Bioaccumulation of chromium, copper and iron in the organs and tissues of *Clarias gariepinus* in the Olifants River, Kruger National Park

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Abstract

An investigation was undertaken into the bioaccumulation of Cr, Cu and Fe in the gills, liver, muscle and skin of the fish *Clarias gariepinus* from two sites on the Olifants River in the Kruger National Park. During 1994, four surveys (February, May, July and November) were undertaken. Metal bioaccumulation was analysed using atomic absorption spectrophotometry and was then applied to differentiate between the concentrations found at the two locations, and between all of the surveys. The greatest concentration of Cr was detected in the gills, suggesting that this was the prime site of absorption and loss of Cr to and from the aquatic environment. The concentrations of Cu and Fe were highest in the liver, which is a storage and detoxification organ for metal, followed by the gills. Mamba and Balule generally showed very little difference in the concentration of bioaccumulated metal. However, the gills as in the case of Cr generally showed high concentrations at Mamba, while the liver as in the case of Fe, showed consistently higher concentrations at Balule. The possible effects that temperature, pH, hardness and salinity have on the individual metals, as well as bioaccumulation of these metals, are discussed in detail. The continuous monitoring of the quality of water in the Olifants River is imperative for the future sustainability of the Kruger National Park.

Introduction

In nature, aquatic animals are constantly exposed to metals. The species and concentrations of metals in water are determined by geochemical processes and large scale releases into the aquatic environment by human activities (anthropogenic activities) (Wittmann, 1979). Rapid industrial development, as well as the use of metals in production processes have led to the increased discharges of heavy metals into the environment (Koli et al., 1977). According to Förstner and Prosi (1979) the harmful effects of heavy metals as pollutants result from incomplete biological degradation. Therefore, these metals tend to accumulate in the aquatic environment. Since heavy metals are non-biodegradable, they can be bio-accumulated by fish, either directly from the surrounding water or by ingestion of food (Patrick and Loutit, 1978; Kumar and Mathur, 1991). In addition, Heath (1987) indicates that when metals reach sufficiently high concentrations in body cells they can alter the physiological functioning of the fish.

Toxic substances cannot easily be defined due to a number of factors that can influence and modify the toxicity of these substances. Metals are of particular interest in this regard. Some of the factors that can influence the toxicity of metals include:

- the metal species in the water;
- the presence of other metals or pollutants;
- abiotic factors such as temperature, pH, dissolved oxygen, hardness, salinity, etc.;
- biotic factors such as age, size, sex, stage in life history, adaptive capabilities; and
- Behavioural responses (Bryan, 1976b; Van Vuren et al., 1994).

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Chromium (Cr)

In natural waters the concentration of Cr is low and is within the range of between 1 and 2 µg/ℓ dissolved Cr (Moore and Ramamoorthy, 1984). Cr is used in industry for electroplating, steel-making alloys, in chrome plating, rubber manufacturing, leather tanning and for fertilisers (Babich et al., 1982). The toxicity of Cr is dependant on its chemical speciation and thus associated health effects are influenced by the chemical form of exposures (Holdway, 1988). There are four states in which the Cr ion is found: Cr^{2+}, Cr^{3+}, Cr^{5+} and Cr^{6+}. It is in the hexavalent form where Cr is allowed to cross biological membranes of aquatic organisms. Doudoroff and Katz (1953) indicated that hexavalent Cr behaves toxicologically in a manner quite different from most heavy metals. Because hexavalent Cr can readily penetrate gill membranes by passive diffusion and concentrate at higher levels in various organs and tissues, it can manifest its toxic action internally as well as on the gill surface (Knoll and Fromm, 1960; Buhler et al. 1977). Cr is particularly dangerous as it can accumulate in many organisms, sometimes as much as 4 000 times above the level of the surrounding environment as was noted in aquatic algae (Duffus, 1980).

Copper (Cu)

Copper is one of the most abundant trace metals and for almost all organisms it is an essential micronutrient (Duffus, 1980). Natural concentrations in water are at ≤5 µg/ℓ (Alabaster and Lloyd, 1980), but according to the Department of Water Affairs and Forestry (1993b) levels of 12 mg/ℓ have been recorded in South Africa. The increase in Cu pollution can be attributed to geological weathering, atmospheric deposition, municipal and industrial sewage (Moore and Ramamoorthy, 1984), the discharge of mine tailings and fly ash (the major source of solid Cu pollution), fertiliser production and algaecide and molluscicide runoff (Felts and Heath, 1984; Moore and Ramamoorthy, 1984).

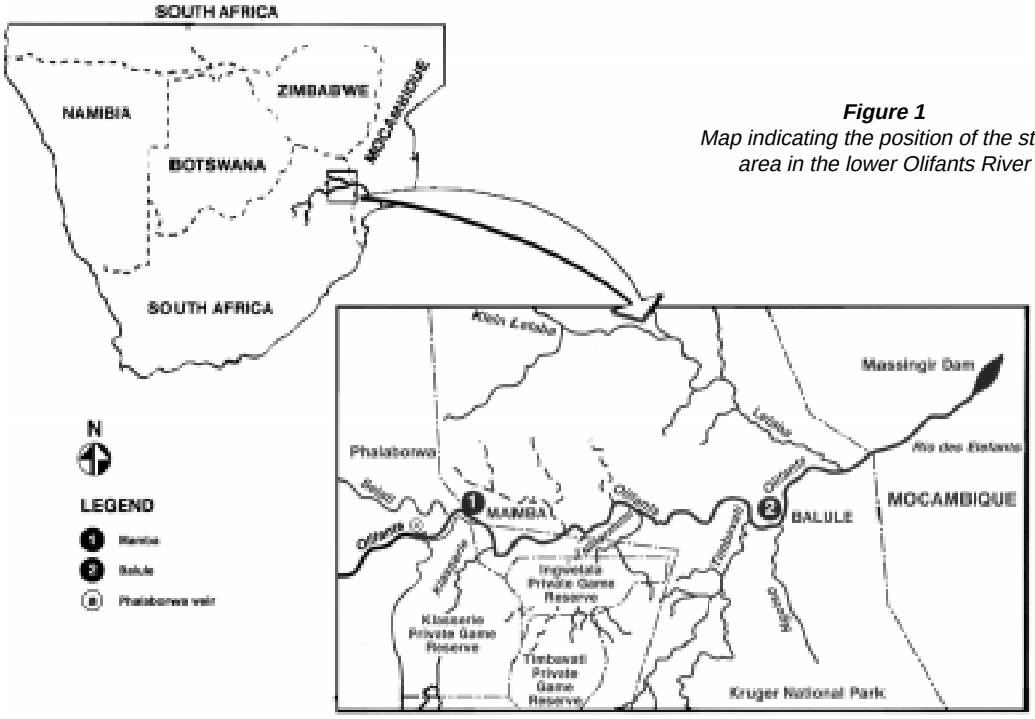

Figure 1
Map indicating the position of the study area in the lower Olifants River

According to Stiff (1971) and Andrew et al., 1977, the soluble forms of Cu are the only forms available to fish. The toxicity of Cu appears to be related to the soluble form, with Cu^{2+} and, to some extent, the Cu hydroxyl ($Cu(OH)_n$) ion being the toxic forms. The free ion rarely occurs in aquatic environments as the soluble form of Cu in river waters and consists mainly of complexed, non-toxic forms. Humic acids, hardness, temperature, pH, dissolved oxygen and suspended solids can have a significant altering effect on the bioavailibility of Cu to fish (Stiff, 1971; EIFAC, 1978; Howarth and Sprague, 1978; Waiwood and Beamish, 1978a; Miller and Mackay, 1980; Moore and Ramamoorthy, 1984).

Iron (Fe)

The main source of increased Fe concentrations in the aquatic environment is acid mine drainage, mineral and steel processing and industrial runoff (Förstner and Wittmann, 1979; Cruywagen et al., 1981). Acid mine drainage releases ferrous and ferric sulphates into the aquatic environment, while coalmine waste dumps and lagoons leach iron into the water (Ellis, 1989; Department of Water Affairs, 1992). Iron ore and magnetite mining in the Phalaborwa area could potentially have a significant effect on the quality of water entering the Olifants River (Cruywagen et al., 1981).

The form of Fe frequently found in solution in groundwater is the ferrous (Fe^{2+}) ion (Hem, 1989), with ferrous compounds which is believed to be toxic. The availability of Fe for aqueous solution is affected by environmental conditions, particularly changes in the degree of oxidation or reduction (Hem, 1989), which is dependent primarily on pH, redox potential and temperature (Wetzel, 1983). Fe is readily oxidised by dissolved oxygen to the ferric form in the neutral to slightly acidic pH range, while the ferrous ion is stable under these conditions (Förstner and Wittmann, 1979). Fe is present in organic wastes and in plant debris in soils and the activities within the biosphere may strongly influence the occurrence of Fe in water (Hem, 1989).

The lower Olifants River has been, as many studies have revealed, subject to contamination input from activities from both its catchment areas, and in particular Phalaborwa, resulting in heavy metal pollution in the Olifants River in the KNP (Du Preez and Steyn, 1992; Wepener et al., 1992; Seymore et al., 1994; Marx, 1996; Marx and Avenant-Oldewage, 1998; Kotze et al., 1999; Nussey et al., 1999). Fish deaths have resulted from high levels of cations (potassium, chloride, sulphate, magnesium and sodium) and high silt loads present in the Olifants River (Moore, 1990). The present study was, therefore undertaken to acquire information about the heavy metal content of natural fish populations of *Clarias gariepinus* from two different study sites along the Olifants River in the Kruger National Park. This paper focuses on the bioaccumulation of Cr, Cu and Fe in the organs and tissues of *C. gariepinus*. From previous studies done by Du Preez and Steyn (1992), Van der Merwe (1992) and Seymore et al. (1994), Marx and Avenant-Oldewage (1998), Kotze et al. (1999) and Nussey et al. (1999) on the Olifants River, it has been revealed that metals are present at relatively high concentrations. The investigation into the possible effects of physical, chemical and biological influences on bioaccumulation has been included. Temporal, as well as seasonal differences of metal accumulation, will also be investigated.

Study area

Two main study sites in the lower catchment area (Fig. 1) of the Olifants River were focused on during this study. The first site, Mamba, is situated on the western border of the Kruger National Park (KNP) in close proximity to the mining town of Phalaborwa. The second site, Balule, is situated approximately 40 km eastwards into the interior of the KNP. Extensive reed beds separate the two

sites. Of major concern is the industrialisation of the Olifants River and the influence it has on the lower reaches of the Olifants River.

Methods

Field procedures

Four surveys were conducted at Mamba and Balule in the Olifants River, KNP, during February, May, July and November of 1994. Water and sediment samples were taken for metal analysis, as well as for physical and chemical water parameters.

A maximum of 20 fish from the species *C. gariepinus* (Burchell, 1822) (Sharptooth catfish) were collected at both Mamba and Balule during each survey undertaken in 1994. They were collected from the water primarily with the aid of hand lines and gill nets with mesh sizes ranging between 70-120 mm. Hand line techniques were, however, the most effective in capturing *C. gariepinus*.

To determine heavy metal accumulation in fish, different tissues such as gills, liver, muscle and skin were analysed separately. These tissues were chosen for the following reasons:

- They are all known to accumulate metals (Seymore et al., 1994).
- The gills and skin are constantly exposed to the metals in the water and the accumulation and subsequent effects that metals have on the gill and skin tissue, being the first route of entry into the body, is of interest.
- As the liver is known as a storage and detoxification organ (Klaassen, 1976) the amount of metal accumulated therein might reflect the severity of the pollution.
- The muscle is the tissue generally consumed by humans and the metal accumulation content is important for the presumed effect on human health (Du Preez et al., 1997).

The fish were placed onto polypropylene dissection boards, immobilised and killed by cutting through the spinal cord behind the head and subsequently dissected. The gills, liver, muscle and skin were dissected out and placed in separate 25 mℓ glass bottles. All samples collected were refrigerated in a portable Coleman refrigerator. Thereafter they were frozen and stored until the time of analysis.

Laboratory procedures

To determine the mass of the pre-thawed tissue samples (gills, liver, muscle and skin) they were placed in Erlenmeyer flasks and weighed again (subtracting the mass of the flask) using a Mettler PK 4800 balance. Approximately 5 g of wet tissue was used and this corresponded to approximately 1 g of dried tissue. The gill filaments were removed from the gill arches and used for the analysis. The subsamples were dried at 60°C in a Haraeus Hanau KB 500 oven for 48 h. The dried samples were then also weighed in order to determine the percentage moisture content thereof. Samples were digested according to methods described by Van Loon (1980) and Du Preez and Steyn (1992). A mixture of 10 mℓ, 55% nitric acid (NHO_3), and 5 mℓ 70% perchloric acid ($HClO_4$) was added to the 1 g of dried sample in the Erlenmeyer flasks. The samples were then placed on a hot plate and allowed to digest at ±200°C until a transparent and clear solution was obtained. Samples were allowed to cool down and then diluted to 50 mℓ with double-distilled water. These solutions were then filtered through a 6 m Millipore acid-resistant membrane filter attached to a vacuum pump. The samples were then carefully poured into presterilised, acid-washed glass bottles rinsed in double-distilled water.

The total concentrations of Cr, Cu and Fe were determined with the aid of a Varian Atomic Absorption Spectrophotometer (SPECTRA AA-10). Calibration of the spectrophotometer for each metal was carried out with Holpro stock solutions. A 2:1 nitric and perchloric acid solution was made up and its absorbency read. The metal concentrations, which were determined in the acid solution, were subtracted from the value determined for each tissue metal concentration. This was done in order to eliminate the concentrations of metals found in the acid solution, which could have caused inaccuracies in the concentrations of the tissue metal readings. Individual metal concentrations in the tissue samples were then read at specified absorbencies for each metal. Metal concentrations of all tissues were calculated as follows:

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

Du Preez et al. (1997) obtained metal bioaccummulation values from wet tissue. In the current study and subsequent studies it was obtained from 1 g of dried tissue. This method is commonly used in fish studies (Marais and Erasmus, 1997). In order to compare the values from wet tissue with those obtained in the present study it was necessary to calculate the metal concentration. It was done by modifying the formula:

$$\%\text{Moisture} = \frac{\text{Wet mass of sample(g) - Dry mass of sample(g) x100}}{\text{Wet mass of sample (g)}}$$

(when 1 g of dried tissues was used) to:

$$x = \frac{1}{\frac{-y}{100} + 1}$$

where:

y = % moisture calculated when drying the tissue
x = wet mass of the tissue

$$\mu\ \text{gram}_{\text{metal}} = \frac{x}{\text{metal mass obtained for dry tissue}}$$

where:

μ gram$_{metal}$ i.e the amount of metal in 1 g of wet tissue.

Statistical analysis

Metal data were statistically analysed with the aid of Statsgraphics 7 computer software. The summary statistics determined included the mean, standard deviation, standard error, coefficient of variation and the minimum and maximum values.

The multivariate analysis of variance (MANOVA) was carried out to see if any significant differences existed between groups. This determines the relevance of carrying out any univariate analysis of variance tests (ANOVA). MANOVA is used when three or more variables are being compared; this was done when seasons and tissue types were compared with each other. The MANOVA test used during the present study was the Wilks' Lambda test. When only two variables are being compared (e.g. Sample sites, Mamba and Balule) a different multivariate test is used. The one chosen during the present study was the Hotelling T^2 test. ANOVA is used when three or more variables are being

TABLE 1
Mean water quality variables from the Olifants River, at Mamba (Loc 1) and Balule (Loc 2), Kruger National Park, Feb. 1994 – Nov. 1994

	Survey1 (Feb)		Survey 2 (May)		Survey 3 (July)		Survey 4 (Nov)	
	Loc 1	Loc 2	Loc 1	Loc 2	Loc 1	Loc 2	Loc 1	Loc 2
Chemical & physical water variables								
Temperature (°C)	23.0	24.2	20.0	24.8	16.9	19.2	25.0	27.8
pH	7.88	6.86	8.32	8.42	9.01	8.26	8.91	8.73
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
Alkalinity (mg/ℓ) $CaCO_3$	387	309	N/A	N/A	N/A	N/A	44	166
TDS (mg/ℓ)	315	786	560	585	1644	1401	1069	848
Metal concentrations (mgl ℓ) of water								
Chromium	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Copper	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
Iron	0.003	0.003	0.003	0.003	0.003	0.059	0.166	0.129
Metal concentrations (mglg) of sediment								
Chromium	0.218	0.360	0.249	0.356	0.532	0.335	0.259	0.105
Copper	0.004	0.023	0.041	0.056	0.036	0.209	0.049	0.010
Iron	45.23	52.91	38.83	54.38	62.53	76.75	33.23	34.48
N/A = Not available								

compared. This was done when seasons and tissue types were compared with each other. To do a univariate analysis of only two groups (e.g. sample sites, Mamba and Balule) the t-test was employed.

Results

Table 1 summarises the information gathered, during this study, on the physical and chemical water parameters, as well as water and sediment metal concentrations. The complete set of data is contained in Marx (1996). 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).

Bioaccumulation of metals in tissue

The four different tissue types gills, liver, muscle and skin were analysed for the three metals specified and the results for both localities were summarised and tabulated (Table 2). There is a large amount of variation amongst the different organs and tissues with regard to each metal. Therefore, the results for each metal will be explained individually. The different organs and tissues sampled, differed in their moisture content. As a result of the variation in moisture content of the different organs and tissues, metal concentrations in these tissues were calculated on a dry weight basis. In order to compare these to the values obtained by Du Preez et al. (1994) the metal content in 1 g of wet tissue was calculated and is shown in comparison to their results in Table 3.

Chromium

Bioaccumulation of chromium (Table 2)

The degree and site of bioaccumulation for Cr in the four tissue and organ samples for all four surveys and two locations varied a great deal. For Survey 1, Mamba showed that the largest mean bio-accumulation and the site with the highest mean concentration was the muscle (117.22±44.25 μg/g), whereas at Balule it was the gills (118.34±38.00 μg/g). The results of Survey 2 were converse to that found in Survey 1 with the gills (45.19±36.05 μg/g) having the highest mean concentration at Mamba, while for Balule the mean concentration in muscle (69.62±11.49 μg/g) was the highest. The degree of bioaccumulation in the different organs and tissues for both Mamba and Balule during Survey 3 corresponded with each other, with the gills being the highest accumulation site, 52.96±25.44 μg/g and 29.77±14.74 μg/g, respectively. During Survey 4 the gills (39.00±12.06 μg/g) concentrated the highest mean amount of Cr at Mamba, while at Balule the liver (25.78±6.50 μg/g) showed the highest mean concentration of Cr. The general degree of Cr concentration in the tissues for both Mamba and Balule was found to be in the following order: gills > liver > muscle > skin.

Variation between the Cr tissue and organ concentrations for individual specimens at the same locality was pronounced. For example, at Mamba during Survey 3 (July 1994) the concentration of Cr in the gills for the 20 fish sampled ranged between a minimum of 17.50 μg/g to a maximum of 112.10 μg/g. The value of the standard deviation indicates the degree of variation among individuals, the greater the standard deviation, the larger the variation.

TABLE 2

Heavy metal concentrations (μg/g) in gills, liver, muscle and skin of *Clarias gariepinus*, from the Olifants River, Kruger National Park

Metal concentration (μg/g)	Tissue types				Tissue types			
	Gills	**Liver**	**Muscle**	**Skin**	**Gills**	**Liver**	**Muscle**	**Skin**
Survey 1, February 1994								
	Location One (Mamba) (n=20)				**Location Two (Balule) (n=20)**			
Cr Mean ± sd	77.2±36.6	67.9±27.9	117.2±44.3	69.7±23.4	118.3±38.0	67.1±15.1	68.3±23.7	77.5±27.1
Min/Max	(31.9-156.9)	(40.9-142.0)	(42.2-245.2)	(33.0-114.1)	(70.7-227.3)	(35.3-101.1)	(33.5-134.8)	(46.1-162.7)
se	8.2	6.2	9.9	5.2	8.5	3.4	5.3	6.1
cv	47.4	41.1	37.8	33.5	32.1	22.4	34.7	34.9
Cu Mean ± sd	12.2±4.8	152.8±70.2	12.5±4.5	10.9±4.3	33.0±13.6	108.9±39.5	4.6±1.5	5.6±1.8
Min/Max	(5.5-23.4)	(76.8-300.8)	(5.6-23.2)	(6.3-22.5)	(17.1-67.6)	(42.6-203.8)	(1.5-6.3)	(3.3-11.2)
se	1.1	15.7	1.1	0.9	3.0	8.8	0.3	0.4
cv	39.5	45.9	36.2	38.8	38.9	36.3	32.5	32.4
Fe Mean ± sd	646.5±215.2	2380.6±1648.5	675.0±877.8	546.2±305.2	1303.7±634.6	2573.5±608.5	1641.8±584.7	1729.0±532.8
Min/Max	(174.7-1045.6)	(943.0-6673.2)	(200.2-4333.0)	(309.9-1335.7)	(619.4-2766.0)	(1848-4290.3)	(802.9-3298.2)	(1128.0-3548)
se	48.2	368.6	196.3	68.3	141.9	136.1	130.8	532.8
cv	33.4	69.3	130.1	55.9	48.7	23.7	35.6	30.8
Survey 2, May 1994								
	(Mamba) (n=18)				**(Balule) (n=20)**			
Cr Mean ± sd	45.2±36.1	29.1±28.2	25.3±19.3	19.4±16.0	27.1±16.6	28.0±14.3	69.6±11.5	39.0±10.4
Min/Max	(10.6-113.9)	(0.8-77.3)	(3.3-71.7)	(3.1-47.9)	(7.6-63.8)	(13.3-60.8)	(51.2-87.5)	(27.9-74.1)
se	8.5	6.6	4.5	3.8	3.7	3.2	2.6	2.3
cv	79.8	97.0	76.2	82.7	61.3	51.2	16.5	26.7
Cu Mean ± sd	10.8±3.9	42.8±26.3	5.1±3.0	3.1±1.2	22.6±11.7	100.5±40.1	3.7±0.7	5.1±1.7
Min/Max	(6.2-18.9)	(13.5-114.2)	(0.8-12.1)	(1.3-5.7)	(8.4-50.7)	(25.7-197.5)	(2.8-5.2)	(3.7-9.9)
se	0.9	6.2	0.7	0.3	2.6	9.0	0.2	0.4
cv	95.0	61.4	59.7	38.3	51.8	39.9	19.6	32.5
Fe Mean ± sd	1033.1±552.4	1382±981.5	231.0±132.4	168.6±97.5	1491.0±970.5	2500.7±1153	148.1±71.6	168.8±95.3
Min/Max	(490.2-2590.3)	(322.2-2998.2)	(76.8-479.8)	(47.0-422.7)	(73.8-3044.7)	(257.8-838.6)	(80.4-390.6)	(82.5-379.6)
se	130.2	231.4	31.2	23.0	217.0	257.8	16.0	82.5
cv	53.5	71.0	59.3	57.8	65.1	46.1	48.4	56.4
Survey 3, July 1994								
	(Mamba) (n=20)				**(Balule) (n=20)**			
Cr Mean ± sd	53.0±25.4	25.9±14.4	11.5±1.1	12.7±3.3	29.8±14.7	20.2±12.7	10.3±0.7	11.7±3.3
Min/Max	(17.5-112.1)	(10.9-50.7)	(9.0-14.0)	(9.7-20.6)	(13.1-59.2)	(10.5-50.8)	(9.0-11.2)	(7.6-18.0)
se	5.8	3.2	0.3	0.7	3.3	2.9	0.2	0.7
cv	48.0	55.5	9.9	26.1	49.5	63.0	6.6	28.2
Cu Mean ± sd	9.5±2.0	60.0±26.0	2.3±0.5	2.6±0.9	9.0±3.3	94.9±23.8	1.9±0.6	2.7±1.3
Min/Max	(6.7-14.2)	(12.9-102.7)	(1.6-3.5)	(1.7-4.7)	(2.8-16.4)	(51.6-131.1)	(1.4-3.3)	(1.5-6.4)
se	0.5	5.8	0.1	0.2	0.7	5.3	0.1	0.3
cv	20.7	43.3	20.7	33.9	36.8	25.1	30.3	49.4
Fe Mean ± sd	2267.0±968.4	1737.9±827.8	132.9±42.8	166.1±86.2	1216.0±634.9	2373.6±1221.0	79.5±28.1	146.0±93.9
Min/Max	(948.2-4475.5)	(754.6-3425.3)	(74.7-218.6)	(72.7-420.7)	(550.5-2736.2)	(858.2-4858.2)	(50.2-183.7)	(67.7-375.4)
se	222.2	185.1	9.6	19.3	142.0	273.0	6.3	21.0
cv	42.7	47.6	32.2	51.9	52.2	51.4	35.4	64.3
Survey 4, November 1994								
	(Mamba) (n=19)				**(Balule) (n=20)**			
Cr Mean ± sd	39.0±12.1	16.1±6.0	12.1±3.1	13.6±4.3	21.9±5.6	25.8±6.5	12.5±3.7	15.7±6.6
Min/Max	(17.2-63.0)	(9.1-32.1)	(9.7-19.2)	(8.7-22.9)	(11.7-31.5)	(17.2-35.8)	(9.2-19.7)	(7.7-34.6)
se	2.8	1.4	0.7	1.0	1.3	1.5	0.8	1.5
cv	30.9	37.5	25.8	31.6	25.5	25.2	29.3	42.2
Cu Mean ± sd	11.9±3.1	44.1±20.3	2.7±1.1	2.5±0.8	5.7±1.5	42.7±23.3	1.5±0.7	1.4±0.6
Min/Max	(6.4-16.6)	(23.3-81.5)	(1.4-5.4)	(0.9-3.9)	(2.9-8.2)	(18.2-92.9)	(90.4-2.9)	(0.4-3.3)
se	0.7	4.7	0.2	0.2	0.3	5.2	0.2	0.1
cv	26.0	45.9	39.5	31.3	26.7	54.6	45.5	45.7
Fe Mean ± sd	1973.0±772.0	1431.0±1064.2	193.6±63.9	236.9±102.3	1263.1±723.3	1012.8±584.9	199.3±88.5	250.1±154.5
Min/Max	(963.4-4339.7)	(420.7-353.7)	(109.2-317.7)	(121.8-509.7)	(367.0-2708.2)	(426.2-2618.2)	(87.2-448.7)	(99.7-633.7)
se	177.1	244.2	14.7	23.5	161.7	130.8	19.8	34.6
cv	39.1	74.3	33.0	43.2	57.3	57.8	44.4	61.8

Cr (μg/g) = chromium concentration; **Cu** (μg/g) = copper concentration; **Fe** (μg/g) = iron concentration; **sd** = standard deviation; **se** = standard error; **cv** = coefficient of variation.

Table 3
Mean metal concentration in selected tissue as obtained in the present study in comparison with values obtained by du Preez et al. (1994), following conversion of dry tissue results (Highest value in bold)

Location	Organ	Metal	% Moisture	Mean Metal Mass (μg/g)	Mean Metal Mass (μg/g)
		Present study			Du Preez et al. (1997)
		Survey Feb-94			Feb -90
Mamba	Gills	Cr	85.26%	11.4	**52**
Mamba	Liver	Cr	76.30%	16.1	**52**
Mamba	Muscle	Cr	83.47%	19.4	**50**
Mamba	Skin	Cr	79.01%	14.6	N/A
Balule	Gills	Cr	86.34%	16.2	**18**
Balule	Liver	Cr	78.19%	14.6	**26**
Balule	Muscle	Cr	86.87%	9.0	**23**
Balule	Skin	Cr	80.42%	15.2	N/A
Mamba	Gills	Cu	85.26%	1.8	**5**
Mamba	Liver	Cu	76.30%	**36.2**	21
Mamba	Muscle	Cu	83.47%	2.1	**4**
Mamba	Skin	Cu	79.01%	2.3	N/A
Balule	Gills	Cu	86.34%	4.5	**63**
Balule	Liver	Cu	78.19%	23.8	**140**
Balule	Muscle	Cu	86.87%	0.6	**26**
Balule	Skin	Cu	80.42%	1.1	N/A
Mamba	Gills	Fe	85.26%	95.3	N/A
Mamba	Liver	Fe	76.30%	564.2	N/A
Mamba	Muscle	Fe	83.47%	111.6	N/A
Mamba	Skin	Fe	79.01%	114.7	N/A
Balule	Gills	Fe	86.34%	178.1	N/A
Balule	Liver	Fe	78.19%	561.3	N/A
Balule	Muscle	Fe	86.87%	215.6	N/A
Balule	Skin	Fe	80.42%	338.5	N/A
		May-94			Jun-90
Mamba	Gills	Cr	87.43%	5.7	**34**
Mamba	Liver	Cr	79.12%	6.1	**21**
Mamba	Muscle	Cr	88.58%	2.9	**23**
Mamba	Skin	Cr	80.64%	3.8	N/A
Balule	Gills	Cr	88.29%	3.2	**27**
Balule	Liver	Cr	76.38%	6.6	**17**
Balule	Muscle	Cr	84.21%	11.0	**20**
Balule	Skin	Cr	79.34%	8.1	N/A
Mamba	Gills	Cu	87.43%	1.4	**7**
Mamba	Liver	Cu	79.12%	8.9	**24**
Mamba	Muscle	Cu	88.58%	0.6	**5**
Mamba	Skin	Cu	80.64%	0.6	N/A
Balule	Gills	Cu	88.29%	2.7	**3**
Balule	Liver	Cu	76.38%	23.7	**25**
Balule	Muscle	Cu	84.21%	0.6	**2**
Balule	Skin	Cu	79.34%	1.1	N/A
Mamba	Gills	Fe	87.43%	129.9	N/A
Mamba	Liver	Fe	79.12%	288.6	N/A
Mamba	Muscle	Fe	88.58%	26.4	N/A
Mamba	Skin	Fe	80.64%	32.6	N/A
Balule	Gills	Fe	88.29%	174.6	N/A
Balule	Liver	Fe	76.38%	590.7	N/A
Balule	Muscle	Fe	84.21%	23.4	N/A
Balule	Skin	Fe	79.34%	34.9	N/A
		Jul-94			Aug . 90
Mamba	Gills	Cr	84.69%	8.1143	**40**
Mamba	Liver	Cr	77.55%	5.8	**45**
Mamba	Muscle	Cr	93.07%	0.8	**21**
Mamba	Skin	Cr	80.97%	2.4	N/A
Balule	Gills	Cr	86.78%	3.9	**34**
Balule	Liver	Cr	80.62%	3.9	**39**
Balule	Muscle	Cr	91.45%	0.9	**20**
Balule	Skin	Cr	80.56%	2.3	N/A
Mamba	Gills	Cu	84.69%	1.5	**5**
Mamba	Liver	Cu	77.55%	13.5	**25**
Mamba	Muscle	Cu	93.07%	0.2	**4**
Mamba	Skin	Cu	80.97%	0.5	N/A
Balule	Gills	Cu	86.78%	1.2	**20**
Balule	Liver	Cu	80.62%	**18.4**	10
Balule	Muscle	Cu	91.45%	0.2	**5**
Balule	Skin	Cu	80.56%	0.5	N/A
Mamba	Gills	Fe	84.69%	347.1	N/A
Mamba	Liver	Fe	77.55%	390.0	N/A
Mamba	Muscle	Fe	93.07%	9.2	N/A
Mamba	Skin	Fe	80.97%	31.6	N/A
Balule	Gills	Fe	86.78%	160.8	N/A
Balule	Liver	Fe	80.62%	460.0	N/A
Balule	Muscle	Fe	91.45%	6.8	N/A
Balule	Skin	Fe	80.56%	28.4	N/A
		Nov-94			Dec-90
Mamba	Gills	Cr	87.87%	4.7	**29**
Mamba	Liver	Cr	78.78%	3.4	**26**
Mamba	Muscle	Cr	91.93%	1.0	**7**
Mamba	Skin	Cr	82.31%	2.4	N/A
Balule	Gills	Cr	87.23%	2.8	**21**
Balule	Liver	Cr	77.76%	5.7	**21**
Balule	Muscle	Cr	94.50%	0.7	**14**
Balule	Skin	Cr	81.02%	3.0	N/A
Mamba	Gills	Cu	87.87%	1.4	**7**
Mamba	Liver	Cu	78.78%	9.4	**10**
Mamba	Muscle	Cu	91.93%	0.2	**2**
Mamba	Skin	Cu	82.31%	0.4	N/A
Balule	Gills	Cu	87.23%	0.7	**8**
Balule	Liver	Cu	77.76%	**9.5**	8
Balule	Muscle	Cu	94.50%	0.1	**2**
Balule	Skin	Cu	81.02%	0.3	N/A
Mamba	Gills	Fe	87.87%	239.3	N/A
Mamba	Liver	Fe	78.78%	303.7	N/A
Mamba	Muscle	Fe	91.93%	15.6	N/A
Mamba	Skin	Fe	82.31%	41.9	N/A
Balule	Gills	Fe	87.23%	161.3	N/A
Balule	Liver	Fe	77.76%	225.1	N/A
Balule	Muscle	Fe	94.50%	11.0	N/A
Balule	Skin	Fe	81.02%	47.5	N/A

N/A not available

Concentration differences between the two localities

For Survey 1 (February 1994) the concentrations of Cr differed a great deal between the first two locations, especially for the gills and the muscle. Very little difference could separate the concentrations in the liver and skin for both localities. Of the four organs and tissues the liver and muscle mean concentrations were higher at Mamba than at Balule and the mean concentrations of the skin and gills lower at Mamba than at Balule. Survey 2 (May 1994), showed large differences between the concentrations of the gills and muscle for both localities, with the gills and liver exhibiting higher mean concentrations at Mamba. The results for Survey 3 (July 1994) showed higher mean Cr concentrations overall for all organs and tissues sampled from Mamba as compared to Balule. Mean gill concentration was far higher here than at Balule. Except for the gills, the results for Survey 4 (November 1994) showed higher mean concentrations for liver, muscle and skin at Balule.

Seasonal variation (comparison between the different surveys)

As in the case of the first four surveys, the sets of data on the concentration in the gills from both locations showed the similar increase-decrease fluctuating pattern from Survey 1 (late summer) to Survey 4 (early summer). This was also true for muscle results

obtained at Balule. The concentration in the muscle at Mamba showed some type of pattern, where the maximum concentration levels in Survey 1 (late summer) were subsequently followed by a decrease in concentration observed for autumn (May 1994) and winter (July 1994) with a slight increase in summer (November 1994). The mean liver concentration at Mamba showed a decrease from Survey 1 (late summer) to Survey 4 at the outset of summer. The mean concentration in the liver at Balule, as well as the mean concentrations in the skin at both Mamba and Balule, exhibited the same pattern. A high concentration for Survey 1 (late summer) was followed by a decrease in concentration up until Survey 3 (winter). Thereafter a slight increase in concentration was noted.

Copper

Bioaccumulation of copper (Table 2)

Variation between locations and surveys regarding the site and degree of accumulation of copper in the organs and tissues was diminutive. The results for Survey 1 showed that the highest concentration site for both locations was the liver, exhibiting concentrations of 152.84±70.17 μg/g and 108.91±39.48 μg/g, respectively. Survey 2 showed that the mean concentration of Cu was highest in the liver (42.84±26.32 μg/g) at Mamba and in the liver at Balule (100.49±40.11 μg/g). The order of bioaccumulation for Cu in the different organs and tissues was equivalent for both Mamba and Balule during Survey 3. The liver was shown to have a maximum mean concentration of 59.97±25.98 μg/g at Mamba and 94.87±23.79 μg/g at Balule. As with Survey 3, Survey 4 revealed that the order of bioaccumulation was identical for both locations. Similarly, the liver had the highest Cu concentration, with a maximum mean concentration of 44.14±20.26 at Mamba and 42.69±23.30 μg/g at Balule. The general degree of Cu concentration in the organs and tissues for both Mamba and Balule was found to have the following order: liver > gills > muscle > skin. Individual organ and tissue concentrations at the same locality varied. The liver concentrations at Mamba during Survey 3, for example, had concentrations ranging from 12.87 μg/g to 102.66 μg/g.

Concentration differences between the two localities

For Survey 1 (February 1994), it was concluded that the mean concentrations of Cu in the liver, muscle and skin were higher at Mamba than at Balule. A similar, but opposite, pattern was evident for Survey 2 (May 1994), where the mean concentrations in the gills, liver and skin at Mamba were much lower than those found at Balule. Survey 3 (July 1994) revealed that the mean concentrations in the gills and muscle were higher at Mamba than at Balule, whilst the mean concentrations in the liver and skin were lower. The data obtained for Survey 4 (November 1994) showed higher mean Cu concentrations for all the organs and tissues at Mamba relative to those found at Balule.

Seasonal variation (comparison between the different surveys)

There was variation with regard to the mean concentrations for the individual organs and tissues found at the different surveys throughout the year. A reduction in the mean concentration levels in the gills and muscle at Mamba was evident from the first survey (late summer) to the second survey (autumn) with the minimum concentration being attained during winter (Survey 3). The mean concentration in the gills, liver, muscle and skin at Balule, as well as the concentration in the skin at Mamba, presented the same seasonal pattern. A maximum concentration during Survey 1 (late summer), with a subsequent decrease in concentration for the second (autumn), third (winter) and fourth surveys (early summer) were recorded. The concentration in the liver at Mamba was the only organ to show fluctuating results throughout the study period.

Iron

Bioaccumulation of iron (Table 2)

There was variation in the accumulated concentrations of Fe between the different organs and tissues. However, in the first three surveys at both locations (February, May and July 1994), one aspect was constant throughout: the fact that the liver accumulated the most Fe. From Survey 1 to three the mean Fe concentrations for Mamba were the following: 2380.58±1648.5 μg/g; 1382±981.52 μg/g and 1737±827.81 μg/g and for Balule the concentrations of Fe in the liver were the following: 2573.51±608.50 μg/g; 2500.73±1153 μg/g and 2373.57±1221.10 μg/g. During Survey 4 (November 1994) both locations showed that the gills had the maximum mean concentrations of Fe 1973±772.02 μg/g and 1263.05±723.30 μg/g, respectively, with liver being a close second. The general degree of Fe concentration in the organs and tissues sampled at Mamba was observed to be in the following sequence: gills $\geq$ liver > muscle $\geq$ skin, while at Balule it was found to be: liver > gills > skin > muscle.

The mean concentration of Fe in each organ and tissue sampled, differed greatly. At Mamba for example, during Survey 3, the concentration in the liver varied from 754.62 μg/g to 3425.30 μg/g. The standard deviation for the concentration of iron in various individuals was extremely high, indicating large variation between individuals.

Concentration differences between the two localities

There was no distinctive trend as to which location generally had the highest mean concentration. For Survey 1 (February 1994) the mean concentrations of all the organs and tissues were lower at Mamba, with large differences in concentrations separating the locations. In Survey 2 (May 1994), with the exception of the muscle, the remaining organs all exhibited lower concentrations at Mamba, the mean concentrations being slightly higher at Balule. The reverse was evident for Survey 3 (July 1994) where, with the exception of the liver, the mean concentrations in all the other organs and tissues were highest at Mamba. For Survey 4 (November 1994), the concentrations in the gills and liver were higher at Mamba, whilst the muscle and skin concentrations were lower.

Seasonal variation (comparison between the different surveys)

In the different organs and tissues variation occurred during the different seasons at the respective locations. At Mamba the mean concentration in the gills showed an increase from Survey 1 (late summer), through to Survey 3 (winter), where it peaked and then decreased slightly, as was observed from the results of Survey 4 (early summer). At Balule, however, the mean concentration in the gills fluctuated slightly, with a low concentration in Survey 1 (late summer), a high concentration in Survey 2 (autumn), etc. Generally, concentrations remained similar. The liver concentration at Mamba showed no general seasonal pattern. The mean concentration in the liver at Balule showed a decrease for subsequent surveys, from Survey 1 (late summer) to Survey 4 (early summer). The seasonal pattern for the mean muscle concentration, as depicted for 1994, showed that at Mamba and Balule the concentration decreased from Survey 1 (late summer) to Survey 3 (winter). Thereafter, it increased slightly as was observed in Survey 4 (early summer). At

TABLE 4
Table showing the ranking of metal concentrations in the different organs and tissues

Metal		Ranking of accumulation: the highest to the lowest concentration			
		Organ	Organ	Organ	Organ
Chromium	(Cr μg/g)	⇒ gills	>liver	>muscle	>skin
Copper	(Cu μg/g)	⇒ liver	>gills	>muscle	>skin
Iron	(Fe μg/g)	⇒ liver	>gills	>muscle	>skin

Mamba and Balule a maximum concentration was detected during Survey 1 (late summer). The concentration of the Fe in the skin was seen to have lowered nominally by Survey 3 (winter). Thereafter, it increased marginally in the early summer during Survey 4.

Summary of metal bioaccumulation

All the metals tested for were detected in reasonably high concentrations in *C. gariepinus*. The general pattern with regard to metal bioaccumulation was that in the case of Cr and Cu, highest concentrations were evident at Mamba. Contrary to this, the concentration of iron was found to be higher at Balule. Seasonal patterns showed variation among the different metals. The data obtained for Mamba and Balule revealed that Cr exhibited highest concentrations in the gills, with concentrations of Cu and Fe peaking in the liver. The order of the degree of concentration for the three metals in the different organs is shown in Table 4.

MANOVA/ Multivariate analysis

Tissue types
A value of 0.000 was recorded as a significant p-value when the Wilks' Lambda test was employed for tissue types. This indicates an absolute significant difference between tissue types with a 99% confidence limit.

Seasons
A value of 0.000 was recorded as a significant p-value when the Wilks' Lambda test was employed for seasons. This indicated an absolute significant difference between the seasons sampled with a 99% confidence limit.

Sample sites
A value of 0.118 was recorded as a significant p-value when the Hotelling T^2 test was employed for sample sites. The high value recorded indicated that no significant difference was present between sample sites as the significant p-value is greater than the 0.01 significance limit.

ANOVA (Tables 5-7)

Tissues
Significant p-values were recorded for Cu above the 0.000 significance level when muscle was compared to gills at 0.001 and when skin was compared to muscle at 0.007.

Seasons
Significant p-values were recorded above the 0.000 significance level for Cu when autumn was compared to summer at 0.008 and winter was compared to summer at 0.003. Furthermore, during the spring autumn comparison a p-value above 0.000 was recorded (0.014). Likewise, during the spring winter comparison at 0.039 no p-values were recorded for Cr above the 0.000 significance level indicating a confidence limit of 95% for this metal during all four seasons. For Fe a significant p-value (above the 0.000 significance) was only recorded above the 0.000 significant level when summer was compared to winter when a value of 0.006 was obtained.

Sample sites (Table 8)
The ANOVA test was not employed as only two sites were used. The Levene's test for equality indicated no significant differences between the two sites for these metals with 0.312 recorded for Cu, 0.975 for Cr and 0.018 for Fe. Because the Multivariate analysis (equivalent to the MANOVA test) done for sample sites indicated no significant difference between sample sites, the value obtained for iron may not be significant, but just be due to the sample group tested, i.e. by chance. This means that one could probably ignore this value and assume that no significant difference exists between the sample sites for iron.

Discussion

Significant variation among individuals was observed for all bioaccumulated metals tested and in all the organs and tissues sampled. Possible reasons for the variation are:

1) Variation in the individual's optimum for essential elements due to age, size or genetic variability.
2) Variation in fish size or health.
3) Time of residence, in particular collection area.
4) Differential stress during capture and retrieval or further undocumented events such as feeding habits or previous disease (Pinder and Giesy, 1981).

Concentration differences were also observed for the individual metals in the same organs and tissues. From literature cited (Matthiessen and Brafield, 1977; Holcombe et al., 1979), differences in tissue metal concentrations of fish from a specific locality and in experimental conditions where fish were exposed to metal, were recorded. Another important factor that must be considered is that metal concentrations in fish are the result of complex processes associated with uptake, excretion rates and homeostasis in fish (Giesy and Wiener, 1977; Heath, 1987). The coefficient of variation (cv) which reflects variation among individual fish was relatively high. Generally, it was the result of mostly single organ or tissue samples that contained high concentration levels. Other scientists have observed similar large variations in related studies (Pagenkopf and Neuman, 1974; Ray, 1978; Du Preez and Steyn, 1992).

Chromium

In this study the Cr levels in the different organs and tissues sampled revealed high individual variations. The Cr concentrations in the tissues of *C. gariepinus* recorded, were lower during all seasons and in all tissues types than for comparative studies done at Mamba and Balule on *C. gariepinus* by Van der Merwe (1992) in 1990/1991 and shown in Table 3. The general ratio of the Cr concentrations between the various organs and tissues being, gills > liver > skin > muscle, agrees with results obtained in similar studies by Kuhnert and Kuhnert (1976) on *Salmo gairdneri* and

TABLE 5
Table showing significance levels for tissue types and seasons. The significant p-values above 0.000 are highlighted in bold, bold and italics, bold italics and underline or in italics and underline. This table should be used in conjunction with Tables 4 and 5.

Legend	Tissue type Significance values	Seasons Significance values
Copper (Cu)	0.000/**0.001**/***0.007***	**0.008**/***0.003***/0.000/***0.014***/*0.039*
Chromium (Cr)	0.000	0.000
Iron (Fe)	0.000	0.000/**0.006**

TABLE 6
Table showing the metals, which recorded a significant difference when compared with the different tissue types separately. The metals highlighted in bold, bold and italics, bold italics and underline or in italics and underline recorded significance values above 0.000.

Tissue type	Gills	Muscle	Liver	Skin
Gills		**Cu**, Fe	Cu, Cr, Fe	Cu, Cr, Fe
Muscle	**Cu**, Fe		Cu, Fe	***Cu***
Liver	Cu, Cr, Fe	Cu, Fe		Cu, Fe
Skin	Cu, Cr, Fe	***Cu***	Cu, Fe	

Table 7
Table showing the metals, which recorded a significant difference when compared with the different seasons separately. The metals highlighted in bold, bold and italics, bold italics and underline or in italics and underline, recorded significance values above 0.000.

Seasons	Summer	Autumn	Winter	Spring
Summer		**Cu**, Cr, Fe	***Cu***, Cr, **Fe**	Cu, Cr, Fe
Autumn	**Cu**, Cr, Fe		Cr	***Cu***, Cr
Winter	***Cu***, Cr, **Fe**	Cr		*Cu*
Spring	*Cu*, Cr, Fe	***Cu***, Cr	*Cu*	

TABLE 8
Table showing the sample site according to the Levene's test for equality of variance

Metal	Value
Cu	0.312 (Equal variance not assumed)
Cr	0.975 (Equal variance assumed)
Fe	0.018 (Equal variance not assumed)

$p < 0.05$ therefore 5% significance level applies = 95% confidence limit.

Van der Merwe (1992) for *C. gariepinus*. The degree of Cr bioaccumulation in tissues suggests that Cr was taken up more readily by the gills. Sellers et al. (1975), reported on findings where Cr readily accumulates in the gill tissue, increasing gill metal concentration levels above that of the other organs and tissues. This, however, is regulated by water pH levels as pointed out by Van der Putte et al. (1981a & b). They observed that the gills were the primary site of toxic action at a pH value of 6.5. Increasing the exposure water to a pH of 7.8 resulted in higher accumulation of Cr in the internal organs than in the gills. This, however, was not the case in the present study. Many of the gill samples analysed had a higher bioaccumulation of Cr compared to tissues tested at the different locations and surveys, even with three of the four surveys showing a pH well over 8.00. Van der Putte et al. (1981a) determined that decreasing the pH of the exposure water from 7.8 to 6.5 increased the toxicity of hexavalent Cr to rainbow trout, *Salmo gairdneri* by a factor of between 3.2 and 3.6 times.

Liver was the tissue with the second highest Cr accumulation. The liver is a primary storage and detoxification site for Cr (Klaassen, 1976). It is suggested that liver chromium is stored linked to proteins and smaller peptides such as glutathione (Gaugl-hofer and Bianchi, 1991). According to Mertz (1969), fish excrete chromium via their faeces, as was shown by high levels in the bile of fish during and after the ingestion of contaminated food or contaminated water (Heath, 1987). However, high concentrations, particularly in the gills and liver, may be as a result of the slow elimination rate of chromium by the fish once it has accumulated (Buhler et al., 1977).

Generally, the sites and level of Cr bioaccumulation did not differ much between Mamba and Balule, except for the mean concentration in the gills. The concentration in the gills at Mamba showed that this location was more polluted than at Balule. This points to the bioavailability of chromium being lower at Balule. The alkalinity ($CaCO_3$) or hardness has a definite effect on the amount of chromium which is bioavailable to the fish (Prosi, 1979). The harder the water, the less chromium is bioavailable as was observed for the water at Balule, which was much harder than at Mamba. Studies by Pickering and Henderson (1966) showed that trivalent chromium was significantly more toxic to both the fathead minnow and the bluegill sunfish in soft water than in hard water. Furthermore, water hardness affects the gill permeability to water and ions so that the harder the water, the less permeable the tissue becomes. The calcium ion responsible for the hardness of water

also causes the electrical charge on the outside of the gills to be more positive, causing further repulsion of positively charged molecules (Mc Williams and Potts, 1978).

Temperature is known to have an effect on metabolism (Cairns et al., 1975), influencing the rate of metabolic processes including the uptake, metabolism and excretion of metals. For example, 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 the release of heavy metals by organisms (Prosi, 1979; Abel, 1989). This could explain the low mean concentration rates in all the organs and tissues during winter as the temperatures at both locations were lower, by between 3°C and 5°C as compared to the rest of the surveys. The fact that an increase in the bioaccumulation of chromium was observed in three of the four organs and tissues during the warmest sampling periods (February and November), shows a seasonal pattern, with highs in summer and lows in winter. Prosi (1979), also notes that the toxicity of metals to fish increases with higher water temperatures and a reduction in dissolved oxygen because the tendency of increased respiration rates in fish results in an increase in Cr uptake. Smith and Heath (1979 demonstrated that with channel catfish and other fish species, slightly higher LC_{50}s are achieved at higher temperatures.

High salinity levels in the water could also have contributed to the 'type' of seasonal pattern observed. During winter the total dissolved solids, sodium and chloride concentrations were at a maximum while the bioaccumulation concentration was at a minimum. This agrees with Barron's (1990) and Phillips' (1980) findings that the metal uptake rate increases as salinity decreases, which could be due to the fact that freshwater fish are hyperosmotic to a low saline environment and, therefore, the influx into the fish may facilitate chemical uptake.

In summer, rain dilutes the concentrations of metals in the water and might decrease the Cr concentration available for uptake. However, from the water data it was evident that the Cr concentration in the water remained constant throughout the sampling period of 1994. Therefore, with the water variables contributing largely to the rate of metal uptake by the fish, it is obvious that the seasonal pattern in the bioaccumulation concentrations shows the variable effects of bioavailability to be true.

Chromium levels were relatively high in tissues as compared to the concentration found in the water. However, the Cr concentration in the sediment was much higher. This could be suggestive of a large quantity of Cr uptake via the food chain because of the omnivorous and bottom feeding habits of *C. gariepinus*. The uptake route via the food chain was ruled out by Knoll and Fromm (1960). These authors experimentally determined that Cr pumped into the gut was eliminated almost immediately, with no Cr being distributed to other tissues, thereby implicating the gills as the major source of Cr uptake. According to Eisler (1986), no biomagnification of Cr has been observed in food chains, with the highest concentrations being at the lowest trophic levels. It has been found that some fish are capable of bio-accumulating Cr levels nearly 100 times the concentration of Cr in the water. Exposure, as well as unknown factors, influences the time required to reach equilibrium tissue levels of Cr. With fluctuating Cr levels in the water (which was not detected because water was monitored only four times in one year), fish will not experience mass cumulative Cr uptake as it is rapidly eliminated by fish in water with a low concentration or in uncontaminated water (Phillips and Russo, 1978).

Copper

From the results it was apparent that high individual variation for the Cu concentrations in the different organs and tissues exists. However, results on the ratio of the Cu concentrations between the various tissues were uniform throughout all the surveys. The concentration in tissues was found to be in the following sequence, liver > gills > muscle > skin. The sequence resulting from the degree of concentration was supported by the findings of Buckley et al. (1982), Du Preez and Steyn (1992), Miller et al. (1992) and Van der Merwe (1992). Thus the gills are the primary uptake site of Cu from the water. Similar studies done by Van der Merwe (1992) show higher levels of Cu bioaccumulation in *C. gariepinus* during the 1990/1991 study of the Olifants River in the KNP, when compared to the results obtained in the present study in all but three occasions (Table 3).

However, with the greatest accumulation of Cu occurring in the liver, it reinforces the view that the liver in fish plays a protective role against chronic heavy metal exposures by producing metallo-thioneins (Mc Carter and Roch, 1983), acting as a storage site, and being a vital organ in the regulation of copper (Buckley et al., 1982). Metallothioneins have already been isolated from fish liver (Carpene and Vašák, 1989) and are low molecular-weight cytosolic proteins. These metallothioneins have high affinities for Cu and other heavy metals, and in doing so, concentrate and regulate these metals (Klerks and Levinton, 1989; Carpene and Vašák, 1989). Wittmann (1979) stated that the liver proteins, haemocuprein and hepatocuprein, as well as several oxidative enzymes, need Cu as an important component to function. Some evidence has shown that, of the concentrated Cu levels in the liver of the fish, at least some copper undergoes urinary and biliary excretion (Dixon and Sprague, 1981; Heath, 1987).

According to Stokes (1979), fish muscle normally contains low concentrations of Cu and, even at high levels of Cu exposure, muscle does not often reflect increases in the external environment. In contrast, the gill tissue of the fish tends to concentrate Cu from the water. The gills represent the area of closest proximity between the internal and external environments, due to the short diffusion distance from the water to the blood, as well as the large surface area exposed to the water. The resultant absorption and binding of Cu ions to the branchial surface would increase the concentration of Cu in the gills (Stagg and Shuttleworth, 1982). Furthermore, the gills possess an extensive vascular network, which, according to Laurent and Dunel (1980), brings the gill tissue into close contact with any blood-borne metal. It has been shown that Cu accumulation essentially occurs in osmotic and ionic regulating organs such as the gills (Benoit, 1975; Stagg and Shuttleworth, 1982). Data obtained on Cu exposure to the gills of the eel, *Anguilla anguilla*, revealed that, due to the induction of elevated ambient copper, metallothionein was produced which, in turn, binds to Cu (Noël-Lambot et al., 1978). When fish are exposed to high Cu levels, the gills are the first organs that are affected by this increase. Mucous cells respond by increasing in activity, size and abundance. Histological damage (Cardeilhac et al., 1979) and impaired physiological function (Sellers et al., 1975; Cardeilhac et al., 1979) indicates that Cu binds to gill tissue and result in tissue damage. It has been concluded from the results of many studies that the accumulation of Cu in the gill is possibly due to its binding to haemopoeitic tissue, mucous and metallothioneins implicated with excretion and detoxification.

With the exception of the fourth survey (November 1994), where all the organs and tissues showed bioaccumulation levels higher at Mamba than at Balule, the level of bioaccumulation did

not differ much between the two locations. The mean concentration of Cu in the gills and the muscle, with the exception of Survey 2, continually exhibited higher concentrations at Mamba for 1994, indicating higher Cu bioavailability to the fish at this site. The results for Survey 2 (May 1994) conflicted with the general findings of higher bioaccumulation at Mamba. Here, the Cu bioaccumulation for the gills, liver and muscle was much higher at Balule.

Bioavailability of Cu to the fish is influenced by a number of factors: alkalinity, hardness and pH being of primary importance, as well as chemical processes, including: absorption onto particulate matter, precipitation and complexation with inorganic and organic ligands (Stiff, 1971). Howarth and Sprague (1978) determined, by experimenting with rainbow trout, that high hardness decreased toxicity at any pH. However, at a high pH of 9 and at high hardness levels, Cu's toxicity increases. Lloyd and Herbert (1962), postulated that hydroxyl Cu complexes accumulate between the gill filaments and the consequent excretion of carbon dioxides at the gill surface lowers the surrounding pH, ionizes hydroxides, liberating considerable amounts of cupric ion which is taken in by the gill. This scenario could well have contributed to the higher bioaccumulation of Cu in the gills at Mamba, as the pH here was higher than at Balule. Lloyd and Herbert (1962), and Howarth and Sprague (1978), demonstrated that increasing hardness, as was observed at Balule, displays a protective effect due to Cu complexing with OH^- and CO_3^{2-} anions, thereby decreasing the bioavailability of Cu to the fish.

Higher temperatures result in decreasing oxygen levels, leading to increases in the metabolic rate. Because of this, the fish take up greater amounts of Cu as a result of the increased diffusion or active uptake associated with higher rates of water movement across the gills or other cell membranes (Prosi, 1979). Furthermore, the properties of the copper metal itself may be directly influenced by temperature, by changing the equilibrium effect between molecular and ionised forms (Cairns et al., 1975). The higher the temperature, the more ionised copper forms are produced resulting in greater toxicity to the fish (Prosi, 1979). Lower temperature would induce lower metabolism as observed during winter (Survey 3). All bioaccumulation results were lowest during this period, with high concentrations being recorded for Survey 1 (February 1994) and occasionally for Survey 4 (November 1994), which were the warmest sampling periods for 1994. The seasonal 'type' pattern observed showed that in winter bioaccumulation was at a minimum, while generally in summer bioaccumulation rates were at a maximum.

According to Moore and Ramamoorthy (1984), the amount of Cu taken in by food consumption is immense and is probably a more important source of Cu than water. Therefore, bioaccumulation in fish cannot be consistently related to the ambient pollution levels. This statement is backed by the findings of Du Preez and Steyn (1992), and Seymore (1994), who found the concentration of Cu to be much higher in the gut than in the gills. Several authors (Knox et al., 1982; Gatlin et al., 1989) have shown that increased concentrations in the liver and gills were due to increased Cu in the diet. The resultant biomagnification of Cu will increase the concentration in the internal organs, as the liver takes up the majority of Cu to detoxify it. According to Miller et al. (1993), diet appeared to be the dominant source of Cu to rainbow trout, particularly in the liver. This, too, can justify the findings of far higher liver Cu concentrations.

Iron

Iron was detected in all tissues sampled and it was observed that *C. gariepinus* is able to bioaccumulate high levels of Fe. Great variation exists in the level of iron concentration tissues, with the degree of Fe concentration being present producing the following general sequence: liver > gills > muscle ≥ skin. Rehwoldt et al. (1976), Du Preez and Steyn (1992) and Seymore (1994) found coinciding sequences for the accumulation of Fe. These results conclude that the liver is the primary organ for Fe bioaccumulation. The levels of Fe in different tissues in fish are mainly due to the presence and metabolism of haemoglobin (Bryan, 1976a). The liver has a vast vascular network where blood passes through. Iron released from the breakdown of haemoglobin, as well as excess Fe found in the body, is stored and detoxified in the liver (Buckley et al., 1982; Schmidt-Nielson, 1991).

The gills, which are continually exposed to the external environment, also exhibited high accumulation levels, which could be attributed to a number of factors. Firstly, Rehwoldt et al. (1972), proposed that the gill system acts as a filter, thus allowing high concentrations of metals which are bound to particles (suspended solids) to become embedded on the gill surfaces and not in the tissue itself. However, Heath (1987) suggests that Fe complexes with the mucous around the gills. The high concentrations of Fe found in the sediment could have resulted in the high Fe levels in the gills during the process of bottom feeding of *C. gariepinus*. With an immense vascular network in close proximity to the gill tissue, the gills will be in continual contact with the blood-borne Fe (Laurent and Dunel, 1980). A distinct preference as to which location had the highest bioaccumulation could be established. However, results from Survey 1 (February 1994), show bioaccumulation levels in all the organs and tissues at Balule to be far greater than at Mamba. The concentration of Fe in the liver, with the exception of Survey 4, was consistently much higher at Balule than at Mamba. Concentration differences between the skin and muscle at these locations were minimal.

One of the reasons that these higher levels at Balule exist could be attributed to the surrounding rock formations which produce Fe through the process of weathering. The Fe concentration in the sediment at Balule could substantiate this, as the concentrations are far higher at Balule than at Mamba. Additionally, Wetzel (1983) stated that Fe enrichment is commonly found in water where the content of organic matter is high. Consistent with the previous statement, Shapiro (1957) established that high concentrations of complexed soluble Fe are found to be associated with high levels of humic acids. Humic acids form as a result of decomposed vegetation and as Balule has extensive reed bed colonies upstream of and at the sampling site, Fe concentrations would be much higher. Another underlying reason could be the significance of abiotic factors affecting the bioavailability of Fe to the fish. According to Hem (1989), apart from the fact that ferric iron forms inorganic complexes with hydroxyl-anions, the inorganic complexes it has with many other anions such as chloride, fluoride, sulphate and phosphate are important in regulating Fe in natural systems. The concentration of these abiotic factors was generally higher at Mamba than at Balule (Table 1 and Marx, 1996), rendering iron less bioavailable to fish at Mamba. Results from studies done by Seymore (1994), showed the bioaccumulation of iron in *B. marequensis* was higher at Balule than at Mamba, with the exception of a few cases, supporting the results obtained in this study.

No clearly defined seasonal pattern was evident for the organs and tissues. The concentration in the liver at Balule, as well as in the skin and muscle at both Mamba and Balule, showed a seasonal

pattern: maximum concentrations during summer (February and November 1994) and minimum concentrations during winter (July 1994). These patterns, as previously mentioned, result from differences in temperature and salinity (total dissolved solids, sodium, chloride). However, according to the Department of Water Affairs and Forestry (1993a), increasing water temperatures and elevated alkalinity levels enhance the oxidation of the soluble Fe^{2+} to the insoluble Fe^{3+} iron states. The abiotic factors mentioned above are also at a maximum level during winter (July 1994), further decreasing the bioavailability of iron to fish during this period.

ANOVA

Tissue types

Copper

All four tissue types tested reflected a significant difference when compared to each other, indicating a high degree of variability between these tissue types. Certain physiological processes may be stimulated by copper uptake, causing variable activity in various organs and tissue. This is particularly evident during blood production where copper is involved in haemoglobin synthesis.

Iron

The skin/muscle comparison was the only statistical analysis which did not provide a significant difference. This is possibly an indication that the same rate of storage in muscle and excretion via the skin took place. Iron is a primary component of haemoglobin (Friberg et al., 1986). Iron is also required for enzymatic pathways of protein synthesis and in respiratory enzymes (Wetzel, 1983). This suggests that the gills are the primary sites of iron activity, because they are the primary sites of respiration, continually circulated with blood. This too may indicate that gills, rather than skin, are the site of iron excretion. This low storage of iron in muscle would be accompanied by low iron concentrations in the skin. This further substantiates the low significant differences observed when these two tissue types are compared.

Chromium

Significant differences were observed during the gill/liver and gill/skin comparisons. This may indicate that gills are both the site of uptake and excretion of this metal. This is indicated, firstly, by the differences observed between gill and liver concentrations, with detoxification appearing not to equal uptake, as well as by the fact that significant differences were also observed between gill and skin concentrations. This suggests that different rates of excretion took place between these sites. Maximum concentrations were recorded in the gills with skin recording the minimum concentrations when all four tissue types were compared. This suggests that gills too may well be the site of excretion of this metal rather than the skin. This further explains why it appears as if the rate of detoxification did not equal the rate of uptake.

Seasons

Significance values recorded for Cu and Cr appeared variable between seasons, recording significant differences between almost all comparisons. Significant differences were however only recorded for iron when summer was compared with the other three seasons. According to Luoma (1983) temperature could affect the quantity of metal accumulation by organisms, and this may affect metal bioavailability. This indicates that temperature may play a pivotal role in iron bioaccumulation because of the high temperatures experienced at the sites during summer months.

Conclusion

From the findings of this study it is concluded that metals at elevated levels in the aquatic environment can accumulate in tissues of fish. *C. gariepinus* generally bioaccumulated the highest amount of metals in its gills followed by its liver, skin and muscle. The high metal concentrations detected in the gills and liver might indicate long-term (chronic) exposure of the fish to these metals. Only Cr and Cu could be compared to previous studies. In the vast majority of cases the metal accumulation was far lower than that recorded by Du Preez *et al.* (1994) for 1991-1992.

Abiotic factors such as temperature, pH and hardness have shown to influence the bioavailability of metals to the fish. Through routine monitoring of the water at the sampling locations, these abiotic factors should be maintained at constant and, if possible, acceptable levels. However, if this is not achieved, it is highly probable that an increase in metal accumulation and toxicity would result which, ultimately, could be detrimental to the health of the fish.

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