (J'), as well as the sensitivity scores.

Taxa		Site											Sensitivity
		1A	2A	3A	4A	5A	1B	2B	3B	4B	5B	1C	
Cladocera													
Daphniidae	<i>Daphnia magna</i>			7			695	125	76				
	<i>Simocephalus</i> sp.						289						
Decapoda													
Atyidae	<i>Caridina nilotica</i>	48	11	1	9	1	1				6	423	**
Potamonautidae	<i>Potamonautes warreni</i>		4		3	2					2		*
Cnidaria													
Hydridae	<i>Hydra</i> sp.							1	1				*
Turbellaria													
Rhabdocoela	<i>Mesostoma</i> sp.		21		1		5	12					*
Nematoda													
Oligochaeta													
Tubificidae	<i>Tubifex</i> sp.			123		11	19	76	12	10	3	2	*
	<i>Branchiura</i> sp.			19				13					*
Hirudinea													
Glossiphoniidae	<i>Alboglossiphonia</i> sp.		1	1			12	41	9				*
	<i>Batracobdelloides tricarinata</i>							5					*
	<i>Marsupiobdella africana</i>						2		1				*
	<i>Placobdelloides jaegerskioeldi</i>		7	2			21	89	10				*
Trombidiformes													
Hydrachnidae			4										**
Mollusca													
Lymnaeidae	<i>Lymnaea natalensis</i>	1	3								1	1	*
	<i>Lymnaea truncatula</i>			2									*
Ancylidae	<i>Burnupia mooiensis</i>	1				10					1		**

Table 4.7 continued

	<i>Ferrissia cawstoni</i>								1				**
Planorbidae	<i>Ceratophallus natalensis</i>		12	121	11				2	7	1	1	*
	<i>Gyraulus connollyi</i>		3		5	7					6	14	*
	<i>Gyraulus costulatus</i>	4			1								*
	<i>Bulinus africanus</i>	1											*
	<i>Bulinus tropicus</i>		1	89			9	8		4			*
Physidae	<i>Physa acuta</i>			163	1		2	42	16	97			*
Corbiculidae	<i>Corbicula fluminalis africana</i>		2						14				*
Sphaeriidae	<i>Pisidium costulosum</i>				2	3							*
	<i>Pisidium langleyanum</i>				3	87					3		*
	<i>Pisidium viridarium</i>				2	5					2		*
Ephemeroptera													
Baetidae	Baetidae sp.	67	10		161	63			1		12	3	**
	<i>Acanthiops erepens</i>				110								**
	<i>Acanthiops</i> sp.				102	51			49				**
Caenidae	<i>Caenis</i> sp.	6	23	12	210	41					3	3	**
Odonata													
Zygoptera													
Chlorocyphidae	<i>Platycypha caligata</i>					1							**
Coenagrionidae	<i>Pseudagrion</i> sp.	49	225	6	2	4			4	1	3	6	*
	<i>Enallagma</i> sp.	1	385	8									*
Anisoptera													
Gomphidae	Gomphidae sp.										1		**
	<i>Gomphidia</i> sp.										1		**
	<i>Notogomphus</i> sp.				1	5				1			**
Aeshnidae	<i>Anax</i> sp.		2	2									**
	<i>Aeshna</i> sp.			1									**
Libellulidae	<i>Trithemis</i> sp.		1	5	4	2						1	*

Table 4.7 continued

Hemiptera													
Veliidae	<i>Rhagovelia</i> sp.	9			1								*
Gerridae	<i>Neogerris</i> sp.											2	*
Paraphrynoveliidae	<i>Paraphrynovelia</i> sp.	2											
Corixidae	<i>Micronecta</i> sp.			1			4			2			*
	<i>Sigara</i> sp.		3	1			17	7	1	1			*
	<i>Agraptocorixa</i> sp.		2				1	6	8	17			*
Notonectidae	<i>Anisops</i> sp.								1				*
Pleidae	<i>Plea pullula</i>		2										*
	<i>Plea piccanina</i>	1							1	1			*
Naucoridae	<i>Neomacrocoris</i> sp.	1											**
	<i>Laccocoris</i> sp.					3		1					**
Belostomatidae	<i>Appasus</i> sp.	3	3					2				7	*
Trichoptera													
Hydropsychidae	<i>Cheumatopsyche</i> sp.		5	1	11	2			2				**
	<i>Cheumatopsyche thomasseti</i>		12	4	1	6							**
Hydroptilidae	<i>Hydroptila</i> sp.					1						24	**
Ecnomidae	<i>Ecnomus thomasseti</i>					2							**
Lepidoptera													
Crambidae	Schoenobiinae sp.	4				1							***
Diptera													
Tipulidae				2			3						*
Dixidae	Dixidae sp.	1				1							**
Chaoboridae	<i>Chaoborus</i> sp.		6									1	
Ceratopogonidae	<i>Bezzia</i> sp.			1	5	16				14	3		*
Culicidae	Culicinae sp.				2				1				*
Simuliidae	<i>Simulium adersi</i>		3		3	3	11	1	17				*
	<i>Simulium hargreavesi</i>	1				3							*

Table 4.7 continued

	<i>Simulium chatteri</i>					4			45				*
	<i>Simulium nigrirtarse</i>		9		37	15			11	1			*
Chironomidae	Chironominae	29	5	6	2	2	319	53	48		36	14	*
	Orthocladiinae			1			18	11	5				*
	Tanypodinae	1	43	10	21	21	4	38			2	2	*
Tabanidae		1			2			1					*
Dolichopodidae								1					
Ephydriidae				1						2			*
Muscidae				3				2		7	1		*
Coleoptera													
Gyrinidae	<i>Orectogyrus</i> sp.			55	107	7		16					*
	<i>Dineutus</i> sp.				3	2			1				*
Dytiscidae	<i>Dytiscidae</i> sp.								1				*
	<i>Derovatellus</i> sp.			26	2	2	1				1		*
	<i>Laccophilus</i> sp.							1					*
	<i>Neptosternus</i> sp.							1					*
	<i>Philodytes</i> sp.		1						1				*
	<i>Africophilus</i> sp.							1					*
	<i>Philaccolus</i> sp.	1			1				2			4	*
Hydrophilidae	<i>Amphiops</i> sp.											1	*
	<i>Berosus</i> sp.			1									*
	<i>Laccobius</i> sp.							1					*
	<i>Enochrus</i> sp.									1			*
Hydraenidae	<i>Ochthebius</i> sp.				2			1					**
	<i>Hydraena</i> sp.											5	**
Elmidae	Elminae sp.											1	**
Curculionidae	<i>Pseudobagous</i> sp.	1											
	<i>Bagous</i> sp.	2								11	1		

Table 4.7 continued

	Number of organisms (n)	235	809	675	828	384	1433	556	341	177	89	515	
	Species richness (SR)	23	29	30	32	32	19	27	28	16	20	19	
	Shannon-Wiener index (H')	2.0	1.7	2.2	2.2	2.6	1.4	2.4	2.5	1.7	2.2	0.9	
	Pielou's Evenness index (J')	0.7	0.5	0.7	0.6	0.8	0.5	0.7	0.8	0.6	0.7	0.3	

*Highly tolerant, **Moderately sensitive, ***Highly sensitive.

Table 4.8: Biodiversity list of all macroinvertebrates collected at each site during Survey 3, the number of organisms (n), the species richness (SR), Shannon-Wiener index (H'), Pielou's evenness index (J'), as well as the sensitivity scores.

Taxa		Site											Sensitivity
		1A	2A	3A*	4A	5A	1B	2B	3B	4B	5B	1C	
Cladocera													
Daphniidae	<i>Daphnia magna</i>						37	4					
	<i>Simocephalus</i> sp.						13						
Ostracoda													
Cypridoidea	<i>Parastenocypris junodi</i>	3											
Decapoda													
Atyidae	<i>Caridina nilotica</i>	95	31		249	2					40	179	**
Potamonautidae	<i>Potamonautes warreni</i>	1	15		20	7		1			3	3	*
Turbellaria													
Rhabdocoela	<i>Mesostoma</i> sp.					5	80	1					*
Nematoda									1				
Oligochaeta													
Tubificidae	<i>Tubifex</i> sp.	2	17		7	8	55	156	4	9	7		*
	<i>Branchiura</i> sp.							13					*
Hirudinea													
Glossiphoniidae	<i>Alboglossiphonia</i> sp.						1	1	1				*
	<i>Placobdelloides jaegerskioeldi</i>						19		2				*
Trombidiformes													
Eylaidae										1			**
Mollusca													
Lymnaeidae	<i>Lymnaea columella</i>									1			*
	<i>Lymnaea natalensis</i>	10	19			1			6	29	8	5	*
Ancylidae	<i>Burnupia mooiensis</i>	2	8		1	24	52			3		4	**
	<i>Ferrissia cawstoni</i>								7				**
Planorbidae	<i>Ceratophallus natalensis</i>		22		9					59	2		*

Table 4.8 continued

	<i>Gyraulus connollyi</i>	18	27						1	45	85	7	*
	<i>Gyraulus costulatus</i>	3			12	14							*
	<i>Bulinus africanus</i>							4	1		1		*
	<i>Bulinus depressus</i>		25			1			1	2			*
	<i>Bulinus tropicus</i>				2		46	12	2	4			*
Physidae	<i>Physa acuta</i>		3				2	2		11	1		*
Corbiculidae	<i>Corbicula fluminalis africana</i>	1	37		5				4				*
Sphaeriidae	<i>Pisidium costulosum</i>		8		2	22					1	3	*
	<i>Pisidium langleyanum</i>		11		2	2			1				*
	<i>Pisidium viridarium</i>	3	5		5	5				4			*
Ephemeroptera													
Baetidae	Baetidae sp.	65	5		120	70	55	1	40	18	32	47	**
	<i>Acanthiops erepens</i>							4					**
	<i>Acanthiops</i> sp.				21	32	19		80				**
Caenidae	<i>Caenis</i> sp.	10	21		113	28				1	6	26	**
Polymitarcyidae	<i>Povilla</i> sp.		2										**
Odonata													
Zygoptera													
Coenagrionidae	<i>Pseudagrion</i> sp.	136	31		7	2	8	18	1	59	21	37	*
	<i>Ceriagrion</i> sp.									9			*
	<i>Enallagma</i> sp.						1	1					*
Protoneuridae	<i>Elatoneura glauca</i>											1	**
Anisoptera													
Gomphidae	<i>Paragomphus</i> sp.					1							**
	<i>Notogomphus</i> sp.	1	1			9				1			**
Aeshnidae	<i>Anax</i> sp.	2	9							11	4	2	**
	<i>Aeshna</i> sp.		2										**
Libellulidae	<i>Orthetrum</i> sp.		1							2			*

Table 4.8 continued

	<i>Diplacodes</i> sp.		12							8			*
	<i>Trithemis</i> sp.	4	13							4		1	*
Hemiptera													
Mesoveliidae	<i>Mesovelia</i> sp.	5					1			9	1		*
Veliidae	<i>Rhagovelia</i> sp.	2					1						*
	<i>Tenagovelia</i> sp.				1								*
Gerridae	<i>Neogerris</i> sp.									1			*
	<i>Eurymetra</i> sp.	2	1										*
Corixidae	<i>Micronecta</i> sp.		1			1	254	1	5		1	3	*
	<i>Sigara</i> sp.		1										*
	<i>Agraptocorixa</i> sp.					7		9					*
Notonectidae	<i>Anisops</i> sp.						5						*
Pleidae	<i>Plea pullula</i>		1										*
	<i>Plea piccanina</i>									27			*
Naucoridae	<i>Naucoris</i> sp.	1											**
	<i>Laccocoris</i> sp.	1					48	1			1	14	**
	<i>Macrocoris</i> sp.		1								2	6	**
Nepidae	<i>Ranatra</i> sp.											1	*
	<i>Laccotrephes</i> sp.	1						2					*
Belostomatidae	<i>Appasus</i> sp.	10					5	12	7	13	1		*
Trichoptera													
Hydropsychidae	<i>Cheumatopsyche</i> sp.				12	18		24					**
	<i>Cheumatopsyche thomasseti</i>				10	13	49		63				**
Hydroptilidae	<i>Thricholeiochiton</i> sp.												**
	<i>Oxyethira</i> sp.												**
	<i>Hydroptila</i> sp.				1		101	6	1				**
Ecnomidae	<i>Ecnomus thomasseti</i>	1				8			1				**

Table 4.8 continued

Lepidoptera													
Crambidae	Schoenobiinae sp.									1			***
Diptera													
Psychodidae	<i>Clogmia albipunctata</i>									1			*
Dixidae	<i>Dixa</i> sp.											2	**
Ceratopogonidae	<i>Bezzia</i> sp.	1								2			*
Culicidae	Culicinae sp.									9		1	*
	Anophelinae sp.	11							1	3			*
Simuliidae	<i>Simulium adersi</i>				16	5	61	51	207	1			*
	<i>Simulium ruficorne</i>								4				*
	<i>Simulium chatteri</i>				3	15			74			1	*
	<i>Simulium nigrirtarse</i>				9	4			54				*
	<i>Simulium rotundum</i>				1								*
Chironomidae	Chironominae	40	54				21	2	64	9	25	6	*
	Orthocladiinae	5	1		8	4		2	31		3	1	*
	Tanypodinae	1	21		7	4	14	2	68	12	6	9	*
Stratiomyidae					1								
Empididae						1							**
Ephydriidae					1			17	2			1	*
Muscidae			5		1	2	27	2				1	*
Coleoptera													
Gyrinidae	Gyrinidae sp.								1				*
	<i>Aulonogyrus</i> sp.				4		13	62					*
Haliplidae	<i>Haliphus</i> sp.				1				1				*
Dytiscidae	<i>Derovatellus</i> sp.	1	2		4		1						*
	<i>Laccophilus</i> sp.		1							1			*
	<i>Philodytes</i> sp.				1								*
	<i>Philaccolus</i> sp.	1							1				*

Table 4.8 continued

	<i>Canthyporus</i> sp.									2			*
Hydrophilidae	<i>Amphiops</i> sp.											1	*
	<i>Berosus</i> sp.	1	1						1	2			*
	<i>Laccobius</i> sp.							1				1	*
Hydraenidae	<i>Ochthebius</i> sp.	3	1						1	2			**
	<i>Hydraena</i> sp.				2				1				**
	<i>Discozantaena</i> sp.		1										**
	<i>Parasthetops</i> sp.							2					**
Elmidae	Elminae sp.	1			62	1				1			**
	<i>Leptelmis</i> sp.				1								**
Chrysomelidae	<i>Donicia</i> sp.	1											
Curculionidae	<i>Bagous</i> sp.									4		1	
	<i>Stenopelmus</i> sp.									1			
	Number of organisms (n)	445	417		721	316	989	414	741	381	251	364	
	Species richness (SR)	35	37		35	30	27	29	36	38	21	27	
	Shannon-Wiener index (H')	2.3	3.0		2.3	2.8	2.6	2.2	2.4	2.9	2.2	1.9	
	Pielou's Evenness index (J')	0.6	0.8		0.6	0.8	0.8	0.7	0.7	0.8	0.7	0.6	

*Highly tolerant, **Moderately sensitive, ***Highly sensitive, Site 3A dry.

Figure 4.2 shows the mean with standard error of the number of specimens (n), species richness (SR), Shannon-Wiener diversity index (H') and Pielou's evenness index (J') for each site, over the three surveys. The mean number of organisms ranged between 160 at Site 1B and 140 at Site 5B (Figure 4.2A). After a one-way ANOVA was conducted, it is further evident that Site 1B differed significantly from Site 4B, as well as Site 5B, with regard to the number of specimens, as these sites showed the lowest number of specimens collected throughout the three surveys. This may be an indication of the suitability of Site 1B, in relation to species survival and reproduction.

Although it is well known that the Wonderfontein Spruit is highly impacted and transformed due to mining activities (DWS, 2014; Hamman, 2012; Van Veelen, 2009), it is evident that Site 1B yielded the highest number of organisms. This may be attributed, again, to the suitability of Site 1B, due to a variety of biotopes, as well as the availability of an adequate supply of nutrients, a surmise which is further supported by the elevated EC values for this site during the three surveys (Figure 4.1B; Tables 4.2, 4.3 and 4.4). The finding that Site 5B showed the lowest mean species richness (SR = 20.0) was probably due to the lack of nutrient sources, as this site is one of the springs in the Mooi River catchment, with naturally low organic enrichment.

The highest mean SR was calculated for Sites 2A and 3B (SR = 32.0) and can possibly be ascribed to the diversity of suitable biotopes present at both sites. Site 3B also showed the highest mean biodiversity index value ($H' = 2.5$). This high value can most probably also be ascribed to the diversity of biotopes at this site, as it was characterised by stones in streambed, marginal and aquatic vegetation, algae, stones in current, stones out of current, run, riffle and pool biotopes. The low mean values calculated for biodiversity ($H' = 1.4$) and species evenness ($J' = 0.4$) at Site 1C could be ascribed to the abundance of *Caridina nilotica* (family Atyidae). The highest species evenness was calculated for Site 5B, the Turffontein Spring, indicating an even distribution of species and an absence of any dominant species.

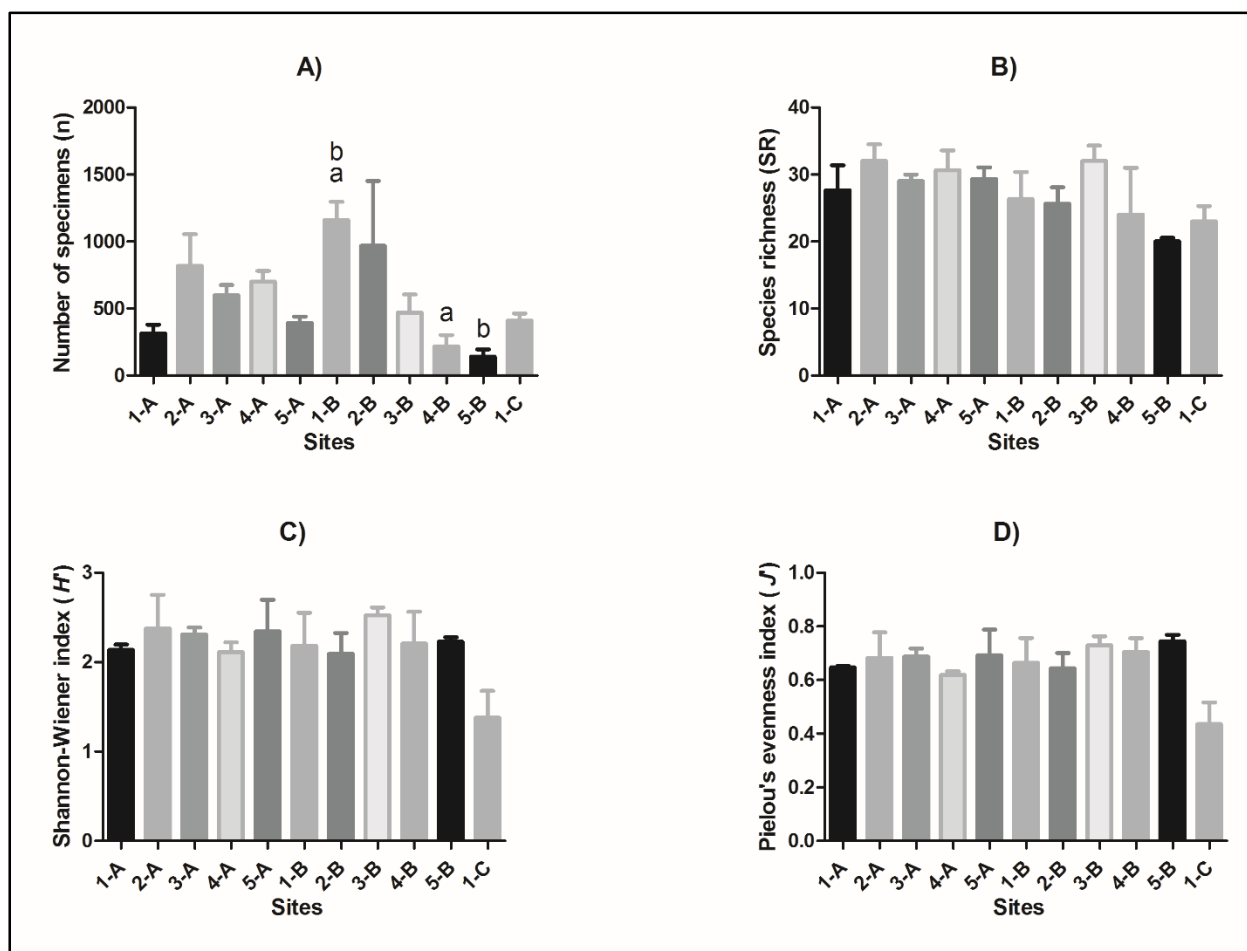


Figure 4.2: The mean with standard error of the A) number of specimens, B) species richness, C) Shannon-Wiener diversity index and D) Pielou's evenness index at each of the selected sites, over the three surveys. Common superscripts indicate significant difference. Bars with similar superscript differ significantly ($p < 0.05$).

Regarding the percentage composition of highly sensitive, moderately sensitive and highly tolerant taxa collected throughout the study (Figure 4.3, 4.4 and 4.5), the majority were classified as highly tolerant. The family Crambidae, specifically the subfamilies Schoenobiinae and Nymphulinae, were the only highly sensitive taxa collected during this study. Only 94 of the 104 taxa collected during Survey 1 were allocated with sensitivity scores based on the SASS 5 sensitivity values. Approximately 70 % of the 94 macroinvertebrate taxa collected during this survey were classified as highly tolerant, 28.7 % as moderately sensitive and 2.1 % as highly sensitive (Figure 4.3). During the first survey, Nymphulinae were collected at Sites 5A and 1C, while Schoenobiinae were collected at Site 4A (Table 4.6). The presence of this family at Sites 4A and 1C can possibly be ascribed to the low organic enrichment and availability of suitable biotopes (i.e. marginal and aquatic vegetation) (Henning, 2003; Thirion, 2007) at these sites.

Although organic enrichment occurred at Site 5A, it was probably not to such an extent as to exclude the highly sensitive subfamily Nymphulinae. From Figure 4.3 it is further evident that the majority of macroinvertebrates collected at Site 2B were classified as highly tolerant and included Daphniidae and Hydridae, both families tolerant to lower water quality (Ebert, 2005; Griffiths *et al.*, 2015).

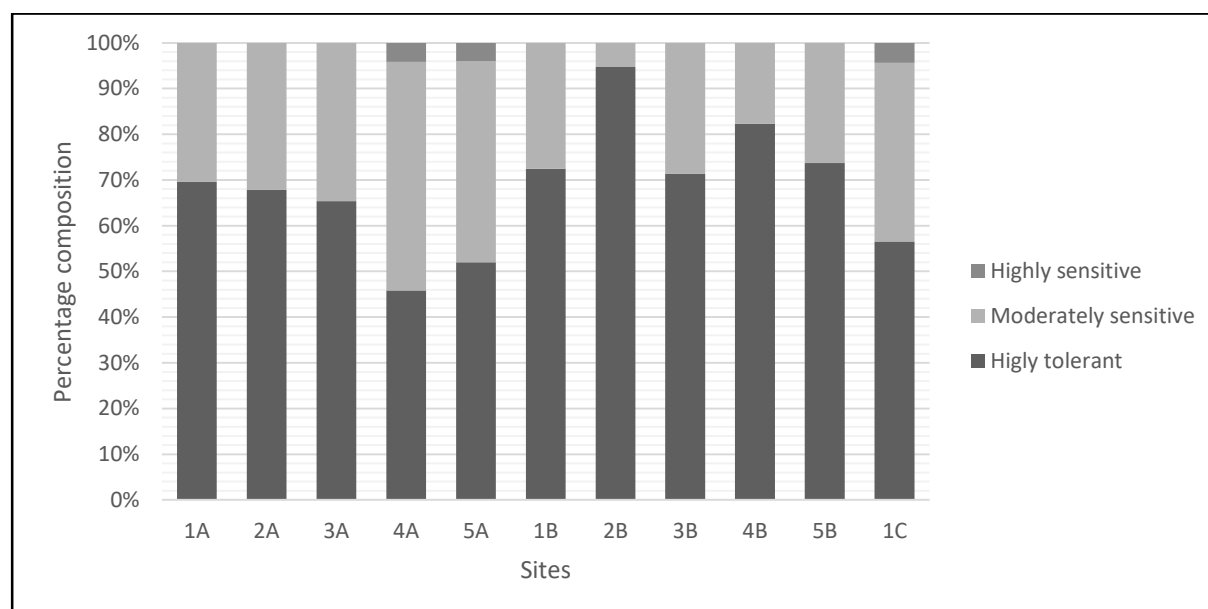


Figure 4.3: Percentage composition of highly sensitive, moderately sensitive and highly tolerant taxa collected during Survey 1.

During the second survey, only 84 of the 91 taxa collected were allocated with a sensitivity scores, of these 70.2 % were classified as highly tolerant, 28.6 % as moderately sensitive and 1.2 % as highly sensitive (Figure 4.4). Schonobiinae was collected at Sites 1A and 5A during this survey (Table 4.7). The occurrence of this family at Site 1A can be attributed to the availability of abundant marginal and aquatic vegetation, the preferred biotope of this family (Henning, 2003; Thirion, 2007). Regarding Site 5A, the levels of organic enrichment was probably not to such an extent as to exclude the highly sensitive family, Schoenobiinae. Figure 4.4 shows that the majority of taxa collected at Sites 1B, 2B and 4B were classified as highly tolerant taxa and included Daphniidae, Physidae, and Chironomidae (Table 4.7). These three families are also tolerant to organically enriched waters (Brown, 1994; Ebert, 2005; Griffiths *et al.*, 2015; Thirion, 2007), conditions encountered at Sites 1B, 2B and 4B.

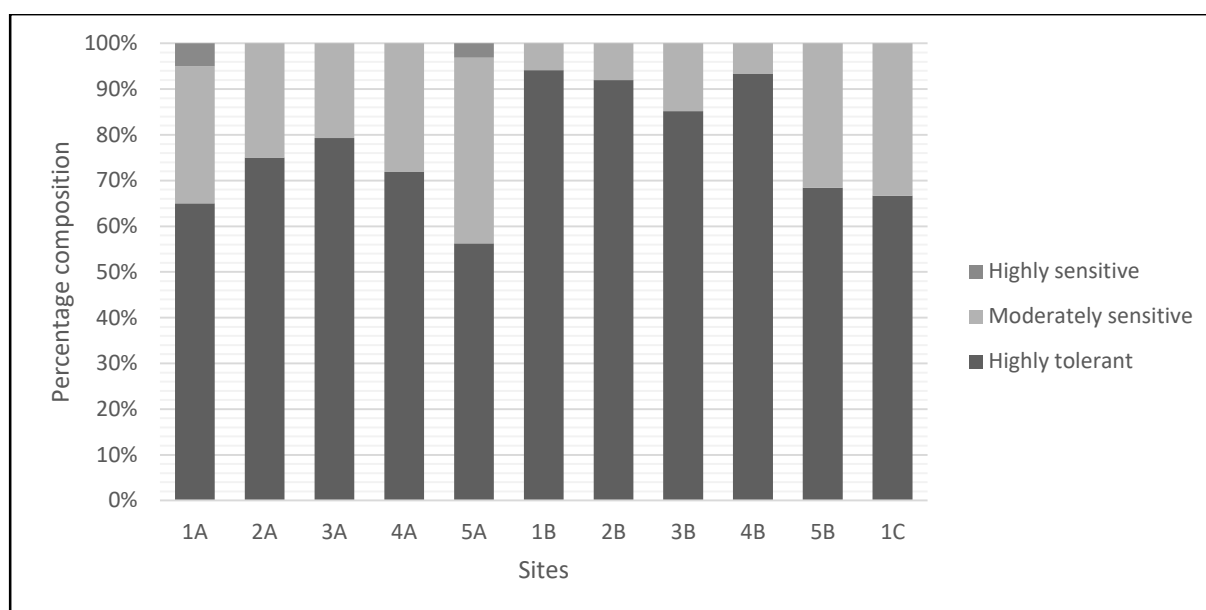


Figure 4.4: Percentage composition of highly sensitive, moderately sensitive and highly tolerant taxa collected during Survey 2.

A total of 104 taxa was collected during the last survey and sensitivity scores were allocated to only 96. From the 96 taxa, 66.7 % were classified as highly tolerant, 32.3 % as moderately sensitive and 1.0 % as highly sensitive (Figure 4.5). In contrast to the literature (Dickens & Graham, 2002; Thirion, 2007), the highly sensitive subfamily, Schoenobiinae, was collected at Site 4B (Table 4.4), a highly organic enriched site. No plausible explanation for the occurrence of Schoenobiinae at this site could be found.

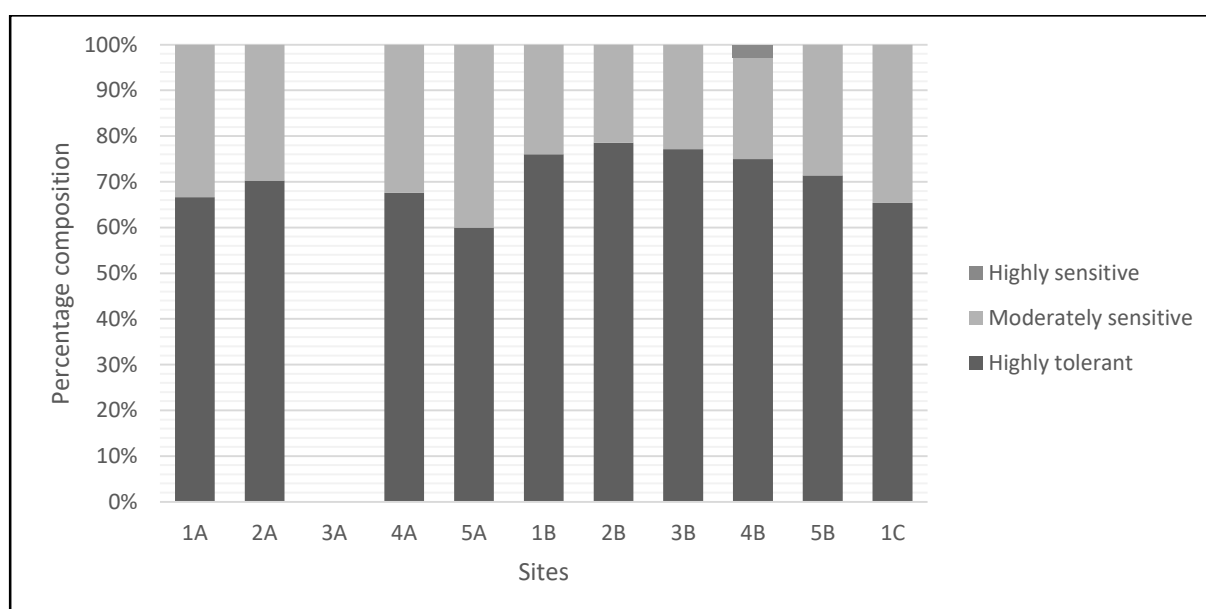


Figure 4.5: Percentage composition of highly sensitive, moderately sensitive and highly tolerant taxa collected during Survey 3 (Site 3A dry).

4.3.3 Spatial and temporal macroinvertebrate community structure

Figure 4.6 shows a RDA tri-plot illustrating associations between macroinvertebrates, selected abiotic factors and biotopes of all three surveys. Based on the RDA, it is evident that some biotopes and abiotic factors were the most important factors determining the distribution of the families collected during this study. A strong association between Simuliidae and flow-rate, riffle, stones in current and run biotopes were found. According to Thirion (2007) and De Moor (2003), these macroinvertebrates are best adapted to life in fast flowing water and often lodges itself on cobbles, stones or submerged and floating vegetation. Hydropsychidae predominantly associated with flow-rate, and to a lesser extent with the riffle and stones in current biotopes. This family is commonly found on and around stones and cobbles and are specialised for rheophilic (fast-flowing) waters (De Moor & Scott, 2003; Thirion, 2007). The RDA shows that pH had a limited effect on the dispersal of families and had limited associations between some sites and surveys, whereas flow-rate and EC showed to have the greatest influence on the families. A spatial, rather than a temporal variation occurred. Associations were found between Tubificidae, Daphniidae, Glossiphoniidae and Corixidae and Sites 1B and 2B, both during Survey 2, as well as Site 3B, during all three surveys. These associations may possibly be attributed to the higher numbers of these families present at these sites during the second survey. These four families are also tolerant to lower water quality and organically enriched waters (Ebert, 2005; Thirion, 2007), conditions encountered at Sites 1B, 2B and 3B. An association is also evident between EC and Site 2B, Survey 1. This association may be ascribed to the fact that the highest EC value was measured at this site during Survey 1 (Table 4.2). In the bottom right quadrant, turbidity associated with Site 4B due to the fact that the highest values of turbidity (27.0 NTU) was measured at this site.

The family Curculionidae strongly associated with the pool biotope, as well as Site 4B, Survey 2. These aquatic weevils are considered as phytophilous and are found in still or very slow moving water (Stals, 2007b), which was also the case at Site 4B where the highest number of these beetles were collected. From Figure 4.6, it is further evident that associations were found between Sites 1C (Surveys 1 and 3) and 5B (all three surveys), temperature, as well as aquatic vegetation and to a lesser extent, the pool biotope. This association can be explained by the fact that the highest temperatures were recorded at both Sites 5B and 1C (Figure 4.1; Tables 4.2, 4.3 and 4.4), and that aquatic vegetation was present at these sites during all three surveys. The pool biotope

were also present at these sites. Further associations were found between stones out of current, Sites 1A (all three surveys), as well as the family Atyidae. This site associated with the stones out of current biotope due to the fact that this biotope was present at Site 1A during all three surveys. The family Atyidae was collected in the largest numbers at Sites 1A, and according to Dallas (2007), can be found in the stones biotope. Baetidae, Hydrachnidae and, to a lesser extent, Caenidae, associated with marginal vegetation in the upper left quadrant. According to the literature (Barber-James & Lugo-Ortiz, 2003; Jansen van Rensburg & Day 2002; Thirion, 2007) these macroinvertebrates can populate this biotope. Baetidae strongly associated with Site 3A, Survey 1, as some of the highest numbers of this family were collected at this site during this survey.

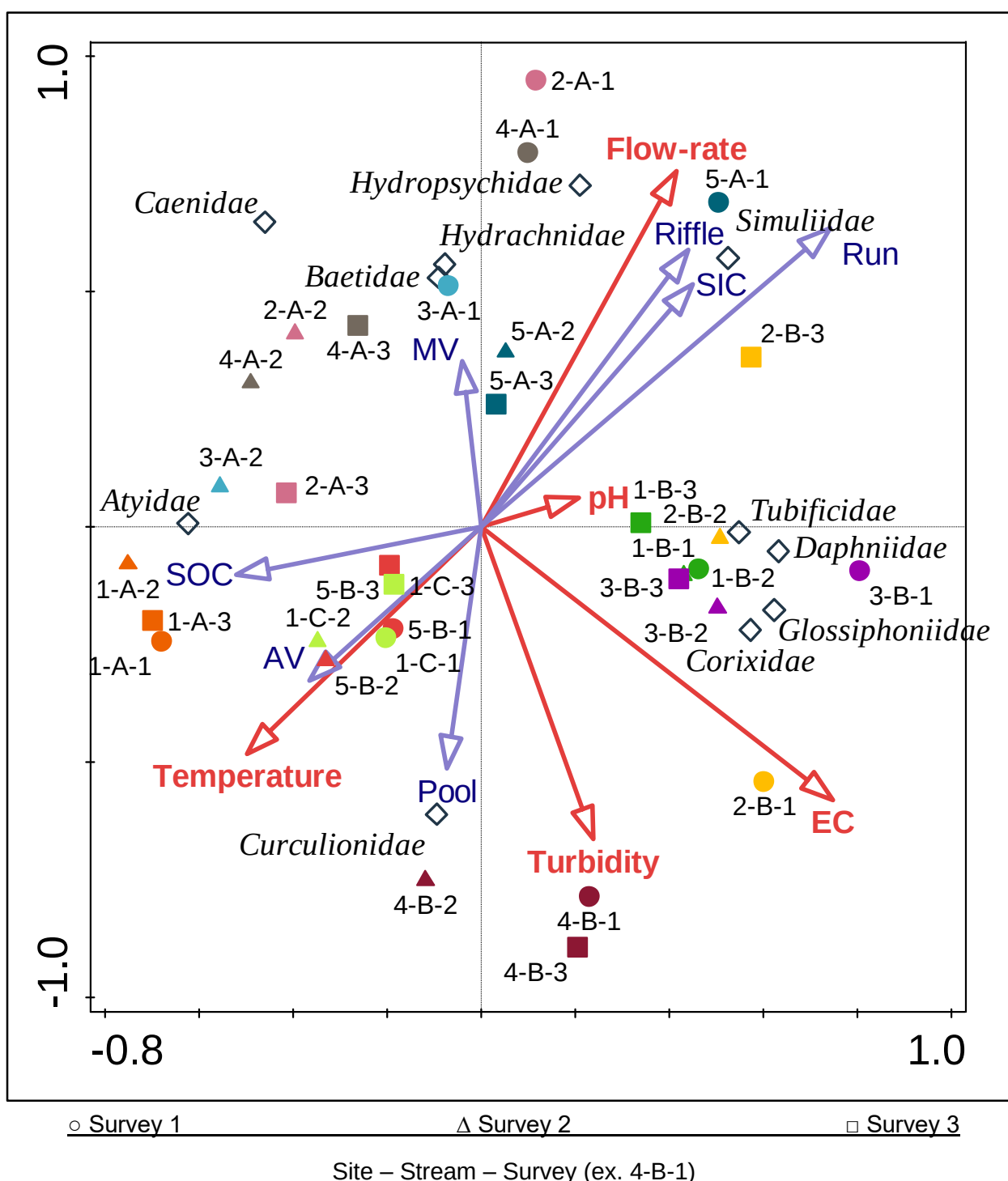


Figure 4.6: RDA tri-plot (with supplementary variables) illustrating associations between macroinvertebrate families, electrical conductivity (EC), turbidity, flow-rate, pH, temperature, riffle, run, stones in current (SIC), stones out of current (SOC), pool, marginal vegetation (MV) and aquatic vegetation (AV) biotopes, from Surveys 1, 2 and 3. The tri-plot describes 27.1 % of the variation, with 15.7 % on the first axis and 11.4 % on the second axis.

4.4 Conclusion

From this study it is evident that some variation occurred with regard to the families found between the various sampling sites, as well as the three surveys. These

variations can most probably be attributed to a combination of physico-chemical conditions, which include limited variation in water parameters, the availability of suitable habitats and biotopes, and variation in macroinvertebrate communities. Different biotope types and selected parameters (specifically flow-rate and EC) played a prominent role in the distribution of the families in the Mooi River catchment area.

The majority of the families collected throughout this study were classified as highly tolerant. Only one highly sensitive family was collected during this study, indicating that organic enrichment is present throughout the catchment area. Although the levels of organic enrichment observed at some sites during this study could be advantageous for those families known to be tolerant to, or have a preference for such environments or conditions, these levels were probably not to such an extent as to exclude the less tolerant families from these sites. From these observations it is evident that, although various springs feed the Mooi River catchment, the waters of this catchment is organically enriched. The highest SR and number of organisms were collected at sites impacted by mining effluent (Sites 1B, 2B and 3B) and therefore, the hypothesis that anthropogenic activities in the Wonderfontein Spruit has a detrimental effect on the macroinvertebrate assemblages, is rejected.

Chapter 5: The accumulation of metals by macroinvertebrates

5.1 Introduction

The ability of macroinvertebrates to accumulate metals in aquatic environments is well known (Goodyear & McNeil, 1999; Rainbow, 2002; Rainbow 2007). Different taxa of macroinvertebrates can, due to specific morphological or physiological features, accumulate metals in different concentrations in their tissues or by adsorption onto their bodies (Eisler, 1981; Goodyear & McNeill, 1999; Hoffman *et al.*, 2003; Rainbow, 2002). Therefore macroinvertebrates inhabiting the same environment may have very different concentrations of metals in the organism itself (Hare, 1992). Macroinvertebrates may accumulate metals from various sources, including from the surrounding water, sediments, as well as from the food they consume (Wang & Fisher, 1999).

The degree of accumulation of waterborne metals is influenced by various factors, which include, amongst others, the concentrations to which an organism is exposed to, the presence of metals bound to suspended particles and the species of the specific metal (Hoffman *et al.*, 2003). In the aquatic environment, potential absorption sites of contaminants, such as metals, include the skin, gills and digestive tract of the organism (Hoffman *et al.*, 2003). The accumulation of sediment-related metals is, on the other hand, influenced by the metal-sediment interaction, as well as processes regarding uptake and exposure, where routes of uptake may include ingestion of sediment or organic particles, contact with interstitial water, as well as direct contact with sediments (Hoffman *et al.*, 2003). The uptake of metals may also be influenced by the feeding habits of macroinvertebrates and can be classified into the following Functional Feeding Groups (FFGs): filter-feeders (F-F), scraper-grazers (S-G), collector-gatherers (C-G), shredders (Sh) and predators (Pr) (Begon *et al.*, 1990; Cummins, 1973; Cummins & Klug, 1979; Goodyear & McNeill, 1999; Ramirez & Gutiérrez-Fonseca, 2014; Wallace & Webster, 1996).

The following hypotheses will be tested during this chapter:

1. Trophic transfer of metals will be reflected in the FFGs, with higher concentrations in the predators compared to collector-gatherers and scraper-grazers.
2. Macroinvertebrates associating with the benthic environment, will display higher metal concentrations than those associated with the water column.

The aim of this chapter was thus to determine the accumulation potential of metals by the above mentioned feeding groups in the benthos, as well as in the water column.

5.2 Material and methods

5.2.1 Macroinvertebrate collection identification

For the methods on the collection and identification of macroinvertebrates, refer to Chapter 4, where these methods are discussed in detail. After sampling and identification, macroinvertebrates collected from Site 1B, during Survey 3, conducted in May 2015 (Figure 2.1), were used for metal concentration analysis. This site was selected based on a number of factors, including the presence of a large diversity of macroinvertebrate taxa, agricultural activities in the surrounding area, as well as mining effluent.

5.2.2 Laboratory methods

5.2.2.1 Macroinvertebrate digestion

The digestion of macroinvertebrates is based on methods and techniques described in Wolmarans & Van Aardt (1985). The samples were selected from the taxa Coenagrionidae, Notonectidae, Naucoridae, Belostomatidae, Muscidae, Gyrinidae, Planorbidae, Baetidae, Simuliidae, Chironomidae, Tubificidae, Hydropsychidae, Rhabdocoela, Hirudinea and Corixidae. Organisms were pooled to provide sufficient biomass for three replicates per family. Samples were weighed and transferred to a multiple cell Teflon container consisting 100 digestion cells, each with a diameter of 9 mm and depth of 21 mm. Each cell was provided with a tight-fitting lid. After each sample was added to a digestion cell, 1 ml of 65 % nitric acid was added, where after the samples were digested under pressure at 70 °C for 24 hours. After digestion, the samples were diluted with MiliQ water and analysed with an ICP-MS to determine the metal concentrations. Metal concentrations were expressed as µg/g wet weight. Certified reference materials (Certified Reference Material for Mussel Tissue (ERM-CE278k)) were used and standard calibration runs were performed. The percentage recoveries for the standards and certified reference materials were within an acceptable range (< 10% deviation) (Table 5.1).

Table 5.1: Metal concentrations ($\mu\text{g/g}$) recovered from Certified Reference Material (CRM).

	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

5.2.3.1 Data compilation

For the purpose of this study, macroinvertebrate taxa were sorted into the following different FFGs, as well as according to their primary biotope preferences (i.e. benthic or the water column) (Appleton, 2002a; Appleton, 2002b; Barber-James & Lugo-Ortiz, 2003; Brown, 1994; Dallas & Day, 2004; De Moor, 2003; De Moor & Scott, 2003; Goodyear & McNiell, 1999; Harrison, 2003; Harrison *et al.*, 2003b; Oosthuizen & Siddall, 2002; Ramirez & Gutiérrez-Fonseca, 2014; Reavell, 2003; Samways & Wilmot, 2003; Stals, 2007a; Thirion, 2007).

1. Filter-feeder (F-F): An organism with modified structures or mouth parts, which feed on suspended particles in the water column.
2. Scraper-grazer (S-G): An organism with mouth parts modified to scrape resources, such as algae, micro-organisms and films off substrates.
3. Collector-gatherer (C-G): An organism with adapted mouth parts to collect or gather small particles on bottom sediments.
4. Shredder (Sh): An organism adapted to cut and crush coarse particles of organic matter and leaf litter.
5. Predator (Pr): An organism consuming other living organisms.

Table 5.2: The Functional Feeding Groups (FFGs) of selected macroinvertebrate taxa and their primary biotope preferences.

Taxa	Functional Feeding Groups (FFGs)					Habitat	
	Filter-feeder (F-F)	Scraper-grazer (S-G)	Collector-gatherer (C-G)	Shredder (Sh)	Predator (Pr)	Pelagic	Benthic
Coenagrionidae					X	X	
Notonectidae					X	X	
Naucoridae					X	X	
Belostomatidae					X	X	
Muscidae					X	X	
Gyrinidae					X	X	
Planorbidae		X				X	X
Baetidae			X			X	X
Simuliidae	X						X
Chironomidae	X						X
Tubificidae			X				X
Hydropsychidae			X	X			X
Rhabdozoa					X		X
Hirudinea					X		X
Corixidae					X		X

5.2.3.2 Univariate and multivariate statistical analyses

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-Lillie for P value), respectively. When data were parametric, analysis of variance (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. Graphical presentations of the data were performed using GraphPad Prism (mean and standard error). A Principal Component Analysis (PCA) was conducted by making use of Canoco (version 5) to determine the possible associations between macroinvertebrate taxa, metal concentrations, FFGs, as well as the two primary habitats.

5.3 Results and discussion

5.3.1 Metal concentrations in macroinvertebrates

It is evident that all the metal concentrations measured in the family Simuliidae were markedly higher than those measured in any other macroinvertebrate family, followed by the family Chironomidae (Figures 5.1 to 5.6; Table B1 (Appendix B)). The concentrations of Ti, Co, Ni, As and Pb measured in the family Simuliidae were significantly higher than the majority of the remaining macroinvertebrate families ($p < 0.05$). Both the families Simuliidae and Chironomidae are primarily filter-feeders on particles suspended in water (De Moor, 2003; Goodyear & McNiel, 1999; Harrison, 2003; Griffiths *et al.*, 2015). These aquatic larvae (Simuliidae and Chironomidae) mostly feed on diatoms, algae, microscopic invertebrates, as well as bacteria (De Moor, 2003; Harrison, 2003). Due to the fact that metals readily adsorb onto the above mentioned suspended particles (Binning & Baird, 2001; Inengite *et al.*, 2010; Lin & Chen, 1998; Madkour *et al.*, 2011; Newman & Watling, 2007; Vuković *et al.*, 2011), filter feeding may be the primary route for ingestion of metals by these families and probably the reason for the elevated metal concentrations. However, the high concentrations of metals found in Simuliidae may also be attributed to the fact that the suspended particles may become lodged in the cephalic fans of these macroinvertebrates' feeding structures. As it is well-known that metals can accumulate in sediments (Eggleton & Thomas, 2004; Jain & Ram, 1997; Lin & Chen, 1998; Smith, 1999; Westrup *et al.*, 2005; Yao *et al.*, 2015), it was not surprising that the family Chironomidae, which prefers the GSM biotopes, had high metal concentrations as demonstrated during this study.

Coenagrionidae and Rhabdocoela also showed some of the highest metal concentrations (Figures 5.1 to 5.6), when compared to the remaining macroinvertebrate taxa. These concentrations were, however, not significantly higher ($p > 0.05$). Both taxa are considered as predators, feeding on other, smaller macroinvertebrates, including protozoans, insect larvae, small crustaceans and oligochaetes (Appleton, 2002a; Griffiths *et al.*, 2015; Samways & Wilmot, 2003). The family Coenagrionidae has up to 17 instar larval stages (Samways & Wilmot, 2003), which may have contributed to the high metal concentrations measured in this family, as they are exposed to the conditions (metal concentrations) in the water for a prolonged period of time. Regarding the taxon Rhabdocoela, the elevated metal concentrations may be attributed to its close

association with sediments, where these macroinvertebrates slowly “glide” over the substratum through cilia located on the epidermis (Appleton, 2002a).

Low concentrations of most of the metals were present in macroinvertebrates generally associated with the water column, however, Hirudinea, which are predominantly benthic organisms, were also amongst those that presented with the lowest metal concentrations (Figures 5.1 to 5.6). Moderate to high concentrations of Co, Ni, Cu, and Pb were measured in this taxon. The lowest concentrations of the majority of the metals (Al, Cr, Fe, Co, Ni, Zn, As and Cd) were measured in the family Gyrinidae, which may be attributed to the fact that these aquatic beetles are usually found swimming on the water surface and are only submerged for short periods at a time (Stals, 2007a). Low concentrations of the remaining metals (Ti, Mn, Cu, and Pb) were also measured in the taxa Muscidae, Planorbidae, Belostomatidae and Hirudinea. Except for the family Planorbidae, the remaining families are all categorized as predators (Table 5.2), suggesting that a biomagnification phenomenon does not take place in these instances, as the majority of these metals do not biomagnify in the food chain (Rainbow, 2002; Yu *et al.*, 2013). The varying metal concentrations measured in the different macroinvertebrates belonging to different FFGs, may be ascribed to various factors which include, amongst others, the different diet requirements of the organism within a feeding group, the specific metal species involved, the length of life stages and the age of the organism, as well as the variation in physico-chemical factors (Goodyear & McNiell, 1999).

With regard to the metals accumulated in the highest concentrations (Al, Mn, Fe and Zn) (Figure 5.1A, 5.2B, 5.3A and 5.5A), it is well known that Al is generally accumulated on ion-regulatory surfaces, and is usually not biomagnified (Gensemer & Playle, 1999). The high concentrations of Al generally present in most of the families may be ascribed to the adsorption onto the iron-regulatory surfaces. The uptake of Mn by aquatic macroinvertebrates are influenced by temperature and pH, where the uptake of Mn increases with temperature and decreases with pH (Howe *et al.*, 2004). In the study of Dittman and Buchwalter (2010) it was found that Mn is absorbed onto tissues through calcium transporters. The uptake of Fe by aquatic invertebrates occurs either through water or food sources, but is not readily absorbed through the gastro-intestinal tract (Vuori, 1995). According to Eisler (1993) and Ward *et al.* (1986) the concentrations of Zn may be elevated in the tissues of aquatic organisms near point sources of

anthropogenic activities, which may be the case during this study at the selected site (Site 1B, Figure 2.1 and Table 2.6). Although all the metals measured during this study may be potentially toxic to macroinvertebrates (DWAF, 1996; Jonh & Leventhal, 1995; Tchounwou *et al.*, 1995), these concentrations may not have had a harmful effect, possibly due to mechanisms, which include metabolism, detoxification and sequestration, as well as elimination (Ahearn *et al.*, 2004; Hoffman *et al.*, 2003 Phillips & Rainbow, 1989).

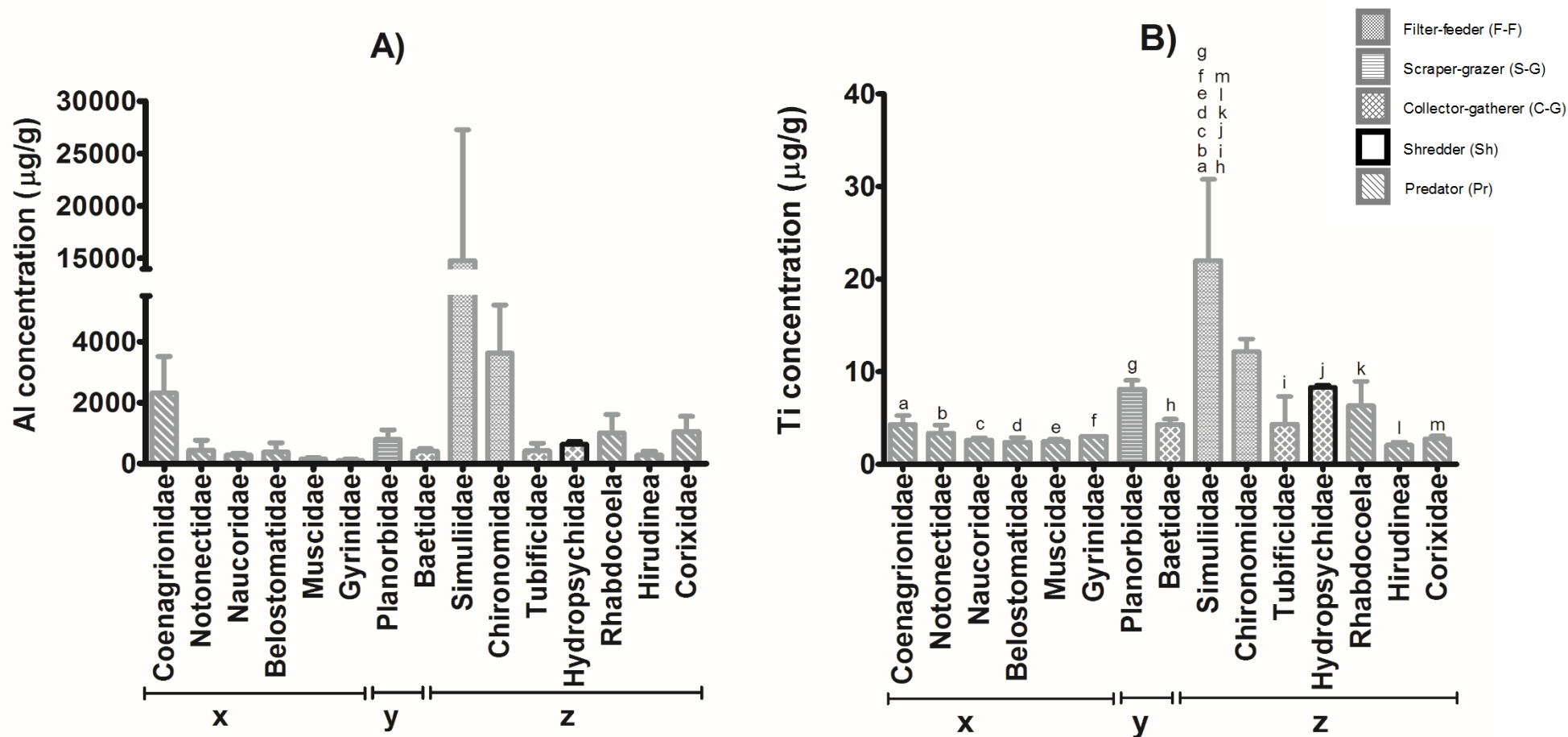


Figure 5.1: Mean and standard error of A) Al and B) Ti concentrations (µg/g) measured in the selected macroinvertebrate taxa (x = pelagic macroinvertebrates; y = no specific preference with regard to water or benthic habitats; z = benthic macroinvertebrates). Common alphabetical superscripts indicate significant difference ($p < 0.05$).

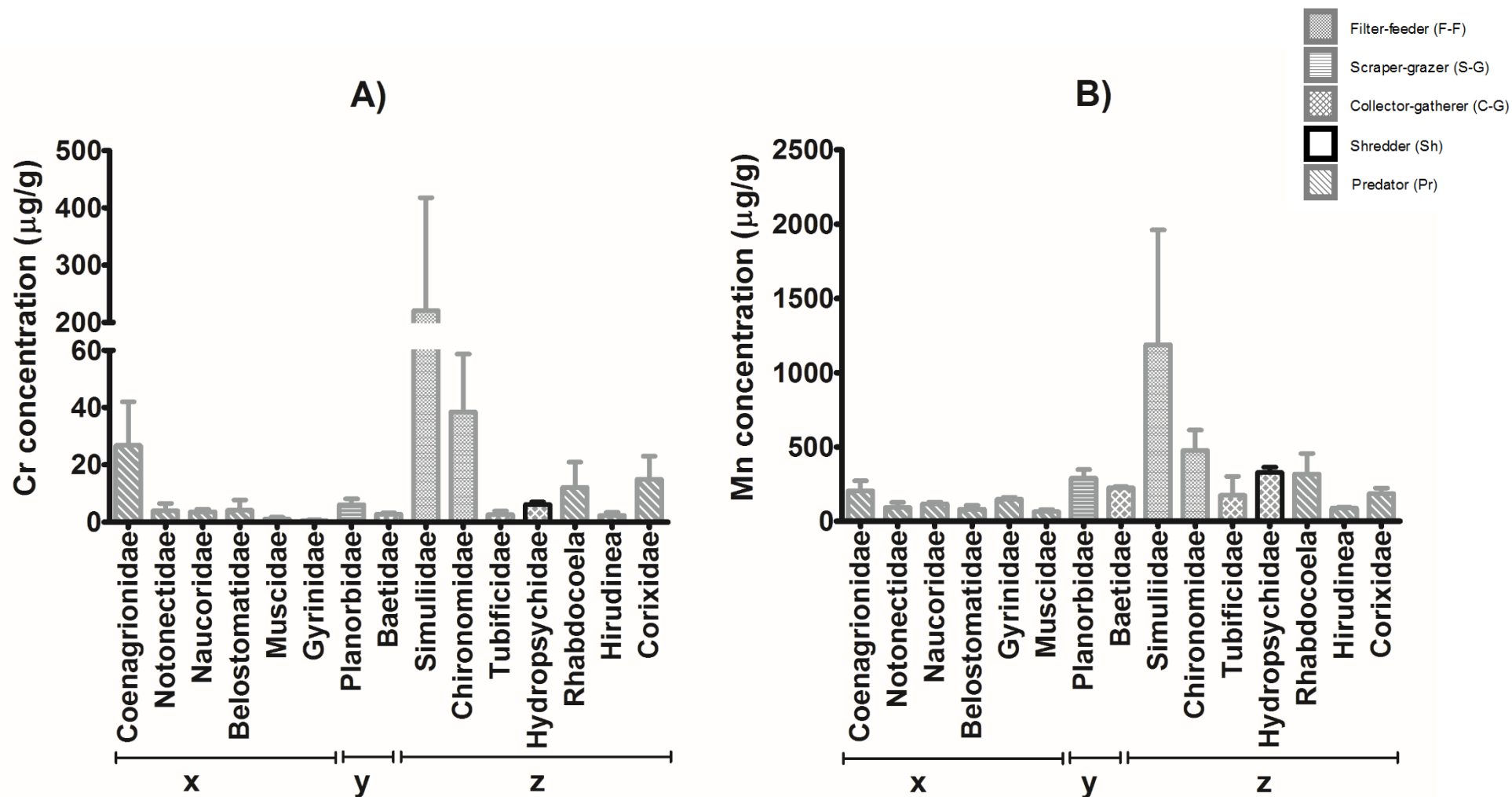


Figure 5.2: Mean and standard error of A) Cr and B) Mn concentrations ($\mu\text{g/g}$) measured in the selected macroinvertebrate families taxa (x = pelagic macroinvertebrates; y = no specific preference with regard to water or benthic habitats; z = benthic macroinvertebrates). Common alphabetical superscripts indicate significant difference ($p < 0.05$).

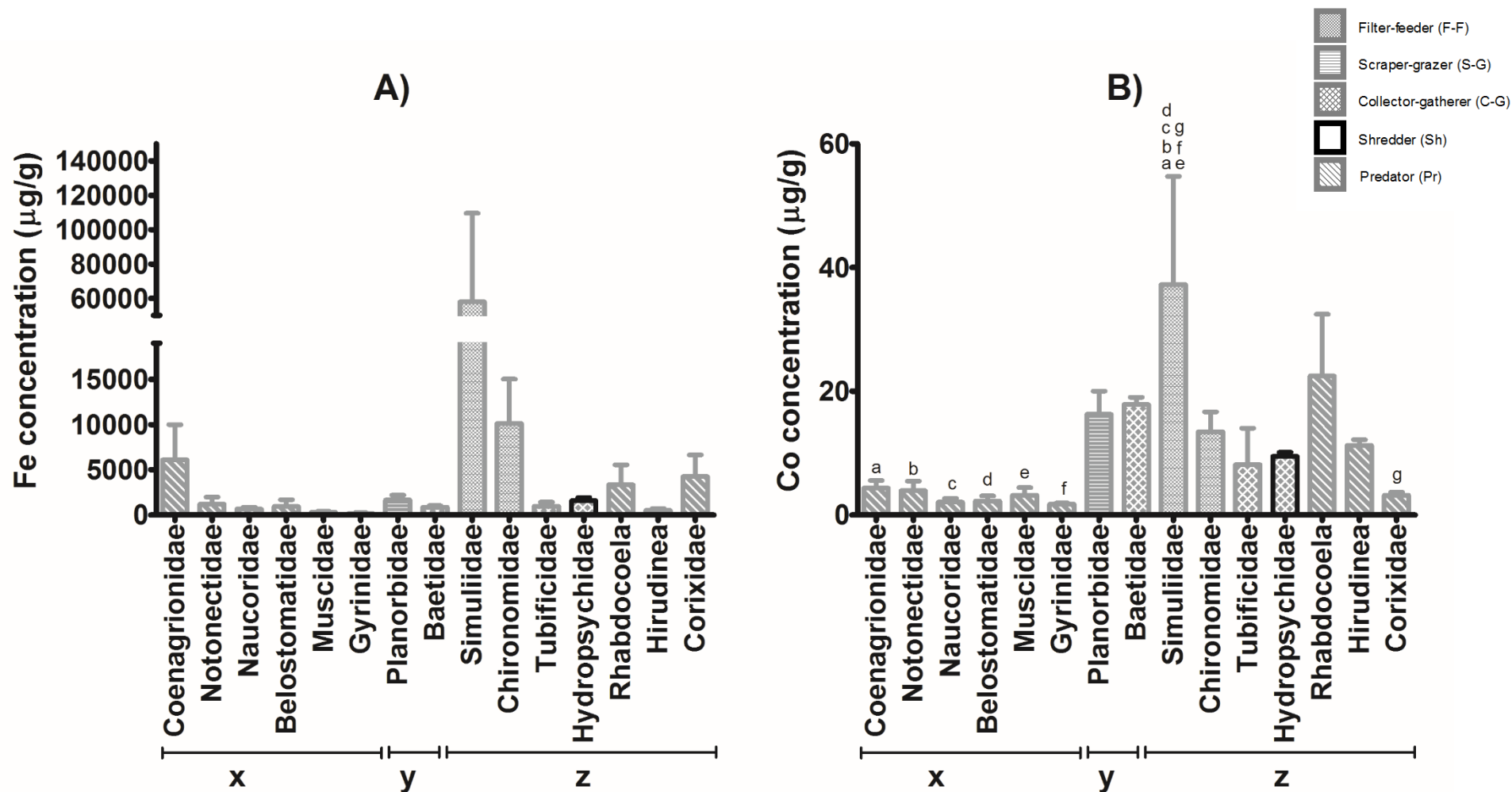


Figure 5.3: Mean and standard error of A) Fe and B) Co concentrations (µg/g) measured in the selected macroinvertebrate families taxa (x = pelagic macroinvertebrates; y = no specific preference with regard to water or benthic habitats; z = benthic macroinvertebrates). Common alphabetical superscripts indicate significant difference ($p < 0.05$).

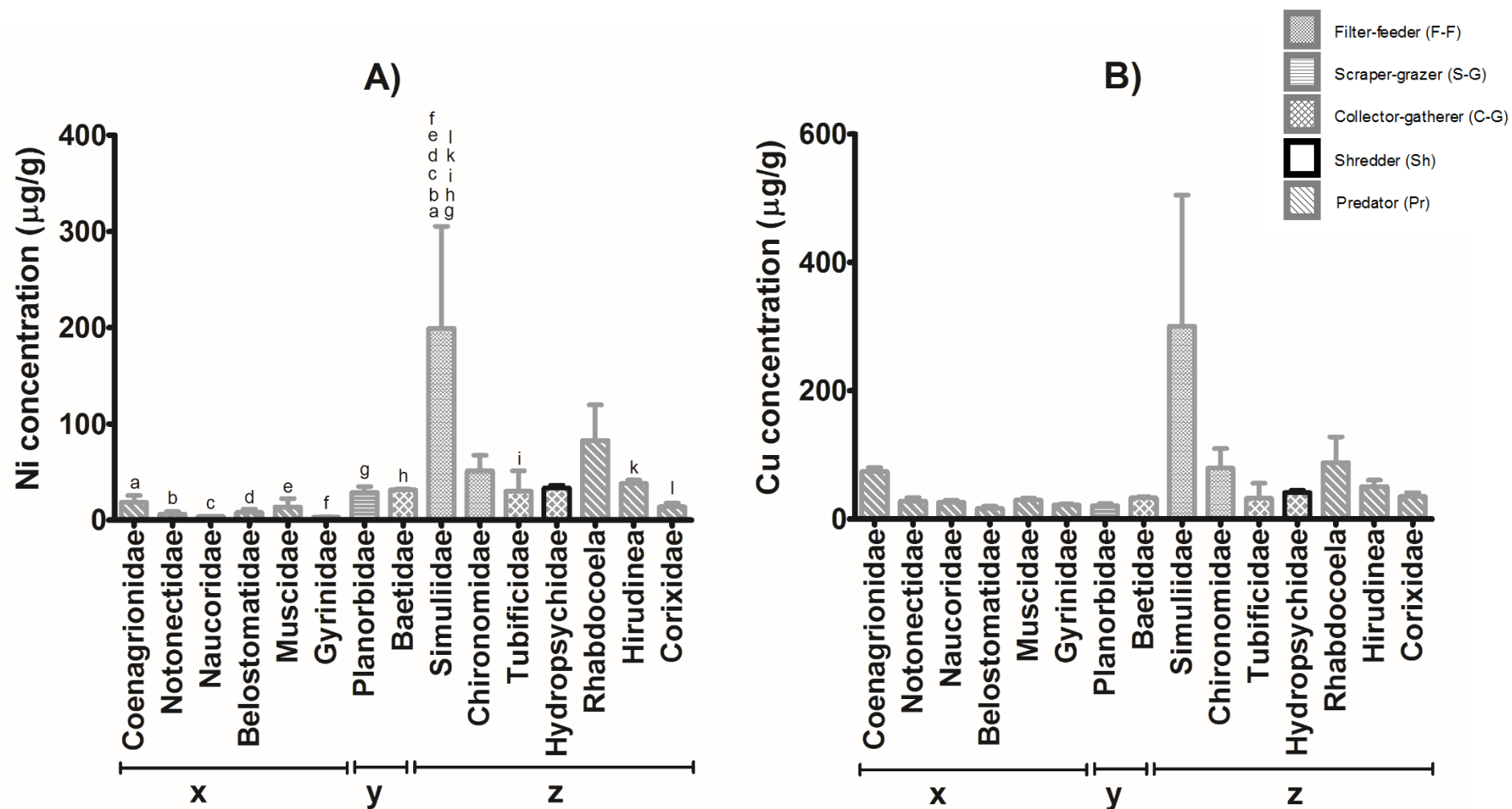


Figure 5.4: Mean and standard error of A) Ni and B) Cu concentrations (µg/g) measured in the selected macroinvertebrate families taxa (x = pelagic macroinvertebrates; y = no specific preference with regard to water or benthic habitats; z = benthic macroinvertebrates). Common alphabetical superscripts indicate significant difference ($p < 0.05$).

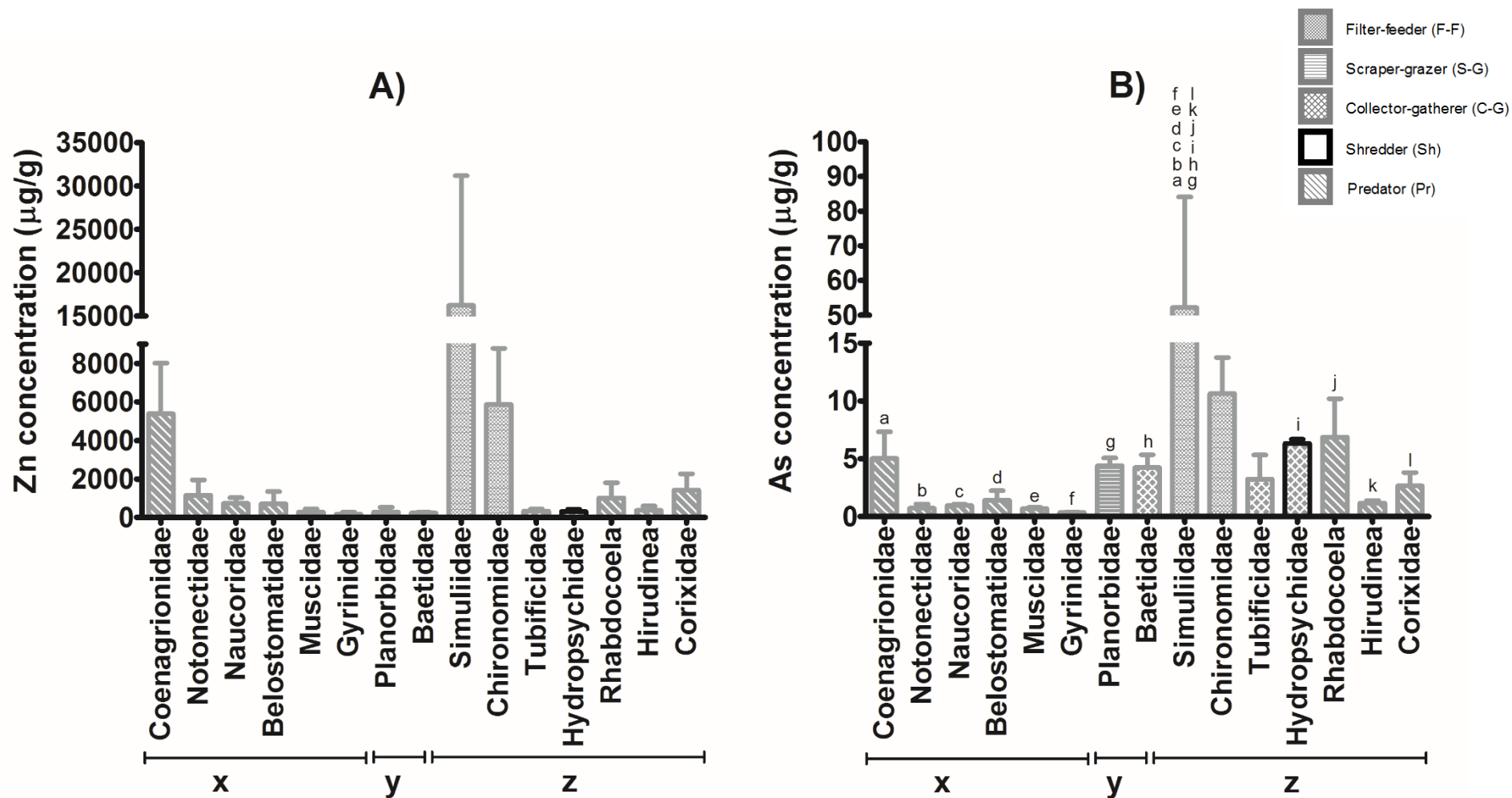


Figure 5.5: Mean and standard error of A) Zn and B) As concentrations (µg/g) measured in the selected macroinvertebrate families taxa (x = pelagic macroinvertebrates; y = no specific preference with regard to water or benthic habitats; z = benthic macroinvertebrates). Common alphabetical superscripts indicate significant difference ($p < 0.05$).

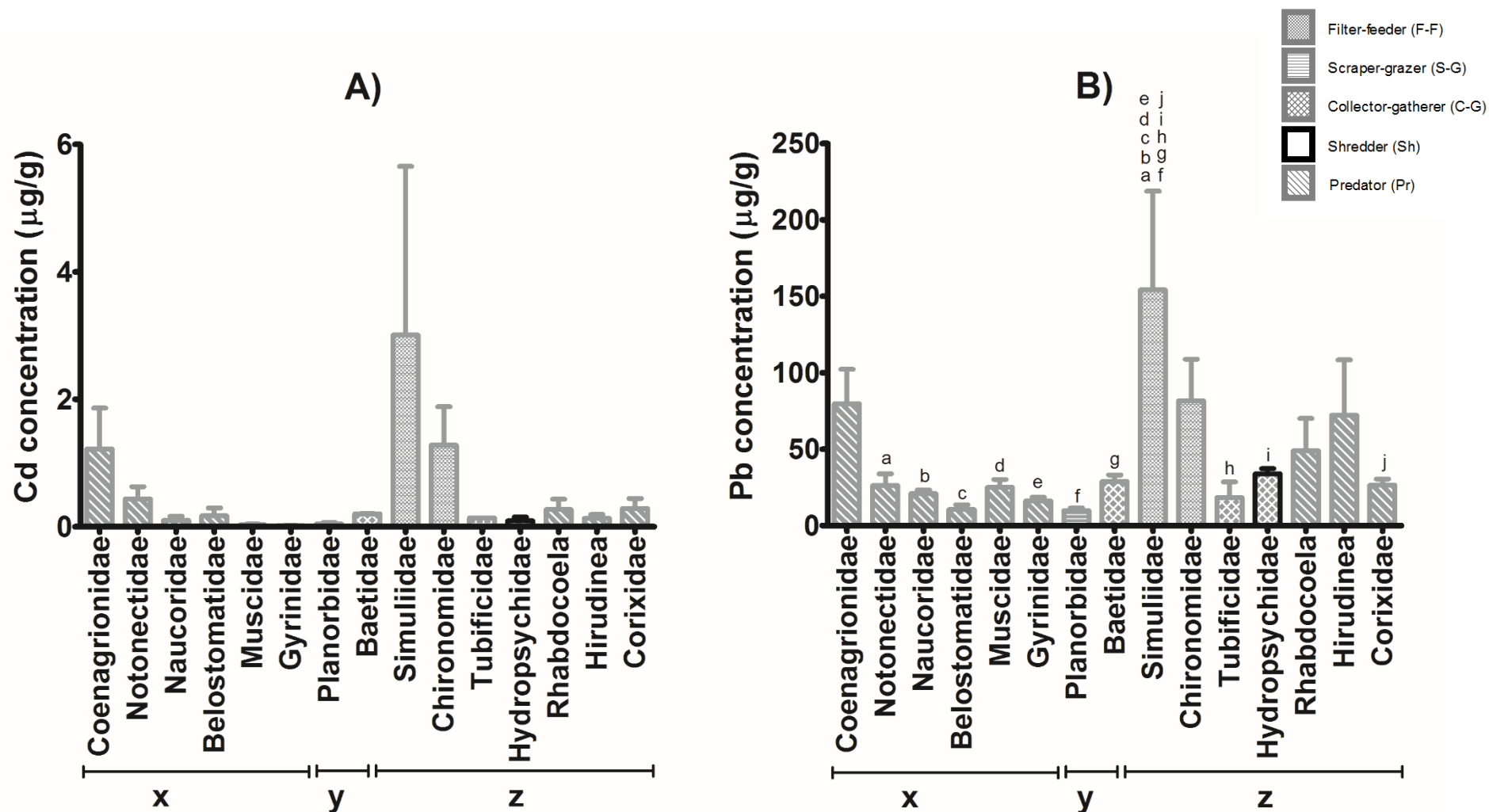


Figure 5.6: Mean and standard error of A) Cd and B) Pb concentrations (µg/g) measured in the selected macroinvertebrate families taxa (x = pelagic macroinvertebrates; y = no specific preference with regard to water or benthic habitats; z = benthic macroinvertebrates). Common alphabetical superscripts indicate significant difference ($p < 0.05$).

5.3.2 Associations between macroinvertebrates and metal concentrations

The PCA tri-plot (Figure 5.7) displays possible associations between metal concentrations and macroinvertebrate families. From this it is evident that Simuliidae and Chironomidae, both classified as F-F, showed strong associations between metal concentrations, as the highest metal concentrations were measured in both families, which is supported by the results depicted in and Figures 5.1 to 5.6. The taxa Rhabdocoela (Pr), Hydropsychidae (C-G/ Sh), Baetidae (C-G), Planorbidae (S-G), Hirudinea (Pr) and Tubificidae (C-G) associated in the bottom left and right quadrants of the PCA tri-plot, which can be explained by the fact that these taxa are all classified as benthic macroinvertebrates (Table 5.2). Due to the fact that the families Gyrinidae (Pr) and Muscidae (Pr) had the lowest metal concentrations, they associated in the opposite quadrants of the PCA tri-plot. All the families primarily found in the water column and classified as predators, i.e. Naucoridae, Belostomatidae, Notonectidae and Coenagrionidae, except for the family Corixidae, associated together.

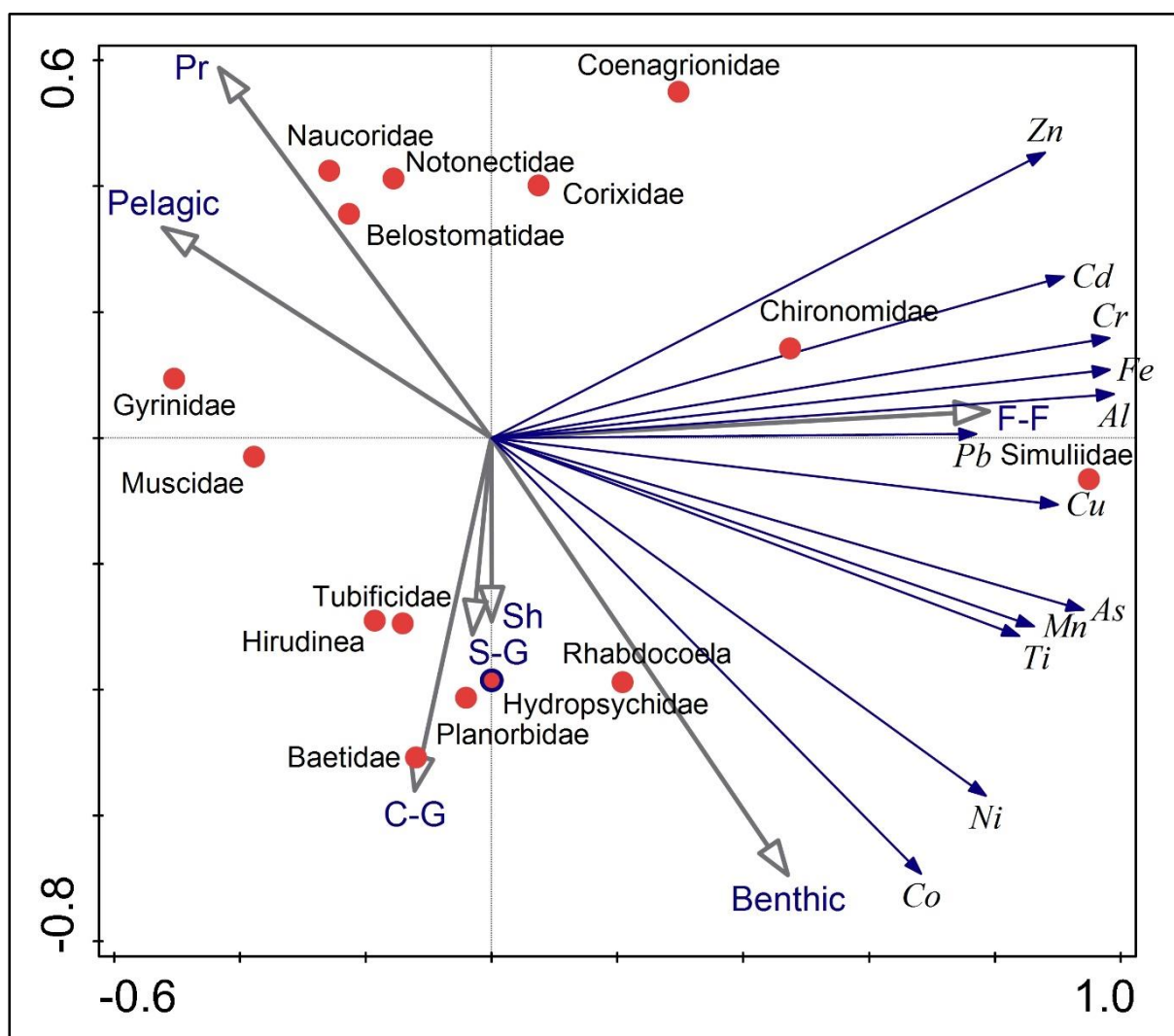


Figure 5.7: PCA tri-plot (with supplementary variables) illustrating possible associations between metal concentrations of Al, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd and Pb measured in the different macroinvertebrate taxa, the different feeding groups (filter-feeders(F-F), scraper-grazers (S-G), collector-gatherer (C-G), shredders (Sh) and predators(Pr)), as well as the two primary habitats i.e. benthic or pelagic. The tri-plot describes 93.6 % of the variation, with 82.8 % on the first axis and 10.8 % on the second axis.

5.4 Conclusion

In contrast to the limited variations in metal concentrations measured in the pelagic organisms, of which the majority were classified as predators, a substantial variation in the metal concentrations measured in the benthic macroinvertebrates, is evident. The metal concentrations varied considerably between the different feeding groups. Filter-feeders (Simuliidae and Chironomidae) had the highest metal concentrations, while the majority of the taxa classified as predators, had the lowest metal concentrations. Simuliidae showed significantly higher metal concentrations, when compared to the

majority of the families ($p < 0.05$). The metal concentrations in the collector-gatherers and scraper-grazer varied considerably between the taxa and the metals measured. From this it is evident the first hypothesis was not accepted.

It is further evident that the majority of macroinvertebrates that associated with the benthic environment had the highest metal concentrations. This is possibly due to the ingestion of sediment and organic particles, direct contact with sediments or contact with interstitial water. Therefore, the second hypothesis that higher metal concentrations will be present in benthic macroinvertebrates than in those associated with the water column, can be accepted. This is also supported by the literature (Kalman *et al.*, 2011), which states that metal concentrations in organisms associating with the sediment are usually considerably higher than in those in the overlying water column.

Chapter 6: Conclusion and recommendations

6.1 Conclusion

Due to the increasing demand for freshwater and human mediated activities, the state of freshwater in South Africa is quickly deteriorating. These activities threaten the integrity of natural ecosystems and aquatic life and therefore, the use of this valuable commodity needs to be carefully managed and monitored.

The aim of this study, to determine the aquatic macroinvertebrate diversity and the possible influences that selected physico-chemical factors may have on the macroinvertebrates, was successfully achieved. The aim was attained by 1) determining the physico-chemical characteristics in water and sediment; 2) determining the aquatic macroinvertebrate diversity of the Mooi River and Wonderfontein Spruit and 3) determining the interaction between the physico-chemical parameters and the macroinvertebrates.

The hypothesis from Chapter 3, that mining effluent from the Wonderfontein Spruit is responsible for an increase in metal contamination of the water and sediment of the Mooi River, was rejected. From the results, it was concluded that the majority of the metal concentrations measured during this study was probably as a result of natural weathering of the minerals and rocks present in the catchment area, as well as habitat modification and soil erosion due to the agricultural activities around the rivers.

The results from Chapter 4 indicated that the majority of the families collected during this study were classified as highly tolerant, however, the levels of organic enrichment observed at some sites were not to such an extent as to exclude the less tolerant families. Although some sites in the Mooi River catchment area are impacted by activities such as mining and agriculture, it appeared that the macroinvertebrate assemblages were not adversely affected by these activities. The hypothesis that these activities have a detrimental effect on the macroinvertebrate assemblages was thus rejected. The availability of suitable habitats and selected parameters, such as the range of the flow-rate and EC, also played a prominent role in the distribution of the families in the Mooi River catchment area.

Furthermore, the results of Chapter 5 revealed that metal concentrations varied considerably between different FFGs, where the filter-feeders had the highest metal concentrations, while the majority of the taxa classified as predators, had the lowest metal

concentrations. The hypothesis that trophic transfer of metals will be reflected in the FFGs, with higher concentrations in the predators compared to collector-gatherers and scraper-grazers, was thus rejected. The second hypothesis of Chapter 5, that macroinvertebrates associating with the benthic environment, will display higher metal concentrations than those associated with the water column, was accepted. This may be attributed to the ingestion of sediment and organic particles, direct contact with sediments or contact with interstitial water by benthic macroinvertebrates.

6.2 Recommendations for future studies

Based on the findings of this study, recommendations for future research can be made:

- For monitoring purposes, sampling can be done in the different biotopes and be kept separate in order to obtain full understanding of the macroinvertebrate assemblages occupying every biotope.
- A broader range of physico-chemical parameters, such as nutrients and dissolved oxygen should be assessed in the Mooi River catchment area in order to obtain a holistic insight of the state of this catchment area.
- Surveys could be made during periods of normal flow conditions and be compared to this current study, to assess the effect of drought on the state of the river.
- Metal analysis can be conducted in additional macroinvertebrate families collected from more sites and surveys.

Appendix A

Table A1: Mean and standard deviation of metal concentrations (µg/L) and selected abiotic factors measured in water samples during Survey 1, as well as the Target Water Quality Range (TWQR) values, Chronic Effect Values (CEV) and Acute Effect Values (AEV) of the metals toxic to aquatic ecosystems.

	Site													
	TWQR	CEV	AEV	1A	2A	3A	4A	5A	1B	2B	3B	4B	5B	1C
Be				0.1 ± 0.1	0.1 ± 0.04	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.09	0.1 ± 0.01	0.1 ± 0.04	0.1 ± 0.01	0.1 ± 0.03
B				3.3 ± 0.2	1.6 ± 0.1	0.3 ± 0.1	0.0	2.6 ± 0.1	56.1 ± 5.0	58.6 ± 4.2	52.9 ± 5.6	49.0 ± 4.6	10.6 ± 0.7	2.4 ± 1.0
Na				3 258.9 ± 26.6	4 947.4 ± 36.7	5 340.7 ± 30.9	5 750.4 ± 47.8	23 835.9 ± 55.6	33 607.7 ± 75.9	33 615.1 ± 67.7	33 603.1 ± 83.7	33 618.4 ± 64.4	32 644.6 ± 56.8	26 040.8 ± 55.1
Mg				26 289.6 ± 76.8	29 348.6 ± 68.3	29 990.1 ± 64.9	31 376.5 ± 74.7	39 081.9 ± 102.3	42 098.6 ± 126.8	40 318.5 ± 71.9	36 251.2 ± 46.6	37 900.5 ± 86.5	36 329.8 ± 83.4	39 203.3 ± 83.7
Al	10.0	20.0	150.0	23.8 ± 5.5	42.7 ± 5.7	31.5 ± 4.2	27.8 ± 3.4	14.3 ± 2.0	61.7 ± 7.5	46.4 ± 5.0	28.9 ± 3.8	32.1 ± 4.8	19.9 ± 1.4	29.8 ± 4.4
P				46.1 ± 6.8	48.6 ± 7.6	44.8 ± 4.3	49.7 ± 4.3	45.7 ± 6.6	577.2 ± 10.6	645.4 ± 11.7	319.7 ± 4.2	66.7 ± 4.5	44.3 ± 4.2	46.6 ± 4.9
K				5 383.9 ± 15.9	4 370.1 ± 37.7	7 280.1 ± 50.3	6 637.8 ± 38.5	3 803.1 ± 40.0	12 652.5 ± 48.0	24 238.3 ± 66.9	14 739.1 ± 51.1	11 975.8 ± 66.6	6 139.7 ± 17.8	3 024.1 ± 18.6
Ca				39 545.3 ± 53.5	44 599.3 ± 86.5	44 662.5 ± 61.5	47 056.9 ± 80.3	63 252.5 ± 48.0	79 639.6 ± 55.5	77 438.3 ± 62.0	78 330.5 ± 71.1	67 774.1 ± 74.8	65 820.8 ± 80.1	64 488.5 ± 66.5
Ti				1.5 ± 0.5	1.4 ± 0.1	1.6 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	2.7 ± 0.1	2.3 ± 0.1	1.2 ± 0.1	0.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.4
V				0.5 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	2.6 ± 0.1	4.5 ± 0.6	0.8 ± 0.1	0.4 ± 0.1
Cr	7.0	14.0	200.0	4.2 ± 1.0	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	2.4 ± 0.1	1.5 ± 0.5	1.6 ± 0.1	1.3 ± 0.1	1.2 ± 0.02	2.5 ± 0.3	4.7 ± 0.3
Mn	180.0	37.0	1 300.0	0.9 ± 0.1	1.1 ± 0.02	1.5 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	1.4 ± 0.1	1.8 ± 0.1	1.5 ± 0.03	1.0 ± 0.1	0.8 ± 0.3	1.1 ± 0.04
Fe				65.8 ± 10.7	65.5 ± 6.9	88.7 ± 7.4	97.7 ± 6.0	96.8 ± 8.1	141.0 ± 5.8	152.3 ± 4.8	107.7 ± 4.4	93.8 ± 1.8	82.3 ± 7.2	87.0 ± 5.1
Co				0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.04	0.2 ± 0.1	2.9 ± 0.2	13.4 ± 0.8	10.4 ± 0.8	7.8 ± 0.6	6.6 ± 0.3	1.2 ± 0.03	6.3 ± 1.1

Table A1 continued

Ni				2.3 ± 0.1	2.1 ± 0.1	2.6 ± 0.1	7.0 ± 0.1	4.4 ± 0.1	57.9 ± 4.5	52.5 ± 5.2	17.8 ± 1.0	28.8 ± 3.7	2.7 ± 0.5	3.5 ± 0.5
Cu	1.4	2.8	12.0	2.0 ± 0.1	2.4 ± 0.6	2.5 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	5.2 ± 0.1	4.5 ± 0.6	4.5 ± 0.7	4.4 ± 1.2	2.2 ± 0.3	2.1 ± 0.6
Zn	2.0	3.6	36.0	50.9 ± 3.4	37.6 ± 4.5	43.0 ± 4.5	39.3 ± 7.0	36.9 ± 5.5	63.9 ± 4.0	51.9 ± 6.0	52.1 ± 2.8	50.2 ± 4.0	47.9 ± 5.9	38.7 ± 4.0
As	10.0	20.0	130.0	0.04 ± 0.01	0.2 ± 0.1	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.01	12.6 ± 0.7	13.2 ± 0.3	14.0 ± 1.6	3.5 ± 0.6	0.02 ± 0.0	0.0
Se				0.0	0.0	0.0	0.0	0.0	0.7 ± 0.1	0.3 ± 0.03	0.0	0.0	0.0	0.0
Rb				2.3 ± 0.1	2.3 ± 0.2	2.6 ± 0.1	2.6 ± 0.1	3.6 ± 0.4	8.7 ± 0.4	9.5 ± 0.8	10.9 ± 1.4	10.8 ± 0.8	4.7 ± 0.5	3.6 ± 0.3
Sr				34.1 ± 3.7	43.4 ± 3.1	46.2 ± 6.1	44.8 ± 11.6	60.6 ± 3.5	233.4 ± 6.1	243.4 ± 4.5	238.9 ± 3.1	241.2 ± 4.2	72.2 ± 6.5	44.1 ± 5.8
Mo				0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	2.4 ± 0.1	2.2 ± 0.2	2.9 ± 0.1	3.4 ± 0.3	0.2 ± 0.01	0.2 ± 0.1
Pd				1.1 ± 0.0	1.1 ± 0.01	1.3 ± 0.3	1.1 ± 0.02	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.2	1.6 ± 0.3	1.3 ± 0.1	1.1 ± 0.03
Ag				0.0	0.0	0.0	0.0	0.2 ± 0.03	0.0	0.0	0.0	0.0	0.0	0.0
Cd	0.4	0.8	13.0	0.03 ± 0.01	0.03 ± 0.0	0.03 ± 0.01	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.01
Sb				0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	1.1 ± 0.01	1.0 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
Ba				3.7 ± 0.47	11.6 ± 2.0	14.2 ± 2.0	14.3 ± 3.1	7.2 ± 0.9	13.6 ± 0.5	14.3 ± 1.9	12.0 ± 1.7	15.7 ± 0.6	4.4 ± 1.2	2.5 ± 0.1
Pt				0.0	0.0	0.0	0.0	0.0	0.0	0.1 ± 0.01	0.0	0.0	0.3 ± 0.1	0.0
Au				1.6 ± 0.4	1.7 ± 0.1	2.7 ± 0.4	1.7 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.3 ± 0.1	1.7 ± 0.2	1.9 ± 0.1	2.2 ± 0.2	1.7 ± 0.2
Tl				0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.1 ± 0.0	0.1 ± 0.01	0.04 ± 0.01	0.03 ± 0.0	0.03 ± 0.01	0.03 ± 0.0

Table A1 continued

Pb	1.2	2.4	16.0	0.1 ± 0.01	0.2 ± 0.04	0.2 ± 0.01	0.1 ± 0.0	0.04 ± 0.0	0.2 ± 0.02	0.2 ± 0.02	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
Bi				0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.1	0.1 ± 0.01
Th				0.5 ± 0.1	0.5 ± 0.03	0.5 ± 0.1	0.5 ± 0.02	0.5 ± 0.04	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.3	0.6 ± 0.1	0.5 ± 0.03
U				0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	5.5 ± 0.7	52.2 ± 4.8	57.4 ± 3.5	101.1 ± 3.9	106.7 ± 3.5	0.6 ± 0.1	0.7 ± 0.1
Tempera- ture (°C)				19.2	12.0	9.6	10.2	11.3	13.9	12.5	6.7	15.6	19.8	19.7
EC (µS/cm)				457.0	438.0	441.0	456.0	697.0	1 001.0	1 055.0	974.0	996.0	798.0	785.0
pH				6.8	7.7	7.8	7.8	7.4	7.5	7.1	7.0	8.0	7.2	7.1
Turbidity (NTU)				5.0	5.0	5.0	5.0	5.0	7.0	15.0	5.0	27.0	5.0	5.0
Flow-rate (m/s)				0.1	1.2	0.3	0.8	1.1	0.1	0.2	0.1	0.1	0.1	0.1

Table A2: Mean and standard deviation of metal concentrations (µg/L) and selected abiotic factors measured in water samples during Survey 2, as well as the Target Water Quality Range (TWQR) values, Chronic Effect Values (CEV) and Acute Effect Values (AEV) of the metals toxic to aquatic ecosystems.

	Site													
	TWQR	CEV	AEV	1A	2A	3A	4A	5A	1B	2B	3B	4B	5B	1C
Be				0.1 ± 0.01	0.1 ± 0.02	0.2 ± 0.03	0.1 ± 0.01	0.2 ± 0.02	0.2 ± 0.04	0.2 ± 0.02	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
B				0.0	0.0	0.0	0.0	0.0	49.7 ± 4.5	50.7 ± 3.5	95.8 ± 6.3	68.1 ± 4.1	6.2 ± 1.6	0.0
Na				3 727.2 ± 14.2	4 796.5 ± 30.1	5 316.7 ± 17.5	5 530.1 ± 14.2	27 673.7 ± 113.4	43 703.9 ± 93.6	43 703.8 ± 80.5	43 702.9 ± 92.0	43 703.2 ± 81.5	42 773.8 ± 73.4	28 601.2 ± 87.5
Mg				27 630.1 ± 64.0	32 605.2 ± 90.6	33 938.2 ± 52.0	32 458.2 ± 61.0	41 708.0 ± 82.0	42 117.7 ± 72.1	43 646.5 ± 48.8	62 153.1 ± 43.6	43 406.4 ± 86.6	46 832.6 ± 51.7	42 869.7 ± 98.0
Al	10.0	20.0	150.0	20.9 ± 2.5	5.6 ± 0.1	22.5 ± 5.0	22.2 ± 3.7	12.8 ± 2.5	36.3 ± 3.8	11.6 ± 1.9	5.8 ± 0.3	17.9 ± 1.8	47.7 ± 3.4	71.5 ± 1.8
P				0.0	0.0	2.2 ± 0.04	0.0	0.0	519.5 ± 4.7	559.7 ± 8.1	543.3 ± 5.0	9.2 ± 1.0	0.0	0.0
K				3 908.4 ± 35.0	4 556.1 ± 13.0	4 415.4 ± 15.6	3 072.7 ± 41.6	3 177.1 ± 15.9	9 174.6 ± 14.0	9 746.5 ± 16.0	18 973.2 ± 73.7	12 213.9 ± 59.6	4 578.4 ± 21.0	2 974.1 ± 14.3
Ca				38 189.1 ± 36.6	44 257.3 ± 52.8	45 258.8 ± 57.1	50 639.1 ± 111.8	73 244.1 ± 50.2	88 958.1 ± 42.0	92 063.9 ± 55.6	77 183.2 ± 70.6	85 313.5 ± 66.0	67 159.8 ± 47.4	65 580.6 ± 71.8
Ti				2.0 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	2.0 ± 0.1	2.8 ± 0.1	1.8 ± 0.2	1.9 ± 0.1	0.7 ± 0.04	2.1 ± 0.03	1.9 ± 0.1
V				0.7 ± 0.1	1.1 ± 0.01	1.2 ± 0.04	1.5 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	3.2 ± 0.1	7.5 ± 1.1	0.9 ± 0.1	0.9 ± 0.1
Cr	7.0	14.0	200.0	5.0 ± 0.1	1.2 ± 0.03	1.1 ± 0.04	1.1 ± 0.04	3.3 ± 0.3	2.1 ± 0.1	1.8 ± 0.1	2.4 ± 0.1	1.6 ± 0.1	4.5 ± 1.0	6.2 ± 1.0
Mn	180.0	37.0	1 300.0	3.3 ± 1.0	3.2 ± 0.8	1.8 ± 0.1	2.2 ± 0.03	7.0 ± 0.1	2.9 ± 0.1	2.5 ± 0.1	3.9 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	5.5 ± 0.6
Fe				889.6 ± 14.6	1 015.1 ± 19.4	197.9 ± 10.1	274.9 ± 7.0	2 723.3 ± 10.4	401.3 ± 5.6	359.1 ± 6.6	355.3 ± 8.5	300.3 ± 4.0	319.1 ± 2.0	1 305.7 ± 19.3
Co				0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.03	3.5 ± 0.2	12.5 ± 2.8	12.6 ± 0.8	12.3 ± 1.2	5.7 ± 1.1	1.9 ± 0.1	7.5 ± 0.9
Ni				1.8 ± 0.4	1.9 ± 0.1	1.6 ± 0.04	1.6 ± 0.1	3.1 ± 0.1	32.9 ± 4.6	29.9 ± 4.0	24.8 ± 2.8	36.2 ± 3.1	1.4 ± 0.1	1.8 ± 0.04

Table A2 continued

Cu	1.4	2.8	12.0	2.4 ± 0.6	2.1 ± 0.04	2.1 ± 0.04	2.4 ± 0.1	2.8 ± 0.2	4.2 ± 0.1	4.8 ± 0.5	3.5 ± 1.1	3.6 ± 1.0	2.5 ± 0.5	2.7 ± 0.5
Zn	2.0	3.6	36.0	60.0 ± 4.8	30.3 ± 2.8	34.3 ± 9.9	52.7 ± 6.8	40.8 ± 4.5	42.3 ± 5.8	42.3 ± 2.2	33.3 ± 3.5	41.2 ± 4.1	46.9 ± 2.7	51.6 ± 4.7
As	10.0	20.0	130.0	0.8 ± 0.04	0.9 ± 0.1	1.1 ± 0.04	0.9 ± 0.1	0.9 ± 0.1	18.9 ± 2.4	23.3 ± 3.4	18.4 ± 1.6	7.2 ± 1.0	0.7 ± 0.03	0.7 ± 0.04
Se				0.9 ± 0.04	0.8 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.5 ± 0.1
Rb				2.8 ± 0.2	2.6 ± 1.3	4.4 ± 1.2	3.1 ± 0.1	4.8 ± 0.1	11.6 ± 2.1	12.2 ± 0.9	20.2 ± 1.5	16.1 ± 1.1	7.3 ± 0.7	4.5 ± 1.0
Sr				37.9 ± 5.1	43.5 ± 4.3	46.4 ± 6.1	46.3 ± 5.0	69.0 ± 3.1	257.7 ± 4.5	273.8 ± 5.6	358.2 ± 4.1	282.2 ± 3.2	91.8 ± 3.6	49.9 ± 2.2
Mo				0.4 ± 0.04	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.03	0.6 ± 0.1	3.2 ± 1.0	2.8 ± 0.1	3.5 ± 0.6	3.9 ± 1.0	0.3 ± 0.02	0.4 ± 0.04
Pd				1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.04	1.7 ± 0.1	1.6 ± 0.04	1.8 ± 0.1	1.7 ± 0.1	1.4 ± 0.03	1.4 ± 0.1
Ag				0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cd	0.4	0.8	13.0	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.0	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.03
Sb				0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.03	1.1 ± 0.02	1.2 ± 0.0	1.0 ± 0.1	1.4 ± 0.04	0.5 ± 0.1	0.4 ± 0.04
Ba				1.9 ± 0.1	16.2 ± 0.9	21.5 ± 3.0	18.6 ± 1.9	8.4 ± 1.2	16.5 ± 2.3	16.7 ± 0.8	25.5 ± 1.5	16.7 ± 1.1	1.8 ± 0.04	2.3 ± 0.8
Pt				0.04 ± 0.0	0.04 ± 0.0	0.1 ± 0.02	0.04 ± 0.04	0.04 ± 0.03	0.04 ± 0.03	0.04 ± 0.03	0.04 ± 0.03	0.1 ± 0.02	0.04 ± 0.02	0.04 ± 0.03
Au				1.95 ± 0.05	1.94 ± 0.06	1.95 ± 0.05	1.94 ± 0.06	2.02 ± 0.13	2.04 ± 0.07	1.97 ± 0.03	1.96 ± 0.05	2.09 ± 0.02	1.93 ± 0.07	2.03 ± 0.18
Tl				0.0	0.0	0.0	0.0	0.0	0.01 ± 0.0	0.0	0.01 ± 0.0	0.0	0.0	0.0
Pb	1.2	2.4	16.0	0.6 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.04	0.7 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.1 ± 0.02	0.2 ± 0.04	1.0 ± 0.0	1.8 ± 0.2
Bi				1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.3 ± 0.04	1.4 ± 0.1	1.3 ± 0.04	1.4 ± 0.3	1.3 ± 0.1

Table A2 continued

Th				0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.04	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.04	0.6 ± 0.1	0.6 ± 0.04
U				1.4 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	0.5 ± 0.03	8.1 ± 1.0	75.7 ± 4.2	72.0 ± 6.7	79.7 ± 2.4	107.6 ± 2.5	0.8 ± 0.03	1.0 ± 0.1
Tempera- ture (°C)				20.3	14.8	17.4	19.7	18.0	17.7	17.3	14.7	22.9	21.5	21.9
EC (µS/cm)				443.0	430.0	448.0	476.0	704.0	1 027.0	1 027.0	1 033.0	1 017.0	764.0	766.0
pH				7.2	7.8	7.4	7.5	7.3	7.2	7.5	7.2	8.4	7.1	7.2
Turbidity (NTU)				5.0	5.0	6.0	6.0	5.0	8.0	12.0	6.0	27.0	5.0	5.0
Flow-rate (m/s)				0.1	0.2	0.1	0.5	0.8	0.5	0.7	0.2	0.1	0.1	0.1

Table A3: Mean and standard deviation of metal concentrations (µg/L) and selected abiotic factors measured in water samples during Survey 3, as well as the Target Water Quality Range (TWQR) values, Chronic Effect Values (CEV) and Acute Effect Values (AEV) of the metals toxic to aquatic ecosystems.

	Site													
	TWQR	CEV	AEV	1A	2A	3A*	4A	5A	1B	2B	3B	4B	5B	1C
Be				0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.2 ± 0.02	0.0	0.0
B				7.7 ± 0.9	11.7 ± 0.8		7.7 ± 0.7	14.8 ± 0.9	74.9 ± 2.8	82.5 ± 3.9	72.3 ± 2.2	68.4 ± 4.4	23.9 ± 3.9	8.7 ± 1.0
Na				4 902.5 ± 85.3	6 858.9 ± 44.6		7 224.5 ± 23.5	34 229.9 ± 66.5	89 565.3 ± 67.2	99 507.2 ± 80.6	102 899.5 ± 96.9	0.0	46 756.0 ± 46.9	33 722.5 ± 101.6
Mg				38 188.2 ± 67.4	43 398.8 ± 88.1		42 027.5 ± 60.1	53 761.2 ± 56.1	49 582.5 ± 76.8	54 911.3 ± 87.6	50 261.8 ± 49.6	43 392.7 ± 88.7	50 534.2 ± 65.3	49 758.3 ± 43.7
Al	10.0	20.0	150.0	36.6 ± 2.2	49.7 ± 2.4		30.5 ± 2.1	34.7 ± 2.8	64.2 ± 3.6	66.4 ± 3.1	50.8 ± 2.7	17.9 ± 2.9	151.5 ± 7.3	50.8 ± 3.3
P				105.4 ± 2.1	120.7 ± 2.4		104.2 ± 2.0	109.3 ± 4.0	613.4 ± 11.5	645.1 ± 6.4	1 217.5 ± 12.2	9.4 ± 0.8	112.0 ± 3.3	98.3 ± 5.3
K				5 798.8 ± 13.0	6 142.7 ± 42.9		16 408.2 ± 82.0	2 760.2 ± 6.1	6 262.5 ± 24.5	8 643.0 ± 7.3	9 720.0 ± 33.8	12 211.2 ± 56.5	3 440.5 ± 5.5	2 266.5 ± 4.5
Ca				62 210.4 ± 93.7	53 490.1 ± 78.2		60 682.5 ± 72.5	85 646.9 ± 41.9	100 900.3 ± 95.8	111 600.1 ± 99.2	105 897.5 ± 94.0	85 298.8 ± 47.0	76 744.9 ± 65.1	10 435.9 ± 60.6
Ti				2.6 ± 0.6	2.6 ± 0.5		2.7 ± 0.6	3.1 ± 0.9	3.2 ± 1.0	3.0 ± 0.6	3.7 ± 0.6	0.7 ± 0.04	5.4 ± 1.1	0.9 ± 0.04
V				0.3 ± 0.02	0.5 ± 0.1		1.6 ± 0.1	1.1 ± 0.1	1.1 ± 0.03	1.2 ± 0.1	3.5 ± 1.0	7.6 ± 0.9	0.8 ± 0.1	0.5 ± 0.03
Cr	7.0	14.0	200.0	1.9 ± 0.1	0.03 ± 0.01		0.03 ± 0.0	0.03 ± 0.0	0.9 ± 0.1	0.03 ± 0.0	0.03 ± 0.01	1.7 ± 0.1	2.4 ± 1.2	2.2 ± 1.0
Mn	180.0	37.0	1 300.0	1.6 ± 0.3	2.8 ± 0.7		2.6 ± 0.6	1.8 ± 0.04	2.5 ± 0.7	2.2 ± 1.0	2.3 ± 0.9	1.3 ± 0.04	2.4 ± 1.2	1.4 ± 0.1
Fe				307.3 ± 3.1	323.6 ± 5.0		253.5 ± 6.2	388.9 ± 4.3	441.7 ± 4.4	413.5 ± 8.4	405.3 ± 18.3	303.0 ± 18.1	578.4 ± 4.5	151.8 ± 4.2

Table A3 continued

Co				0.4 ± 0.1	0.4 ± 0.1		0.4 ± 0.1	3.7 ± 0.5	15.2 ± 2.8	15.8 ± 1.6	7.7 ± 2.0	5.6 ± 0.9	2.1 ± 1.0	7.3 ± 1.0
Ni				1.3 ± 0.1	2.1 ± 1.0		1.7 ± 0.04	3.9 ± 0.4	61.2 ± 2.3	65.0 ± 3.4	12.8 ± 2.5	36.9 ± 3.1	2.0 ± 0.1	1.1 ± 0.1
Cu	1.4	2.8	12.0	8.7 ± 1.6	14.1 ± 1.1		9.8 ± 1.2	11.5 ± 2.0	14.7 ± 1.3	12.3 ± 1.2	12.8 ± 2.8	3.6 ± 0.4	10.5 ± 0.8	7.3 ± 1.1
Zn	2.0	3.6	36.0	127.2 ± 7.4	232.4 ± 4.0		136.5 ± 2.2	214.7 ± 8.0	252.0 ± 5.5	162.3 ± 5.0	178.7 ± 7.1	40.8 ± 5.0	173.1 ± 3.7	112.5 ± 3.0
As	10.0	20.0	130.0	0.4 ± 0.04	0.4 ± 0.03		0.2 ± 0.1	0.4 ± 0.1	21.3 ± 3.2	23.5 ± 1.7	16.5 ± 1.7	7.3 ± 0.8	0.3 ± 0.1	0.1 ± 0.01
Se				0.0	0.0		0.0	0.5 ± 0.04	1.2 ± 0.1	1.5 ± 0.02	1.0 ± 0.1	1.3 ± 0.04	0.6 ± 0.03	1.1 ± 0.03
Rb				3.3 ± 0.8	3.2 ± 1.0		3.4 ± 0.9	4.5 ± 1.2	10.6 ± 1.7	11.4 ± 1.1	12.5 ± 2.2	16.3 ± 3.2	6.5 ± 1.4	4.5 ± 0.8
Sr				45.4 ± 4.1	51.2 ± 2.5		47.7 ± 2.5	73.5 ± 3.0	272.7 ± 8.5	301.8 ± 3.4	281.3 ± 6.1	282.0 ± 5.6	86.9 ± 5.6	7.4 ± 0.9
Mo				0.1 ± 0.04	0.3 ± 0.03		0.1 ± 0.02	0.2 ± 0.02	2.2 ± 1.0	2.3 ± 1.0	2.3 ± 1.0	3.4 ± 0.5	0.1 ± 0.03	0.2 ± 0.03
Pd				0.2 ± 0.03	1.3 ± 0.1		0.2 ± 0.02	0.5 ± 0.04	0.6 ± 0.04	0.7 ± 0.03	0.7 ± 0.1	1.7 ± 0.1	0.2 ± 0.1	0.2 ± 0.02
Ag				0.0	0.0		0.1 ± 0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cd	0.4	0.8	13.0	0.03 ± 0.0	0.03 ± 0.0		0.03 ± 0.01	0.03 ± 0.0	0.01 ± 0.0	0.01 ± 0.0	0.03 ± 0.01	0.1 ± 0.02	0.03 ± 0.0	0.03 ± 0.0
Sb				0.5 ± 0.04	0.6 ± 0.03		0.5 ± 0.03	0.5 ± 0.03	1.0 ± 0.04	1.1 ± 0.1	1.1 ± 0.02	1.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.04
Ba				1.7 ± 0.04	40.8 ± 2.5		20.2 ± 2.2	7.5 ± 1.1	13.5 ± 1.1	15.3 ± 0.9	21.1 ± 2.8	16.5 ± 1.5	2.2 ± 0.9	1.1 ± 0.03
Pt				0.6 ± 0.03	0.2 ± 0.02		0.0	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.0	0.04 ± 0.01	0.0	0.3 ± 0.1
Au				0.2 ± 0.03	1.1 ± 0.1		0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.02	0.2 ± 0.03	2.1 ± 1.1	0.2 ± 0.02	0.4 ± 0.02

Table A3 continued

Tl				0.1 ± 0.04	0.1 ± 0.02		0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.02	0.0	0. ± 0.01	0.1 ± 0.03
Pb	1.2	2.4	16.0	20.1 ± 2.4	26.5 ± 1.1		23.5 ± 2.0	18.1 ± 2.2	22.9 ± 3.3	19.6 ± 1.6	22.8 ± 4.9	21.2 ± 0.04	21.7 ± 1.5	17.6 ± 2.7
Bi				0.1 ± 0.04	0.2 ± 0.02		0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.02	1.3 ± 0.03	0.1 ± 0.01	0.1 ± 0.03
Th				0.03 ± 0.01	0.1 ± 0.03		0.03 ± 0.03	0.04 ± 0.01	0.04 ± 0.03	0.04 ± 0.02	0.04 ± 0.03	0.53 ± 0.1	0.1 ± 0.02	0.02 ± 0.01
U				0.3 ± 0.04	0.3 ± 0.02		0.3 ± 0.03	5.2 ± 1.1	57.1 ± 4.0	62.2 ± 2.0	67.9 ± 5.1	107.7 ± 3.4	0.5 ± 0.1	0.1 ± 0.02
Tempera- ture (°C)				19.5	14.0		13.3	16.4	19.1	18.5	14.7	18.9	19.7	19.8
EC (µS/cm)				453.0	422.0		444.0	704.0	1038.0	1046.0	975.0	1096.0	786.0	796.0
pH				6.9	7.1		7.7	7.4	7.9	7.7	7.2	8.0	7.5	7.4
Turbidity (NTU)				5.0	5.0		5.0	5.0	10.0	7.0	5.0	27.0	5.0	5.0
Flow-rate (m/s)				0.1	0.1		0.3	0.5	0.4	0.9	0.2	0.1	0.1	0.1

*Site 3A dry.

Table A4: Mean and standard deviation of selected metal concentrations (mg/kg) measured in the sediment samples during Survey 1, as well as the percentage composition of gravel (> 2000 µm), sand (> 500 µm), silt (53 – 212 µm) and clay (< 53 µm) of each selected site.

	Site										
	1A	2A	3A	4A	5A	1B	2B	3B	4B	5B	1C
Be	1.3 ± 0.1	1.6 ± 0.5	2.4 ± 0.3	1.4 ± 0.2	2.4 ± 0.1	0.9 ± 0.1	6.8 ± 1.3	2.9 ± 0.3	2.0 ± 0.4	0.6 ± 0.2	1.8 ± 0.2
B	0.1 ± 0.1	0.0	0.0	0.0	0.0	0.0	14.3 ± 2.4	0.0	0.0	0.0	0.0
Na	169.2 ± 6.8	171.1 ± 5.1	347.8 ± 9.3	258.7 ± 8.2	1 296.3 ± 7.7	234.8 ± 8.1	2 705.0 ± 7.7	1 287.8 ± 29.7	2 021.2 ± 56.5	198.5 ± 15.2	214.6 ± 7.3
Mg	3 089.5 ± 102.2	5 359.8 ± 34.5	5 428.0 ± 85.9	4 768.3 ± 2.8	17 470.0 ± 16.0	1 416.6 ± 18.0	20 352.2 ± 18.0	3 866.9 ± 10.9	5 198.0 ± 25.4	919.2 ± 22.1	7 199.1 ± 25.5
Al	7 073.9 ± 70.6	17 797.1 ± 13.9	58 678.0 ± 18.9	40 965.8 ± 5.8	104 199.7 ± 14.2	12 964.1 ± 10.6	149 825.5 ± 20.5	84 079.6 ± 30.9	84 962.7 ± 101.8	9 904.8 ± 29.2	18 306.1 ± 83.5
P	961.0 ± 25.0	692.9 ± 9.6	1 698.8 ± 5.4	461.2 ± 15.3	1 788.5 ± 10.1	814.5 ± 8.6	15 860.1 ± 9.72	2 539.5 ± 25.8	1 291.6 ± 36.6	416.4 ± 35.3	1 569.3 ± 27.0
K	456.9 ± 77.6	2 077.1 ± 32.1	3 405.8 ± 66.1	2 236.2 ± 9.4	6 721.6 ± 14.0	727.3 ± 15.4	1 0531.9 ± 57.9	3 772.1 ± 23.8	5 878.3 ± 30.7	1 021.9 ± 17.8	1 521.8 ± 30.2
Ca	8 399.8 ± 172.9	77 678.8 ± 46.4	15 703.1 ± 15.0	11 132.0 ± 26.3	104 103.0 ± 83.2	12 695.9 ± 20.5	110 088.2 ± 93.6	18 909.6 ± 157.5	49 276.3 ± 82.6	2 851.1 ± 30.7	18 635.8 ± 104.5
Ti	136.6 ± 5.7	177.3 ± 6.9	415.7 ± 8.9	243.1 ± 12.3	761.3 ± 19.1	331.0 ± 12.3	1 015.5 ± 71.9	990.9 ± 24.3	797.6 ± 25.6	225.7 ± 27.9	329.3 ± 25.7
V	123.7 ± 11.8	97.6 ± 7.9	230.2 ± 14.9	67.1 ± 7.5	144.4 ± 12.2	74.8 ± 7.1	206.1 ± 18.4	352.0 ± 29.9	216.4 ± 35.7	33.1 ± 11.7	90.0 ± 19.3
Cr	376.6 ± 32.7	722.3 ± 7.9	745.5 ± 35.4	454.3 ± 18.2	715.6 ± 8.3	380.0 ± 7.1	768.2 ± 16.9	701.5 ± 14.0	616.4 ± 22.5	256.2 ± 13.8	426.2 ± 26.3
Mn	6 501.3 ± 282.6	7 658.9 ± 34.6	18 807.9 ± 15.8	3 440.4 ± 40.7	4 883.3 ± 6.2	4 200.3 ± 13.4	18 835.7 ± 50.8	15 867.9 ± 56.6	406.2 ± 27.1	396.5 ± 14.1	6 002.3 ± 96.6
Fe	55 948.3 ± 891.5	105 122.8 ± 60.0	116 814.5 ± 14.3	33 351.7 ± 24.0	89 698.1 ± 15.4	34 181.8 ± 6.7	233 225.8 ± 131.1	257 629.3 ± 139.4	67 623.6 ± 39.7	9 442.0 ± 41.5	86 142.3 ± 138.7
Co	46.2 ± 3.7	77.0 ± 7.6	97.2 ± 3.9	27.7 ± 7.4	61.0 ± 7.4	266.7 ± 7.8	1 770.9 ± 15.0	151.6 ± 16.5	88.8 ± 6.9	7.0 ± 2.8	33.8 ± 11.5
Ni	104.1 ± 77.7	307.6 ± 12.2	198.6 ± 3.3	96.4 ± 6.0	159.7 ± 4.0	576.6 ± 8.4	5 012.2 ± 38.7	286.9 ± 20.4	593.7 ± 10.6	27.5 ± 9.8	66.6 ± 13.2

Table A4 continued

Cu	50.9 ± 4.6	97.3 ± 8.2	147.6 ± 4.6	58.6 ± 6.4	110.4 ± 5.6	61.6 ± 10.1	1 311.8 ± 29.0	227.3 ± 26.1	128.5 ± 15.2	14.9 ± 5.0	46.5 ± 9.5
Zn	61.7 ± 7.1	270.7 ± 17.9	159.1 ± 7.1	58.1 ± 3.8	169.2 ± 6.2	194.2 ± 9.6	1 964.8 ± 23.9	479.0 ± 15.5	288.4 ± 12.0	22.5 ± 6.4	144.5 ± 20.6
As	20.0 ± 2.6	63.4 ± 5.1	27.1 ± 2.3	4.9 ± 0.8	9.7 ± 3.3	66.4 ± 6.0	810.6 ± 40.7	81.1 ± 9.6	31.7 ± 6.5	5.5 ± 1.6	17.0 ± 7.1
Se	1.0 ± 0.1	1.7 ± 0.2	3.4 ± 0.5	1.4 ± 0.2	9.1 ± 3.0	1.0 ± 0.1	16.1 ± 4.0	3.4 ± 0.2	4.7 ± 2.5	0.7 ± 0.2	1.7 ± 0.2
Sr	23.9 ± 4.5	49.6 ± 5.5	26.2 ± 2.0	15.4 ± 0.5	80.9 ± 8.5	33.9 ± 7.9	305.8 ± 27.8	97.5 ± 17.2	139.6 ± 8.1	11.0 ± 2.9	17.5 ± 3.5
Mo	0.9 ± 0.1	2.3 ± 0.1	1.5 ± 0.3	0.6 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	8.7 ± 3.1	6.2 ± 2.3	3.8 ± 1.5	0.4 ± 0.1	1.3 ± 0.1
Pd	1.4 ± 0.1	1.9 ± 0.3	2.4 ± 0.4	1.7 ± 0.1	2.5 ± 0.5	1.7 ± 0.3	6.8 ± 3.6	3.8 ± 1.5	2.6 ± 0.6	1.6 ± 0.2	1.8 ± 0.1
Ag	0.1 ± 0.1	1.4 ± 0.4	0.4 ± 0.1	0.03 ± 0.01	0.6 ± 0.3	0.2 ± 0.1	6.7 ± 1.5	1.4 ± 0.6	0.6 ± 0.1	0.2 ± 0.02	0.3 ± 0.1
Cd	0.2 ± 0.03	0.4 ± 0.3	0.4 ± 0.1	0.1 ± 0.01	0.3 ± 0.1	0.7 ± 0.2	5.4 ± 1.7	0.7 ± 0.2	0.4 ± 0.1	0.03 ± 0.01	0.1 ± 0.02
Sb	0.5 ± 0.2	0.9 ± 0.2	0.5 ± 0.1	0.6 ± 0.03	0.4 ± 0.2	0.6 ± 0.3	1.3 ± 0.6	0.7 ± 0.1	0.8 ± 0.2	0.6 ± 0.2	0.6 ± 0.1
Ba	620.2 ± 68.1	162.7 ± 7.3	1 215.8 ± 9.3	389.6 ± 5.6	472.1 ± 15.0	89.2 ± 6.2	753.7 ± 42.3	2 440.0 ± 23.3	277.8 ± 10.3	19.3 ± 3.0	327.1 ± 9.3
Pt	0.1 ± 0.02	0.1 ± 0.03	0.2 ± 0.07	0.1 ± 0.01	0.1 ± 0.01	0.5 ± 0.3	0.2 ± 0.1	0.1 ± 0.03	0.2 ± 0.1	0.1 ± 0.04	0.1 ± 0.0
Au	2.1 ± 0.5	2.1 ± 0.2	2.7 ± 0.8	2.3 ± 0.3	2.8 ± 0.2	2.5 ± 0.4	4.9 ± 1.3	2.7 ± 0.5	8.6 ± 1.4	2.5 ± 0.6	3.9 ± 0.9
Tl	0.4 ± 0.04	0.7 ± 0.2	1.4 ± 0.2	0.6 ± 0.07	0.9 ± 0.2	0.4 ± 0.2	1.5 ± 0.3	2.5 ± 0.4	0.9 ± 0.2	0.4 ± 0.04	0.6 ± 0.1
Pb	38.3 ± 2.9	213.0 ± 12.3	78.4 ± 3.9	36.0 ± 2.8	49.6 ± 4.5	17.2 ± 6.4	174.5 ± 21.1	183.9 ± 22.1	50.5 ± 3.7	12.6 ± 2.7	58.9 ± 16.9
U	0.6 ± 0.02	2.3 ± 0.06	1.9 ± 0.3	0.9 ± 0.2	6.4 ± 0.2	19.9 ± 2.6	252.1 ± 12.0	64.8 ± 15.9	218.4 ± 17.4	0.8 ± 0.1	1.3 ± 0.6

Table A4 continued

% Gravel	14.7	51.1	24.4	30.0	6.3	3.0	5.0	31.8	13.4	26.4	23.6
% Sand	34.8	33.4	15.4	31.3	22.1	8.2	30.5	22.9	32.5	18.1	15.9
%Silt	50.3	14.5	54.2	37.7	62.9	87.3	45.3	41.8	48.1	51.2	57.3
% Clay	0.2	1.0	6.0	1.0	8.7	1.5	19.2	3.5	6.0	4.3	3.2

Table A5: Mean and standard deviation of selected metal concentrations (mg/kg) measured in the sediment samples during Survey 2, as well as the percentage composition of gravel (> 2000 µm), sand (> 500 µm), silt (53 – 212 µm) and clay (< 53 µm) of each selected site.

	Site										
	1A	2A	3A	4A	5A	1B	2B	3B	4B	5B	1C
Be	1.1 ± 0.02	2.3 ± 1.0	2.5 ± 0.5	0.7 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	9.5 ± 1.4	4.1 ± 0.1	3.5 ± 0.6	0.5 ± 0.1	1.5 ± 0.1
B	0.0	0.0	33.0 ± 5.9	0.0	1.9 ± 0.1	0.8 ± 0.1	55.2 ± 4.2	0.0	76.8 ± 8.9	0.0	0.0
Na	138.0 ± 12.8	125.0 ± 18.9	448.8 ± 25.2	255.5 ± 11.8	520.7 ± 7.6	512.9 ± 10.4	3 295.9 ± 19.5	334.6 ± 12.7	1 979.4 ± 18.5	171.8 ± 9.4	176.8 ± 11.4
Mg	2 465.8 ± 16.9	3 869.5 ± 25.5	7 859.1 ± 14.2	3 949.2 ± 46.3	7 382.2 ± 128.0	3 194.4 ± 27.3	23 611.8 ± 63.0	1 333.2 ± 8.6	8 090.4 ± 23.9	770.3 ± 8.0	3 964.9 ± 23.3
Al	14 568.3 ± 104.9	36 782.0 ± 77.7	131 601.5 ± 95.0	26 271.6 ± 72.1	70 868.4 ± 66.0	21 326.4 ± 52.5	182 692.6 ± 93.5	46 626.3 ± 43.1	225 734.6 ± 56.0	14 159.2 ± 51.3	25 225.4 ± 20.3
P	878.9 ± 22.8	630.9 ± 13.7	1 645.8 ± 17.8	219.3 ± 22.5	555.8 ± 11.9	1 936.8 ± 15.7	16 820.5 ± 67.2	734.8 ± 13.5	1 526.1 ± 15.4	154.7 ± 13.6	921.9 ± 25.8
K	1 014.1 ± 25.8	3 851.1 ± 35.1	13 102.8 ± 81.3	2 574.9 ± 21.4	6 053.0 ± 57.4	1 466.3 ± 21.3	17 925.1 ± 74.8	1 770.7 ± 20.4	22 077.2 ± 39.6	1 559.4 ± 15.1	3 451.7 ± 56.6
Ca	7 833.4 ± 92.1	13 727.1 ± 74.7	34 773.1 ± 50.2	25 582.9 ± 98.4	51 924.4 ± 34.1	25 718.1 ± 17.4	99 485.4 ± 82.4	4 042.8 ± 52.8	49 221.5 ± 62.6	2 337.3 ± 15.0	11 956.5 ± 50.4
Ti	177.4 ± 22.0	244.0 ± 38.1	547.8 ± 26.6	228.0 ± 15.8	460.8 ± 10.7	339.4 ± 16.1	1 267.4 ± 18.1	650.1 ± 11.2	782.8 ± 15.6	221.1 ± 10.4	241.9 ± 20.4
V	98.1 ± 10.0	246.0 ± 18.0	206.8 ± 24.2	72.7 ± 11.4	114.3 ± 13.0	78.9 ± 8.1	291.2 ± 9.4	495.7 ± 10.5	310.6 ± 23.4	50.3 ± 8.7	99.1 ± 13.3
Cr	269.1 ± 17.3	826.0 ± 52.1	452.9 ± 18.3	133.7 ± 13.4	337.3 ± 12.2	294.9 ± 11.8	790.7 ± 12.4	1 645.5 ± 14.9	516.0 ± 13.2	285.0 ± 8.8	280.4 ± 15.2
Mn	20 008.4 ± 104.5	24 759.8 ± 73.8	2 790.8 ± 50.3	6 099.2 ± 17.9	4 297.2 ± 14.1	3 068.2 ± 10.2	16 714.9 ± 41.6	35 078.1 ± 73.5	1 025.9 ± 27.3	398.5 ± 11.7	5 763.3 ± 24.4
Fe	36 837.7 ± 105.3	194 526.8 ± 196.4	90 910.2 ± 45.9	47 324.4 ± 102.1	56 391.0 ± 64.1	36 210.8 ± 23.3	232 190.8 ± 111.2	281 141.2 ± 110.7	78 411.6 ± 78.2	11 795.9 ± 59.9	99 955.0 ± 49.1
Co	47.1 ± 16.2	98.1 ± 15.7	25.2 ± 8.8	27.2 ± 6.5	32.7 ± 6.1	245.1 ± 12.9	1 883.5 ± 21.7	187.9 ± 13.2	155.5 ± 13.1	6.2 ± 0.9	22.4 ± 7.5
Ni	269.8 ± 16.9	638.2 ± 33.8	167.8 ± 17.0	106.6 ± 10.4	119.7 ± 6.6	633.5 ± 11.4	5 515.8 ± 28.2	299.9 ± 15.6	1 448.3 ± 27.4	39.8 ± 7.5	82.2 ± 12.2

Table A5 continued

Cu	63.4 ± 10.4	103.8 ± 13.8	66.3 ± 10.9	34.3 ± 10.8	53.1 ± 6.3	169.9 ± 8.2	1 817.6 ± 35.3	185.9 ± 10.5	187.0 ± 10.6	14.6 ± 2.3	41.7 ± 6.1
Zn	55.8 ± 7.4	197.7 ± 26.5	183.8 ± 8.8	62.7 ± 9.1	103.7 ± 7.7	318.1 ± 8.2	2 728.3 ± 26.4	338.4 ± 13.5	1 045.9 ± 27.8	14.1 ± 1.6	165.6 ± 13.0
As	20.0 ± 8.1	74.5 ± 10.2	13.9 ± 4.5	10.1 ± 5.1	8.6 ± 0.5	68.5 ± 7.2	649.1 ± 18.1	83.4 ± 8.7	66.0 ± 12.0	6.4 ± 0.5	24.1 ± 5.1
Se	1.4 ± 0.3	2.5 ± 0.6	2.9 ± 0.8	1.5 ± 0.1	3.5 ± 0.6	3.0 ± 0.1	15.3 ± 3.8	3.7 ± 1.3	7.1 ± 0.2	1.5 ± 0.5	2.1 ± 0.1
Sr	32.4 ± 8.4	24.3 ± 5.7	33.9 ± 8.3	13.4 ± 3.2	31.9 ± 5.3	48.2 ± 7.7	265.0 ± 12.4	43.4 ± 10.6	136.3 ± 8.8	3.7 ± 0.7	12.4 ± 1.2
Mo	1.4 ± 0.2	2.0 ± 0.04	0.3 ± 0.2	0.5 ± 0.04	0.04 ± 0.01	2.2 ± 0.1	6.6 ± 1.5	4.3 ± 1.0	0.9 ± 0.1	0.0	1.0 ± 0.1
Pd	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.3 ± 0.01	0.6 ± 0.02	0.8 ± 0.1	3.3 ± 0.6	0.8 ± 0.03	1.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Ag	0.0	0.0	0.0	0.0	0.0	0.5 ± 0.04	12.8 ± 2.3	0.1 ± 0.01	0.7 ± 0.1	0.0	0.0
Cd	0.2 ± 0.02	0.6 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.01	0.9 ± 0.1	5.7 ± 1.3	0.6 ± 0.04	0.7 ± 0.1	0.1 ± 0.0	0.2 ± 0.01
Sb	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	1.6 ± 0.3	1.6 ± 0.2	0.4 ± 0.02	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.02
Ba	1 197.4 ± 14.3	837.8 ± 15.2	235.3 ± 9.0	225.9 ± 3.2	158.4 ± 7.6	153.1 ± 8.6	915.4 ± 7.1	4 421.9 ± 22.2	265.3 ± 12.3	20.5 ± 2.3	278.3 ± 10.6
Pt	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.03	0.2 ± 0.1	0.2 ± 0.01	0.2 ± 0.01
Au	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.04	1.8 ± 0.1	4.2 ± 1.1	0.6 ± 0.04	13.5 ± 1.6	0.7 ± 0.1	1.3 ± 0.1
Tl	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.1 ± 0.01	0.2 ± 0.02	0.1 ± 0.01	1.8 ± 0.2	4.1 ± 1.0	1.3 ± 0.1	0.03 ± 0.01	0.2 ± 0.01
Pb	40.3 ± 8.4	79.4 ± 8.8	33.9 ± 7.7	22.1 ± 2.8	19.8 ± 2.5	31.4 ± 6.1	232.5 ± 12.1	143.0 ± 9.4	79.3 ± 8.2	11.5 ± 0.9	93.9 ± 8.0
U	0.6 ± 0.1	2.4 ± 0.6	1.2 ± 0.01	0.1 ± 0.01	3.5 ± 0.5	35.8 ± 3.6	276.0 ± 12.2	25.9 ± 5.1	145.4 ± 14.2	0.3 ± 0.01	0.7 ± 0.1

Table A5 continued

% Gravel	24.2	43.7	7.0	46.4	16.4	0.6	1.4	19.9	13.6	22.9	60.5
% Sand	43.3	40.5	20.4	34.4	9.0	5.9	11.7	26.2	39.8	21.8	15.8
%Silt	32.4	14.9	65.0	19.0	72.1	91.3	47.0	52.3	36.7	52.3	23.3
% Clay	0.1	0.9	7.6	0.2	2.5	2.2	39.9	1.6	9.9	3.0	0.4

Table A6: Mean and standard deviation of selected metal concentrations (mg/kg) measured in the sediment samples during Survey 3, as well as the percentage composition of gravel (> 2000 µm), sand (> 500 µm), silt (53 – 212 µm) and clay (< 53 µm) of each selected site.

	Site										
	1A	2A	3A*	4A	5A	1B	2B	3B	4B	5B	1C
Be	1.9 ± 0.03	2.6 ± 0.1		3.6 ± 0.5	3.5 ± 0.2	3.2 ± 0.3	4.5 ± 0.4	4.8 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	2.6 ± 0.1
B	3.6 ± 0.7	3.6 ± 0.4		2.9 ± 0.4	10.9 ± 1.4	3.4 ± 0.3	12.8 ± 0.8	17.6 ± 1.3	4.6 ± 0.1	1.5 ± 0.1	6.0 ± 0.03
Na	203.8 ± 13.2	310.7 ± 10.1		635.5 ± 7.4	1 083.7 ± 8.5	515.2 ± 6.5	761.2 ± 9.5	938.7 ± 6.6	504.4 ± 8.0	403.5 ± 7.5	579.2 ± 4.6
Mg	3 016.1 ± 27.9	11 804.9 ± 40.3		6 830.1 ± 10.4	20 447.5 ± 51.4	10 533.0 ± 58.7	7 989.7 ± 20.7	3 828.5 ± 26.9	1 143.0 ± 11.2	1 034.7 ± 22.0	82 681.0 ± 66.7
Al	9 417.8 ± 66.8	25 068.5 ± 62.3		23 549.3 ± 47.5	96 018.2 ± 56.7	45 583.8 ± 38.1	27 356.1 ± 51.3	35 392.0 ± 51.9	11 154.1 ± 43.5	6 606.0 ± 19.4	30 161.7 ± 37.4
P	1 322.3 ± 32.3	1 661.7 ± 32.9		1 053.2 ± 13.5	2 069.2 ± 20.0	3 463.2 ± 20.4	3 919.1 ± 17.9	3 706.7 ± 16.5	791.5 ± 10.6	485.8 ± 10.0	928.7 ± 16.6
K	598.9 ± 15.5	3 626.6 ± 15.1		1 166.1 ± 12.1	5 570.5 ± 22.4	989.1 ± 15.5	1 261.9 ± 16.7	2 589.5 ± 29.1	592.9 ± 11.4	578.9 ± 5.0	1 510.5 ± 18.2
Ca	6 429.9 ± 27.6	86 372.2 ± 57.1		76 139.9 ± 74.5	138 076.3 ± 77.3	72 153.9 ± 13.2	77 644.2 ± 60.8	16 144.0 ± 53.5	7 196.8 ± 16.1	2 558.8 ± 6.9	228 483.6 ± 77.4
Ti	160.3 ± 17.9	329.1 ± 7.6		123.9 ± 12.3	130.6 ± 6.6	407.5 ± 23.4	697.7 ± 12.8	764.9 ± 12.6	157.9 ± 11.7	126.0 ± 6.2	579.9 ± 15.6
V	103.9 ± 9.5	139.5 ± 8.2		85.6 ± 8.8	109.4 ± 6.6	348.5 ± 7.6	352.1 ± 7.0	288.8 ± 7.4	84.2 ± 5.5	32.1 ± 6.0	119.7 ± 5.3
Cr	535.1 ± 18.2	935.7 ± 27.7		294.0 ± 6.5	838.0 ± 7.0	1 564.7 ± 10.2	1 473.7 ± 12.6	1 054.5 ± 9.4	698.8 ± 14.0	515.8 ± 10.3	519.7 ± 12.0
Mn	25 824.3 ± 72.9	20 843.9 ± 41.9		87 756.2 ± 55.1	4 884.1 ± 24.7	23 150.9 ± 48.7	30 204.4 ± 30.3	56 212.5 ± 70.6	523.1 ± 12.5	779.2 ± 6.5	26 865.9 ± 55.6
Fe	65 429.7 ± 111.6	111 538.8 ± 72.1		143 990.0 ± 79.0	90 620.7 ± 52.1	248 294.1 ± 41.0	302 169.3 ± 87.0	352 708.8 ± 55.9	50 132.7 ± 46.6	14 618.6 ± 35.4	79 098.2 ± 92.6
Co	62.5 ± 7.8	73.6 ± 8.5		145.6 ± 9.4	64.0 ± 6.1	560.3 ± 18.1	598.0 ± 12.0	219.0 ± 6.1	30.3 ± 9.2	7.3 ± 0.7	80.6 ± 8.5
Ni	203.5 ± 11.7	314.5 ± 9.0		378.7 ± 7.7	162.7 ± 6.3	1 619.0 ± 17.1	1 852.7 ± 21.4	386.6 ± 10.4	185.8 ± 10.1	28.4 ± 7.2	124.1 ± 12.0

Table A6 continued

Cu	56.7 ± 11.1	96.7 ± 8.7		179.1 ± 7.9	129.2 ± 8.4	293.7 ± 9.8	355.2 ± 12.8	352.3 ± 8.3	41.1 ± 8.2	22.5 ± 5.7	83.0 ± 7.5
Zn	74.2 ± 6.2	174.2 ± 5.7		142.5 ± 7.5	139.4 ± 5.4	566.1 ± 10.0	982.0 ± 18.2	868.0 ± 17.1	175.8 ± 10.6	119.1 ± 7.6	162.1 ± 9.6
As	23.4 ± 5.5	28.7 ± 4.7		15.8 ± 3.9	9.5 ± 1.7	183.8 ± 8.0	304.7 ± 7.8	128.7 ± 6.7	16.7 ± 1.1	5.4 ± 0.5	20.2 ± 5.2
Se	0.8 ± 0.1	1.7 ± 0.3		4.3 ± 0.9	8.8 ± 0.8	1.2 ± 0.1	2.8 ± 0.2	3.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	2.4 ± 0.2
Sr	46.5 ± 7.1	52.9 ± 6.7		121.4 ± 4.6	69.6 ± 3.4	103.6 ± 6.9	119.5 ± 8.3	89.7 ± 6.4	22.7 ± 7.0	2.8 ± 0.6	84.8 ± 6.2
Mo	1.4 ± 0.5	2.4 ± 0.5		1.8 ± 0.1	0.3 ± 0.04	2.3 ± 0.1	7.1 ± 0.1	5.2 ± 0.2	0.9 ± 0.1	0.3 ± 0.2	1.8 ± 0.1
Pd	1.4 ± 0.1	1.6 ± 0.4		3.2 ± 0.6	2.5 ± 0.5	1.2 ± 0.04	3.4 ± 0.2	2.4 ± 0.1	1.0 ± 0.1	1.8 ± 0.2	1.6 ± 0.1
Ag	0.0	0.0		0.4 ± 0.1	0.2 ± 0.03	0.5 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.02 ± 0.01	0.3 ± 0.1	0.2 ± 0.0
Cd	0.1 ± 0.03	0.4 ± 0.1		0.3 ± 0.03	0.2 ± 0.1	1.1 ± 0.1	1.8 ± 0.1	1.1 ± 0.1	0.04 ± 0.01	0.0	0.2 ± 0.0
Sb	0.4 ± 0.1	0.5 ± 0.1		0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	1.6 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.8 ± 0.1	0.4 ± 0.1
Ba	1 848.9 ± 13.6	355.7 ± 8.1		2 882.7 ± 26.8	229.8 ± 3.6	759.6 ± 8.2	1 245.1 ± 7.0	2 980.2 ± 10.5	261.5 ± 7.9	20.7 ± 1.9	1 946.7 ± 9.8
Pt	0.02 ± 0.01	0.2 ± 0.1		0.1 ± 0.1	0.05 ± 0.0	0.0	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.04 ± 0.01	0.03 ± 0.01
Au	0.7 ± 0.02	0.7 ± 0.04		0.5 ± 0.03	1.3 ± 0.1	0.4 ± 0.1	3.6 ± 0.5	1.6 ± 0.2	2.5 ± 0.1	1.0 ± 0.01	0.8 ± 0.1
Tl	0.8 ± 0.1	0.9 ± 0.1		1.3 ± 0.1	0.8 ± 0.04	0.6 ± 0.1	0.6 ± 0.1	3.8 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	2.1 ± 0.1
Pb	92.4 ± 8.0	231.2 ± 6.9		147.1 ± 5.0	58.0 ± 3.1	118.9 ± 5.1	143.9 ± 5.4	285.9 ± 10.9	66.8 ± 5.6	63.9 ± 6.3	146.4 ± 6.5
U	0.5 ± 0.1	2.4 ± 0.1		1.5 ± 0.1	7.1 ± 0.1	42.2 ± 6.5	51.3 ± 4.5	49.4 ± 7.0	44.9 ± 3.8	0.4 ± 0.1	3.9 ± 0.1

Table A6 continued

% Gravel	20.6	30.6		38.4	5.3	31.1	15.2	25.1	10.0	10.3	35.3
% Sand	27.6	23.6		38.0	9.6	34.8	28.8	19.8	18.7	16.9	29.6
%Silt	51.5	45.7		23.5	76.1	33.6	39.5	52.5	69.6	69.0	30.2
% Clay	0.3	0.1		0.1	9.0	0.5	16.5	2.6	1.7	3.8	4.9

*Site 3A dry.

Appendix B

Table B1: Mean and standard deviation of metal concentrations (µg/g) measured in different macroinvertebrate taxa.

Taxa	Al	Ti	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb
Muscidae	157.9 ± 79.3	2.5 ± 0.4	1.1 ± 1.1	65.3 ± 22.5	293 ± 179.7	3.2 ± 2.2	13.7 ± 15.4	29.6 ± 5.2	279.2 ± 283.3	0.7 ± 0.2	0.03 ± 0.02	25.1 ± 9.0
Gyrinidae	105.2 ± 87.3	3.0 ± 0.1	0.6 ± 0.5	147.1 ± 24.7	176.1 ± 120.0	1.7 ± 0.3	3.1 ± 0.6	21.9 ± 3.1	179.9 ± 167.2	0.3 ± 0.1	0.02 ± 0.01	16.2 ± 4.2
Naucoridae	277.9 ± 110.1	2.6 ± 0.4	3.6 ± 1.5	115.9 ± 21.4	667.3 ± 307.4	2.1 ± 1.0	3.8 ± 0.3	26.1 ± 5.5	745.9 ± 493.6	0.9 ± 0.2	0.1 ± 0.1	20.9 ± 4.3
Belostomatidae	381.8 ± 524.1	2.4 ± 0.9	4.2 ± 6.2	81.1 ± 44.5	939.1 ± 1 291.9	2.3 ± 1.4	7.8 ± 6.2	16.6 ± 6.5	715.9 ± 1 101.5	1.4 ± 1.4	0.2 ± 0.2	10.7 ± 5.2
Notonectidae	448.9 ± 559.3	3.4 ± 1.5	4.0 ± 4.3	93.3 ± 57.9	1 184.7 ± 1 363.8	3.9 ± 2.7	6.3 ± 4.6	27.8 ± 9.5	1 153.6 ± 1 389.6	0.7 ± 0.6	0.4 ± 0.3	26.3 ± 13.4
Coenagrionidae	2 318.8 ± 2 071.4	4.3 ± 1.6	26.8 ± 26.3	204.6 ± 117.3	6 099.7 ± 6 742.5	4.4 ± 2.1	18.6 ± 11.8	74.1 ± 10.5	5 399.4 ± 4 536.8	5.0 ± 4.0	1.2 ± 1.0	79.7 ± 39.2
Planorbidae	805.9 ± 528.5	8.1 ± 1.7	6.1 ± 3.7	290.5 ± 98.1	1 644.9 ± 965.6	16.3 ± 6.5	29.1 ± 10.2	21.0 ± 5.3	283.4 ± 427.7	4.4 ± 1.1	0.04 ± 0.01	9.8 ± 3.2
Baetidae	413.2 ± 135.5	4.3 ± 1.0	2.8 ± 0.8	224.4 ± 16.2	861.2 ± 268.9	17.8 ± 2.1	31.5 ± 1.8	33.1 ± 2.2	238.0 ± 73.8	4.2 ± 1.9	0.2 ± 0.0	28.9 ± 7.3
Corixidae	1 060.5 ± 857.6	2.7 ± 0.5	15.0 ± 13.9	187.2 ± 63.3	4 246.4 ± 4 161.0	3.2 ± 0.9	14.1 ± 6.1	35.1 ± 10.5	1 434.2 ± 1 457.3	2.7 ± 2.0	0.3 ± 0.3	26.6 ± 6.8
Hydropsychidae	641.6 ± 153.4	8.3 ± 0.5	6.2 ± 1.6	327.3 ± 62.5	1 575.3 ± 648.2	9.5 ± 1.1	33.3 ± 5.5	41.5 ± 6.0	316.4 ± 209.6	6.3 ± 0.6	0.1 ± 0.1	34.0 ± 5.7
Hirudinea	278.7 ± 224.7	2.1 ± 0.5	2.3 ± 1.8	89.1 ± 8.5	524.0 ± 320.7	11.2 ± 1.7	38.3 ± 6.0	50.7 ± 17.4	377.2 ± 396.0	1.2 ± 0.4	0.1 ± 0.1	72.2 ± 62.7
Rhabdocoela	1 016.3 ± 1 037.6	6.3 ± 4.5	12.2 ± 15.3	317.3 ± 240.0	3 343.2 ± 3 822.7	22.5 ± 17.2	82.9 ± 64.1	88.1 ± 69.7	1 028.5 ± 1 342.6	6.9 ± 5.8	0.3 ± 0.3	49.0 ± 36.7
Chironomidae	3 628.8 ± 2 731.2	12.2 ± 2.3	38.5 ± 35.0	476.3 ± 237.3	10 116.3 ± 8 527.9	13.5 ± 5.5	51.3 ± 28.2	79.5 ± 52.8	5 870.3 ± 5 037.0	10.6 ± 5.4	1.3 ± 1.1	81.7 ± 47.1
Simuliidae	14 789.0 ± 21 622.2	22.0 ± 15.2	221.0 ± 341.3	1 188.9 ± 1 337.6	57 901.7 ± 89 371.2	37.2 ± 30.3	199.4 ± 183.3	300.4 ± 354.7	16 229.1 ± 25 902.2	52.1 ± 55.5	3.0 ± 4.6	154.3 ± 111.7
Tubificidae	420.0 ± 358.5	4.3 ± 4.2	2.6 ± 1.8	174.9 ± 178.9	942.8 ± 694.4	8.1 ± 8.3	30.1 ± 29.8	32.5 ± 33.0	334.7 ± 154.6	3.2 ± 3.0	0.1 ± 0.0	18.5 ± 14.4

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