# Manganese, lead and strontium bioaccumulation in the tissues of the yellowfish, *Barbus marequensis* from the lower Olifants River, Eastern Transvaal

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## Abstract

The bioaccumulation of manganese, lead and strontium in the freshwater fish (*Barbus marequensis*) from the lower Olifants River, E. Transvaal, was investigated. The highest concentrations of these metals were detected in the vertebrae and gills. The localities in the Kruger National Park did not differ significantly from each other and therefore no clear indication as to where the highest bioaccumulation had occurred, could be established. However, the highest manganese and strontium levels occurred in fish from the Selati River. For the future monitoring of manganese, lead and strontium levels in bony fish, it is suggested that bony tissues (e.g. vertebrae, opercular bone or scales), gills, liver and muscle tissue are used.

## Introduction

Manganese, lead and strontium appear to be metabolised via calcium metabolic pathways (Hammond and Beliles, 1980) and, therefore, accumulate mainly in the skeletal tissues of fish (Paul and Pillai, 1983; Patterson and Settle, 1977; Bagenal et al., 1973). Manganese is an essential trace element and shows relatively low toxicity to aquatic biota. Lead is a non-essential metal and is known to be toxic to aquatic organisms, especially fish (Klein, 1962). The requirement of strontium by fish has not been established, but appears to be a non-essential metal, for although it is a bone-seeking element, strontium is not essential for bone formation (Sauer and Watabe, 1989).

In the natural freshwaters, manganese is rarely found at concentrations above 1 mg/ℓ (Hellawell, 1986), while concentrations of soluble lead are generally less ≤3µg/ℓ (Förstner and Wittmann, 1979). Strontium values in South African surface waters typically range from 50 to several hundredµg/ℓ (Kempster, 1994). The forms in which manganese and lead occur in freshwater are mainly particulate or complexed forms (Seenayya and Prahalad, 1987; Moore and Ramamoorthy, 1984), decreasing the bioavailability of these metals to the fish. As the pH of the water decreases, however, the ionic state of the metals becomes more prevalent and toxicity increases (Wang, 1987). Strontium, on the other hand, is found in water in solution rather than in particulate form (Carraca et al.,1990) and might, therefore, be more bioavailable to fish for uptake. Nevertheless, in calcium-rich waters calcium will compete with strontium in the uptake process, resulting in lower strontium accumulation by the fish (Phillips and Russo, 1978). Factors such as the water pH, water hardness, organic materials and other metals will, therefore, influence the toxicity of these metals, but there also seems to be a relation between the concentrations of these metals in the water and the accumulation thereof by freshwater fish (Bermane, 1969).

The manganese, lead and strontium concentrations in the water can increase to quite an extent due to the influence of industrial wastes and mining effluents on the river. The combustion of oil and gasoline accounts for more than 50% of anthropogenic lead emissions and therefore atmospheric fall-out is usually the most important source of lead in freshwaters (Moore and Ramamoorthy, 1984). Fish can be affected sublethally when they are chronically exposed to lead concentrations ranging between 5 and 5 000 µg/ℓ inorganic lead (Haux et al., 1986). Two distinctive characteristics of chronic lead poisoning in fish are black tails, also an early symptom of spinal deformities (Hodson et al., 1979), and a strong inhibition of the aminolevulinic acid dehydratase (ALA-D) activity in erythrocytes (Haux et al., 1986). The 96-h $LC_{50}$ value of total lead for freshwater fish varies from 0.5 to 482 mg/ℓ Pb, depending on the water hardness and life stage of the fish (Moore and Ramamoorthy, 1984; Pickering and Henderson, 1966).

Manganese and strontium can also affect fish adversely at elevated levels, but limited research has been done in this field. Sublethal effects can occur at a manganese concentration of 0.278 g/ℓ (Seymore, 1994), while the 96-h $LC_{50}$ value can vary from 1.723 to 3.230 g/ℓ Mn (Nath and Kumar, 1987). For strontium the 96-h $LC_{50}$ value for fish has been determined to be greater than 92.8 mg/ℓ Sr (Dwyer et al., 1992). The general order in which the relevant three metals can affect fish, is therefore: Pb > Mn > Sr. Associated factors, such as environmental conditions, should, however, be taken into consideration when assessing the toxicity of these metals to fish.

The objective of this study was to determine the extent of bioaccumulation (with respect to site, seasons, years, age, tissues) of manganese, lead and strontium in the yellow fish, *Barbus marequensis* from the lower Olifants River, E. Transvaal. The data were also used to establish which of the tissues contained the highest and lowest concentrations of these metals, respectively. The Olifants River was selected as study area because it is one of the most important rivers that flows through the Kruger National Park. Furthermore, anthropogenic activities in the catchment of this river may effect the quality of the water flowing through the Kruger National Park.

## Materials and methods

Large-scaled yellowfish (*B. marequensis*) were sampled with gill nets (70 to120 mm stretched mesh size) and standard cast nets

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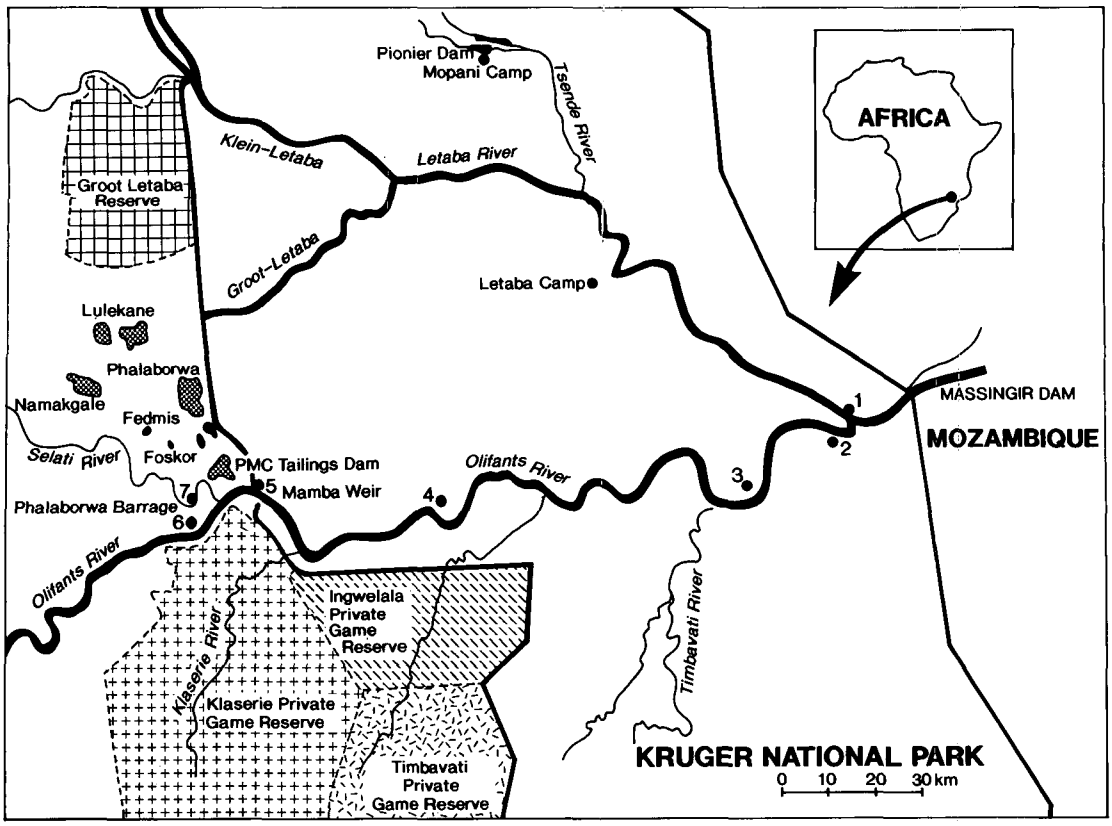


***Figure 1***

*The study area in the lower Olifants River catchment indicating the sampling localities during 1990 to 1992. The sampling localities correspond to the localities 1 to 7 indicated by Seymore et al. (1994)*

every alternate 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 (Fig. 1). In February 1992 ten fish were also collected at Pionier Dam to establish reference concentrations. This dam only receives water from inside the Kruger National Park and should, therefore, be the least contaminated site. The mass and fork length of each fish were recorded. Fish scales were collected for age determination and blood samples were drawn for metal analysis. The fish were then dissected on a polyethylene work surface, using stainless steel tools (Heit and 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 thawing the tissue samples, all the tissues (except for the bile and blood) were dried in an oven at 60°C for 48h. The wet and dry sample mass was recorded in order to calculate the percentage of moisture of each sample. Ten m$\ell$ concentrated nitric acid (55%) and 5 m$\ell$ perchloric acid (70%) were added to 1 g dry tissue in a 100 m$\ell$ Erlenmeyer flask. Digestion was performed on a hotplate (200 to 250°C) for at least 4 h, until the solutions were clear (Van Loon, 1980). The undried bile was digested in a similar manner. For the blood digestion, 5 m$\ell$ each of concentrated nitric (55%) and perchloric acid (70%) were added to 0.5 m$\ell$ blood in a 100 m$\ell$ Erlenmeyer flask and digestion similar to the other samples was then performed. For the analysis of strontium an additional 0.5 m$\ell$ of a 2.682 M potassium chloride solution (200g KCl per litre distilled water) was added to the digested 50 m$\ell$ samples in order to suppress the ionisation of strontium (Varian, 1989). After digestion each solution was filtered using an acid-resistant 0.45 µm filter paper and a vacuum pump. The filter system was then rinsed with doubly distilled water, whereafter the samples were made up to 50 m$\ell$ each with doubly distilled water. The samples were stored in glass bottles, until the metal concentrations could be determined. Prior to use, all glassware was soaked in a 2% Contrad soap solution (Merck chemicals) for 24 h, rinsed in doubly distilled water, acid-washed in 1M HCl for 24 h and rinsed again in doubly distilled water (Giesy and Wiener, 1977). A Varian atomic absorption spectrophotometer (Spectra AA-10) was used to determine the metal concentrations in the tissue samples of the fish. Analytical standards were prepared from Holpro stock solutions.

Bioconcentration factors (Wiener and Giesy 1979) between the fish tissues and the water ($BF_w$) and sediment ($BF_s$) were determined, using the mean metal concentration in each tissue. The metal concentrations in water and sediment for the Olifants River from the publication by Seymore et al., 1994 were used to calculate $BF_w$ and $BF_s$. The scales were washed with warm water and soap and were placed between two objective slides which were then tightened with masking tape. The circuli were counted under a microprojector (Nielsen and Johnson, 1983). Statistical analysis (Hotelling $T^2$ and Scheffè tests) was performed by using the BMDP 2V statistical program. The significance level was $P \leq 0.05$.

## Results

### Fish size and age

The mean mass and length of the fish caught at the different localities for each month are presented in Table 1. In general, the female fish were larger than the male fish at each locality. The

**TABLE 1**
**LENGTHS AND WEIGHTS OF *BARBUS MAREQUENSIS* CAUGHT IN THE OLIFANTS RIVER (KRUGER NATIONAL PARK), DURING THE PERIOD APRIL 1990 - FEBRUARY 1992**

| Month | Locality | N | Mass (g) | | Length (cm) | | Month | Locality | N | Mass (g) | | Length (cm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Range | $\bar{X}$± SD | Range | $\bar{X}$± SD | | | | Range | $\bar{X}$± SD | Range | $\bar{X}$± SD |
| April 1990 | 3 | 1 F# | 800 | | 35.5 | | April 1991 | 3 | 6 F# | 205-470 | 304.3±94.2 | 23.6-94.2 | 25.9±1.9 |
| | | | | | | | | | 2 M* | 125-193 | 159.0±48.1 | 20.0-22.4 | 21.2±1.7 |
| | 4 | 7 F | 186-701 | 353.1±169.3 | 23.8-36.5 | 28.8±3.9 | | 4 | 9 F | 33-470 | 161.4±154.7 | 13.5-30.4 | 20.1±6.3 |
| | | 2 M* | 201-199 | 150.5±68.6 | 19.1-23.9 | 21.5±3.4 | | | 1 M | 220 | | 24.9 | |
| | 5 | 0 | - | - | - | - | | 5 | 7 F | 205-900 | 452.1±255.1 | 23.0-38.5 | 29.0±5.5 |
| | | | | | | | | | 3 F/M* | 93-135 | 116.3±21.4 | 17.0-20.0 | 18.7±1.5 |
| | 7 | 10 F/M+ | 46-134 | 64.8±27.5 | 15.0-21.4 | 16.4±2.0 | | 7 | 1 M | 240 | | 25.0 | |
| | | | | | | | | | 1 F/M | 45 | | 14.5 | |
| June 1990 | 3 | 2 F | 216-222 | 219.0±4.2 | 26.5-28.0 | 27.3±1.1 | June 1991 | 3 | 9 F | 215-720 | 319.4±154.6 | 28-33.3 | 25.7±3.1 |
| | 4 | 0 | - | - | - | - | | 4 | 1 F | 457 | | 28.7 | |
| | | | | | | | | | 6 M | 230-360 | 309.2±52.7 | 23.1-27.9 | 25.6±2.0 |
| | 5 | 0 | - | - | - | - | | 5 | 3 M | 200-330 | 261.7±65.3 | 22.7-26.3 | 24.5±1.8 |
| | 7 | 0 | - | - | - | - | | 7 | 0 | - | - | - | - |
| Aug 1990 | 3 | 4 F | 81-509 | 254.8±181.4 | 17.3-32.5 | 24.8±6.2 | Aug 1991 | 3 | 1 F | 400 | | 27.2 | |
| | | 3 M | 50-176 | 115.0±63.1 | 15.1-23.8 | 19.6±4.4 | | | | | | | |
| | | 2 F/M | 61-182 | 121.5±85.6 | 17.3-22.5 | 19.9±3.7 | | | | | | | |
| | 4 | 6 F | 116-352 | 244.0±91.9 | 24.3-30.5 | 26.7±2.6 | | 4 | 8 F | 290-550 | 404.8±76.6 | 24.5-31.1 | 28.0±1.9 |
| | | 3 M | 391-592 | 507.7±104.3 | 30.5-35.5 | 33.0±2.5 | | | | | | | |
| | 5 | 2 F | 262-356 | 26.0±28.5 | 26.0-28.5 | 27.3±1.8 | | 5 | 11 F | 610-1110 | 872.7±178.5 | 30.6-40.0 | 35.4±3.0 |
| | | 1 M | 573 | | 32.0 | | | | 1 M | 510 | | 27.5 | |
| | | 3 F/M | 227-246 | 237.0±9.5 | 25.0-25.9 | 25.5±0.5 | | | | | | | |
| | | 5 F/M | 24-44 | 34.2±9.6 | 12.5-15.2 | 14.0±1.3 | | | | | | | |
| | 7 | 0 | | | | | | 7 | 1 F/M | 120 | | 20.6 | |
| Oct 1990 | 3 | 2 F | 383-545 | 464.0±114.6 | 28.3-30.9 | 29.6±1.8 | Oct 1991 | 3 | 4 F | 390-793 | 540.5±177.0 | 28.0-34.6 | 30.8±2.8 |
| | | 1 M | 1000 | | 35.9 | | | | 2 M | 269-400 | 334.5±92.6 | 25.0-27.2 | 26.1±1.6 |
| | | 4 F/M | 122-463 | 282.0±140.9 | 19.4-28.0 | 24.1±3.6 | | | | | | | |
| | 4 | 3 F | 392-600 | 480.7±107.3 | 21.1-29.5 | 24.7±4.3 | | 4 | 9 F | 155-889 | 603.9±206.3 | 21.0-36.0 | 31.1±4.4 |
| | | 7 M | 550-800 | 636.3±98.0 | 27.5-33.7 | 30.2±2.5 | | | 2M | 400-459 | 429.5±41.7 | 26.8-28.5 | 27.7±1.2 |
| | 5 | 1 F | 592.0 | | 31.2 | | | 5 | 12 F | 474-800 | 655.9±114.8 | 27.9-34.0 | 31.0±1.9 |
| | | 6 M | 166-1050 | 477.3±323.8 | 20.3-38.7 | 30.6±6.9 | | | 3 M | 400-617 | 502.3±109.0 | 28.5-31.0 | 29.7±1.3 |
| | | 3 F/M | 169-272 | 235.7±57.8 | 21.5-23.5 | 22.8±1.1 | | | | | | | |
| | 7 | 1 F | 900 | | 34.0 | | | 7 | 1 F | 188 | | 23.0 | |
| Dec 1990 | 3 | 4 F | 171-549 | 302.0±175.0 | 22.0-32.3 | 26.0±4.7 | Jan 1992 | 3 | 4 F | 451-641 | 567.3±84.2 | 29.1-32.0 | 30.7±1.3 |
| | | 1 M | 254 | | 24.2 | | | | 2 F/M | 117-148 | 132.5±21.9 | 19.3-20.8 | 20.1±1.1 |
| | | 2 F/M | 70-80 | 75.0±7.1 | 16.6-18.6 | 17.6±1.4 | | | | | | | |
| | 4 | 0 | - | | - | - | | 4 | 7 F | 98-965 | 386.6±315.8 | 17.9-38.0 | 26.5±7.3 |
| | | | | | | | | | 1 M | 120 | | 18.6 | |
| | | | | | | | | | 3 F/M | 99-150 | 118.3±27.6 | 17.8-21.0 | 19.0±1.7 |
| | 5 | 1 F/M | 80 | | 17 | | | 5 | 8 F | 439-944 | 699.5±163.9 | 29.2-34.7 | 32.4±1.7 |
| | | | | | | | | | 4 M | 368-520 | 456.0±63.6 | 29.2-34.7 | 28.7±1.3 |
| | 7 | 0 | - | - | - | - | | 7 | 8 F/M | 46-245 | 90.3±69.1 | 27.0-29.9 | 17.8±3.8 |
| Feb 1991 | 3 | 0 | - | - | - | - | Feb 1992 | 3 | 4 F | 135-216 | 184.5±35.5 | 22.3-23.0 | 22.7±0.34 |
| | | | | | | | | | 1 M | 188 | | 22.4 | |
| | | | | | | | | | 1 F/M | 151 | | 20.4 | |
| | 4 | 0 | - | - | - | - | | 4 | 6 F | 138-1108 | 583.8±343.3 | 20.8-40.5 | 31.4±7.0 |
| | | | | | | | | | 4 M | 190-410 | 286.5±99.9 | 23.8-28.2 | 25.2±2.0 |
| | 5 | 1 M | 220 | | 24.0 | | | 5 | 10 F | 399-1211 | 659.9±242.6 | 29.0-40.5 | 33.1±3.6 |
| | 7 | 4 F/M | 71-225 | 125.9±69.8 | 16.9-23.5 | 19.5±3.0 | | 7 | 0 | - | - | - | - |
| | | | | | | | | Pionier Dam | 5 F | 1035-1679 | 1408±300.7 | 35.4-43.5 | 40.2±3.5 |
| | | | | | | | | | 5 M | 710-845 | 806.6±55.9 | 33.3-34.6 | 34.2±0.5 |

# Female, * Male, + Female and Male (fish immature)

largest fish were usually caught at Locality 5 (Mamba Weir), except in February 1992 when the largest fish were caught at Pionier Dam. The breeding season stretches from October to April and it was noted that the largest fish were caught during the month of October. Age determination was difficult due to unclear circuli which were formed during the dry periods and also because no sharp difference in water temperature had occurred between the different seasons for this area (300 to 900 m above sea level). Nevertheless, the data indicated that the fish were 1 to 2 years of age at a fork length of 14 to 20 cm, 2 to 3 years at 20 to 30 cm fork length, 3 to 4 years at 30 to 34 cm fork length and 4 to 6 years at 34 to 40 cm fork length.

### Bioaccumulation

The moisture content in the tissues differed, with the mean percentage of moisture being 79 ± 3% in the gut, 77 ± 5% in the gonads, 75 ± 2% in the muscle, 74 ± 3% in the gills, 69 ± 5% in the kidney, 67 ± 5% in the liver, 62 ± 3% in the skin, 42 ± 2% in the vertebrae and 10 ± 8% in the fat. Due to this variation in moisture content, the metal concentrations in the different organs and tissues were calculated on a dry-mass basis.

Manganese, lead and strontium accumulated mostly in the vertebrae and gills of *B. marequensis* (Figs. 2, 3 and 4). High concentrations were also detected in the gut content of the fish. Variation in the metal concentrations of individuals was detected, but it was more pronounced in manganese and strontium than in lead. The largest variation in manganese concentration was detected in the gut contents (e.g. 978 to 4576 µg/g Mn at Locality 5 in October 1991) and, in the first year, also in the gills (e.g. 23 to 123µg/g Mn at Locality 4 in April 1990). For strontium, the largest variation was detected in the vertebrae (e.g. 1 403 to 3 925 µg/g Sr at locality 7 in January 1992), gills (e.g. 601 to 2 116 µg/g Sr at locality 5 in January 1992) and gut contents (e.g. 132 to 1 326 µg/g Sr at locality 4 in August 1991).

The general order of bioaccumulation for manganese was: hindgut contents > foregut contents > gills > vertebrae > hindgut > foregut > liver > kidney > blood > ovaries > fat = bile > skin > muscle > testes. For lead the order was: foregut contents > hindgut contents = vertebrae > hindgut > gills > foregut > blood > bile > testes > kidney = liver > fat > ovaries > skin > muscle; and for strontium it was: vertebrae > gills > foregut contents > hindgut contents > hindgut > muscle > foregut > liver > ovaries > bile > kidney > testes > skin > blood > fat. Statistically the gut contents, vertebrae and gills differed significantly ($P \leq 0.05$) from the other organs with respect to the manganese, lead and strontium concentrations as indicated in Table 2. In addition, the liver and blood differed significantly from some organs with respect to the manganese and lead concentrations respectively (Table 2), but only during the summer of 1992.

The calculated bioconcentration factors between water and tissues ($BF_w$) were higher than the bioconcentration factors between sediment and tissues ($BF_s$). Manganese $BF_w$ values ranged from 0.7 (calculated for bile in February 1992) to 3 593 (calculated for the hindgut in April 1991), while the $BF_s$ values ranged from 0.001 (calculated for bile in February 1992) to 1.51 (calculated for the gills in December 1990). Lead $BF_w$ values ranged from 1 (calculated for fat in October 1990) to 2 610 (calculated for bile in June 1991), while the $BF_s$ values ranged from 0.08 (calculated for fat in December 1990) to 137 (calculated for the gills in October 1991). Strontium $BF_w$ values ranged from 2 (calculated for blood in January 1992) to 25 533 (calculated for the vertebrae in June 1991), while the $BF_s$ values ranged from 0.005 (calculated for blood in January 1992) to 7 (calculated for the gills in December 1990).

### Locality differences

Although the manganese, lead and strontium concentrations in the fish tissues were mostly in the same range at each locality, significant differences ($P \leq 0.05$) did occur between localities (Table 3). Higher manganese and strontium concentrations were detected in the fish tissues at Locality 7 than at the other localities, while lower strontium concentrations occurred in the fish tissues at Pionier Dam.

### Seasonal differences

Significant differences was evident in the mean seasonal manganese, lead and strontium concentrations in various organs, but no clear trend emerged (Table 4). In the case of manganese, the summer of 1990/91 and winter of 1991 differed significantly from all the other seasons. Additional seasonal differences regarding the mean manganese concentrations are indicated in Table 4. Nearly all the seasons differed from each other with respect to the mean lead concentrations detected in various organs, but not with respect to the mean strontium concentrations (Table 4).

The mean seasonal manganese, lead and strontium concentrations, as determined separately for male and female organs and tissues, are indicated in Figs. 2 to 4. There were no clear-cut and continuous differences in metal accumulation between the two genders. The males did, however, have higher manganese and lead concentrations in their gut contents than the females (Fig. 2).

### Annual differences

The first and second year differed significantly with respect to the manganese concentrations in the gills, muscle and gonads (Fig. 5); and also with respect to the lead and strontium concentrations in the gills, liver, muscle and gonads (Fig. 5). Using the mean manganese, lead and strontium concentrations detected in the fish organs during the second year (Fig. 5), the order of metal accumulation of *B. marequensis* was determined, and it differed slightly from the order based on the monthly data. For manganese it was: hindgut contents > foregut contents > gills > hindgut > vertebrae > foregut > liver > kidney > ovaries = testes > blood = muscle > skin = fat > bile; for lead the order was: foregut contents > vertebrae > hindgut contents > hindgut > testes> gills > foregut > blood = kidney > bile > skin > liver > fat > muscle = ovaries; and for strontium it was: vertebrae > gills > hindgut contents > foregut contents > hindgut > foregut = muscle > testes > liver = kidney > bile = ovaries > skin > fat > blood.

## Discussion

### Bioaccumulation

The uptake and excretion of metals by fish is a subject of interest to many researchers, but little is known about the exact routes in fish. Existing literature indicates that manganese, lead and strontium can be taken up indirectly from food and ingested sediments via the gut, or directly through the gills (Bendell-Young and Harvey, 1986; Hodson et al., 1978; Carraca et al., 1990; Wren et al., 1983). The gills, however, seem to be the main route of uptake of these metals, especially in the case of manganese and strontium, for little

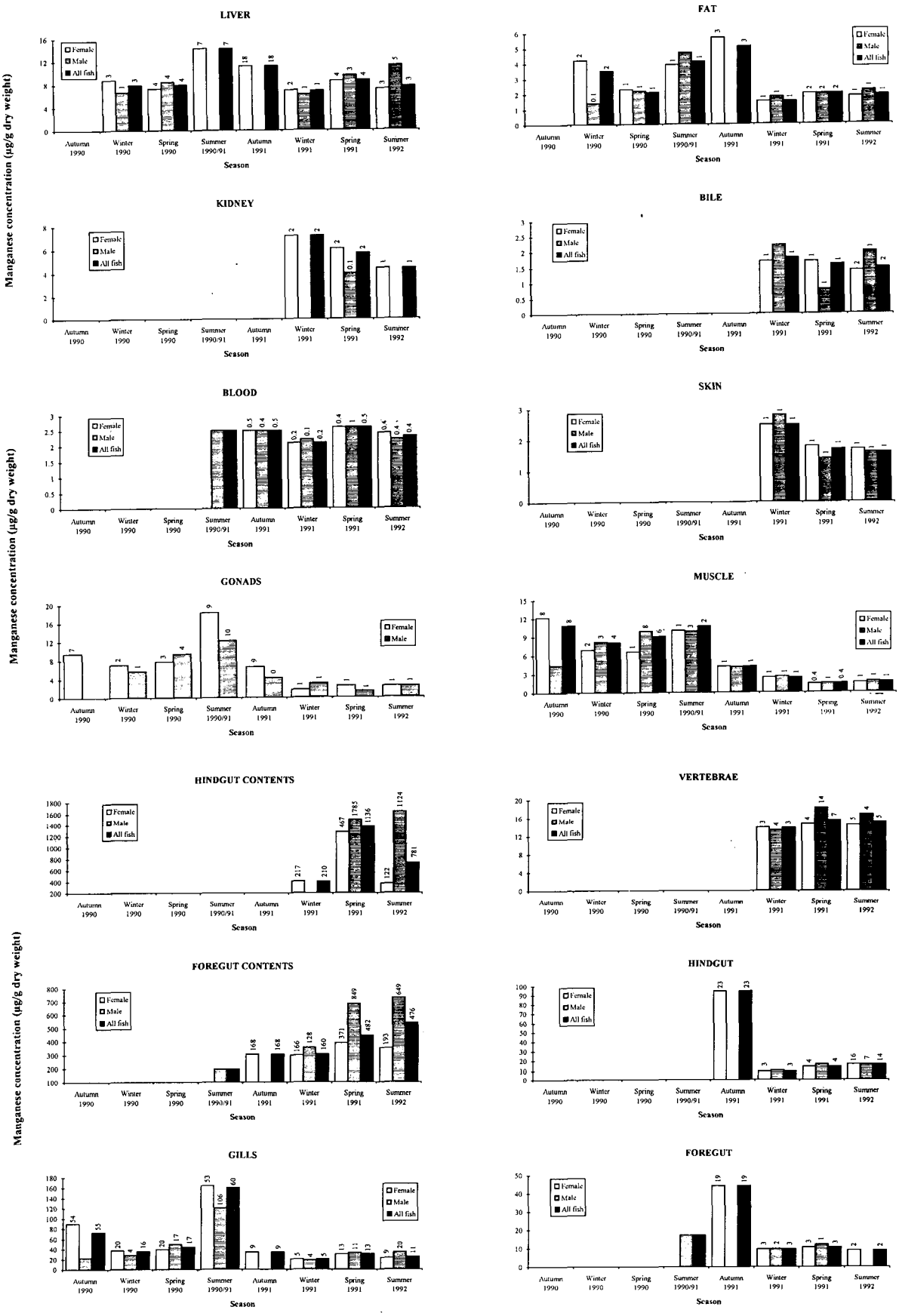


***Figure 2***

*Mean seasonal manganese concentrations (µg/g dry mass) in the liver, kidney, blood, gonads, fat, bile, skin and muscle of* Barbus marequensis *for males and females separately, as well as the sexes combined (Standard deviations are indicated above each bar)*

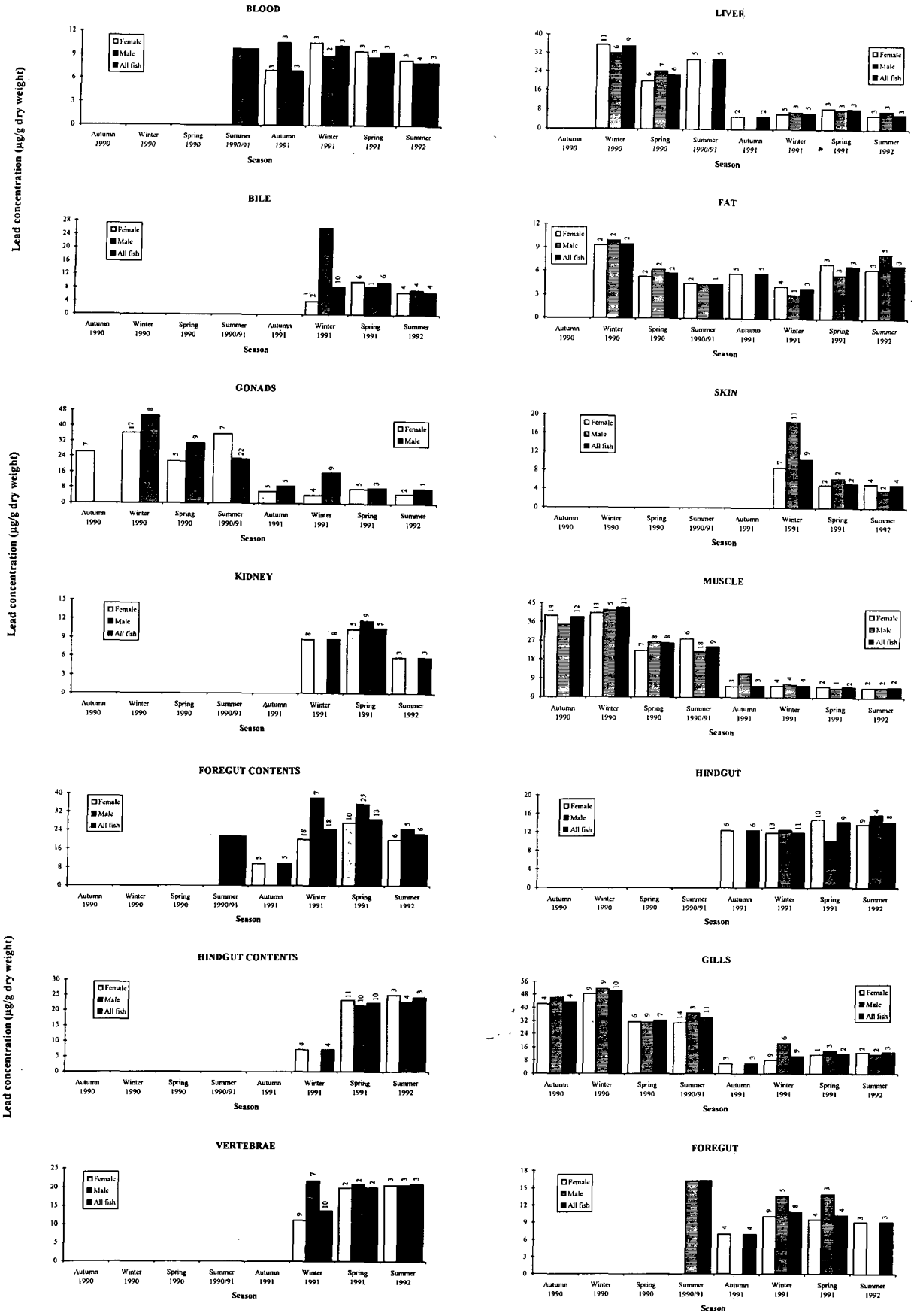


***Figure 3***

*Mean seasonal lead concentrations (µg/g dry mass) in the foregut contents, vertebrae, hindgut, gills and foregut of* Barbus marequensis *for males and females separately, as well as the sexes combined (Standard deviations are indicated above each bar)*

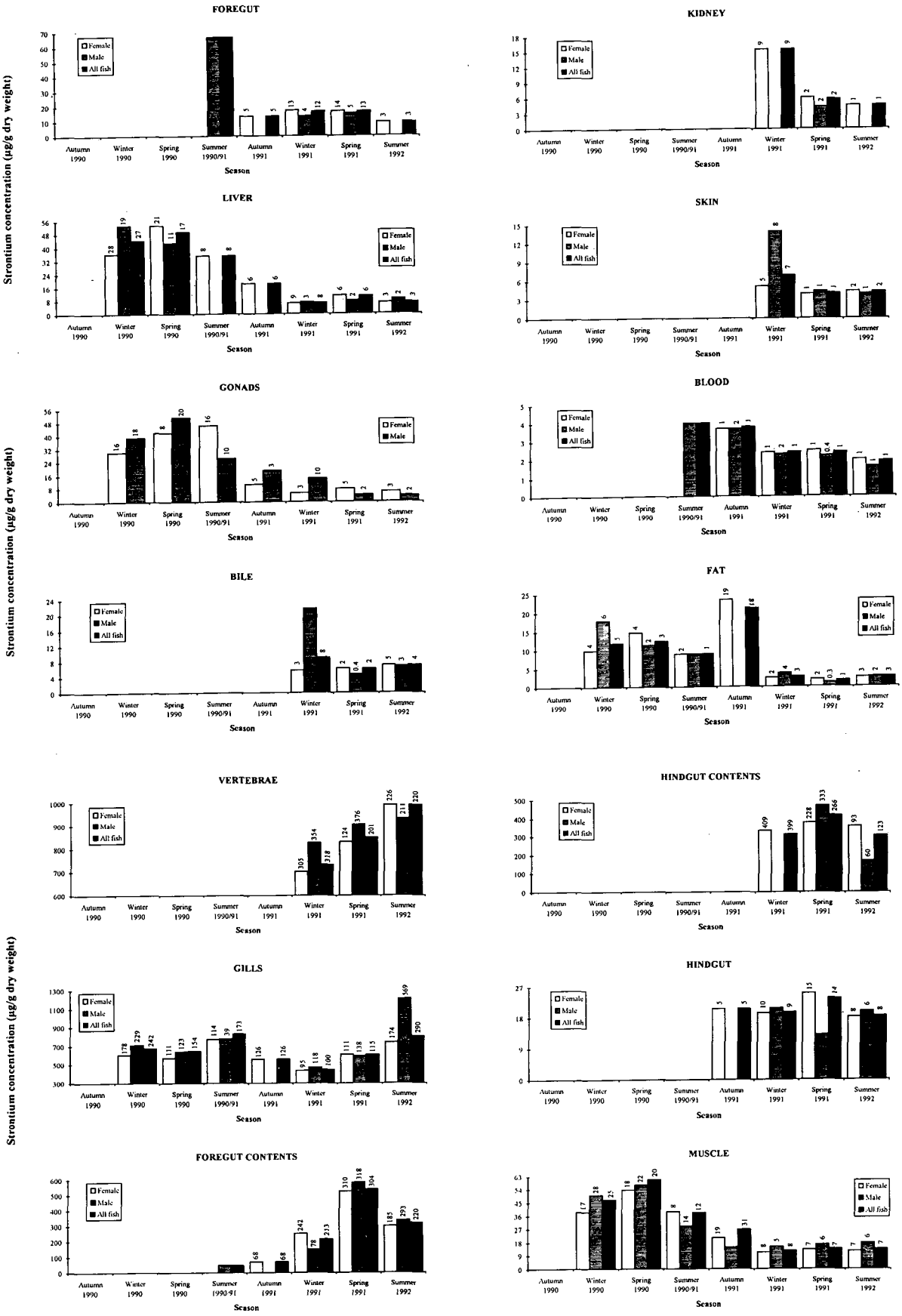


***Figure 4***

*Mean seasonal strontium concentrations (µg/g dry mass) in the foregut, liver, gonads, bile, kidney, skin, blood and fat of* Barbus marequensis *for males and females separately, as well as the sexes combined (Standard deviations are indicated above each bar)*

**TABLE 2**
**SUMMARY OF STATISTICAL DIFFERENCES (P≤0.05) BETWEEN THE MANGANESE, LEAD AND STRONTIUM 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 | Testes | Fat | Liver | Muscle | Skin | Gut | Gut content | Verte-brae | Kidney | Bile | Blood |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MANGANESE** | | | | | | | | | | | | | |
| Gill | | | | | | | | | | | | | |
| Ovary | S2 | | | | | | | | | | | | |
| Testes | S2 | | | | | | | | | | | | |
| Fat | S2 | | | | | | | | | | | | |
| Liver | S2 | | | S2 | | | | | | | | | |
| Muscle | S2 | | | | S2 | | | | | | | | |
| Skin | S2 | | | | S2 | | | | | | | | |
| Gut | | | | | | | | | | | | | |
| Gut content | W2,SP2,S2 | W2,SP2 | W2 | W2,SP2,S2 | W2,SP2,S2 | W2,SP2 | S2,SP2,S2 | W2,SP2,S2 | | | | | |
| Vertebrae | S2 | S2 | S2 | S2 | S2 | S2 | S2 | | W2,SP2,S2 | | | | |
| Kidney | | | | | | | | | SP2,S2 | | | | |
| Bile | S2 | | | | S2 | | | | SP2,S2 | S2 | | | |
| Blood | S2 | | | | S2 | | | | W2,SP2 | S2 | | | |
| **LEAD** | | | | | | | | | | | | | |
| Gill | | | | | | | | | | | | | |
| Ovary | SP2,S2 | | | | | | | | | | | | |
| Testes | | | | | | | | | | | | | |
| Fat | SP2,S2 | | | | | | | | | | | | |
| Liver | S2 | | | | | | | | | | | | |
| Muscle | SP2,S2 | | | | | | | | | | | | |
| Skin | SP2,S2 | | | | | | | | | | | | |
| Gut | | | | | | | | | | | | | |
| Gut content | W2,SP2 | W2,SP2 | SP2 | W2,SP2,S2 | W2,SP2,S2 | W2,SP2 | W2,SP2,S2 | W2,SP2,S2 | | | | | |
| Vertebrae | SP2,S2 | W2,SP2,S2 | SP2,S2 | W2,SP2,S2 | SP2,S2 | SP2,S2 | SP2,S2 | SP2 | W2,SP2 | | | | |
| Kidney | | | | | | | | | SP2,S2 | S2 | | | |
| Bile | S2 | | | | | | | | SP2,S2 | SP2,S2 | | | |
| Blood | S2 | S2 | | | | S2 | S2 | | W2,SP2 | SP2,S2 | | | |
| **STRONTIUM** | | | | | | | | | | | | | |
| Gill | | | | | | | | | | | | | |
| Ovary | W2,SP2,S2 | | | | | | | | | | | | |
| Testes | W2,SP2,S2 | | | | | | | | | | | | |
| Fat | W2,SP2,S2 | | | | | | | | | | | | |
| Liver | W2,SP2,S2 | | | | | | | | | | | | |
| Muscle | W2,SP2,S2 | | | | | | | | | | | | |
| Skin | W2,SP2,S2 | | | | | | | | | | | | |
| Gut | WS,SP2,S2 | | | | | | | | | | | | |
| Gut content | W2,S2 | W2,SP2 | W2,SP2 | W2,SP2,S2 | W2,SP2,S2 | W2,SP2 | W2,SP2,S2 | W2,SP2,S2 | | | | | |
| Vertebrae | W2,SP2,S2 | W2,SP2,S2 | W2,SP2,S2 | W2,SP2,S2 | S2,SP2,S2 | W2,SP2,S2 | W2,SP2,S2 | W2,SP2,S2 | W2,SP2,S2 | | | | |
| Kidney | S2 | | | | | | | | SP2,S2 | S2 | | | |
| Bile | SP2,S2 | | | | | | | | SP2,S2 | SP2,S2 | | | |
| Blood | SW2,SP2,S2 | | | | | | | | W2,SP2 | W2,SP2,S2 | | | |

**TABLE 3**
**SUMMARY OF STATISTICAL DIFFERENCES (P≤0.05) BETWEEN LOCALITIES WITH RESPECT TO MANGANESE (#), LEAD(+) AND STRONTIUM (*) CONCENTRATIONS IN THE TISSUES OF *BARBUS MAREQUENSIS***

| Locality and sampling date | Locality number | | |
|---|---|---|---|
| | 3 | 4 | 5 |
| **October 1990**<br>3<br>5<br>7 | gill#/liver#/muscle# | liver*<br>gill+/liver+/muscle+<br>muscle# | muscle*/liver*+<br><br>gill#/muscle# |
| **June 1991**<br>4 | muscle#+/fat+ | muscle#+/fat | |
| **October 1991**<br>5 | fat+/vertebrae* | | |
| **January 1992**<br>5<br>7 | blood*/vertebrae*fat# | muscle+/vertebrae+/blood*<br>blood*/vertebrae*/fat# | blood*/vertebrae+*/fat# |
| **February 1992**<br>3<br>5 | | vertebrae+/blood*<br>muscle#/blood# | vertebrae*+ |
| **Pionier Dam** | vertebrae+/fat*/muscle*<br>vertebrae*/blood* | muscle#*/blood#/vertebrae* | fat#/vertebrae#*/blood#*<br>/muscle* |

absorption of these two metals occurs through the gut from the food (Katz et al., 1972). These were also the findings in the present study, where higher manganese and strontium concentrations were detected in the gills than in the gut. It has been demonstrated that water-borne lead was readily taken up by fish resulting in subtle sublethal physiological responses, while dietary lead was not taken up and therefore did not affect the fish (Hodson et al., 1978). If the calcium concentrations of the water were low, however, these would probably have enhanced the dietary uptake of lead by fish due to the more effective uptake of aqueous lead by organisms in the lower trophic levels, leading in turn to a greater dietary absorption by fish (Spry and Wiener, 1991). Lead concentrations were very similar in the gills and in the gut of *B. marequensis* indicating that both routes must have been utilised to the same extent in the uptake of lead.

Apart from being uptake routes of manganese, lead and strontium, the gills and gut have also been suggested to be excretion routes, especially for lead (Klaassen, 1976; Latif et al., 1982). The gills, as well as the skin, have an abundance of mucus and therefore, excretion through these routes would probably involve the sloughing off of mucus (Varanasi and Markey, 1978). Other possible routes of excretion are the urine and bile of the fish. In this study, the higher manganese concentrations in the kidneys compared to the bile of *B. marequensis* suggested urinary excretion rather than biliary excretion of manganese. On the other hand, excretion of lead and strontium seemed to be biliary and urinary. However, the biliary excretion of lead has been reported to be quantitatively more important than urinary lead excretion (Klaassen, 1976).

After absorption, metals are distributed to various tissues in the body of the fish. The importance of each tissue in the storage and detoxification of a metal differs from metal to metal. The high manganese, lead and strontium concentrations in the vertebrae of *B. marequensis* indicated that these metals were primarily distributed to the skeletal tissues. Manganese is a normal constituent of vertebrate skeletal tissues and is thought to be essential to the normal mineralisation process (Guggenheim and Gaster, 1973; Love, 1980). Lead and strontium, on the other hand, are not essential for bone formation, but they accumulate in bony tissues due to their similarity to calcium (Moore and Ramamoorthy, 1984; Phillips and Russo, 1978). The retention of strontium can be long, because it interchanges with calcium (Radtke, 1989). Older fish will, therefore, have higher strontium concentrations in their bony tissues than younger ones. This might explain the large variation that was detected in the vertebrae strontium concentrations of *B. marequensis*, as the age of the fish that were caught during the study varied from one to six years. Scales have also been reported to be major storage sites of manganese, lead and strontium (Sauer and Watabe, 1989). Bony tissues of fish (e.g. vertebrae, scales and opercular bone) will, therefore, be good indicators of exposure to sublethal concentrations of manganese, lead and strontium.

Other tissues in *B. marequensis* also accumulated manganese, lead and strontium, although to a much lesser degree than the skeletal tissues. Blood, the distributor of these metals, is a good indicator of lead uptake by the fish, for the activity of the erythrocyte enzyme ALA-D is inhibited by the presence of lead. Furthermore, the ALA-D activity is negatively correlated with the lead concentration in the blood (Dwyer et al., 1988). The muscle tissue of *B. marequensis* accumulated relatively high strontium concentrations which would probably render this tissue a good indicator of strontium exposure. Lead concentrations in the muscle

**TABLE 4**

**SUMMARY OF STATISTICAL DIFFERENCES (P ≤0.05) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN MANGANESE, LEAD AND STRONTIUM 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 (SHADED BLOCKS). BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE**

| | Autumn 1990 | Winter 1990 | Spring 1990 | Summer 1990/91 | Autumn 1991 | Winter 1991 | Spring 1991 | Summer 1992 |
|---|---|---|---|---|---|---|---|---|
| **MANGANESE** | | | | | | | | |
| Autumn 1990 | Female<br>Male | G,M | G,M | G | M | G,M | G,M | G,M |
| Winter 1990 | | Female<br>Male | | G<br>G | | M | M | M |
| Spring 1990 | | | Female<br>Male | G<br>G | | M<br>M | M<br>M | M<br>M |
| Summer 1990/91 | G* | G* | G* | Female<br>Male | M | G,M<br>G | G,M<br>G | G,M<br>G |
| Autumn 1991 | M* | | M* | M* | Female<br>Male | B | M | M |
| Winter 1991 | G*,M* | M* | G*,M* | G*,M* | B* | Female<br>Male | B | B,S |
| Spring 1991 | G*,M* | M* | M* | G*,M* | | S*,B* | Female<br>Male | |
| Summer 1992 | G*,M* | M* | G*,M* | G*,M* | | S*,B* | B* | |
| **LEAD** | | | | | | | | |
| Autumn 1990 | Female<br>Male | | M | | M | G,M<br>M | G,M<br>M | G,M<br>M |
| Winter 1990 | | Female<br>Male | G,M<br>G,M | G,M<br>M | M,L<br>M | G,M,L<br>G,M,L | G,M,L<br>G,M,L | G,M,L<br>G,M |
| Spring 1990 | M* | G*,M*,L* | Female<br>Male | | M,L | G,M,L | G,M | G,M |
| Summer 1990/91 | M* | G*,M* | | Female<br>Male | M | G,M | G,M | G,M<br>G |
| Autumn 1991 | M* | M*,L* | M*,L* | M* | Female<br>Male | B | | |
| Winter 1991 | G*,M* | G*,M*,L* | G*,M*,L* | G*,M* | B* | Female<br>Male | V,S | V,B,S |
| Spring 1991 | G*,M* | G,M*,L* | G*,M*,L* | G*,M* | G*,B* | V*,S* | Female<br>Male | |
| Summer 1992 | G*,M* | G*,M*,L* | G*,M*,L* | G*,M* | G* | V*,S*,B* | | |
| **STRONTIUM** | | | | | | | | |
| Autumn 1990 | Female<br>Male | | | | | | | |
| Winter 1990 | | Female<br>Male | L | | M | G,M,L<br>M,L | M,L<br>M,L | M,L<br>G,M |
| Spring 1990 | | | Female<br>Male | | M,L | M,L<br>M,L | M,L<br>M | M,L<br>G,M |
| Summer 1990/91 | | | M* | Female<br>Male | M | G,M | M | M |
| Autumn 1991 | | M*,L* | M*,L* | | Female<br>Male | B,L | B<br>B | B,L<br>B |
| Winter 1991 | | G*,M*,L* | G*,M*,L* | G*,M* | B* | Female<br>Male | G<br>S | G,V<br>G,S |
| Spring 1991 | | M*,L* | M*,L* | M* | B* | S* | Female<br>Male | V<br>G |
| Summer 1992 | | M*,L* | M*,L* | M* | G*,B* | V*,S*,G* | G* | |

differed only slightly from the lead concentrations in some other tissues, such as the liver. This might have reflected the relatively low rate of binding to SH groups and, in addition, the low solubility of lead salts might have restricted movement across cell membranes (Moore and Ramamoorthy, 1984). In the first year the muscle lead concentrations ranged from 13 to 57 µg/g Pb dry mass, exceeding the maximum allowable concentration for human consumption of lead in fish flesh, which is 2µg/g Pb wet mass or 8µg/g Pb dry mass (assuming the moisture percentage of the muscle was 75%) (Brown et al., 1984). The fish were, therefore, exposed to higher lead concentrations in the first year than in the second year (Seymore et al., 1994) and these were probably sublethal concentrations. No "normal" or allowable values are available for manganese and strontium concentrations in fish flesh. The detected concentrations of these metals in the muscle tissues during the first year were, however, also higher than the muscle concentrations in the second year. Fish were therefore exposed to higher manganese and strontium concentrations in the first year, which is also evident from the study by Seymore et al. (1994).

The manganese and lead $BF_s$ recorded for *B. marequensis* in October 1990 at Locality 3 in this study, were mostly higher than the manganese and lead $BF_s$ recorded for *Hydrocynus vittatus* in October 1990 at the same locality (Du Preez and Steyn, 1992), which ranged from 29 to 157 and 21 to 41 respectively. It was only the $BF_s$ regarding the manganese concentrations in the gonads and fat, as well as the lead concentrations in the fat of *B. marequensis* that were lower than the $BF_s$ recorded for *H. vittatus*. It must be emphasised, however, that the $BF_s$ for *B. marequensis* were calculated on a dry mass basis and for *H. vittatus* on a wet mass basis, making direct comparisons difficult.

The manganese and lead concentrations in the organs and tissues of *B. marequensis* (recorded in summer 1992 in the Olifants River, KNP) were generally lower than the concentrations in the organs and tissues of *Clarias gariepinus* (summer 1988/89) from the industrial and mine-polluted Germiston Lake in the Transvaal (De Wet, 1990). The fish caught at Locality 7 in the Selati River (*B. marequensis*) did, however, accumulate more manganese in their organs than *C. gariepinus* did and the average water manganese concentration at Locality 7 (230 ± 2 µg/ℓ Mn) was, in fact, higher than the average manganese concentration in Germiston Lake (36 ± 31 µg/ℓ Mn). In general, *B. marequensis* accumulated more manganese in their gut than *C. gariepinus* did. This suggests that conditions in the Olifants and Selati Rivers were more favourable for manganese to be taken up through the gut of the fish than was the case in Germiston Lake.

### Locality differences

The localities inside the Kruger National Park (Localities 3, 4, 5 and Pionier Dam) did not differ significantly from each other and therefore, no definite trend as to where the highest bioaccumulation had occurred could be established. The fish at Pionier Dam did, however, have the lowest strontium levels. The highest strontium, as well as manganese levels, were detected in the fish at Locality 7 (in the Selati River). These findings coincided with the manganese and strontium concentrations in the water of the study area, which were also the highest at Locality 7 (Seymore et al., 1994). Indications are, therefore, that manganese and strontium originated from a source before or at Locality 7, that is in the catchment of the Selati River which is a tributary of the Olifants River outside the Kruger National Park.

### Seasonal differences

The high manganese concentrations in the organs of *B. marequensis* during the summer of 1990/91 (Fig. 2) might have been due to the heavy rainfall (Deacon, 1994) in December 1990. Under high rainfall conditions, leaching is more pronounced and systems usually have lower pH values (Hahne and Kroontje, 1973). More hydrogen ions will, therefore, be available to compete with manganese for binding sites on particle surfaces and solution ligands, thereby increasing the bioavailability of manganese to fish. Lead and strontium accumulation did not, however, seem to be directly affected by the rainfall (Fig. 3), but was rather mediated by the lead and strontium concentrations in the water (Seymore et al., 1994). The seasonal trend regarding accumulated concentrations of manganese in the gonads was similar to that of iron (Seymore, 1994), while lead accumulation (Fig. 3) was similar to that of chromium and strontium accumulation (Fig. 4) was similar to that of copper and iron in the gonads. It is not certain what role, if any, manganese, lead and strontium played in gonad development, but no relationship seemed to exist between the concentrations in the gonads and the concentrations in the liver. Strontium has, however, been reported to increase in concentration in the ovaries of *Oncorhynchus mykiss* throughout maturation, while the manganese concentrations increased only during early maturation before it declined rapidly as the GSI increased (Shearer, 1984). The strontium levels in the liver were observed to decrease significantly during the sexual maturation of *O. mykiss* (Shearer, 1984).

Seasonal differences that occurred between the males and females in the accumulated concentrations of manganese, lead and strontium in their organs were such that no definite pattern could be established to relate the differences to physiological processes. The requirements of the two genders regarding manganese, lead and strontium could, therefore, not be established, except that there was a difference in metal levels between the two groups at times.

### Annual differences

The accumulation of manganese, lead and strontium in the organs of freshwater fish is related to the concentrations of these metals in the surrounding water. Due to generally higher concentrations of these metals in the water of the study area in the first year (Seymore et al., 1994), more manganese, lead and strontium were accumulated by *B. marequensis* during this year (Fig. 5). It was only the gut contents that did not necessarily accumulate higher manganese, lead and strontium levels in the first year (Fig. 5), for there was no direct relation between the gut contents concentrations and the water concentrations.

## Conclusions

*Barbus marequensis* accumulated the highest manganese, lead and strontium concentrations in its vertebrae and gills. The high strontium concentrations that were detected in the fish organs, especially in the first year, indicated that the fish were exposed to elevated strontium levels. The detected lead and manganese concentrations in the fish tissue suggested no serious lead and manganese pollution in the study area, although the fish did seem to have been chronically exposed to sublethal lead concentrations in the first year. In addition, the fish at Locality 7 might have been exposed to sublethal manganese concentrations. The source of these metals, especially in the Selati River catchment needs to be identified in future monitoring programmes and, if necessary,

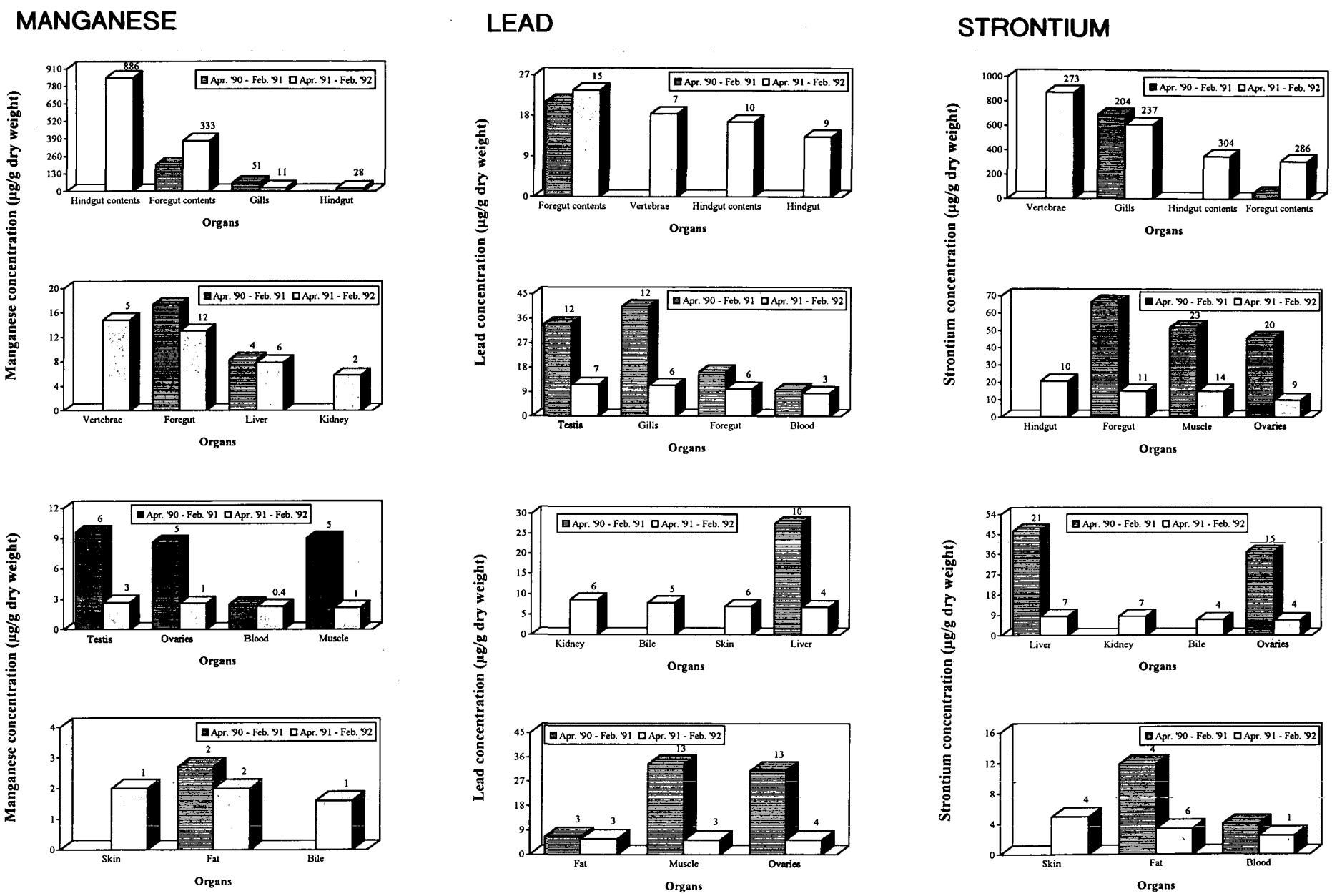


***Figure 5***

*Mean manganese, lead and strontium concentrations (µg/g dry mass) for the two years in the different organs and tissues of* Barbus marequensis
*(Standard deviations are indicated above each bar)*

measures should be taken in order to reduce the levels. Suggested organs and tissues to sample for the analysis of manganese, lead and strontium in fish, are: bony tissues (e.g. scales, vertebrae and opercular bone), gills, liver and muscle tissue (to test its fitness for human consumption). In addition, blood should also be sampled for the analysis of lead, in order to determine the lead concentrations, as well as the ALA-D activity in the erythrocytes.

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