Metal concentrations in *Clarias gariepinus* and *Labeo umbratus* from the Olifants and Klein Olifants River, Mpumalanga, South Africa: Zinc, copper, manganese, lead, chromium, nickel, aluminium and iron

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Abstract

The upper catchment of the Olifants River, from its origin near Bethal to its confluence with the Wilge River, north of Witbank, and its tributaries are being subjected to increasing afforestation, mining, power generation, irrigation, domestic and industrial activities. These activities have a profound effect on the water quality. The major point sources of pollution in the study area include mines, industries and very importantly, combined sewage purification works that are located alongside the river. In addition to oxidizable material, these sources contain detergents, nutrients and metals. It was therefore necessary to determine the extent to which these activities affect the water quality of the system. The bioaccumulation of zinc (Zn), copper(Cu), manganese (Mn), lead (Pb), chromium (Cr), nickel (Ni), aluminium (Al) and iron (Fe) in the skin, muscle, liver and gill tissues of *Clarias gariepinus* and *Labeo umbratus* from the Upper Olifants River and Klein Olifants River was investigated over the period February 1994 to May 1995. The highest concentrations of these metals were found in the gill and liver tissues of both species, with lower concentrations in the skin and muscle tissues. Bioaccumulation patterns were found to vary according to the species of the fish, mainly according to their different feeding habits and the routes of metal uptake. It also varied as a function of the different localities. Accumulation was size specific, with higher concentrations of metals found in smaller fish.

Introduction

All metals are natural constituents of the environment and are found in varying levels in all ground and surface waters (Martin and Coughtrey, 1982). Some are essential, required for the normal metabolism of aquatic organisms, while others are non-essential and play no significant biological roles (Prosi, 1979; Rainbow and White 1989). In addition to their natural occurrence, metals may enter and contaminate the environment from five general sources namely geological weathering (the source of natural/background levels), industrial processing of metals and ores, the use of metals and their compounds, leaching of metals from municipal and solid waste dumps - especially mine dumps, and animal and human excretions (Förstner and Prosi, 1979; Witman, 1979). Metal pollution in South Africa and especially in the Upper Olifants River catchment area, is mainly attributed to water use for afforestation, mining and power generation, irrigation as well as domestic and industrial purposes (Coetzee, 1996).

Metals are persistent and tend to accumulate in the environment, especially in the sediments (Tessier and Campbell, 1990; Birch et al., 1996). The chemical characteristics of metals are responsible for the fact that all metals ultimately become toxic at some elevated concentration (Rainbow, 1985). Abnormally high concentrations can cause the inability of organisms to excrete, sequester or otherwise detoxify themselves, especially in the case of non-essential metals (Thorp et al., 1979). They can also become strongly enriched in the aquatic food chain, through a process referred to as biomagnification (Förstner and Müller, 1976).

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Organisms can accumulate metals to levels above those which are required for normal physiological functioning. The measurement of metal concentrations in these organisms provides the basis for the use of bioaccumulative indicators of the degree of metal pollution in various aquatic ecosystems. The objective of this study was to determine the extent of zinc (Zn), copper (Cu), manganese (Mn), lead (Pb), chromium (Cr), nickel (Ni), aluminium (Al) and iron (Fe) bioaccumulation in different tissues of the African Sharptooth Catfish, *Clarias gariepinus* and the Moggel, *Labeo umbratus* from the Upper Olifants River Catchment (Klein Olifants River - Locality KOR1 and the Olifants River -Locality OR1) (Fig. 1). These data were required to assess the possibility of elevated levels of these metals in fish due to point and diffuse sources of pollution in the Upper Catchment of the Olifants River. The dependence of bioaccumulation in different tissues on the species, size, gender and locality differences of these fish were specifically addressed.

Experimental method

Description of catchment and localities

The Olifants River originates in the Bethal-Trichardt area and flows in an easterly direction before crossing the Kruger National Park into Mozambique (Fig. 1). The Olifants River basin in the Transvaal drains a large area of over 54 575 km^2 (Theron et al., 1991). Although conditions are not ideal for afforestation due to low rainfall, approximately 17 680 ha of exotic afforestation occurs in the upper catchment, which is concentrated around Witbank and Middelburg in the catchments of the Olifants and Klein Olifants Rivers respectively. The upper catchment, with its tributaries, drains a portion of the Mpumalanga highveld where most of the

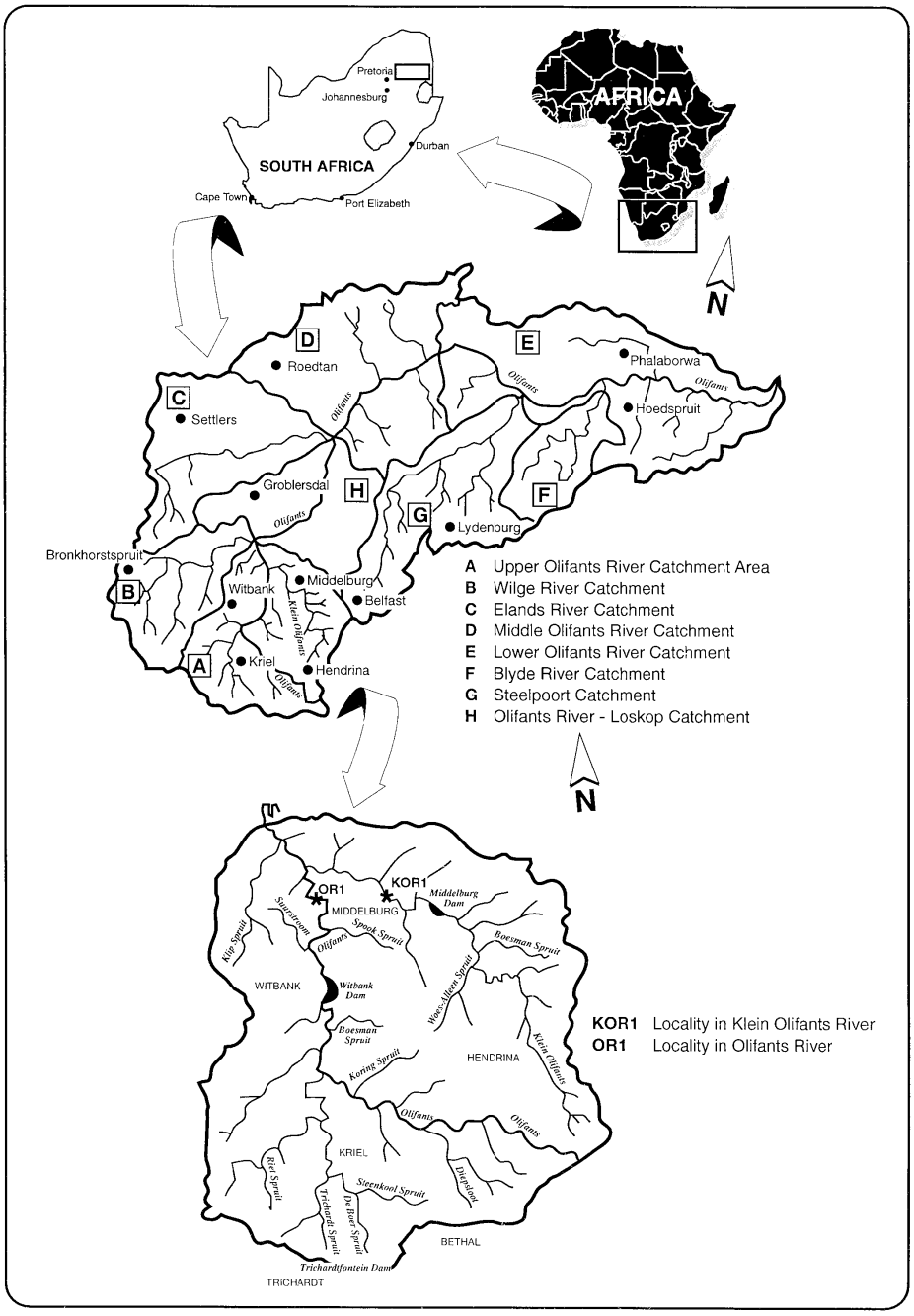

Figure 1

Localities of the sampling sites in the Olifants River (OR1) and Klein Olifants River (KOR1) in the Upper Olifants River Catchment

thermal power stations in the country are located (six of the eight). These power stations receive imported water, with the Komati-, Hendrina-, Arnot- and Duhva Power Stations receiving water from the Komati system and the Kriel- and Matla Power Stations from the Usutu system. The operating mines in this region comprise of 37 coal, 6 brick, 17 sand, 4 felsite and 7 clay mines. Irrigation is a major water use sector in the upper catchment and at the time of the study, 4 760 ha of land was irrigated. Seven towns are situated in the upper catchment. The Witbank/Middelburg complex is a major industrial area with smaller towns Davel, Kinross, Trichardt, Hendrina and Ogies. Several magisterial districts fall partly inside the catchment boundaries, namely Bethal, Belfast, Ermelo, Highveld Ridge, Middelburg and Witbank.

The study area includes two sampling sites namely Locality KOR1 (Locality 13 in Coetzee, 1996) in the Klein Olifants River and Locality OR1 (Locality 14 in Coetzee,1996) in the Olifants River. These were chosen to determine the water quality and the effect of the different effluent discharges in the Klein Olifants River before its confluence with the Olifants River (KOR1) as well as in the Olifants River after its confluence with all the streams receiving effluent from mines, industries, towns and power stations in the area, before it reaches the Klein Olifants River. Locality KOR1 is dependent on water from the Middelburg Dam and the sewage works located upstream from this locality. The point sources of pollution in this area are mainly the combined sewage treatment works and domestic and industrial effluent. The sewage

treatment works and a number of informal settlements alongside the river are responsible for the nutrient load in the river (e.g. phosphates and nitrates) which can lead to eutrophication. The industries in the area are point sources containing constituents such as calcium, sulphates, potassium, sodium and metals. Industries in the Middelburg area include steelworks and engineering firms providing services to the steelworks and two large paint factories, which may contribute to surface runoff. The non-point sources in this area are mainly atmospheric deposition, rural and urban runoff and agriculture. Polluted groundwater seeping to surface streams can also be a source of pollution.

Locality OR1 is located at the Olifants River Lodge holiday resort. Aquatic plants (*Salvelinus* and *Potamageton* spp.) and algae are common in this area. The pollution point sources above this sampling point are mainly the sewage works, mines and industries containing constituents such as fluorides, metals, phosphates, sulphates, calcium, magnesium, sodium and potassium. This locality receives water from the Witbank Dam and streams such as Suurstroom and Spook Spruit (receiving effluents from mines such as the Middelburg Mine Services) which flow into the Olifants River. Industries located alongside the river in the Witbank area include steelworks like Highveld Steel, Ferro Metals and Trans Alloys as well as service industries for the mines located in the area, a petrol depot and a brewery. Non-point sources include runoff from towns and agricultural activities along the river.

Field sampling

Two freshwater fish species (*C. gariepinus* and *L. umbratus*) were collected during the period February 1994 to May 1995 at localities KOR1 and OR1 by means of gill nets (70 to120 mm stretched mesh size) in the Klein Olifants River and Olifants River respectively. For *C. gariepinus* the mass and total lengths of the fish were recorded after capture and for *L. umbratus* the mass, fork length, and total length. The fish were dissected on a polythene dissection board, using clean stainless steel tools, wearing surgical gloves. Skin and muscle tissues were removed, placed in clean, pre-washed glass bottles and frozen until further analysis in the laboratory. The liver and gills were removed, the total mass determined and the gill filaments removed, placed in clean pre-washed glass bottles and frozen until metal concentration analyses.

Laboratory procedures

All glassware were soaked in a 2% Contrad soap solution (Merck chemicals) for 24 h, rinsed in distilled water, acid-washed in 1 M HCl for 24 h and rinsed again in distilled water (Giesy and Wiener, 1977) prior to use. The tissues were thawed and rinsed in distilled water to remove excess mucus or other foreign particles that could have adsorbed metals. Approximately 5g of each sample was dried in an oven at 60°C for 48 h. In order to determine the percentage of moisture for each sample, the wet and dry mass of the samples was recorded. The samples were digested by adding concentrated nitric acid (55%) and perchloric acid (70%) to 1 g of dried tissue, in a 2:1 ratio in a 50 mℓ Erlenmeyer. Digestion was performed on a hotplate at 200 to 250°C for ± 4 h until the solutions were clear (Van Loon, 1980). After digestion, each solution was filtered through an acid resistant 0.45 µm filter paper under vacuum. The filtering system was rinsed with distilled water and each sample was made up to 50 mℓ with distilled water and stored in pre-washed bottles until metal concentration analysis.

The metal concentrations were determined using a Varian Atomic Absorption Spectrophotometer (Spectra AA-10) (Varian, 1989). Analytical standards were prepared from Holpro stock solutions. To assure accurate and precise determination of trace elements in freshwater biological samples, a standard tissue sample (IAEA/R1/64) was used. Metal contamination from the laboratory was avoided. A triplicate acid blank was also analysed.

Statistical procedures

Statistical analyses were performed using the STATISTICA program. The different statistical analyses were only performed on certain data, as sample sizes did not allow the analyses of all the data for all the cases. The capturing success varied and it was not possible to obtain large numbers (preferable >20 individuals, Seymore, 1994) of each fish species at both localities during a specific survey. The low capturing success and the capturing of fish which varied in mass and length did not enable the selection of fish of a specific size and gender for analyses. Because the sample sizes in this study were small, non-parametric methods were used. For the comparison of two groups, for example, gender, localities and species, the Kolmogorov-Smirnov two-sample test was used. This test is sensitive to differences in the general shapes of the distributions in the two groups, i.e. to differences in, for example, dispersion and skewness. For the comparison of multiple groups, in this case the bioaccumulation of metals in the skin, muscle, liver and gills, the Kruskal-Wallis analysis of ranks was used. The interpretation of this test is basically identical to that of the parametric one-way ANOVA, except that it is based on ranks rather than means.

Results

Fish size

The total lengths of the fish ranged between 40.0 cm and 68.5 cm for *C. gariepinus* and 43.0 cm and 77.0 cm for *L. umbratus*, while the mass of the fish was in the range of 0.2 to 2.8 kg for *C. gariepinus* and 0.8 to 3.5 kg for *L. umbratus.*

Bioaccumulation of Zn, Cu, Mn, Pb, Cr, Ni, Al and Fe in the skin, muscle, liver and gill tissue of *C. gariepinus* and *L. umbratus*

The moisture content of the individual tissues differed from one another and to some extent also from one individual to the next, with the mean percentages being 69±2%, 73±3%, 80±2% and 80±2% for the skin, liver, muscle and gills of *L. umbratus* respectively. For *C. gariepinus* the moisture contents were 72±3%, 77±1%, 80±2% and 80±2% in the skin, liver, muscle and gills respectively. As it has been demonstrated that the variations in metal concentrations of a particular organ can be ascribed in large measure to the varying moisture content of that organ, the samples were handled on a dry mass basis (Adrian and Stevens, 1979).

Tissue differences in the Zn bioaccumulation for *C. gariepinus* were not clearly distinguishable (Table 1). Nevertheless, the order ranged between the highest levels in the gills (G) and the liver (L), followed by lower levels in the skin (S) and muscle (M). For *L. umbratus* the order of bioaccumulation for Zn was more apparent, being G>L>S>M. Zn concentrations ranged from 29 µg/g dry mass in the muscle tissue of *L. umbratus* to 257 µg/g dry mass in the gills *of C. gariepinus*. The general order of bioaccumulation for Cu by both species was L>G>M»S with very high concentrations in the liver (Table 2). Cu concentrations fluctuated between 2 µg/g dry mass in the skin tissue of *C. gariepinus* and 568 µg/g dry mass

TABLE 1
Mean zinc concentrations (µg/g dry mass) in the tissues and organs of *C. gariepinus* and *L. umbratus* at localities KOR1 and OR1 from February 1994 to May 1995 (month, locality, species, number) and the water (Bf_w) and sediment (Bf_s) bioconcentration factors for these tissues/organs

		Skin	Liver	Muscle	Gills
Feb 1994/KOR1	Mean±S_d	127±46	99±27	31±8	126±21
C. gariepinus (20)	Bf_w/Bf_s	632/1.6	521/1.3	163/0.4	668/1.7
Feb 1994/OR1	Mean±S_d	90±25	136±26	33±8	131±52
C. gariepinus (10)	Bf_w/Bf_s	1800/0.2	2520/0.2	640/0.1	2640/0.2
May 1994/OR1	Mean±S_d	87±35	115±29	31±12	88±22
C. gariepinus (20)	Bf_w/Bf_s	1740/0.4	2300/0.5	620/0.1	1760/0.4
Aug 1994/KOR1	Mean±S_d	94±33	127±31	29±7	144±76
L. umbratus (20)	Bf_w/Bf_s	1880/0.6	2540/0.8	580/0.2	2860/0.9
Aug 1994/OR1	Mean±S_d	111±11	144±19	36±7	104±9
C. gariepinus (6)	Bf_w/Bf_s	2775/0.9	3600/0.1	900/0.3	2600/0.8
Aug 1994/OR1	Mean±S_d	87±22	137±29	36±18	165±45
C. gariepinus (8)	Bf_w/Bf_s	2175/0.7	3425/1.1	900/0.3	4100/1.3
Nov 1994/KOR1	Mean±S_d	92±25	98±18	46±13	200±63
C. gariepinus (14)	Bf_w/Bf_s	613/0.3	653/0.3	307/0.1	1333/0.6
Nov 1994/KOR1	Mean±S_d	41±11	90±12	30±2	162±21
L. umbratus (3)	Bf_w/Bf_s	273/0.1	600/0.3	200/0.1	1080/0.5
Nov 1994/OR1	Mean±S_d	133±35	125±11	73±9	189±9
C. gariepinus (3)	Bf_w/Bf_s	1330/1.1	1250/1.1	730/0.6	1890/1.6
Nov 1994/OR1	Mean±S_d	74±24	115±28	38±12	179±46
L. umbratus (20)	Bf_w/Bf_s	740/0.6	1150/1.0	380/0.3	1790/1.5
Feb 1995/KOR1	Mean±S_d	75±31	100±28	53±15	257±196
C. gariepinus (4)	Bf_w/Bf_s	625/0.3	833/0.5	442/0.2	2142/1.2
Feb 1995/KOR1	Mean±S_d	122±54	145±77	46±14	175±100
L. umbratus (20)	Bf_w/Bf_s	1017/0.6	1208/0.7	383/0.2	1458/0.8
Feb 1995/OR1	Mean±S_d	126±38	110±49	47±11	142±47
C. gariepinus (13)	Bf_w/Bf_s	1800/0.7	2143/0.8	671/0.3	2029/0.8
Feb 1995/OR1	Mean±S_d	110±41	90±53	47±22	184±41
L. umbratus (20)	Bf_w/Bf_s	1571/0.6	1286/0.5	671/0.3	2629/1.3
May 1995/KOR1	Mean±S_d	118±35	122±33	44±10	169±95
C. gariepinus (3)	Bf_w/Bf_s	843/1.1	871/1.1	314/0.4	1207/1.5
May 1995/KOR1	Mean±S_d	160±40	182±31	52±16	143±21
L. umbratus (7)	Bf_w/Bf_s	1143/1.5	1014/1.3	371/0.5	1021/1.3
May 1995/OR1	Mean±S_d	122±44	149±28	42±11	117±14
C. gariepinus (20)	Bf_w/Bf_s	2033/NA	2483/NA	700/NA	1950/NA
May 1995/OR1	Mean±S_d	100±42	104±24	36±14	139±20
L. umbratus (7)	Bf_w/Bf_s	1667/NA	1733/NA	600/NA	1317/NA

in the liver of the same species. The highest concentrations of Mn in the tissues/organs of both species were clearly found in the gill tissue, followed by the liver and then the skin and muscle tissue. The differences between these tissues were mostly significant (Table 3). The lowest Mn concentration (2±0.5 µg/g dry mass) was found in the muscle tissue of *C. gariepinus* and the highest concentration (167 µg/g dry mass) in the gill tissue of *L. umbratus*. For Pb, however, the order of bioaccumulation was not clearly distinguishable, but it was evident that the highest concentrations (values ranged between 2 µg/g dry mass in the muscle tissue of *L. umbratus* and 32 µg/g dry mass in the gill tissue of the same species) were found in the gill tissue for both species (Table 4).

The bioaccumulation pattern of Cr (minimum 10 µg/g dry mass - in the liver of *C. gariepinus* - and maximum 130 µg/g dry mass - in the gill tissue of *C. gariepinus*) and Ni (minimum 8 µg/g dry mass - in the skin tissue of *C. gariepinus* - and maximum 71 µg/g dry mass - in the gill tissue of *C. gariepinus*) was not clear, but it seems as if predominantly, higher concentrations of these metals were found in the gills, followed by the liver, muscle and skin tissues of *C. gariepinus* and *L. umbratus* (Tables 5 and 6). Al concentrations (ranging between 11 µg/g dry mass in the muscle tissue of *C. gariepinus* and 371 µg/g dry mass in the gill tissue of *C. gariepinus*) in the tissues of both species showed high variations (Table 7). Al accumulated mostly in the gill and liver tissue, followed by the muscle and skin tissues. The highest Fe concentrations were found in the liver of both species, with the exception in August 1994 at locality KOR1 for *L. umbratus*, where the gill tissue accumulated higher concentrations (Table 8). Fe concentrations fluctuated between 72 µg/g dry mass in the muscle tissue of *C. gariepinus* and 3 963 µg/g dry mass in the liver tissue of *L. umbratus*.

The highest and lowest bioconcentration factors (Bf_w) for the eight selected metals compared to

the metal concentrations in the water at the corresponding periods are presented in Table 9.

Differences in bioaccumulation patterns between species

Statistical analyses were performed only on data from August 1994 at Locality OR1 onward, as sample sizes for the remaining were too small. Zn and Cu bioaccumulation in the different tissues of the two species varied significantly in most cases. In May 1994 at Locality OR1, significant differences (P±0.05) in the Zn concentrations between the species were found in the skin and gill tissues, with higher levels of Zn in the skin tissue of *C. gariepinus*, while *L. umbratus* showed higher levels in the gills. In May and November 1994 and February and May 1995, the Cu concentrations in the liver and muscle tissue concentrations were significantly higher in *L. umbratus* than in *C. gariepinus*. The Cu gill concentrations were, however, higher in *C. gariepinus* in November 1994 at Locality KOR1. Mn and Pb bioaccumulation in the different tissues and organs showed significant differences (P±0.05) between the two species. Mn concentrations in the muscle, liver and gill tissues of *L. umbratus* were mostly very high compared to those in the same tissues of *C. gariepinus* in May 1994 at Locality OR1, November 1994 at Locality KOR1, February 1995 at Locality OR1 and May 1995 at Locality OR1. In May 1995 at Locality OR1, however, the Mn muscle tissue concentrations were higher for *C. gariepinus* than *L. umbratus.* Pb muscle and liver tissue concentrations were higher in *L. umbratus* in May 1995 at Locality OR1, but *C. gariepinus* accumulated more Pb in the liver, gill and skin tissues in November 1994 at both localities, February 1995 at Locality OR1 and May 1995 at Locality OR1 respectively.

The only significant (P±0.05) species differences for Cr and Ni were found in the gill tissue, with

TABLE 2
Mean copper concentrations (μg/g dry mass) in the tissues and organs of *C. gariepinus* and *L. umbratus* at localities KOR1 and OR1 from February 1994 to May 1995 (month, locality, species, number) and the water (Bf_w) and sediment (Bf_s) bioconcentration factors for these tissues/organs

		Skin	Liver	Muscle	Gills
Feb 1994/KOR1	Mean±Sd	14±9	42±17	14±10	29±22
C. gariepinus (20)	Bf_w/Bf_s	20/0.1	60/0.3	20/0.1	41/0.2
Feb 1994/OR1	Mean±Sd	4±0.8	54±17	4±0.6	13±11
C. gariepinus (10)	Bf_w/Bf_s	100/0.03	1350/0.4	75/0.03	325/0.1
May 1994/OR1	Mean±Sd	2±0.5	43±17	2±1	5±1
C. gariepinus (20)	Bf_w/Bf_s	20/0.6	430/0.2	20/0.01	50/0.02
Aug 1994/KOR1	Mean±Sd	4±2	286±152	7±4	8±3
L. umbratus (20)	Bf_w/Bf_s	40/0.05	2860/4.0	70/0.1	80/0.1
Aug 1994/OR1	Mean±Sd	2±0.6	64±15	4±0.7	6±1
C. gariepinus (6)	Bf_w/Bf_s	100/0.02	3200/0.7	200/0.04	300/0.1
Aug 1994/OR1	Mean±Sd	2±0.4	568±196	19±9	9±5
C. gariepinus (8)	Bf_w/Bf_s	100/0.02	28400/6.0	950/0.2	450/0.1
Nov 1994/KOR1	Mean±Sd	5±2	20±7	6±3	22±14
C. gariepinus (14)	Bf_w/Bf_s	500/0.01	2400/0.04	600/0.01	2200/0.04
Nov 1994/KOR1	Mean±Sd	5±9	57±22	5±0.7	6±0.6
L. umbratus (3)	Bf_w/Bf_s	800/0.01	5700/0.1	500/0.01	600/0.01
Nov 1994/OR1	Mean±Sd	8±105	28±5	4±0.5	8±0.4
C. gariepinus (3)	Bf_w/Bf_s	800/0.1	2800/0.2	400/0.02	800/0.1
Nov 1994/OR1	Mean±Sd	6±3	503±174	11±8	12±6
L. umbratus (20)	Bf_w/Bf_s	600/0.04	50300/3.0	1100/0.1	1200/0.1
Feb 1995/KOR1	Mean±Sd	4±0.2	24±5	5±1	13±5
C. gariepinus (4)	Bf_w/Bf_s	133/0.1	800/0.3	167/0.1	433/0.2
Feb 1995/KOR1	Mean±Sd	5±4	360±374	6±4	11±7
L. umbratus (20)	Bf_w/Bf_s	167/0.1	12000/5.0	214/0.1	337/0.2
Feb 1995/OR1	Mean±Sd	9±10	30±38	4±3	11±9
C. gariepinus (13)	Bf_w/Bf_s	900/0.1	3000/0.4	400/0.1	1100/0.1
Feb 1995/OR1	Mean±Sd	7±5	527±425	7±4	8±2
L. umbratus (20)	Bf_w/Bf_s	700/0.1	52700/6.0	700/0.1	800/0.1
May 1995/KOR1	Mean±Sd	4±1	40±6	3±0.8	6±1
C. gariepinus (3)	Bf_w/Bf_s	21/0.1	211/0.6	16/0.04	32/0.1
May 1995/KOR1	Mean±Sd	6±4	187±89	5±3	7±5
L. umbratus (7)	Bf_w/Bf_s	32/0.1	984/3.0	26/0.1	37/0.1
May 1995/OR1	Mean±Sd	3±2	35±9	4±3	6±1
C. gariepinus (20)	Bf_w/Bf_s	150/NA	1750/NA	181/NA	300/NA
May 1995/OR1	Mean±Sd	3±0.6	50±16	5±2	8±3
L. umbratus (7)	Bf_w/Bf_s	150/NA	2500/NA	350/NA	400/NA

NA=Not available

TABLE 3
Mean manganese concentrations (μg/g dry mass) in the tissues and organs of *C. gariepinus* and *L. umbratus* at localities KOR1 and OR1 from February 1994 to May 1995 (month, locality, species, number) and the water (Bf_w) and sediment (Bf_s) bioconcentration factors for these tissues/organs

		Skin	Liver	Muscle	Gills
Feb 1994/KOR1 *C. gariepinus* (20)	Mean±Sd Bfw/Bfs	4±1 200/0.02	10±2 500/0.06	4±0.8 200/0.02	11±7 550/0.06
Feb 1994/OR1 *C. gariepinus* (10)	Mean±Sd Bfw/Bfs	4±7 200/0.02	7±2 350/0.04	2±0.4 100/0.01	43±24 2150/0.3
May 1994/OR1 *C. gariepinus* (20)	Mean±Sd Bfw/Bfs	3±3 14/0.002	4±2 18/0.002	2±1 9/0.001	29±12 132/0.02
Aug 1994/KOR1 *L. umbratus* (20)	Mean±Sd Bfw/Bfs	2±0.6 100/0.006	3±0.8 150/0.008	2±0.8 100/0.006	34±12 1700/0.09
Aug 1994/OR1 *C. gariepinus* (6)	Mean±Sd Bfw/Bfs	3±2 150/0.006	3±2 150/0.006	2±0.5 100/0.004	25±12 1250/0.05
Aug 1994/OR1 *C. gariepinus* (8)	Mean±Sd Bfw/Bfs	3±1 150/0.006	4±1 200/0.008	3±1 150/0.006	73±19 3650/0.1
Nov 1994/KOR1 *C. gariepinus* (14)	Mean±Sd Bfw/Bfs	9±5 900/0.004	10±4 1000/0.005	3±1 300/0.001	85±39 8500/0.04
Nov 1994/KOR1 *L. umbratus* (3)	Mean±Sd Bfw/Bfs	7±6 700/0.003	9±1 900/0.004	6±1 600/0.003	59±10 5900/0.03
Nov 1994/OR1 *C. gariepinus* (3)	Mean±Sd Bfw/Bfs	28±24 2800/0.01	10±7 1000/0.005	5±0.7 500/0.003	50±38 5000/0.03
Nov 1994/OR1 *L. umbratus* (20)	Mean±Sd Bfw/Bfs	6±3 600/0.003	14±12 1400/0.007	5±2 500/0.003	118±50 11800/0.06
Feb 1995/KOR1 *C. gariepinus* (4)	Mean±Sd Bfw/Bfs	2±0.7 33/0.004	5±3 83/0.009	4±1 67/0.007	59±29 983/0.1
Feb 1995/KOR1 *L. umbratus* (20)	Mean±Sd Bfw/Bfs	2±1 33/0.004	4±2 67/0.007	3±2 50/0.005	60±41 1000/0.1
Feb 1995/OR1 *C. gariepinus* (13)	Mean±Sd Bfw/Bfs	6±2 300/0.02	11±4 550/0.03	5±3 250/0.02	80±32 4000/0.3
Feb 1995/OR1 *L. umbratus* (20)	Mean±Sd Bfw/Bfs	13±14 650/0.04	23±25 1150/0.07	9±6 450/0.03	167±53 8350/0.5
May 1995/KOR1 *C. gariepinus* (3)	Mean±Sd Bfw/Bfs	5±3 46/0.01	4±1 36/0.01	3±3 27/0.008	23±8 209/0.06
May 1995/KOR1 *L. umbratus* (7)	Mean±Sd Bfw/Bfs	6±5 55/0.02	8±6 73/0.02	3±2 27/0.008	39±6 355/0.1
May 1995/OR1 *C. gariepinus* (20)	Mean±Sd Bfw/Bfs	7±3 23/NA	11±7 36/NA	8±5 26/NA	40±12 129/NA
May 1995/OR1 *L. umbratus* (7)	Mean±Sd Bfw/Bfs	6±4 19/NA	12±5 39/NA	4±2 13/NA	83±9 268/NA

NA=Not available

the highest concentrations found in *C. gariepinus* in November 1994 at both localities. In the case of Ni, *L. umbratus* accumulated significantly (P±0.05) more of the metal in the skin, liver and muscle tissues in November 1994 at Locality OR1 than *C. gariepinus*, which accumulated more in the gill tissue in November 1994 at both localities. No definite trend could be established for Al between the two species. For instance, the highest concentrations in the skin tissue were found for *C. gariepinus* in May and November 1994, while *L. umbratus* also accumulated more Al in the skin in May 1995 at Locality KOR1. In the case of Fe, a trend could be established to some degree. *L. umbratus* accumulated higher concentrations of the metal in the skin and muscle tissues in November 1994 at Locality OR1. Higher concentrations of Fe were found in the gill tissue of *C. gariepinus* in May and November 1994, but in May 1995 at Locality OR1, the highest concentrations were found for *L. umbratus*. *C. gariepinus* accumulated more Fe in the liver in November 1994, but higher concentrations were found in the liver tissue of *L. umbratus* in November 1994 and February 1995 at Locality OR1.

Relationship between metal concentrations and total fish lengths

Because the sample sizes were too small for individual analysis of the different species at the different localities for each month, these variables had to be grouped together in order to obtain statistically verifiable results. The Zn muscle and skin tissue concentrations showed negative correlations with the lengths of the fish. Thus, the larger the fish, the lower the Zn concentrations. The Zn concentrations in the liver and gill tissues showed a positive correlation with total fish lengths, with higher concentrations associated with larger fish. The Cu concentrations in the skin, muscle

and liver tissues showed significant negative correlations with the lengths of the fish. The bioaccumulation of Mn in the skin, liver, muscle and gill tissues showed significant negative correlations with the lengths of the fish, with correlation coefficients ranging between –0.03 and –0.27 and P-values ± 0.02. There was also a negative correlation between the Pb concentration in the liver, skin and muscle tissues and the lengths of the fish, but a significant positive correlation was found in the gills, with a correlation coefficient of 0.57.

Predominantly significant (P±0.05) negative correlations were found for the bioaccumulation of Cr and Ni and the lengths of the fish. The concentrations of Cr and Ni in the gills, however, showed significant (P±0.05) positive correlations, as did the Ni concentrations in the muscle tissue. Significant (P±0.05) negative correlations were also found between the Al concentrations in the skin, liver, muscle and gill tissues of the two species and the lengths of the fish. Fe showed significant negative correlations between the lengths of the fish and the concentrations in the skin and gill tissues, while insignificant correlations for the liver and muscle tissues were found.

Gender differences

Generally, few significant (P±0.05) differences in the accumulation of Zn and Cu in the different tissues/organs were found for both species between males and females. Significant (P±0.05) gender differences were found for Cu, with higher concentrations in the liver tissue of the females at Locality KOR1 in February 1994 for *C. gariepinus*. No significant gender differences were found for Mn and Pb bioaccumulation. The only significant gender difference was found for Ni in the muscle tissue of *C. gariepinus* in May 1995 at Locality OR1 with the highest concentrations detected in the females.

TABLE 4
Mean lead concentrations (µg/g dry mass) in the tissues and organs of *C. gariepinus* and *L. umbratus* at localities KOR1 and OR1 from February 1994 to May 1995 (month, locality, species, number) and the water (Bf_w) and sediment (Bf_s) bioconcentration factors for these tissues/organs

		Skin	Liver	Muscle	Gills
Feb 1994/KOR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	8±2 62/0.1	8±2 62/0.1	8±1 62/0.1	19±6 146/0.3
Feb 1994/OR1 *C. gariepinus* (10)	Mean±S_d Bfw/Bfs	5±1 25/0.03	10±4 50/0.07	5±1 25/0.03	27±26 135/0.2
May 1994/OR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	3±2 19/0.02	3±2 19/0.02	5±2 31/0.03	9±4 56/0.05
Aug 1994/KOR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	8±3 35/0.06	9±3 39/0.07	9±3 39/0.07	16±5 70/0.1
Aug 1994/OR1 *C. gariepinus* (6)	Mean±S_d Bf_w/Bf_s	6±3 29/0.04	8±6 38/0.06	5±1 24/0.04	15±7 71/0.1
Aug 1994/OR1 *C. gariepinus* (8)	Mean±S_d Bf_w/Bf_s	6±1 29/0.04	8±1 38/0.06	6±1 29/0.04	15±4 71/0.1
Nov 1994/KOR1 *C. gariepinus* (14)	Mean±S_d Bf_w/Bf_s	6±3 25/0.02	8±3 33/0.02	6±3 25/0.02	39±18 163/0.1
Nov 1994/KOR1 *L. umbratus* (3)	Mean±S_d Bf_w/Bf_s	3±1 13/0.01	5±4 21/0.01	4±2 17/0.01	10±0.5 42/0.03
Nov 1994/OR1 *C. gariepinus* (3)	Mean±S_d Bf_w/Bf_s	3±1 12/0.03	3±2 12/0.03	3±0.6 12/0.03	22±4 85/0.2
Nov 1994/OR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	4±1 15/0.03	5±2 19/0.04	4±3 15/0.03	11±3 42/0.1
Feb 1995/KOR1 *C. gariepinus* (4)	Mean±S_d Bf_w/Bf_s	7±1 29/0.03	9±0.1 38/0.04	11±3 46/0.05	31±13 129/0.2
Feb 1995/KOR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	8±3 33/0.04	8±2 33/0.04	8±2 33/0.04	15±8 63/0.07
Feb 1995/OR1 *C. gariepinus* (13)	Mean±S_d Bf_w/Bf_s	9±5 450/0.04	11±5 550/0.05	8±3 400/0.3	22±10 1100/0.09
Feb 1995/OR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	8±8 400/0.03	12±22 600/0.05	8±3 400/0.03	15±5 750/0.06
May 1995/KOR1 *C. gariepinus* (3)	Mean±S_d Bf_w/Bf_s	5±2 24/0.04	5±3 24/0.04	5±2 24/0.04	14±6 71/0.1
May 1995/KOR1 *L. umbratus* (7)	Mean±S_d Bf_w/Bf_s	3±1 14/0.03	3±1 14/0.03	2±0.6 10/0.02	9±2 43/0.08
May 1995/OR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	5±3 46/NA	5±3 46/NA	6±3 55/NA	10±3 91/NA
May 1995/OR1 *L. umbratus* (7)	Mean±S_d Bf_w/Bf_s	14±12 127/NA	21±12 191/NA	12±8 109/NA	32±37 291/NA

NA=Not available

TABLE 5
Mean chromium concentrations (µg/g dry mass) in the tissues and organs of *C. gariepinus* and *L. umbratus* at localities KOR1 and OR1 from February 1994 to May 1995 (month, locality, species, number) and the water (Bf_w) and sediment (Bf_s) bioconcentration factors for these tissues/organs

		Skin	Liver	Muscle	Gills
Feb 1994/KOR1 *C. gariepinus* (20)	Mean±Sd Bfw/Bfs	19±7 70/1	19±5 71/1	16±6 59/1	29±8 107/1
Feb 1994/OR1 *C. gariepinus* (10)	Mean±Sd Bfw/Bfs	16±4 49/0.3	27±8 82/0.5	14±2 42/0.3	50±59 152/1
May 1994/OR1 *C. gariepinus* (20)	Mean±Sd Bfw/Bfs	11±3 37/0.2	14±12 47/0.2	14±4 43/0.2	17±55 57/0.3
Aug 1994/KOR1 *L. umbratus* (20)	Mean±Sd Bfw/Bfs	15±5 52/0.4	19±7 66/0.5	16±6 55/0.4	24±20 83/0.6
Aug 1994/OR1 *C. gariepinus* (6)	Mean±Sd Bfw/Bfs	14±4 50/0.4	16±13 57/0.5	11±0.5 39/0.3	25±12 89/1
Aug 1994/OR1 *C. gariepinus* (8)	Mean±Sd Bfw/Bfs	13±3 46/0.4	10±2 36/0.3	13±4 46/0.4	18±6 64/0.5
Nov 1994/KOR1 *C. gariepinus* (14)	Mean±Sd Bfw/Bfs	27±9 150/0.5	33±15 183/0.6	26±10 144/0.5	130±97 722/2
Nov 1994/KOR1 *L. umbratus* (3)	Mean±Sd Bfw/Bfs	20±5 111/0.4	30±9 167/0.5	23±4 128/0.4	26±3 144/0.5
Nov 1994/OR1 *C. gariepinus* (3)	Mean±Sd Bf_w/Bf_s	30±2 130/1	27±4 130/1	26±3 113/1	54±4 235/2
Nov 1994/OR1 *L. umbratus* (20)	Mean±Sd Bf_w/Bf_s	30±11 130/1	29±12 126/1	28±9 122/1	33±11 144/1
Feb 1995/KOR1 *C. gariepinus* (4)	Mean±Sd Bf_w/Bf_s	19±1 106/0.3	22±3 122/0.4	28±6 156/0.5	62±13 344/1
Feb 1995/KOR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	25±9 139/0.4	23±6 128/0.4	22±9 122/0.4	28±13 156/0.5
Feb 1995/OR1 *C. gariepinus* (13)	Mean±Sd Bf_w/Bf_s	30±13 19/0.4	40±18 25/1	33±17 20/1	56±25 35/2
Feb 1995/OR1 *L. umbratus* (20)	Mean±Sd Bf_w/Bf_s	47±34 29/1	58±48 36/2	48±42 30/1	47±27 29/1
May 1995/KOR1 *C. gariepinus* (3)	Mean±Sd Bf_w/Bf_s	58±24 200/2	49±2 200/2	54±29 186/1	86±48 297/2
May 1995/KOR1 *L. umbratus* (7)	Mean±S_d Bf_w/Bf_s	43±16 148/1	60±29 207/2	44±18 152/1	48±22 166/1
May 1995/OR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	56±22 55/NA	59±25 58/NA	56±28 55/NA	70±31 69/NA
May 1995/OR1 *L. umbratus* (7)	Mean±S_d Bf_w/Bf_s	59±47 58/NA	68±28 67/NA	49±28 48/NA	67±31 66/NA

NA=Not available

Differences between localities

The data indicate several significant (P±0.05) differences between the two localities with regard to the bioaccumulation of the selected metals in the tissues of the two species. These differences were found in February 1994 (higher tissue concentrations at Locality OR1), February 1995 and May 1995 (higher tissue concentrations at Locality KOR1) for Zn (Zn concentrations in the water for these periods were much higher at Locality KOR1 - Table 9). For Cu significant (P±0.05) differences were found in August 1994 (higher tissue concentrations at Locality OR1; equal Cu concentrations in the water at both localities) and May 1995 (higher tissue concentrations at Locality KOR1; higher Cu concentration in the water at this locality). In February 1994 the highest Cu concentrations were found at Locality KOR1 (Cu concentrations in the water equal at both localities). In August 1994 the highest Cu tissue concentrations were found at Locality OR1 (Cu concentrations in the water equal at both localities). Higher concentrations of Mn were found at Locality KOR1 (equal Mn concentrations in the water at both localities). Mn tissue concentrations were higher at Locality OR1 during February 1995 as well as in May 1995 (Mn concentrations in the water were higher at this locality during these periods). Pb tissue concentrations were higher at Locality KOR1 during February 1994 (Pb concentrations in the water higher at Locality OR1). During February and May 1995 Pb tissue concentrations were higher at Locality OR1 (Pb concentrations in the water higher at Locality KOR1).

Significantly (P±0.05) higher tissue concentrations of Cr at Locality OR1 were found during February 1995 (also higher Cr concentrations in the water at this locality), while higher Cr tissue concentrations

were found at Locality KOR1 during February and August 1994 (Cr concentrations in the water in the same range at both localities during these periods - Table 9). Ni tissue concentrations were significantly (P±0.05) higher at Locality OR1 during February 1995 (with corresponding higher Ni concentrations in the water), while higher Ni tissue concentrations were found at Locality KOR1 during February 1994 (equal Ni concentrations in the water). Al tissue concentrations were higher at Locality OR1 during May 1994 (higher Al concentrations in the water at Locality KOR1) and higher at Locality KOR1 during February and May 1995 (with corresponding higher concentrations in the water). Fe concentrations were significantly higher at Locality OR1 during February and May 1995 (higher Fe concentrations in the water during these periods) and significantly higher at Locality KOR1 during May 1994 (higher Fe concentration in the water at Locality OR1).

TABLE 6
Mean nickel concentrations (µg/g dry mass) in the tissues and organs of *C. gariepinus* and *L. umbratus* at localities KOR1 and OR1 from February 1994 to May 1995 (month, locality, species, number) and the water (Bf_w) and sediment (Bf_s) bioconcentration factors for these tissues/organs

		Skin	Liver	Muscle	Gills
Feb 1994/KOR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	15±5 71/0.3	16±4 76/0.3	14±5 67/0.3	26±8 124/0.5
Feb 1994/OR1 *C. gariepinus* (10)	Mean±S_d Bf_w/Bf_s	11±4 50/0.1	19±6 86/0.2	9±2 41/0.1	34±36 155/0.4
May 1994/OR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	12±5 60/0.1	12±4 60/0.1	15±5 75/0.1	18±5 90/0.1
Aug 1994/KOR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	9±3 90/0.1	10±35 100/0.1	10±3 100/0.1	14±5 140/0.2
Aug 1994/OR1 *C. gariepinus* (6)	Mean±S_d Bf_w/Bf_s	9±3 90/0.1	11±8 110/0.1	8±1 80/0.1	18±9 180/0.2
Aug 1994/OR1 *C. gariepinus* (8)	Mean±S_d Bf_w/Bf_s	8±2 80/0.1	9±2 90/0.1	9±3 90/0.1	15±4 150/0.1
Nov 1994/KOR1 *C. gariepinus* (14)	Mean±S_d Bf_w/Bf_s	14±5 467/0.1	17±7 567/0.1	14±6 467/0.1	71±48 1367/0.4
Nov 1994/KOR1 *L. umbratus* (3)	Mean±S_d Bf_w/Bf_s	10±3 333/0.1	14±6 467/0.1	11±2 367/0.1	13±1 433/0.1
Nov 1994/OR1 *C. gariepinus* (3)	Mean±S_d Bf_w/Bf_s	10±0.4 250/0.1	11±0.6 275/0.1	12±2 300/0.1	27±1 675/0.3
Nov 1994/OR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	19±8 475/0.2	27±8 675/0.3	23±8 575/0.3	25±9 625/0.3
Feb 1995/KOR1 *C. gariepinus* (4)	Mean±S_d Bf_w/Bf_s	9±1 39/0.1	11±3 48/0.1	14±4 61/0.1	31±14 135/0.3
Feb 1995/KOR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	11±6 39/0.1	10±4 44/0.1	10±5 43/0.1	14±7 61/0.1
Feb 1995/OR1 *C. gariepinus* (13)	Mean±S_d Bf_w/Bf_s	20±11 54/0.3	27±15 73/0.4	23±15 62/0.3	40±23 108/0.5
Feb 1995/OR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	31±20 84/0.4	39±22 105/0.5	31±20 84/0.4	39±24 105/0.5
May 1995/KOR1 *C. gariepinus* (3)	Mean±S_d Bf_w/Bf_s	24±16 104/0.3	16±2 113/0.4	21±19 91/0.3	32±21 139/0.4
May 1995/KOR1 *L. umbratus* (7)	Mean±S_d Bf_w/Bf_s	21±12 91/0.3	35±22 152/0.5	24±15 104/0.3	34±19 148/0.5
May 1995/OR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	30±17 120/NA	34±21 136/NA	31±18 124/NA	40±21 160/NA
May 1995/OR1 *L. umbratus* (7)	Mean±S_d Bf_w/Bf_s	42±46 168/NA	48±27 168/NA	27±25 112/NA	44±34 176/NA

NA=Not available

Discussion

Bioaccumulation of Zn, Cu, Mn, Pb, Cr, Ni, Al and Fe in the skin, muscle, liver and gill tissue of *C. gariepinus* and *L. umbratus*

Metal contamination of aquatic ecosystems has long been recognised as a serious pollution problem. Metals, released into surface water, tend to accumulate in the sediments through adsorption and precipitation processes. It can be reintroduced into the water in a bio-available form with changing water quality conditions to fish which absorbs it from the water by means of gills or epithelial tissues and concentrates it in the body. Metal concentrations in fish have been related to morphometry and pH (Wren et al., 1983), alkalinity (Scheider et al., 1979), dissolved organic matter

TABLE 7
Mean aluminium concentrations (µg/g dry mass) in the tissues and organs of *C. gariepinus* and *L. umbratus* at localities KOR1 and OR1 from February 1994 to May 1995 (month, locality, species, number) and the water (Bf_w) and sediment (Bf_s) bioconcentration factors for these tissues/organs

		Skin	Liver	Muscle	Gills
Feb 1994/KOR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	35±11 90/0.001	46±12 118/0.002	28±11 72/0.001	110±36 282/0.004
Feb 1994/OR1 *C. gariepinus* (10)	Mean±S_d Bf_w/Bf_s	33±18 275/0.001	53±31 440/0.001	26±16 217/0.001	166±127 1383/0.01
May 1994/OR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	13±9 34/0.0003	33±19 87/0.001	11±5 29/0.0002	76±43 200/0.002
Aug 1994/KOR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	26±11 74/0.001	23±16 66/0.001	19±9 54/0.001	74±44 211/0.002
Aug 1994/OR1 *C. gariepinus* (6)	Mean±S_d Bf_w/Bf_s	29±12 78/0.001	31±14 84/0.001	23±14 84/0.001	147±103 397/0.01
Aug 1994/OR1 *C. gariepinus* (8)	Mean±S_d Bf_w/Bf_s	18±8 49/0.001	28±20 76/0.001	42±16 114/0.002	111±38 300/0.004
Nov 1994/KOR1 *C. gariepinus* (14)	Mean±S_d Bf_w/Bf_s	209±203 290/0.01	35±5 49/0.001	26±14 36/0.001	122±38 169/0.003
Nov 1994/KOR1 *L. umbratus* (3)	Mean±S_d Bf_w/Bf_s	53±12 74/0.001	32±9 44/0.001	58±30 81/0.002	114±28 158/0.003
Nov 1994/OR1 *C. gariepinus* (3)	Mean±S_d Bf_w/Bf_s	52±9 69/0.001	27±3 36/0.001	54±32 72/0.001	234±72 312/0.01
Nov 1994/OR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	28±10 37/0.001	38±25 51/0.001	34±20 45/0.001	158±99 211/0.003
Feb 1995/KOR1 *C. gariepinus* (4)	Mean±S_d Bf_w/Bf_s	98±21 86/0.002	109±49 96/0.002	109±20 96/0.002	371±212 325/0.01
Feb 1995/KOR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	61±23 54/0.001	79±37 69/0.002	59±25 51/0.001	172±102 151/0.003
Feb 1995/OR1 *C. gariepinus* (13)	Mean±S_d Bf_w/Bf_s	40±21 191/0.001	47±30 224/0.002	41±21 195/0.001	137±113 652/0.01
Feb 1995/OR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	40±26 191/0.001	68±86 324/0.002	26±9 124/0.001	113±117 538/0.004
May 1995/KOR1 *C. gariepinus* (3)	Mean±S_d Bf_w/Bf_s	53±2 14/0.001	36±12 10/0.001	43±10 12/0.002	150±14 40/0.004
May 1995/KOR1 *L. umbratus* (7)	Mean±S_d Bf_w/Bf_s	57±38 15/0.001	72±86 19/0.002	31±11 8/0.001	173±60 46/0.004
May 1995/OR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	19±9 158/NA	18±10 150/NA	23±17 192/NA	58±26 483/NA
May 1995/OR1 *L. umbratus* (7)	Mean±S_d Bf_w/Bf_s	29±10 242/NA	34±15 283/NA	21±4 175/NA	79±20 658/NA

NA=Not available

(Wiener and Giesy, 1979), trophic relationships of fish (Rodgers and Quadri, 1982) as well as differences among species and fish mass within populations (Wren et al., 1983) and usually exhibit positive skewness and are frequently non-normal (Wiener and Giesy, 1977), a finding which is supported by data from the present study. Once absorbed, the blood transports the element to a storage point like fat, muscle and bone, or the kidney and liver for storage and/or transformation (Heath, 1987). Elements transformed by the liver may be stored there and excreted in the bile or stored in extrahepatic tissue like fat. It may also be passed back into the blood for possible excretion by the kidney or gills. In the present study, metal concentrations in the different tissues were variable, but it was clear that the highest concentrations were found in the gills and liver tissues, followed by the muscle and skin tissues.

The gills are very important due to their close contact with the external environment and thus intimate ionic regulation and it is clear that Zn, Mn, Pb, Cr, Ni and Al uptake occurs primarily through the gills. The gill can act as a depot tissue, where the uptake of metals significantly exceeds the elimination and therefore metals will be accumulated.

Zn has a twofold influence on the gills, namely bioconcentration of metals and the structural cellular alterations that were noted on the gills of *Tilapia sparmanii* (Van Rensburg, 1989). Zn concentrations in the gill tissue of *C. gariepinus* and *L. umbratus* ranged between 88 and 257 µg/g dry mass and 139 and 184 µg/g dry mass respectively. When these results are compared with levels reported by Bezuidenhout *et. al.,* (1990) collected for *C. gariepinus* from the Germiston Lake in Gauteng, which was also affected by mining and industrial effluents, similar levels were detected in the gill tissue between the two studies. Cu concentrations on

the other hand, fluctuated between 5 and 20 µg/g dry mass for *C. gariepinus* during the present study, which were lower than levels recorded by Bezuidenhout et al., (1990) in the Germiston Lake. The highest Mn concentrations were detected in the gill tissue of *L. umbratus* which is the main route of uptake of Mn, as little absorption of this metal occurs through the gut from food (Katz *et. al.*, 1972). Pb was also noted for its specific accumulation in the gill tissue (also noted by Brooks and Rumsy, 1974). The mean Pb concentrations found in the gill tissue of the two species were higher than concentrations of this metal found in the gill tissue of *B. marequensis* in the lower Olifants River Catchment (Seymore *et.al.,* 1995), but lower than concentrations found by Du Preez and Steyn (1992) in the Olifants River, Kruger National Park (assuming a moisture content of 80%).

The liver is the site of high bioaccumulation of especially Cu (50 to 568 µg/g dry mass) and also Fe (344 to 3963 µg/g dry mass). These values for Cu were very high compared to values found for *Barbus marequensis* (Seymore, 1994), which also accumulated the highest Cu concentrations in its liver. The higher Fe concentrations in the liver tissue from this study (Seymore, 1994) also compared well to high Fe levels in the liver tissue from the present study. These high Fe concentrations in the liver tissue may be due to iron-containing enzymes and the extensive vascular system of the liver, as the haemoglobin in the blood binds approximately three-quarters of the Fe in the body (Voynar, 1960). The liver tissue also accumulated the second highest concentrations of Mn, Pb, Cr, Ni and Al. The ability of fish to regulate essential metals should be taken into account. The liver is in an advantageous position for clearing the blood of substances entering the circulation from the gastro-

TABLE 8
Mean iron concentrations (µg/g dry mass) in the tissues and organs of *C. gariepinus* and *L. umbratus* at localities KOR1 and OR1 from February 1994 to May 1995 (month, locality, species, number) and the water (Bf_w) and sediment (Bf_s) bioconcentration factors for these tissues/organs

		Skin	Liver	Muscle	Gills
Feb 1994/KOR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	147±49 149/0.01	1201±757 1213/0.04	121±38 122/0.004	324±109 327/0.02
Feb 1994/OR1 *C. gariepinus* (10)	Mean±S_d Bf_w/Bf_s	104±40 63/0.002	1586±556 955/0.03	72±18 43/0.001	466±290 281/0.01
May 1994/OR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	101±40 58/0.001	1514±756 875/0.02	149±36 86/0.002	347±96 201/0.004
Aug 1994/KOR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	82±41 65/0.004	398±152 313/0.02	92±25 72/0.004	337±125 265/0.02
Aug 1994/OR1 *C. gariepinus* (6)	Mean±S_d Bf_w/Bf_s	169±80 132/0.01	1074±246 839/0.03	140±35 109/0.004	447±75 349/0.01
Aug 1994/OR1 *C. gariepinus* (8)	Mean±S_d Bf_w/Bf_s	101±13 79/0.003	2218±1168 1733/0.06	141±31 110/0.004	329±61 257/0.01
Nov 1994/KOR1 *C. gariepinus* (14)	Mean±S_d Bf_w/Bf_s	469±446 347/0.01	1997±200 1479/0.04	184±48 136/0.004	1303±769 965/0.03
Nov 1994/KOR1 *L. umbratus* (3)	Mean±S_d Bf_w/Bf_s	280±191 207/0.01	348±109 258/0.01	195±21 144/0.004	437±34 324/0.01
Nov 1994/OR1 *C. gariepinus* (3)	Mean±S_d Bf_w/Bf_s	216±71 190/0.01	1531±858 1343/0.04	255±88 224/0.01	737±115 647/0.20
Nov 1994/OR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	620±261 544/0.01	3963±1770 3476/0.1	739±275 648/0.02	1234±608 1083/0.03
Feb 1995/KOR1 *C. gariepinus* (4)	Mean±S_d Bf_w/Bf_s	140±10 100/0.003	892±187 637/0.02	211±41 151/0.01	733±419 524/0.02
Feb 1995/KOR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	145±43 104/0.003	344±131 243/0.01	139±46 99/0.003	607±423 434/0.01
Feb 1995/OR1 *C. gariepinus* (13)	Mean±S_d Bf_w/Bf_s	206±79 62/0.01	1073±396 322/0.03	214±111 64/0.01	564±254 169/0.02
Feb 1995/OR1 *L. umbratus* (20)	Mean±S_d Bf_w/Bf_s	366±375 110/0.01	2461±1944 739/0.1	275±179 83/0.01	583±192 175/0.02
May 1995/KOR1 *C. gariepinus* (3)	Mean±S_d Bf_w/Bf_s	266±114 83/0.01	646±60 201/0.02	246±152 77/0.01	515±135 160/0.02
May 1995/KOR1 *L. umbratus* (7)	Mean±S_d Bf_w/Bf_s	216±65 67/0.01	647±400 210/0.02	208±67 65/0.01	595±209 185/0.02
May 1995/OR1 *C. gariepinus* (20)	Mean±S_d Bf_w/Bf_s	238±103 68/NA	1366±959 389/NA	272±131 78/NA	491±148 140/NA
May 1995/OR1 *L. umbratus* (7)	Mean±S_d Bf_w/Bf_s	289±210 82/NA	2225±1261 634/NA	245±104 70/NA	1547±1288 441/NA

NA=Not available

TABLE 9
Highest and lowest water bioconcentration factors (Bf_w) compared to metal concentrations in the water

Metal	Lowest	Highest
Zn Bf_w	May 1994 - KOR1	Aug 1994 - OR1
Concentration in water	163 0.19	3 600 0.04
Cu Bf_w	May 1995 - KOR1	Feb 1995 - KOR1
Concentration in water	16 0.11	52 700 0.06
Mn Bf_w	May 1995 - OR1	Nov 1994 - OR1
Concentration in water	13 0.31	11 800 0.01
Pb Bf_w	Nov 1994 - OR1	Feb 1995 - OR1
Concentration in water	12 0.26	1 100 0.02
Cr Bf_w	Feb 1995 - OR1	Nov 1994 - KOR1
Concentration in water	19 1.62	722 0.21
Ni Bf_w	Feb 1995 - KOR1	Nov 1994 - KOR1
Concentration in water	39 0.23	1 367 0.03
Al Bf_w	May 1995 - KOR1	Feb 1994 - OR1
Concentration in water	8 3.73	1 383 0.12
Fe Bf_w	Feb 1994 - OR1	Nov 1994 - OR1
Concentration in water	43 1.66	3 476 1.14

intestinal tract. Since the blood from this system passes though the liver before reaching the systemic circulation, theoretically the liver can remove toxicants from the blood, biotransform them or excrete them into the bile and thus prevent their distribution to other parts of the body. High metal concentrations therefore reflect its multifunctional role in detoxification and storage and for that reason fish livers were analysed to prove their use as indicators of trace element pollution in the freshwater environment.

The lowest concentrations of Zn, Cu, Mn, Pb, Cr, Ni, Al and Fe were found in the muscle and skin tissues. The metal concentrations in these tissues are very important as these are the edible parts of the fish. Lower concentrations in these tissues can possibly indicate that the skin is an important excretory organ for these metals (Khalaf et al., 1985), presumably by means of the mucus (Heath, 1987). The skin tissue, together with the gill tissue, is characterized by a mucus layer on the outer surface. This can indicate them as excretion routes involving the sloughing off of mucus from these surfaces (Varanasi and Markey, 1978). In August 1994 and February 1995 at Locality KOR1 and in May 1995 at Locality OR1, the mean Pb concentrations in the muscle tissue for both species, ranging between 9 and 12 µg/g dry mass, exceeded the limit for human consumption (8µg/g dry mass, assuming a 75% moisture content) (Brown et al.,1984). It is therefore proposed that additional research should be done regarding this issue, as the fish at Locality OR1 are consumed by humans. The estimated intake of Cr should average at ± 150 µg Cr per day (Snyder et al., 1975) and in the present study the average Cr concentration in the muscle tissue for the two species was 29 µg/g dry mass and therefore pose no serious health risk to the consumer. These findings are in contrast to values obtained by Van den Heever and Frey (1996) for *C. gariepinus* in treated sewage water and natural dam water, with values of 157.3 µg/g wet mass (768.5 µg/g dry mass with moisture content assumed to be 80%) in treated sewage water and 151.9 µg/g wet mass (759.5 µg/g dry mass with 80% moisture content) in natural dam water.

The bioavailability of these metals should also be taken into account. The bioaccumulation factor (BF) can be seen as a constant of proportionality between the concentration of the metal in the fish and the concentration in the water (BF_w) and/or sediment (BF_s). Thus, the calculated values provide some indication of the bioavailability of metals to the fish from the water and/or sediment. The total concentration of the metal in the water does not play the major role in availability of that metal. This was clear from the present study, as higher bioconcentration factors did not coincide with higher metal concentrations in the water (Table 9). Metal species as well as physico-chemical conditions of the waters such as pH, are very important, as this determines the toxicity and speciation of the metals. It is also important to remember that bioconcentration factors would not give an accurate indication of the relative bioavailability of metals for uptake, if these metals were regulated in the fish, which might have been the case in the present study. Also, the kinetics of uptake play a crucial role. If a steady state is not reached, the bioconcentration factor in itself is a function of time. It must be noted that only one water sample was collected once every three months, stressing the importance of more regular monitoring of the system. More research is therefore needed regarding the bioavailability of metals and the different

TABLE 10
Summary of statistical differences regarding the bioaccumulation in the skin, liver, muscle and gill tissues between the species *Clarias gariepinus* (*C.g*) and *Labeo umbratus* (*L.u*), localities KOR1 and OR1 and the correlations between the metal concentrations in the tissues and the total lengths of the fish

Tissue type		Mn	Cu	Pb	Cr	Zn	Fe	Ni	Al
Skin	Bioaccumulation	✳	✳	✳	✳	✳	✳	✳	✳
	Species			*C.g*		*C.g*	*L.u*	*L.u*	
	Localities	OR1			OR1			OR1	
	Relationship	-corr	-corr	-corr	-corr	-corr	-corr	-corr	-corr
Liver	Bioaccumulation	▲	▲	▲	▲	▲	▲	▲	▲
	Species	*L.u*	*L.u*	*L.u*				*L.u*	
	Localities		OR1			OR1		OR1	
	Relationship	-corr	-corr	-corr	-corr	+corr	-corr	-corr	-corr
Muscle	Bioaccumulation	✳	✳	✳	✳	✳	✳	✳	✳
	Species		*L.u*	*L.u*		*C.g*	*L.u*	*L.u*	
	Localities		OR1		OR1				
	Relationship	-corr	-corr	-corr	-corr	-corr	-corr	-corr	-corr
Gills	Bioaccumulation	▲	▲	▲	▲	▲	▲	▲	▲
	Species	*L.u*		*C.g*	*C.g*	*L.u*		*C.g*	
	Localities								
	Relationship	-corr		+corr	+corr	+corr	-corr	+corr	-corr

✳ Low bioaccumulation
▲ High bioaccumulation

forms of these metals in the water and sediment at the specific localities.

Differences in bioaccumulation patterns between species

Although there were several significant (P±0.05) differences between the two species regarding the bioaccumulation of the selected metals, no definite trend could be established. Differences could, however, be attributed to the different feeding habits of the two species, as uptake of metals via the food plays an important role. Mathis and Cummins (1973) found that significantly higher concentrations of Zn were present in omnivorous fish. Thus, the feeding patterns of *C. gariepinus*, which is omnivorous, catches living prey and eats any organic material (including aquatic weeds, detritus as well as fish, birds, frogs, small mammals, reptiles, snails, crabs, insects and other plant material), are important (Van der Waal, 1972). *L. umbratus,* on the other hand, feeds on soft sediment and organic material (Skelton, 1993) and this can be the main reason for the significant differences between the two species with higher Zn concentrations in the skin, liver and muscle tissue of *C. gariepinus.* Higher metal concentrations in the skin & muscle tissue of *C. gariepinus* can also be ascribed to the fact that the catfish does not have scales, facilitating the uptake of the metal through these tissues. High metal concentrations in the gills of *L. umbratus* can be expected as this fish is dependent only on its gills for breathing and the gills are an important route for metal uptake and the metals are absorbed in the ionic form by the gills in high concentrations. *C. gariepinus* is also air breathing - using branched air breathing organs situated in the cavities above the gill arches - contributing, together with loss of metals occurring via the gills by the mucus layer covering this organ (Varanasi et al., 1978) to lower metal concentrations in the gills.

Relationships between metal concentrations and total fish lengths

There was a relationship between the bioaccumulation of Zn, Cu, Mn, Pb, Cr, Ni, Al and Fe concentrations and the lengths of the fish ranging between 40 and 77 cm. The concentrations were generally lower with increasing fish size, which can be related to new tissues being incorporated at a greater rate than metals can be actively transported into the tissues to establish a steady state concentration dilution by growth (Cross et al., 1973), changes in proximal composition of the muscle tissue or decreased dietary intake, contributing to the negative correlation between the bioaccumulation of these metals in the muscle tissue and the size of the fish, which supports findings of Bohn and Mc Elroy (1976), where the same relationship was found for Al and Fe. Small fish also have a higher metabolic rate per unit body mass than large fish (Patrick and Loulit, 1978) and hence require relatively more oxygen (Winberg, 1956; Matthiessen and Bradfield, 1977) and this requirement is met via a higher rate of flow over the gills and by having a larger gill surface area/g body mass than larger fish (Hughes, 1970). Thus, increasing metal concentrations can be related to decreasing fish size. Although Cu concentrations in the liver showed a negative correlation, the Zn concentration in this organ increased with fish size. This increase can be accounted for by physiological requirements for survival. It must be stressed that the comparison was based on the fish combined over a time period which may

influence the comparison. A more accurate evaluation would be possible if a definite size range of fish was captured at the same time. Nevertheless, the present data and those of previous studies (De Wet et al., 1994) indicate that the size of the fish must be considered, especially when dealing with juveniles compared to adult fish.

Gender differences

The accumulation of metals in the different tissues of the two species showed some difference between male and female fish. No definite trend could, however, be established. It is suggested that the gonads of the different sexes should be examined to determine the actual differences between the males and females regarding the bioaccumulation of these metals in the two species. The developmental stages of the gonads should, however, be considered as this may greatly influence the data. This is supported by findings of Du Preez and Steyn (1992) in a study performed on the concentrations of metals in the tissues of the tigerfish, *Hydrocynus vittatus* from the Olifants River, Kruger National Park and also by a study performed on other aquatic animals, including tissues of the freshwater crab, *Potamonautes warreni* from industrial, mine and sewage-polluted freshwater ecosystems (Steenkamp et al., 1994). Seymore (1994) suggested that female fish require greater amounts of Zn, necessary for gonad development. The concentrations in the gonads therefore increased until the fish were sexually mature. These findings (Seymore, 1994) also showed that when the Zn concentrations in the gonads decreased, Zn concentrations increased in the internal sources (skin, muscle and liver tissues) and the other way around.

Differences between localities

A definite trend in the different bioaccumulation patterns at the two localities was not always evident, but *C. gariepinus* accumulated more Zn in the liver tissue at Locality OR1 in February 1994. This could possibly be attributed to the absence of dilution, since the river experienced low flows during that time as a result of drier conditions. *L. umbratus* accumulated more Zn in the liver at Locality KOR1 in February and May 1995, which coincides with higher Zn concentrations in the water at Locality KOR1 in those months (Table 9) due to direct input of point source pollution from the combined sewage purification works located upstream from this locality and less rainfall and the low flow of the river which concentrated these pollutants. The lower Zn concentration at this locality for *C. gariepinus* can be attributed to accumulation of Zn by the liver to the maximum concentration and the consequent regulation thereof by the liver. The higher Zn concentration in the liver was also found for *C. gariepinus* in treated sewage water in a study regarding the differences in bioaccumulation of Zn and Cu between different tissue types in dam water and treated sewage water (Van den Heever and Frey, 1994). The Cu skin and muscle tissue concentrations were the highest for *C. gariepinus* at Locality KOR1 in February 1994. In August 1994 the Cu liver and muscle tissue concentrations were the highest at locality OR1 and for *L. umbratus* the highest at Locality KOR1 in May 1995.

The Mn bioaccumulation seemed to be the highest at Locality OR1 in the skin, liver, gill and muscle tissues, with the exceptions in February 1994 for *C. gariepinus*, where the highest Mn concentrations were found at Locality KOR1 in the muscle and liver tissues. This coincides with the higher Mn Bf_w at Locality KOR1 at that time (Table 3). The high Mn concentrations may be attributed to industrial activities in the catchment area as well as to effluents from the combined sewage purification works located upstream. The data presented indicate that the fish are exposed to sublethal levels of Mn and Pb, resulting in the observed concentrations. In the case of Pb, the bioaccumulation in the skin, liver and muscle tissues of *L. umbratus* was the highest at Locality OR1, receiving effluent from mines and sewage purification works and surface runoff from industries such as paint factories. Once again, *C. gariepinus* accumulated more Pb at Locality KOR1 in February 1994.

The increase of Cr and Ni concentrations in the tissues of both species and in the water at Locality OR1 may be due to the different industries in the Witbank area and effluent received from the Witbank Dam and the Suurstroom and Spook Spruit. Both of these receive effluent from mines in their areas and flowing into the Olifants River before it reaches this locality. The metal concentrations were not necessarily higher in the water of Locality OR1 in February 1995, thus the significant differences in the bioaccumulation of these metals could be attributed to the physical/chemical properties of the water at that time. The Cr and Ni concentrations in the skin, liver, muscle and gill tissues were mostly higher than the concentrations in these tissues obtained by Seymore (1994) for *B. marequensis* in the lower catchment of the Olifants River. This points to more serious contamination by greater input from point and diffuse sources of pollution in the Upper Catchment of the Olifants River and more intense investigations regarding this is suggested.

In general, fish at Locality OR1, which receives water from various industries and mines in the catchment area, accumulated more Al and Fe than those at Locality KOR1 in August 1994. The Al concentrations in the water in August 1994 were slightly higher at Locality OR1, but for Fe the concentrations in the water were similar at both localities (0.35 and 0.37 mg/l) (Coetzee, 1996). In May 1994, *C. gariepinus* accumulated more Fe at Locality KOR1, despite the lower Fe concentrations in the water (Coetzee, 1996). In these cases, elevated accumulation of the metal may be due to different physical/chemical properties of the water at that time. The highest Fe concentrations were found in fish at Locality OR1 in February 1995, which coincided with much higher Fe concentrations in the water at locality OR1 (Coetzee, 1996). This may be due to point and diffuse source effluent from the coal mines in the catchment area. For Al the fish at Locality KOR1 accumulated higher concentrations in February 1995, which can be explained by the much higher Al concentrations in the water at this locality at this time (Coetzee,1996). Fish at Locality KOR1 in May 1995 also accumulated higher levels of Al with the concentrations in the water being much higher at this locality.

Conclusion

There was a large variation in the metal concentrations in the different tissues of *C. gariepinus* and *L. umbratus* during the period February 1994 to May 1995. This suggests that the number of fish sampled at each locality should be increased in order to minimize this variation and to be able to select fish of certain size ranges, as the results pointed towards metal accumulation patterns in the muscle, skin, liver and gill tissues, varying as a function of the lengths of the fish, with higher concentrations in smaller fish. The metals mainly accumulated in the gill and liver tissue, followed by the muscle and skin tissue (Table 10). Very high concentrations of Cu and Fe were found in the liver tissue, which could indicate that the fish have been exposed to sublethal levels of the metal. The highest concentrations of Zn, Mn, Pb, Cr, Ni and Al were accumulated in the gill tissue, but in a general monitoring program,

the muscle and skin tissues should also be included as fish are consumed by humans and in this case, by people from the informal settlements, as well as anglers at Locality OR1.

The accumulation patterns varied as a function of the species, probably according to the different feeding habits of the two species and the routes of metal uptake by the fish, as well as the localities where the fish were collected (Table 10). The higher concentrations at the different localities mainly coincided with the higher metal concentrations in the water at the time, with the treated sewage water being a major problem, especially at Locality KOR1 during the drier periods and low flows. In order to have obtained more detailed information with regards to the accumulation of metals according to gender, the gonads of both sexes should have been examined, together with the different tissues/organs used in this study.

The establishment of a general biomonitoring program is needed where the hydrological and geomorphological characteristics, the chemical and physical water quality and the riparian vegetation are also considered as these all affect the aquatic system.

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