Bioaccumulation of chromium and nickel in the tissues of *Barbus marequensis* A. Smith, 1841 from the Lower Olifants River, Mpumalanga

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Bioaccumulation of chromium and nickel in selected tissues and organs of the freshwater fish *Barbus marequensis* was investigated. According to the monthly data, the blood accumulated the highest amount of chromium, followed by the bile and vertebrae, while the skin accumulated the lowest amount. Nickel mainly accumulated in the blood, followed by the vertebrae and gills, while the lowest nickel concentrations occurred in the fat tissue. Although significant differences ($p \leq 0,05$) between localities were detected, no definite trend as to where the highest bioaccumulation had occurred could be established. The levels in the tissues and organs of *B. marequensis* suggested no serious chromium or nickel pollution in the study area.

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Although chromium and nickel are regarded as essential elements, they are virtually absent from living organisms (Moore & Ramamoorthy 1984; Vos, Hovens & Hagel 1986). Even in natural waters, under normal conditions, chromium and nickel occur in low concentrations, ranging from 1 to 2 µg/l dissolved chromium (Moore & Ramamoorthy 1984) and 1 to 3 µg/l dissolved nickel (Snodgrass 1980). Anthropogenic sources, such as industrial effluents from metal plating, iron and steel manufacture, chrome tanning, anodising and rubber manufacture (Hellawell 1986) can, however, increase the nickel and chromium levels in the water to levels that can be harmful to the aquatic life.

The two important oxidation states of chromium in natural waters are III and VI, of which chromium (VI) is the more toxic form. Interconversions between Cr (VI) and Cr (III) do occur, but most of the time anthropogenically introduced soluble Cr (VI) is reduced to Cr (III). The dominating fraction of chromium in fresh waters will be in the particulate matter (Moore & Ramamoorthy 1984). Particulates also play a vital role in sequestering and transporting nickel. Nickel (II) forms stable complexes with inorganic and organic ligands in natural waters (Moore & Ramamoorthy 1984).

The toxicity of chromium and nickel to fish, as individual elements, is generally low (Khangarot & Ray 1990), but when combined in a mixture, synergism exists between the two elements, with nickel toxicity increasing approximately ten-fold in the presence of chromium (Hellawell 1986). Although fish are generally not very sensitive to chromium, they can be affected sub-lethally when exposed to concentrations ranging from 0.013 to 50 mg/l Cr (Olson & Foster 1956; Van der Putte, Laurier & Van Eijk 1982) and lethally when exposed to concentrations ranging from 3.5 to 280 mg/l Cr (Moore & Ramamoorthy 1984; Van der Putte, Brinkhorst & Koeman 1981). This variability in exposure concentration can, in many instances, be attributed to differential species response and a difference in the water chemistry. Sub-lethal chromium concentrations can affect the blood physiology, growth and certain enzyme activities of a fish, while lethal chromium concentrations can cause histological damage to the kidneys, intestine and gills of a fish (Van der Putte *et al.* 1982; Olson & Foster 1956; Heath 1987). The site of toxic action during lethal exposures depends on the pH of the water. Van der Putte *et al.* (1981) observed that at pH 6.5 the gill was the primary site of toxic action, whereas at pH 7.8 more chromium (VI) accumulated in the internal organs (kidney and stomach) than in the gills. The distribution and toxicity of nickel in freshwater fish is poorly documented, although the metal appears to have an affinity for tissues participating in hemopoiesis (Tjälve, Gottofrey & Borg 1988). The toxicity of nickel has been attributed to a variety of causes, one of which is the replacement of some of the other elements with similar physiological characteristics such as cobalt or iron in various metabolic processes (Ray, Banerjee & Chatterjee 1990). Sub-lethal concentrations seem to range from 40 to 6000 µg/l Ni (Dave & Xiu 1991; Baylock & Frank 1979), affecting spawning, hatchability of eggs, blood physiology and histology of the gonads and gills of the fish (Pickering 1974; Agrawal, Srivastava & Chaudhry 1979; Nath & Kumar 1990). Lethal concentrations range from 4.4 to 118 mg/l Ni (Pickering & Henderson 1996) causing severe morphological and physiological changes, such as extensive gill damage, especially when the water has a pH value less than 6.5 (Van Hoof & Nauwelaers 1984).

Very little information regarding the metal concentrations in fish of the Lower Olifants River, Eastern Transvaal, is available. In this study, the extent of chromium and nickel bioaccumulation in the organs and tissues of the largescale yellowfish, *Barbus marequensis*, was determined.

Materials and Methods

Barbus marequensis was sampled with gill and cast nets every alternative month from April 1990 to February 1992 at localities 3, 4 and 5 in the Olifants River and at locality 7 in the Selati River (Figure 1). In February 1992 10 fish were also collected at Pionier Dam (Kruger National Park), the natural reference point used in this study. At the other localities only water and sediment samples were collected (Seymore, Du Preez & Van Vuren 1994). After capture, the weight and

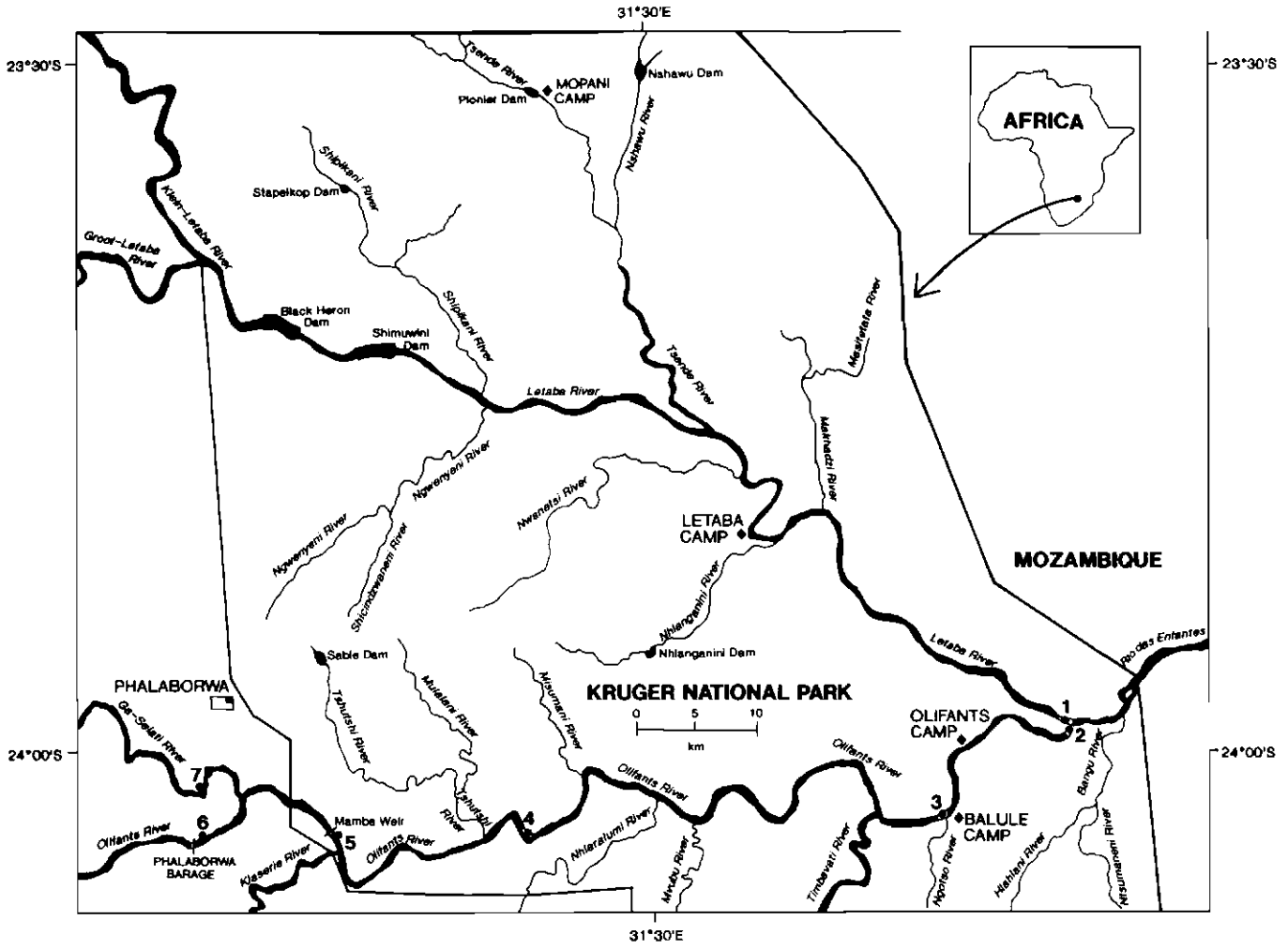

Figure 1 The study area in the Lower Olifants River Catchment, indicating the sampling localities.

fork length of each fish were recorded. Fish scales were collected for age determination (see Seymore, Du Preez & Van Vuren 1995) and blood samples were drawn for metal analysis. The fish were then dissected on a polyethylene work-surface, using stainless steel tools (Heit & Klusek 1982) and wearing surgical gloves. The gut contents, as well as the following organs and tissues were removed for metal analysis: skin, axial muscle, gills, gonads, fat, liver, kidney, gut (fore and hind separately), bile and vertebrae. All the samples were kept frozen, until they could be subjected to metal concentration analysis in the laboratory.

After the tissue samples were thawed in the laboratory, they were prepared and analysed with a Varian atomic absorption spectrophotometer (Spectra AA-10) to determine the chromium and nickel concentrations. Procedures were similar to those described for manganese and lead analysis in Seymore *et al.* (1995).

Bioconcentration factors between the fish tissues and the water (BF_W) and sediment (BF_S) were determined, using only the mean chromium and nickel concentration in each organ. The formula (Wiener & Giesy 1979) is:

$$BF_w \text{ or } BF_s = \frac{[\text{metal}] \text{ in organ } (\mu g/g \text{ dry wt})}{[\text{metal}] \text{ in water } (\mu g/ml) \text{ or sediment } (\mu g/g)}$$

The concentrations of chromium and nickel in the water and sediment were obtained from the publication by Seymore *et al.* (1994).

Statistical analyses of the data were performed by using the Hotelling T^2 and Scheffe tests of the BMDP 2V statistical program (see Seymore 1994). The significance level was $p \leq 0.05$.

Results

The fork length and weight of the fish ranged from 13 to 44 cm and 24 to 1679 g, respectively. The age of the fish that were caught during the study varied from one to six years. A detailed summary of the size and age of the fish is presented by Seymore *et al.* (1995).

The order of bioaccumulation of chromium and nickel in the different organs and tissues and gut contents of *B. marequensis* was not clearly distinguishable, but the highest concentrations of both metals were detected in the gut contents and blood of the fish, as well as in the vertebrae in the case of nickel (Figures 2 & 3). Variation in metal concentration, especially in chromium concentration, was mostly detected in the gut contents. The general order of bioaccumulation for chromium was: hindgut contents > foregut contents > blood > bile > vertebrae > hindgut > gill > foregut ≈ kidney > liver > testes ≈ fat > ovaries ≈ muscle > skin. Statistically the gut contents differed significantly ($p \leq 0.05$) from all the organs

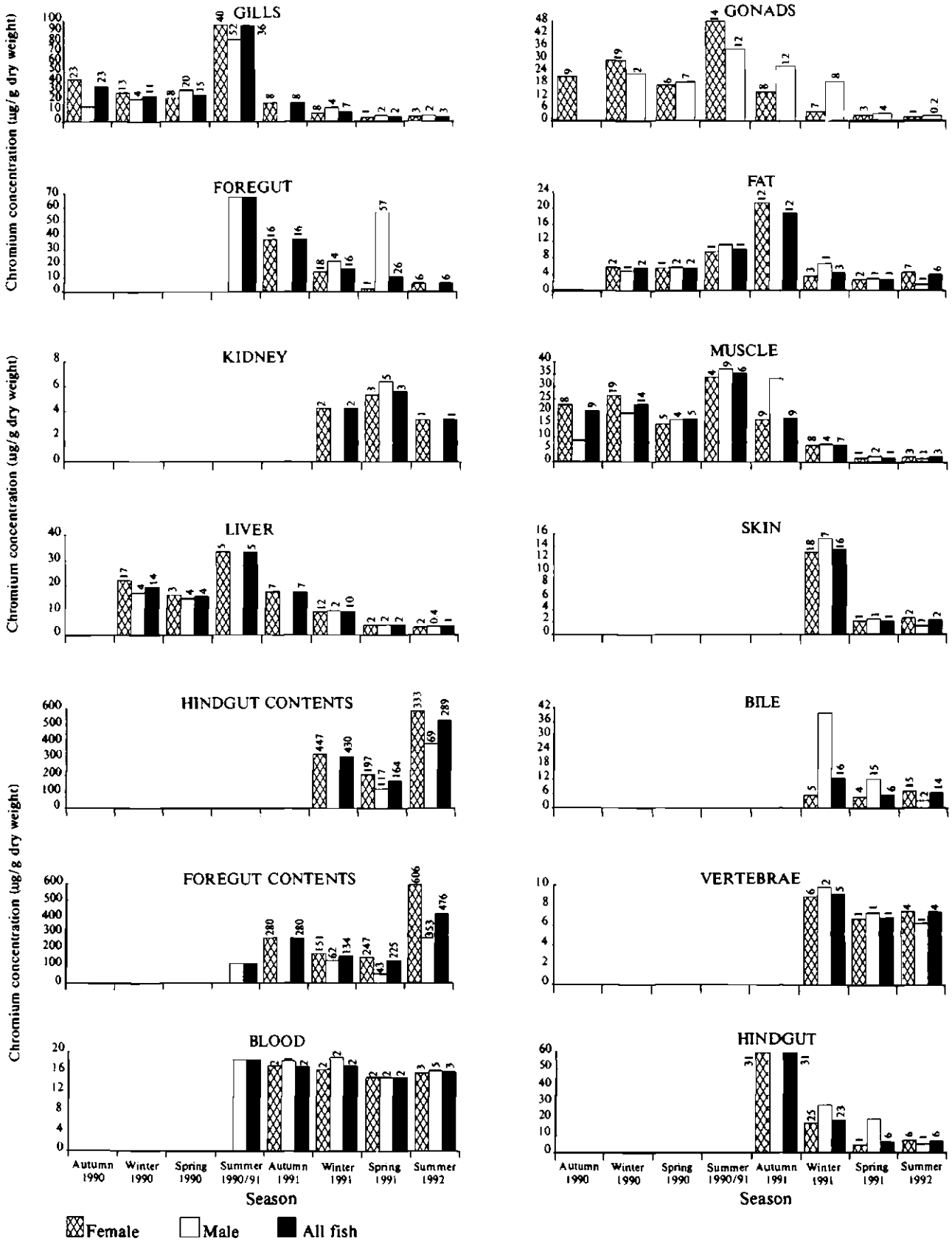

Figure 2 Mean seasonal chromium concentrations (µg/g dry wt.) in the different organs and tissues of *Barbus marequensis* for males and females separately as well as the sexes combined. (Standard deviations are indicated above each bar).

with respect to the accumulated chromium concentrations. In addition, the blood and vertebrae differed significantly from most of the other organs with respect to the accumulated chromium concentrations, but only in the summer of 1992 (Table 1). In the case of nickel, the general order of bioaccumulation was: hindgut contents > foregut contents ≈ blood > vertebrae > gill > hindgut > bile > kidney > foregut > liver > muscle ≈ ovaries > testes ≈ skin > fat. Statistically the gills,

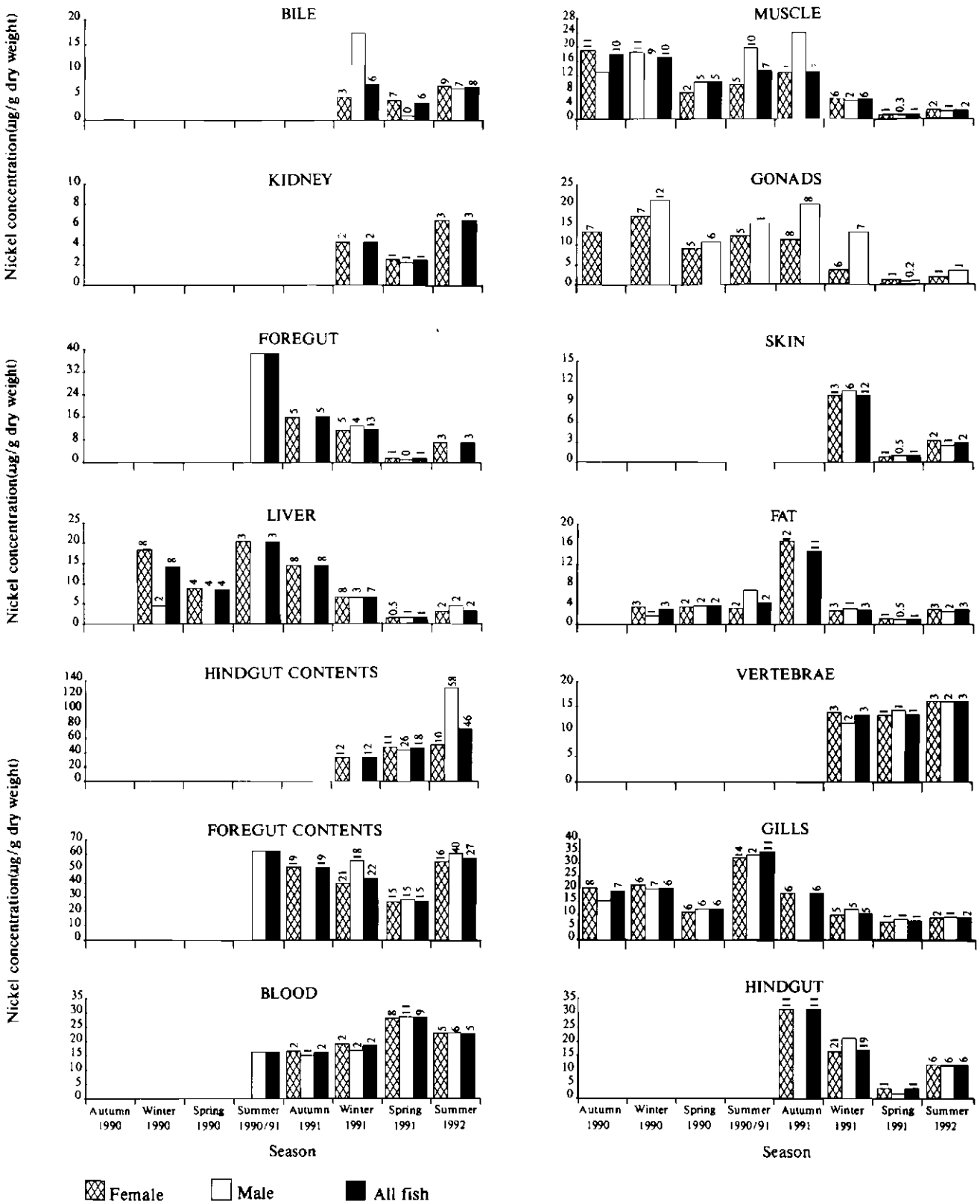

Figure 3 Mean seasonal nickel concentrations (µg/g dry wt.) in the different organs and tissues of *Barbus marequensis* for males and females separately as well as the sexes combined. (Standard deviations are indicated above each bar).

blood and vertebrae differed significantly from most of the other organs with respect to the accumulated nickel concentrations, while the gut contents differed significantly from all the organs with respect to the accumulated nickel concentrations (Table 1).

The calculated bioconcentration factors between the water and the organs (BF_W) were higher than the bioconcentration factors between the sediment and the organs (BF_S). The chromium BF_W values ranged from 5 (calculated for ovaries) to 2314 (calculated for blood), while the BF_S values ranged from 0.001 (calulated for various tissues) to 3.5 (calculated for the gills). Nickel BF_W values ranged from 3 (calculated for fat tis-

Table 1 Summary of statistical differences ($p \leq 0.05$) between the chromium and nickel concentrations in the organs, tissues and gut contents of *Barbus marequensis* during the seasons Winter 1991 (W2), Spring 1991 (SP2) and Summer 1992 (S2). (Blank spaces indicate no significant difference)

	Gill	Ovary	Testis	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Chromium													
Gill													
Ovary													
Testis													
Fat													
Liver													
Muscle	S2												
Skin													
Gut													
Gut cont.	W2,SP2,S2	W2,SP2	W2	W2,SP2	W2,SP2,S2	W2,SP2	W2,SP2,S2	W2,SP2,S2					
Vertebrae		S2		S2	S2	S2	S2		W2,SP2				
Kidney									SP2,S2				
Bile									SP2,S2	S2			
Blood	S2	S2	S2	S2	S2	S2	S2		W2,SP2	S2		S2	
Nickel													
Gill													
Ovary	S2												
Testis													
Fat	SP2,S2												
Liver	S2												
Muscle	SP2,S2												
Skin	SP2,S2												
Gut				W2									
Gut cont.	W2,SP2,S2	W2,SP2	W2,SP2	W2,SP2,S2	W2,SP2,S2	W2,SP2	W2,SP2,S2	W2,SP2,S2					
Vertebrae	S2	SP2,S2	S2	W2,SP2,S2	SP2,S2	SP2,S2	SP2,S2		W2,SP2				
Kidney									SP2,S2				
Bile	S2								SP2,S2	SP2,S2			
Blood	SP2,S2	W2,SP2,S2	SP2,S2	W2,SP2,S2	W2,SP2,S2	W2,SP2,S2	SP2,S2	SP2	W2	SP2,S2		SP2,S2	

sue) to 1090 (calculated for blood), while the BF_S values ranged from 0.01 (calculated for various tissues) to 1.7 (calculated for blood). A detailed summary of these bioconcentration factors is presented by Seymore (1994).

Although the chromium and nickel concentrations in the fish organs were in the same range at each locality, significant differences ($p \leq 0.05$) between the localities did occur. These differences are outlined in the document of Seymore (1994).

Significant seasonal differences ($p \leq 0.05$) with regard to the mean chromium and nickel concentrations in various organs were detected and are summarised in Table 2.

The first and second year differed significantly with respect to the chromium concentrations in the gill, liver, muscle and male and female gonads and also with respect to the nickel concentrations in the gill, liver, muscle and female gonads (Figure 4). The mean metal concentrations in the fish organs during the second year were also used to determine the order of bioaccumulation, which differed slightly from the order based on the monthly data. For chromium it was: hindgut contents > foregut contents > hindgut ≈ blood > foregut > testes > vertebrae ≈ gills > bile > liver > skin ~ muscle > kidney ≈ fat > ovaries; and for nickel, hindgut contents > foregut contents > blood > vertebrae > hindgut > gills > foregut > testes > bile > liver > skin > muscle > kidney > ovaries > fat.

Discussion

Limited research has been undertaken on the uptake, distribution and excretion of chromium and nickel by freshwater fish. The role of each fish organ in these processes has therefore not yet been ascertained. From this study it seemed, according to the order of chromium and nickel bioaccumulation in the organs and tissues, that these metals were taken up by the gills and/or the gut *via* the gut contents. It is important to note that the high metal levels in the gut contents were not necessarily due to the accumulated metal levels in the food, but rather to the metal rich bottom sediments associated with the food (Wren, Maccrimmon & Loescher 1983). A large variation in the chromium and nickel concentrations of the gut

Table 2 Summary of statistical differences ($p \leq 0,05$) between the various seasons with respect to the mean chromium and nickel concentrations in the muscle (M), gill (G), liver (L), vertebrae (V), skin (S) and blood (B) of *B. marequensis* for sexes combined (*), as well as for males and females separately. (Blank spaces indicate no significant difference)

	Autumn 1990	Winter 1990	Spring 1990	Summer 1990/91	Autumn 1991	Winter 1991	Spring 1991	Summer 1992
Chromium								
Autumn	Female			G		G,M	G,M	G,M
1990	Male			M	M			
Winter		Female		G		G,M	G,M	M
1990		Male		G,M		M,L	M,L	
Spring			Female	G			G	G
1990			Male	G,M	M	M,L	M,L	M
Summer	M*,G*	M*,G*	M*,G*	Female		G,M	G,M	G,M
1990/91				Male		G,M	G,M	G,M
Autumn				M*	Female	M	M,L,B	M,L
1991					Male	M	M	M
Winter	M*,G*	M*,G*	M*	M*,G*	M*	Female	S	S
1991						Male	S,V	S,V
Spring	M*,G*	M*,G*,L	M*,G*,L	M*,G*	M*,G*, L*,B*	B*,S*	Female	
1991							Male	
Summer	M*,G*	M*,G*,L*	M*,G*,L*	M*,G*	M*,G*,L*	L*,S*		
1992								
Nickel								
Autumn	Female					M	G,M	G,M
1990	Male							
Winter		Female				M	M	M
1990		Male						
Spring		G*,M*	Female	G				
1990			Male	G			M	M
Summer	G*	G*	G*	Female		G	G	G
1990/91				Male		G,M	G,M	G,M
Autumn					Female	M	M,L,B	M,L,B
1991					Male		M,B	M
Winter	M*	G*,M*		G*,M*	M*,L*	Female	B,S	B,S,V
1991						Male	B,S,V	V
Spring	G*,M*	G*,M*,L*	M*,L*	G*,M*	G*,M*	L*,B*,S*	Female	B,V
1991					L*B*		Male	
Summer	G*,M*	G*,M*,L*	M*	G*,M*	G*,M*, L*,B*	B*,S*,V*	B*,V*	
1992								

contents of *B. marequensis* can be expected, because they feed on a variety of food items (Skelton 1993) and also ingest metal rich sediments. Furthermore, varied foraging habits can occur in a specific population. Excretion was mainly biliary, especially in the case of chromium (Figure 2). It has been suggested by Flos, Riva & Balasch (1983), who experimented with chromium accumulation in goldfish (*Carassius auratus*), that biliary excretion was more important in small than in large fish. *Barbus marequensis,* therefore, probably also excreted chromium and nickel through the gills, kidneys and in the faeces.

The blood of *B. marequensis* accumulated chromium and nickel levels that were higher than the levels in the surrounding water (Seymore *et al.* 1994). It was also noticed that the chromium and nickel concentrations (especially nickel) increased in the blood when the primary uptake route of these metals was through the gills, which was the case in August 1991 and October 1991. A relationship between the gill uptake of chromium and nickel and the consequent concentrations of these metals in the blood is therefore suggested. This suggestion, as well as an observation made by Van der Putte *et al.* (1981) that hydrochromate and chromate ions

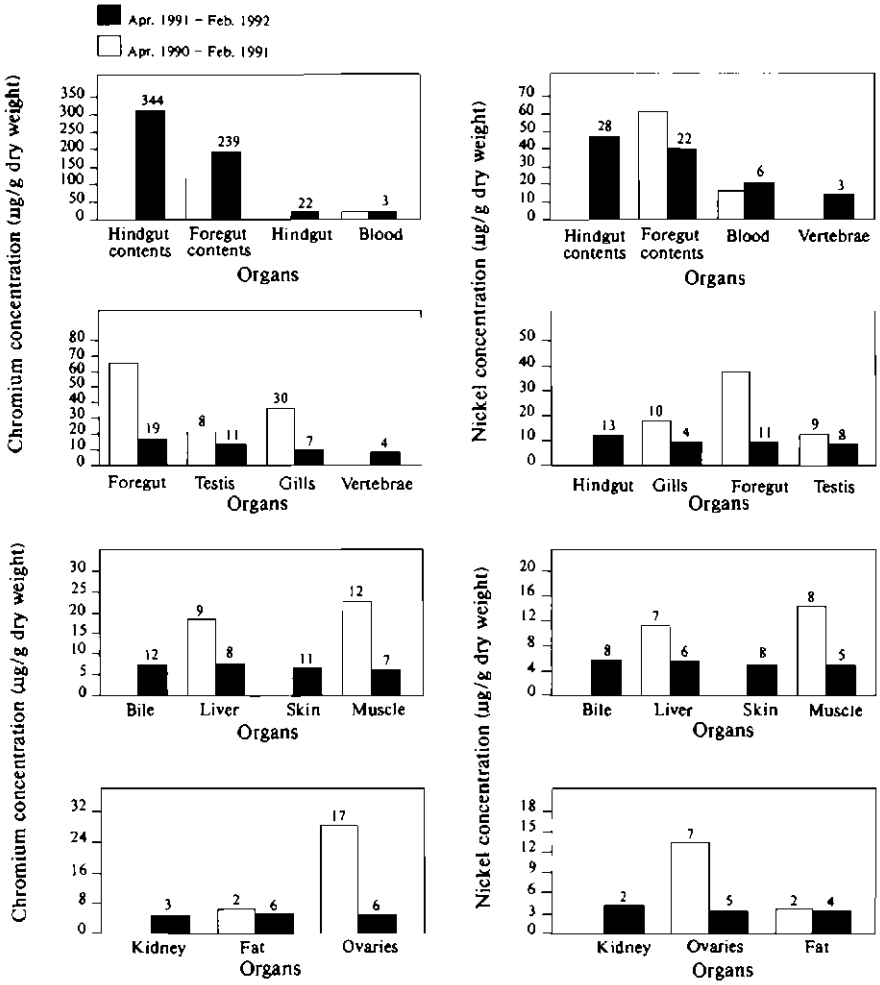

Figure 4 Mean chromium and nickel concentrations (µg/g dry wt.) for the two years in the different organs and tissues of *Barbus marequensis*. (Standard deviations are indicated above each bar).

caused common effects in the blood of *Oncorhynchus mykiss* when acutely exposed, may render blood a good indicator of chromium and nickel poisoning in fish. Furthermore, sub-lethal concentrations of hexavalent chromium (0.098 mg/l) at different pH values have been shown to alter the haematology of *Tilapia sparrmanii* in such a way that they were potentially hazardous (Wepener, Van Vuren & Du Preez 1992). Hexavalent chromium did, for instance, decrease the clotting ability of the blood, causing internal bleeding which can ultimately lead to death (Gey van Pittius, Van Vuren & Du Preez 1992).

Apart from accumulating chromium and nickel, blood also distributes these metals to the different organs and tissues, where they are accumulated to some degree. In this study, chromium and especially nickel were mainly stored in the vertebrae (Figures 2 & 3) and, other than that, accumulation was preferentially by the kidneys rather than the liver, which is in accordance with previous reports (N.R.C.C. 1981). According to the concentrations in, for example, the muscle tissue, *B. marequensis* was exposed to higher chromium and nickel levels from April 1990 to June 1991 than from August 1991 to February 1992. The suggested chromium concentrations in the muscle of freshwater fish from industrialised areas is below 0.25 µg/g Cr wet weight (Moore & Ramamoorthy 1984) or in this case 1 µg/g Cr dry weight (the moisture percentage of the muscle was 75%), which was not the case from April 1990 to June 1991 when the chromium concentrations in the muscle ranged from 6 (June 1991) to 43 (February 1991) µg/g Cr dry weight (see Seymore 1994). Suggested nickel concentrations in the muscle of freshwater fish from industrialised areas were not available

The localities did not differ that much from each other (see Seymore 1994) and therefore no definite trend as to where the highest bioaccumulation had occurred could be established. In February 1992 the fish at Pionier Dam did, however, accumulate slightly more chromium and nickel in their organs (with the exception of the blood) than the fish at the other localities. Lower chromium and nickel concentrations were detected in the gills of the fish from Pionier Dam than in their gut and, therefore, the gills did not play a major role in the uptake of these metals, which was not the case at the other localities. This might be a reason why less chromium and nickel were detected in the blood of the fish from Pionier Dam than in the blood of the fish from the other localities. The chromium and nickel concentrations in the fish did not seem to be related to the metal concentrations in the water. It must be stressed, however, that water samples were only collected every second month, making comparisons difficult.

The high chromium and nickel concentrations in the gills of *B. marequensis* during the summer of 1990/91 might have

been due to the heavy rainfall in December 1990, but the concentrations of these metals in the water were not necessarily higher during that period (Seymore *et al.* 1994). Instead, the concentrations in the gills seemed to have been related to the concentrations in the gut, for similar seasonal trends were observed in these tissues, as well as in the liver and muscle tissues (Figures 2&3).

Higher chromium and nickel concentrations were detected in the water of the study area in the first year than in the second year (Seymore *et al.* 1994), but this is not necessarily the main reason why the organs of *B. marequensis* accumulated higher chromium and nickel concentrations in the first than in the second year (Figure 4). As mentioned before, there was no direct relationship between the monthly water data and the monthly fish data and, therefore, annual differences in accumulation might rather have been related to chromium and nickel uptake through the gut. Unlike the majority of organs and tissues, the blood accumulated less nickel in the first than in the second year (Figure 4). This can be explained by assuming that fish have a mechanism to prevent excess bioconcentration of nickel in blood (Grobler-Van Heerden, Van Vuren & Du Preez 1991).

Conclusions

Suggested organs and tissues to sample for chromium and nickel analysis in fish are: blood, vertebrae, the gall-bladder for bile, the gut, gills, kidney, liver and muscle tissue (to test its fitness for human consumption). One should also remember to take the water pH into consideration, because acidic water would necessitate additional histopathological studies on the gills for reasons already mentioned. The detected concentrations in the fish organs suggested no serious chromium and nickel pollution problem in the study area, but the fish did seem to have been exposed to chronic sub-lethal concentrations, especially from April 1990 to June 1991, which might have caused sub-lethal effects.

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