A further investigation into the bioaccumulation of lead and zinc in the organs and tissues of the African sharptooth catfish, *Clarias gariepinus* from two localities in the Olifants River, Kruger National Park

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The bioaccumulation of lead and zinc in the gills, liver, muscle and skin of *Clarias gariepinus* from two locations, Mamba and Balule, on the Olifants River within the Kruger National Park is reported here. Over a one year period (1994), four surveys (February, May, July and November) were undertaken. Atomic absorption spectrophotometry was used to determine the concentrations of both metals in the tissues. The gills were the major route of uptake, with the liver accumulating high lead and zinc concentrations. The concentration of metals in the fish found at Mamba and Balule were found to be significantly different from each other. However, it could not be established at which location the greatest amount of bioaccumulation had taken place. The influences of temperature, alkalinity, salinity and pH on metal toxicity, bioavailability and bioaccumulation rates are discussed in detail. It is imperative that pollution levels in the Olifants River and its effect on fish is continually monitored and captured, so as to maintain and conserve this river and the biota dependant on it.

Key words: bioaccumulation, *Clarias gariepinus*, Olifants River, lead, zinc.

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Introduction

The presence of pollutants in the environment of the air, land and water is reflected in the accumulation in and effects on aquatic organisms. This is because the aquatic section of the biosphere is often the sink for human generated pollutants (Couch 1982). Biological communities are accurate indicators of overall environmental conditions as they are affected by chemical and physical influences and integrate these effects over time (Lawrence & Williams 1991). Discharges of heavy metals into aquatic ecosystems may result in numerous physical, chemical and biological responses, which are separated into two categories: (1) the effect of the environment on the metal, (2) the effect of the metal on the environment. The first category stresses the importance of conditions in the water that may lead to a change in the speciation and toxicity of metals. In the second category, the biological responses depending on environmental conditions include a change in density, diversity, community structure and species composition of populations. The type and extent of the change depends primarily on the heavy metal concentration in the water and sediment. This, in turn, is governed by the physiochemical processes in the polluted water, thereby having an indirect effect on biological responses (Moore & Ramamoorthy 1984).

The primary source of water for the Kruger National Park (KNP) is the Olifants River. As one of the many conservation areas in the Olifants River basin and the fact that the KNP represents a highly unique conservation area preserving all natural ecosystems (Moore 1990), it is imperative that the effects of all anthropogenic activities on the water quality and aquatic biota of this river be

determined. This must be done to secure the ecosystem and sustain species diversity for future generations. Within the KNP, the major threat to the quality of water in the Olifants River is as a result of point sources of industrial activity, municipal sewage treatment plants, smelting activities and, in particular, mining at Phalaborwa which may increase metal levels in the water, such as lead, zinc, copper, manganese, chromium, nickel and iron, seriously affecting fish.

The metals selected for this study included lead (Pb) and zinc (Zn). From previous studies done (Du Preez & Steyn 1992; Wepener *et al.* 1992; Seymore *et al.* 1995; Du Preez *et al.* 1997) on the Olifants River, it has been shown that these metals are present at relatively high concentrations, generally higher than the guideline values stipulate for the protection of aquatic life. A number of studies, which are listed in Table 1, have been done on the water quality and biota affected by pollution in the Olifants River, Kruger National Park. However, not much work has been done on the bioaccumulation and the affect of metals on *Clarias gariepinus* in this region.

The present study was undertaken to acquire information about lead and zinc content in the organs and tissues of natural populations of *Clarias gariepinus* from two different study sites on the Olifants River in the Kruger National Park and to establish a history of succession of lead and zinc bioaccumulation in fish from this region. In addition, the effect of physical, chemical and biological influences on the metal concentration levels were studied. Locality and seasonal differences of metal accumulation were investigated.

In order to reveal the history of pollution and the pattern of heavy metal accumulation, samples from the gills, liver, muscle and skin were separately analysed. These organs and tissues were chosen for the following reasons:

- they are all known to accumulate metals;
- the gills and skin are constantly exposed to the metals in the water and the accumulation and effect that metals have on the gill and skin tissue is of interest;
- the liver is known as a storage and detoxification organ (Klaassen 1976) and the amount of metal accumulated in it might reflect the severity of the pollution; and
- muscle is the tissue generally consumed by humans and the metal content is important for the presumed effect on human health.

Lead

The concentration of soluble lead in uncontaminated freshwater is normally 3 µg/l (Förstner 1979). The main dissolved inorganic forms of lead are the free ion Pb^{2+}, hydroxide complexes and probably the carbonate and sulphate ion pairs (Hem 1989). The absorption of lead by biota, from environmental and anthropogenic sources, is not only dependent on the amount of lead presented to the passage of entry per unit time, but on the physical and chemical state in which the metal is present. In addition, it is also influenced by host factors such as physiological status. On entering the bloodstream, lead binds to blood cells and plasma proteins, with 90 % of the lead in blood being bound to erythrocytes, primarily to the haemoglobin within the cell (Ewers & Schlipköter 1991b). From here it is distributed to various organs and tissues according to the relative affinity of each tissue for lead (Ewers & Schlipköter 1991a).

Evidence on the effects of chronic toxicity of lead on fish has been extensively discussed by Davies *et al.* (1976), and Holcombe *et al.* (1976). The following suggested aberrations resulting from lead toxicity were revealed:

- the "blacktail" phenomenon (the caudal region posterior to the dorsal fin was blackened), also observed in experimentation done by Sippel *et al.* (1983), generally followed by;

Table 1
Summary of information and studies done on pollution in the Olifants River, Kruger National Park

Reference	Subject
Moore 1990	Water quality requirements of the biota of the Kruger National Park Rivers
Van Veelen 1990	Preliminary water quality guidelines and assessment of the current water quality status
Van Veelen 1991	Water quality assessment: the concept of fitness for use
Moore *et al.* 1991	Preliminary water quality guidelines for the Kruger National Park Rivers
Du Preez & Steyn 1992	Bioaccumulation of Fe, Zn, Pb, Ni, Cu, Cd and Mn in the organs and tissues of tiger fish (*Hydrocynus vittatus*)
Wepener *et al.* 1992	The development of an aquatic toxicity index as a tool in the operational management of water quality in the Olifants River Kruger National Park
Avenant-Oldewage 1994	Parasites as indicators of heavy metal pollution
Buermann 1994	Silt concentrations in the Olifants River (Dissertation)
Seymore *et al.* 1994	Water quality variables and metal concentrations in the sediment of the lower Olifants and Selati rivers
Seymore 1994	Bioaccumulation of 6 metals in *Barbus marequensis* (Dissertation)
Van Vuren *et al.* 1994	Effect of pollutants on the physiology of fish in the Olifants River
Seymore *et al.* 1995	Bioaccumulation of Mn, Pb and Sr in the tissues of the yellow fish (*Barbus marequensis*)
Avenant-Oldewage *et al.* 1995	Development of a fish health and condition procedure
Marx 1996	Health Assessment Index and bioaccumulation of Cr, Cu, Fe, Mn, Ni, Pb, Sr, Zn in the sharptooth catfish (*Clarias gariepinus*) (Dissertation)
Robinson 1996	Health Assessment Index and bioaccumulation of Cr, Cu, Fe, Mn, Ni, Pb, Sr, Zn in *Oreochromis mossambicus* (Dissertation)
Du Preez *et al.* 1997	Bioaccumulation of Cr, Cu, Mn, Ni, Pb and Zn in the African sharptooth catfish (*Clarias gariepinus*)
Luus-Powell 1997	Health Assessment Index and bioaccumulation of Cr, Cu, Fe, Mn, Ni, Pb, Sr, Zn in *Labeo rosae* (Dissertation).

- lordoscoliosis (spinal curvatures) which, in turn, prevents successful reproduction;
- paralysis;
- muscular atrophy; and
- degeneration of the caudal fin.

From the abnormalities observed, Davies *et al.* (1976), have concluded that these abnormalities are highly suggestive of direct neurological damage or biochemical inhibition in the metabolism of fish chronically exposed to lead.

Zinc

The normal levels of zinc found in unpolluted water sources range between 0.5 µg/l and 15 µg/l (Moore & Ramamoorthy 1984). The zinc oxidation state Zn^{2+}, has a strong tendency to react with acidic, alkaline and inor-

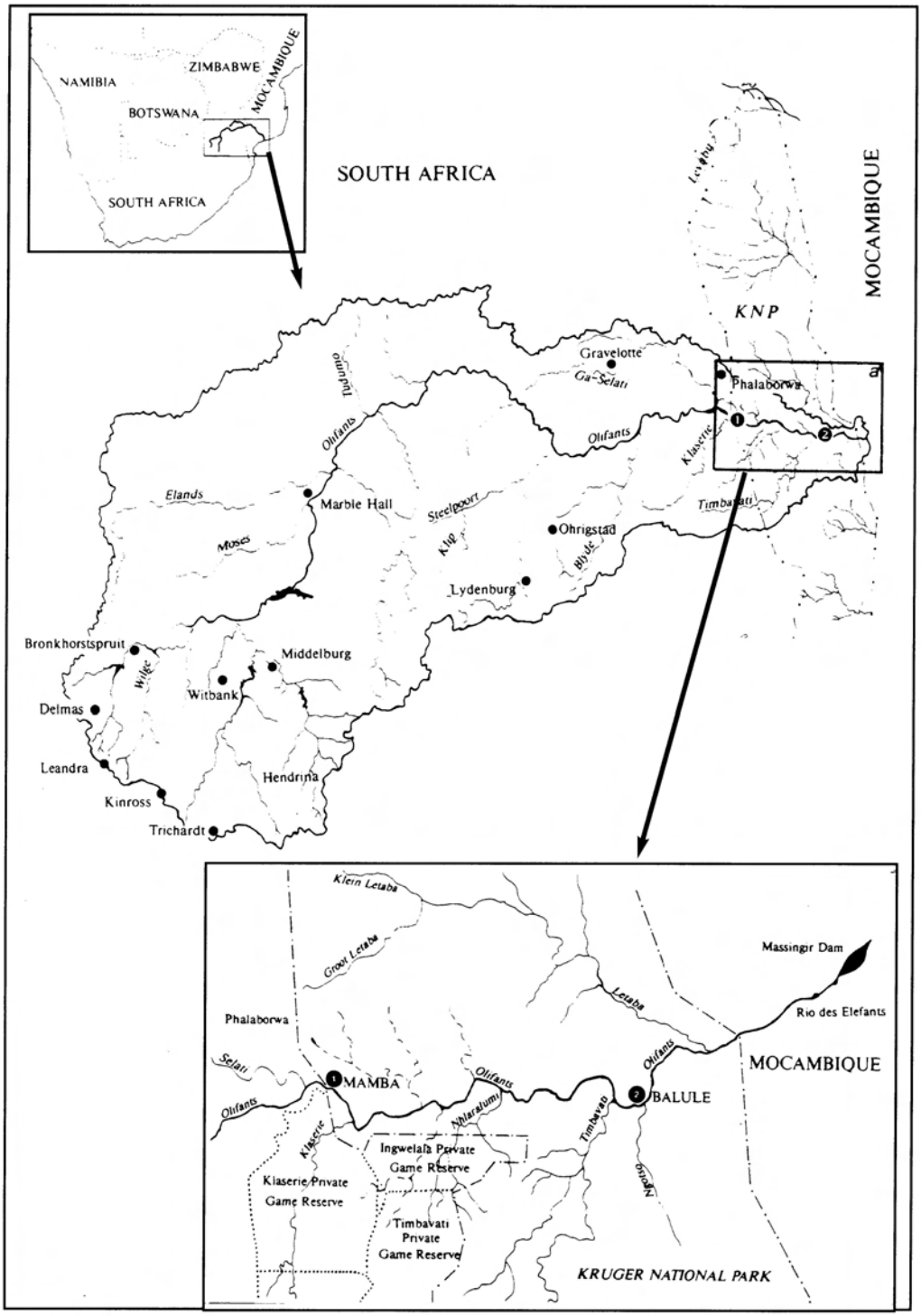

Fig. 1. Map indicating the position of the study area in the lower Olifants River catchment.

ganic compounds (Ohnesorge & Wilhelm 1991). The solubility of zinc (Zn^{2+}), is essentially controlled by the solubility of zinc carbonate, which is a function of the concentration of carbonate ion and is dependent upon the pH value and the concentration of bicarbonate ion in the solution (Solbé 1974). The stability of the zinc-organic complexes is enhanced by the presence of nitrogen and sulphur donor atoms in the ligand. The presence of organic and inorganic chelators in solution may significantly reduce absorption by fish (Moore & Ramamoorthy 1984). According to these authors, zinc's behaviour is inconsistent when binding to particulates as this is dependent on the physico-chemical characteristics of the aquatic environment.

Zinc is an element that is essential for the correct functioning of the body and is a metal which is homeostatically controlled in fish (Giesy & Weiner 1977). Many zinc metalloenzymes, as well as their biological role in zinc metabolism, have been identified (Ohnesorge & Wilhelm 1991). Zinc is an important factor in the healing process of body tissues and is required for physiological processes, such as hormone metabolism, immune response and stabilisation of membranes (Moore & Ramamoorthy 1984). In high concentrations zinc is toxic to fish.

The exposure of fish to high levels of zinc leads to a number of physical and biochemical changes in the fish, which could be detrimental (Hodson & Sprague 1975; Moore & Ramamoorthy 1984). These departures from normality are:

- gill damage –
  i) separation of epithelium, ii) enlargement of central and marginal channels, iii) occlusion of central blood spaces, iv) resulting in decreased oxygen consumption, inability to transport ions across the gill surface and an increase in hypoxia, ventilation frequency and coughing frequency (Matthiessen & Brafield 1977; Hodson & Sprague 1975; Lloyd 1992),
- increase in lactic and pyruvic acid, decreasing blood pH,
- non-functioning of kidney tissue and enzymes,
- decrease in growth, size and fecundity (Pierson 1981), and
- alterations in reproductive behaviour and inhibition of production (Hodson & Sprague 1975).

Study area

Two locations in the lower catchment area of the Olifants River were selected for the purpose of this study (Fig. 1). The first location, Mamba (Fig. 1), is situated on the western boundary of the Kruger National Park (KNP), in close proximity to the mining town of Phalaborwa. The second location Balule (Fig. 1), is situated approximately 40 km downstream, in the interior of the park.

Materials and Methods

Field procedures

Four surveys were conducted at Mamba and Balule in the Olifants River, Kruger National Park, during 1994 (February, May, July and November). Water and sediment were analysed for metals and physical and chemical water parameters (Table 2). A maximum of twenty fish of the species *Clarias gariepinus* (Burchell, 1822) (Sharptooth catfish) were collected separately at Mamba and Balule during each survey. They were caught with hand lines and gill nets with mesh sizes ranging between 70-120 mm. The fish (individually placed on to polypropylene dissection boards) were immobilised and killed by severing the spinal cord behind the head and dissected. Samples from the gills, liver, muscle and skin were dissected out and placed separately in 25 ml glass bottles and kept in a portable Coleman refrigerator to be frozen later that day.

Laboratory procedures

The frozen tissue samples were first thawed and placed in Erlyn Myer flasks and weighed (subtracting the weight of the flask) using a Mettler PK 4800 scale. Approximately five grams of wet tissue were weighed out which corresponds to approximately one gram of dried tissue. The gill filaments were removed from the gill arches and only the gill filaments were weighed. The weighed samples were

Table 2
Water quality variables recorded in 1994 in the Olifants River,Kkruger National Park at Mamba and Balule

Physical and chemical water variables	[a]Survey 1 (Feb, 1994)		[a]Survey 2 (May, 1994)		[a]Survey 3 (July, 1994)		[a]Survey 4 (Nov, 1994)	
	Mamba	Balule	Mamba	Balule	Mamba	Balule	Mamba	Balule
Temperature (°C)	23	24.2	20	24.8	16.9	19.2	25	27.8
pH	7.88	6.86	8.32	8.42	9.01	8.26	8.91	8.73
Conductivity (µS/m)	690	580	960	933	1980	1745	1786	1327
Oxygen (mg/l)	7.9	8.5	8.8	9.6	12.3	8.9	N/A	N/A
Oxygen saturation (%)	94	100	97	118	128	100	N/A	N/A
Turbidity (secchi disk, cm)	13	25	26	23	19	48	28	25
[a]Alkalinity (mg/l) $CaCO_3$	147	309	N/A	N/A	N/A	N/A	44	166
[a]Sulphates (mg/l) SO_4	34	22	149	172	723	572	582	536
[a]Ammonium (mg/l)NH_4	0.10	0.19	0.04	0.03	0.03	0.05	0.04	0.04
[a]Nitrates (mg/l) (NO_3^-+NO_2^-)	0.08	0.04	0.48	0.03	0.12	0.03	0.04	0.31
[a]TDS	315	789	560	585	1644	1401	1069	848
[a]EC (mS/m)	42.6	98.6	76.60	70.8	204	187	174	114.5
[a]Chloride (mg/l) Cl	21	15	52	52	170	145	120	79
[a]Calcium (mg/l) Ca	26	26	38	42	107	81	33	44
Lead (Pb) conc. (mg/l) in water	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
Lead conc. (mg/g)in sediment	0.018	0.012	0.020	0.020	0.020	0.020	0.020	0.020
Zinc (Zn) conc. (mg/l) in water	0.036	0.003	0.003	0.003	0.003	0.003	0.048	0.039
Zinc conc. (mg/g) in sediment	0.079	0.072	0.348	0.063	0.067	0.280	0.066	0.069

TDS = Total dissolved solids; EC = Electrical conductivity;
[a] Analysed water quality data as supplied by the Institute for Water Quality Studies
N/A = Not available

dehydrated at 60 °C in a Haraeus Hanau KB 500 oven for 48 hours. Samples were digested according to methods described by Van Loon (1980) using a mixture of 10 ml, 55 % nitric acid (NHO_3), and 5 ml, 70 % perchloric acid ($HClO_4$) which was then added to the one gram of dried tissue in the Erlyn Myer flasks. The samples were subsequently placed on a hot plate at ±200 °C and allowed to digest until transparent and clear. Samples were removed to cool down and subsequently diluted to 50 ml with doubly distilled water. These samples were then filtered individually through a 6 µm Millipore acid-resistant membrane filter attached to a vacuum pump. The samples were then carefully poured into pre-sterilised, acid-washed glass bottles rinsed in doubly distilled water (Giesy & Wiener 1977).

The total metal concentrations of lead (Pb) and zinc (Zn) were determined with the aid of a Varian Atomic Absorption Spectrophotometer (SPECTRA AA-10). Calibration was ensured with the preparation of four to five analytical standards using Holpro stock solutions. A 2:1 nitric and perchloric acid solution was prepared and its absorbency read to determine if the acid solution was contaminated with metals. From this metal concentrations from the samples could be corrected to reflect accurate readings. Individual concentrations of the tissue samples were then read against particular absorbencies defined for each metal. Metal concentrations of all tissues were calculated as follows:

$$MC = AAS/M \times V - AM, \quad \text{where}$$

MC = Metal concentration
AAS = AAS reading (µg/ml)
M = Sample mass (g) dry wt.
V = Sample volume (50 ml)
AM = Acid metal concentration

The bioconcentration factor between the mean metal concentration found in the fish tissues and organs and the metal concentrations found in the sediment were determined. The calculation used to determine the bioconcentration factor was as proposed by Veith *et al.* (1979) and Wiener & Giesy (1979).

Metal data was statistically analysed with the aid of Statgraphics 7 computer software and Microsoft Excel statistical software (*t*-test). The variables determined included the mean, standard deviation, standard error, coefficient of variation, variance, the

minimum and maximum values and probability.

Results and discussion

A table (Table 2) documenting the information gathered, during this study, on the physical and chemical water parameters, as well as water and sediment metal concentrations is included. The results obtained for the water and sediment study done at Mamba and Balule, showed that the water quality at Mamba was poorer than the water quality at Balule, with sediment contamination higher at Mamba (Marx 1996).

Variation in the concentration of lead and zinc in the same organs and tissues were observed , with the coefficient of variation being relatively high (Table 3). Large variation between concentrations of zinc in individual fish organs and tissues occurred relatively seldom. One of the few examples of large variation between individual fish was observed for the zinc concentration in the muscle at Mamba, during survey two (May 1994), with one sample analysed at 13.11 µg/g and another at 191.03 µg/g with a mean muscle concentration of 76.10 µg/g. These individual variations of concentration could potentially be as a result of i) variation in the individual's optimum for essential elements due to age, size or genetic variability, ii) variation in fish size or health, iii) time of residence, in particular collection area, iv) differential stress during capture and retrieval or further undocumented events such as feeding habits or previous disease (Pinder & Giesy 1981) and v) variation in levels of lead and zinc in the water. Another important factor, which must be borne in mind, is that metal concentrations in fish are the result of complex processes associated with uptake and excretion rates, detoxification and homeostasis in fish (Giesy & Wiener 1977; Heath 1987). Similar large variations have been observed by other scientists in related studies (Pagenkopf & Neuman 1974; Du Preez & Steyn 1992).

Lead and zinc

The data presented in the present study showed that *C. gariepinus* bioaccumulated lead in the different organs and tissues, with the degree of concentration being in the following order, gills > liver > muscle ≥ skin (Fig 2). The results for survey one (Table 3) showed that the organs with the highest lead concentration for both Mamba and Balule, was the liver (53.96 ± 24.29 µg/g) and the muscle (39.06 ± 12.04 µg/g) respectively. In all subsequent surveys the gills showed the highest concentration. Much the same pattern for lead bioaccumulation was found in studies done by Seymore (1994) and Du Preez *et al.* (1997). In general, lead concentrations observed by Du Preez *et al.* (1997) in *C. gariepinus*, were higher at Mamba and Balule compared to the present study's findings. The low lead concentrations observed in the water and sediment data can, therefore, account for the low lead bioaccumulation levels observed in the current study. High concentrations of zinc were found in all organs and tissues tested (Table 3). Much higher zinc concentrations in the organs and tissues of *C. gariepinus* were recorded in the present study, when compared to the findings of Du Preez *et al.* (1997). There was slight variation with regard to which organ or tissue had the highest degree of bioaccumulation, the gills or the liver, with a greater difference being the particular sequence of bioaccumulation in the different organs and tissues. The ranking of zinc concentrations from the highest to the lowest, in the organs and tissues was found to be liver > gills > skin > muscle (Fig 2). This particular ranking was confirmed by the results of Bezuidenhout *et al.* (1990) and Du Preez *et al.* (1997).

For the four surveys collectively, statistical comparisons of the lead concentrations for comparable tissue showed that there was a significant difference ($P \leq 0.05$) between the two locations with exceptions highlighted in Table 4. Except for the mean lead concentration for all the organs in survey 2, the muscle for survey 1 and the skin for survey

Table 3

Lead and zinc concentrations (µg/g) and other statistical variables in gills, liver, muscle and skin of Clarias gariepinus, *from the Olifants River, Kruger national Park*

Survey 1, February 1994								
	(Mamba) (*n*=20)				(Balule) (*n*=20)			
Metal Conc.	Tissue Types				Tissue Types			
	Gills	Liver	Muscle	Skin	Gills	Liver	Muscle	Skin
Pb (µg/g)								
Mean ± sd	35.97±10.36	53.96±24.29	30.93±14.73	40.51±21.94	28.82±13.03	34.81±14.60	39.06±12.04	19.96±4.84
Min/Max	(6.91-53.09)	(24.17-112.58)	(16.35-81.52)	(4.91-21.60)	(15.28-69.02)	(10.82-65.41)	(25.57-77.58)	(13.14-32.35)
se	2.32	5.43	3.29	4.91	2.91	3.26	2.69	1.08
cv	28.80	45.01	47.63	54.15	45.20	41.93	30.81	24.26
Variance	101.31	560.23	206.98	457.62	169.66	213.04	144.86	23.46
BcFw	1.80	2.70	1.55	2.03	1.44	1.74	1.95	0.998
BcFs	1.80	2.70	1.55	2.03	1.44	1.74	1.95	0.998
Zn (µg/g)								
Mean ± sd	124.93±36.06	287.53±156.76	110±64.10	138.28±49.68	224±144.2	154.39±64.36	82.44±22.23	138.59±80.73
Min/Max	(50.07-210.41)	(107.05-688.6)	(40.67-257.71)	(73.15-244.76)	(108.3-676.06)	(81.5-366.01)	(54.44-125.99)	(62.59-368.79)
se	8.06	35.05	14.02	11.11	32.25	14.39	4.97	18.05
cv	28.86	54.52	54.08	35.93	64.34	41.69	26.96	58.25
Variance	1300	2457.2	4108.95	16079	20803.8	4142.47	494.20	6517.92
BcFw	3.47	7.99	3.06	3.84	74.67	51.46	27.48	46.20
BcFs	0.419	0.965	1.39	0.464	1.37	0.947	0.506	0.850
Survey 2, May 1994								
	(Mamba) (*n*=18)				(Balule) (*n*=20)			
Pb (µg/g)								
Mean ± sd	14.27±5.21	11.48±1.04	1.10±0.933	0.867±0.560	30.78±17.84	18.01±9.28	11.37±1.49	13.10±3.72
Min/Max	(6.79-29.76)	(0.087-3.65)	(0.19-3.13)	(0.03-1.51)	(11.40-73.42)	(9.09-37.35)	(8.81-15.39)	(9.15-24.32)
se	1.23	0.244	0.220	0.132	3.99	2.08	0.334	0.832
cv	36.54	69.98	84.69	64.59	57.97	51.53	13.13	28.40
Variance	27.18	1.04	0.87	0.31	318.43	86.12	2.23	13.85
BcFw	0.714	0.074	0.055	0.043	1.54	0.90	0.569	0.655
BcFs	0.714	0.074	0.055	0.043	1.54	0.90	0.569	0.655
Zn (µg/g)								
Mean ± sd	112.05±32.81	73.38±47.71	76.10±51.20	81.78±32.66	145.13±77.44	243.02±143.48	27.04±6.66	86.15±27.88
Min/Max	(66.67-194.96)	(22.48-166.84)	(13.11-191.03)	(30.3-159.78)	(49.98-363.56)	(672-84-118.4)	(19.63-45.48)	(44.53-151.7)
se	7.73	11.25	12.07	7.70	17.32	17.34	1.49	6.23
cv	29.28	65.02	67.28	39.93	53.36	34.60	24.64	32.36
Variance	1076.4	2276.03	2621.68	1066.5	5997.67	2621.65	44.40	777.21
BcFw	37.35	24.46	25.37	27.26	48.38	81.01	9.01	28.72
BcFs	0.322	0.211	0.219	0.235	2.31	3.86	0.429	1.37
Survey 3, July 1994								
	(Mamba) (*n*=20)				(Balule) (*n*=20)			
Pb (µg/g)								
Mean ± sd	20.559.51	8.214.95	5.240.347	16.103.47	23.009.20	9.454.74	5.540.64	6.251.50
Min/Max	(9.16-42.31)	(3.34-17.99)	(4.55-5.82)	(10.11-23.31)	(9.22-42.94)	(5.29-19.93)	(4.47-6.86)	(4.12-8.77)
se	2.18	1.11	0.078	0.777	2.06	1.06	0.143	0.334
cv	46.27	60.26	6.61	21.58	40.03	50.10	11.54	23.93
Variance	90.43	24.45	0.12	12.08	84.71	22.42	0.41	2.24
BcFw	1.03	0.411	0.262	0.805	1.15	0.473	0.277	0.313
BcFs	1.03	0.411	0.262	0.805	1.15	0.473	0.277	0.313
Zn (µg/g)								
Mean ± sd	139.0045.52	150.7729.35	32.304.39	87.6019.93	144.1539.15	226.8183.43	26.764.34	104.0143.66
Min/Max	(99.78-276.81)	(101.33-217.4)	(25.47-40.57)	(55.37-124.81)	(8.75-85.51)	(96.93-451.62)	(21.35-38.63)	(52.37-241.3)
se	10.44	6.56	0.983	4.46	8.75	18.65	0.969	9.76
cv	32.74	19.47	13.60	22.75	27.16	36.78	16.20	41.98
Variance	2071.62	861.54	19.31	397.06	1532.81	6960.04	18.80	1906.35
BcFw	46.33	50	10.77	29.20	48.05	75.60	8.92	34.67
BcFs	2.08	2.25	0.482	1.31	0.515	0.810	0.096	0.371

Table 3 (continued)

Survey 4, November 1994								
	(Mamba) (n=19)				(Balule) (n=20)			
Pb (µg/g)								
Mean ± sd	31.0810.71	11.21±3.53	11.21±1.44	4.972.29	14.143.41	6.631.93	6.061.70	7.781.77
Min/Max	(16.58-50.29)	(6.29-18.35)	(9.88-15.28)	(2.08-10.32)	(8.68-22.41)	(3.88-9.64)	(2.83-8.53)	(3.13-11.64)
se	2.46	0.810	0.331	0.525	0.762	0.431	0.379	0.395
cv	34.45	31.49	12.89	46.07	24.10	29.05	28.01	22.70
Variance	114.66	12.46	2.09	5.25	12.46	3.72	2.88	3.12
BFw	1.55	0.561	0.561	0.249	0.707	0.332	0.303	0.389
BcFs	1.55	0.561	0.561	0.249	0.707	0.332	0.303	0.389
Zn (µg/g)								
Mean ± sd	164.7722.58	142.633.97	33.649.55	97.3737.91	103.8918.83	112.9630.43	31.649.73	69.6316.61
Min/Max	(126.31-200.13)	(98.7-22.98)	(22.88-57.58)	(47.53-180.38)	(74.23-145.46)	(69.93-164.49)	(18.83-58.18)	(50.93-103.42)
se	5.18	7.79	2.19	8.70	4.21	6.80	2.17	3.71
cv	13.71	23.82	28.41	38.94	18.12	26.93	30.74	23.85
Variance	510.08	1154.29	91.30	1437.47	354.43	925.71	94.59	275.76
BcFw	3.43	2.97	0.701	2.03	2.66	2.90	0.811	1.79
BcFs	2.50	2.16	0.510	1.48	1.51	1.64	0.459	1.01

Pb (µg/g) = lead concentration; Zn (µg/g) = zinc concentration; sd = standard deviation; se = standard error; cv = coefficient of variation.

$$\text{Bioconcentration factor } (BcF_{water})/(BcF_{sediment}) = \frac{\text{Metal concentration (µg/g) in tissue (dry weight)}}{\text{Metal concentration in sediment (µg/g)/water (µg/ml)}}$$

4, the mean lead concentration in the gills, liver, muscle and skin for the entire study period at Mamba and Balule, showed higher concentrations at Mamba. Generally, there was a significant difference ($P \leq 0.05$) between the concentration of zinc in related tissue at Mamba and Balule with the exceptions being tabulated (Table 4). A possible explanation for this variation of concentration between the two locations, could be as a result of a number of factors such as the effect of the physical and chemical variables on metal speciation and bioavailability, the points of individual variation listed above, and the relevance of the reeds found upstream from Balule. These reeds absorb a large amount of metal and when they die off and decompose, they release this metal into the aquatic environment for uptake by the fish (De Wet *et al.* 1990).

The gills proved to be the primary route for the uptake of lead and zinc (Fig. 2). High concentrations of lead found particularly in the gills and kidneys, according to Hodson *et al.* (1978), is reflective of the direct contact of the gills with lead contaminated water and their ability to actively take up particular ions and to excrete metabolic products and the possible excretion of lead via urine. The binding of lead to the mucous and structures of the gills, could also have contributed to the high concentrations analysed (Moore & Ramamoorthy 1984). The high lead and zinc concentrations found in the gills could be due to a number of factors. Firstly, if gills become damaged the local accumulation of these metals at the gill surface will increase. Secondly, a reduction in the pH of the microenvironment at the gill surface may facilitate the release of toxic soluble lead and zinc from the accumulated precipitates (Everall *et al.* 1989a). Hughes & Flos (1978), also suggest that zinc in food can increase gill concentrations. This occurs after absorption of zinc through the alimentary canal, where it is taken up and transported by the blood system and deposited in the gills where it forms part of an equilibrium action involved in the ionic regulatory function of

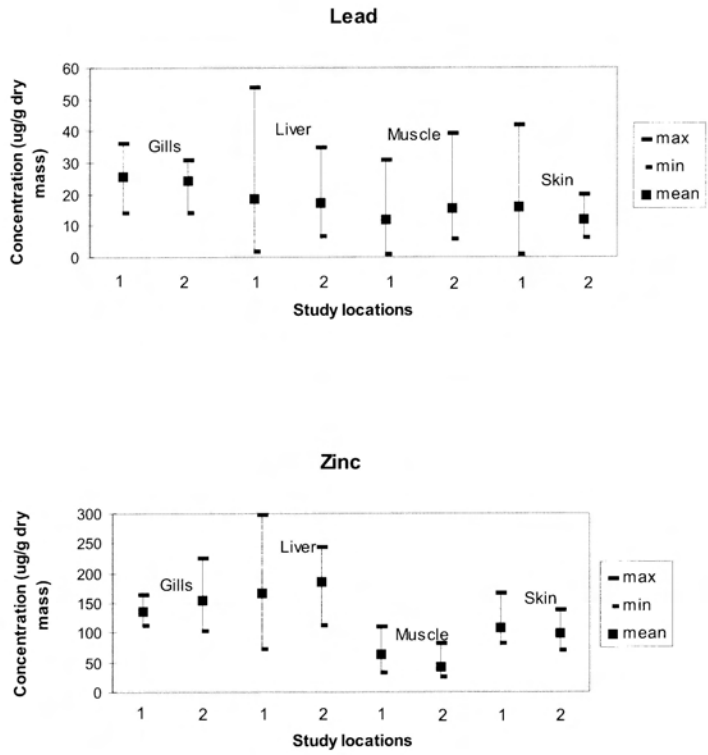

Fig. 2. Mean metal concentrations in the gills, liver, muscle and skin of *Clarias gariepinus* for the 1994 sampling period at Mamba (location 1) and Balule (location 2).

the gills. After the exposure of small amounts of zinc and its subsequent uptake by the gills, the zinc concentrations are transferred to the blood (unbound) (Pentreath 1973) which leads to higher bioconcentration levels in the blood than in the water (Grobler-Van Heerden *et al.* 1991). An explanation offered by Grobler-Van Heerden *et al.* (1991), suggests that regulatory mechanisms are not activated for these blood zinc concentrations, however, as soon as the zinc concentrations become too high, detoxification of surplus zinc occurs, which verifies homeostatic control of zinc.

The liver was found to be the organ to contain the highest concentration of zinc and the second highest concentration of lead (Fig. 2). The liver has a high capacity to bind metals, which might be related to the fact that the blood flow through the liver is very high and that these blood vessels are easily permeable to hydrophilic molecules and ions. In addition, lead concentrates within the liver and this occurs due to the functioning of the intracellular binding proteins, which accumulate the amount of lead in the liver (Ewers & Schlipköter 1991b). After chronic exposure of lead (119 µg/l) to brook trout, Holcombe *et al.* (1976) showed that gills, liver and kidney accumulated over 100 µg/g of lead after 38 weeks. The liver and bile fluid have previously been found to contain high concentrations of zinc (Du Preez *et al.* 1997), indicating a detoxification function and excretory function via the intestines

Table 4
Probability values comparing the concentrations of lead and zinc from fish caught at Mamba and Balule

Survey	Gills	Liver	Muscle	Skin
		Lead		
1	*0.031281228	*0.002431874	*0.03184078	*0.000267936
2	*0.000323527	*8.14708E-08	*2.3606E-23	*2.25217E-12
3	0.273315055	0.21039487	*0.03827039	*4.47321E-12
4	*6.51665E-07	*1.50663E-05	*1.4618E-12	*5.94222E-05
		Zinc		
1	*0.003479746	*0.000844858	*0.038827009	0.494528444
2	*0.045867204	*2.24254E-05	*0.000407868	0.330756016
3	0.324466459	*0.000397629	0.311858912	*0.069059857
4	*5.44652E-11	*0.003171458	0.257744771	*0.003596446

*Significant difference

(Everall *et al.* 1989b). These findings were in agreement with those by Romanenko *et al.* (1985), who found high concentrations of zinc excreted via the faeces. They suggest that the liver breaks down zinc complexes, liberating zinc into the intestinal cavity in bile and, in doing so, zinc is detoxified and excreted. In liver metallothioneins, zinc is the predominant metallic species. Because of this, excessive concentrations of zinc become bound to these proteins for metabolism. This excess zinc is either detoxified in the liver or becomes stored in the liver with a consequent increase in zinc concentration (Noël-Lambot *et al.* 1978).

Metals are readily complexed in natural waters, with the degree being largely dependent on the alkalinity (bicarbonate-carbonate concentrations) in the prevailing water. Alkalinity or water hardness decreases the bioavailability and toxicity of lead and zinc to fish (Alabaster & Lloyd 1980). Since the water at Balule tends to have a much higher concentration of alkalinity (hardness) than at Mamba (Table 2), a decrease in metal bioavailability and toxicity is expected. Calcium, according to Phillips & Russo (1978), decreases lead accumulation by fishes and lead may inhibit calcium accumulation and deposition. The protective effect of water hardness on fish exposed to metal and the mechanism behind it, is very complex. Several theories suggest that the process is either biological or chemical or both. The biological explanation involves the influence of calcium and/or magnesium on branchial permeability (Bradley & Sprague 1985). An increased external calcium level in hard water reduces gill surface permeability and hence metal uptake (Calamari *et al.* 1980). The chemical explanations involve changes in the speciation of the metal or competition for biological uptake sites (Bradley & Sprague 1985). Calcium, like zinc, is a divalent ion and both compete for metal uptake sites at the fish gill and for binding sites on protein molecules (internal or external) (Everall *et al.* 1989a; Lloyd 1992).

Three major interrelated factors may contribute to the seasonality of pollutants in aquatic biota i) the amount of pollutants delivered to the aquatic environment, ii) organism physiology, with particular reference to the sexual cycle, iii) changes in ambient water quality variables such as temperature, salinity and pH (Phillips 1980), and iv) dilution/run-off due to rainstorms. Temperature influences the rate of metabolic

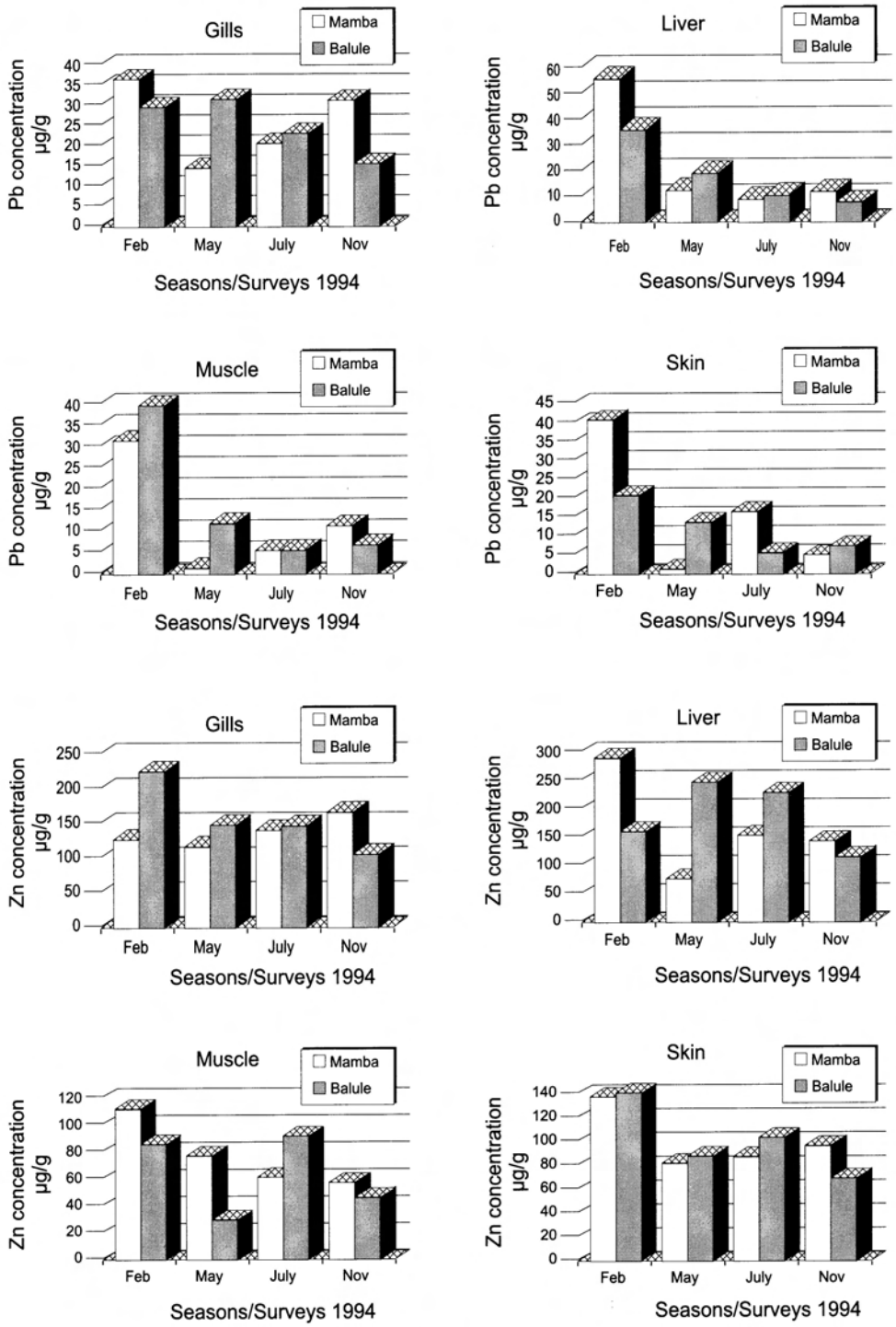

Fig. 3. Graphic illustrations of the seasonal bioaccumulation of lead and zinc in the gills, liver, muscle and skin observed at both locations.

processes including the uptake, metabolism and excretion of metals. When the ambient temperature drops, a drop in the biological activity of organisms results, which could lead to a change in the rate of incorporation and release of heavy metals by organisms (Prosi 1979; Abel 1989). This could explain the lower mean tissue concentration of lead and zinc from the summer to the winter season. Higher concentrations of lead were accumulated during February 1994, while lower accumulation was noted for the cooler months of May and July 1994 (Fig. 3). This shows that seasonal variation could influence the rate of bioaccumulation of lead. With regard to temperature, Somero *et al.* (1977), exposed the estuarine fish, *Gillichthys mirabilis*, to lead concentrations over a 42-day period and detected a two-fold increase in lead uptake between 10 °C and 20-25 °C in the muscle.

The different organs showed variations in seasonal patterns (Fig. 3) with regard to zinc concentration. The seasonal pattern observed, showed high bioaccumulation during late summer (February 1994), followed by a decline in autumn (May 1994) and winter (July 1994). Elevated temperatures, according to Cairns *et al.* (1975), decrease survival time because the consequent increase in respiratory rate increases the amount of zinc in contact with the gills and, in combination with a declining blood oxygen, the resultant situation occurs. Badsha & Sainsbury (1978), have reported on fish species exhibiting seasonal variation in zinc concentration levels of muscle and organs. Five-bearded rockling from the Severn Estuary, exhibited whole body concentrations at peaks during the spring and early summer and a decline in autumn. The reason given was a possible change in diet during the summer. During the colder months of May and July, the concentrations of lead and zinc in fish sampled at Balule was much higher than those sampled at Mamba, this could possibly be attributed to a higher temperature at Balule and a higher calcium concentration in the water at Mamba.

Du Preez *et al.* (1997) noted that as *C. gariepinus* is omnivorous and feeds on zooplankton, benthic organisms, aquatic hydrophytes, detritus and fish, the uptake of metals via the ingestion of food could be significant. A study undertaken by Singh & Ferns (1978), showed that diet-related lead accumulation in the rainbow trout (*Salmo gairdeneri*) does occur, but within specific limits. These authors observed that a peak in lead accumulation was reached after the eighteenth day of a 70-day experiment, where-after it rapidly declined. From the results, these authors suggest that some process operates to regulate body levels of lead to a certain degree, although there is a delay before this begins to operate as metal excretion may not begin until the body levels exceed certain limits. They also think it likely that this regulatory mechanism involves an increased rate of loss rather than a reduced rate of uptake. Findings from studies done by Spry *et al.* (1988), showed that a zinc-deficient diet leads to zinc-deficient fish. However, when these fish were fed a diet with zinc, excretion of the metal far outweighed the retention thereof. It therefore appears that zinc is taken up from both water and dietary sources. However, distinction between the two routes is dependent on the concentrations in the two sources (i.e. the higher the ambient concentration the greater the uptake of zinc through water). As the zinc concentration in the water was not excessively high, biomagnification cannot be discarded as an important source of zinc to the fish.

Identical lead bioconcentration factors for water and sediment were determined. As both these values were low, it suggests that only a small fraction of lead found in the sediment and water was bioavailable to the fish for uptake. Since lead is greatly associated with particulate matter (organic matter, mostly humic and fulvic acids) and is generally not available in the ionic form, Pb^{2+} (Moore & Ramamoorthy 1984) and hence, bioaccumulation is limited. The BcFs and especially the BcFw values were lower when compared to findings by Du Preez *et al.*

(1997) on *C. gariepinus* from the Olifants River. For surveys two and three, the zinc bioconcentration factors between the water (BcFw) and the tissues were relatively high at Mamba and Balule, which suggests that the zinc in the water was readily available for uptake by the fish. However, the bioavailability of zinc from the sediment is minimised due to the low sediment bioconcentration factors determined. Comparisons made with Seymore's (1994) findings show both water and sediment bioconcentration factors at much higher levels than those recorded in the present study. The BcFw calculated in the present study were generally lower than that calculated for *C. gariepinus* in the Olifants River by Du Preez *et al.* (1997).

Conclusion

Lead and zinc have generated a great deal of concern with regard to studies on the effect of pollution on fish physiology (Heath 1987). Once metals have accumulated in an organism, they may cause biochemical (metabolic pathways), physiological, morphological and genetic transformations in fish. These transformations can ultimately influence specific performances of the organisms by reducing their survival and disrupting their development, growth and reproductive potential (Nagel 1991). Determining the dose of metals to which aquatic environments are exposed is not yet possible but concentrations of metals in sediments, water and, in particular, biological tissues can be employed as indicators of exposure. Metal analyses of tissues are especially valuable as an indicator of bioavailable metal exposure (Nagel 1991).

An important question to address is the high metal concentrations detected in both the gills and the livers of the fish examined at Mamba and Balule. This might indicate long-term (chronic) exposure of the fish to these metals. After reviewing the study undertaken during 1991 by Du Preez *et al.* (1997) and compared to the present study's results, a decline in lead concentration as well as a significant rise in zinc concentration in fish was noted after a three year period. Even though there is no direct relationship between metal concentrations found in the organs and tissues of *C. gariepinus* and the metal levels to which the fish were exposed, it could be used as an indication of increased metal loads. It is important that regular sampling of the Olifants River is undertaken, to understand the dynamics of this river and to further prevent the decline of the integrity of its biota.

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