Chromium, copper, iron and manganese bioaccumulation in some organs and tissues of *Oreochromis mossambicus* from the lower Olifants River, inside the Kruger National Park

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Abstract

The bioaccumulation of chromium, copper, iron and manganese by *Oreochromis mossambicus* was investigated at two localities (Mamba and Balule) in the lower Olifants River, inside the boundaries of the Kruger National Park. The Cr, Cu, Fe and Mn concentrations, recorded in the tissues of *O. mossambicus* at Mamba and Balule, did not differ much from each other. However, it was generally found that the highest accumulation of metals by the fish occurred at Mamba, except for iron, where the opposite occurred. The metals investigated during this study mainly accumulated in the liver and gills, followed by the skin, and lastly the muscle.

Introduction

Biological systems function within relatively narrow physical and chemical limits. Pollution of natural aquatic ecosystems occurs frequently as population densities lead to increasing mining and industrial activities (Ellis, 1989). The release of metal ions into river systems poses a serious threat to aquatic life, and causes secondary effects upon water quality (Rehwoldt et al., 1971). Two factors which contribute to the damaging effect of metals as environmental pollutants are: Firstly, the inadequacy of biological degradation to inert metals - as in the case of most organic pollutants - and secondly, the trend of metals to accumulate and largely remain in the aquatic environment (Förstner and Prosi, 1979).

Metal ions are commonly present and are readily taken in by aquatic organisms through the digestive system, gills and integument. These ions are individually, or in combination, important factors of water pollution (Calamari and Marchetti, 1973). Ellis (1989) states that the effect of two or more toxicants may be additive, antagonistic or even synergistic. There are several factors which affect the toxicity of pollutants to aquatic organisms, and they can be divided into biotic factors, such as tolerance (Sprague, 1971), growth and reproduction (Cross et al., 1973), species variation, inter- and intraspecific variation (Abel, 1989) and nutrition (Lanno et al., 1989), and abiotic factors, such as organics (Mouvet and Bourg, 1983), pH (Alabaster and Lloyd, 1980), temperature (Cairns et al., 1975), alkalinity and hardness (Sprague, 1970), metal interactions (Lloyd, 1965), sediment (Burton et al., 1992) and dissolved oxygen (DO) (Lloyd, 1992).

A typical "dynamic" environment is a system where mechanical, biological and chemical phenomena take place, with strong gradients of chemical parameters (E_h, pH) and variations in time (e.g. biological activities). For example, changes in water hardness may lead to the release of certain metals into the aquatic environment (Lloyd, 1992), and furthermore, the position that an organism occupies in the food chain may also play a role (Aoyama et al., 1978). The presence of heavy metals in the aquatic ecosystem becomes harmful to organisms when the concentrations rise above the natural background in water and sediment. These metals must be in a bioavailable chemical state to cause significant heavy metal uptake by organisms (Abel, 1989). It is thus clear that the physico-chemical parameters which influence metal bioavailability, play an essential role in the bioaccumulation and toxicity of metals in aquatic organisms.

In the aquatic environment Cr, Cu, Fe and Mn are commonly present. Cr is essentially present in the oxidation states 3+ and 6+, which are pH dependent (Dyg et al., 1990). It is, furthermore, known that Cr is one of the trace metals which, at low concentrations, is least toxic, and that the hexavalant ion (Cr^{6+}) is the most toxic in elevated concentrations (Kraybill et al., 1978; Wittmann, 1979; Duffus, 1980; Dallas and Day, 1993). Copper, a common metal (i.e. >5 $g \cdot cm^{-3}$) in the environment (Duffus, 1980), occurs in nature as Cu^{+}, Cu^{2+} and Cu^{3+} oxidation states (Leckie and Davis, 1979; Thornton, 1979; Cole, 1983), and is toxic at high concentrations (Lee et al., 1990), as is the case with most other metals. The toxicity of Cu is largely attributed to Cu^{2+} (Sylva, 1976; Luoma, 1983; Abel, 1989) and $CuOH^{+}$ (Luoma, 1983), which is only present in small quantities in freshwater (Boyle, 1979). The cupric form of Cu speedily forms complexes with inorganic and organic substances and can be adsorbed onto particulate matter. As a result, free Cu ions rarely occur freely in water, except in acidic soft waters (Alabaster and Lloyd, 1980).

Fe exists commonly in two oxidation states in solution, i.e. the ferrous (Fe^{2+}) and the ferric (Fe^{3+}) (Wetzel, 1975; Cole, 1983; Department of Water Affairs and Forestry, 1993), with the latter the most common state found in surface waters (Department of Water Affairs and Forestry, 1993). The concentration and chemical behaviour of dissolved Fe is greatly influenced by organic complexing agents. According to Förstner (1979) and the Department of Water Affairs and Forestry (1993), Fe only exists as Fe^{3+} under highly oxygenated and in slightly acidic to alkaline conditions. On the other hand, the solubility of Fe^{2+} is facilitated by the presence of dissolved oxygen under strongly acidic conditions, usually at pH 3, as in the case of acid mine drainage (Förstner, 1979; Department of Water Affairs and Forestry, 1993). According to Wetzel (1975), the cycling of Fe is largely attributed to the oxidation-reduction conversion reactions, which are interdependent on other constituents and properties, of which microorganisms - belonging to the *Thiobacillus-Ferrobacillus* group - act as major mediators (Duffus, 1980; Cole, 1983; Department of

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Water Affairs and Forestry, 1993).

Manganese, the second most abundant metal in nature (Wittmann, 1979), may exist in several oxidation states, ranging from -3 to +7 (Environment Canada, 1987), of which manganous Mn(II) and manganic Mn(IV) are the most important states (Environment Canada, 1987). Soluble ionic divalent Mn^{2+} persists in water at low redox potential (E_h), pH (Förstner, 1979) and oxygen concentrations (Department of Water Affairs and Forestry, 1993). Mn does not carry much weight as a hazardous pollutant in aquatic ecosystems (Hellawell, 1986; Wepener et al., 1992; Seymore et al., 1995) and rarely exceeds concentrations of 1.0 $mg \cdot \ell^{-1}$ in water (Hellawell, 1986; Department of Water Affairs and Forestry, 1993).

Most metal ions taken in by aquatic animals are essential micronutrients for life. Duffus (1980) states that Cr is essential in trace quantities, for fat and carbohydrate metabolism in most organisms. Steenkamp et al. (1994), on the other hand, state that life processes will not function at their maximum efficiency if metals such as Cu are only available in limited quantities. Cu is actively involved in many physiological and biochemical reactions in aquatic organisms. For example, Cu is involved in essential redox reactions, forms an essential part of cytochrome oxidase and is a constituent of a number of enzymes, to name but a few. This means that normal Cu metabolism should be closely related to both high and low concentrations within the fish's body (Dallas and Day, 1993).

Fe is associated with various enzymes in living systems, such as peroxidase, catalase, cytochrome, oxidase, and nitrogenase, to name but a few (Cole, 1983). Fe is also a basic component of haemoglobin (respiratory pigment) in blood - an oxygen-carrying protein molecule of blood - and is regarded as the most essential Fe^{2+} complex consisting of the globin protein with four heme units attached to it (Wetzel, 1975; Wittmann, 1979; Dallas and Day, 1993). According to several authors, Mn is an essential element in micro-organisms, plants, animals and man. Mn is a co-factor and essential catalyst in various enzyme systems, for example, the functioning of flavoprotein and the synthesis of sulphates, mucopolysaccharides and cholesterol, an essential component in numerous metabolic processes in animals and bacteria (Wetzel, 1975; Förstner and Prosi, 1979; Wittmann, 1979; Duffus, 1980; Dallas and Day, 1993; Department of Water Affairs and Forestry, 1993; Seymore et al., 1995).

Although these metal ions are essential for aquatic life, excessive amounts may be detrimental. In general it is maintained that chronic exposure of fish to Cr has a detrimental effect on immunological responses (O'Neill, 1981; Zeeman and Brindley, 1981). O'Neill (1981) demonstrated significant weight loss and reduced haematocrit and serum protein levels in fish exposed to Cr, whilst Beisel (1982) established that Cr inhibits RNA synthesis and the proliferative stimulation of lymphocytes at concentrations as low as 10^{-7} M. According to Stiff (1971), Cu may have serious damaging effects on aquatic life. Cu^{2+} causes cell damage by altering the active sites of cellular enzymes and the peroxidation of membranes, thus causing serious modifications in the cell and environment/cell interface. Furthermore, Cu^{2+} could have damaging effects on the organs of fish. For example, Zeeman and Brindley (1981) found that fish exposed to 1 to 3.2 mg/kg Cu^{2+} showed a marked increase in the necrosis of the interstitial hematopoietic tissues in the kidney of fish.

The toxicity of Fe to the aquatic environment is greatly influenced by the chemical state in which the metal finds itself - i.e. either the ferrous (Fe^{2+}) or ferric (Fe^{3+}) state, as well as whether Fe is in suspension or in solution (Dallas and Day, 1993). For example, Fe compounds are readily oxidised, and high concentrations of reduced Fe ions may result in oxygen depletion in the environment (Dallas and Day, 1993), which will ultimately cause oxygen deficiencies in fish. Furthermore, Fe has an inhibiting effect on various enzymes (Singh and Sivalingam, 1982). According to Dallas and Day (1993), the threshold concentration of Fe to fish is 0.2 $mg \cdot \ell^{-1}$. Wetzel (1975), Förstner and Prosi (1979) and Dallas and Day (1993) state that Mn, which is toxic at high concentrations, may have detrimental effects on aquatic organisms. Wepener et al. (1992) observed a decrease in the amount of red blood cells and haematocrit of *Tilapia sparmanii* after exposure to Mn at pH values 5 and 7.4. This is a result of internal haemorrhaging, which probably occurred as a result of necrosis of the internal mucosa and kidneys. The anaemic conditions observed by Wepener et al. (1992) resulted from damage of heamapoietic tissue, such as the spleen and kidney. According to Wepener et al. (1992), Mn-induced anaemia could thus be seen as a secondary effect to Mn pollution of the aquatic environment.

Dallas and Day (1993) define bioaccumulation as the ability of an organism to accumulate and concentrate substances directly from the surrounding water medium - i.e. bioconcentration - or indirectly through the food chain, i.e. biomagnification. An increase in the available level of the above-mentioned metals, which are typically present in natural ecosystems in both water and sediment, may lead to the accumulation of these metals in the tissues and organs of aquatic organisms.

The objective of this study was to determine the extent (with respect to site, season and tissues) of bioaccumulation of Cr, Cu, Fe and Mn in *O. mossambicus* from the lower Olifants River, inside the boundaries of the Kruger National Park. Furthermore, the tissues containing the highest and lowest concentrations of these metals were established. The Olifants River was selected as study area as it is one of the most important rivers flowing through the Kruger National Park. It has increasing industrial, mining and agricultural activities in its catchment area, which already have detrimental effects on the water quality of the river flowing through the Park. During this study water and sediment analysis was done by the Institute for Water Quality Studies (IWQS), of which the chemical and physical parameters most commonly referred to in the discussion are tabulated in Table 1 in the results. Furthermore, a similar paper on the extent of bioaccumulation of nickel (Ni), lead (Pb), strontium (Sr) and zinc (Zn) by *O. mossambicus*, from the same study area, is in preparation.

Material and methods

Selection of sampling sites

O. mossambicus was sampled (n=20, seasonally) using 70 to 120 mm stretched mesh size gill nets, in February, May, July and November 1994, at two sites, i.e. Mamba (Location 1) and Balule (Location 2), in the lower Olifants River Catchment, inside the boundaries of the Kruger National Park (KNP) (Fig. 1). Surveys 1 to 4 were respectively undertaken in late summer (February), autumn (May), winter (July) and early summer (November). This river system was chosen, for it is known that the copper-mining activities in the Olifants River region have the potential of increasing the levels of Cu, Cr, Fe and Mn in the river water (Van Vuren et al., 1994). Furthermore, previous studies revealed that these metals are prevalent at high concentrations in the Olifants River (Du Preez and Steyn, 1992; Seymore, 1994; Van Vuren et al., 1994).

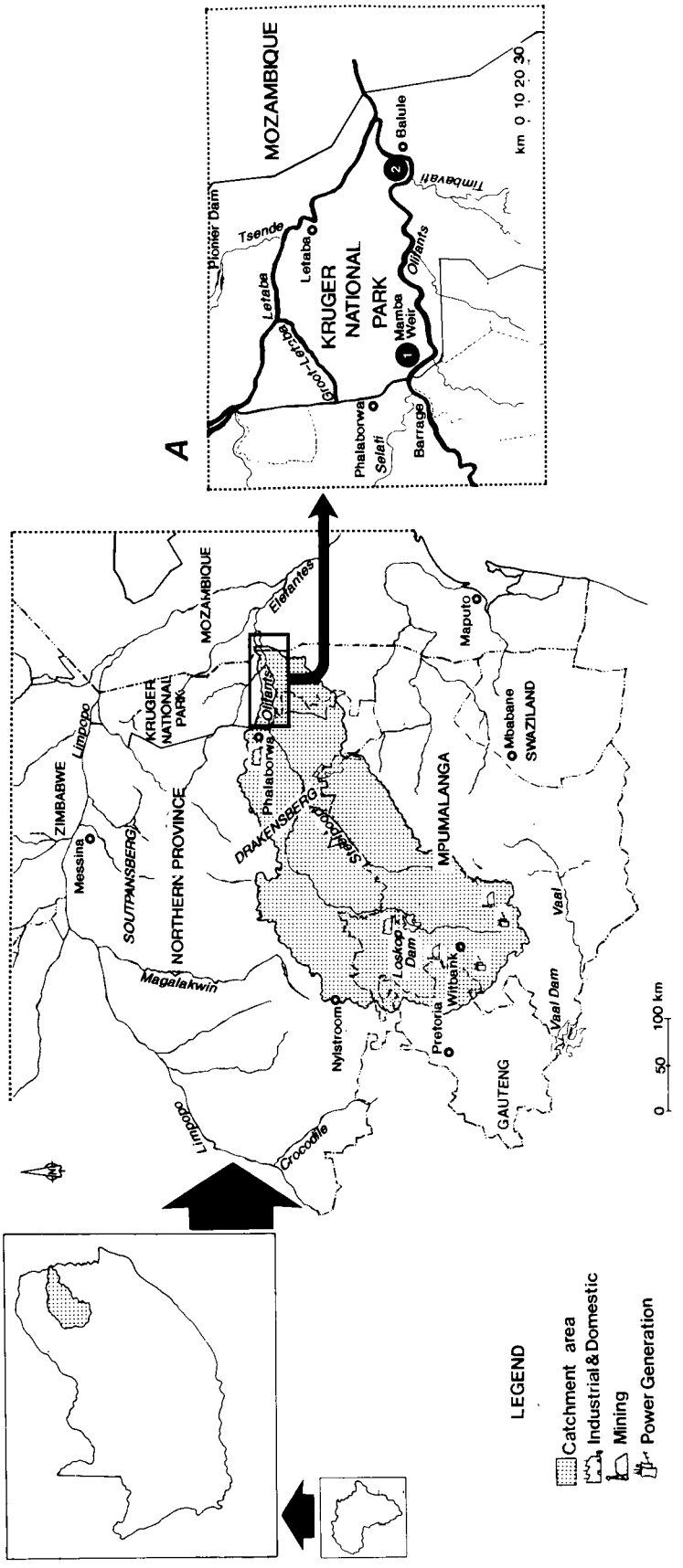

Figure 1

The Olifants River catchment area, with involved co-basin states.

***A**: The study area in the Lower Olifants River catchment, indicating sampling sites 1 (Mamba) and 2 (Balule) inside the Kruger National Park*

Field sampling

The fish were placed on a polypropylene board and killed by cutting through the spinal cord behind the head, after which each individual fish was dissected, using stainless steel dissecting tools. Gills, liver, muscle and skin samples were removed and placed in 25 mℓ glass bottles. All samples were kept frozen until determination of metal concentrations in the laboratory. These tissues were chosen for the following reasons:

- They are known to accumulate metals.
- The gills and skin are in direct contact with the aquatic environment, and are directly influenced by the chemical composition thereof.
- The liver is known as a detoxification organ (Klaassen, 1976) and is therefore a major site of bioaccumulation.
- The muscle, which is consumed by humans, should be monitored as protection against consumption of contaminated food (Du Preez et al., 1997).

All water and sediment samples, which were collected during the surveys, were preserved with mercuric chloride ($HgCl_2$), refrigerated and sent to the IWQS for analysis of macro variables and metal concentrations. All the water and sediment data are available from Avenant-Oldewage et al. (1995).

Laboratory procedures

To facilitate discussion of results, the organs and tissues studied will be referred to as tissues or tissue samples. The tissue samples (gills, liver, muscle, skin) were thawed and placed in Erlen Meyer flasks, whereafter the wet mass of each sample was accurately recorded by means of a Mettler PK 4800 scale. The samples were then dried at 60°C in a HARAEUS HANAU KB 500 oven for 48 h, after which the dry tissue mass of all the samples was again recorded using the same balance. Ten milliliter concentrated nitric acid (55% - NHO_3) and 5 mℓ perchloric acid (70% - $HClO_4$) were added to approximately 1 g of dried tissue and the material digested on a hot plate at ± 200 to 250°C for at least 4 to 6 h. Samples were digested until the solutions were clear and then left to cool. Each sample was made up to 50 mℓ with doubly distilled water and

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

Chemical and physical water variables	Survey 1 (Feb)		Survey 2 (May)		Survey 3 (Ju ly)		Survey 4 (Nov)	
	Loc1	Loc2	Loc1	Loc2	Loc1	Loc2	Loc1	Loc2
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
Oxygen ($mg \cdot \ell^{-1}$)	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 ($mg \cdot \ell^{-1}$) $CaCO_3$	387	309	N/A	N/A	N/A	N/A	44	166
TDS ($mg \cdot \ell^{-1}$)	315	786	560	585	1644	1401	1069	848
Metal concentartions ($mg \cdot \ell^{-1}$) 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
Manganese	0.618	0.949	0.001	0.001	0.001	0.001	0.009	0.001
Metal concentrations (mg/g) 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
Manganese	0.820	1.045	1.60	0.832	1.18	1.19	0.611	0.375

then filtered, using an acid-resistant 6 µm Millipore filter membrane attached to a vacuum pump. The filter system was rinsed with doubly distilled water before each sample was filtered. The samples were stored in glass bottles which were soaked in 1M HCl for 24 h and rinsed in doubly distilled water prior to use (Giesy and Wiener, 1977).

After analytical standards were prepared from Holpro solutions, the atomic absorption spectrophotometer (AAS) SPECTRA AA-10 was calibrated, using five standard concentrations per individual metal. The SPECTRA AA-10 was then used to determine the Cr, Cu, Fe and Mn concentrations in the tissue samples of the fish. Bioconcentration factors between fish tissue and water (BcF_w) and fish tissue and sediment (BcF_s) were determined (Wiener and Giesy, 1979), using the mean metal concentration in each tissue. The metal concentrations in the water and sediment from the Olifants River, used to calculated the BcF_w and BcF_s, were taken from Avenant-Oldewage et al. (1995). Statistical analysis was done using a "Statgraphics 7" computer program to determine the mean, minimum and maximum values, standard deviation, standard error and coefficient of variation.

Results

Chemical and physical water and sediment varibles, obtained from Avenant-Oldewage et al. (1995), are summarised in Table 1. The analysis and statistics of the bioaccumulation in the different tissue types for the four specified metals, are summarised in Tables 2 and 3. Locality differences, as well as seasonal differences, for each individual metal, together with bioconcentration in the water and sediment will be discussed, as regards the degree of bioaccumulation. Figures 2.1a and b and Figs. 2.2c and d give a graphic representation of the seasonal variations which occurred in the different metal concentrations in the gills (*A*), liver (*B*), muscle (*C*) and skin (*D*), for Mamba and Balule, 1994.

Bioaccumulation in different tissues

The bioaccumulation of Cr in the different tissues during Survey 1 corresponded with each other, with the highest bioaccumulation site the gills, i.e. 225.79±72.56 $\mu g \cdot g^{-1}$ and 103.47±22.59 $\mu g \cdot g^{-1}$ for Mamba and Balule respectively (Table 2). For Survey 2 the highest bioaccumulation levels for both Mamba, i.e. 55.35±78.02 $\mu g \cdot g^{-1}$ and Balule, i.e. 224.81±147.89 $\mu g \cdot g^{-1}$, were evident in the liver (Table 2). As seen from Table 3, the same trend was found during Survey 3 for both locations, with the liver showing the highest bioaccumulation, with values of 35.33±46.96 $\mu g \cdot g^{-1}$ and 25.36±14.23 $\mu g \cdot g^{-1}$ for Mamba and Balule respectively. The bioaccumulation position for Survey 4 differed, with the highest mean Cr concentration at Mamba occurring in the gills (38.05±16.85 $\mu g \cdot g^{-1}$) and at Balule in the liver (56.12±62.32 $\mu g \cdot g^{-1}$) (Table 3). High variation did occur between the Cr concentrations in the individual tissue types of individuals at the same locations. For example, the Cr content in the gills during Survey 1 at Mamba, for all 20 fish sampled, ranged between 144.35 $\mu g \cdot g^{-1}$ and 437.95 $\mu g \cdot g^{-1}$ with the standard deviation (s_d) 72.56 (Table 2). The s_d is indicative of the degree of variation which occurred between individuals in a sample. Although variation occurred in the Cr concentration of the different tissues, the degree of Cr concentration in *O. mossambicus* for Mamba and Balule was in

TABLE 2
HEAVY METAL CONCENTRATIONS (µg/g) IN GILLS, LIVER, MUSCLE AND SKIN OF *O. MOSSAMBICUS*, FROM THE OLIFANTS RIVER, KRUGER NATIONAL PARK, FEBRUARY (SURVEY 1) AND MAY (SURVEY 2), 1994

Survey one	Location One (Mamba) (n=20)				Location Two (Balule) (n=20)			
Conc. (µg/g)	Gills	Liver	Muscle	Skin	Gills	Liver	Muscle	Skin
Cr (µg/g)	❶	③	④	②	❶	③	④	②
Mean ± s_d	225.79±72.56	109.36±68.12	98.09±47.49	120.00±67.36	103.47±22.59	86.69±54.07	84.14±15.74	87.19±54.18
Min/Max	(144.35-437.95)	(32.26-287.76)	(27.16-271.02)	(43.27-256.44)	(60.80-154.38)	(38.67-288.34)	(55.52-106.99)	(47.66-302.63)
s_e	16.23	15.23	10.62	15.06	5.05	12.09	3.52	12.11
cv	32.14	62.29	48.42	56.13	21.82	62.37	18.71	62.14
BcF_w	75.26	36.45	32.70	40.00	34.49	28.90	28.05	29.06
BcF_s	1.036	0.502	0.459	0.550	0.287	0.241	0.234	0.242
Cu (µg/g)	③	❶	④	②	②	❶	④	③
Mean ± s_d	32.30±39.16	305.30±352.44	12.18±3.59	35.69±50.17	28.81±16.63	151.19±157.34	8.09±1.53	9.67±6.57
Min/Max	(11.75-149.09)	(49.67-1329.93)	(6.32-19.30)	(8.75-238.26)	(12.87-83.88)	(35.99-564.03)	(4.93-11.04)	(4.49-36.36)
s_e	8.76	78.81	0.80	11.22	3.71	35.18	0.34	1.47
cv	121.24	115.44	29.46	140.55	57.70	104.06	18.96	67.90
BcF_w	8.075	76.325	3.045	8.922	7.203	37.80	2.022	2.426
BcF_s	8.075	76.325	3.045	8.922	1.253	6.573	0.352	0.420
Fe (µg/g)	②	❶	④	③	④	❶	②	③
Mean ± s_d	947.35±364.71	2282.02±1860.1	360.62±164.82	690.01±443.76	802.98±389.37	2507.9±1164.68	1759.34±341.51	1722.11±568.89
Min/Max	(514.22-1673.2)	(319.64-6908.2)	(195.43-966.97)	(228.0-1681.55)	(18.84-1990.52)	(1032.4-6226.8)	(1178.2-2180.6)	(1007.6-3298.2)
s_e	81.55	415.94	36.85	99.23	87.07	260.43	76.36	127.21
cv	38.50	81.51	45.70	64.31	48.49	46.44	19.41	33.03
BcF_w	315.78	760.67	120.21	230.00	267.66	835.97	586.45	574.04
BcF_s	0.021	0.050	0.008	0.015	0.015	0.047	0.033	0.033
Mn (µg/g)	❶	②	④	③	❶	②	④	③
Mean ± sd	40.12±16.34	19.17±24.38	9.28±5.45	11.48±11.31	33.46±9.70	27.75±13.99	16.80±2.58	19.67±11.46
Min/Max	(13.88-80.42)	(1.68-100.96)	(4.39-29.96)	(2.59-51.63)	(20.11-54.74)	(9.97-68.54)	(11.97-20.52)	(10.49-64.97)
s_e	3.62	5.45	1.22	2.53	2.17	3.13	0.58	2.56
cv	40.73	127.22	58.73	98.44	28.98	50.42	15.34	58.27
BcF_w	0.065	0.031	0.015	0.019	0.035	0.029	0.018	0.021
BcF_s	0.049	0.023	0.011	0.014	0.032	0.027	0.016	0.019

Survey two	Location One (Mamba) (n=20)				Location Two (Balule) (n=15)			
Conc. (µg/g)	Gills	Liver	Muscle	Skin	Gills	Liver	Muscle	Skin
Cr (µg/g)	②	❶	④	③	②	❶	④	③
Mean ± s_d	46.43±52.41	55.35±78.02	20.86±19.11	22.74±25.62	75.62±41.58	224.81±147.89	21.11±14.25	38.13±13.51
Min/Max	(1.79-177.42)	(0.36-242.98)	(0.65-60.94)	(0.41-77.19)	(23.49-166.55)	(45.83-618.10)	(13.70-72.18)	(20.19-67.04)
s_e	11.72	17.44	4.27	5.73	10.74	38.18	3.68	3.49
cv	112.87	140.97	91.63	112.67	54.99	65.78	67.49	35.45
BcF_w	15.48	18.45	6.953	7.58	25.21	74.94	7.037	12.71
BcF_s	0.186	0.222	0.084	0.091	0.212	0.631	0.059	0.107
Cu (µg/g)	②	❶	③	④	②	❶	④	③
Mean ± s_d	4.94±1.86	49.51±46.37	4.90±4.45	2.66±1.36	28.96±15.77	102.56±139.07	4.88±4.66	6.36±2.11
Min/Max	(1.20-8.86)	(8.36-179.56)	(1.01-16.36)	(0.54-6.20)	(12.15-60.29)	(11.45-549.17)	(2.06-20.67)	(3.36-9.93)
s_e	0.42	10.37	1.00	0.31	4.17	35.91	1.20	0.52
cv	37.59	93.66	91.05	51.28	54.47	135.60	95.49	31.49
BcF_w	1.235	12.38	1.225	0.665	7.24	25.64	0.25	1.59
BcF_s	0.120	1.208	0.120	0.065	0.517	1.831	0.087	0.114
Fe (µg/g)	❶	②	③	④	❶	②	④	③
Mean ± s_d	765.26±359.20	416.42±382.18	312.52±270.40	259.75±268.22	1289.44±1341.6	1162.10±414.41	164.09±97.85	254.03±101.66
Min/Max	(190.9-1558.93)	(61.71-1144.65)	(71.01-1262.22)	(14.15-978.92)	(270.89-5819.7)	(586.75-1801.6)	(95.81-463.77)	(103.96-422.91)
s_e	80.32	85.46	60.46	59.98	346.41	107.00	25.26	26.25
cv	46.94	91.78	86.82	103.26	104.05	35.66	59.63	40.02
BcF_w	255.09	138.81	104.17	86.58	429.67	387.37	54.70	84.68
BcF_s	0.020	0.011	0.008	0.007	0.024	0.021	0.003	0.005
Mn (µg/g)	❶	②	③	④	❶	②	③	④
Mean ± sd	30.66±11.23	22.25±50.37	7.62±6.48	7.53±5.91	68.74±64.43	41.60±20.47	5.21±3.81	4.29±2.37
Min/Max	(5.80-50.11)	(1.30-228.54)	(2.74-31.57)	(0.68-27.47)	(15.11-265.68)	(15.57-81.30)	(2.30-15.87)	(1.87-11.36)
s_e	2.51	11.26	1.45	1.32	16.64	5.29	0.98	0.61
cv	36.63	226.35	85.06	78.56	93.74	49.21	73.26	55.17
BcF_w	30.66	22.25	7.62	7.53	68.74	41.60	5.21	4.29
BcF_s	0.019	0.014	0.005	0.005	0.083	0.05	0.006	0.005

Cr (µg/g) = chromium concentration; **Cu** (µg/g) = copper concentration; **Fe** (µg/g) = iron concentration; **Mn** (µg/g) = manganese concentration; s_d = standard deviation; s_e = standard error; cv = coefficient of variation.
❶ > ② > ③ > ④ = order of the degree of bioaccumulation in the different tissue types of *O. mossambicus*.

TABLE 3

HEAVY METAL CONCENTRATIONS (µg/g) IN GILLS, LIVER, MUSCLE AND SKIN OF *O. MOSSAMBICUS*, FROM THE OLIFANTS RIVER, KRUGER NATIONAL PARK, JULY (SURVEY 3) AND NOVEMBER (SURVEY 4), 1994.

Survey three	Location One (Mamba) (n=18)				Location Two (Balule) (n=20)			
Conc. (µg/g)	Gills	Liver	Muscle	Skin	Gills	Liver	Muscle	Skin
Cr (µg/g)	②	❶	③	④	②	❶	④	③
Mean ± s_d	34.57±17.09	35.33±46.96	11.60±1.05	11.58±5.39	20.29±7.12	25.36±14.23	5.15±1.81	15.42±6.74
Min/Max	(15.97-75.92)	(9.75-183.17)	(9.55-13.65)	(5.33-27.39)	(5.43-36.03)	(10.68-73.80)	(1.80-8.29)	(9.11-34.37)
s_e	3.82	10.50	0.23	1.19	1.59	3.18	0.10	1.51
cv	49.44	132.92	9.05	45.79	35.09	56.13	35.12	43.73
BcF_w	11.52	11.78	3.867	3.86	6.763	8.453	1.717	5.14
BcF_s	0.065	0.066	0.022	0.022	0.061	0.076	0.015	0.046
Cu (µg/g)	②	❶	④	③	②	❶	④	③
Mean ± s_d	8.78±3.14	163.20±213.40	3.45±4.70	4.51±2.57	8.36±2.41	53.84±75.23	2.31±0.91	4.18±4.13
Min/Max	(5.41-16.60)	(10.28-652.02)	(1.33-22.93)	(2.38-11.11)	(5.70-14.54)	(3.33-272.46)	(1.57-5.38)	(2.03-18.57)
s_e	0.70	47.72	1.05	0.57	0.54	16.82	0.20	0.92
cv	35.70	130.76	136.11	57.06	28.78	139.72	39.43	98.52
BcF_w	2.195	40.8	0.863	4.51	2.09	13.46	0.578	1.045
BcF_s	0.244	4.533	0.096	0.125	0.029	0.186	0.008	0.014
Fe (µg/g)	❶	②	④	③	❶	②	④	③
Mean ± s_d	1035.75±349.16	677.47±459.99	84.69±24.12	243.32±233.60	1100.02±706.31	435.93±245.44	86.59±35.06	235.76±139.04
Min/Max	(542.94-1781.9)	(162.7-1776.78)	(60.21-161.21)	(79.76-1157.30)	(383.11-2942.1)	(187.8-1164.87)	(53.16-178.41)	(94.78-598.21)
s_e	78.07	102.86	5.39	51.23	157.94	54.88	7.84	31.09
cv	33.71	67.90	28.48	96.01	64.21	56.30	40.49	58.98
BcF_w	345.25	225.82	28.23	81.11	18.64	7.389	1.468	3.996
BcF_s	0.016	0.011	0.001	0.004	0.014	0.006	0.001	0.003
Mn (µg/g)	❶	②	④	③	❶	③	④	②
Mean ± sd	17.58±10.42	13.03±14.30	2.01±1.43	9.21±19.32	32.10±11.66	10.78±6.95	4.62±3.46	12.12±14.71
Min/Max	(3.71-37.64)	(1.86-50.85)	(0.85-7.76)	(2.34-90.87)	(16.32-60.28)	(2.23-31.49)	(2.56-18.71)	(3.46-70.65)
s_e	2.33	3.20	0.32	4.32	2.61	1.55	0.77	3.29
cv	59.27	109.78	71.35	209.69	36.33	64.45	75.01	121.40
BcF_w	17.58	13.03	2.01	9.21	32.10	10.78	4.62	12.12
BcF_s	0.015	0.011	0.002	0.008	0.027	0.009	0.004	0.010
Survey four	**Location One (Mamba) (n=20)**				**Location Two (Balule) (n=20)**			
Conc. (µg/g)	Gills	Liver	Muscle	Skin	Gills	Liver	Muscle	Skin
Cr (µg/g)	❶	②	④	③	②	❶	④	③
Mean ± s_d	38.05±16.85	36.67±29.91	15.59±0.73	21.73±6.74	34.95±14.74	56.12±62.32	14.55±2.00	20.01±10.06
Min/Max	(18.29-88.94)	(8.92-116.33)	(14.16-16.86)	(14.86-38.94)	(15.50-61.20)	(10.06-262.16)	(11.54-17.48)	(11.91-56.56)
s_e	3.77	6.69	0.16	1.51	3.29	13.93	0.45	2.25
cv	44.28	81.57	4.68	31.03	42.16	111.04	13.77	50.28
BcF_w	12.68	12.22	5.197	7.243	11.65	18.71	4.85	6.67
BcF_s	0.147	0.142	0.060	0.084	0.333	0.534	0.139	0.191
Cu (µg/g)	②	❶	④	③	②	❶	④	③
Mean ± s_d	9.27±3.84	152.46±106.78	2.04±0.58	2.28±0.72	5.94±2.79	69.10±70.34	1.63±0.66	1.85±1.60
Min/Max	(4.91-21.34)	(14.26-445.36)	(1.39-3.37)	(1.38-4.28)	(3.42-14.19)	(8.80-238.30)	(0.88-3.41)	(0.58-6.81)
s_e	0.86	23.88	0.13	0.16	0.62	15.73	0.15	0.36
cv	41.46	70.04	28.38	31.72	47.07	101.79	40.59	86.47
BcF_w	2.318	38.12	0.51	0.57	1.485	17.28	0.408	0.463
BcF_s	0.189	3.111	0.042	0.047	0.594	6.910	0.163	0.185
Fe (µg/g)	❶	②	④	③	❶	②	④	③
Mean ± s_d	1847.17±935.32	996.83±709.62	106.77±26.77	275.05±164.37	1462.45±840.49	1253.66±787.61	103.77±11.86	196.54±64.34
Min/Max	(814.0-3737.10)	(266.73-3346.0)	(81.71-194.21)	(100.74-680.35)	(670.71-3873.21	(293.31-3398.2)	(80.71-124.94)	(119.50-362.01)
s_e	209.14	158.68	5.99	36.75	187.94	176.11	2.65	14.39
cv	50.64	71.19	25.07	59.76	57.47	62.82	11.43	32.74
BcF_w	11.127	6.005	0.643	1.657	11.34	9.718	0.804	1.526
BcF_s	0.056	0.030	0.003	0.008	0.042	0.036	0.003	0.006
Mn (µg/g)	❶	②	④	③	❶	②	④	③
Mean ± sd	50.90±19.38	35.25±32.38	3.95±0.80	9.55±69.76	53.66±26.12	35.74±20.95	3.77±0.69	5.59±1.95
Min/Max	(10.68-83.20)	(10.47-149.00)	(3.09-5.63)	(10.47-311,87)	(19.13-121.57)	(11.75-86.12)	(2.88-5.27)	(3.23-10.21)
s_e	4.33	7.24	0.18	15.60	5.84	4.69	0.15	0.44
cv	38.07	91.84	20.38	141.53	48.67	58.62	18.20	34.88
BcF_w	5.66	3.926	0.439	1.061	53.66	35.74	3.77	5.59
BcF_s	0.083	0.058	0.006	0.016	0.143	0.095	0.010	0.015

Cr (µg/g) = chromium concentration; **Cu** (µg/g) = copper concentration; **Fe** (µg/g) = iron concentration; **Mn** (µg/g) = manganese concentration; s_d = standard deviation; s_e = standard error; cv = coefficient of variation.

❶ > ② > ③ > ④ = order of the degree of bioaccumulation in the different tissue types of *O. mossambicus*.

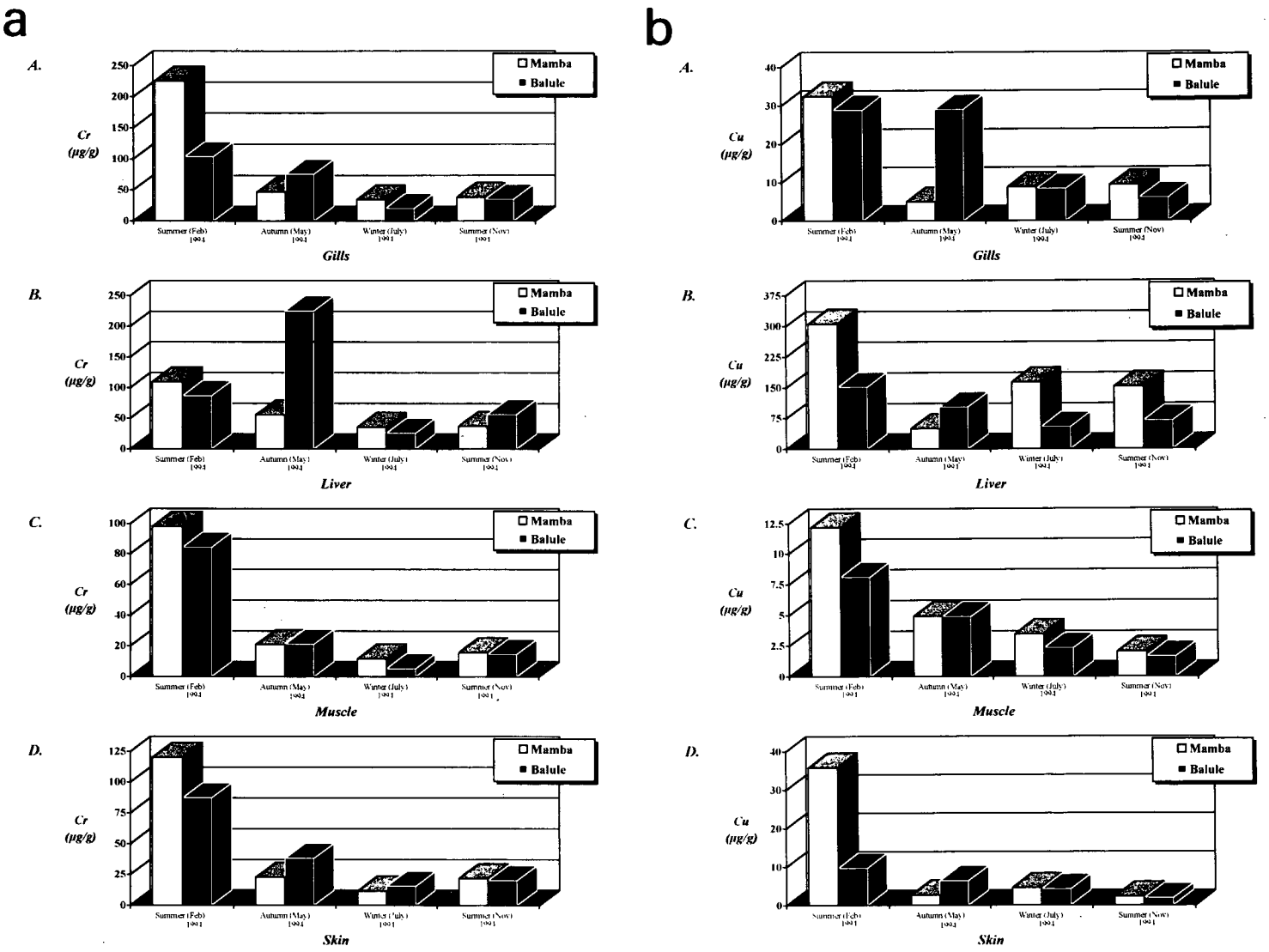

Figure 2.1

*Mean seasonal Cr (**a**) and Cu (**b**) concentrations (µg·g⁻¹ dry mass) in the **A**: gillls, **B**: liver, **C**: muscle and **D**: skin of* Oreochromis mossambicus, *from Mamba and Balule 1994*

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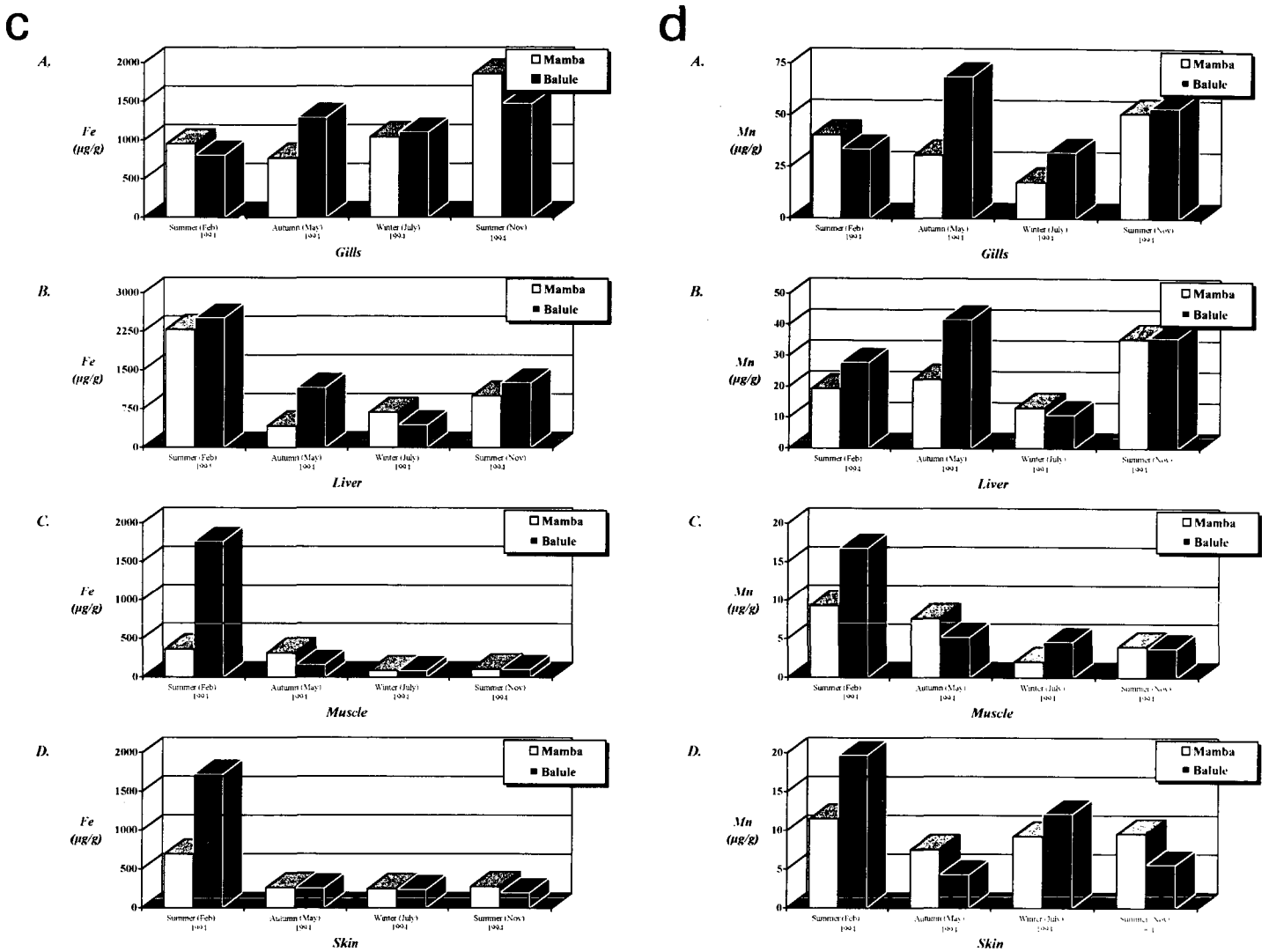

Figure 2.2

*Mean seasonal Fe (**c**) and Mn (**d**) concentrations (µg·g⁻¹ dry mass) in the **A**: gillls, **B**: liver, **C**: muscle and **D**: skin of* Oreochromis mossambicus, *from Mamba and Balule 1994*

the following order: Liver > gills > skin > muscle (Table 2 and 3).

During all four surveys, at both Mamba and Balule, most Cu was bioaccumulated in the liver (Table 2 and 3): The values recorded during Survey 1 was 305.30±352.44 $\mu g \cdot g^{-1}$ and 151.19±157.34 $\mu g \cdot g^{-1}$, during Survey 2: 49.51±46.37 $\mu g \cdot g^{-1}$ and 102.56139.07 $\mu g \cdot g^{-1}$, during Survey 3: 163.20±213.40 $\mu g \cdot g^{-1}$ and 53.84±75.23 $\mu g \cdot g^{-1}$, and during Survey 4: 152.46±106.78 $\mu g \cdot g^{-1}$ and 69.10±70.34 $\mu g \cdot g^{-1}$ for Mamba and Balule respectively. Concentration values of Cu for the individuals at the same locations differed considerably for different individuals, for example, the Cu concentration recorded during Survey 1 in the liver at location one - for the 20 fish caught - varied between 49.67 $\mu g \cdot g^{-1}$ and 1 329.93 $\mu g \cdot g^{-1}$, with a s_d of 352.44 (Table 2). Although variation occurred in the Cu concentrations of all the tissue types, the degree of bioaccumulation for both Mamba and Balule was in the following order: Liver > gills > skin > muscle (Table 2 and 3).

The mean Fe concentrations in the different tissues varied considerably. The Fe concentrations were, throughout the year, consistently higher in the gills, except for Survey 1 where the preferred bioaccumulation site was the liver, with values of 2282.02±1860.10 $\mu g \cdot g^{-1}$ and 2507.90±1164.68 $\mu g \cdot g^{-1}$ for Location 1 and 2 respectively (Table 2). The mean Fe concentration at Mamba and Balule for Surveys 2 (Table 2), 3 (Table 3) and 4 (Table 3) were: 765.26±359.20 $\mu g \cdot g^{-1}$ and 1 289.44±1 341.60 $\mu g \cdot g^{-1}$, 1 035.75±349.16 $\mu g \cdot g^{-1}$ and 1 100.02±706.31 $\mu g \cdot g^{-1}$, and 1 847.17±935.32 $\mu g \cdot g^{-1}$ and 1 462.45±840.49 $\mu g \cdot g^{-1}$ for the two locations respectively. The Fe concentrations in individual tissues, within the 20 sampled at Mamba and Balule, varied considerably, with the s_d ranging between 11.86 (Mamba, Survey 4) and 1860.10 (Mamba, Survey 1) for the liver. The degree of bioaccumulation in the tissues of *O. mossambicus* at Mamba and Balule, was in the following order: Gills > liver > skin > muscle (Tables 2 and 3).

Bioaccumulation of Mn in the different tissues from Mamba and Balule was very consistent, with the gills being the position showing the highest concentration, throughout the year (Table 2 and 3). The mean concentrations of Mn in the gills, recorded during Survey 1, were 40.12±16.34 $\mu g \cdot g^{-1}$ and 33.46±9.70 $\mu g \cdot g^{-1}$, during Survey 2: 30.66±11.23 $\mu g \cdot g^{-1}$ and 68.74±64.43 $\mu g \cdot g^{-1}$, during Survey 3: 17.58±10.42 $\mu g \cdot g^{-1}$ and 32.10±11.66 $\mu g \cdot g^{-1}$, and during Survey 4: 50.90±19.38 $\mu g \cdot g^{-1}$ and 53.66±26.12 $\mu g \cdot g^{-1}$, for Mamba and Balule respectively. High variations in the Mn concentrations were recorded between individuals, and between the different tissue types, especially the liver, where the s_d ranged between 6.95 at Balule during the July survey (Table 3), and 50.37 at Mamba during May (Table 2). The variations in the degree of bioaccumulation found in the different tissues were minimal, with the only variation found during Survey 2 (May), where the bioaccumulation was higher in the muscle than in the skin, with the situation converse for the other surveys. The degree of Mn bioaccumulation in *O. mossambicus* at both Mamba and Balule was in the following order: Gills > liver > skin > muscle (Table 2 and 3).

Bioconcentration factor in water and sediment

Cr bioconcentration factor (BcF) values between tissues and water (BcF_w) were much higher than those of the sediment for all four surveys in the KNP, with the BcF_w ranging from 1.717 for the muscle (July at Balule) to 75.26 for the gills (February at Mamba). The BcF values between the tissues and the sediment (BcF_s) for Mamba and Balule were much lower and ranged from 0.015 for the muscle (July at Balule) to 1.036 for the gills (February at Mamba). The BcF values for both water and sediment recorded at Mamba and Balule were similar, except during Survey 1, where the values were considerably higher at Mamba. The highest chromium BcF values, for both water and sediment, were observed in the gills at both locations.

Copper BcF values for water were consistently higher than in sediment for both locations, except during February at Mamba, where the BcF_w and BcF_s were the same. The BcF_w ranged between 0.25 for muscle (May at Balule) and 76.325 for liver (February at Mamba), and the BcF_s between 0.014 for the skin (July at Balule) and 76.325 for the liver (February at Mamba). No significant differences could be established between values from Mamba and Balule, although the general trend seemed to indicate slightly higher values at Mamba. The highest copper BcF_w and BcF_s, for both locations, was recorded in liver.

Iron BcF values were, during all sampling periods, much higher for tissue/water ratios than for tissue/sediment ratios, with the BcF_w ranging between 0.643 for muscle (November at Mamba) and 760.67 for liver (February at Mamba) and the BcF_s ranging between 0.003 for muscle (November at Mamba and Balule) and 0.050 for liver (February at Mamba). The BcF values for water and sediment, recorded from Mamba and Balule, were fairly similar, except during the July survey where the BcF_w was significantly higher at Mamba than at Balule. The highest iron BcF values, for both water and sediment, were recorded from liver at both locations.

Manganese BcF_w values were, during all sampling periods, higher than the BcF_s for both Mamba and Balule. High variation occurred between the BcF_w and BcF_s values for all four surveys, except in Survey 1 where the variation in the bioconcentration values found in the gills was minimal, with BcF_w being 0.16 higher than the BcF_s. The BcF_w ranged from 0.015 for muscle (February at Mamba) to 68.74 for gill (May at Balule), and the BcF_s between 0.002 for muscle (July at Mamba) and 0.095 for liver (November at Balule). No general trend could be established for the manganese BcF_w and BcF_s values recorded at Mamba and Balule. It was, however, noted that the BcF_w recorded at Balule during November, were much higher than those from Mamba during the same survey. The minimum Mn bioconcentration values in the tissues, for water and sediment, at both locations, were recorded in the muscle, and the highest values predominantly found in the gills.

Locality differences

During Survey 1 (February, 1994), the Cr concentrations for all four tissue types (gills, liver, muscle and skin) were higher at Mamba than at Balule, with the opposite trend occurring during Survey 2 (May, 1994) (Table 2). The Cr concentration in the liver during Survey 2 was considerably higher at Balule than the value recorded at Mamba, with the values of the other tissues fairly comparative. The Cr concentrations in the gills, liver and muscle of Survey 3 (July, 1994) were higher at Mamba than at Balule, with the opposite trend evident in the skin (Table 3). During Survey 4 (November, 1994) - with the exception of the liver - the gills, muscle and skin had higher Cr concentrations at Mamba, although the concentration differences between the two locations were minimal (Table 3).

During Survey 1 (February, 1994), the mean Cu concentrations were higher at Mamba then at Balule for all four tissue types (Table 2). However, during Survey 2 (May, 1994), the opposite

occurred, with the mean Cu concentrations higher at Balule for all the tissue types except for muscle, where the Cu concentration was 0.02 $\mu g \cdot g^{-1}$ higher at Mamba (Table 2). As seen from Table 3, the mean Cu concentrations in the gills, liver, muscle and skin recorded during Surveys 3 (July, 1994) and 4 (November, 1994) were higher at Mamba than at Balule.

No general trend could be established as to which location showed the highest rate of Fe bioaccumulation. During Survey 1 (February, 1994), values from the liver, muscle and skin were higher at Balule than at Mamba,with the opposite in the gills (Table 2). During Survey 2 (May, 1994), however, the gills and liver values were higher at Balule and the muscle and skin higher at Mamba (Table 2). During the third survey (July, 1994), the liver and skin values showed higher concentration values at Mamba, whilst the values in the gills and muscles were lower (Table 3). With the exception of the liver, the Fe concentrations in the gills, muscle and skin recorded during Survey 4 (November, 1994) were, throughout the year, higher at Mamba than at Balule (Table 3).

During Survey 1 (February, 1994), the mean Mn concentrations were higher at Balule for all tissue types, except for the gills which showed a higher concentration at Mamba (Table 2). As seen from Table 2, the Mn concentrations in the gills and liver, recorded during Survey 2 (May, 1994) at Mamba, were higher than the concentrations found at Balule, with the opposite tendency in muscle and skin. During Survey 3 (July, 1994), the Mn concentrations in the gills, muscle and skin were higher at Balule, with liver having a higher concentration at Mamba (Table 3). The bioaccumulation tendency during Survey 4 (November, 1994), was converse to that found in the second survey, with the gills and liver higher at Balule and the muscle and skin higher at Mamba (Table 3).

Seasonal differences

The Cr concentrations in the gills, muscle and skin recorded at Location 1 and 2, as well as the concentrations in liver at Mamba, showed the following tendency: The highest concentrations occurred in late summer (February) followed by a steady decrease during autumn (May) and up to winter (July), after which an increase occurred during early summer (November) (Fig. 2.1a ***A***, ***B***, ***C*** and ***D***). The liver Cr concentrations at Balule showed an increase from late summer (February), to autumn (May), followed by a sharp decrease until winter (July), after which the concentration increased again towards early summer (November) (Fig. 2.1a ***B***).

The Cu concentrations in the different tissues varied considerably throughout the year. The bioaccumulation concentration of the Cu in the gills of fish, recorded at Mamba, showed the following seasonal variation: The highest Cu concentration occurred in late summer (February), after which a decrease in concentration towards autumn (May), followed by a steady increase in concentration up until early summer (November) of the same year (Fig. 2.1b ***A***). As seen from Fig. 2.1b ***B*** and ***D*** the Cu concentration recorded at Mamba in the liver and skin of fish showed the same variation, with the highest concentration recorded in late summer (February), followed by a decrease in concentration during autumn (May), after which an increase occurred towards winter (July) and a further decline towards early summer (November). The Cu concentrations recorded in the muscle of fish at Mamba and Balule, as well as those concentrations recorded in the skin of fish at Balule, showed a steady decrease in concentration from late summer (February), through autumn (May) and winter (July) and up until early summer (November) (Fig. 2.1b ***C*** and ***D***). The Cu concentrations in the gills of fish, recorded at Balule, showed a slight increase of 0.15 $\mu g \cdot g^{-1}$ from late summer (February) to autumn (May), followed by a decline in concentration during winter (July) and towards early summer (November), when the lowest value was recorded (Fig. 2.1b ***A***). As seen from Fig. 2.1b ***B***, the Cu concentrations recorded in the liver at Balule, decreased from late summer (February) to autumn (May), with the minimum value obtained in winter (July).

The mean Fe concentration values in the muscle of the fish from Mamba and Balule, the skin of fish at Mamba and the liver of fish at Balule showed the same seasonal variation: the highest concentrations were recorded in late summer (February), followed by a decline in concentration through autumn (May) up until winter (July), after which an increase in concentration occurred during early summer (November) (Fig. 2.2c ***B***, ***C*** and ***D***). The Fe concentrations recorded from the gills of fish from Mamba showed a decline from late summer (February) towards autumn (May), whereafter a steady increase in concentration occurred towards early summer (November)of the same year, when the highest concentration was recorded (Fig. 2.2c ***A***). As seen from Fig. 2.2c ***A*** and ***B***, the concentrations in the liver at Mamba followed the same seasonal variation as that of the gills, except that the highest concentration was recorded during late summer (February) instead of early summer (November), as was the case with the gills. The Fe concentrations recorded from gills at Balule had the following seasonal variation (Fig. 2.2c ***A***): An increase in concentration from late summer (February) to autumn (May), followed by a decrease in concentration up until winter (July), after which an increase occurred toward early summer (November). The highest Fe concentration value in the skin, at Balule, was recorded during late summer (February), after which a steady decline in concentration is evident up until early summer (November) of the same year (Fig. 2.2c ***D***).

The seasonal variations in different tissue types showed noticeable differences. The Mn concentrations found in the gills and muscle of fish from Mamba showed a decrease in concentration from late summer (February) - when the highest value was recorded in the gills - , through autumn (May) and up until winter (July), after which an increase in values occurred in the early summer (November) - at which time the highest value was recorded in the muscle (Fig. 2.2d***A*** and ***C***). As seen from Fig. 2.2d ***A*** and ***B***, the Mn concentrations recorded from liver of fish from both locations, as well as the concentration found in the gills at Balule, showed the following seasonal variation: An increase in concentration was observed from late summer (February) to autumn (May), after which a decline in concentration occurred until winter (July), followed by an increase in concentration in early summer (November). The highest value in the liver at Mamba was recorded during early summer (November), and the highest values in the gills and liver from Balule during autumn (May). The skin from both locations showed a decline in concentration from late summer (February) to autumn (May), after which the value increased during winter (July). After the winter months, however, the value recorded for skin at Mamba showed a further increase towards early summer (November), whilst the value recorded for skin at Balule showed a decrease in concentration (Fig. 2.2d ***D***). The Mn concentration recorded from the muscle showed a decline in concentration from late summer (February), right up until early summer (November), of the same year (Fig. 2.2d ***C***).

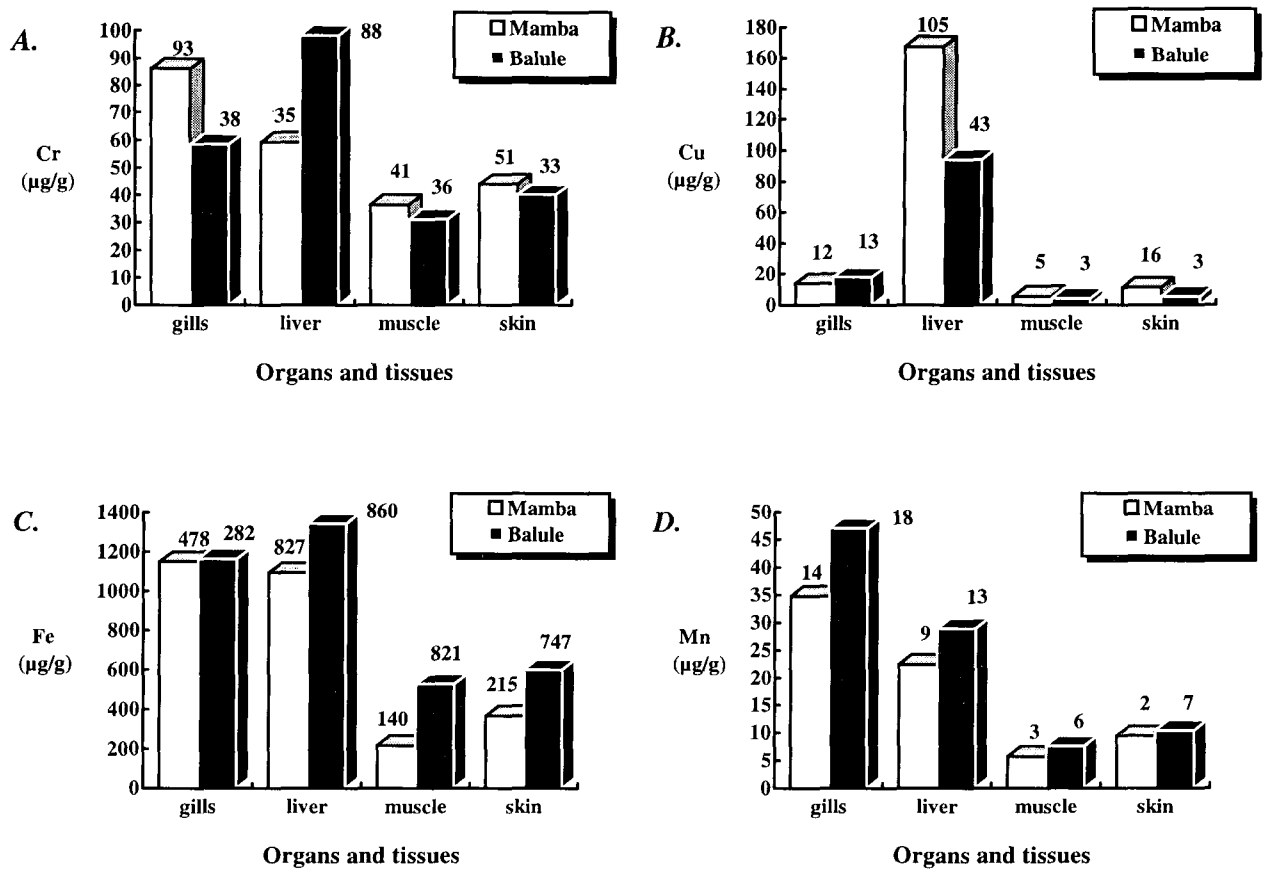

Figure 3

*Mean Cr (**A**), Cu (**B**), Fe (**C**) and Mn (**D**) concentrations (mg·g^{-1} dry mass) for Mamba and Balule 1994, in different tissues of* Oreochromis mossambicus *(Standard deviations are indicated above each bar)*

Discussion

Each individual metal will be discussed in the following manner:

- Bioaccumulation of the metal.
- Effect of major inorganic constituents on the toxicity of the metal.

Figure 3 shows the average concentrations of Cr, Cu, Fe and Mn in the different tissues of *O. mossambicus*, for Mamba and Balule throughout 1994. The main objective of this figure is to give a graphic representation of the order of the degree of bioaccumulation in the gills, liver, muscle and skin for each individual metal.

Chromium

Bioaccumulation - Limited research has been done on the bioaccumulation and excretion of Cr in freshwater fish. Duffus (1980) and Paasivirta (1991) both regard Cr in its salt form as highly bioaccumulative at high concentrations, and particularly dangerous because of the ability of Cr to accumulate in many organisms. Eisler (1986) found that adverse effects of Cr to sensitive species have been reported at 10.0 µg·ℓ^{-1} (ppb) for Cr^{6+} and 30.0 µg·ℓ^{-1} for Cr^{3+}, and that tissue levels exceeding4.0 mg of total Cr/kg dry mass, should be evidence of Cr contamination. The values obtained during this study were well under this limit.

In this study, high variation occurred in Cr concentrations found in individual fish, as well as in the different tissues. The Cr bioaccumulation in gills and liver seemed closely related, with the general degree of bioaccumulation for Mamba and Balule being, liver > gills > skin > muscle in that order (Fig. 3 *A*). In studies done by Ten Holder et al. (1978), it was found that the degree of bioaccumulation of Cr in different organs and tissues were the kidneys > liver > gills, which agrees with results obtained in similar studies done by Seymore (1994) on *Barbus marequensis*. Therefore, gills are important in the uptake of metals in fish. Rapid bioaccumulation of metals in the gills is usually associated with structural gill damage (Holdway, 1988). This was confirmed in studies completed by Taylor et al. (1985) where the grey mullet and the dab both suffered respiratory problems after exposure to hexavalent Cr.

A possible explanation for the higher Cr bioaccumulation in the liver - generally found at Mamba and Balule - could be due to the fact that *O. mossambicus* generally maintain bottom feeding habits, ingesting sludge rich in organic matter and green algae, as well as feeding on macro-invertebrates (Pienaar, 1978; Skelton, 1993). According to Hodson (1988), the liver of fish receives its blood supply directly from the intestinal portal system, in contrast to other tissues which are supplied by the gills. Dietary Cr would thus pass through the liver after intestinal absorption before reaching other tissues, thus making liver bioaccumulation most likely. However, studies done by Ten Holder et al. (1978)

showed that Cr concentrations in the digestive tract of fish are rapidly excreted, whilst Cr concentrations in the gills, muscle and skin decrease slower. Another explanation for the relatively high Cr concentrations found in liver could be the fact that liver is the detoxification organ of the body.

It is possible for aquatic organisms to accumulate high, even lethal concentrations of heavy metals, over a long period of time, from an environment with extremely low concentrations. For example, Ellis (1989) states that the brown bullhead (*Ictalurus nebulosus*) accumulated Cr concentrations more than 2 600 times the concentration found in the water. This phenomenon was repeated in results from this study where the Cr concentrations in organs and tissues were higher than those found in the water (Table 1). For example, it was found that the mean Cr level recorded at Mamba in February, was over 200 times the concentration recorded (Table 1) in the water. The Cr concentrations in the sediment (Table 1) were, however, much higher than the concentrations in the tissues. This may suggest that the uptake of Cr by *O. mossambicus* was not only through bioconcentration directly from the water medium, but also through biomagnification, i.e. through the food chain, due to the fact that *O. mossambicus* mainly feed on sludge and green algae on the bottom of the river, thus explaining the higher Cr values recorded in the tissues than in the water.

According to Holdway (1988), bioconcentration of Cr in fish is not a serious problem, for fish possess mechanisms to regulate Cr. Holdway (1988) further states that the maximum BcF_w, with elevated Cr levels, seldom reach a value higher than 10.00, except in the direct uptake, via diet or gills, from contaminated sediment. It was the case in most instances during this study, where higher values were obtained in the gills and liver (Fig. 3 ***A***), which correlates with the high values in the sediment (Table 1), and the fact that *O. mossambicus* is a bottom feeder.

The Cr concentrations in the different tissues obtained at Mamba and Balule did not differ much, with the concentrations at Mamba mostly higher than at Balule (Fig. 3 ***A***), except during the second survey where the opposite occurred. The higher Cr levels found at Mamba indicate that the bioavailability of Cr to fish may be higher at Mamba than at Balule. This should be expected, for it has been established that the water quality at Balule is better than at Mamba (Avenant-Oldewage et al., 1995). Furthermore, the water hardness ($CaCO_3$) is higher at Mamba than at Balule (Table 1), thus causing a higher bioaccumulation rate at Mamba. A possible explanation for the higher Cr levels recorded in *O. mossambicus* at Balule during the second survey, could be the release of metal ions from decaying reeds, which occurs in the Balule area. Reeds are known to accumulate metal ions (Seymore, 1994) and the decay thereof could lead to the release of metal ions into the water, which will result in a higher bioaccumulation rate at this locality.

Effect of major inorganic constituents on toxicity and accumulation of Cr - The toxicity of various metal compounds is strongly affected by water quality, especially by factors such as pH, temperature, water hardness and dissolved oxygen concentration (Alabaster and Lloyd, 1980; Abel, 1989; Lloyd, 1992). The toxicity of chromates is pH dependent, and according to Ellis (1989), Cr increases in toxicity with a reduction in pH. For instance, a 50 to 200-fold increase in the toxicity of Cr as observed by Hogendoorn-Roozemond et al. (1978) when the pH decreased from 7.9-6.8. Furthermore, the site of toxic action during lethal Cr exposure to fish varies with different pH values. Van der Putte et al. (1981) found that the primary site of toxic action is in the gills at a pH of 6.5, whilst at a pH of 7.8, Cr (VI) tends to accumulate more readily in the internal organs. This was also evident in this study where most of the Cr concentrated in the liver, with the corresponding pH values of the water ranging from 6.86 to 9.01 (Table 1).

Temperature does not only affect the toxicity of Cr (VI) (Wang, 1987), but also the rate of metabolic processes. For example, Cairns et al. (1975) found that a rise in temperature lowered the resistance time of fish to higher Cr concentrations. This may explain the lower Cr concentrations recorded in the tissues of *O. mossambicus* during July (winter), when the temperature is considerably lower than temperatures recorded in the other surveys (Fig. 2.1a ***A-D***). As water temperature rises, the respiratory rate in fish will increase, thus causing an increase in Cr uptake. This was evident in the results. The effect of water hardness on the toxicity of Cr was illustrated by Ellis (1989), among others. Ellis (1989) found that the LC-50 values for Cr (VI) increased with a decrease in the water hardness. Furthermore, Trama and Benoit (1960) and Holdway (1988) state that total alkalinity significantly reduces the toxic effect of Cr (VI) on fish. One should be cautious in making deductions from the results obtained in this study, for the $CaCO_3$ (total alkalinity) values for May and July, 1994 were not available.

Copper

Bioaccumulation - According to Stokes (1979), the distribution of Cu in fish tissue is as follows:

Fish muscle has poor accumulative properties, with low concentrations of Cu found in the muscle, even in systems containing high Cu levels.

Contrary to this, gill tissue tends to accumulate Cu from water. This could be attributed to the large surface area available for adsorption and the volume of water which passes through the gills. Furthermore, during acute Cu stress it is found that the gills produce excessive amounts of mucus, which may block the gills and cause instant death.

Liver and kidneys have Cu bioaccumulation properties, with the accumulative capacity much greater in the liver than in the kidney.

In Fig. 3 ***B*** it is shown that Cu bioaccumulated in all the tissues of *O. mossambicus*. It was found that of all tissue types, the highest concentrations of Cu were found in the liver, at both locations, followed by the gills (Fig. 3 ***B***). The same pattern of the degree of bioaccumulation in organs was established in studies done by Brooks et al. (1976), Wiener and Giesy (1979), Buckley et al. (1982), Nemcsók et al. (1987), Hogstrand and Haux (1991), and Seymore (1994). Fish minimise the metabolic effects caused by elevated metal concentrations by regulating the bioaccumulation rates, or by forming complexes. The elevated Cu concentrations found in the liver can be ascribed to the binding of Cu to metallothionein (MT) to form metallothionein complexes (Buckley et al., 1982), which act as a detoxification mechanism (Nemcsók et al., 1987; Hogstrand and Haux, 1991).

Metallothionein, which occurs in most organisms, is a low molecular weight protein with a high cysteine content, which renders metallothionein as a potential, preferred binding site for metals (Hodson, 1988). According to Hodson (1988), low metallothionein levels occur in the liver, but considerable induction occurs when exposed to cadmium, Cu, Zn and mercury. According to Singh and Sivalingam (1982), Cu is the strongest inhibitor of catalase activities and McCarter et al. (1982) state that the synthesis of metallothionein reduces the exposure of

organisms to metal ions.

Metallothionein creates a protective mechanism by binding to these metal ions - thus protecting the metabolic activity of cellular proteins from metal poisoning. This was confirmed in a study done by McCarter et al. (1982), where the data indicated that the formation of Cu-MT complexes in the liver cytosol preceded the elevation of Cu levels in high-molecular-mass protein fractions in fish. It is, however, important to realise that as metal exposure increases and the rate of metallothionein synthesis reaches a maximum, excess free metal ions can no longer form complexes with metallothionein, and bind with other proteins, thereby causing enzyme inhibition and toxicity.

The accumulation of Cu in the gills of fish could be related to the fact that the gills play an essential role in Cu uptake (Nemcsók et al., 1987). Nemcsók et al. (1987) found damage to the gills, liver, kidney and nervous system of the carp, exposed to a Cu concentration of 1.5 mg/ℓ. Histological examinations done by Lloyd (1965) and Sellers et al. (1975) on the gills of rainbow trout showed swollen epithelial cells, which separated from the pilaster cells of the lamellae and finally sloughed off, causing death. This suggests that the toxic action of Cu is not only internal but that it may affect the epithelial cells of the gill lamellae.

The Cu concentrations in the various tissues of the fish were generally higher at Mamba than at Balule, except for Survey 2, where the opposite occurred. The values at the two localities were, however, very similar. The higher concentrations at Mamba could possibly indicate a higher bioavailability of Cu to fish in this area. The BcF values calculated for the tissue/water ratio were higher than those of the sediment, which suggest possibly a higher degree of bioavailability of metals to fish in the water, although the water chemistry, as well as regulatory mechanisms of Cu in the fish, should be considered when determining the actual degree of metal bioaccumulation.

Effect of major inorganic constituents on the toxicity and accumulation of copper - There are various factors affecting acutely lethal levels of Cu. Cu in the aquatic environment is mobile and soluble at a low pH (Alabaster and Lloyd, 1980). In alkaline conditions Cu precipitates and is non-toxic (Dallas and Day, 1993). Andrew et al. (1977) found that a slight increase in H^+ concentration (pH 8.0 to 7.6) leads to a decrease in the toxicity of free Cu^{2+}. According to Campbell and Stokes (1985), the pH dependency of Cu toxicity is due to competition for a binding site on the gill surface of fish. As would be expected that bioaccumulation levels in tissues of Mamba during February, July and November were higher than those found at Balule - due to the lower pH values recorded at Mamba during these seasons (Table 1).

According to Cairns et al. (1975) and Alabaster and Lloyd (1980), the survival time of fish exposed to Cu are shortened by an increase in temperature. It was confirmed by results from this study, where lower Cu bioaccumulation levels were detected in winter (Fig. 2.1b ***A-D***). The concentration of dissolved salts are generally higher in hard than in soft water. As a result of this, the availibility of Cu ions will be lower in hard than in soft water, for Cu form complexes with carbonate. Thus, the toxicity of Cu is reduced with an increase in water hardness (Förstner, 1979; Alabaster and Lloyd, 1980; Ellis, 1989), and the reduction of dissolved oxygen levels (Lloyd, 1965; Ellis, 1989). This was depicted in the results where the observed $CaCO_3$ value recorded at Balule in November was higher than that at Mamba (Table 1). Thus CO_3^{2+} ions are available for Cu complexing, which will result in a lower bioavailability of Cu to fish at Balule.

Iron

Bioaccumulation - Although Fe may be toxic to the aquatic environment at high concentrations, it is also advantageous to both the aquatic habitat and its inhabitants. For example, metal accumulation by aquatic organisms is influenced by protective and/or competitive effects of sediment constituents, of which amorphous Fe oxyhydroxides are an important contributing factor (Tessier et al., 1984). In studies done by Johnson (1986) it was found that the adsorption of Cu and Zn to solid phases does appear to control these metal concentrations in solution in the aquatic environment. Furthermore, Johnson (1986) established that the biogenic particles of Fe oxyhydroxides are relatively abundant in the aquatic environment, and have particularly suitable surface areas with a high binding affinity for Cu and Zn. Therefore, Fe - in its hydrous oxide form in sediment - decreases the availability of certain trace metals to aquatic organisms by adsorption of these metals onto the Fe compound.

In the surveys conducted, high variation occurred in the bioaccumulation of Fe in individuals of *O. mossambicus*, as well as in different tissues. Of all tissues, liver accumulated the highest concentrations of Fe in *O. mossambicus*. The degree of bioaccumulation found in *O. mossambicus* at Mamba and Balule was in the following order: liver > gills > skin > muscle (Fig. 3 ***C***). According to Brooks et al. (1976), the distribution of Fe in tissues of trout is similar to that of Cu. Brooks et al. (1976) found that the highest Fe concentrations were found in the spleen of trout, followed by the liver, with concentrations in the muscle being much lower. In studies done by Seymore (1994) the highest Fe bioaccumulation appeared in the gut in *B. marequensis* - with a similar finding by Cheggour et al. (1990) in *Scrobicularia plana* (bivalve mollusc) - followed by the liver.

Furthermore, Du Preez and Steyn (1992) established that the highest bioaccumulation of Fe in the tigerfish *(Hydrocynus vittatus)*, caught in the Olifants River, occurred in the liver. In this study the bioaccumulation values coincide with findings of the above mentioned authors. According to Hodson (1988), dietary metals generally pass through the liver after intestinal adsorption before accumulating in other organs and/or tissues. This would explain the high Fe levels in the gut content found by both Cheggour et al. (1990) and Seymore (1994), and may explain the higher bioaccumulation levels found in the liver of *O. mossambicus* during this study. The BcF_w was much higher than the BcF_s at Mamba and Balule. Therefore the bioavailability of Fe in water should be higher than in sediment, which may be due to the formation of inorganic complexes, such as carbonate, in the sediment of river systems which have high $CaCO_3$ levels, such as in the Olifants River system.

Accumulation values for Fe in the different tissue types of *O. mossambicus* were generally higher at Balule than at Mamba (Fig. 3 ***C***). This could partly be attributed to the underlying basalt formation which occur in the Balule area, and, as stated by the Department of Water Affairs and Forestry (1993) and Seymore (1994), the weathering of the rock formation will lead to the release of Fe into the aquatic environment. The weathering of the basalt rock formation will result in the release of acidic H^+ and ionic Fe^{2+} (Förstner and Wittmann, 1981), which are bioavailable for accumulation by aquatic organisms.

Effect of major inorganic constituents on the toxicity and bioaccumulation of Fe - The behaviour of Fe in the aquatic environment, i.e. the amount of Fe in solution and the rate of oxidation of Fe^{2+} to Fe^{3+}, is greatly dependent on the redox-

potential (E_h), pH and temperature (Wetzel, 1975). Changes in the redox-potential, which usually occur in conjunction with a decline in oxygen potential, may result in the dissolution and release of hydroxides of trace metals such as Fe (Dallas and Day, 1993). Moss (1988) states that Fe may be toxic at a modest concentration of ± 100 $\mu g \cdot \ell^{-1}$ if it is present as Fe^{2+} in low dissolved oxygen concentrations, or as Fe^{3+} at a pH of 3 to 4. Similar concentrations, however, may form complexes with colloidal hydroxides at a pH of 7 to 9, which are largely inert to living organisms. Cole (1983) found that at a pH of 7.5 to 7.7 a threshold is reached where Fe precipitates automatically to $Fe(OH)_3$. Furthermore, Wang (1987) states that the toxicity of Fe to the survival of zebrafish increased at pH 4, in contrast to more alkaline conditions.

With the pH slightly more acidic at Balule in most cases (Table 1), it would be expected that bioaccumulation at this location may be higher than at Mamba, as was the case in this study. However, the difference in pH between the two localities is minimal (i.e. < 1.0 difference) (Table 1), and the higher accumulation values found at Balule should rather be attributed to the geological formations found in this area.

According to Luoma (1983), temperature could affect the quantity of metal accumulated by organisms, and thus affect metal bioavailability. A ±10°C increase in temperature generally causes the metabolic process rate to double (Luoma, 1983), as was illustrated during the present study where the highest accumulation of Fe generally occurred in February and November during 1994 (Fig. 2.2c ***A-D***). Due to the increase in temperature during the summer months, an increase in metabolic activity in fish is expected, which could result in an increase in Fe uptake.

Manganese

Bioaccumulation - According to the Department of Water Affairs and Forestry (1993), the main route of Mn adsorption occurs through the respiratory and gastrointestinal tracts, with the adsorption of Mn in the digestive tract closely linked to that of Fe. The adsorption of Mn in the digestive tract is inversely related to Ca^{2+} levels in the diet of the organism, and directly to the levels of potassium (Department of Water Affairs and Forestry, 1993). The Department of Water Affairs and Forestry (1993) further states that conditions such as anaemia result in increasing adsorption of both Mn and Fe, with the adsorption rate of Mn increasing more than two fold.

No *in situ* or experimental data for Mn accumulation in the food chain exist, and in only a few heavy metal studies the pattern of bioconcentration within fish species has been investigated (Seenayya and Prahalad, 1987). Furthermore, little is known about the regulative properties of fish to elevated Mn concentrations. Some regulation of Mn has been observed in decapod crustaceans by Bryan (1976). Even though Mn inhibits enzymes, astonishingly high concentrations of Mn have been detected in some aquatic species (Bryan, 1976). The Department of Water Affairs and Forestry (1993) states that Mn in the body of fish is primarily regulated by excretion, rather than by excretion and adsorption. Seymore et al. (1995) further found higher Mn concentrations in the kidney than in the bile of *B. marequensis*, which implies urinary excretion rather than biliary excretion of Mn.

The degree of bioaccumulation of Mn in *O. mossambicus* at Mamba and Balule was similar. Highest Mn bioaccumulation occurred in the gills, followed by the liver, skin and the lowest values found in the muscle (Fig. 3 ***D***). In studies by De Wet (1990), *C. gariepinus* also showed the highest accumulation of Mn in the gills, and, similarly, Brooks et al. (1976) found the highest Mn accumulation in gills of 21 trout from Lake Taupa, followed by gonads and then liver, which contained ± twice the amount of Mn than muscle. Wiener and Giesy (1979) also found that the mean Mn concentration in the liver was greater than in the axial muscle tissue of the bluegill, largemouth bass, chain pickerel and bowfin. Brooks et al. (1976) further established that the Mn concentrations in trout was extremely low (average 0.5 $\mu g \cdot g^{-1}$), with the mean average Mn content in rainbow trout never exceeding 1.0 $\mu g \cdot g^{-1}$. This was not the case in this study, where much higher values were evident in all tissues tested (values ranged between 17.58 $\mu g \cdot g^{-1}$ and 179.62 $\mu g \cdot g^{-1}$). This could imply that Mn was more bioavailable for uptake in the Olifants River system than in the area studied by Brooks et al. (1976).

In studies by Du Preez and Steyn (1992) on tigerfish (*H. vittatus*), the highest accumulation of Mn was found in the stomach tissue, and in similar studies by Seymore et al. (1995) on *B. marequensis*, it was found that accumulation of Mn was highest in the gills, closely followed by the gut content. The results from this study, and existing literature, indicate that the uptake of Mn by aquatic organisms, particularly fish, occurs mainly directly through concentrations of dissolved metals via the gills, or through indirect food uptake and sediment ingestion. The bioconcentration factors for Mn, calculated between the water and tissues, were higher than the bioconcentration factors for the sediment and tissues. This suggests that Mn was more bioavailable in the water, which partially explains the high accumulation levels found in gills, which is a direct uptake route of Mn from water.

Mn bioaccumulation values in the various tissues, at Mamba and Balule, did not differ much from each other, and no definite trend could be established (Fig. 3 ***D***). The high Mn concentrations found in the tissues of *O. mossambicus* during the summer months (Fig. 2.2d ***A-D***) could be due to more pronounced leaching occurring during this time of the year as a result of the summer rainfall. Hahne and Kroontje (1973) state that systems subjected to rainfall and increased leaching usually have lower pH values. This will result in more hydrogen ions available, to compete with Mn ions in the water, for binding sites on particle surfaces and solution ligand, thus increasing the availability of Mn to fish.

Effect of major inorganic constituents on the toxicity and bioaccumulation of Mn - According to Wang (1987), several reports indicate that toxicity of Mn increases with a decrease in pH. Reader and Dempsey (1989) found that experimental exposure of brown trout (*Salmo trutta*) to decreased pH and increased Mn concentrations resulted in a 100% mortality rate. Most pH values, recorded in this study, were alkaline (Table 1), and no trend between pH and accumulation could be established for Mamba and Balule. Mn accumulation and toxicity are also influenced by temperature. Cairns et al. (1975) found that Mn uptake in blue-green algae (*Plectonema boryanum*) increased to 35°C, then decreased at higher temperatures. Additionally Braginskiy and Shcherban (1978) found Mn to be non-toxic at 10 to 15°C for six species of freshwater invertebrates, but toxicity increased sharply at 25 to 30°C. This should, affect the degree of bioaccumulation in *O. mossambicus*, because *O. mossambicus* feeds on both algae and invertebrates (Pienaar, 1978; Skelton, 1993). As would be expected, higher accumulation of Mn in *O. mossambicus* occurred during summer (Fig. 2.2d ***A-D***), with no sign of chronic exposure evident in the fish sampled. Salinity

plays an important role in Mn uptake, due to the relationship between Mn absorption and Ca^{2+} (Cheggour et al., 1990). The total dissolved solids (TDS) recorded at Mamba were higher than at Balule, except during May (Table 1), which suggests a higher Ca^{2+} concentration in the water at Mamba. More Ca^{2+} ions will compete withMn^{2+} ions for absorption through the gill surface, thus decreasing the bioavailability of Mn^{2+} to fish at Mamba, when compared to Balule. However, when the results in this study are studied, no trend could be established between the TDS values and the bioaccumulation values.

Conclusion

It appeared that the metals tested in this study mainly accumulated in the liver and gills, followed by the skin, and lastly the muscle. The concentrations found in the tissues of *O. mossambicus* suggest no serious metal contamination. Fish, however, could have been exposed to chronic sublethal concentrations, mostly during February 1994, which could possibly have led to sublethal effects in the fish. In summary, certain factors should be considered when surveys on trace metals concentrations in fish are conducted. For example, the sample size must be large, and caution should be taken in editing of statistical data. Furthermore, trace metal concentrations in fishes result from a great variety of complex probability functions, which describe the input-output phenomena and homeostasis of the organism. These functions and external factors should be accounted for when interpreting the results.

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