Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepinus*, from the Olifants River, Mpumalanga, South Africa

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Abstract

The upper and lower catchments of the Olifants River are characterised by many anthropogenic activities that adversely impact on the water quality of the river. The present study indicated that both Cu and Zn are present in elevated levels as reflected in the bioaccumulation of these metals in the fish. Bioaccumulation differences in the different age and gender groups were generally not significant (P>0.05). This was due to the sampling of mostly adult fish and the exclusion of reproductive organs (gonads). Copper content in the organs and tissues indicated the following pattern: liver>gills>skin>muscle, but no specific pattern for Zn content was observed. The extent of accumulation differed in many cases between the two species, possibly due to differences in behaviour and feeding. Temporal variation in bioaccumulation occurred and generally indicated increased levels of bioaccumulation during periods of high flows. This phenomenon was ascribed to the influence of sediment-bound metals, being brought into contact with the fish at a higher intensity during these periods. Both Loskop Dam and Mamba Weir, the latter to a greater extent, are at present being exposed to levels of Cu and Zn which cause bioaccumulation. A more holistic biomonitoring approach is proposed for these impacted areas in an attempt to guide managers in a direction of improvement.

Introduction

As a result of mining, industrial and other anthropogenic activities in the catchment of the Olifants River over the past decade, this aquatic ecosystem has been degraded and increasingly contaminated by pollutants such as metals. This poses stress to aquatic organisms in particular and to the whole ecosystem in general. Copper and Zn are two of the metals occurring in elevated levels in the water of the Olifants River, Mpumalanga (Grobler et al., 1994; Seymore et al., 1994). This is of concern since they are tentatively classified as highly toxic metals by Hellawell (1986) and are also bioaccumulated by aquatic organisms (Alabaster and Lloyd, 1980; Latif et al., 1982; Villarreal-Treviño et al., 1986; Seymore et al., 1996). Due to the deleterious effects of metals on aquatic ecosystems, it is important to monitor the bioaccumulation of metals in an aquatic system. This will give an indication of the temporal and spatial extent of metal accumulation, as well as an assessment of the potential impact on human health (fish consumed) and organism health (if they have been exposed to elevated levels of a pollutant or if consumed by predators).

This study investigated levels of Cu and Zn in two species of fish, namely *Oreochromis mossambicus* and *Clarias gariepinus*, collected in the Olifants River, Mpumalanga (Fig. 1). The data will give some indication of the extent of metal contamination in these two fish species. The data from Loskop Dam are particularly important since this impoundment is commonly used for angling purposes. It is therefore important to determine the extent of the metal bioaccumulation of these two fish species as they are common angling species consumed by humans. Mamba Weir is situated inside the Kruger National Park (KNP) and it is the National Parks Board's view that water quality has to be managed in order to maintain essential ecological processes. These ecological processes will preserve the genetic diversity and ensure sustainable utilisation of both species and ecosystems (Venter and Deacon, 1992). Monitoring of the metal pollution at this locality is thus important for the mentioned conservation objectives.

Materials and methods

Field sampling

Field surveys were undertaken seasonally at Loskop Dam and Mamba Weir (KNP) during the period February 1994 to May 1995 (Fig. 1). During the final survey (May 1995) Phalaborwa Barrage was also included so as to investigate the effect of the metal-polluted Selati River on the water quality of the Olifants River before it enters the KNP. Nhlanganini Dam was sampled as a control because its catchment lies within a relatively natural area (KNP). *Oreochromis mossambicus* (Mozambique tilapia) and *C. gariepinus* (Sharptooth catfish) were sampled with gill nets (70 to 120 mm stretched mesh size), cast nets and fishing rods. After capture the mass, length and gender of each fish were recorded. The fish were then killed by a blow on the head and a cut of the spinal cord behind the head. Fish were dissected on a polyethylene work-surface using stainless steel tools while taking care to prevent any contamination of the samples (Heit and Klusek, 1982). Muscle, gill, liver and skin tissues were removed from each fish and frozen until metal analyses could be performed.

Laboratory procedures

The samples were thawed in the laboratory and dried in an oven at 60°C for a period of 48 h. In order to calculate the moisture content of each sample, the dry and wet mass of each sample was recorded. Twenty mℓ of concentrated nitric acid (55%) and 10 mℓ

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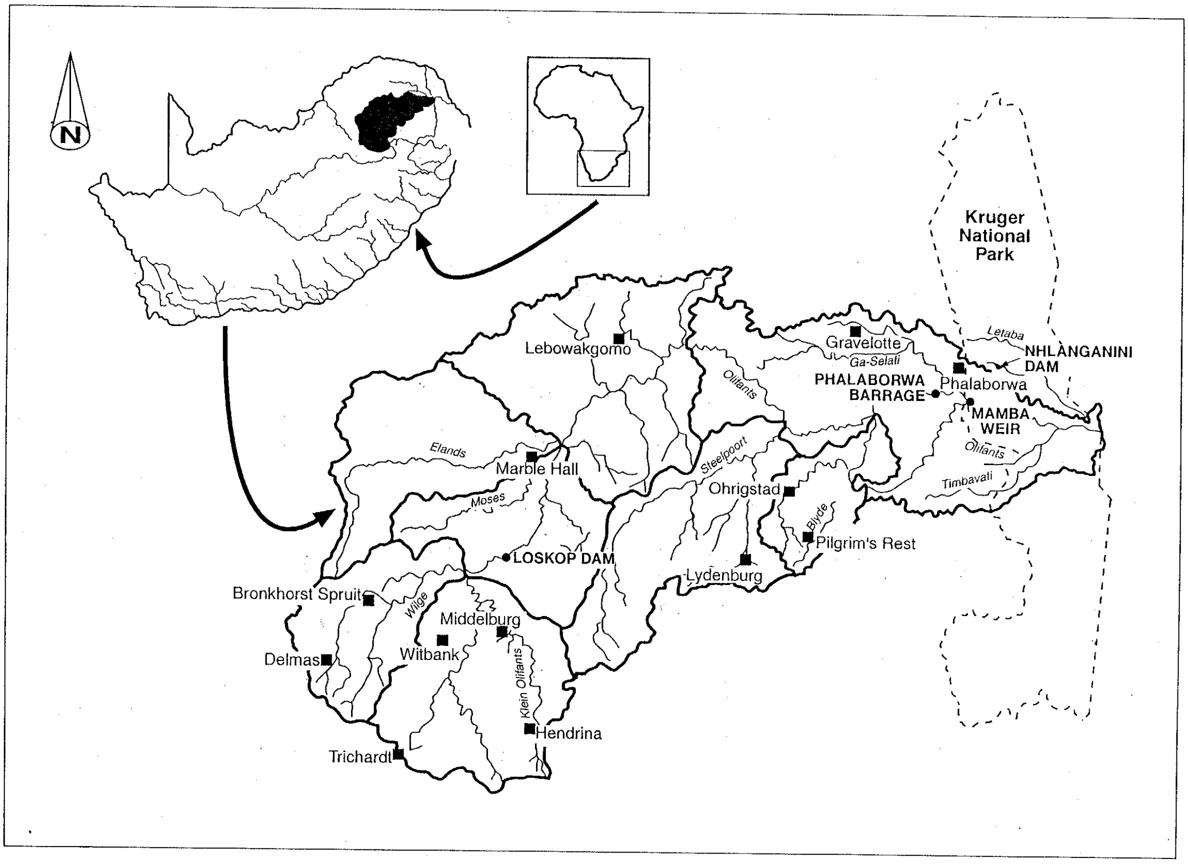

Figure 1
The Olifants River catchment and study area with selected localities

of perchloric acid (70%) were added to approximately 1 g tissue (dry mass) in a 100 mℓ Erlenmeyer flask. Digestion was performed on a hotplate (200 to 250°C) until the solutions were clear (Van Loon, 1980). The solutions were then filtered through an acid-resistant 0.45 µm filter paper and made up to 50 mℓ each with doubly distilled water. The samples were stored in clean glass bottles until the metal concentration could be determined using a Spectra AA-10 Varian atomic absorption spectrophotometer. The detection limit for Cu was 0.003 $\mu g \cdot g^{1}$ and for Zn it was 0.001 $\mu g \cdot g^{1}$. A standard sample, consisting of tuna homogenate (sample IAEA-350) from the International Atomic Energy Agency Marine Environment Laboratory, was prepared as a control in line with the above-mentioned procedures with every set of samples, to ensure accuracy of data through comparison. Analytical standards were prepared from Holpro stock solutions. Prior to use all glassware was soaked in a 2% Contrad soap solution (Merck chemicals) for 24 h, rinsed in distilled water, acid-washed in 1 M HCl for another 24 h and rinsed again in distilled water (Giesy and Wiener, 1977).

Statistical procedures

Statistical analysis was performed using the *Statgraphics Version 7* computer package. The mean, median, range and standard deviation (SD) of the data were determined through summary statistics. Logarithmic transformation had to be performed for normalisation of the data. Analyses of variance (ANOVA) were used to determine differences between various data sets.

Results

Fish size, age and gender differences

Oreochromis mossambicus

The general size of specimens of *O. mossambicus* ranged from 16.8 to 43.0 cm and 70 to 1 569 g. The fish sampled fell in the age groups of 2 years and older (Table 1). The overall ratio of females to males captured during the study period and at all localities sampled was 1:1.6. More males than females were collected at all the localities, except for Mamba Weir. Female to male ratios for each locality was 1:1.1 at Loskop Dam; 1:2.8 at Mamba Weir; 1.2:1 at Phalaborwa Barrage and 1:1.4 at Nhlanganini Dam.

Clarias gariepinus

The length range of *C. gariepinus* sampled at all localities over the entire study period was 11.5 to 95.0 cm and the mass varied between 170 and 7 250 g. Specimens of *C. gariepinus* ranged from 1 to 9 years of age (Table 1). The female to male ratio of specimens of *C. gariepinus* sampled during the study period was 1:1.2. For Loskop Dam the female to male ratio was 1:1, for Mamba Weir it was 1:1.06 and for Nhlanganini Dam 1:7.5.

The moisture content of the different tissues sampled (muscle, gills, liver and skin) differed from each other as well as between the two species. The mean percentage of moisture for *O. mossambicus* and *C. gariepinus* was respectively calculated to be 76±5% and 81±5% for muscle tissue, 69±4% and 72±6% for skin tissue, 79±5% and 77±6% for the gills and 63±6% and

73±2% for the liver tissue. Because of this variation in moisture between the different tissues and organs, the metal concentrations were calculated on a dry mass basis.

One-way ANOVA of the concentration of Cu and Zn detected in males and females during each survey investigated separately, usually indicated no significant (P>0.05) gender differences. As a result of this and the usually non-statistically significant differences (P>0.05) between the different age groups, no further distinctions were made between age and gender. The rest of the results are based on the grouped data of all ages and the two genders together.

TABLE 1
NUMBERS OF *OREOCHROMIS MOSSAMBICUS* AND *CLARIAS GARIEPINUS* IN EACH AGE CLASS AND SIZE RANGE, SAMPLED AT VARIOUS LOCALITIES

Age class (year)	Size range (cm)	Loskop Dam	Mamba Weir	Phalaborwa Barrage	Nhlanga-nini Dam	Total
		Number of *O. mossambicus*				
<1	<9.0	0	0	0	0	0
1-2	9.0-15.0	0	0	0	0	0
2-3	15.1-24.0	0	74	8	0	82
3-4	24.1-29.0	2	35	10	3	50
4-5	29.1-31.5	9	2	2	6	19
5-6	31.6-33.0	13	2	0	5	20
6-7	33.1-34.5	22	0	0	2	24
7-8	34.6-35.5	17	0	0	2	19
8+	>35.5	57	0	0	2	59
		Number of *C. gariepinus*				
1	< 29.5	1	3	*	0	4
2	29.6-37.0	1	16	*	0	17
3	37.1-44.0	1	34	*	2	37
4	44.1-54.5	4	26	*	1	31
5	54.6-65.0	25	1	*	12	38
6	65.1-73.0	18	4	*	2	24
7	73.1-79.0	3	3	*	0	6
8	79.1-87.0	1	2	*	0	3
9	> 87.1	1	0	*	0	1

* No fish sampled

Cu and Zn bioaccumulation in different organs and tissues

The general pattern of Cu concentrations detected in different organs/tissues was liver > gills > skin > muscle. This was similar for both species and at all the localities surveyed (Fig. 2). Liver tissue always contained Cu levels significantly higher (P<0.05) than the gills, which again contained significantly higher levels of Cu than the skin and muscle tissues (Tables 2 and 3). The level of Cu in the skin tissue was slightly higher than that in the muscle tissue (Fig. 2).

A large variation occurred in the pattern of the Zn accumulation in the different organs and tissues (Fig. 3). In both species, muscle tissue always contained significantly lower (P<0.05) levels of Zn than any of the other tissues and organs sampled. Gill, skin and liver tissues of *O. mossambicus* all contained relatively high levels of Zn (Fig. 3). For *C. gariepinus* the gills and liver usually had the highest levels of Zn accumulation, with the skin containing slightly lower levels (Tables 2 and 3). However, at Nhlanganini Dam the bioaccumulation pattern was different, namely skin > liver > gills > muscle (Fig. 3). *Clarias gariepinus* accumulated the highest levels of Zn in their gills and livers, with slightly lower levels in their skin tissue (Fig. 3).

Species differences for Cu and Zn bioaccumulation

At both Loskop Dam and Mamba Weir the level of Cu in the combined organs/tissues of *O. mossambicus* was usually slightly higher (P>0.05) than those of *C. gariepinus*. These differences were significant at Loskop Dam during February 1995, and at Mamba Weir during February 1994 and May 1995 (Fig. 4). The levels of Zn detected in the fish sampled at Loskop Dam did not indicate a specific trend for bioaccumulation between the two different species (Fig. 4). *O. mossambicus* sampled at Mamba Weir accumulated significantly higher levels (P<0.05) of Zn during February 1994, but had lower levels for the rest of the surveys (Fig. 5). Generally, at both these localities, the highest levels of Zn were usually detected in *C. gariepinus*.

Temporal differences in Cu and Zn bioaccumulation

Loskop Dam

The Cu and Zn levels detected in a specific organ/tissue during a specific survey differed, in many cases significantly (P<0.05), from the level detected in the same organ/tissue during another survey (Tables 4 and 5). This was also the case in the levels of Cu and Zn detected in the combined organs/tissues of different surveys (Tables 4 and 5). For *O. mossambicus* the Cu content in muscle, gills and skin all showed similar temporal trends. The Cu concentrations in the organs/tissues of *C. gariepinus* (combined as well as separate) did not differ significantly (P>0.05) between surveys (Table 4). *Oreochromis mossambicus* usually accumulated higher levels of Cu during summer months. In both species, the concentration of Zn in the organs/ tissues combined, did not differ significantly (P>0.05) between different surveys (Table 5 and Fig. 4). Muscle and liver tissues indicated similar temporal trends in Zn accumulation while the temporal trends in gill and skin tissues usually agreed.

Mamba Weir, KNP

Temporal variation of the Cu and Zn content in the combined organs/tissues differed between the two species of fish investigated at this locality (Fig. 5). Similar to the trends in Cu accumulation witnessed at Loskop Dam, the temporal variation in the muscle, gill and skin tissues correlated to a large extent, while trends for liver tissue were different. For *C. gariepinus* muscle and skin tissue showed similar temporal trends in Cu bioaccumulation. In both *O. mossambicus* and *C. gariepinus* the

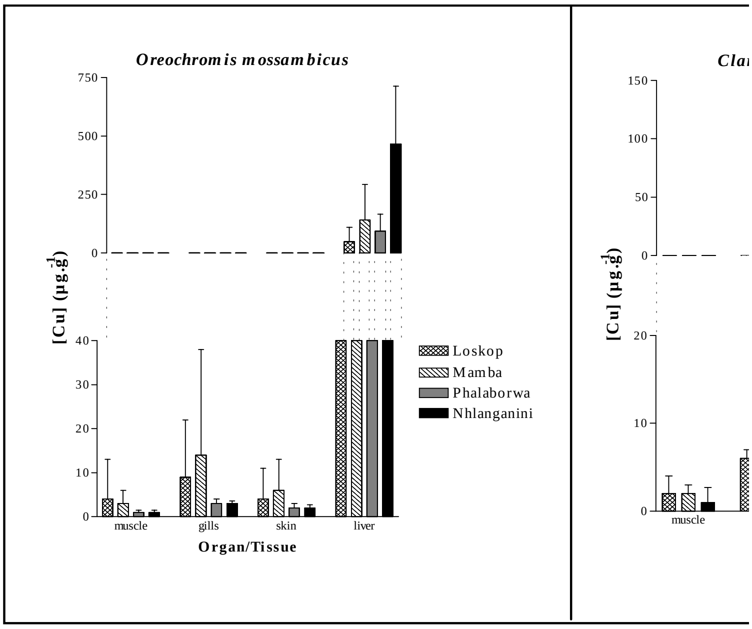

Figure 2
Mean copper concentration (µg·g[1] *dry mass) in the organs and tissues of* O. mossambicus *and* C. gariepinus

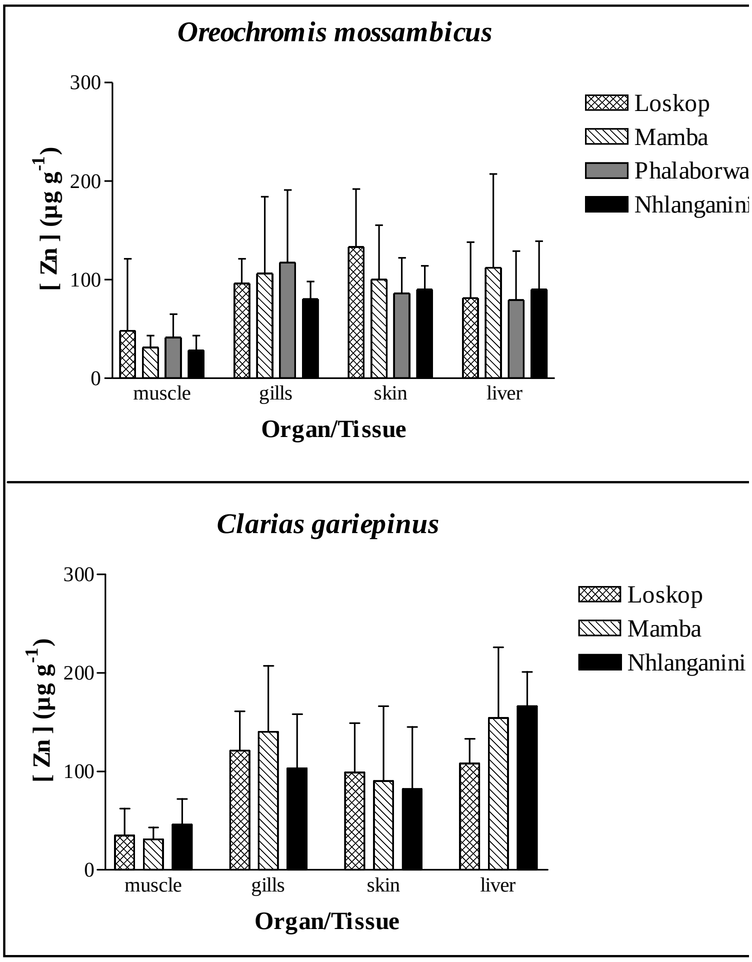

Figure 3
Mean zinc concentration (µg·g[1] *dry mass) in the organs and tissues of* O. mossambicus *and* C. gariepinus

organs/tissues (combined as well as separate) usually indicated a similar Zn bioaccumulation trend (especially between gill and skin tissues of *O. mossambicus*).

Spatial bioaccumulation differences in Cu and Zn

The concentrations of Cu and Zn in the different organs/tissues of the fish generally showed variation between the different localities (Figs. 2 and 3). For Cu accumulation, muscle, gill and skin tissues generally differed from the trend observed in liver tissue. The Cu concentration in the organs/tissues for both species of fish, generally indicated that fish at Mamba Weir accumulated significantly higher ($P<0.05$) levels of Cu than the fish at Loskop Dam and Phalaborwa Barrage. Muscle, gill and skin tissues indicated that fish at Nhlanganini Dam accumulated significantly lower levels of Cu than fish at Loskop Dam and Mamba Weir. Liver tissue, on the other hand, indicated the opposite trends with hepatic levels of Cu detected in both species at Nhlanganini Dam (control) being significantly higher ($P<0.05$) than those at the other localities (Table 6).

Zinc levels in fish, when all organs/tissues were combined, were generally not significantly different ($P>0.05$) between different localities (Table 5). Spatial trends of Cu accumulation also varied to a great extent between the different organs and tissues. The Zn content of both the muscle and skin tissues of fish sampled at Loskop Dam was usually significantly higher ($P<0.05$) than that at Mamba Weir (Table 7). Liver Zn concentrations at Mamba Weir were, however, significantly higher ($P<0.05$) than those at Loskop Dam while the gill Zn content was slightly higher at Mamba Weir. The gill Zn content of *O. mossambicus* sampled at Phalaborwa Barrage was the highest Zn level detected in the gills of

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TABLE 2
STATISTICS OF COPPER AND ZINC CONCENTRATIONS ($\mu g \cdot g^{-1}$ DRY MASS) IN THE DIFFERENT ORGANS AND TISSUE OF *O. MOSSAMBICUS*

Locality		Copper concentration				Zinc concentration			
		Muscle	Gills	Liver	Skin	Muscle	Gills	Liver	Skin
Loskop	**n**	120	120	120	119	120	120	120	119
	Range	0.8-67	1-86	1-325	1-69	9-613	16-166	24-514	23-387
	Median	2	5	27	2	32	98	65	130
	Mean	4	9	48	4	48	96	81	133
	SD	9	13	62	7	73	25	57	59
Mamba	**n**	113	112	102	113	113	105	106	113
	Range	0.2-23	1-160	2-843	0.5-43	2-86	13-625	4-781	10-342
	Median	2	8	88	3	28	91	85	87
	Mean	3	14	141	6	31	106	112	100
	SD	3	24	152	7	12	78	95	55
Phal.	**n**	20	20	20	20	20	20	20	20
	Range	# -2	1-8	8-243	0.1-8	13-132	56-392	20-233	6-152
	Median	1	3	76	1	39	99	67	84
	Mean	1	3	93	2	41	117	79	86
	SD	0.5	1	73	1	24	74	50	36
Nhlang.	**n**	20	19	19	20	20	19	20	20
	Range	0.8-3	2-4	117-989	1-3	12-82	48-115	46-213	51-137
	Median	1	4	442	1	25	76	73	84
	Mean	1	3	466	2	28	80	90	90
	SD	0.5	0.6	248	0,7	15	18	49	24

Below detection limit

TABLE 3
STATISTIC OF COPPER AND ZINC CONCENTRATIONS ($\mu g \cdot g^{-1}$ DRY MASS) IN THE DIFFERENT ORGANS AND TISSUE OF *C. GARIEPINUS*

Locality		Copper concentration				Zinc concentration			
		Muscle	Gills	Liver	Skin	Muscle	Gills	Liver	Skin
Loskop	**n**	54	54	53	55	54	54	54	55
	Range	1-17	1-12	6-74	1-27	14-207	18-328	53-188	22-227
	Median	2	6	29	2	29	124	108	85
	Mean	2	6	30	3	35	121	108	99
	SD	2	1	13	3	27	40	25	50
Mamba	**N**	88	88	88	88	88	87	88	89
	Range	0.3-6	0.7-61	1.2-135	0.9-41	4-73	7-421	27-378	17-713
	Median	2	9	53	2	31	133	147	80
	Mean	2	11	53	3	31	140	154	90
	SD	1	7	29	4	12	67	72	76
Nhlang.	**n**	17	17	17	17	17	17	17	17
	Range	0.4-8	3-5	51-135	0.8-3	18-110	12-197	120-245	32-262
	Median	1	4	69	1	36	126	164	59
	Mean	1	4	79	1	46	103	166	82
	SD	1.7	0.8	25	0.6	26	55	35	63

TABLE 4
SUMMARY OF SIGNIFICANT DIFFERENCES (P<0.05) BETWEEN DIFFERENT SURVEYS WITH RESPECT TO THE COPPER CONCENTRATION IN THE MUSCLE (M), GILLS (G), LIVER (L), SKIN (S) AND COMBINED ORGANS/TISSUES (C) FOR *O. MOSSAMBICUS* (*O.MOS*) AND *C. GARIEPINUS* (*C.GAR*). THE SHADED RIGHT HAND TRIANGLE DEALS WITH LOSKOP DAM AND THE SHADED LEFT HAND TRIANGLE WITH MAMBA WEIR. BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCES (P>0.05) WHILE (-) INDICATES SIGNIFICANT DIFFERENCES (P<0.05) BETWEEN LOCALITIES DURING A SPECIFIC SURVEY.

Survey	Spp.	Summer '94	Autumn '94	Winter '94	Spring '94	Summer '95	Autumn '95
Summer '94	*O.mos.*	15-17	M,L		S	C,M,G,L,S	M
	C.gar.	15-17					
Autumn '94	*O.mos.*	C,M,G,L,S		M,L	M,L,S	C,G,L	M,L,S
	C.gar.	C,M,G,L					
Winter '94	*O.mos.*	C,M,G,L,S	G,S			C,M,G,L,S	
	C.gar.	C,M,G,L,S	M,S				
Spring '94	*O.mos.*	C,M,G,S	G	L	15-17	C,M,G,L,S	
	C.gar.	C,M,G,L					
Summer '95	*O.mos.*	C,G,L,S	C,M,G,S	M	M,L,S		C,M,G,L,S
	C.gar.	C,M,G,L,S	M	M,S	M,L,S		
Autumn '95	*O.mos.*	G	C,M,G,L,S	C,M,L,S	C,M,S	L,M	15-17,17-19, 17-20,19-20
	C.gar.	C,M,G,L,S	L	S	L,S		17-20

TABLE 5
SUMMARY OF SIGNIFICANT DIFFERENCES (P<0.05) BETWEEN DIFFERENT SURVEYS WITH RESPECT TO THE ZINC CONCENTRATION IN THE MUSCLE (M), GILLS (G), LIVER (L), SKIN (S) AND COMBINED ORGANS/TISSUES (C) FOR *O. MOSSAMBICUS (O.MOS)* AND *C. GARIEPINUS (C.GAR)*. THE SHADED RIGHT HAND TRIANGLE DEALS WITH LOSKOP DAM AND THE SHADED LEFT HAND TRIANGLE WITH MAMBA WEIR. BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCES (P>0.05) WHILE (-) INDICATES SIGNIFICANT DIFFERENCES (P<0.05) BETWEEN LOCALITIES DURING A SPECIFIC SURVEY.

Survey	Spp.	Summer '94	Autumn '94	Winter '94	Spring '94	Summer '95	Autumn '95
Summer '94	*O.mos.*	15-17	M,S	M,G	M,G	C,M,G	M,G,L
	C.gar.	15-17	S	M,S	M,S	M	
Autumn '94	*O.mos.*	C,M,G,L,S	15-17	M,G,L,S	M,G,S	M,G,S	M,G,L,S
	C.gar.	C,M,G,L,S		M	M	M,S	
Winter '94	*O.mos.*	C,G	C,M,G,L,S			M	
	C.gar.	C,M,G,L,S				M	
Spring '94	*O.mos.*	C,M,G	C,G,L,S	S		M	L,S
	C.gar.	C,M,G,L,S					
Summer '95	*O.mos.*	C,G,L,S		C,G,S	C,G,L,S	15-17	L
	C.gar.	C,M,G,L,S	L	L	L		
Autumn '95	*O.mos.*		C,M,G,L,S	C,M,L	C,M,G	C,M,G,L,S	15-17,15-20, 17-19,17-20
	C.gar.	C,M,G,L,S	C,M,L	C,M,L	M,L		17-20

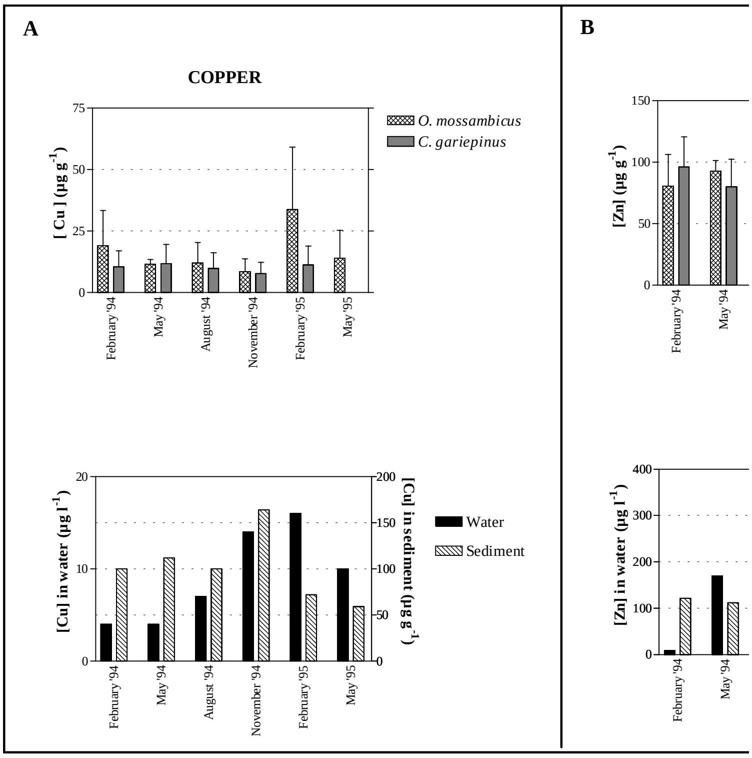

Figure 4
Temporal variation in copper (A) and zinc (B) concentrations of some biotic and abiotic (Kotze, 1997) components of Loskop Dam

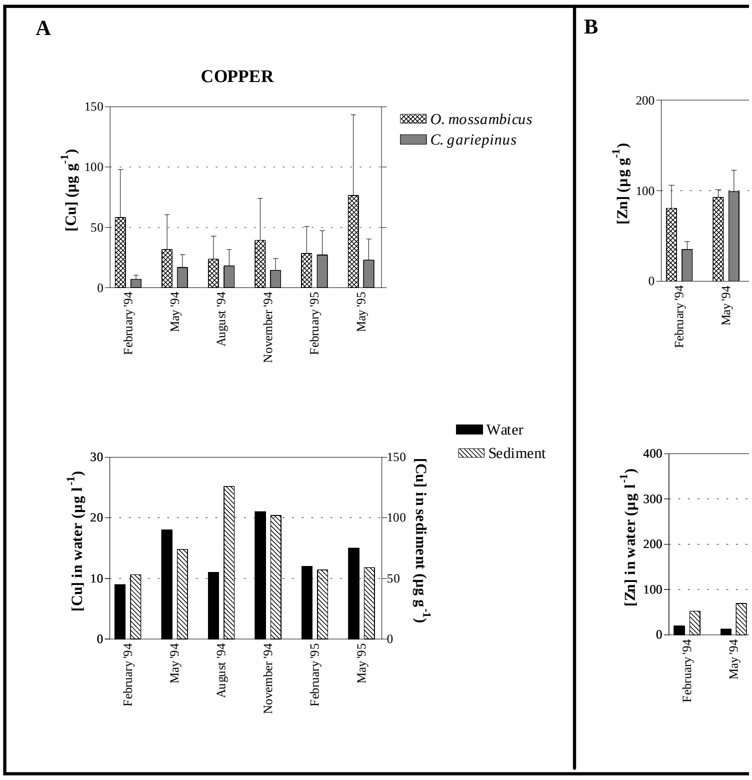

Figure 5
Temporal variation in copper (A) and zinc (B) concentration of some biotic and abiotic (Kotze, 1997) components of Mamba Weir, Kruger National Park

TABLE 6
SUMMARY OF SIGNIFICANT DIFFERENCES (P<0.05) BETWEEN VARIOUS LOCALITIES WITH RESPECT TO THE COPPER CONCENTRATION IN THE MUSCLE (M), GILLS (G), LIVER (L), SKIN (S) AND COMBINED ORGANS/TISSUES (C) OF *O. MOSSAMBICUS* (*O.MOS*) AND *C. GARIEPINUS (C.GAR)*. BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCES (P>0.05) AND (-) INDICATES SIGNIFICANT DIFFERENCES (P<0.05) BETWEEN DIFFERENT TISSUES/ORGANS AT A SPECIFIC LOCALITY.

Locality	Spp.	Loskop Dam	Mamba Weir	Phalaborwa Barrage	Nhlanganini Dam
Loskop	*O.mos.*	M-G,M-L,G-L,G-S,L-S	C		C
	C.gar.	M-G,M-L,G-L,G-S,L-S	C		
Mamba	*O.mos.*	G,L,S	ALL	C	C
	C.gar.	G,L	M-G,M-L,G-L,G-S,L-S		C
Phal.	*O.mos.*	M,G,L,S	M,G	M-G,M-L,M-S,G-L,L-S	
Nhlang.	*O.mos.*	M,G,L,S	M,G,L,S	M,G,L	ALL
	C.gar.	M,G,L,S	M,G,L,S		M-G,M-L,S-G,S-L

fish from all localities. Skin and gill Zn content of both species was the lowest at Nhlanganini Dam (Table 7). Muscle tissue of *C. gariepinus* sampled at Nhlanganini Dam had the highest Zn level of all localities (significantly higher than Mamba Weir). Similar to the trend observed in Cu accumulation, *C. gariepinus* sampled at Nhlanganini Dam had the highest liver Zn content (significantly higher than Loskop Dam). *Oreochromis mossambicus* at Nhlanganini Dam had Zn levels in their livers slightly lower than those at Mamba Weir and slightly higher than those at Loskop Dam.

Discussion

When fish are exposed to elevated levels of metals in a polluted aquatic ecosystem like the Olifants River (Du Preez and Steyn, 1992; Seymore, 1994), they tend to take these metals up from their direct environment. It is assumed that most metals are taken up in the ionic form and that this uptake is influenced by various environmental factors such as pH and temperature. The metals enter the body of the fish via the gills and skin, or through the intake of contaminated food or drinking water. Transport of metals in the fish occurs through the blood where ions are usually bound to proteins. The metals are thus brought into contact with the organs and tissues of the fish and consequently accumulated to a different extent in different organs/tissues. All the metals taken up are not accumulated because fish have the ability to regulate their body metal concentration to a certain extent. Excretion of metals can occur through the gills, bile (via faeces), kidney and skin (Romanenko et al., 1986; Heath, 1987). The amount of a metal bioaccumulated is influenced by various environmental and biological factors, leading to differences in metal bioaccumulation between different individuals, species, seasons and sites.

Fish age and gender differences for Cu and Zn bioaccumulation

Although the lethal and sub-lethal effects of Cu and Zn are different for different age groups of aquatic biota (McFarlane and Franzin, 1978; Munkittrich and Dixon, 1988), accumulation of these metals by different age groups seems to be relatively stable (Latif et al., 1982). According to Pentreath (1976) the stable element analyses of liver, muscle and bone samples from fish of different mass and age show that the concentrations in adult fish remain fairly constant. This was also the case generally observed during this study. It should also be noted that fish sampled were generally adults and very few young specimens were included.

In fish, gender differences in Zn and Cu accumulation seem to occur only in certain organs (gonads) and during certain stages of its reproductive cycles (e.g. spawning). Zinc for instance is necessary for gonadal development in fish. Dietary Zn sources are, however, not adequate during this period and alternative internal resources such as the liver, skin, muscle and vertebrae are then utilised. Furthermore, Zn is also deposited in the gonads during their development and then lost during spawning. On the other hand, males do not require such a high Zn concentration, resulting in a more stable Zn concentration in the body (Seymore et al., 1996). These factors can result in different metal concentrations in different sexes. However, reproductive organs (gonads) were not included in this study and this could have been the cause for the usually insignificant (P>0.05) gender differences in Cu and Zn concentrations. Thus, the different capture ratios of males to females do not influence non-sexual comparisons.

Cu and Zn bioaccumulation in different organs and tissues

The phenomenon that different metals are accumulated at different concentrations in the various organs and tissues of fish was also observed in this study (Figs. 2 and 3). According to the literature, Cu and Zn mainly accumulate in the skin, bone, liver, gill, heart, kidney and muscle tissues of fish (Hughes and Flos, 1978; Bezuidenhout et al., 1990; Seymore, 1994; Seymore et al., 1996). The bioaccumulation pattern of Cu and Zn in different organs/tissues of both species of fish observed during this study agreed closely with the studies done by Van Hoof and Van San (1981), Latif et al. (1982), Stagg and Shuttleworth (1982), Du Preez et al. (1993) and Seymore (1994). The differences in the levels of accumulation in the different organs/tissues of a fish can primarily be attributed to the differences in the physiological role of each organ. Regulatory ability, behaviour and feeding habits are other factors that could influence the accumulation differences in the different organs. The metal concentration in the liver (not in direct contact with the metal in the water) which plays a

TABLE 7
SUMMARY OF SIGNIFICANT DIFFERENCES (P<0.05) BETWEEN VARIOUS LOCALITIES WITH RESPECT TO THE ZINC CONCENTRATION IN THE MUSCLE (M), GILLS (G), LIVER (L), SKIN (S) AND COMBINED ORGANS/TISSUES (C) FOR *O. MOSSAMBICUS (O.MOS)* AND *C. GARIEPINUS (C.GAR)*. BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCES (P>0.05) AND (-) INDICATES SIGNIFICANT DIFFERENCES (P<0.05) BETWEEN DIFFERENT TISSUES/ORGANS AT A SPECIFIC LOCALITY.

Locality	Spp.	Loskop Dam	Mamba Weir	Phalaborwa Barrage	Nhlanganini dam
Loskop	*O. mos.*	ALL			
	C. gar.	M-G,M-L,M-S,G-S,L-S			
Mamba	*O. mos.*	M,L,S	M-G,M-L,M-S		
	C. gar.	L	M-G,M-L,M-S,G-S,L-S		
Phal.	*O. mos.*	S		M-G,M-L,M-S,G-L,G-S	
Nhlang.	*O. mos.*	M,S		M,G	M-G,M-L,M-S
	C.gar.	G,L	M,G		M-S,M-G,M-L,S-L,G-L

major role in detoxification as well as storage, would therefore differ from the concentrations detected in the gills and skin tissue (in direct contact with the metals in the water) which play a role in the uptake and excretion of the metal.

The Cu and Zn contents in the muscle was usually the lowest of all the organs/tissues examined (Figs. 2 and 3). Although muscle tissue is capable of regulating its Cu and Zn content, it will accumulate these metals when chronic exposure to elevated levels occurs. Fish in an ecosystem contaminated by trace metals are known to accumulate significantly more metals in edible muscle tissue than do fish in an uncontaminated ecosystem (Murphy et al., 1978; Du Preez et al., 1993). Murphy et al. (1978) state that whole fish from relatively uncontaminated aquatic ecosystems should contain Zn concentrations in the range of 48 to 173 μg Zn·g^{-1} dry mass. Mathis and Cummings (1973), however, recorded maximum values of 40 μg Zn·g^{-1} in muscle tissue of fish from a contaminated river system. A mean muscle concentration of only 28 μg Zn·g^{-1} dry mass was detected in Nhlanganini Dam (control). Muscle tissue at Loskop Dam contained a mean level of 48 μg Zn·g^{-1} dry mass (Tables 2 and 3) which could thus be an indication of a Zn polluted system.

Zinc enters the body mainly via ingestion and absorption through the gills and the skin (Romanenko et al., 1986). Apart from this function, the skin also performs a role in the loss of metals from fish and will therefore give a good indication of the metal levels to which fish were exposed in their immediate environment (Varanasi and Markey, 1978; Bezuidenhout et al.,1990). The levels of Cu detected in the skin tissue during this study were usually slightly higher than that of the muscle tissue (Fig. 2) and corresponded with the levels observed by Seymore et al. (1994) for *Barbus marequensis* in the Olifants River.

Liver and gills are target organs for Cu toxicity in fish and there is usually a positive correlation between the Cu concentrations in the liver and gills and in the environment. The fact that fish are able to regulate metal uptake to a certain extent, complicates this correlation (Segner, 1986). The levels of Cu in the gills of fish were usually significantly higher (P<0.05) than those in muscle and skin tissue while Zn levels were generally only significantly higher than those in muscle tissue. These high Cu and Zn levels in the gill tissue can possibly be due to the fact that fish gills play a distinct role in metal uptake from the environment. The gills are in direct contact with the contaminated medium (water) and have the thinnest epithelium of all the organs. This is due to its respiratory function which causes it to be optimised for gaseous exchange with the environment (large surface area, short diffusion distances between the body and the water). Metal penetration through the epithelial cell is suggested to be through simple diffusion, facilitated diffusion, endocytosis of particulate fractions or via nutrient carriers (Pärt, 1987). Furthermore, the mucus layer on the gills also most probably plays a role in the loss of metals (Varanasi and Markey, 1978; Heath, 1987; Segner, 1986). Metals such as Cu and Zn are thus in continuous interaction with the gills of the fish and can therefore be valuable indicators of acute lethal exposure (Van Hoof and Van San, 1981).

In the present investigation, the liver tissue always contained significantly higher Cu levels than any of the other organs or tissues (Fig. 2). The Zn levels in the liver tissue were also significantly higher than that in the muscle tissue and usually similar to the Zn levels detected in the skin and gill tissues (Fig. 3), which were in accordance with the findings of Heath (1987) and Seymore (1994). The high levels of Cu and Zn generally detected in the livers of fish can be ascribed to the binding of Cu to metallothionein (MT), which serves as a detoxification mechanism. Cu is also part of the liver proteins hemocuprien and hepacuprien and several other oxidative enzymes (Hogstrand and Haux, 1991). Exposure of fish to sublethal levels of other metals such as Zn can also increase the hepatic levels of Cu by mechanisms yet unknown (Sanperra et al., 1983).

Species differences for Cu and Zn bioaccumulation

A different trend of bioaccumulation in different species of fish as stated by Murphy et al. (1978) and Vos and Hovens (1986) was prominent in the results of this study, and in particular for Cu bioaccumulation (Figs. 4 and 5). Species differences were also prominent in the accumulation of Cu and Zn in the different organs/tissues sampled, and the highest level of Zn was usually accumulated in the gills and skin of *O. mossambicus* and in the gills and liver of *C. gariepinus* (Fig. 3). A small species difference for Cu and Zn levels in muscle tissue was also evident, similar to the findings of Eustace (1974).

Species differences in Cu and Zn bioaccumulation could be ascribed to differences in feeding habits and behaviour of the two species. *Oreochromis mossambicus* feeds primarily on plant material and detritus (Deacon, 1988). Juveniles take many small crustaceans but mainly feed on algae, especially unicellular diatoms. Larger specimens feed on filamentous green algae and adults also ingest aquatic insects, crustaceans, earth worms, small fish as well as bottom sludge rich in organic matter. *Clarias gariepinus* on the other hand, is an omnivorous scavenger and predator of other fish and is mainly associated with the bottom (especially when muddy). *Daphnia pullex* and other planktonic organisms are important food items for younger specimens of *C. gariepinus* (Pienaar, 1978; Skelton, 1993). Copper and Zn are known to have an effect and also to be accumulated by aquatic biota such as fish and crustaceans (Memmert, 1987; Zou and Bu, 1994) and aquatic plants (Von Ayfer, 1977; Ward et al., 1986). Aquatic plants, algae and invertebrates generally seem to be more resistant to Cu than fish (Alabaster and Lloyd, 1980) and they all contribute a large amount to the diet of both species sampled. Large amounts of silt are also taken in with the food sources by both species, and especially by *C. gariepinus*. Silt particles play an important role in the transport and availability of metals as they are adsorbed onto the silt particles (Von Ayfer, 1977; Giesy and Wiener, 1977; Ward et al., 1986; Heath, 1987). Elevated metals were also detected in the sediment samples collected during this study (Kotze, 1997). Most metals are furthermore known to become more bioavailable and have increased toxicity with decreasing pH (Shaw and Brown, 1973). The pH in the stomach of *O. mossambicus* decreases significantly (from above 6 to as low as 2.9) after feeding commences (Deacon, 1988). This reduction in pH could thus result in metals becoming more bioavailable from the food sources and silt in the stomach. The metals could therefore be taken up via the intestine, causing increased metal levels in the fish which could result in bioaccumulation. Biomagnification of metals is thus also a possibility in this aquatic ecosystem but an investigation will have to be undertaken to evaluate the metal concentrations and their subsequent release in the different food sources of fish. The above-mentioned factors may well play a definite role in the differences of the metal levels accumulated by the two species.

Temporal differences in Cu and Zn bioaccumulation

The temporal variation of metal levels in fish tissue is usually ascribed to the variation in climatic conditions (e.g. rainfall) as well as fluctuation in pollutant input into a system. The temporal accumulation of Cu and Zn differed between Loskop Dam and Mamba Weir (Figs. 4 and 5). These two localities are distantly separated as Loskop Dam is situated in the upper catchment and Mamba Weir in the lower catchment of the Olifants River (Fig. 1). Their individual subcatchments vary in pollutant inputs via point and non-point sources, as well as flow regimes to a lesser extent. Temporal accumulation trends at these localities are therefore discussed separately.

In general, the temporal trends of Cu and Zn bioaccumulation in both species did not correlate with the temporal trends witnessed for the total Cu and Zn concentrations in the water. This is possibly due to the fact that the metal concentrations detected in fish tissues are due to the exposure of fish to concentrations of metals previous to the time of sampling. Levels of metal bioaccumulation in fish thus give an indication of exposure of fish to metal levels in their immediate environment over time, while water samples are merely a snapshot of a condition at the time of sampling.

Loskop Dam

The Cu levels in *O. mossambicus* tended to decrease with reduced flows (February 1994 to November 1994) and to increase with increased flows (November 1994 to February 1995). This observation opposes most trends often described in the literature for the relation between flow and metal concentrations in fish (Seymore, 1994). This is because metal concentrations in the water tend to be diluted by higher flows during the rainy seasons. Increased Cu input into the system by point or diffuse sources during the times of higher flows could explain the higher levels of Cu detected in the water during these periods (Fig. 4). An increase in the Zn concentrations in the water during February 1995 caused a great increase to occur in the sediment Zn concentration. During this period an increase in Zn accumulation was also detected in the fish, possibly due to this increased Zn exposure. As the Zn precipitated out and was adsorbed onto the sediment particles, it caused very high levels of Zn to be detected during the next survey (May 1995).

Mamba Weir

The higher Cu levels detected in the fish at this locality during periods of higher flow could possibly be ascribed to the following phenomenon: The Olifants River and Mamba Weir especially, is known to be affected by siltation during high flow periods (Buermann et al., 1995). As previously mentioned, both Cu and Zn tend to be adsorbed onto sediment particles, thus reducing the levels of these metals in the water. Increased turbidity is, however, caused by higher flows and also by the flushing of the Phalaborwa Barrage. This results in fish being brought into contact with the silt in the water. Gills are known to be clogged by these fine sediment particles and the situation can even lead to the suffocation of the fish (Buermann et al., 1995). This stressful situation will also cause an increase in respiration and other metabolic processes, increasing uptake of pollutants from the water and thus causing increased metal levels in the fish during higher flows. Increased water temperature and flows during this period could also have caused increased metabolic rates with resultant elevated bioaccumulation of metals. The gradual increase of Zn in *C. gariepinus* over the study period could be an indication of chronic exposure to elevated Zn concentrations at this locality. This also coincides with the Zn levels detected in the water and sediment (Fig. 5), indicating continued and progressive Zn pollution at this site.

Spatial differences in Cu and Zn bioaccumulation

Because levels of metal bioaccumulation in fish give an indication of the extent of chronic exposure of fish to metals, it can be used effectively to evaluate the health of an aquatic ecosystem by identifying potentially impacted areas. Copper and Zn levels detected in the fish during this study also indicated different impacts of these metals in the different areas investigated. Gills, skin and liver tissue of both fish species sampled at Mamba Weir contained higher concentrations of Cu than fish sampled at Loskop Dam (Fig. 2). Copper levels in the water and sediment were also higher at Mamba Weir than Loskop Dam. These results thus indicate Mamba Weir to be more Cu polluted than Loskop Dam. The elevated levels of Cu in the biotic and abiotic components of Mamba Weir could be a result of the mining activities in the Phalaborwa area upstream of this locality. *Oreochromis mossambicus* sampled at Phalaborwa Barrage had accumulated significantly lower levels of Cu than at Mamba Weir, which is approximately 20 km downstream. This difference could possibly be ascribed to the influence of the Selati

River, a highly polluted tributary of the Olifants River, which converges with the Olifants River before it enters the KNP at Mamba Weir. The Selati River drains the mining areas of Phalaborwa and receives effluent from these mines and is the main contributor to the degradation of the Olifants River water quality in this section of the river. This negative impact of the Selati River on the Olifants River was also detected by Seymore et al. (1994).

The control site (Nhlanganini Dam) should have indicated metal levels in a natural and "unpolluted' system. This was, however, not always the case. Copper levels detected in the water and sediment were relatively high when compared to the other localities. It has to be stressed that these findings were based on the data of a single water and sediment sample. The higher Cu levels at Nhlanganini Dam could, however, be due to natural causes such as geological impacts and polluted groundwater. Furthermore, the area of this locality is known to be impacted by air pollution from the mining industry in the Phalaborwa area and high levels of Cu depositioning have been recorded in the area (Grobler, 1996). There were, however, some definite differences between Nhlanganini Dam and the rest of the sites sampled. Muscle, gill and skin tissues of both species sampled at Nhlanganini Dam had significantly lower levels of Cu than at Loskop Dam and Mamba Weir, but Cu and Zn levels in the liver tissue of fish sampled at Nhlanganini Dam were usually significantly higher ($P<0.05$). This could possibly indicate that fish at Loskop Dam and Mamba Weir have been chronically exposed to these metals and thus have had to regulate their levels to a much greater extent than fish at Nhlanganini Dam. When fish are exposed to elevated metals they will strive to detoxify their bodies of the pollutant as effectively as possible. It is possible that they will over-compensate and thus decrease the metal concentration in their liver (detoxifying organ) to a level, lower than those usually detected under unpolluted conditions. The liver will therefore contain higher metal levels during normal conditions (liver acts as a storage organ for metals through the binding with metallothioniens) than under polluted conditions (liver acts as detoxifying organ in an attempt to decrease body metal concentration). This could have been the cause of fish at Nhlanganini Dam containing significantly higher ($P<0.05$) Cu and Zn levels in their livers than fish at Loskop Dam and Mamba Weir.

Similar to Cu, Zn tended to be adsorbed onto suspended particles in the water and thus precipitated out at the bottom (Skidmore, 1964). Changes in water quality, like decreasing pH, causes this bound Zn to become bioavailable again. The levels of Zn to which fish sampled in this study have been exposed are much higher than the target water quality range value of 2 g Zn·ℓ^{-1} for South African aquatic ecosystems (DWAF, 1996). The fact that Zn is less toxic to fish in hard water (e.g. the Olifants River) could have reduced the impact as the amount of bioavailable Zn could have been decreased (Mattheissen and Brafield, 1975; Alabaster and Lloyd, 1980). Muscle and skin Zn levels detected at Loskop Dam were usually significantly higher ($P<0.05$) than at Mamba Weir, but gills and liver tissues indicated the opposite trend (Fig. 3). The levels of Zn in the water and sediment at Mamba Weir were higher than those at Loskop Dam, thus corresponding in this case with observed trends for liver and gill Zn concentrations. Muscle and gill tissues of fish sampled at Phalaborwa Barrage contained higher levels of Zn than fish at Loskop Dam (Fig. 3). A study by Grobler et al. (1994) also indicated similar results with *C. gariepinus* at Phalaborwa Barrage, accumulating higher levels than the same species in Loskop Dam. Muscle, gills and skin tissues usually indicated that Nhlanganini Dam was the least Zn polluted and this also agreed with the levels of Zn detected in the water and sediment (being generally lower than the other localities).

From these results it is thus evident that both Loskop Dam and Mamba Weir, but the latter to a greater extend, are being impacted by elevated levels of Cu and Zn. When compared to the levels of Cu and Zn detected in *C. gariepinus*, sampled in an industrial and mine-polluted lake (Gauteng) by Bezuidenhout et al. (1990), all localities surveyed seemed less impacted. Only liver tissue contained higher levels of Cu and Zn than the levels detected by Bezuidenhout et al. (1990) for the polluted Germiston Lake. Again this could indicate that fish in highly polluted systems decrease their liver metal concentrations to a greater extent than fish in a less polluted system, through regulation of their metal concentrations. Seymore (1994) also investigated the extent of metal pollution in the Olifants River at Mamba Weir in *Barbus marequensis*. Copper levels detected during this study, in all tissues except muscle, were usually lower than the levels detected by Seymore (1994). Zinc levels were, however, usually higher.

Conclusion

This investigation of the bioaccumulation of Cu and Zn gave a good indication of the present state of metal contamination of fish in the upper and lower Olifants River. Bioaccumulation differences for Cu and Zn were usually not significant between different age groups or between males and females. Species differences in Cu and Zn accumulation occurred and were primarily ascribed to the differences in the feeding habits and behaviour of the two species sampled. The pattern for Cu accumulation in the different organs/tissues was liver > gills > skin > muscle and this generally agrees with the literature. No definite trend for Zn bioaccumulation in the different organs/tissues was observed. Liver, gill and skin tissues all accumulated high levels of Zn. Generally, muscle, gill and skin tissues indicated similar trends of accumulation but differed from liver tissue. Both Loskop Dam and Mamba Weir were found to be impacted by sources of Cu and Zn pollution. The situation at Mamba Weir was ascribed to the impact of the highly polluted Selati River. This influence could be highly negative to the aquatic biota and affect the biotic integrity of the Olifants River and thus needs to be urgently addressed. Mamba Weir and riverine sites upstream of Loskop Dam should be monitored more regularly and by means of an integrated approach, including the investigation of fish, macro-invertebrates and aquatic and riparian habitat. This level of monitoring could produce sufficient information to implement better management strategies which would reduce the present negative impacts.

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References

ALABASTER JS and LLOYD R (ed.) (1980) *Water Quality Criteria for Freshwater Fish.* FAO and Butterworths, London. 297 pp.

BEZUIDENHOUT LM, SCHOONBEE HJ and DE WET LPD (1990) Heavy metal content in organs of the African sharptooth catfish, *Clarias gariepinus* (Burchell), from a Transvaal lake affected by mine and industrial effluents. Part 1. Zinc and copper. *Water SA* **16** (2) 125-129.

BUERMANN Y, DU PREEZ HH, STEYN GJ, HARMSE JT and DEACON A (1995) Suspended silt concentrations in the lower Olifants River (Mpumalanga) and the impact of silt releases from the Phalaborwa Barrage on the water quality and fish survival. *Koedoe* **38**(2) 11-34.

DEACON AR (1988) Die voedingsekologie en voedingsfisiologie van die kurpers, *Tilapia rendalli* (Bloulenger) en *Oreochromis mossambicus* (Peters) in 'n warm, rioolverrykte habitat. Ph.D. Thesis, RAU, Johannesburg, South Africa. 193 pp.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF) (1996) *South African Water Quality Guidelines, Volume 7: Aquatic Ecosystems*.

DU PREEZ HH and STEYN GJ (1992) A preliminary investigation of the concentration of selected metals in the tissues and organs of the tigerfish (*Hydrocynus vittatus)* from the Olifants River, Kruger National Park, South Africa. *Water SA* **18** (2) 131-136.

DU PREEZ HH, VAN RENSBURG E and VAN VUREN JHJ (1993) Preliminary laboratory investigation of the bioaccumulation of Zn and Fe in selected tissues of the Banded Tilapia, *Tilapia sparmanii* (Chichlidae). *Bull. Environ. Contam. Toxicol*. **50** 676-681.

EUSTACE IJ (1974) Zinc, cadmium, Cu and manganese in species of finfish and shellfish caught in the Derwent Estuary, Tasmania. *Aust. J. Mar. Freshwat. Res***.** **25** 209-220.

GIESY JP and WIENER JG (1977) Frequency distributions of trace metal concentrations in five freshwater fishes. *Trans. Am. Fish. Soc*. **106** 393-403.

GROBLER D (1996) Personal communication. Veterinarian, Skukuza, Kruger National Park .

GROBLER DF, KEMPSTER PL and VAN DER MERWE L (1994) A note on the occurrence of metals in the Olifants River, Eastern Transvaal, South Africa. *Water SA* **20** (3) 195-204.

HEATH AG (ed.) (1987*) Water Pollution and Fish Physiology.* CRC Press, Inc., Florida, USA. 245 pp.

HEIT M and KLUSEK CS (1982) The effects of dissecting tools on the trace element concentrations of fish and mussel tissues. *Sci. Total Environ.* **24** 129-134.

HELLAWELL JM (ed.) (1986*) Biological Indicators of Freshwater Pollution and Environmental Management.* Elsevier Applied Science Publishers Ltd., London and New York. 546 pp.

HOGSTRAND C and HAUX C (1991) Mini-review: Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionien. *Comp. Biochem. Physiol.***100C** (½) 383-390.

HUGHES GM and FLOS R (1978) Zinc content of the gills of rainbow trout (*S. gairdneri*) after treatment with zinc solutions under normoxic and hypoxic conditions*. J. Fish Biol.* **13** 717-728.

KOTZE PJ (1997) Aspects of Water Quality, Metal Contamination of Sediment and Fish in the Olifants River, Mpumalanga. M.Sc. Thesis, Rand Afrikaans University, South Africa.

LATIF A, KHALAF AN and KHALID BY (1982) Bioaccumulation of Cu, Cd, Pb, and Zn in two cyprinid fishes of Iraq. *J. Biol. Sci*. **13** (2) 45-64.

MATHEISSEN P and BRAFIELD AE (1975) Uptake and loss of dissolved zinc by the stickleback, *Gasterosteus aculeatus* L. *J. Fish Biol*. **10** 399-414.

MATHIS BJ and CUMMINGS TF (1973) Selected metals in sediment, water and biota in the Illinois River. *J. Water Pollut. Cont. Fed*. **45** 1573-1583.

McFARLANE GA and FRANZIN WG (1978) Elevated heavy metals: A stress on a population of white Suckers, *Catastomus commersoni*, in Hamell Lake, Saskatchewan. *J. Fish. Res. Board Can*. **35** 963-970.

MEMMERT U (1987) Bioaccumulation of zinc in two freshwater organisms (*Daphnia magna*, Crustacea and *Brachydanio rerio*, Pisces). *Water Res.* **21**(1) 99-106.

MUNKITTRICH KR and DIXON DG (1988) Growth, fecundity, and energy status of White Sucker (*Catostomus commersoni*) from lakes containing elevated levels of Cu and Zn. *Can. J. Fish. Aquat. Sci.* **45** 1355-1365.

MURPHY BR, ATCCHISON GJ and McINTOSH AW (1978) Cd and Zn in muscle of Bluegill (*Lepomis macrochirus*) and Largemouth bass (*Micropterus salmoides*) from an industrially contaminated lake*. Environ. Pollut*. **17** 253-257.

PÄRT P (1987) Testing the bioavailibility of metals in natural waters. In: *Lectures Notes in Earth Sciences*. Springer-Verlag, Germany.

PENTREATH RJ (1976) Some further studies on the accumulation and retention of ^{65}Zn and ^{54}Mn by the Plaice, *Pleuronectes platessa* L. *J. Exp. Mar. Biol. Ecol*. **21** 179-189.

PIENAAR U de V (1978) *The Freshwater Fishes of the Kruger National Park.* Sigma Press Ltd., Pretoria. 91 pp.

ROMANENKO VD, MALYZHEVA TD and YU YEVTUSHENKO N (1986) The role of various organs in regulating zinc metabolism in fish. *Hydrobiol. J.* **21** (3) 7-12.

SANPERRA C, VALLRIBERA M and CRESPO S (1983) Zn, Cu and Mn levels in the liver of the Dogfish exposed to Zn. *Bull. Environ. Contam. Toxicol*. **31** 415-417.

SEGNER H (1986) Response of fed and starved roach, *Rutilus rutilus*, to sublethal Cu contamination. *J. Fish Biol.* **30** 423-437.

SEYMORE T (1994) Bioaccumulation of Metals in *Barbus marequensis* from the Olifants River, Kruger National Park and Lethal Levels of Manganese to Juvenile *Oreochromis mossambicus*. M.Sc. Thesis, Rand Afrikaans University, South Africa.

SEYMORE T, DU PREEZ HH, VAN VUREN JHJ, DEACON A and STRYDOM G (1994) Variation in selected water quality variables and the metal concentrations in the sediment of the lower Olifants and Selati Rivers, South Africa*. Koedoe* **37**(2) 1-18.

SEYMORE T, DU PREEZ HH and VAN VUREN JHJ (1996) Concentrations of zinc in *Barbus marequensis* from the lower Olifants River, Mpumalanga, South Africa. *Hydrobiol.* **332** 141-150.

SHAW TL and BROWN VM (1973) The toxicity of some forms of Cu to rainbow trout. *Water Res.* **8** 377-382.

SKELTON P (1993) *'n Volledige Gids tot die Varswatervisse van Suider-Afrika*. Southern Book Publishers (Pty) Ltd., Pta., South Africa. 387 pp.

SKIDMORE JF (1964) Toxicity of Zn compounds to aquatic animals, with special reference to fish. *The Quarterly Rev. of Biol.* **39** (3) 227-248.

STAGG RM and SHUTTLEWORTH TJ (1982) The accumulation of Cu in *Platichthys flesus* L. and its effect on plasma electrolyte concentrations*. J. Fish. Biol.* **20** 491-500.

VAN HOOF F and VAN SAN M (1981) Analysis of Cu, zinc, cadmium and chromium in fish tissues. A tool for detecting metal caused fish kills*. Chemosphere* **10** (10) 1127-1135.

VAN LOON TC (ed.) (1980) *Analytical Atomic Absorption Spectroscopy. Selected Methods.* Academic Press, New York. 337.

VARANASI U and MARKEY YD (1978) Uptake and release of lead and cadmium in skin and mucus of coho salmon (*Oncorhynchus kisutch*). *Comp. Biochem. Physiol.* **60C** 187-191.

VENTER FJ and DEACON AR (1992) Management Objectives of the National Parks Board Regarding the Water Quality Requirements of Rivers in the Kruger National Park. Report No. 2/92, Scientific Services Section, Skukuza. 13 pp.

VILLARREAL-TREVIÑO CM, OBREGON-MORALES ME, LOZANO-MORALES JF and VILLEGAS-NAVARRO A (1986) Bioaccumulation of lead, copper and zinc by fish in a transect of the Santa Catarina River in Cadereyta Jimenez, Nuevo Leon, Mexico. *Bull. Environ. Contam. Toxicol.* **37** 395-401.

VON AYFER A (1977) Die Akkumulation von Zink in Pflanzen, Sediment und Fischen*. Z F Wasser- und Abwasser- Forschung*. **11**(1/78) 11-13.

VOS G and HOVENS JPC (1986) Chromium, Ni, Cu, Zn, As, Se, Cd, Hg and Pb in Dutch fishery products 1977-1984*. The Sci. of the Total Environ.* **52** 25-40.

WARD TJ, CORRELL RL and ANDERSON RB (1986) Distribution of Cd, Pb and Zn amongst the marine sediments, seagrasses and fauna, and the selection of sentinel accumulators, near a Pb smelter in South Australia. *Aust. J. Mar. Freshwater Res*. **37** 567-585.

ZOU E and BU S (1994) Acute toxicity of Cu, cadmium, and zinc to the water flea, *Moinairrasa* (Cladocera). *Bull. Environ. Contam. Toxicol.* **52** 742-748.