Bioaccumulation of non-essential trace metals in tissues and organs of *Clarias gariepinus* (sharptooth catfish) from the Vaal River system – strontium, aluminium, lead and nickel

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Abstract

This is the first paper reporting on results obtained in a metal bioaccumulation study in the Vaal River system. It discusses concentrations of four non-essential elements (strontium, aluminium, lead and nickel) in water, sediment and various fish tissues. A second paper will report on concentration levels of the remaining five essential trace metals (chrome, copper, iron, manganese and zinc) studied. While heavy metals did accumulate in *C. gariepinus* tissues, no clear trends emerged with regard to differences between localities (Vaal Dam and Vaal River Barrage) or surveys. The highest non-essential element metal concentrations were generally recorded in gill (filaments and arches), followed by muscle, liver and lastly skin. This general trend appears to be in agreement with trends observed by other workers and reported in the literature. Variability in tissue metal concentrations in *C. gariepinus* within locality and seasons observed in this study is also reflected in results from available literature. This accentuates the importance of factors that influence the concentrations and bioavailability of trace metals.

Keywords: Heavy metal pollution, bioaccumulation, *Clarias gariepinus*, Vaal River Barrage, Vaal Dam, strontium, aluminium, lead, nickel

Introduction

Synopsis

With South Africa's unallocated water supplies dwindling (Blignaut and van Heerden, 2009), pollution levels (including heavy metal pollution) will influence future water conservation and management policy-making decisions. Furthermore, metal pollution may globally have a severe negative impact on human health (Castro-González and Méndez-Armenta, 2008), with South Africa being no exception (Strydom et al., 2006). In fish, lesions caused by low concentrations of trace metals could lead to functional alterations and interference with fundamental processes such as osmoregulation, gas exchange and metabolism (Pandey et al., 2008), and subsequently also affect community structure (Bervoets et al., 2005). Heavy metals can be defined as 'electropositive elements having a density of greater than five' (Roebuck, 1992). This definition invariably lumps together 40 metals of different properties and degrees of acute and chronic toxicity (Roebuck, 1992). With regards to the periodic table, the transition elements are generally termed 'heavy metals' or simply 'metals' (Sorensen, 1991). Possible sources include wastewater arising from informal settlements (Jackson et al., 2007), leachates from domestic and industrial landfill sites (Moodley et al., 2007), mining activities, disposal of metal-containing industrial effluents (Lloyd, 1992; Purves, 1985; Phuong et al., 1998), municipal wastewater, dry docking companies and petrol filling stations (Shriadah, 1998). Presence of metals in natural waters may either result in positive effects when the metals present are essential for life in low concentrations, or negative (i.e. toxicologically undesirable) effects (Galvin, 1996). Toxicity of metals is dependent on the availability of the metal in the ionised form (Sorensen, 1991). This in turn is influenced by factors such as hardness, pH, dissolved oxygen, temperature, salinity, interactions with heavy metal salts and other particles such as suspended solids (Hartung, 1973; Mance, 1987; Wang, 1987; Burton et al., 1972). Synergistic combined effects or additive joint actions of metals have been documented (Enserink et al., 1991; Palaniappan and Karthikeyan, 2009). Antagonistic interactions also occur (e.g. Kwong and Niyogi, 2009). Sorption of several metals to hydrous ferric oxide (Johnson, 1986; Hem, 1977; Swallow et al., 1980) and manganese oxide (Loganathan and Burau, 1973) has been studied. Another factor affecting toxicity of metals is alkalinity (Köck et al., 1995). It is, however, not only water quality variables and chemical elements that are important determinants of metal availability and toxicity. A large number of biological variables also play a significant role with regard to metal accumulation. These include interspecies variations (Giesy and Wiener, 1977; Lowe et al., 1985), orientation to the sediment and behaviour (Kligour, 1991; Kidwell et al., 1995), as well as life stages present (Mance, 1987). Synthetic chelating agents (e.g. ethylene diamine tetraacetic acid) can also reduce metal toxicity (Muramoto, 1980; James et al., 1998). Effects of metal toxicity are varied, ranging from gross pathologies like scale deformation (Yoshitomi et al., 1998) and liver degeneration (Woodward et al., 1994), to the disruption of metabolic

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processes (Barnhoorn et al., 1999) and behaviour (e.g. spawning) (Benoit, 1975).

Metal accumulation is affected by some of the same parameters that affect toxicity (water chemistry and particulate matter). As such it is potentially one of the most valuable tools for identifying and quantifying the impact of metals in aquatic environments (Borgmann and Norwood, 1994; Birungi et al., 2007; Shinn et al., 2009; Allert et al., 2009; Otero-Muras et al., 2010). This impact has been widely investigated in studies examining accumulation in sediments (Rosales-Hoz and Carranza-Edwards, 1998), crustaceans (Zou, 1997; Steenkamp et al., 1994), aquatic insects (Hall et al., 1998), amphipods (Borgmann and Norwood, 1994), mammals associated with water (Gutleb et al., 1998), aquatic macrophytes (van der Merwe et al., 1990), algae (Carr et al., 1998), plankton (Ghatak et al., 1987) and several species of fish (Maage, 1990; Sultana and Rao, 1998; Ward and Neumann, 1999; Annune et al., 1994; Lange et al., 1993; Neuman et al., 1991; Prasath and Khan, 2008). The fish tissues examined to a large extent depend on the objective of the research. If the potential effect of consumption of fish on human health is to be determined the tissue analysed is usually muscle, with the skin either included or removed. When the objective of the research is to determine effects of a contaminant on individual fish health, organs such as kidney, gill or liver are analysed. Whole body analysis will be performed if the objectives include trophic interactions, bioconcentration and bioaccumulation (Goldstein et al., 1995). In biomagnification studies both tissue samples and whole body analysis are often employed, as was demonstrated by Retief et al. (2009). They performed selective host tissue and whole body parasite analysis to examine metal accumulation in the cestode *Bothriocephalus acheilognathi*. The parasite had higher metal concentrations than host tissue, indicating the potential for biomagnification.

Absorption of heavy metals can occur via 2 pathways, as discussed by Bryan (1976) and demonstrated in a comparative study by Alquezar et al. (2008). The first is absorption from solution. Ion transfer through the gills serves as a good example. Metals may, however, also diffuse passively through skin and gills as a soluble complex down gradients created by adsorption at the surface. The second pathway is absorption from food or particles. After reviewing available literature, Bryan (1976) came to the conclusion that, in the majority of cases, food is a much more important source of metals than the water. After these trace elements are absorbed, it is transferred from the gills and intestine to the blood and distributed to other parts of the body (Hogstrand and Haux, 1991).

Essential trace metals (like zinc and copper) are better regulated than nonessential trace metals (like lead and cadmium), since detoxification of nonessential elements may involve sequestering, compared to elimination of essential elements (Giesy and Wiener, 1977). As a result zinc is believed not to accumulate in food chains (van den Heever and Frey, 1994), being lost primarily via the gills (Bryan, 1976). Some metals are also excreted into bile (Klaasen, 1976). Excretion via both the gills and biliary route seem to be quantitatively more important than the urinary route (Klaasen, 1976; Bryan, 1976). Metallothionein, a low molecular weight protein, also plays a role in the binding and detoxification of heavy metals (Hodson, 1988; Hogstrand and Haux, 1991). It has been found that nonessential elements, on the other hand, tend to accumulate in fish year to year in a stepwise manner, since fish are not able to eliminate the metals completely (Köck et al., 1996). In fact, Sorensen (1991) states: 'Loading of aquatic habitats with nondegradable, nonnutritive, cumulative pollutants such as As, Pb, Cd and Hg can result in undeniably complex alterations of numerous teleost trophic levels'. All metals, however, are elements and hence cannot disappear from a system, but can only be transferred from one place to another (Lloyd, 1992). It is the build-up of these metals over many decades in sediment 'sinks' that is of major concern today (Lloyd, 1992). Sediment can affect water quality in many ways. Sorption of pollutants on sediments alters their positional- and bioavailability, and under certain circumstances pollutants can be remobilised (Coetzee, 1993; Grobler et al., 1987). A number of mechanisms regarding metal binding to, and release from, sediments have been identified. These include acid-volatile sulphides in anaerobic sediments, particulate organic carbons in aerobic sediments, iron and manganese oxyhydroxides in aerobic sediments (all metal-binding phases), complexation by ligands and oxidation caused by physical, biological and human activities (Chapman et al., 1998), such as re-suspension of sediment during flood conditions (Literathy and Laszlo, 1977).

Water bodies in the Vaal River system are generally shallow and seldom develop anaerobic hypolimnia (Grobler et al., 1987). As a result re-suspension (i.e. oxidation caused by physical and biological activities) rather than chemical release could be the dominant mechanism for returning pollutants to the water column (Grobler et al., 1987). Gouws and Coetzee (1997) found the extractable metal content (Ni, Mn, Co, Cr, Zn, Fe, Ca, Sn, Cd, Pb, Al and Cu) of Vaal Dam sediment to be low. Furthermore major proportions of most metals seemed to be associated with the inert phase and could therefore be classified as being of geochemical origin (Gouws and Coetzee, 1997).

Scope of study

In the current study the concentrations of 9 metals (strontium, aluminium, chromium, copper, zinc, manganese, iron, lead and nickel) were determined in 5 tissue types, namely, skin, muscle, gill arch, gill filament and liver of the sharptooth catfish (*Clarias gariepinus*). In doing so the possible effect of consumption on human health, as well as effects on fish health, could be discussed. Ultimately the range of tissues used also provided a good indication of the degree of bioaccumulation by the sampled fish populations. Accumulation of trace metals is influenced by feeding behaviour (e.g. Ali and Fishar, 2005). In this respect catfish are particularly suited to bioaccumulation studies. Bottom feeders are readily exposed to metals that accumulate in sediment, while predators accumulate metals from surrounding water or from feeding on other fish (Kidwell et al., 1995). Catfish are bottom-dwelling omnivores, but also actively feed on a wide variety of prey including small fish (Skelton, 2001).

The data gathered during the study was compared between a polluted (Vaal River Barrage affected by industrial, mining and domestic effluent) and less polluted (Vaal Dam located in the upper catchment) locality. Trace metal concentrations in sediment and water samples (Institute for Water Quality Studies, Department of Water Affairs and Rand Water Board database) were also recorded during each survey.

Of the 9 metals for which analysis was performed, chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) are regarded as essential trace metals (Galvin, 1996). These metals play a vital role in many physiological processes, but have a toxic effect when present in high enough concentrations. Aluminium (Al), lead (Pb), nickel (Ni) and strontium (Sr), on the other hand, are considered to be non-essential elements. This, the first paper reporting on results obtained in the study, shall deal only with the latter.

Strontium

The chemistry of strontium (Sr) is similar to that of calcium (Hem, 1989). As such it can be of use in the field of freshwater fisheries management. Schroder et al. (1995) used rings of deposited strontium in otoliths as tags in salmon fry. Strontium was also found to accumulate in the vertebrae and opercula. Radtke (1989) also proposed the use of strontium-calcium concentration ratios in otoliths as environmental indicators. Strontium can affect fish adversely at elevated levels, but, prior to a short review of available literature, Seymore et al. (1995) stated that limited research has been conducted in this field.

Aluminium

Aluminium (Al) is one of the more common elements in the earth’s crust (8.1% by mass) (Galvin, 1996). Dissolved aluminium concentrations are inversely correlated with pH (i.e. high dissolved aluminium concentrations in waters with high acid loads) (Dickson, 1983; Baker and Schofield, 1982). Dissolved aluminium is also more toxic in acidic environments (Buergel and Soltero, 1983; Norrgren et al., 1991). Metal accumulation is affected by some of the same parameters that affects toxicity (Borgmann and Norwood, 1994), and as such the two should be considered together.

Lead

Lead (Pb) is widely used as an additive in (among others) some fuels, production of batteries, paints, anti-radiation armour and pesticide formulations (Galvin, 1996). Sorensen (1991) describes Pb as a ‘bone-seeking’ element. Lead poisoning in fish result in haematological (e.g. higher blood glucose levels), neuronal, muscular and other effects like black tails, lordoscoliosis, pigment alterations and coagulation of surface mucus (Sorensen, 1991; Haux et al., 1986). It can also result in a delay in larval development (Benijts-Claus and Benijts, 1975). Toxicity of Pb is reduced in hard water (i.e. high concentration of $CaCO_3$) (Galvin, 1996) as Ca has the ability to antagonise some of the toxic effects of Pb (Roebuck, 1992). Furthermore Pb accumulation exhibits a negative correlation with pH (Merlini and Pozzi, 1977).

Nickel

Nickel (Ni) is used in the melting process of metallic alloys, as catalyst and in pesticide formulations (Galvin, 1996). Other sources include alkaline storage batteries and the production and utilisation of coal (Birge and Black, 1980). The metal is generally considered not to be very toxic to man (Galvin, 1996) since it is regarded as an essential element (Moore and Ramamoorthy, 1984). Certain salts can however have potentially carcinogenic and mutagenic effects through interactions between the metal and cellular DNA (Galvin, 1996). In humans high ingestion of Ni can also cause renal problems and skin allergies by contact (Galvin, 1996). As is the case with other essential elements like Zn, Ni is toxic to fish when present in high enough concentrations (Pickering, 1974).

Aim

The objectives of this study were to determine to what extent:

- Heavy metal accumulation occurs in *Clarias gariepinus* tissues, and in which tissues (i.e. ranking concentrations in skin, muscle, gill arch, gill filament and liver)
- Metal concentrations differ between localities (Vaal Dam and Vaal River Barrage)
- Metal concentrations differ between surveys and seasons

Materials and methods

Study sites

Surveys were conducted bimonthly at 2 localities: the former RAU Island (now UJ Island) in the Vaal Dam (S 26° 52.249´, E 28° 10.249´) and the Vaal River Barrage near the Barrage wall (S 26° 45.786´, E 27° 41.280´). Water quality in the Vaal River Barrage is poorer than that of the Vaal Dam, due to increased levels of urban, industrial, mining and agricultural effluent and runoff. Surveys were conducted during the following months: November 1998, January 1999, March 1999, June 1999, August 1999, October 1999 and February 2000.

Water and sediment analyses

Integrated water samples from 0 to 5 m were obtained with the aid of a modified hosepipe. Sediment samples were obtained with the aid of a sediment corer. The Department of Water Affairs’ Institute for Water Quality Studies (Roodeplaat Dam, Pretoria) performed all water and sediment trace metal analyses. Rand Water Board also made data available on trace metal analyses resulting from their monitoring activities. Data from the following Rand Water Board sampling points were used: C-VD1I (Rand Water Board Inlet at the Vaal Dam wall) and C-VRB0T (upstream at the Vaal River Barrage).

Tissue and organ collection

During each survey 20 *Clarias gariepinus* (Burchell, 1822) were collected at both localities with the aid of gill nets (stretched mesh sizes 90, 110 and 130 mm.). Fish were transported back to land in a live well and transferred to a holding tank through which dam or river water was continuously circulated. Fish were weighed, measured, placed on a polypropylene dissection board and killed by severing the spinal cord behind the head. The following tissues or organs were removed: liver, skin, muscles and gills (filaments attached to arch). Enough tissue was collected to fill the 25 mℓ glass bottle in which it was stored. Samples were kept on ice before being transferred to a deep freeze at the end of each day. The frozen samples were transported to the laboratory.

Laboratory analysis

In the laboratory samples were allowed to thaw at room temperature. Approximately 5 g of wet tissue (liver, skin and muscle) were weighed in an Erlenmeyer flask with the aid of a Mettler PK 4800 scale. This corresponded to approximately 1 g dry weight. All glassware was soaked in 1M hydrochloric acid (HCL) for 24 h and rinsed in doubly-distilled water prior to use (Giesy and Wiener, 1977). The samples were subsequently placed in a Haraeus Hanau KB 500 oven at 60°C for 48 h or until completely dehydrated. In previous studies (Marx, 1996) only the gill filaments were used for metal analysis after being removed from the arches using small scissors. Seymore et al. (1995) suggested that bony tissue should be included in metal accumulation studies. As a result both gill filaments and gill arches were used in the current study. It was found that not all filament tissue could be successfully removed from the arches using dissection equipment. Whole gill arches with filaments still attached were placed in the Haraeus Hanau KB 500 oven until completely dehydrated. Once dehydrated the filaments could effortlessly be removed from the arches. The dehydrated skin, liver and muscle tissue were weighed again and the weight in grams noted. Dehydrated gill filament (1 g) and gill arch tissue (1 g) were weighed out and the exact weight was recorded. A 2:1 mixture of 55% nitric acid (NHO_3) and 70% perchloric acid ($HCLO_4$) was then added to the 1 g of dried tissue in the Erlenmeyer flask (Van Loon, 1980; Houba et al., 1983). Marx (1996) used a mixture of 10 mℓ NHO_3 and 5 mℓ $HCLO_4$. In the current study a mixture of 14 mℓ NHO_3 and 7 mℓ $HCLO_4$ was used. This allowed the complete digestion of most tissue samples without having to add more of the acid mixture due to evaporation. The results are still comparable, however, since the formula used allows for the subtraction of acid metal concentrations. After adding the acid mixture the samples were placed on a hot plate at approximately 200°C and allowed to digest until transparent (Van Loon, 1980). After the digested samples were removed from the hot plate and allowed to cool to room temperature, they were diluted to 50 mℓ with distilled water. These samples were subsequently filtered individually with the aid of a 6 µm Millipore acid-resistant membrane filter attached to a vacuum pump. The filtered samples were poured into acid-washed glass bottles rinsed in distilled water. Following each filtration the filter system and membrane pump were also rinsed in distilled water. The total metal concentrations of Sr, Al, Pb and Ni were determined with the aid of a Varian Atomic Absorption Spectrophotometer (SPECTRA AA-10). For each metal, Holpro stock solutions were used to prepare 4 different concentrations of analytical standards. Calibration of the spectrophotometer, for each metal individually, was subsequently conducted with the aid of these standards. Individual concentrations of the digested tissue samples were then read against particular absorbencies defined for each metal. An Air-Acetylene flame was used to read strontium and aluminium, while an Air-Nitrous flame was used for all other metals. Both strontium and aluminium was only analysed after adding 0.5 mℓ of a 2.682 M potassium chloride (200 g KCl per 1 000 mℓ distilled water) to the 50 mℓ sample. Potassium chloride is added to keep these metals from ionising (Varian, 1989). A 2:1 (14 mℓ: 7 mℓ) solution of nitric and phosphoric acid was also made up and its absorbency read using the spectrophotometer. The metal concentrations thus determined could be subtracted from the concentrations determined for the digested 50 mℓ samples, using the following formula:

$$\begin{array}{c}\text{Metal}\\ \text{concentration}\\ (\mu g/g)\end{array} = \frac{\text{AAS reading } (\mu g/m\ell)}{\text{Sample mass (g) dry wt.}} \times \begin{array}{c}\text{Sample volume}\\ (50\ m\ell)\end{array} - \begin{array}{c}\text{Acid metal}\\ \text{concentration}\end{array}$$

Statistical significance of metal concentrations between localities and surveys respectively was investigated and tabulated as T-test p-values.

Results

Strontium

Water and sediment

Table 1
Strontium concentrations in water and sediment of the Vaal Dam and Vaal River Barrage, obtained during 7 surveys conducted between 1998 and 2000

Month of survey	Water or sediment sample	Locality	
		Vaal Dam	Vaal River Barrage
November (1998)	Water (mg/ℓ)	N/A	N/A
	Sediment (mg/g)	0.277	N/A
January (1999)	Water (mg/ℓ)	N/A	N/A
	Sediment (mg/g)	0.184	0.161
March (1999)	Water (mg/ℓ)	N/A	N/A
	Sediment (mg/g)	0.116	0.165
June (1999)	Water (mg/ℓ)	N/A	0.168
	Sediment (mg/g)	0.207	0.165
August (1999)	Water (mg/ℓ)	0.125	0.181
	Sediment (mg/g)	0.111	0.371
October (1999)	Water (mg/ℓ)	0.146	0.204
	Sediment (mg/g)	0.054	0.296
February (2000)	Water (mg/ℓ)	0.106	0.138
	Sediment (mg/g)	0.231	0.088

All analyses by Department of Water Affairs (Institute for Water Quality Studies)
N/A = Not available

Differences in Sr concentrations recorded from sediment and water between surveys were not reflected in concentrations recorded from fish tissues. In the Vaal Dam Sr was accumulated in the different tissues (Table 2), in order of decreasing concentration, as follows: gill arch > gill filament > skin > liver > muscle. In the Vaal River Barrage, the decreasing order of Sr concentrations in tissues was: gill arch > gill filament > muscle $\geq$ liver $\geq$ skin. No clear trends regarding seasonal Sr concentrations could be identified (Table 3).

Table 2

Strontium concentrations (µg/g) in 5 different tissue types of *Clarias gariepinus* from the Vaal Dam and Vaal River Barrage (shaded blocks). T-test p-values indicate whether concentrations recorded in each tissue type differed significantly between localities.

Month	Locality	Statistical variable	Tissue type					
			Skin	Liver	Muscle	Gill arch	Gill filament	Whole gill
November (1998)	**Vaal Dam**	**Mean**	2.23	2.94	1.11	-	-	102.66
	(n = 20)	Std. deviation	1.76	2.32	1.22	-	-	25.57
January (1999)	**Vaal Dam**	**Mean**	2.33	3.39	0.42	117.31	77.10	-
	(n = 6)	Std. deviation	1.64	1.80	0.27	42.69	21.35	-
		p-value	ns	**< 0.010**	**< 0.001**	**< 0.010**	**< 0.001**	-
	Barrage	**Mean**	1.83	1.46	2.58	73.48	35.37	-
	(n = 20)	Std. deviation	1.32	1.30	1.59	24.27	13.76	-
March (1999)	**Vaal Dam**	**Mean**	0.83	2.13	2.34	162.24	74.76	-
	(n = 9)	Std. deviation	0.93	1.98	1.10	28.08	12.56	-
		p-value	ns	**< 0.050**	ns	**< 0.001**	**< 0.001**	-
	Barrage	**Mean**	3.10	3.48	2.40	91.85	46.99	-
	(n = 17)	Std. deviation	3.24	1.21	1.59	15.99	8.57	-
June (1999)	**Vaal Dam**	**Mean**	3.90	2.31	2.67	166.46	112.06	-
	(n = 17)	Std. deviation	3.17	1.28	1.44	20.55	34.02	-
		p-value	ns	ns	ns	**< 0.001**	**< 0.001**	-
	Barrage	**Mean**	3.67	1.18	3.74	71.86	32.00	-
	(n = 6)	Std. deviation	1.39	1.72	1.66	12.01	11.68	-
August (1999)	**Vaal Dam**	**Mean**	2.25	1.59	3.43	149.93	111.49	-
	(n = 18)	Std. deviation	1.83	1.08	1.95	16.75	32.73	-
		p-value	ns	ns	ns	**< 0.001**	**< 0.001**	-
	Barrage	**Mean**	2.11	2.31	3.15	84.84	58.76	-
	(n = 20)	Std. deviation	2.12	1.41	1.76	10.05	17.11	-
October (1999)	**Vaal Dam**	**Mean**	2.16	1.97	2.57	148.76	96.45	-
	(n = 20)	Std. deviation	1.27	1.90	2.67	23.46	32.01	-
		p-value	**< 0.050**	**< 0.050**	ns	**< 0.001**	**< 0.001**	-
	Barrage	**Mean**	3.10	3.12	2.97	76.78	49.70	-
	(n = 20)	Std. deviation	1.10	1.54	0.89	16.99	15.86	-
February (2000)	**Vaal Dam**	**Mean**	3.70	2.89	1.89	139.83	83.78	-
	(n = 20)	Std. deviation	2.50	2.37	1.42	28.34	17.48	-
		p-value	ns	ns	ns	**< 0.001**	**< 0.001**	-
	Barrage	**Mean**	2.79	3.70	1.81	63.82	39.41	-
	(n = 17)	Std. deviation	1.71	1.14	1.70	13.15	12.62	-
Pooled for all surveys	**Vaal Dam**	**Mean**	2.64	2.40	2.20	149.60	96.13	-
	(n = 110)	Std. deviation	2.22	1.92	1.89	27.46	30.62	-
		p-value	ns	ns	ns	**< 0.001**	**< 0.001**	-
	Barrage	**Mean**	2.63	2.67	2.68	77.79	45.37	-
	(n = 100)	Std. deviation	2.01	1.60	1.59	18.65	16.21	-

ns = not significant

Table 3
Summary of statistically significant differences between 7 surveys, with reference to bioaccumulation of strontium in *Clarias gariepinus* from the Vaal Dam (blank blocks) and Vaal River Barrage (shaded blocks).

Survey	Tissue type	Jan 1999	Mar 1999	Jun 1999	Aug 1999	Oct 1999	Feb 2000
Nov 1998	Skin	ns	ns	ns	ns	ns	ns
	Liver	ns	ns	ns	ns	ns	ns
	Muscle	ns	ns	**< 0.050**	**< 0.010**	**< 0.050**	ns
Jan 1999	Skin		ns	ns	ns	ns	ns
	Liver		ns	ns	ns	ns	ns
	Muscle		ns	ns	ns	ns	ns
	Gill arch		**0.05**	**< 0.010**	ns	ns	ns
	Gill filament		ns	ns	ns	ns	ns
Mar 1999	Skin	ns		ns	ns	ns	ns
	Liver	**< 0.010**		ns	ns	ns	ns
	Muscle	ns		ns	ns	ns	ns
	Gill arch	ns		ns	ns	ns	ns
	Gill filament	ns		ns	ns	ns	ns
Jun 1999	Skin	ns	ns		ns	ns	ns
	Liver	ns	**< 0.050**		ns	ns	ns
	Muscle	ns	ns		ns	ns	ns
	Gill arch	ns	ns		ns	ns	ns
	Gill filament	ns	ns		ns	ns	ns
Aug 1999	Skin	ns	ns	ns		ns	ns
	Liver	ns	ns	ns		ns	ns
	Muscle	ns	ns	ns		ns	ns
	Gill arch	ns	ns	ns		ns	ns
	Gill filament	**< 0.001**	ns	**< 0.010**		ns	ns
Oct 1999	Skin	**< 0.050**	ns	ns	ns		ns
	Liver	**< 0.050**	ns	ns	ns		ns
	Muscle	ns	ns	ns	ns		ns
	Gill arch	ns	ns	ns	ns		ns
	Gill filament	ns	ns	ns	ns		ns
Feb 2000	Skin	ns	ns	ns	ns	ns	
	Liver	**< 0.001**	ns	**< 0.050**	ns	ns	
	Muscle	ns	ns	ns	ns	ns	
	Gill arch	ns	**0.001**	ns	**< 0.050**	ns	
	Gill filament	ns	ns	ns	**< 0.010**	ns	

Vaal Dam

Vaal River Barrage

ns = not significant

Nickel

Water and sediment

Concentrations recorded from tissues were generally higher than or equal to concentrations recorded in water, but lower than concentrations recorded in sediment. Tissues were ranked according to decreasing Al concentrations (Table 5) as follows: Vaal Dam – gill filament > skin > liver ≥ gill arch > muscle; Vaal River Barrage – gill filament > skin ≥ gill arch > liver > muscle. At both localities significantly higher Al concentrations in muscle were recorded during the June 1999 survey compared to other surveys (Table 6).

Lead

Water and sediment

All Pb concentrations were below the detection limit in both water (0.1 mg/ℓ) and sediment (0.05 mg/g) respectively. The ranking of tissues and organs in terms of Pb concentration (Table 8) were: Vaal Dam – gill arch ≥ gill filament > liver ≥ muscle ≥ skin; Vaal River Barrage – gill arch > gill filament ≥ muscle ≥ liver ≥ skin. Seasonal trends in differences in metal tissue concentrations between localities were not evident (Table 9).

Table 4
Aluminium concentrations in water and sediment of the Vaal Dam and Vaal River Barrage, obtained during 7 surveys conducted between 1998 and 2000

Month of survey	Water or sediment sample	Locality	
		Vaal Dam	Vaal River Barrage
November (1998)	Water (mg/ℓ)	0.481	N/A
	Sediment (mg/g)	41.657	N/A
January (1999)	Water (mg/ℓ)	0.101	0.361
	Sediment (mg/g)	51.798	54.336
March (1999)	Water (mg/ℓ)	0.141	< 0.050
	Sediment (mg/g)	52.787	51.819
June (1999)	Water (mg/ℓ)	0.161	< 0.035
	Sediment (mg/g)	60.564	50.387
August (1999)	Water (mg/ℓ)	< 0.035	< 0.035
	Sediment (mg/g)	35.751	51.017
October (1999)	Water (mg/ℓ)	< 0.035	< 0.035
	Sediment (mg/g)	12.311	52.787
February (2000)	Water (mg/ℓ)	< 0.035	< 0.035
	Sediment (mg/g)	33.386	60.541

Analyses by Department of Water Affairs and Forestry (Institute for Water Quality Studies)
N/A = Not available

Data provided by Rand Water Board

TABLE 5
Aluminium concentrations (µg/g) in 5 different tissue types of *Clarias gariepinus* from the Vaal Dam and Vaal River Barrage (shaded blocks). T-test p-values indicate whether concentrations recorded in each tissue type differed significantly between localities.

Month	Locality	Statistical variable	Tissue type					
			Skin	Liver	Muscle	Gill arch	Gill filament	Whole gill
November (1998)	**Vaal Dam**	**Mean**	40.93	34.30	27.67	-	-	129.19
	(n = 20)	Std. deviation	37.04	23.42	33.74	-	-	159.40
January (1999)	**Vaal Dam**	**Mean**	52.94	47.05	19.85	29.45	95.27	-
	(n = 6)	Std. deviation	36.86	30.24	22.02	34.09	52.42	-
		p-value	ns	ns	ns	ns	ns	-
	Barrage	**Mean**	33.37	35.77	21.94	39.13	72.41	-
	(n = 20)	Std. deviation	23.45	26.78	15.02	30.50	57.09	-
March (1999)	**Vaal Dam**	**Mean**	23.35	82.99	17.50	42.77	99.37	-
	(n = 9)	Std. deviation	13.14	61.93	14.65	18.35	83.42	-
		p-value	ns	ns	**< 0.050**	ns	ns	-
	Barrage	**Mean**	23.74	48.58	32.93	42.08	162.57	-
	(n = 17)	Std. deviation	18.69	20.34	17.56	27.47	84.63	-
June (1999)	**Vaal Dam**	**Mean**	128.58	55.85	57.69	74.02	112.94	-
	(n = 17)	Std. deviation	117.41	26.30	19.29	16.34	46.75	-
		p-value	ns	**< 0.050**	ns	**< 0.010**	**< 0.001**	-
	Barrage	**Mean**	55.24	40.48	52.95	51.97	55.81	-
	(n = 6)	Std. deviation	20.82	2.72	19.99	4.26	9.55	-
August (1999)	**Vaal Dam**	**Mean**	58.97	90.61	33.44	27.84	105.83	-
	(n = 18)	Std. deviation	92.44	159.09	12.36	29.89	75.65	-
		p-value	ns	ns	ns	**< 0.010**	ns	-
	Barrage	**Mean**	48.97	40.20	33.96	53.48	65.57	-
	(n = 20)	Std. deviation	11.59	18.51	15.63	7.33	32.07	-

Table 5 (continued)

October (1999)	**Vaal Dam**	**Mean**	44.78	28.28	23.24	48.68	86.02	-
	(n = 20)	Std. deviation	34.35	25.31	26.03	15.25	33.85	-
		p-value	ns	ns	ns	**< 0.010**	ns	-
	Barrage	**Mean**	53.47	24.62	34.66	35.78	53.92	-
	(n = 20)	Std. deviation	31.17	14.65	8.80	13.44	29.12	-
February (2000)	**Vaal Dam**	**Mean**	68.21	29.36	36.17	43.90	39.05	-
	(n = 20)	Std. deviation	73.11	25.64	23.97	25.72	30.81	-
		p-value	ns	ns	**< 0.050**	ns	**< 0.010**	-
	Barrage	**Mean**	50.77	27.92	22.90	42.25	110.05	-
	(n = 17)	Std. deviation	35.88	12.37	9.50	18.58	93.26	-
Pooled for all surveys	**Vaal Dam**	**Mean**	62.30	49.53	32.73	46.36	94.21	-
	(n = 110)	Std. deviation	75.97	72.61	26.12	27.27	92.36	-
		p-value	**< 0.010**	**< 0.050**	ns	ns	ns	-
	Barrage	**Mean**	43.14	36.08	30.78	43.13	88.07	-
	(n = 100)	Std. deviation	27.12	19.89	15.87	21.05	70.99	-

ns = not significant

Table 6
Summary of statistically significant differences between 7 surveys, with reference to bioaccumulation of aluminium in *Clarias gariepinus* from the Vaal Dam (blank blocks) and Vaal River Barrage (shaded blocks)

Survey	Tissue type	Jan 1999	Mar 1999	Jun 1999	Aug 1999	Oct 1999	Feb 2000
Nov 1998	Skin	ns	ns	ns	ns	ns	ns
	Liver	ns	ns	ns	ns	ns	ns
	Muscle	ns	ns	**< 0.050**	ns	ns	ns
Jan 1999	Skin		ns	ns	ns	ns	ns
	Liver		ns	ns	ns	ns	ns
	Muscle		ns	**< 0.001**	ns	ns	ns
	Gill arch		ns	ns	ns	ns	ns
	Gill filament		ns	ns	ns	ns	ns
Mar 1999	Skin	ns		**< 0.050**	ns	ns	ns
	Liver	ns		ns	ns	ns	ns
	Muscle	ns		**< 0.001**	ns	ns	ns
	Gill arch	ns		**< 0.010**	ns	ns	ns
	Gill filament	**< 0.050**		ns	ns	ns	ns
Jun 1999	Skin	ns	ns		ns	ns	ns
	Liver	ns	ns		ns	ns	ns
	Muscle	**0.001**	ns		**< 0.001**	**< 0.001**	**< 0.001**
	Gill arch	ns	ns		**< 0.001**	**< 0.001**	ns
	Gill filament	ns	**0.001**		ns	ns	ns
Aug 1999	Skin	ns	**0.001**	ns		ns	ns
	Liver	ns	ns	ns		ns	ns
	Muscle	ns	ns	ns		ns	ns
	Gill arch	ns	ns	ns		ns	ns
	Gill filament	ns	ns	ns		ns	ns
Oct 1999	Skin	ns	**< 0.050**	ns	ns		ns
	Liver	ns	**< 0.001**	**< 0.010**	ns		ns
	Muscle	ns	ns	ns	ns		ns
	Gill arch	ns	ns	**0.001**	**< 0.001**		ns
	Gill filament	ns	**0.001**	ns	ns		ns
Feb 2000	Skin	ns	ns	ns	ns	ns	
	Liver	ns	**0.001**	**< 0.050**	ns	ns	
	Muscle	ns	ns	**< 0.010**	ns	ns	
	Gill arch	ns	ns	ns	ns	ns	
	Gill filament	ns	ns	ns	ns	ns	

Vaal Dam

Vaal River Barrage

Table 7
Lead concentrations in water and sediment of the Vaal Dam and Vaal River Barrage, obtained during 7 surveys conducted between 1998 and 2000

Month of survey	Water or sediment sample	Locality	
		Vaal Dam	Vaal River Barrage
November (1998)	Water (mg/ℓ)	< 0.100	N/A
	Sediment (mg/g)	< 0.050	N/A
January (1999)	Water (mg/ℓ)	< 0.100	< 0.100
	Sediment (mg/g)	< 0.050	< 0.050
March (1999)	Water (mg/ℓ)	< 0.100	< 0.100
	Sediment (mg/g)	< 0.050	< 0.050
June (1999)	Water (mg/ℓ)	< 0.100	< 0.100
	Sediment (mg/g)	< 0.050	< 0.050
August (1999)	Water (mg/ℓ)	< 0.100	< 0.100
	Sediment (mg/g)	< 0.050	< 0.050
October (1999)	Water (mg/ℓ)	< 0.100	< 0.100
	Sediment (mg/g)	< 0.050	< 0.050
February (2000)	Water (mg/ℓ)	< 0.100	< 0.100
	Sediment (mg/g)	< 0.050	< 0.050

Analyses by Department of Water Affairs and Forestry (Institute for Water Quality Studies)

Data provided by Rand Water Board

All concentrations are below the detection limit
N/A = Not available

Table 8
Lead concentrations (µg/g) in 5 different tissue types of *Clarias gariepinus* from the Vaal Dam and Vaal River Barrage (shaded blocks). T-test p-values indicate whether concentrations recorded in each tissue type differed significantly between localities.

Month	Locality	Statistical variable	Tissue type					
			Skin	Liver	Muscle	Gill arch	Gill filament	Whole gill
November (1998)	Vaal Dam	**Mean**	1.14	10.37	0.59	-	-	10.29
	(n = 20)	Std. deviation	1.09	2.85	0.67	-	-	8.23
January (1999)	Vaal Dam	**Mean**	9.21	2.57	5.67	8.90	14.82	-
	(n = 6)	Std. deviation	2.12	1.73	0.87	5.96	3.23	-
		p-value	**< 0.001**	**< 0.050**	ns	ns	ns	-
	Barrage	**Mean**	2.26	5.51	5.23	12.93	14.06	-
	(n = 20)	Std. deviation	1.84	2.67	2.15	3.78	5.11	-
March (1999)	Vaal Dam	**Mean**	4.79	3.34	2.64	15.02	12.48	-
	(n = 9)	Std. deviation	2.97	2.18	2.28	7.09	6.84	-
		p-value	ns	ns	**< 0.010**	ns	ns	-
	Barrage	**Mean**	4.15	2.96	5.52	14.79	8.75	-
	(n = 17)	Std. deviation	2.35	3.15	1.91	2.23	2.53	-
June (1999)	Vaal Dam	**Mean**	3.51	4.92	4.29	14.77	10.74	-
	(n = 17)	Std. deviation	1.64	3.25	3.46	1.38	2.76	-
		p-value	ns	ns	ns	ns	ns	-
	Barrage	**Mean**	2.90	2.27	4.02	14.74	12.42	-
	(n = 6)	Std. deviation	1.13	0.82	0.89	1.02	2.63	-
August (1999)	Vaal Dam	Mean	2.61	2.33	2.26	12.34	9.65	-
	(n = 18)	Std. deviation	0.73	0.87	0.64	1.56	2.73	-
		p-value	ns	ns	**< 0.001**	**0.001**	ns	-
	Barrage	**Mean**	2.53	1.92	3.67	14.00	8.19	-
	(n = 20)	Std. deviation	1.38	1.32	0.80	1.17	2.40	-

Table 8 (continued)

October (1999)	Vaal Dam (n = 20)	**Mean** Std. deviation	2.19 0.68	3.14 0.64	3.53 0.82	12.76 1.54	9.69 1.74	- -
		p-value	**0.001**	**< 0.010**	ns	**< 0.050**	**< 0.050**	-
	Barrage (n = 20)	**Mean** Std. deviation	3.97 1.94	4.17 1.16	3.87 4.57	11.63 1.66	8.25 2.00	- -
February (2000)	Vaal Dam (n = 20)	**Mean** Std. deviation	2.29 0.41	2.37 1.36	4.09 1.08	11.85 2.10	8.85 1.65	- -
		p-value	**< 0.001**	ns	**< 0.001**	ns	ns	-
	Barrage (n = 17)	**Mean** Std. deviation	3.43 0.52	2.81 0.39	2.75 0.45	11.53 1.55	8.02 1.42	- -
Pooled for all surveys	Vaal Dam (n = 110)	**Mean** Std. deviation	2.89 2.22	4.44 3.55	3.05 2.17	12.82 3.36	10.32 3.37	- -
		p-value	ns	ns	ns	ns	ns	-
	Barrage (n = 100)	**Mean** Std. deviation	3.21 1.81	3.44 2.29	4.20 2.58	13.07 2.54	9.69 3.80	- -

ns = not significant

Table 9
Summary of statistically significant differences between 7 surveys, with reference to bioaccumulation of lead in *Clarias gariepinus* from the Vaal Dam (blank blocks) and Vaal River Barrage (shaded blocks)

Survey	Tissue type	Jan 1999	Mar 1999	Jun 1999	Aug 1999	Oct 1999	Feb 2000
Nov 1998	Skin	**0.001**	ns	**0.001**	**< 0.001**	**< 0.050**	**< 0.010**
	Liver	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	Muscle	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Jan 1999	Skin		ns	**< 0.010**	**< 0.010**	**< 0.010**	**< 0.010**
	Liver		ns	ns	ns	ns	ns
	Muscle		ns	ns	**< 0.001**	ns	**< 0.010**
	Gill arch		ns	ns	ns	ns	ns
	Gill filament		ns	ns	ns	ns	**< 0.050**
Mar 1999	Skin	ns		ns	ns	ns	ns
	Liver	ns		ns	ns	ns	ns
	Muscle	ns		ns	ns	ns	ns
	Gill arch	ns		ns	ns	ns	ns
	Gill filament	**< 0.010**		ns	ns	ns	ns
Jun 1999	Skin	ns	ns		ns	ns	ns
	Liver	**0.001**	ns		ns	ns	ns
	Muscle	ns	ns		ns	ns	ns
	Gill arch	ns	ns		**< 0.001**	**< 0.010**	**< 0.001**
	Gill filament	ns	ns		ns	ns	ns
Aug 1999	Skin	ns	ns	ns		ns	ns
	Liver	**< 0.001**	ns	ns		ns	ns
	Muscle	ns	**< 0.050**	ns		ns	ns
	Gill arch	ns	ns	ns		ns	ns
	Gill filament	**0.001**	ns	ns		ns	ns
Oct 1999	Skin	ns	ns	ns	ns		ns
	Liver	ns	ns	**0.010**	**< 0.001**		ns
	Muscle	ns	ns	ns	ns		ns
	Gill arch	ns	**0.001**	**0.001**	**< 0.001**		ns
	Gill filament	**0.001**	ns	ns	ns		ns
Feb 2000	Skin	ns	ns	ns	ns	ns	
	Liver	**< 0.010**	ns	ns	ns	**0.001**	
	Muscle	**0.001**	**< 0.001**	ns	**< 0.010**	ns	
	Gill arch	ns	**< 0.001**	**0.001**	**< 0.001**	ns	
	Gill filament	**0.001**	ns	ns	ns	ns	

Vaal Dam

Vaal River Barrage

Table 10
Nickel concentrations in water and sediment of the Vaal Dam and Vaal River Barrage, obtained during 7 surveys conducted between 1998 and 2000

Month of survey	Water or sediment sample	Locality	
		Vaal Dam	Vaal River Barrage
November (1998)	Water (mg/ℓ)	< 0.050	N/A
	Sediment (mg/g)	< 0.009	N/A
January (1999)	Water (mg/ℓ)	< 0.050	< 0.050
	Sediment (mg/g)	< 0.009	< 0.009
March (1999)	Water (mg/ℓ)	< 0.050	< 0.050
	Sediment (mg/g)	< 0.009	< 0.009
June (1999)	Water (mg/ℓ)	< 0.050	< 0.011
	Sediment (mg/g)	< 0.009	< 0.009
August (1999)	Water (mg/ℓ)	< 0.011	< 0.011
	Sediment (mg/g)	< 0.009	< 0.009
October (1999)	Water (mg/ℓ)	< 0.011	< 0.011
	Sediment (mg/g)	< 0.009	0.012
February (2000)	Water (mg/ℓ)	< 0.011	< 0.011
	Sediment (mg/g)	< 0.009	< 0.009

Analyses by Department of Water Affairs (Institute for Water Quality Studies)

Data provided by Rand Water Board

N/A = Not available

Nickel concentrations in both water and sediment were below the limits for detection (50 and 11 µg/ℓ, and 9 µg/g respectively). The low concentrations in sediment and water were mirrored by low Ni concentrations in *C. gariepinus* tissues. Concentrations recorded in the different tissues (Table 11), listed in decreasing order, were: Vaal Dam – gill arch ≥ gill filament ≥ muscle ≥ liver ≥ skin; Vaal River Barrage – gill arch ≥ skin ≥ muscle ≥ gill filament ≥ liver. With regards to significant seasonal differences within the localities no clear trends emerged (Table 12).

Factors that may influence metal availability

Readings and concentrations of 6 variables that may influence metal availability are given in Fig. 1 (next page). Peak flow readings in m^3/s were obtained from the Department of Water Affairs database (recorded from the De Vaal Schoemansdrift gauging weir,
station number C2H018-A01).

Summary of results

Water and sediment

Some of the readings, as indicated in the tables listed earlier, have been obtained from Rand Water Board and others from the Department of Water Affairs. As a result, 2 detection limits have been recorded for the same metal in many cases. Please note that this may give rise to 'false' trends.

Concentrations of the 4 metals under review in sediment and water can be divided into 3 classes. For Pb and Ni very low concentrations (mostly below the detection limits) were recorded in both sediment and water at both localities. Strontium concentrations were recorded in both sediment and water, with no readings under the detection limit being recorded. For Al high concentrations were recorded from sediment, while most water concentrations were found to be below detection. The high Al concentrations recorded from the Vaal River Barrage water samples during the October 1999 and January 1999 surveys were possibly the result of effluent or re-suspension from sediment. Jackson et al. (2009) also recorded high Al concentrations in water and sediment from the Plankenburg and Diep rivers (Western Cape). They could also not identify point sources of pollution.

In general, metal concentrations were not much higher at any one locality, as is evident from the similar maximum concentrations recorded at both localities.

Concentrations in fish tissue

Significant seasonal differences in concentration were recorded for all metals, but the same tissues did not always indicate these differences. In terms of significant ($p < 0.05$) differences in metal concentrations in fish tissue recorded from the 2 localities (Table 13), some general trends do emerge.

Strontium concentrations in gill tissue were significantly higher at the Vaal Dam during all individual surveys and when pooled for surveys. A similar trend was observed with regards to Ni concentration. For Al, Pb and, to a lesser extent, Ni, many of the differences between localities related to concentrations in muscle, skin and liver. When considering the number of 'month classes' for which significant differences were detected, concentrations of Sr and Al were higher more often at the Vaal

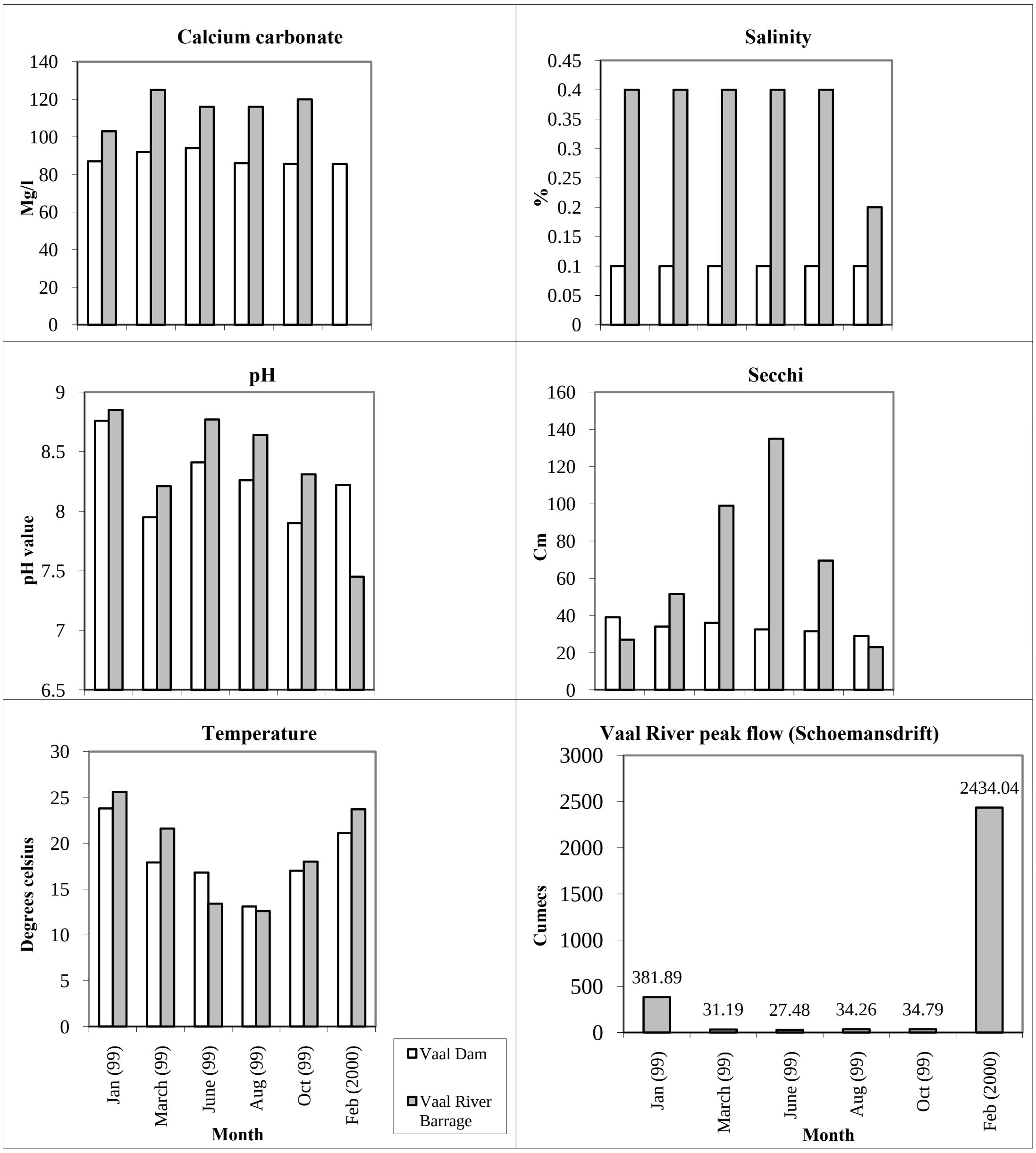

Figure 1

Concentrations and values for variables that affect metal toxicity and availability: water hardness (as calcium carbonate), salinity, pH, suspended solid particles (represented by Secchi disk readings), temperature and peak flow

Dam while Pb and Ni were higher more often at the Vaal River Barrage.

Ranking tissues on the grounds of metal concentrations also allows identification of some trends. At both the Vaal Dam and Vaal River Barrage gill filament tissue was often ranked second with regard to metals concentrations encountered. The highest metal concentrations were most often recorded in gill arch, gill filament or liver tissue, with gill arch being ranked first most often. At both localities the lowest metal concentrations were most often recorded in muscle and skin tissue.

Discussion

The Vaal River Barrage receives domestic and industrial runoff and effluent from the PWV area, through streams like the Rietspruit and the Klip and Suikerbosrand Rivers. In terms of physical variables (e.g. salinity and conductivity) and macro-determinants (phosphates, sulphates etc.), water quality at the Vaal River Barrage is poorer when compared to the Vaal Dam (Crafford and Avenant-Oldewage, 2009). However, contrary to expectations, metal concentrations in sediment and water recorded at both localities were similar. Braune and Rogers (1987) stated that the Vaal River catchment could be divided into 4 zones on the basis of water quality. According to their classification water quality in the Vaal Dam subcatchment is better than water quality in the Vaal River Barrage subcatchment. In view of the trace metal analyses results this is not necessarily the case. They do however continue by saying that ‘this deterioration is fortunately in line with the general distribution of user water quality requirements in the catchment’. They state that water use in the Vaal Dam subcatchment is geared towards power stations and urban use (i.e. drinking water). From this

Table 11
Nickel concentrations (µg/g) in 5 different tissue types of *Clarias gariepinus* from the Vaal Dam and Vaal River Barrage (shaded blocks). T-test p-values indicate whether concentrations recorded in each tissue type differed significantly between localities.

Month	Locality	Statistical variable	Tissue type					
			Skin	Liver	Muscle	Gill arch	Gill filament	Whole gill
November (1998)	Vaal Dam	**Mean**	2.49	0.66	2.02	-	-	6.58
	(n = 20)	Std. deviation	1.25	1.33	0.55	-	-	1.22
January (1999)	**Vaal Dam**	**Mean**	2.08	1.18	0.36	2.06	2.97	-
	(n = 6)	Std. deviation	1.02	1.76	0.54	2.68	3.13	-
		p-value	ns	ns	**< 0.050**	ns	ns	-
	Barrage	**Mean**	3.51	0.09	1.41	3.98	0.81	-
	(n = 20)	Std. deviation	1.92	0.27	1.55	2.41	1.14	-
March (1999)	**Vaal Dam**	**Mean**	0	0.20	8.51	1.07	4.17	-
	(n = 9)	Std. deviation	0	0.33	3.30	2.12	3.92	-
		p-value	**0.001**	ns	**< 0.050**	ns	**< 0.050**	-
	Barrage	**Mean**	4.33	0.52	4.97	1.42	1.34	-
	(n = 17)	Std. deviation	4.64	0.71	4.75	1.75	1.45	-
June (1999)	**Vaal Dam**	**Mean**	2.39	1.89	2.57	4.62	0.73	-
	(n = 17)	Std. deviation	0.83	0.73	1.50	1.19	0.62	-
		p-value	**< 0.010**	ns	**< 0.050**	ns	**< 0.001**	-
	Barrage	**Mean**	1.12	1.55	1.14	4.30	4.72	-
	(n = 6)	Std. deviation	0.57	0.26	0.63	0.55	0.75	-
August (1999)	**Vaal Dam**	**Mean**	1.14	1.21	0.46	8.56	5.00	-
	(n = 18)	Std. deviation	0.37	0.38	0.30	1.12	1.17	-
		p-value	ns	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	-
	Barrage	**Mean**	1.12	2.09	1.47	4.43	2.99	-
	(n = 20)	Std. deviation	0.47	0.59	0.53	0.55	0.70	-
October (1999)	**Vaal Dam**	**Mean**	0.20	2.26	1.38	8.40	4.47	-
	(n = 20)	Std. deviation	0.39	1.18	0.85	4.03	1.89	-
		p-value	**< 0.010**	ns	**0.001**	**< 0.001**	**< 0.010**	-
	Barrage	**Mean**	0.87	2.86	3.20	2.36	2.91	-
	(n = 20)	Std. deviation	0.92	1.23	2.05	3.02	1.54	-
February (2000)	**Vaal Dam**	**Mean**	0.00	2.44	0.58	4.79	3.39	-
	(n = 20)	Std. deviation	0.00	2.22	0.69	2.59	2.76	-
		p-value	**< 0.010**	ns	ns	**< 0.001**	ns	-
	Barrage	**Mean**	4.18	2.97	2.04	1.57	2.28	-
	(n = 17)	Std. deviation	4.79	1.07	3.04	0.98	0.95	-
Pooled for all surveys	**Vaal Dam**	**Mean**	1.16	1.55	1.91	5.76	3.50	-
	(n = 110)	Std. deviation	1.27	1.50	2.44	3.60	2.62	-
		p-value	**< 0.001**	ns	ns	**< 0.001**	**< 0.001**	-
	Barrage	**Mean**	2.61	1.69	2.48	2.92	2.24	-
	(n = 100)	Std. deviation	3.23	1.41	2.87	2.27	1.56	-

ns = not significant

it is concluded that 'water quality', as the term is used by Braune and Rogers (1987), refers to the physical properties of water that determines its acceptability for human consumption, rather than metal pollution. This assumption is confirmed when Braune and Rogers (1987) discuss water quality problems in the Vaal River Barrage subcatchments. They only refer to eutrophication (resulting from organic pollution) and salinity. Organic pollution in the Vaal River Barrage thus appears to take precedence over trace metal pollution. In future, if authors refer to these water quality zones, mention should be made of the specific type(s) of water quality problems addressed. In this respect it may be worthwhile to conduct studies in the 2 lower subcatchments (Bloemhof and Douglas weir subcatchments) to determine the prevalence of heavy metal pollution.

Several possible explanations exist for the fact that significantly higher concentrations of metals were not consistently recorded in tissues from the Vaal River Barrage. In view of sediment and water metal concentrations, the simplest explanation would be that the biologically available metal concentrations at the 2 localities were similar. This is also the most likely

Table 12
Summary of statistically significant differences between 7 surveys, with reference to bioaccumulation of nickel in *Clarias gariepinus* from the Vaal Dam (blank blocks) and Vaal River Barrage (shaded blocks)

Survey	Tissue type	Jan 1999	Mar 1999	Jun 1999	Aug 1999	Oct 1999	Feb 2000
Nov 1998	Skin	ns	**< 0.001**	ns	**< 0.010**	**< 0.001**	**< 0.001**
	Liver	ns	ns	**< 0.050**	ns	**< 0.010**	ns
	Muscle	ns	**< 0.010**	ns	**< 0.001**	ns	ns
Jan 1999	Skin		**< 0.050**	ns	ns	ns	**< 0.050**
	Liver		ns	ns	ns	ns	ns
	Muscle		**< 0.001**	ns	ns	ns	ns
	Gill arch		ns	ns	**< 0.050**	**< 0.010**	ns
	Gill filament		ns	ns	ns	ns	ns
Mar 1999	Skin	ns		**< 0.001**	**< 0.001**	ns	ns
	Liver	ns		**< 0.001**	**< 0.001**	**< 0.001**	**< 0.010**
	Muscle	ns		**< 0.010**	**< 0.001**	**< 0.010**	**< 0.001**
	Gill arch	**0.01**		**< 0.010**	**< 0.001**	**< 0.001**	**< 0.010**
	Gill filament	ns		ns	ns	ns	ns
Jun 1999	Skin	**0.001**	ns		**< 0.001**	**< 0.001**	**< 0.001**
	Liver	**< 0.001**	**0.001**		**< 0.050**	ns	ns
	Muscle	ns	ns		**< 0.050**	ns	ns
	Gill arch	ns	**< 0.001**		**< 0.001**	**< 0.010**	ns
	Gill filament	**< 0.001**	**< 0.001**		**< 0.001**	**< 0.001**	**< 0.010**
Aug 1999	Skin	**< 0.001**	ns	ns		**< 0.001**	**< 0.001**
	Liver	**< 0.001**	**< 0.001**	ns		**< 0.050**	ns
	Muscle	ns	ns	ns		**< 0.010**	ns
	Gill arch	ns	**< 0.001**	ns		ns	**< 0.001**
	Gill filament	**< 0.001**	**< 0.010**	< 0.050		ns	ns
Oct 1999	Skin	**< 0.001**	ns	ns	ns		ns
	Liver	**< 0.001**	**< 0.001**	**< 0.010**	ns		ns
	Muscle	**0.05**	ns	**< 0.010**	**< 0.050**		ns
	Gill arch	ns	ns	ns	ns		**< 0.050**
	Gill filament	**< 0.001**	**< 0.050**	**< 0.050**	ns		ns
Feb 2000	Skin	ns	ns	ns	ns	ns	
	Liver	**< 0.001**	**< 0.001**	**< 0.001**	ns	ns	
	Muscle	ns	ns	ns	ns	ns	
	Gill arch	**< 0.010**	ns	**< 0.001**	**< 0.001**	ns	
	Gill filament	**< 0.010**	ns	**0.001**	ns	ns	

Vaal Dam

Vaal River Barrage

explanation. However, Clements and Rees (1997) warn that bioaccumulation of metals in the field is complex and may be influenced by several factors in addition to levels in food and water. During a metal bioaccumulation study on catfish performed by Avenant-Oldewage and Marx (2000) in the Olifants River, the authors also state that there were no distinctive trends between localities and that variation occurred in different tissues during different seasons at the respective locations. They also stress possible effects of abiotic variables on metal availability and bioaccumulation. In this study higher salinity concentrations were recorded from the Vaal River Barrage compared to the Vaal Dam. High salinity can increase both trace metal availability (Calmano et al., 1992) and toxicity (Dwyer et al., 1992). If salinity did play a role higher metal concentrations would be expected from the Vaal River Barrage, which was not always the case. The higher water hardness recorded from the Vaal River Barrage may, on the other hand, have decreased metal toxicity to fish, and as such may have negatively influenced metal availability (Borgmann and Norwood, 1994). Sorption of heavy metals to sediment particles also reduces their bio-availability. As a result Grobler et al. (1987) believes that the turbid nature of the Vaal River System has a positive effect on water quality. Secci disc readings (Fig. 1) indicate that the Vaal Dam was more turbid during the autumn, winter and spring surveys. Only during the summer surveys were turbid conditions slightly higher at the Vaal River Barrage when compared to the Vaal Dam. If these suspended particles indeed do mitigate metal toxicity, the effect would

Table 13
Table summarising instances where statistically significant differences (T-test) were recorded in *Clarias gariepinus* tissues between localities during the respective surveys

Metal	Survey	[1] Tissue	[2] Locality
Strontium	Jan 1999	# Liver; # Gill arch; * Gill filament	VD
		* Muscle	VRB
	Mar 1999	* Gill arch; * Gill filament	VD
		** Liver	VRB
	Jun 1999 Aug 1999	* Gill arch; * Gill filament	VD
	Oct 1999	* Gill arch; * Gill filament	VD
		** Skin; ** Liver	VRB
	Feb 2000	* Gill arch; * Gill filament	VD
	Pooled		
Aluminium	Mar 1999	** Muscle	VRB
	Jun 1999	**Liver; #Gill arch; * Gill filament	VD
	Aug 1999	# Gill arch	VRB
	Oct 1999	#Gill arch	VD
	Feb 2000	** Muscle # Gill filament	VD VRB
	Pooled	# Skin; ** Liver	VD
Lead	Jan 1999	# Skin ** Liver	VD VRB
	Mar 1999	# Muscle	VRB
	Aug 1999	# Muscle; * Gill Arch	VRB
	Oct 1999	** Gill arch; ** Gill filament *Skin; # Liver	VD VRB
	Feb 2000	*Muscle *Skin	VD VRB
Nickel	Jan 1999	**Muscle	VRB
	Mar 1999	**Muscle; **Gill filament *Skin	VD VRB
	Jun 1999	#Skin; **Muscle *Gill filament	VD VRB
	Aug 1999	*Gill arch; * Gill filament *Liver; *muscle	VD VRB
	Oct 1999	*Gill arch; #Gill filament #Skin; * Muscle	VD VRB
	Feb 2000	*Gill arch #Skin	VD VRB
	Pooled	*Gill arch; *Gill filament *Skin	VD VRB

[1] = *Tissues for which significant differences in metal concentration between localities were recorded.*
[2] = *Locality for which the highest metal concentration was recorded.*
VD = Vaal Dam *VRB = Vaal River Barrage*
** = $p < 0.050$ # = $p < 0.010$ * = $p < 0.001$

thus be greater at the Vaal Dam. Under aquaculture conditions flushing rates have been shown to influence metal accumulation in trout (Davidson et al., 2009) and sea bass (Deviller et al., 2005). Furthermore, flow rates can influence the results obtained when analysing water samples collected from rivers for nickel. Snodgrass (1980) states that particulate matter is flow sensitive and will depend on the amount of rainfall in the river catchment. As a result he believes such samples do not provide a true picture of the total distribution and transport of nickel. The variability in flow rate between surveys (Fig. 1) in the Vaal River Barrage thus may have masked any trends. Increased flow rate results from heavy rain. High rainfall often leads to a lowering of pH, which promotes solubility of heavy metal compounds (Hahne and Kroontje, 1973). The dilution effect (metal concentrations 'diluted' during periods of flooding) must also be kept in mind. Depending on the severity of the flood, the dilution effect will be greater than the re-suspension of metals from sediment resulting from the flood which would normally increase metal concentrations (e.g. Brown, 1977). The effect of the floods is thus expected to have a greater influence on the Vaal River Barrage than the Vaal Dam. Despite the severity of the February 2000 flood (Figure 1), the dilution effect was not pronounced at either the Vaal Dam or Vaal River Barrage. Effects resulting from increased flow rate as a result of the floods are thus unlikely to have contributed to the fact that higher metal concentrations were not recorded from the Vaal River Barrage.

Clements and Rees (1997) discussed other possible explanations that may also shed more light on differences in tissue metal concentrations between localities. They stated that if fish growth or age is not determined, differences in growth rates or age between sites might contribute to differences in metal levels. In the present study fish were collected using gill nets. As a result fish of a specific size were collected. However, growth rate and age may still be important even if fish within the same size-class are selected for metal analysis (Clements and Rees, 1997). This is a possible shortcoming in the current study. In future, age determinations may have to be performed, after which fish will need to be divided into age-classes for metal analysis. The authors further state that potential negative effects of heavy metals may be offset by higher temperatures and increased prey availability. Slightly higher temperatures were recorded in the Vaal River Barrage during the summer, spring and autumn months (Fig. 1). Their study was however concerned with brown trout. It is unlikely that the slight differences in temperature observed in the current study will have any significantly positive effect on fish in the Vaal River Barrage, compared to fish in the Vaal Dam. The higher number of piscivorous birds at the Vaal River Barrage when compared to the Vaal Dam (personal observation) at the time of conduct of the study, may also indicate higher prey availability. This is however unlikely to be the case – a bird sanctuary is located near the Vaal River Barrage which could explain the higher numbers of birds observed. Furthermore, largemouth bass (*Micropterus salmoides*) were collected from the Vaal River Barrage, but not the Vaal Dam during the 1999/2000 surveys. These fish are aggressive predators (Skelton, 1993). The fact that food overlap exists between *C. gariepinus* and other alien predator species (Dörgeloh, 1996; Dörgeloh, 1994; Skelton, 1993), combined with the large amount of piscivorous birds leads to the assumption that prey numbers may well be lower in the Vaal River Barrage as a result of predation pressure. Apart from these 2 factors, Giesy and Wiener (1977) stated that 'limitations of sample sizes and sampling procedures usually make it difficult to determine the degree to which age, location, time of collection, and other factors affect variance in concentrations'. Furthermore, largemouth bass have since been collected from the Vaal Dam locality during recent (2009/2010) parasite

Table 14
Table depicting the ranking of metal concentrations in 5 different tissue types, collected from the Vaal Dam and Vaal River Barrage during 7 surveys conducted between November 1998 and February 2000.

Vaal Dam									
Metal	**Ranking of accumulation from highest to lowest concentration**								
Strontium	Gill arch	>	Gill filament	>	Skin	>	Liver	>	Muscle
Aluminium	Gill filament	>	Skin	>	Liver	>	Gill arch	>	Muscle
Lead	Gill arch	>	Gill filament	>	Liver	>	Muscle	>	Skin
Nickel	Gill arch	>	Gill filament	>	Muscle	>	Liver	>	Skin
Vaal River Barrage									
Metal	**Ranking of accumulation from highest to lowest concentration**								
Strontium	Gill arch	>	Gill filament	>	Muscle	>	Liver	>	Skin
Aluminium	Gill filament	>	Skin	>	Gill arch	>	Liver	>	Muscle
Lead	Gill arch	>	Gill filament	>	Muscle	>	Liver	>	Skin
Nickel	Gill arch	>	Skin	>	Muscle	>	Gill filament	>	Liver

collection surveys (observation by author). Another host-related aspect that could be investigated in future studies is gender-specific interactions (e.g. Pyle et al., 2005). Pollutant concentrations and biological responses in fish relate to cyclic physiological changes that are linked not only to food availability, but also reproduction (Nesto et al., 2007; Marijić and Raspor, 2010). Highest metal concentrations are indeed often found in gonads (e.g. Alquezar et al., 2006). In the current study, tissue concentrations in gonads were however not examined.

Köck et al. (1996) stated that metal uptake may be enhanced during summer as a result of increasing metabolic rates. Most significant differences recorded between surveys were concerned with metal concentrations in muscle and liver tissues. This was however not always the case, as exemplified by the Al concentrations recorded. Possible confounding factors could be any of the variables listed in Fig. 1 or even changes in water or sediment concentrations. It is also possible that decreased metabolism during winter (resulting from low temperatures) may have played a role.

With regard to accumulation of the 4 metals in question in fish tissue, the general order (ranked in order of decreasing concentrations) was found to be: gill arch > gill filament > muscle > liver > skin. Very high standard deviation values were however recorded, indicating that the ranking obtained should be interpreted with caution. Kargin (1998) recorded highest metal concentrations (including Pb) in liver and gills, and lowest concentrations in muscle, of *Capoeta barroisi* from Turkey. Kargin (1998), after reviewing accumulation patterns of metals, further stated that this result is expected. The liver is highly active in the uptake and storage of pollutants and other non-nutritive molecules (Hopson and Wessels, 1990; Sorensen, 1991; Kimball, 1983), while active and passive exchanges occur between the animal and the aquatic environment through the gills (Eckert et al., 1988; Kargin, 1998). Afonso et al. (2007) found highest mercury concentrations in liver (followed by muscle and skin respectively), while Dang and Wang (2009) also found cadmium concentrations to be highest in this organ. Yılmaz et al. (2007) recorded highest Pb concentrations in gill and liver tissue, as did Al-Kahtani (2009), Su et al. (2009) and Pereira et al. (2010a) with reference to liver tissue. Visnjic-Jeftic et al. (2010) confirmed the general trend (i.e. high concentration in gills) for Al and Sr and found lowest concentrations in muscle. Other authors that found high metal concentrations in liver and/or gill tissue include Deviller et al. (2005), Yang et al. (2007), Vinodhini and Narayanan (2008) and Heier et al. (2009). Robinson and Avenant-Oldewage (1997) also speculated that the large surface area of the gills, and the large amount of water that passes over them, further facilitate metal uptake. High metal concentrations were also recorded from gill arches. Similarly Seymore et al. (1995) found high metal concentrations in vertebrae, and recommended the use of bony tissue for the analysis of Pb and Sr in fish. Playle (1998) concludes that metal-gill modelling is indeed a good framework for understanding and predicting metal toxicity to fish. The relevance of this statement was confirmed by Pereira et al. (2010b) who demonstrated that gills do reflect water contamination.

While findings in thus study do appear to concur with the general trend discussed above for the majority of metals (i.e. highest concentration in liver and gill tissues and lowest concentrations in muscle and skin tissues), exceptions have been recorded. Yılmaz et al. (2010) found higher Ni concentrations in skin compared to liver. Reynders et al. (2008) found highest Cd concentrations in the intestine and kidney, followed by gills, liver and finally muscle. Rauf et al. (2009) in turn found highest Cd concentrations in liver and lowest concentrations in gill tissue after examining gills, kidney, liver, skin, muscle and scales. De Boeck et al (2010) found only Cu concentrations to be highest in liver. The remaining metals (including Ni and Pb) were highest in either the kidney or rectal gland. Dural et al. (2007) found highest metal concentrations (including Pb) in muscle tissue after examining muscle, gill, liver and gonad tissues. These apparent discrepancies in available literature are due to variability between fish species, tissues and metals examined (e.g. Mendil et al., 2005; Kraemer et al., 2005; Arain et al., 2008; Uysal et al., 2008; Lakshmanan et al., 2009; Mathews and Fisher, 2009; Mendil et al., 2010). Schmitt et al. (2007) as well as Qiao-qiao et al. (2007) reported within-site variability with regards to metal concentrations in different tissue types. Some fish species also appear to possess an adaptation capability to heavy metal loads (Shah and Altindağ, 2005). The observed variability is however not only restricted to fish species and their tissue. Mohan et al. (2007) found no regular pattern of metal deposition at different trophic levels. Be that as it may, it seems that highest metal concentrations are consistently found in the liver and gills of *C. gariepinus* (e.g. Gbem et al., 2001), as is also reflected in the current study.

A study by Retief et al. (2009) in the Vaal Dam also found no seasonal trend with regards to metal bioaccumulation in host (largemouth yellowfish) tissue. They did however identify a seasonal trend with regards to bioaccumulation in the tapeworm parasite (*Bothriocephalus acheilognathi*). Furthermore, higher mean metal concentration values were recorded from parasite tissue compared to host tissue. Woelfl et al. (2008) also found that *Diphyllobothrium latum* bioconcentrate more trace elements than their host (*Oncorhynchus mykiss*). Furthermore, Pb was found in the parasite but not in any fish organs, indicating that endoparasitic flatworms as sensitive bio-indicators may have an 'early warning' biological monitoring function. Oyoo-Okoth et al. (2010) however caution that partitioning of metals in the parasite/host assemblage may occur. This statement implies a need for baseline data on metal accumulation in both host and parasite species to determine the degree of partitioning. Further studies on other fish species and parasites might not only be useful to confirm trends, but may have interesting implications with regards to biological monitoring practices in the Vaal River system.

Conclusions

Heavy metals did accumulate in *C. gariepinus* tissues. While Sr concentrations in gills were more often significantly (p<0.05) higher in fish from the Vaal Dam, no further clear trends emerged with regards to differences between localities (Vaal Dam and Vaal River Barrage) or surveys. Highest metal concentrations were recorded in gill (both filaments and arches), followed by muscle, liver and lastly skin. This general trend appears to be in agreement with trends observed by other workers and reported in the literature. Comparison of tissue metal concentrations recorded in the current study with that recorded for other species in the literature once again strengthened the notion of interspecies bioaccumulation differences. Differences in tissue metal concentrations in *C. gariepinus* between the current study and results from available literature also accentuated the importance of factors that influence the concentrations and bioavailability of trace metals.

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