Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, *Labeo umbratus* (Cyprinidae), from Witbank Dam, Mpumalanga

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Abstract

This paper focuses on the extent of Cr, Mn, Ni and Pb bioaccumulation in the different tissues of a cyprinid fish, namely the moggel (*Labeo umbratus*) from Witbank Dam in the Upper Olifants River catchment. The dependence of bioaccumulation on size, gender and seasons was specifically addressed. Bioaccumulation of Cr, Mn, Ni and Pb varied between the gills, liver, muscle and skin. The gills generally had the highest metal concentrations, due to their intimate contact with the environment and their importance as an effector of ionic and osmotic regulation. The liver, in its role as a storage and detoxification organ, can also accumulate high levels of metals. Muscles and skin accumulated much less metal concentrations. These two organs must be included in biomonitoring programmes because they are consumed by the general public. Accumulation of the metals decreased with an increase in fish length. Therefore, the smaller the fish the higher the body load of metals due to various bioaccumulation processes. The accumulation of Cr, Mn and Ni in the different tissues of male and female fish did not differ markedly. It is suggested that the male testes and female ovaries should also be compared, in order to obtain further data on the differences of accumulation of metals between males and females. The highest tissue concentrations of Cr, Mn, Ni and Pb with the exception of the muscle and skin tissues, were recorded in the summer of 1995. The higher metal concentrations in the summer, compared to autumn and winter, can possibly be attributed to a varied water temperature.

Introduction

It is well documented that pollutants, such as metals and organic compounds, can be accumulated by aquatic biota (USEPA, 1991). Bioaccumulation measurements refer to studies or methods monitoring the uptake and retention of pollutants like metals or biocides in organs and/or tissues of organisms, such as fish (Roux, 1994). This can only take place if the rate of uptake by the organism exceeds the rate of elimination (Spacie and Hamelink, 1985). There are five potential routes for a pollutant to enter a fish: via the food, non-food particles, gills, oral consumption of water and the skin. Once the pollutant is absorbed, it is transported by the blood to either a storage point (i.e. bone) or to the liver for transformation and/or storage. According to Heath (1991), if the pollutant is transformed by the liver it may be stored there or excreted in the bile or passed back into the blood for possible excretion by the gills or kidneys, or stored in fat, which is an extra-hepatic tissue. Therefore, the concentration found in different tissues, after environmental exposure, for a specific time, depends on several dynamic processes all taking place concurrently.

Chromium (Cr) is a relatively scarce metal, the occurrence and amounts thereof in aquatic ecosystems are generally very low (0.001 to 0.002 mg·ℓ^{-1} - Moore and Ramamoorthy, 1984; DWAF, 1996). However, natural water may receive Cr from anthropogenic sources such as industrial effluents derived from the production of corrosion inhibitors and pigments (Galvin, 1996), which then becomes a pollutant of aquatic ecosystems and thus harmful to aquatic organisms (Srivastava et al., 1979). The toxicity of Cr is affected by species, body size and life stage of the organism as well as the pH of the water and, to a lesser extent, by hardness, salinity and temperature (Holdway, 1988). Fish are usually more resistant to Cr than other aquatic organisms, but they can be affected sublethally when exposed to concentrations ranging from 0.013 to 50 mg·ℓ^{-1} (Olson and Foster, 1956; Van der Putte and Pärt, 1982) and lethally when exposed to concentrations ranging from 3.5 to 280 mg·ℓ^{-1} Cr (Moore and Ramamoorthy, 1984; Van der Putte et al., 1981a; b). Chromium (VI) appears to pass readily through the gill membrane and accumulates rapidly in various tissues at higher levels than in the gills (Holdway, 1988), including the brain, gall bladder, gastro-intestinal tract, intestine, kidney, opercular bone, spleen and stomach (Fromm and Schiffmann, 1958; Buhler et al., 1977; Van der Putte et al., 1981b).

Manganese (Mn) is an essential micronutrient (Dallas and Day, 1993) and does not occur naturally as a metal in aquatic ecosystems (<1.0 mg·ℓ^{-1} – Hellawell, 1986) but is found in various minerals and salts for example, $MnCaCO_3$ (rhodocrosite), MnO_2 (pyrolusite) and $MnSiO_3$ (rhodonite), with oxides being the only important Mn-containing minerals mined (Galvin, 1996). Although Mn demonstrates some significance as a pollutant (Hellawell, 1986), it is one of the first metals to show elevated concentrations in acidified waters (Bendell-Young and Harvey, 1986). According to Kempster et al. (1982), Mn is of moderate toxicity to aquatic organisms. Rouleau et al. (1996) recorded that Mn^{2+} uptake by brown trout was significantly increased at a low pH.

Nickel (Ni) occurs as four basic ores namely, arsenide, laterite, silicate and sulphide (Galvin, 1996). It is a natural ubiquitous element of the earth and in water (0.001 to 0.003 mg·ℓ^{-1} – Snodgrass, 1980). Anthropogenic activities (i.e. mining, electroplating and steel plant operations) can result in Ni discharge into water and air (Galvin, 1996). Nickel ions tend to be soluble at pH values <6.5, and above 6.7 they mostly form insoluble nickel hydroxides (Dallas and Day, 1993). In aquatic ecosystems, dissolved Ni

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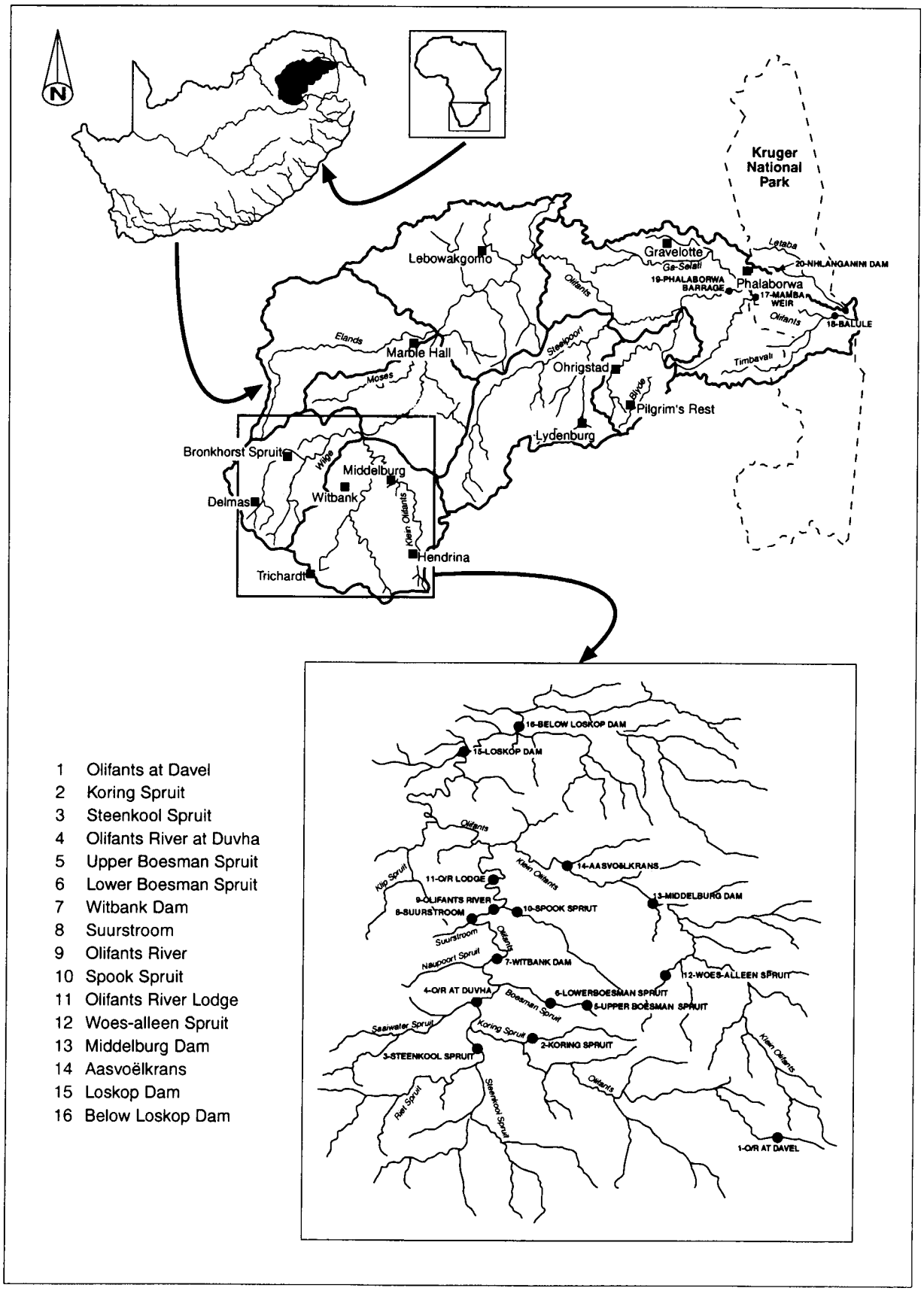

Figure 1
Location of sampling localities in the Upper Catchment of the Olifants River

TABLE 1
METAL CONCENTRATIONS IN THE WATER AND DRIED SEDIMENT OF WITBANK DAM, OBTAINED DURING THE STUDY PERIOD, FEBRUARY 1994 TO MAY 1995

Month of survey	Sample type	Metals			
		Chromium (Cr)	Manganese (Mn)	Nickel (Ni)	Lead (Pb)
February 1994	Water ($mg \cdot \ell^{-1}$) Sediment ($\mu g \cdot g^{-1}$)	0.15 126.92	0.05 298.17	0.16 45.09	0.13 23.15
May 1994	Water ($mg \cdot \ell^{-1}$) Sediment ($\mu g \cdot g^{-1}$)	0.17 102.87	0.13 185.52	0.22 63.04	0.15 29.45
August 1994	Water ($mg \cdot \ell^{-1}$) Sediment ($\mu g \cdot g^{-1}$)	0.25 112.19	0.04 227.08	0.21 76.54	0.16 32.81
November 1994	Water ($mg \cdot \ell^{-1}$) Sediment ($\mu g \cdot g^{-1}$)	0.22 136.99	0.09 204.48	0.17 66.24	0.08 18.83
February 1995	Water ($mg \cdot \ell^{-1}$) Sediment ($\mu g \cdot g^{-1}$)	0.44 86.17	0.25 196.37	0.40 37.14	0.25 18.55
May 1995	Water ($mg \cdot \ell^{-1}$) Sediment ($\mu g \cdot g^{-1}$)	0.25 34.97	0.17 176.97	0.29 33.49	0.08 15.10

concentrations are generally between 0.005 and 0.010 $mg \cdot \ell^{-1}$ (Galvin, 1996). The toxicity of Ni to aquatic life has been shown to vary significantly with organism species, pH and water hardness (Birge and Black, 1980). Nickel toxicity is generally low (Khangarot and Ray, 1990), but elevated concentrations can cause sublethal effects. In freshwater fish recorded Ni accumulation values ranged from 10 to 120 $\mu g \cdot g^{-1}$ (Tong, 1974; Vos and Hovens, 1986), and distribution of Ni varies significantly between the different tissues (Gilmartin and Revelante, 1975).

Lead (Pb) exists in several oxidation states (0, I, II and IV) which are all of environmental importance. The divalent form, Pb(II), is the stable ionic species present in the environment and is thought to be the form in which most Pb is bioaccumulated by aquatic organisms (DWAF, 1996). Lead enters the aquatic environment through erosion and leaching from soil, lead-dust fallout, combustion of gasoline, municipal and industrial waste discharges, runoff of fallout deposits from streets and other surfaces as well as precipitation (DWAF, 1996). In natural water the total Pb concentrations generally range between 0.05 and 10.0 $mg \cdot \ell^{-1}$, whilst the dissolved Pb concentration normally does not exceed 0.01 $mg \cdot \ell^{-1}$ (Galvin, 1996). Lead is known to accumulate in the tissues of fish (Latif et al., 1982; Dallas and Day, 1993), including the bone, gills, kidneys, liver and scales. The uptake of aqueous Pb^{2+} across the gill into the bloodstream is the primary mode of uptake in freshwater fish (Coetzee, 1996). The toxicity of Pb is dependent upon the life stage of the fish, pH and hardness of the water as well as the presence of organic materials (Merlini and Pozzi, 1977; Moore and Ramamoorthy, 1984; Hellawell, 1986).

This paper focuses on the extent of Cr, Mn, Ni and Pb bioaccumulation in the different tissues of a cyprinid fish, namely the moggel (*Labeo umbratus*) from Witbank Dam (Locality 7) in the Upper Olifants River Catchment (Fig. 1). The dependence of bioaccumulation on size, gender and seasons was specifically addressed.

Materials and methods

Description of the sampling site

Witbank Dam (Locality 7 – Fig 1), in the Olifants River, was built in 1949 and has a storage capacity of 10 402 x 10^6 m^3. The catchment area is 3 589 km^2 and comprises part of the most developed region of the Olifants River basin. Mean annual runoff for present land use is estimated at 106.8 x 10^6 m^3. The area under irrigation in the catchment was 2 040 ha in 1988, with an estimated water use of 16.2 x 10^6 $m^3 \cdot a^{-1}$. Users that abstract water from the dam are Witbank Municipality, various mines (Duvha Opencast Services) and Eskom (Duvha power station). Witbank Dam receives water from Greenside Colliery and the Olifants River. Point sources of pollution to Witbank Dam include Greenside Colliery and Duvha Power Station, whilst non-point sources include cattle farming and agricultural runoff, informal rural settlements, atmospheric deposition, surface runoff and polluted groundwater.

Field sampling

Labeo umbratus specimens were captured every three months at Witbank Dam (Locality 7 – Fig. 1) during the study period of February 1994 to May 1995, using gill nets (70 to120 mm stretched mesh size). After capture each fish was individually weighed and total length measured. The fish were then dissected on a polythene work-surface, using stainless steel dissection instruments (Heit and Klusek, 1982) whilst wearing surgical gloves. The following tissues were removed, placed in glass bottles and frozen for metal analysis: gills, liver, muscle and skin. Prior to use, all glassware was soaked in a 2% Contrad soap solution (Merck chemicals) for 24 h, rinsed in distilled water, acid-washed in 1M HCl for 24 h and rinsed in distilled water once again (Giesy and Weiner, 1977).

TABLE 2
MEAN (± SD) CHROMIUM CONCENTRATIONS (µg·g^{-1} DRY MASS) IN THE TISSUES OF *LABEO UMBRATUS* FROM THE WITBANK DAM DURING THE STUDY PERIOD OF FEBRUARY 1994 TO MAY 1995

Month of survey	Variables	Tissues			
		Gills	Liver	Muscle	Skin
February 1994	n	20	20	20	20
	x ± SD	26.21±21.39	28.05±24.53	13.88±7.28	29.21±24.08
	Min/Max	10.62-78.72	9.52-92.33	9.62-38.12	10.17-78.27
	BF_w	174.73	187.00	92.53	194.73
	BF_s	0.21	0.22	0.11	0.23
May 1994	n	20	20	20	20
	x ± SD	12.18±4.13	10.82±2.44	12.39±4.56	10.61±3.24
	Min/Max	6.62-21.12	6.27-19.17	5.57-24.47	4.47-20.62
	BF_w	71.65	63.65	72.88	62.41
	BF_s	0.12	0.11	0.12	0.10
August 1994	n	20	20	20	20
	x ± SD	22.10±6.25	19.92±7.34	21.70±7.09	19.69±7.58
	Min/Max	14.30-32.00	10.90-34.85	12.00-33.75	12.00-33.95
	BF_w	88.40	79.68	86.80	78.76
	BF_s	0.20	0.18	0.19	0.18
November 1994	n	20	20	20	20
	x ± SD	25.99±12.47	19.88±9.61	21.90±6.65	18.77±9.45
	Min/Max	11.34-56.74	8.90-38.55	10.57-37.19	8.49-35.94
	BF_w	118.14	90.36	99.55	85.32
	BF_s	0.19	0.15	0.16	0.14
February 1995	n	20	20	20	20
	x ± SD	60.79±52.61	66.21±45.32	60.31±54.17	123.65±111.18
	Min/Max	22.14-183.75	21.72-170.75	17.67-197.12	16.55-396.12
	BF_w	138.16	150.48	137.07	31.02
	BF_s	0.71	0.77	0.70	0.16
May 1995	n	20	20	20	20
	x ± SD	53.86±9.44	53.60±13.89	49.43±10.61	52.91±12.50
	Min/Max	39.30-66.96	36.04-85.45	36.69-69.20	37.33-75.77
	BF_w	91.29	90.85	83.78	89.68
	BF_s	1.54	1.53	1.41	1.51

Laboratory procedures

After tissue samples were thawed and rinsed in distilled water, approximately 5g of each sample was dried in an oven at 60ºC for a period of 48 h. This was done in order to determine the moisture content of each sample. One gram of dried tissue was then accurately weighed into 100 mℓ Erlenmeyer flasks whereafter 5ml perchloric acid (70%) and 10 mℓ nitric acid (55%) were added. Digestion was performed on a hotplate, at 200 to 250ºC, for at least 4 h or until solutions were clear (Van Loon, 1980). After digestion each sample was filtered using and acid-resistant 0.45µm filter paper and a vacuum pump. After filtration the filtering system was rinsed with distilled water to remove all traces of metals, whereafter the samples were made up to 50 mℓ with distilled water. The samples were stored in amber glass bottles for 2 to 3 weeks until the metal concentrations could be determined. A Varian Atomic Absorption Spectrophotometer (Spectra AA-10) was used to determine the Cr, Mn, Ni and Pb concentrations in the tissue samples of the fish. A range of analytical standards for each metal was prepared from Holpro stock solutions. Bioconcentration factors (Weiner and Giesy, 1979) between the fish tissues and the water (BF_w) as well as the sediment (BF_s) were calculated, using the mean metal concentration in each tissue and the corresponding metal concentration for water and sediments (Table 1) as previously described (Nussey, 1998).

Statistical procedures

Non-parametric statistical analysis was performed using the statistical software package STATISTICA. The Kolmogorov-Smirnov two-sample test was used for the comparison of two groups (e.g. male and female, seasons and different tissues). The Spearman-R test was used to determine the relationship between the metal

TABLE 3
MEAN (±SD) MANGANESE CONCENTRATIONS (µg·g^{-1} DRY MASS) IN THE TISSUES OF *LABEO UMBRATUS* FROM THE WITBANK DAM DURING THE STUDY PERIOD OF FEBRUARY 1994 TO MAY 1995

Month of survey	Variables	Tissues			
		Gills	Liver	Muscle	Skin
February 1994	n	20	20	20	20
	x ± SD	105.67±21.79	16.54±4.45	3.56±0.89	2.77±0.93
	Min/Max	77.42-162.42	10.57-30.12	2.22-6.42	1.57-5.57
	BF_w	2113.40	330.80	71.20	55.40
	BF_s	0.35	0.06	0.01	0.01
May 1994	n	20	20	20	20
	x ± SD	87.30±19.61	7.27±2.44	3.11±1.10	3.65±1.64
	Min/Max	61.52-147.82	2.42-12.32	1.67-5.92	1.27-7.67
	BF_w	671.54	55.92	23.92	28.08
	BF_s	0.47	0.04	0.02	0.02
August 1994	n	20	20	20	20
	x ± SD	106.76±24.86	5.55±1.59	3.84±1.07	2.39±1.04
	Min/Max	59.31-140.36	3.41-9.31	1.21-5.41	1.21-4.11
	BF_w	2669.00	138.75	96.00	59.75
	BF_s	0.47	0.02	0.02	0.01
November 1994	n	20	20	20	20
	x ± SD	77.91±13.64	55.65±16.03	5.35±2.86	4.97±2.27
	Min/Max	52.89-104.73	34.58-92.32	2.09-16.18	1.95-9.05
	BF_w	865.67	618.33	59.44	55.22
	BF_s	0.38	0.27	0.03	0.02
February 1995	n	20	20	20	20
	x ± SD	134.02±32.65	27.40±16.50	9.26±5.15	12.77±9.10
	Min/Max	70.11-192.34	9.00-56.70	3.78-21.67	3.16-26.96
	BF_w	536.08	109.60	37.04	51.08
	BF_s	0.68	0.14	0.05	0.07
May 1995	n	20	20	20	20
	x ± SD	108.77±21.84	12.52±3.15	5.13±1.30	6.29±1.72
	Min/Max	78.52-171.84	6.74-18.14	3.67-8.92	4.02-9.61
	BF_w	639.82	72.06	30.18	37.00
	BF_s	0.61	0.07	0.03	0.04

concentrations in the different tissues and the length of the fish. The significance level used throughout was $P<0.05$.

Results

Differences in Cr, Mn, Ni and Pb bioaccumulation

The mean mass of all captured *L. umbratus* ranged from 925.50 to 1536.45 g, and the mean length from 44.95 to 52.09 cm. The mean percentage moisture content of the tissues was 79.59±1.30% in the gills, 70.54±4.53% in the liver, 75.24±2.31% in the muscle and 61.83±1.62% in the skin.

Chromium concentrations in *L. umbratus* tissues did not fluctuate much during this survey with the possible exception of February 1994 and 1995 (Table 2). The order of Cr bioaccumulation was G≈L≈M≈S. Significant differences ($P<0.05$) were recorded between most tissues, with the exception of muscle/skin and skin/liver. The calculated bioconcentration factors between the tissues and the water (BF_w) ranged from 31.02 (February 1995) to 194.73 (February 1994), both in the skin. The BF_s's ranged from 0.10 in the skin (May 1994) to 1.54 in the gills (May 1995) (Table 2).

The highest Mn concentrations were found in the gills followed by the liver and then the muscle and skin (Table 3). Thus, the prevalent order of Mn bioaccumulation was G>L>M≥S. Manganese concentrations fluctuated from 2.39 µg·g^{-1} dry mass in the skin (August 1994) to 134.02 µg·g^{-1} dry mass in the gills (February 1995) (Table 3). Significant differences ($P<0.05$) were recorded for all comparisons between tissues. The Mn bioconcentration factors (BF_w) between the tissues and water ranged from 23.92 in muscle (May 1994) to 2 669.00 in the gills (August 1994). The bioconcentration factors (BF_s) between tissues and sediment ranged from 0.01 in muscle and skin (February and August 1994) to 0.68 in gills (February 1995) (Table 3).

With the possible exception of the skin Ni concentrations for

TABLE 4
MEAN (± SD) NICKEL CONCENTRATIONS (µg·g^{-1} DRY MASS) IN THE TISSUES OF *LABEO UMBRATUS* FROM THE WITBANK DAM DURING THE STUDY PERIOD OF FEBRUARY 1994 TO MAY 1995

Month of survey	Variables	Tissues			
		Gills	Liver	Muscle	Skin
February 1994	n	20	20	20	20
	x ± SD	11.64±3.14	9.55±3.18	10.01±3.96	9.03±2.44
	Min/Max	2.64-17.84	4.44-16.99	4.74-19.04	6.59-14.44
	BF_w	72.75	59.69	62.56	56.44
	BF_s	0.26	0.21	0.22	0.20
May 1994	n	20	20	20	20
	x ± SD	12.34±3.58	10.46±1.17	13.09±1.98	10.88±0.92
	Min/Max	9.49-20.49	8.04-12.89	9.59-19.39	8.79-12.64
	BF_w	56.09	47.55	59.50	49.45
	BF_s	0.20	0.17	0.21	0.17
August 1994	n	20	20	20	20
	x ± SD	20.47±6.13	15.94±5.76	17.68±5.24	15.62±5.60
	Min/Max	12.51-35.66	9.91-28.41	9.51-23.21	7.56-23.46
	BF_w	97.48	75.90	84.19	74.38
	BF_s	0.27	0.21	0.23	0.20
November 1994	n	20	20	20	20
	x ± SD	17.51±6.07	12.75±5.31	15.55±5.93	10.81±4.96
	Min/Max	9.34-28.69	4.03-22.26	6.41-34.04	5.20-20.79
	BF_w	103.00	75.00	91.47	63.59
	BF_s	0.26	0.19	0.23	0.16
February 1995	n	20	20	20	20
	x ± SD	27.83±15.47	38.29±21.21	35.77±28.24	69.55±56.51
	Min/Max	15.61-69.41	17.31-83.67	12.60-114.51	12.10-159.85
	BF_w	69.78	95.73	89.43	173.88
	BF_s	0.75	1.03	0.96	1.87
May 1995	n	20	20	20	20
	x ± SD	22.67±4.14	23.08±6.47	21.18±4.91	21.63±6.13
	Min/Max	18.02-29.80	14.80-38.16	15.09-31.98	14.84-39.22
	BF_w	78.77	79.59	73.03	74.59
	BF_s	0.68	0.69	0.63	0.65

May 1994 and February 1995, no significant trends were detected and the bioaccumulation order was G≈L≈M≈S. During the February 1995 survey, higher Ni levels were recorded compared to the other surveys (Table 4). There were predominantly significant differences ($P<0.05$), with the only exceptions being skin and liver. The bioconcentration factors (BF_w) for the water and the tissues ranged from 47.55 in the liver (May 1994) to 173.88 in the skin (February 1995). Bioconcentration factors (BF_s) between the tissues and the sediments fluctuated between 0.16 (November 1994) and 1.87 (February 1995), both in the skin (Table 4).

The bioaccumulation of Pb in the tissues also showed no specific trends and the order of bioaccumulation was G>L≈M≈S. It would seem that the highest concentrations were predominantly found in the gills, except during May 1994 where muscle showed the highest level (Table 5). The differences between tissues were significant ($P<0.05$) for most cases except when comparing skin and muscle. The BF_w for Pb fluctuated between 24.04 in skin (February 1995) and 150.13 in the gills (May 1995). The BF_s's ranged from 0.11 in the skin (November 1994) to 1.16 in the gills (February 1995) (Table 5).

Relationships between metal concentrations and total fish length

Data for the study period were grouped together for the statistical analysis of fish length vs. metal concentration (Table 6). Significant ($P<0.05$) negative correlations were recorded between the length of the specimens and the Mn concentrations in the gills and liver as well as for Pb concentrations in the gills, liver and skin. These results indicate that the longer fish usually had the lower tissue metal concentrations. Insignificant negative correlations were recorded between fish length and the Cr concentrations in all four tissue types, the Mn concentration in the skin, the Ni concentrations in the gills, liver and skin as well as the Pb concentration in the muscle. Positive correlations, although insignificant, were found for both the Mn and Ni concentrations in the muscle.

TABLE 5
MEAN (± SD) LEAD CONCENTRATIONS (µg·g^{-1} DRY MASS) IN THE TISSUES OF *LABEO UMBRATUS* FROM THE WITBANK DAM DURING THE STUDY PERIOD OF FEBRUARY 1994 TO MAY 1995

Month of survey	Variables	Tissues			
		Gills	Liver	Muscle	Skin
February 1994	n	20	20	20	20
	x ± SD	18.77±2.30	9.55±3.31	10.01±3.79	8.46±1.97
	Min/Max	15.40-23.25	5.15-17.70	4.35-20.45	13.90-10.90
	BF_w	144.38	73.46	77.00	65.08
	BF_s	0.81	0.41	0.43	0.37
May 1994	n	20	20	20	20
	x ± SD	7.09±3.87	6.46±2.76	11.92±2.30	5.14±2.82
	Min/Max	2.75-18.30	2.35-13.70	6.28-17.15	1.50-12.25
	BF_w	47.27	43.07	79.47	34.27
	BF_s	0.24	0.22	0.40	0.17
August 1994	n	20	20	20	20
	x ± SD	14.93±1.64	8.49±1.32	8.68±1.43	7.31±1.67
	Min/Max	12.06-17.71	6.21-10.71	5.96-12.11	5.06-11.81
	BF_w	93.31	53.06	54.25	45.69
	BF_s	0.46	0.26	0.26	0.22
November 1994	n	20	20	20	20
	x ± SD	10.38±2.51	3.50±1.21	4.02±1.78	2.11±1.37
	Min/Max	7.63-15.83	1.63-5.78	1.18-6.88	0.94-6.79
	BF_w	129.75	43.75	50.25	26.38
	BF_s	0.55	0.19	0.21	0.11
February 1995	n	20	20	20	20
	x ± SD	21.49±2.85	9.86±4.02	7.62±2.85	6.01±2.69
	Min/Max	17.50-28.69	4.52-19.98	2.37-12.61	2.29-11.47
	BF_w	85.96	39.44	30.48	24.04
	BF_s	1.16	0.53	0.41	0.32
May 1995	n	20	20	20	20
	x ± SD	12.01±5.41	4.30±1.15	6.27±4.43	4.27±1.36
	Min/Max	6.55-25.53	1.15-6.10	2.40-18.37	1.52-7.21
	BF_w	150.13	53.75	78.38	53.38
	BF_s	0.80	0.28	0.42	0.28

Differences between males and females

There were no significant differences recorded between males and females regarding Cr (Table 7), Mn (Table 8) and Ni (Table 9) tissues concentrations. Only the May 1995 sample of fish revealed significant differences ($P<0.05$) in the Pb (Table 10) concentrations of muscle and skin, where the higher concentrations were recorded in the females.

Seasonal differences

Statistical analyses were also carried out to establish seasonal differences. During this study significant seasonal differences were recorded in the different tissues of *L. umbratus* for Cr (Table 11), Mn (Table 12), Ni (Table 13) and Pb (Table 14). The Cr concentrations in the different tissue types (gills, liver, muscle and skin) differed significantly ($P<0.05$). These differences were predominantly caused by the Cr concentrations in the skin, followed by that in the muscle, gills and liver. With regard to Mn concentrations, the following comparisons showed significant differences: spring 1994 and summer 1994/winter 1994, winter 1994/summer 1995/autumn 1995 and autumn 1994, summer 1995 and spring 1994 as well as autumn 1995 and summer 1995, and differences ($P<0.05$) appeared to be more likely in the liver, followed by muscle and then gills and skin. Significant seasonal differences ($P<0.05$) in the bioaccumulation of Ni for all tissue types were also found in 10 of the 15 comparisons. The muscle differed significantly in all 15 comparisons, followed by the skin (14), liver (13) and gills (12). For Pb bioaccumulation the gills, followed by the muscle, liver and skin recorded significant differences during the comparisons of the different seasons, e.g. autumn 1994/spring 1994/autumn 1995 and summer 1994, winter 1994/spring 1994 and autumn 1994, spring 1994/autumn 1995 and winter 1994 as well as summer and spring 1994.

TABLE 6
DATA FOR THE CORRELATION BETWEEN THE METAL CONCENTRATIONS IN THE TISSUES OF *LABEO UMBRATUS* AND TOTAL FISH LENGTH (48.57 ± 3.98 cm) FOR THE STUDY PERIOD FEBRUARY 1994 TO MAY 1995

Tissues	Statistics	Metals			
		Chromium	Manganese	Nickel	Lead
Gills	Correlation (Spearman R)	-0.15	-0.32	-0.16	-0.31
	P-value	*0.04	*0.00	*0.03	*0.00
	Calculated t-value	-2.04	-4.39	-2.13	-5.45
Liver	Correlation (Spearman R)	-0.21	-0.44	-0.21	-0.21
	P-value	*0.00	*0.00	*0.00	*0.01
	Calculated t-value	-2.85	-6.44	-2.87	-2.82
Muscle	Correlation (Spearman R)	0.02	0.02	0.13	0.07
	P-value	0.77	0.77	0.10	0.39
	Calculated t-value	0.30	0.29	1.68	0.86
Skin	Correlation (Spearman R)	-0.15	-0.21	-0.16	-0.17
	P-value	0.06	*0.00	*0.04	*0.02
	Calculated t-value	-1.97	-2.87	-2.11	-2.32

*P<0.05

TABLE 7
DIFFERENCES BETWEEN FEMALE AND MALE *LABEO UMBRATUS* REGARDING CHROMIUM BIOACCUMULATION IN THE DIFFERENT TISSUES (FEBRUARY 1994 TO MAY 1995)

Month of survey	Tissues	Variables	Gender		P
			Female	Male	
February 1994 (n: 6F/14M)	Gills	x ± SD	19.35±19.40	24.47±22.44	ns
	Liver	x ± SD	26.27±17.89	25.34±27.32	ns
	Muscle	x ± SD	18.82±17.63	24.94±23.94	ns
	Skin	x ± SD	18.58±23.02	26.10±24.99	ns
May 1994 (n: 13F/7M)	Gills	x ± SD	11.75±4.02	11.63±5.76	ns
	Liver	x ± SD	10.96±2.82	10.55±1.68	ns
	Muscle	x ± SD	12.23±4.05	12.68±5.72	ns
	Skin	x ± SD	11.12±3.71	9.68±2.06	ns
August 1994 (n: 14F/6M)	Gills	x ± SD	23.53±8.08	21.88±5.93	ns
	Liver	x ± SD	19.89±6.71	20.00±9.38	ns
	Muscle	x ± SD	22.44±6.30	19.98±9.11	ns
	Skin	x ± SD	21.84±7.68	14.68±4.67	ns
November 1994 (n: 9F/11M)	Gills	x ± SD	64.17±17.55	56.70±12.04	ns
	Liver	x ± SD	21.57±9.33	16.15±10.15	ns
	Muscle	x ± SD	28.13±13.27	20.34±7.20	ns
	Skin	x ± SD	25.47±11.20	15.75±9.12	ns
February 1995 (n: 2F/18M)	Gills	x ± SD	26.42±4.81	64.61±54.20	ns
	Liver	x ± SD	107.91±88.50	72.69±65.78	ns
	Muscle	x ± SD	117.12±97.07	63.74±62.99	ns
	Skin	x ± SD	180.57±15.95	123.01±16.60	ns
May 1995 (n: 15F/5M)	Gills	x ± SD	443.07±656.55	366.84±457.41	ns
	Liver	x ± SD	56.10±13.80	46.11±12.14	ns
	Muscle	x ± SD	50.54±11.17	46.09±8.92	ns
	Skin	x ± SD	54.54±16.67	48.01±12.95	ns

ns = not significant (P>0.05)

TABLE 8
DIFFERENCES BETWEEN FEMALE AND MALE *LABEO UMBRATUS* REGARDING MANGANESE BIOACCUMULATION IN THE DIFFERENT TISSUES (FEBRUARY 1994 TO MAY 1995)

Month of survey	Tissues	Variables	Gender		P
			Female	Male	
February 1994 (n: 6F/14M)	Gills	x ± SD	77.23±36.52	121.07±42.82	ns
	Liver	x ± SD	17.07±14.18	23.45±26.14	ns
	Muscle	x ± SD	26.96±58.92	4.34±2.16	ns
	Skin	x ± SD	2.65±0.64	3.95±4.36	ns
May 1994 (n: 13F/7M)	Gills	x ± SD	3.14±1.08	3.05±1.24	ns
	Liver	x ± SD	6.97±2.68	9.87±5.46	ns
	Muscle	x ± SD	80.83±20.01	90.61±29.43	ns
	Skin	x ± SD	3.56±1.37	3.80±2.18	ns
August 1994 (n: 14F/6M)	Gills	x ± SD	107.59±25.28	104.83±26.07	ns
	Liver	x ± SD	5.64±1.83	5.35±0.91	ns
	Muscle	x ± SD	4.06±0.93	2.77±1.60	ns
	Skin	x ± SD	2.41±1.21	2.85±3.22	ns
November 1994 (n: 9F/11M)	Gills	x ± SD	75.65±12.63	79.75±14.74	ns
	Liver	x ± SD	52.68±16.14	55.41±35.02	ns
	Muscle	x ± SD	6.73±3.65	4.21±1.33	ns
	Skin	x ± SD	5.53±1.84	5.38±3.87	ns
February 1995 (n: 2F/18M)	Gills	x ± SD	103.91±47.80	144.14±40.67	ns
	Liver	x ± SD	15.89±6.79	32.29±22.20	ns
	Muscle	x ± SD	12.14±9.80	8.94±4.79	ns
	Skin	x ± SD	19.71±1.05	31.36±78.32	ns
May 1995 (n: 15F/5M)	Gills	x ± SD	115.42±38.01	112.62±15.23	ns
	Liver	x ± SD	11.16±3.99	13.36±3.46	ns
	Muscle	x ± SD	4.94±1.36	5.70±0.99	ns
	Skin	x ± SD	6.32±1.79	6.19±1.71	ns

ns = not significant (P>0.05)

Discussion

Metal bioaccumulation in tissues

When fish are exposed to elevated metal levels in an aquatic environment, they can absorb the bioavailable metals directly from the environment via the gills and skin or through the ingestion of contaminated water and food. Metals in the fish are then transported by the bloodstream which brings it into contact with the various organs and tissues (Van der Putte and Pärt, 1982). Fish can regulate metal concentrations to a certain extent, whereafter bioaccumulation will occur (Heath, 1991). Therefore, the ability of each tissue to either regulate or accumulate metals can be directly related to the total amount of metal accumulated in that specific tissue. Furthermore, physiological differences and the position of each tissue in the fish can also influence the bioaccumulation of a particular metal (Kotze, 1997).

The Cr bioaccumulation pattern in the tissues of the selected fish showed no clear pattern. Higher concentrations of Cr were mostly recorded in the gills followed by the liver. The concentrations of Cr found in the gills, liver, muscle and skin during this study can also be supported by various other studies (Buhler et al., 1977; Barnhoorn, 1996; Coetzee, 1996; Kotze, 1997; Wepener, 1997). The accumulation of Cr in gill tissue is usually associated with structural damage to the gill epithelium as well as impaired respiratory and osmoregulatory function. These effects have often been cited as the acute mechanism of metal toxicity (Burton et al., 1972). During this study the Cr concentrations in the gills of fish were lower than gill Cr concentrations found elsewhere in the Upper Olifants River Catchment (Fig.1: Locality 13, 17 to 87 $\mu g \cdot g^{-1}$ dry mass – Barnhoorn, 1996; Locality 11, 17 to 67 $\mu g \cdot g^{-1}$ dry mass and Locality 14, 24 to 130 $\mu g \cdot g^{-1}$ dry mass – Coetzee, 1996; Locality 15, 5 to 120 $\mu g \cdot g^{-1}$ dry mass – Kotze, 1997). Gill Cr concentrations were also lower compared to that found for *Barbus marequensis* in the Lower Olifants River and Selati River (3.1 to 104.0 $\mu g \cdot g^{-1}$ dry mass – Seymore, 1994). Higher Cr concentrations would be expected in the gills of *L. umbratus* from the Witbank Dam if the pH of the dam's water was more acidic. At low pH levels Cr has an increased bioavailability and there is a subsequent increased uptake of the monovalent hydrochromate ion (Van der Putte et al., 1981a; b; Van den Heever and Frey, 1996). The liver also showed high Cr concentrations, which was not unexpected because the

TABLE 9
DIFFERENCES BETWEEN FEMALE AND MALE *LABEO UMBRATUS* REGARDING NICKEL BIOACCUMULATION IN THE DIFFERENT TISSUES (FEBRUARY 1994 TO MAY 1995)

Month of survey	Tissues	Variables	Gender		P
			Female	Male	
February 1994 (n: 6F/14M)	Gills	x ± SD	12.08±3.19	13.18±4.04	ns
	Liver	x ± SD	12.83±5.30	8.95±2.75	ns
	Muscle	x ± SD	9.26±4.21	11.44±5.56	ns
	Skin	x ± SD	9.21±2.59	10.63±6.07	ns
May 1994 (n: 13F/7M)	Gills	x ± SD	12.63±3.81	11.79±3.32	ns
	Liver	x ± SD	10.14±1.14	11.06±1.08	ns
	Muscle	x ± SD	13.22±2.26	12.77±1.44	ns
	Skin	x ± SD	10.52±0.82	11.56±0.72	ns
August 1994 (n: 14F/6M)	Gills	x ± SD	20.42±6.72	20.56±5.03	ns
	Liver	x ± SD	15.50±5.06	16.96±7.60	ns
	Muscle	x ± SD	18.26±5.17	16.29±5.61	ns
	Skin	x ± SD	16.69±5.84	13.09±4.44	ns
November 1994 (n: 9F/11M)	Gills	x ± SD	19.38±6.91	15.99±5.12	ns
	Liver	x ± SD	15.30±4.15	10.66±5.41	ns
	Muscle	x ± SD	18.19±6.48	13.39±4.66	ns
	Skin	x ± SD	14.01±6.61	9.58±4.69	ns
February 1995 (n: 2F/18M)	Gills	x ± SD	16.96±0.10	42.12±33.16	ns
	Liver	x ± SD	69.20±52.74	50.40±40.12	ns
	Muscle	x ± SD	75.80±65.53	42.50±39.01	ns
	Skin	x ± SD	107.75±12.52	77.03±67.84	ns
May 1995 (n: 15F/5M)	Gills	x ± SD	22.98±4.30	21.70±3.90	ns
	Liver	x ± SD	24.09±6.97	20.04±3.71	ns
	Muscle	x ± SD	23.16±7.09	19.17±3.21	ns
	Skin	x ± SD	22.31±6.70	19.57±3.79	ns

ns = not significant ($P>0.05$)

liver is associated with storage and detoxification functions. An experimental bioaccumulation study by Van Hoof and Van San (1981), revealed that the rudd (*Scardinium erythrophtalmus*) bioaccumulated most Cr in the gill tissue, followed by the liver. In this study, the lowest Cr concentrations were mainly found in the muscle (11.02 to 60.31 $\mu g \cdot g^{-1}$ dry mass). This coincided favourably with the results from other studies (Nishihara et al., 1985; Lazos et al., 1989; Seymore, 1994; Coetzee, 1996; Wepener, 1997).

Manganese accumulation was found to be the highest in the gills, followed by the liver, then the muscle and skin. It has been shown that Mn can be taken up directly via the gills or indirectly from food and ingested sediments via the gut (Bendell-Young and Harvey, 1986). The high Mn concentrations, detected in the gills of various species, showed that the main route of Mn uptake was through the gills because little absorption of this metal occurred through the gut via the food (Katz et al., 1972). These results and bioaccumulation patterns coincided with results obtained from other localities (Fig. 1) in the Upper Catchment (Barnhoorn, 1996, Coetzee, 1996; Kotze, 1997) as well as Lower Catchment (Seymore, 1994; Wepener, 1997). Although the present study revealed either higher or lower concentrations in the gills than found in the Upper Catchment (Locality 13, 27 to 72 $\mu g \cdot g^{-1}$ dry mass – Barnhoorn, 1996; Locality 11, 39 to 167 $\mu g \cdot g^{-1}$ dry mass and Locality 14, 11 to 85 $\mu g \cdot g^{-1}$ dry mass – Coetzee, 1996; Locality 15, 21 to 179 $\mu g \cdot g^{-1}$ dry mass – Kotze, 1997) as well as the Lower Catchment, Kruger National Park (16.6 to 123.1 $\mu g \cdot g^{-1}$ dry mass – Seymore et al., 1995), the order of bioaccumulation during all of these studies remained the same: G>L>M≥S. The high Mn levels in the gills can possibly also be ascribed to the fact that the gills are used as an excretion route for this metal (Seymore et al., 1995).

Fish are known to accumulate Ni in different tissues, when they are exposed to elevated levels in their environment (Van Hoof and Nauwelaers, 1984; Vos and Hovens, 1986; Tjälve et al., 1988). Nickel is also extensively bioaccumulated from the intake of contaminated food (Singh and Ferns, 1978). During this study it was found that the selected fish species showed accumulation of Ni in all the tissues, but the data indicated that the gills contained the highest levels, followed by the liver, and then the muscle and skin. Research indicates that Ni is taken up via the gills as a result of its close blood-water contact (Tjälve et al., 1988). Therefore, the gills are the main site for absorption of Ni from the surrounding medium. It should also be remembered that the gills play an important role in the secretion of metals, probably via the secretion of mucus (Heath, 1991). The Ni concentrations in the gills of fish during this

TABLE 10
DIFFERENCES BETWEEN FEMALE AND MALE *LABEO UMBRATUS* REGARDING LEAD BIOACCUMULATION IN THE DIFFERENT TISSUES (FEBRUARY 1994 TO MAY 1995)

Month of survey	Tissues	Variables	Gender		P
			Female	Male	
February 1994 (n: 6F/14M)	Gills	x ± SD	18.52±2.88	18.87±2.12	ns
	Liver	x ± SD	11.84±4.49	8.57±2.18	ns
	Muscle	x ± SD	9.94±5.75	10.85±4.02	ns
	Skin	x ± SD	9.01±2.20	8.93±3.26	ns
May 1994 (n: 13F/7M)	Gills	x ± SD	7.93±4.38	5.54±2.17	ns
	Liver	x ± SD	6.60±3.21	6.05±1.87	ns
	Muscle	x ± SD	10.07±2.59	11.65±1.79	ns
	Skin	x ± SD	6.62±5.95	4.97±1.57	ns
August 1994 (n: 14F/6M)	Gills	x ± SD	14.74±2.20	14.87±1.51	ns
	Liver	x ± SD	8.15±1.26	9.09±1.63	ns
	Muscle	x ± SD	8.71±1.48	17.30±21.70	ns
	Skin	x ± SD	7.92±2.34	7.00±0.87	ns
November 1994 (n: 9F/11M)	Gills	x ± SD	10.10±2.38	10.61±2.70	ns
	Liver	x ± SD	3.37±1.27	5.60±1.20	ns
	Muscle	x ± SD	4.19±1.99	3.87±1.66	ns
	Skin	x ± SD	1.74±1.09	2.40±1.55	ns
February 1995 (n: 2F/18M)	Gills	x ± SD	19.50±1.08	21.16±5.42	ns
	Liver	x ± SD	7.86±0.39	10.08±4.18	ns
	Muscle	x ± SD	7.38±7.10	7.64±2.47	ns
	Skin	x ± SD	5.40±1.25	7.82±7.08	ns
May 1995 (n: 15F/5M)	Gills	x ± SD	12.05±4.73	11.89±7.70	ns
	Liver	x ± SD	6.38±4.51	3.28±1.44	ns
	Muscle	x ± SD	6.42±3.52	5.81±7.02	P<0.05
	Skin	x ± SD	6.81±5.44	2.94±1.01	P<0.05

ns = not significant (P>0.05)

study were lower than concentrations found at other localities in the Upper Catchment, e.g. 15 to 44 $\mu g \cdot g^{-1}$ dry mass at Locality 13 (Barnhoorn, 1996), 16 to 52 $\mu g \cdot g^{-1}$ dry mass at Locality 11 and 13 to 71 $\mu g \cdot g^{-1}$ dry mass at Locality 14 (Coetzee, 1996), and 9 to 71 $\mu g \cdot g^{-1}$ dry mass at Locality 15 (Kotze, 1997). Nickel concentrations found in the gills of fish species in the Lower Olifants River Catchment (1.1 to 37.5 $\mu g \cdot g^{-1}$ dry mass - Seymore, 1994), were also higher than those concentrations recorded at Witbank Dam during this study. The second highest levels of Ni occurred in the liver, and this could again be ascribed to the major role that the liver plays in the storage and detoxification of metals. Experimental bioaccumulation studies conducted on carp (*Cyprinus carpio*) showed that the order of Ni accumulation was G>L>M during sublethal exposures, compared to G>M>L during lethal exposure (Sreedevi et al., 1992).

During the present study Pb bioaccumulation was predominantly found to be the highest in the gill tissue, followed by the other tissues (liver, muscle and skin). This was also confirmed by various other studies (Somero et al., 1977; Latif et al., 1982; Villegas-Navarro and Villareal-Treviño, 1989; Seymore, 1994; Barnhoorn, 1996; Coetzee, 1996; Kotze, 1997; Wepener, 1997). The Pb concentrations in the gills of *L. umbratus* from this study were similar to data obtained at other localities in the Upper Catchment of the Olifants River (10 to 21 $\mu g \cdot g^{-1}$ dry mass at Locality 13 – Barnhoorn, 1996; 9 to 32 $\mu g \cdot g^{-1}$ dry mass at Locality 11 and 9 to 39 at Locality 14 – Coetzee, 1996; 4 to 35 $\mu g \cdot g^{-1}$ dry mass at Locality 15 – Kotze, 1997), but was lower than concentrations found in the Lower Olifants River (1.9 to 58.2 $\mu g \cdot g^{-1}$ dry mass – Seymore et al., 1995). Experimental bioaccumulation studies in carp (*C. carpio*) revealed that Pb concentrations in the tissues were G>L>M as opposed to L>G>M in non-exposed fish (Nishihara et al., 1985).

As was the case with Cr, the lowest concentrations of Mn, Ni and Pb, were detected in the muscle and skin of *L. umbratus*. This is very important because the muscle is the edible part of the fish as generally consumed by South Africans. The lower levels of these metals might indicate that the skin is an important excretory organ for these metals, presumably by means of mucus secretions (Heath, 1991). The skin and the gills are both characterised by a mucus layer on their outer surfaces, indicating that they are possible routes of excretion. This involves the sloughing off of metal-containing mucus from these surfaces (Varanasi and Markey, 1978).

The calculated bioaccumulation factor (BF) values provide

TABLE 11

SUMMARY OF STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN THE VARIOUS SEASONS, REGARDING THE BIOACCUMULATION OF CHROMIUM IN *LABEO UMBRATUS* FROM THE WITBANK DAM DURING THE STUDY PERIOD OF FEBRUARY 1994 TO MAY 1995) (FEBRUARY = SUMMER, MAY = AUTUMN, AUGUST = WINTER, NOVEMBER = SPRING)

Season	Tissues	Summer 1994	Autumn 1994	Winter 1994	Spring 1994	Summer 1995	Autumn 1995
Summer 1994	Gills Liver Muscle Skin		P<0.05 P<0.01 ns P<0.01	P<0.01 ns P<0.01 P<0.01	ns ns P<0.01 ns	P<0.001 P<0.001 P<0.001 P<0.01	P<0.001 P<0.001 P<0.001 P<0.001
Autumn 1994	Gills Liver Muscle Skin			P<0.001 P<0.001 P<0.01 P<0.001	P<0.001 P<0.001 P<0.001 P<0.05	P<0.001 P<0.001 P<0.001 P<0.001	P<0.001 P<0.001 P<0.001 P<0.001
Winter 1994	Gills Liver Muscle Skin				ns ns ns P<0.05	P<0.05 P<0.001 P<0.01 P<0.001	P<0.001 P<0.001 P<0.001 P<0.001
Spring 1994	Gills Liver Muscle Skin					ns P<0.001 P<0.01 P<0.001	P<0.001 P<0.001 P<0.001 P<0.001
Summer 1995	Gills Liver Muscle Skin						P<0.05 ns P<0.05 P<0.01
Autumn 1995	Gills Liver Muscle Skin						

ns = not significant (P>0.05)

some indication of the bioavailability of Cr, Mn, Ni and Pb to the fish from the water and sediment. For Cr, the bioconcentration factors between the different tissues and the water (BF_w) were much lower than bioconcentration factors calculated by Seymore, 1994 (46 to 2 314.3, in the Lower Olifants River) and Coetzee, 1996 (19 to 722, at Aasvoëlkrans and Olifants River Lodge) but higher than values calculated by Barnhoorn, 1996 (51 to 343, in Middelburg Dam) and Kotze, 1997 (131 to 245, in Loskop Dam) for localities within the Olifants River System. These relatively high BF_w values indicated that Cr is available for bioaccumulation. Although the high BF_w values did not correlate with high Cr concentrations in the water at that specific time (Table 1), the values can be ascribed to various physicochemical properties of the water (Heath, 1991). It must be noted that only one water sample was collected once every three months thereby stressing the importance of more regular monitoring of the system, which may not always be cost-effective.

Manganese bioaccumulation factors between the water (BF_w) and the different tissues were higher than BF_w at Middelburg Dam (127 to 1 761 – Barnhoorn, 1996) and Loskop Dam (31 to 535 – Kotze, 1997) but lower than BF_w in the Lower Olifants River and Selati River (0.7 to 3 593.3 – Seymore, 1994) as well as Olifants River Lodge and Aasvoëlkrans (27 to 8 500 – Coetzee, 1996). During the present study and other studies the highest BF_w were calculated for the gills of fish. The high degree of bioavailability of Mn, especially in February 1994, can be due to the physicochemical properties of the water (Heath, 1991) because Mn concentrations in the water (Table 1) were not necessarily higher at that time (Kotze, 1997; Nussey, 1998).

For Ni, the bioconcentration factors between the different tissues and the water (BF_w) were lower than other localities in the Upper Catchment (Localities 11 and 14, 39 to 2 367 - Coetzee, 1996) as well as in the Lower Catchment (3 to 1 090 - Seymore, 1994), but higher than values recorded at Locality 13 (56 to 315 – Barnhoorn, 1996) and Locality 15 (113 to 221 – Kotze, 1997) in the Upper Catchment. The lower BF_w may be attributed to Ni forming complexes with carbonates in the water and therefore becoming less bioavailable for uptake by fish.

Bioconcentration factors for Pb in the different tissues and the water (BF_w) were the lowest in the skin and the highest in the liver (Table 5). These BF_w's were higher than values for Middelburg Dam (Locality 13, 28 to 183 – Barnhoorn, 1996), Aasvoëlkrans (Locality 14, 10 to 146 – Coetzee, 1996) and Loskop Dam (Locality 15, 95 to 240 – Kotze, 1997) but lower than values at Olifants River Lodge (Locality 11, 12 to 1 100 – Coetzee, 1996), all of which occur in the upper reaches of the Olifants River. The lower reaches (Olifants River in the Kruger National Park and Selati River)

TABLE 12

SUMMARY OF STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN THE VARIOUS SEASONS, REGARDING THE BIOACCUMULATION OF MANGANESE IN *LABEO UMBRATUS* FROM THE WITBANK DAM DURING THE STUDY PERIOD OF FEBRUARY 1994 TO MAY 1995) (FEBRUARY = SUMMER, MAY = AUTUMN, AUGUST = WINTER, NOVEMBER = SPRING).

Season	Tissues	Summer 1994	Autumn 1994	Winter 1994	Spring 1994	Summer 1995	Autumn 1995
Summer 1994	Gills Liver Muscle Skin		P<0.001 P<0.001 P<0.001 ns	ns P<0.001 ns ns	P<0.001 P<0.001 P<0.001 P<0.001	P<0.05 ns P<0.001 P<0.001	ns P<0.01 P<0.001 P<0.001
Autumn 1994	Gills Liver Muscle Skin			P<0.001 P<0.05 P<0.001 P<0.01	P<0.001 P<0.001 P<0.001 ns	P<0.001 P<0.001 P<0.001 P<0.01	P<0.001 P<0.01 P<0.001 P<0.01
Winter 1994	Gills Liver Muscle Skin				P<0.01 P<0.001 P<0.05 P<0.01	ns P<0.001 P<0.001 P<0.001	ns P<0.001 P<0.05 P<0.001
Spring 1994	Gills Liver Muscle Skin					P<0.001 P<0.01 P<0.05 P<0.01	P<0.001 P<0.001 ns ns
Summer 1995	Gills Liver Muscle Skin						P<0.05 P<0.001 P<0.05 P<0.01
Autumn 1995	Gills Liver Muscle Skin						

ns = not significant (P>0.05)

recorded much higher BF_w (10.8 to 2 610 – Seymore, 1994) than Witbank Dam. Although the water of the Upper Catchment is considered to be hard (Kotze, 1997; Nussey, 1998), the Lower Catchment recorded higher total hardness values (Wepener, 1997).

The bioconcentration factors between the different tissues of selected fish species and the sediment (BF_s) for Cr, Mn, Ni and Pb were low, indicating that only a small fraction of these metals in the sediment is actually available for accumulation. The low bio-availability of these metals from the sediment, can be ascribed to the formation of complexes with inorganic compounds, e.g., or suspended solids in the fairly hard water (mean alkalinity 90.67 $mg \cdot \ell^{-1}$) of the Olifants River, and therefore reducing their bioavailability. During the present study the calculated BF were in the same range as that found by other researchers in the Upper Catchment of the Olifants River (Barnhoorn, 1996; Coetzee, 1996; Kotze, 1997), but lower than BF_s values calculated for the Lower Catchment of the Olifants River (Seymore, 1994).

Relationships between metal concentrations and fish length

Accumulation of the metals decreased with an increase in fish length. Therefore, the smaller the fish the higher the body load of metals due to various bioaccumulation processes. Smaller fish have higher metabolic rates (per gram of body tissue) and therefore are able to take up metals, via food and water, more rapidly than larger fish (Patrick and Loutit, 1978). The higher ventilation rate of smaller fish might also be another reason for the higher metal concentrations in their tissues.

Differences between males and females

The accumulation of Cr, Mn and Ni in the different tissues of male and female fish did not differ markedly. It is suggested that the male testes and female ovaries should also be compared, in order to obtain further data on the differences of accumulation of metals between males and females.

Seasonal differences

The highest tissue concentrations of Cr, Mn, Ni and Pb with the exception of the muscle and skin tissues, were recorded in the summer of 1995. This could be ascribed to the higher rainfall during this period (Weather Bureau, Department of Environmental Affairs and Tourism, personal communication, 1994 and 1995). The higher rainfall could have caused pronounced leaching of

TABLE 13
SUMMARY OF STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN THE VARIOUS SEASONS, REGARDING THE BIOACCUMULATION OF NICKEL IN *LABEO UMBRATUS* FROM THE WITBANK DAM DURING THE STUDY PERIOD OF FEBRUARY 1994 TO MAY 1995) (FEBRUARY = SUMMER, MAY = AUTUMN, AUGUST = WINTER, NOVEMBER = SPRING)

Season	Tissues	Summer 1994	Autumn 1994	Winter 1994	Spring 1994	Summer 1995	Autumn 1995
Summer 1994	Gills Liver Muscle Skin		P<0.05 P<0.05 P<0.001 P<0.001	P<0.001 P<0.001 P<0.001 P<0.001	P<0.001 ns P<0.05 ns	P<0.01 P<0.001 P<0.001 P<0.001	P<0.001 P<0.001 P<0.001 P<0.001
Autumn 1994	Gills Liver Muscle Skin			P<0.001 P<0.05 P<0.001 P<0.05	P<0.01 P<0.05 P<0.01 P<0.05	P<0.001 P<0.001 P<0.001 P<0.001	P<0.001 P<0.001 P<0.001 P<0.001
Winter 1994	Gills Liver Muscle Skin				P<0.05 ns P<0.05 P<0.05	ns P<0.001 P<0.01 P<0.001	ns P<0.05 P<0.05 P<0.05
Spring 1994	Gills Liver Muscle Skin					P<0.05 P<0.001 P<0.01 P<0.001	ns P<0.001 P<0.001 P<0.001
Summer 1995	Gills Liver Muscle Skin						P<0.05 P<0.05 P<0.05 P<0.001
Autumn 1995	Gills Liver Muscle Skin						

ns = not significant (P>0.05)

metals into the water (Dallas and Day, 1993). These findings can then coincide with the higher metal concentrations in the water (Table 1). The higher metal concentrations in the summer, compared to autumn and winter, can possibly be attributed to a varied water temperature. Higher temperatures in summer can cause higher activity and ventilation rates in fish. This is due to increasing temperatures that lower the oxygen affinity of the blood and increases the rate of pollutant accumulation (Grobler, 1988). A higher metabolic rate may also lead induce more frequent feeding sessions, which in turn might also result in increased metal concentrations, if these metals are taken up via the food chain.

Conclusion

Bioaccumulation of Cr, Mn, Ni and Pb varied between the gills, liver, muscle and skin. The gills generally had the highest metal concentrations, due to their intimate contact with the environment and their importance as an effector of ionic and osmotic regulation. The liver, in its role as a storage and detoxification organ, can also accumulate high levels of metals. Muscles and skin accumulated much less metal concentrations. These two organs must be included in biomonitoring programmes because they are consumed by the general public.

The higher metal concentrations during the present study did not always correlate with the higher metal concentrations in the water at that time and can probably be ascribed to various physico-chemical properties of the water. Although some significant differences were found between males and females, the differences were generally insignificant. Therefore, the gonads of both sexes should be used in order to establish significant differences between the sexes. The bioaccumulation of Cr, Mn, Ni and Pb was dependent on body length, where decreasing metal concentrations are recorded in larger fish. However, if sample sizes can be increased, then a certain size range can be selected for this process, thereby reducing variability.

Lastly, it is impossible to predict from biomonitoring field data at which specific concentration, any toxicological actions of metals are activated. This can be determined through a combination of controlled experimental exposure conditions and biomonitoring field data using an adequate indicator organism. Research in this direction should be supported since metals do accumulate in the aquatic environment. Permits and licences manage the levels of metals in the effluents from anthropogenic activities, should be issued according to scientific information, where available. Re-

TABLE 14

SUMMARY OF STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN THE VARIOUS SEASONS, REGARDING THE BIOACCUMULATION OF LEAD IN *LABEO UMBRATUS* FROM THE WITBANK DAM DURING THE STUDY PERIOD OF FEBRUARY 1994 TO MAY 1995) (FEBRUARY = SUMMER, MAY = AUTUMN, AUGUST = WINTER, NOVEMBER = SPRING)

Season	Tissues	Summer 1994	Autumn 1994	Winter 1994	Spring 1994	Summer 1995	Autumn 1995
Summer 1994	Gills		P<0.001	P<0.001	P<0.001	P<0.05	P<0.001
	Liver		P<0.01	ns	P<0.001	ns	P<0.001
	Muscle		P<0.01	ns	P<0.001	ns	P<0.001
	Skin		P<0.001	ns	P<0.001	P<0.01	P<0.001
Autumn 1994	Gills			P<0.001	P<0.001	P<0.001	P<0.001
	Liver			P<0.001	P<0.001	P<0.001	P<0.05
	Muscle			P<0.001	P<0.001	P<0.001	P<0.001
	Skin			P<0.001	P<0.001	ns	ns
Winter 1994	Gills				P<0.001	P<0.001	P<0.001
	Liver				P<0.001	ns	P<0.001
	Muscle				P<0.001	P<0.05	P<0.001
	Skin				P<0.001	ns	P<0.001
Spring 1994	Gills					P<0.001	ns
	Liver					P<0.001	ns
	Muscle					P<0.001	ns
	Skin					P<0.001	P<0.001
Summer 1995	Gills						P<0.001
	Liver						P<0.001
	Muscle						P<0.001
	Skin						ns
Autumn 1995	Gills						
	Liver						
	Muscle						
	Skin						

ns = not significant (P>0.05)

search on indigenous organisms will contribute to the confidence of levels set for the metals.

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