BIOACCUMULATION OF METALS IN *BARBUS MAREQUENSIS* FROM THE OLIFANTS RIVER, KRUGER NATIONAL PARK AND LETHAL LEVELS OF MANGANESE TO JUVENILE *OREOCHROMIS MOSSAMBICUS*

by

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SUMMARY

The catchment area of the Olifants River is being subjected to increased agricultural and mining activities, industrial development and urbanisation. As a result of this, the water quality of the Olifants River and some of its tributaries (e.g. Selati River) has been deteriorating since 1983. This causes reason for concern as one of the downstream water users in the Olifants River catchment is the Kruger National Park, which requires water of good quality to sustain its terrestrial and aquatic ecosystems. It was therefore necessary to determine to what extent activities upstream of the Olifants River, especially in the Phalaborwa area, influenced the water quality of the Olifants River. In this study, an evaluation was done of the water quality (physically and chemically) of the Lower Olifants River inside of the Kruger National Park, as well as the Lower Selati River, a tributary of the Olifants River which flows through the Phalaborwa area. Special attention was paid to the metal concentrations in the water, sediment and fish.

Water and sediment were sampled every alternate month from April 1990 to February 1992 at six sampling sites along the Lower Olifants River and one in the Selati River. Standard methods were used to determine the physical and chemical characteristics (e.g. pH, TDS, etc.) of the water. The fish species *Barbus marequensis* was also sampled every alternative month, but only at three sampling sites along the Olifants River and one in the Selati River. Organs and tissues that were dissected, included the gills, fat, liver, gonads, gut, muscle, skin, blood, vertebrae, kidney and bile, as well as the gut contents. In February 1992, additional sampling was performed at Pionier Dam, a natural reference point used in this study. Atomic absorption spectrophotometry was used in the laboratory to determine the Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn concentrations in the water, sediment and fish samples.

The water quality of the Selati River was found to be stressful to aquatic life, especially with regard to the sodium, fluoride, chloride, sulphate, potassium, total dissolved salts and metal concentrations (except strontium). The Selati River also influenced the water quality of the Olifants River after the Selati-Olifants confluence. Most of the time the water quality of the Lower Olifants River in the KNP did, however, comply with the recommended guideline limits, except for the metal concentrations. The high metal concentrations detected in the water (in some cases sub-lethal levels) indicated some degree of metal pollution, but, due to the hardness of the Olifants River water, conditions were not necessarily toxic to the aquatic life. The accumulated metals in the organs and tissues of *B. marequensis* gave a good indication of the metal levels to which the fish were exposed. More metals were taken up by the fish from April 1990 to February 1991 than from April 1991 to February 1992. The various metals were distributed differently to the tissues of *B. marequensis*, with the highest zinc concentrations being accumulated by the skin and ovaries; the highest copper and iron by the liver, kidney and gut; the highest chromium and nickel by the blood; and the highest manganese, lead and strontium by the vertebrae and gills. These results afforded guidelines as to the types of tissue which should be sampled as the most suitable for the analysis of the various metals in polluted waters.

Apart from the field study, an acute toxicity test was also performed in the laboratory in order to determine the 96-hour LC50 and incipient LC50 values of manganese for juvenile *Oreochromis mossambicus*. Little is known about the effects that high manganese concentrations have on fish and therefore the fish were exposed to different concentrations of manganous chloride tetrahydrate ($MnCl_2.4H_2O$) in a flow-through system. Visible sub-lethal effects (e.g. opaque eyes and haemorrhaging) started to occur at 0.278 g/l Mn, while the 96-hour LC50 and incipient LC50 values were determined to be 1.723 g/l Mn and 1.46 g/l Mn respectively. Although manganese concentrations as high as the mentioned values would never occur naturally in the environment, mine effluents can contain manganese in sub-lethal concentrations.

Monitoring of the study area should be continued, using the results obtained in this study as a reference for the assessment of the possible changes in the quality of the water of the Olifants River catchment. Particular thorough monitoring should be performed to address problem areas, such as the elevated metal levels.

OPSOMMING

Die Olifantsrivieropvangsgebied word toenemend aan landbou- en mynbou-bedrywighede, nywerheidsontwikkeling en verstedeliking onderwerp. Die waterkwaliteit van die Olifantsrivier en sommige van sy sytakke, bv. die Selati-rivier, het as gevolg hiervan sedert 1983, merkbaar verswak. Aangesien die Nasionale Krugerwildtuin, wat 'n stroom-af gebruiker van die Olifantsrivieropvangsgebied is, water van hoë kwaliteit benodig vir die behoud van gesonde terrestriële en akwatiese ekosisteme, is die afname in die waterkwaliteit kommerwekkend. Dit was dus nodig om te bepaal tot watter mate die aktiwiteite stroom-op in die Olifantsrivier, veral in die Phalaborwagebied, die waterkwaliteit van die Olifantsrivier beïnvloed. Die fisiese en chemiese eienskappe van die water in die laer Olifantsrivier binne die NKW, asook dié in die laer Selati-rivier, 'n sytak van die Olifantsrivier wat deur die Phalaborwagebied vloei, is in hierdie ondersoek geëvalueer. Spesiale aandag is veral aan die metaalkonsentrasies in die water, sediment en vis geskenk.

Water en sediment is elke tweede maand vanaf April 1990 tot Februarie 1992 by ses versamelingslokaliteite in die laer Olifantsrivier en een lokaliteit in die Selati-rivier versamel. Standaardmetodes vir die bepaling van die fisiese en chemiese parameters (bv. pH, TOS, ens.) van die water, is gebruik. Die visspesie *Barbus marequensis* is ook elke tweede maand versamel, maar slegs by drie lokaliteite in die laer Olifantsriver en een lokaliteit in die Selati-rivier. Vis is gedissekteer en die derminhoud, asook geselekteerde weefsels en organe, naamlik kieue, vet, lewer, gonades, derm, spier, vel, bloed, werwels, nier en gal is verwyder. Gedurende Februarie 1992 is monsters ook in Pioniersdam, wat as 'n natuurlike verwysingspunt in die gebied beskou word, versamel. Atoomabsorpsiespektrofotometrie is aangewend vir die analise van die water-, sediment- en vismonsters om die Cr, Cu, Fe, Mn, Ni, Pb, Sr en Zn konsentrasies te bepaal.

Daar is bevind dat die waterkwaliteit van die Selati-rivier stresvol vir akwatiese lewe was, veral ten opsigte van die natrium-, fluoried-, sulfaat-, kalium-, totale opgeloste soute en metaalkonsentrasies (uitsluitende stronsium) en dat dit ook die waterkwaliteit van die Olifantsrivier, na die samevloei, beïnvloed het. Die waterkwaliteit van die Olifantsrivier in die NKW het egter meestal binne die limiete van die waterkwaliteitsriglyne geval, behalwe vir die metaalkonsentrasies. Hoë metaalkonsentrasies was in die water aanwesig (in sommige gevalle sub-letale vlakke), wat wel 'n aanduiding van toksiese toestande kon wees, maar, omdat die water van die Olifantsrivier hard is, sou die toestande nie noodwendig toksies vir akwatiese lewe gewees het nie. Die geakkumuleerde metale in die weefsels en organe van *B. marequensis* het 'n goeie aanduiding van die metaalvlakke waaraan die vis blootgestel was, gegee. Meer metale is deur die vis opgeneem vanaf April 1990 tot Februarie 1991, as vanaf April 1991 tot Februarie 1992. Die onderskeie metale is nie tot dieselfde mate deur die verskillende weefsels van *B. marequensis* geakkumuleer nie. Die hoogste sinkkonsentrasies is in die vel en ovaria waargeneem; die hoogste koper en yster in die lewer, nier en derm; die hoogste chroom en nikkel in die bloed; en die hoogste mangaan, lood en stronsium in die werwels en kieue.

Behalwe vir die veldstudie, is 'n akute toksisiteitstoets ook in die laboratorium uitgevoer, om sodoende die 96-uur LC50 en die aanvangs LC50-waardes van mangaan vir jong *Oreochromis mossambicus* te bepaal. Daar is nog nie veel oor die effek van hoë mangaankonsentrasies op vis bekend nie en daarom is die vis aan verskillende konsentrasies van mangaanchloriedtetrahidraat ($MnCl_2.4H_2O$) in 'n deurvloeisisteem blootgestel. Sigbare sub-letale effekte (bv. swart oë en interne bloeding) is vanaf 0.278 g/l Mn waargeneem, terwyl die 96-uur LC50 en aanvangs LC50-waardes 1.723 g/l Mn en 1.46 g/l Mn, onderskeidelik, was. Alhoewel mangaankonsentrasies van bogenoemde vlakke nooit natuurlik in die omgewing sal voorkom nie, kan mynbou-uitvloeisels tog sub-letale konsentrasies mangaan bevat.

Voortgesette monitering van die studiegebied word as wenslik geag. Die resultate wat in hierdie studie verkry is, kan as basis vir die evaluering van moontlike veranderinge in die waterkwaliteit van die Olifantsrivieropvanggebied dien. Probleemareas, soos onder andere die metaalkonsentrasies, behoort besonder noukeurig gemonitor te word.

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Chapter 1

INTRODUCTION

Metal pollution in rivers is increasing world-wide due to the growth in mining, industrial and agricultural activities, as well as a proliferating human population. The most important metals in water pollution are zinc, copper, lead, cadmium, mercury, nickel and chromium (Abel, 1989). Some of these metals are essential trace elements to living organisms (such as copper and zinc), while others (such as lead and cadmium) are non-essential, having no known biological function. All metals are, however, toxic to aquatic organisms when present at elevated levels, causing direct or indirect effects such as histological damage or a reduction in the survival, growth and reproduction of the species (Heath, 1987). The toxicity of metals can be influenced by various factors, of which environmental conditions (e.g. temperature, pH, water hardness) are the most important ones. These conditions determine the chemical speciation of the metals (Abel, 1989) and consequently the bioavailability of the metals to aquatic organisms. Other factors influencing metal toxicity are interactions between pollutants, the developmental stage of the organism and interspecific variations in susceptibility to metals (Hellawell, 1986).

In view of the consequences of metal pollution in aquatic ecosystems, it is undoubtedly essential to monitor river systems which may be affected directly or indirectly by mining and industrial activities on a regular basis. Metal concentrations in the water can then be compared to the metal concentrations proposed by existing water quality guidelines. The fitness of the aquatic environment to which the aquatic organisms are exposed, can thereby be assessed. In order to obtain a reliable and general assessment of the metal pollution in question, the purely physical and chemical monitoring of the water and sediment should, however, be supported by biological monitoring (Abel, 1989). This supportive monitoring is based on the fact that living organisms can provide useful information on the chemical quality of the water as they have experienced it throughout their lives, while a chemical analysis can only indicate the conditions prevailing at the instant of sampling (Abel, 1989). Fish are good organisms to use in biological monitoring for a number of reasons. They are known to accumulate metals in their organs and tissues, they are readily identified, they can be sampled easily and quantitatively and they have a cosmopolitan distribution (Hellawell, 1986). Their economic importance as a resource is also an added feature of great importance. Fish can therefore provide valuable information in addition to the water and sediment data.

In practice, answers are always sought to the problems raised by water pollution, especially metal pollution. These answers can best be obtained by combining field research with laboratory research. It is only in the laboratory that organisms, such as fish, can be exposed to pollutants under controlled conditions in order to determine the sub-lethal and lethal levels of the pollutants, as well as the effects these pollutants have on the organisms. Toxicity tests can therefore be significant in the determination of water quality guidelines or the verification of existing guidelines.

The Olifants River is the second largest river in the Transvaal province, but, due to increasing mining, industrial and agricultural activities in the whole catchment area, the water quality of this river and also some of its tributaries (e.g. Selati River) has been deteriorating. A downstream water user in the Lower Olifants River catchment that cannot afford a deterioration in water quality, however, is the Kruger National Park. It was therefore deemed necessary to determine the effect of upstream activities, especially mining and industrial activities in the Phalaborwa area adjacent to the

Selati River, on the water quality of and aquatic life in the Lower Olifants River flowing through the KNP. The **objectives** of this study were:

- **to determine the general water quality and extent (if any) of metal pollution in the Lower Selati and Lower Olifants Rivers;**
- **to study the possible effect that the water quality of the Selati River might have on the water quality of the Olifants River;**
- **to determine the extent of metal bioaccumulation in the organs and tissues of *Barbus marequensis*, a sensitive fish species in the Olifants River;**
- **to determine the preferred order of bioaccumulation of selected metals (Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn) in the different organs and tissues of *B. marequensis*;**
- **to determine if there were any differences regarding metal bioaccumulation between the selected sampling localities;**
- **to determine if any seasonal differences regarding metal bioaccumulation existed and**
- **to perform, under controlled laboratory conditions, a 96-hour LC50 toxicity test, exposing juvenile *Oreochromis mossambicus* to the metal manganese, which is commonly found in the effluents of mines.**

This study can therefore be of aid in evaluating the water quality of the Lower Olifants River flowing through the KNP. Furthermore, very little information about the metal concentrations in the water, sediment and fish of the Lower Olifants River is available and the data generated by this study will serve as basic information in future monitoring programmes.

The results of the study are presented in the following chapters. Chapter 2 gives background on the study area, as well as on the Olifants River Catchment. Chapter 3 deals with the water and sediment data of the study area. The data and findings regarding the bioaccumulation of metals in the different organs and tissues of *B. marequensis* are presented in Chapters 4 to 7. These chapters are divided according to the bioaccumulation pattern of the selected metals in the different organs and tissues of *B. marequensis*. Chapter 8 deals with the acute exposure of juvenile *O. mossambicus* to manganese and, finally, the conclusions and suggestions are summarised in Chapter 9.

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CHAPTER 2

Chapter 2

THE OLIFANTS RIVER BASIN AND STUDY AREA

2.1 The Olifants River Basin, with special reference to the Lower Catchment

2.1.1 General description

The Olifants River is the second largest river in the Transvaal province of the Republic of South Africa. Together with its tributaries (of which the major ones are the Wilge, Klein Olifants, Moses, Elands, Steelpoort, Blyde, Selati and Timbavati Rivers) it drains a catchment area of 54 575 km^2, which is approximately 20% of the Transvaal province (Theron, Prinsloo, Grimsehl & Pullen, 1991b). The Olifants River originates in the Witbank-Middelburg area and flows in an easterly direction through the Drakensberg before crossing the Kruger National Park into Mozambique, where it flows into the Indian Ocean after its confluence with the Limpopo River (Fig. 2.1). As illustrated, the water resources of this river system are not only of interest to the Republic of South Africa, but also to the Republics of Bophuthatswana and Mozambique, as well as the self-governing territories of Lebowa, Gazankulu and KwaNdebele.

Topographically, on the basis mainly of altitude and relief, the catchment can be divided into four zones (Steffen, Robertson & Kirsten, 1991). These zones are: the Highveld in the south (1 200 m - 1 800 m above sea level), the Springbok flats in the west (900 m - 1 200 m above sea level), the Transvaal Drakensberg/Strydpoort escarpment zone in the centre of the basin (1 500 m - 2 400 m above sea level) and the Lowveld in the east (300 m - 900 m above sea level) (Fig. 2.2).

The climate of the basin is warm to hot sub-tropical, with seasonal rainfall occurring predominantly during the summer months (October to March), with the peak in January. Rainfall generally varies with altitude. Low rainfall of below 600 mm per annum occurs in the Lowveld and Springbok Flats (Fig. 2.3). Moving towards the Highveld and escarpment zone, the rainfall gradually increases to 800 mm per annum. However, it increases rapidly with altitude along the escarpment to as much as 2 000 mm per annum (Kleynhans, 1992).

In order to describe the natural vegetation occurring in the Olifants River catchment, the biome approach could be used. "A biome is a broad ecological unit which represents large, natural and reasonably homogeneous areas of biotic and abiotic features. The biotic component is closely related to physical factors, particularly soil type and climate" (Steffen *et al.*, 1991). Three of the identified biomes in South Africa occur in the Olifants River catchment, namely the grassland, savanna and forest biomes (Fig. 2.4). The grassland biome comprises the Highveld mainly, as well as the southern and western part of the escarpment. The vegetation is dominated by hemicryptophytes of

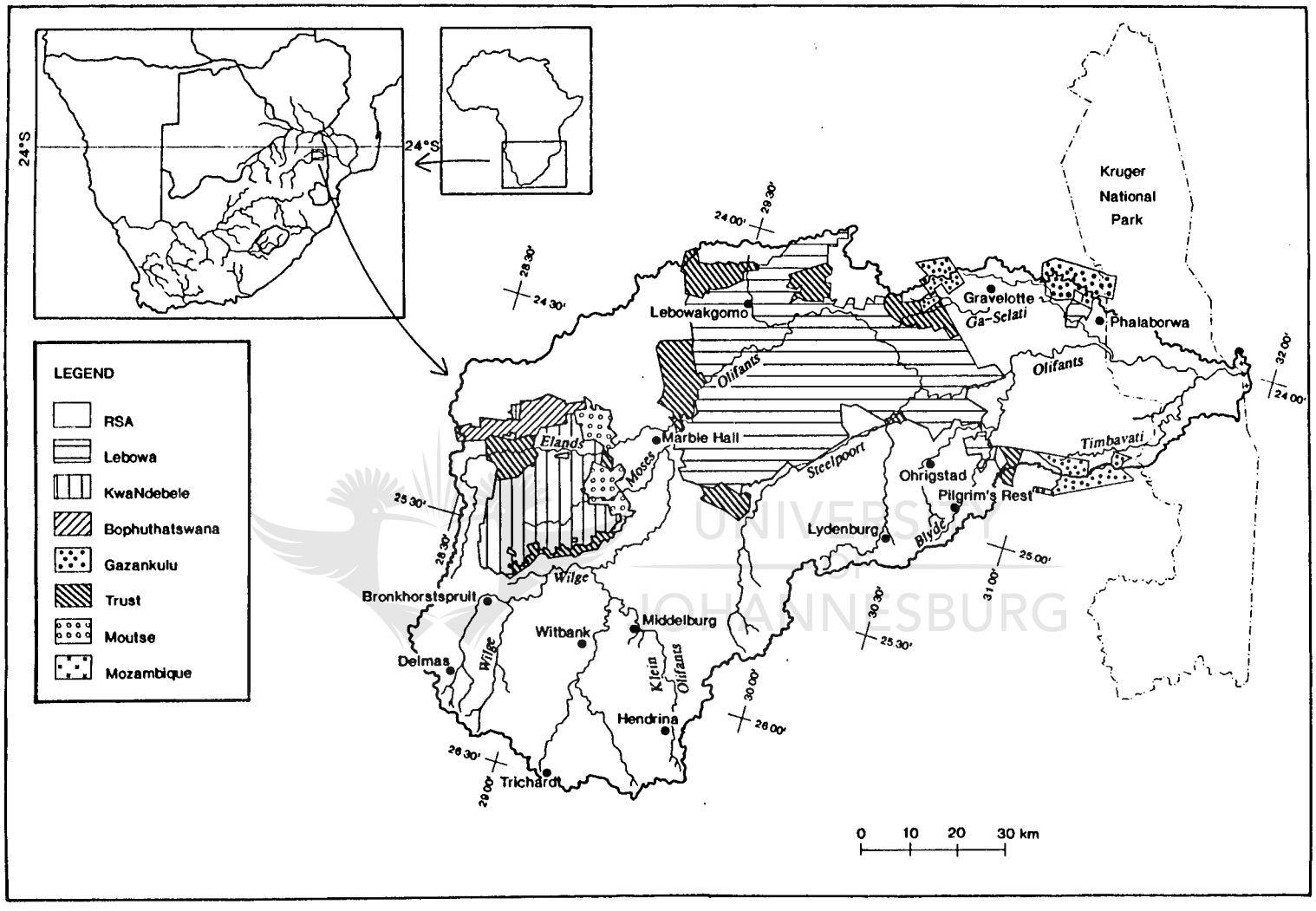

Figure 2.1
The Olifants River Catchment, indicating the involved co-basin states. (From: Theron et al., *1991a)*

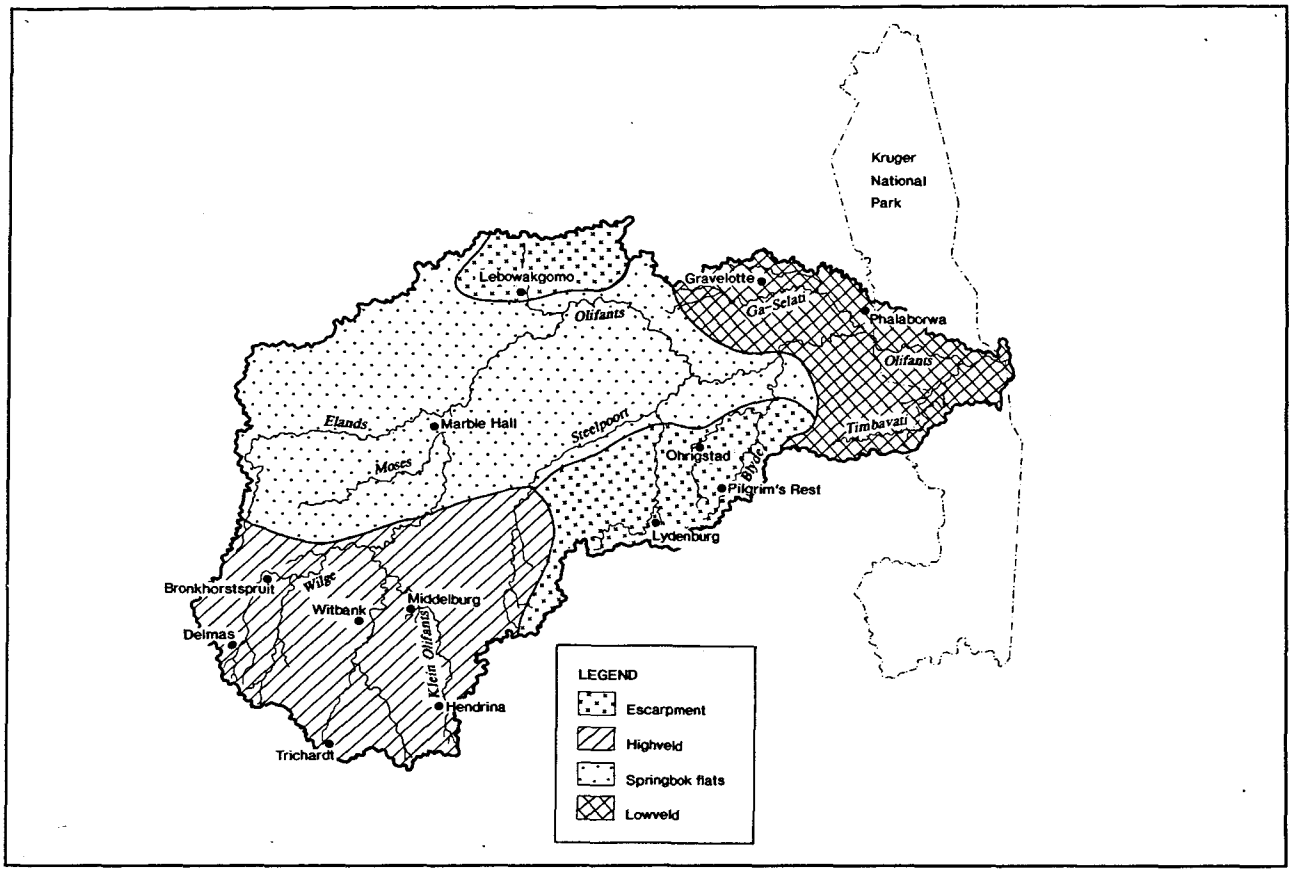

Figure 2.2
The topographical zones of the Olifants River Catchment. (From: Theron et al., *1991a)*

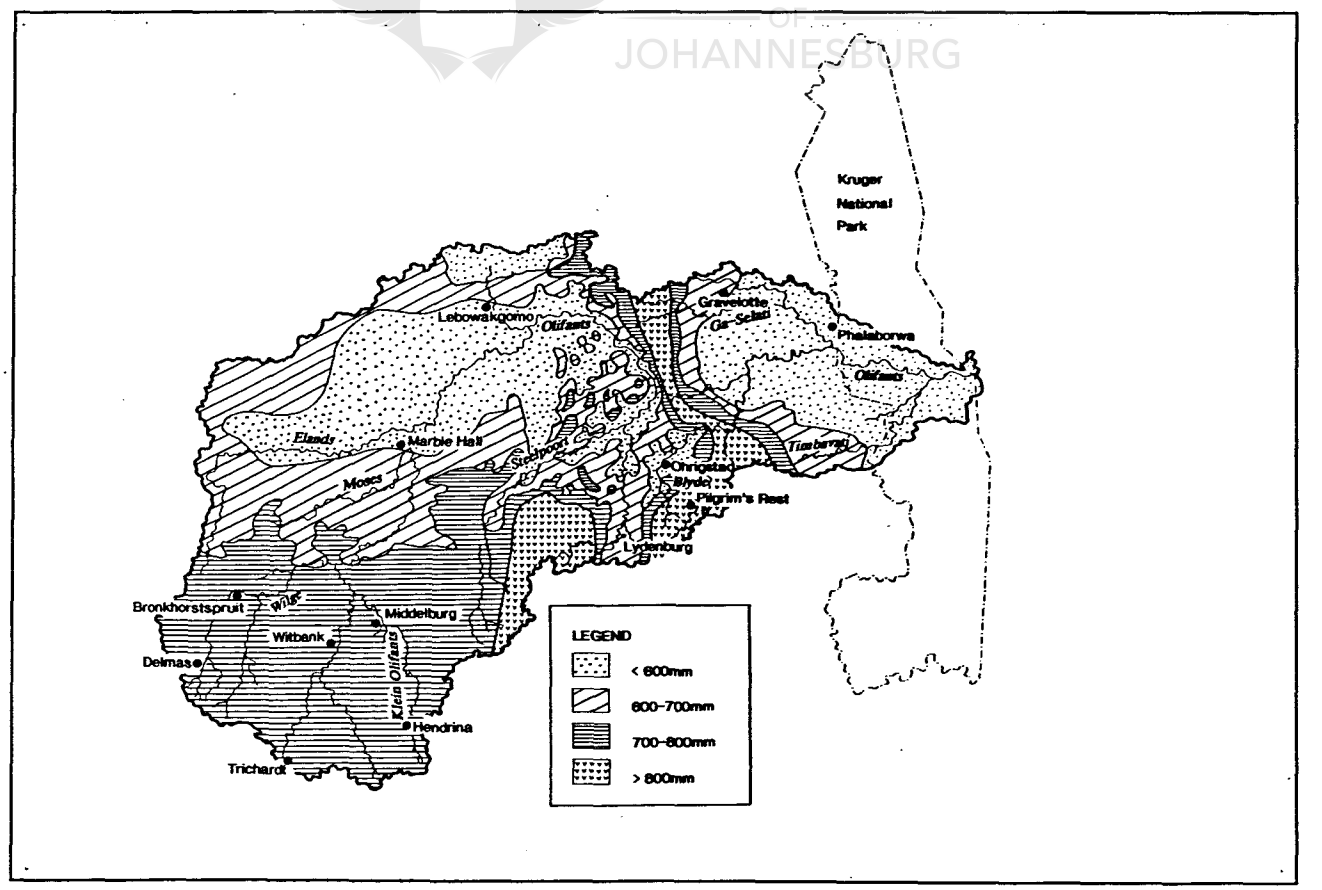

Figure 2.3
The rainfall pattern in the Olifants River Catchment. (From: Theron et al., *1991b)*

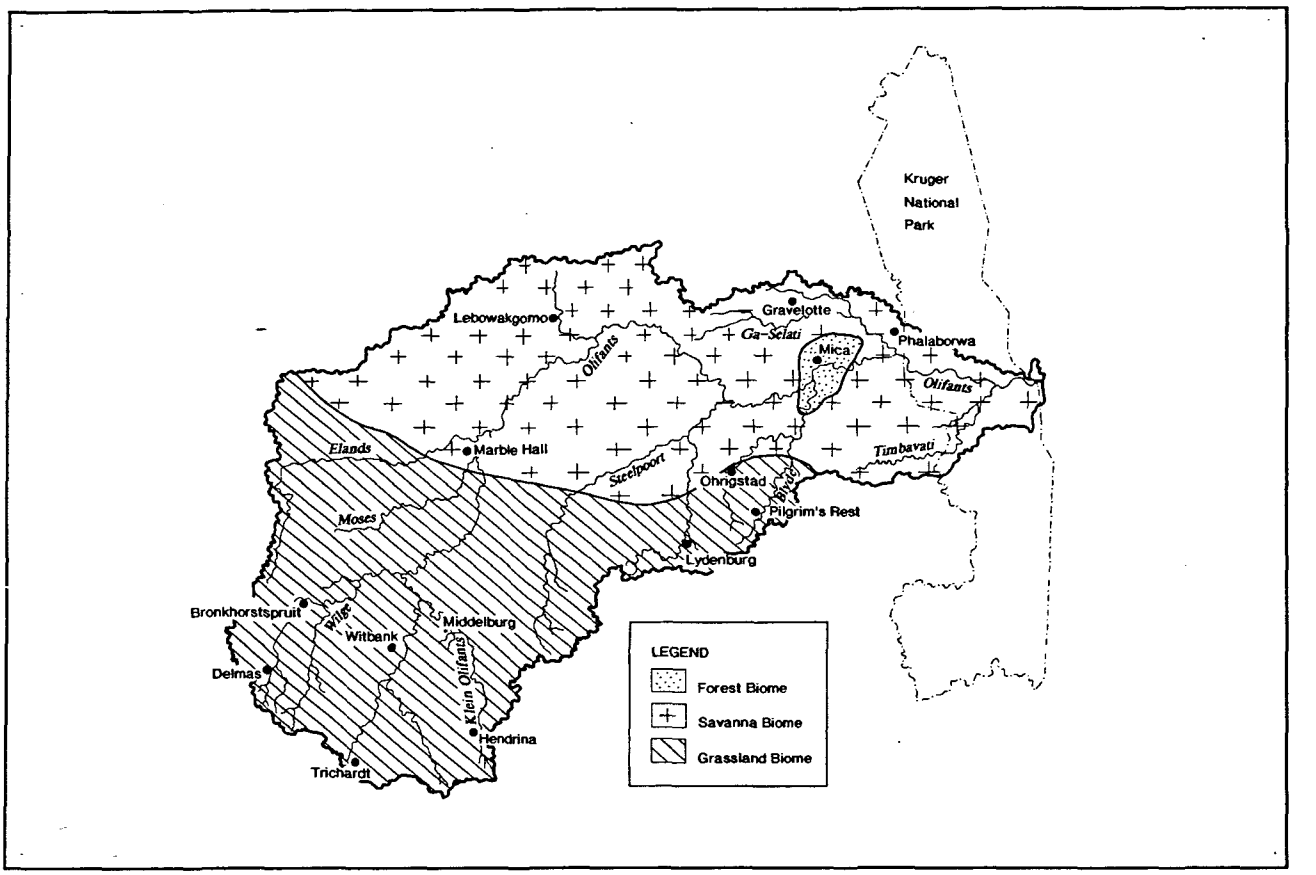

Figure 2.4
Biomes in the Olifants River Catchment. (From: Theron et al., *1991b)*

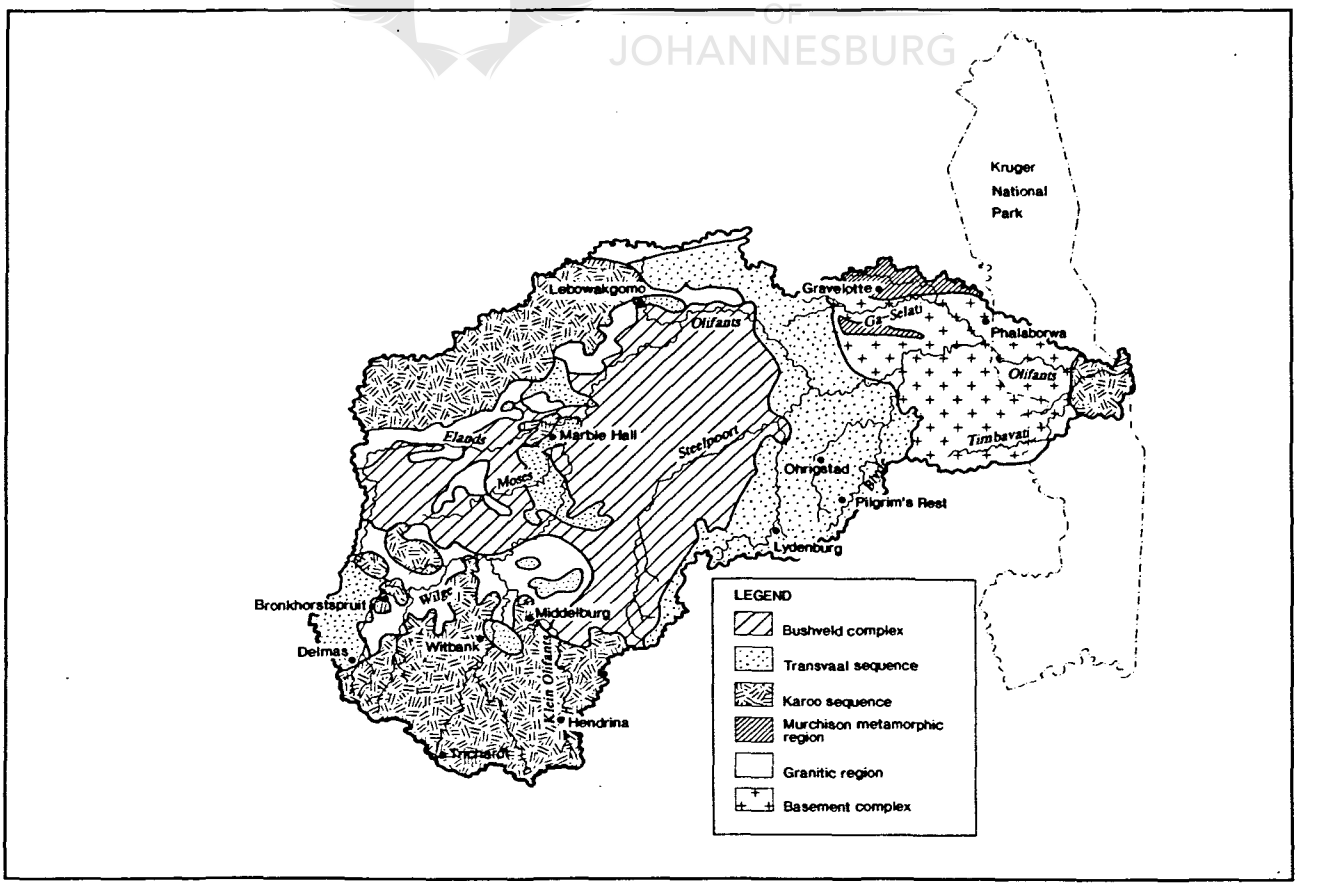

Figure 2.5
Geological regions in the Olifants River Catchment. (From: Theron et al., *1991a)*

the Poaceae, with *Themeda triandra* being the most widespread species. The canopy cover decreases with lower rainfall. Sweet grass occurs in drier regions, while sour grass occurs in areas where the rainfall exceeds 625 mm. Trees are uncommon, although they do occur in high altitude areas east of the escarpment. The savanna biome comprises the greater part of the Springbok Flats and the Lowveld, as well as the north-eastern parts of the escarpment. The vegetation consists of graminoid hemicryptophytes and perennial woody plants. It is well adapted to withstand both drought and fire. Most of the savanna biome is used for livestock grazing and game ranching. The forest biome covers a small portion of the catchment and is more or less centred around Mica. The vegetation consists mainly of evergreen woody plants. A multi-layered structure can be distinguished, with perennial woody plants and herbaceous species as the understorey, while epiphytes, ferns and lianas comprise the sub-canopy (Steffen *et al.*, 1991).

The main geological outcrops in the basin are the Transvaal sequence, Karoo sequence, the Bushveld complex and in the Lower Olifants catchment, the Basement complex. Other lithostratigraphic units are represented as small localised occurrences only (Fig. 2.5). The expected water quality associated with the geology is generally good, but weathering of older granites of the Basement complex, dolomites of the Transvaal sequence and shales and mudstones of the Karoo sequence could produce mineralised waters (Theron *et al.*, 1991b).

The total human population of the Olifants River basin in 1990 was approximately 2.5 million. The distribution of the people among the main co-basin states is illustrated in Figure 2.6. Approximately 66% of the population live in rural or third world conditions, concentrated in settlements with limited infrastructure scattered widely across the various states. The largest urban concentrations are at Witbank and Middelburg, accommodating more than 150 000 people. The population density is approximately 50 - 100 persons/km^2 in the RSA districts, 100 - 150 persons/km^2 on average in the self-governing regions and 350 persons/km^2 in Moutse. An estimation of the future population in the area, taking growth rate and the state of development into account, points to more or less 3.9 million and 4.7 million in the years 2000 and 2010 respectively, with the developing society comprising 94% of the population (Theron *et al.*, 1991b).

2.1.2 Water resources

The Department of Water Affairs and Forestry (1986) recognises five types of water sources, namely surface runoff from rainfall, ground water, unconventional water sources (for instance desalination, rainfall augmentation and reduction of evaporation), reuse of effluent returned to public streams and water imported from other countries. The ground and surface water, however, are at this stage the main water resources of the Olifants River catchment.

GROUND WATER

The geology, slope, rainfall, weathering and structural geology have an influence on the ground water potential. Ground water is an important source of supply for many towns and villages, stock-watering and irrigation, particularly on the Springbok Flats. Ground water recharge in the basin averages between three and six per cent of the mean annual precipitation, although a recharge of eight per cent can be expected in the areas on the north-western fringes of the basin, where deep soil and fractured formations dominate. The total recharge for the basin is estimated to be approximately 1 800 million m^3/a. Areas with high to very high potential yield occur in the vicinity of the Steelpoort River (3 - 20 l/s). However, less than 30% of boreholes are expected to be dry. Roughly half of the catchment west of the Drakensberg mountains is classified as having moderate to high ground water potential (1.5 - 5 l/s), the ground water potential for the Lower Olifants catchment is low to very low, due to the limited ability of granite to store and transmit water (Theron *et al.*, 1991b).

SURFACE WATER

Rainfall is the most important determinant of runoff. Because of the non-uniform distribution of rainfall in the catchment and differences between the physical characteristics of sub-catchments,

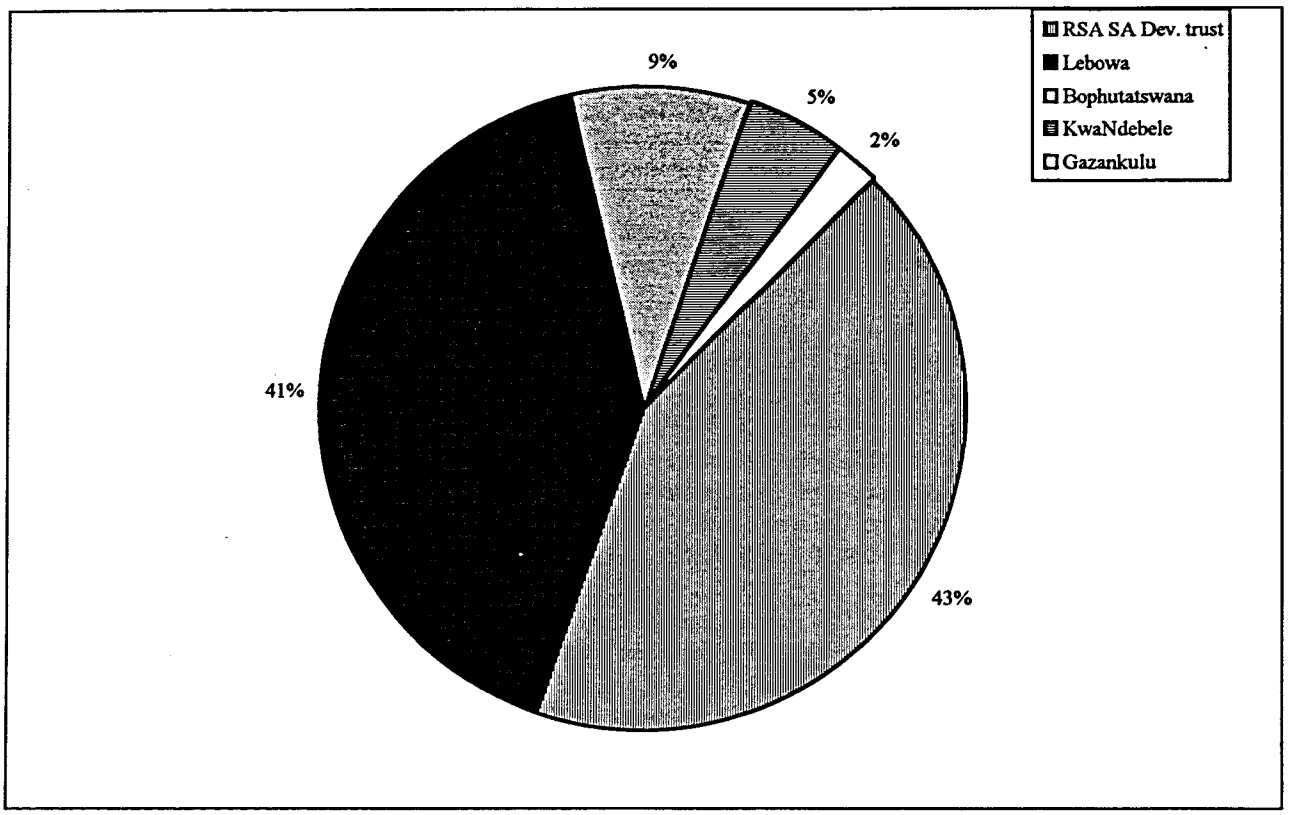

Figure 2.6
Distribution of the people among the main co-basin states in the Olifants River Catchment. (From: Theron et al., *1991a)*

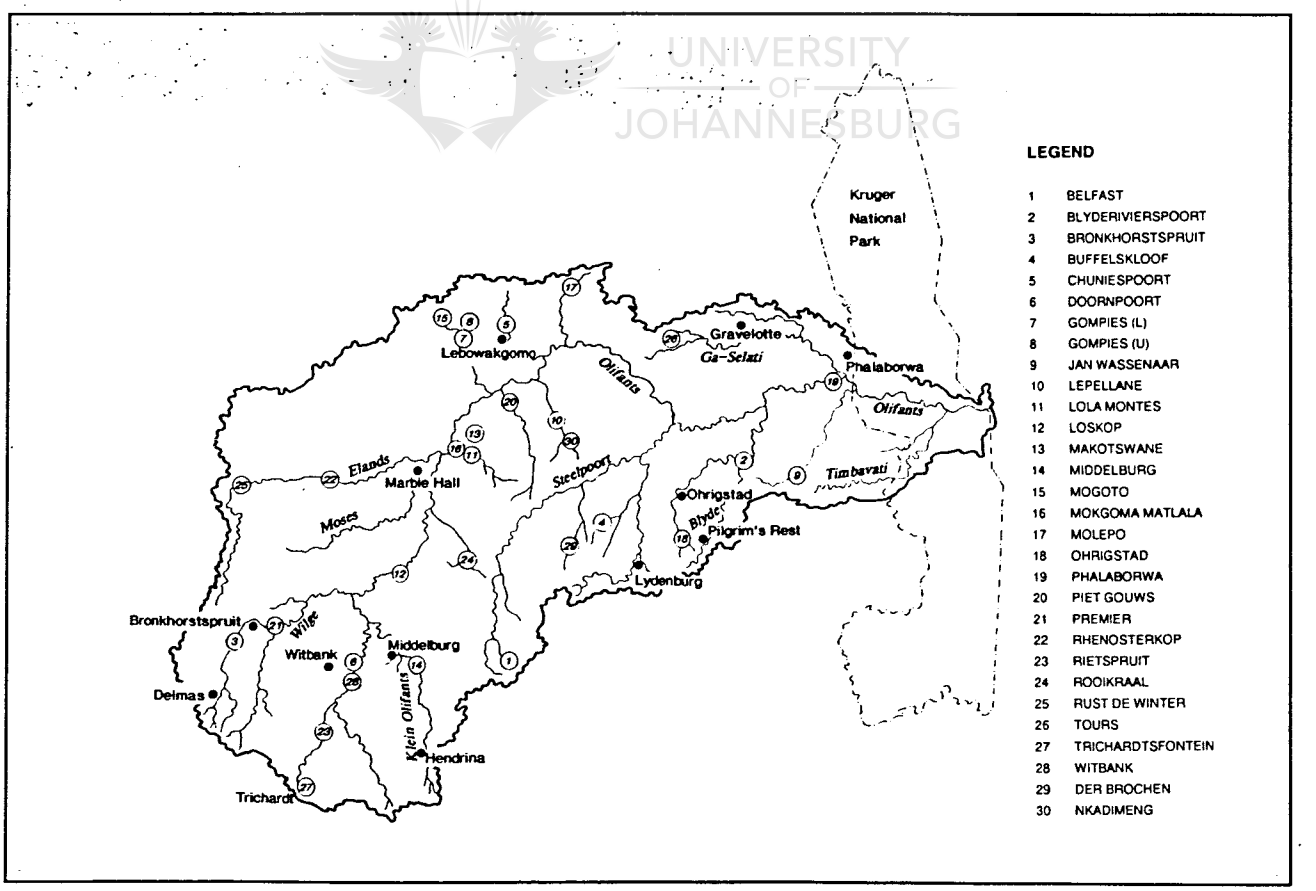

Figure 2.7
Major dams in the Olifants River Catchment. (From: Theron et al., *1991a)*

runoff is not uniformly distributed. The central portion of the basin, with rainfall below 600 mm, produces proportionately the least runoff, while the Steelpoort and Blyde Rivers, which drain the more mountainous sub-catchments, contribute 42% to runoff.

The total natural mean annual runoff at the Mozambique border is estimated to be 1 950 million m^3 per annum. However, changes in the catchment characteristics (for instance through afforestation), abstractions for use (especially for irrigation) and evaporation have decreased the runoff at the outlet of the catchment to an average of 1 235 million m^3 per annum at present (Theron *et al.*, 1991b). Mozambique is of course the water user furthest downstream in the Olifants River basin and therefore its water resources will definitely be affected in quantity and quality by upstream water management.

Dams play an important role in supplying water at a high level of assurance. Two problems that have been encountered, however, are sedimentation and evaporation. As a result of sediment accumulation, an average of 0.5% of the storage capacity of existing dams in the RSA is lost annually and approximately 27% of the water that existing dams can deliver is lost by evaporation (Department of Water Affairs, 1986). There are more than 2 500 dams in the Olifants River catchment, of which more than 90% have a volume of less than 20 000 m^3, while the 30 major dams have capacities greater than two million m^3. According to a survey done in 1987 and 1988, the total storage capacity of minor and small dams is approximately 193 million m^3. These dams regulate 35% of the basin and approximately 87% of the Upper Olifants River catchment. The 30 major dams in the basin (Fig. 2.7) have a combined storage capacity of 1 065 million m^3 and can deliver an assured yield of 645 million m^3/a (Theron *et al.*, 1991b). This annual volume, although sufficient to meet the total present water requirements in the Olifants River basin, is not geographically well distributed relative to demand and consequently shortages and short term surpluses in certain areas do exist. The largest dam is the Loskop dam with a capacity of 348 million m^3, followed by Rhenosterkop dam (205 million m^3), Mokgomo Matlala dam in Lebowa (105 million m^3) and Witbank dam (104 million m^3). In the Lower Olifants River catchment, the major dams are the Tours and Jan Wassenaar Dams and the Phalaborwa Barrage. The flow of the Olifants River in the Kruger National Park is directly related to the operation of the Phalaborwa Barrage. The water level of the barrage is usually kept almost full and the base inflow to the barrage (approximately 1.5 m^3/s) is, as a rule, released as compensation for the Park. During times of low inflow to the barrage, water is released from the Blyderivierspoort dam, to supplement the available water supply (Theron *et al.*, 1991c). Future development (disregarding the impact of additional dams) could, however, result in a zero flow situation where the Olifants River enters the Park for 70% of the time in October. The flow at the 98 percentile would be even more severely affected and the river is predicted to be dry from August to December (Theron *et al.*, 1991b). This situation can be mitigated by compensation releases from Blyderivierspoort dam *via* the Phalaborwa Barrage. Eight potential dam sites were also investigated in order to help the situation, but only the sites at Fountain Gorge and the Strijdom Tunnel in the Ngwabitsi and Olifants Rivers met the feasibility criteria.

2.1.3 Water user sectors

The different water user sectors are always in competition with each other for the limited available water resources. The Department of Water Affairs and Forestry (1986) recognises seven water user sectors. They are: domestic and industrial, power generation, mining, irrigation, stock-watering, afforestation and the environment.

AGRICULTURE (IRRIGATION, STOCK-WATERING & AQUACULTURE)

Irrigation is the major water user sector in the Olifants River basin, utilising approximately 510 million m^3/a or 53% for the irrigation of 103 000 ha. Maize is the dominant crop of the Olifants River catchment. Other crops that are being cultivated in the basin are grain sorghum, wheat, sunflower, cotton, citrus, vegetables, tobacco, ground nuts and deciduous fruit. In the Lower Olifants catchment oranges are the dominant crop, followed by mangoes and avocados.

The water resources that are being used for irrigation, are surface and/or ground water (depending on the area). In the Lower Olifants River catchment surface water is used for irrigation mainly from the

Olifants, Selati and Klaserie Rivers. It is expected that the water demand for irrigation could increase by 90 million m^3/a over the next 20 years. Water supplies to support this growth will thus have to be made available from storage facilities (Theron *et al.*, 1991b). The largest proportion (91%) of the irrigated areas falls within the Republic of South Africa; the balance is situated mainly in Lebowa with only small schemes in Gazankulu and KwaNdebele.

Stock-farming is an important component of the agricultural sector with a population in 1990 of approximately 1.5 million large stock units (±80% cattle, 17% sheep and the balance chickens, goats, game and other species). Stock-watering relies on surface water, springs and boreholes for water supply. Present water use for stock-watering is about 28 million m^3/a and could grow to 40 million m^3/a, limited by the grazing carrying capacity which averages between three and six ha per large stock unit (Theron *et al.*, 1991b).

The production of trout and barbel for commercial purposes is practised at several locations. Water usage by aquaculture is partly consumptive as a result of water being lost through evaporation or seepage, and partly non-consumptive as the water can be returned to the stream of origin, although usually degraded in quality. Aquaculture generally causes nutrient enrichment and bacteriological pollution of water resources. This situation can, however, be mitigated if aquaculture is practised in conjunction with irrigation, for then the enriched water can be used for irrigation (Department of Water Affairs, 1986).

The main threats from agriculture for the aquatic environment include crop spraying (causing organic pollution), leaching of fertilisers (causing eutrophication), erosion (causing siltation), damming (causing changes in aquatic habitats) and water extraction (decreasing water availability to the aquatic environment) (Engelbrecht, 1992). It is therefore essential that the efficiency of irrigation equipment and practices should be improved.

AFFORESTATION

The Olifants River basin has low afforestation potential, as favourable conditions for afforestation are limited to the mountain slopes of the Drakensberg escarpment. The affforested area comprises approximately 72 000 ha or one per cent of the Olifants River catchment, using almost six million m^3 water per annum. Both the surface and ground water potentials are high in the afforested area, in other words both can be used as water sources.

Forests have a negative effect on the hydrology of the catchment by interception and evapotranspiration. Present exotic plantations decrease the natural runoff by about 56 million m^3/a, which approximates three per cent of the natural mean annual runoff. During times of low flow, the impact of forests is more severe (especially on downstream water users), since afforestation occurs in the upper reaches and thus has a first claim on runoff. In the future, if all new afforestation that can be foreseen does materialise, an additional seven million m^3 water on average will annually be taken from natural runoff (Theron *et al.*, 1991b).

DOMESTIC AND INDUSTRIAL

Water used for domestic and industrial purposes comprised approximately 90 million m^3/a in 1990. Improvements in the quality of life and increased urbanisation will have a dramatic impact on the water requirements for domestic use. It is expected that those requirements, including those for industrial use, will grow to between 150 and 230 million m^3/a in the year 2010. This represents an annual growth of almost three per cent in the water demand. About 80% of the projected domestic and industrial water demand for the Olifants River basin will be by the three major industrial centres - Witbank, Middelburg and Phalaborwa - and by villages in the self-governing territories (Theron *et al.*, 1991b).

Less than 20% of the population in the Olifants River basin has fully reticulated water supply systems. The remaining almost two million people obtain water from street pipes, hand pumps or by hand from wells or streams. Ground water resources are thus being utilised fully. At present Phalaborwa and a few towns and villages in the Lebowa and Gazankulu districts use surface water, which is abstracted

by various schemes from the Olifants River and its tributaries. Owing to the expected rapid increase in the population and standard of living in developing areas, increased pressure will be exerted on the existing surface water resources, especially on the Olifants River.

Industries in the Olifants River catchment are related to mining and agricultural activities. Several saw mills, canneries, flour mills, manufacturers of furniture and of agricultural equipment are also found across the basin (Theron *et al.*, 1991b). The industrial water use for the basin is included in the domestic water use figures as they are invariably supplied from municipal water supply schemes and often the end users are not differentiated.

MINING AND POWER GENERATION

The Olifants River basin has considerable mineral deposits (such as chrome, gold, vanadium and platinum) and the historical development and future growth of the area are closely linked to the mining activities. Mining is concentrated on the eastern Transvaal coal fields, in the Steelpoort River valley and at Phalaborwa. Of the more than 200 mines presently productive, more than 50 are coal mines.

The water requirements for the mining sector are expected to grow from about 80 million m^3/a at present to 100 million m^3/a by the year 2010, of which almost 70 million m^3/a would be from surface water. Other water sources for mining activities are borehole water and imported water (water abstracted from adjacent river basins) (Theron *et al.*, 1991b).

The mines, situated in the Lower Olifants catchment, amount to a total of 45, with ten closed down, six that do not use much water and six that have not been commissioned. The existing mines that consume water mine copper, emerald, asbestos, magnetite, phosphate, clay, feldspar, slate and fertilisers (one of each), while there are two gold mines, two mica mines, two crushed-stone mines, two platinum mines, three andalusite mines and three chrome mines. Palaborwa Mining Company (PMC) and Foskor (which extract mainly copper and phosphorus respectively), receive water from the Phalaborwa Water Board at present. These two mines use 84% of the total water consumption of mines in this region. According to projections made by the Phalaborwa Water Board, these mines will use their maximum total allowance permitted, in the future (Theron *et al.*, 1991c).

Eight Eskom power stations are situated in the Witbank-Middelburg region due to the abundance of coal reserves. Since the available water in the Olifants River basin is insufficient to cater for the requirements of these stations (approximately 208 million m^3/a), interbasin transfer schemes were developed to import water from the adjacent Komati, Usutu and Vaal Rivers (Theron *et al.*, 1991a).

Unfortunately, mining and power generation can have detrimental effects on the aquatic environment. Coal mining produces minerals such as pyrite which decompose into acid-forming compounds, and these are released into the environment or atmosphere from waste dumps or slime dumps. The phenomenon is known as acid mine drainage and it can be chronically or lethally toxic to the aquatic environment, depending on the extent to which it leads to release of heavy metals into the system (Kemp, 1965; Steffen Robertson & Kirsten, 1990; United States Department of the Interior, 1978). The acidification of the aquatic environment that has already occurred in the Olifants River has resulted in a reduction of biotic productivity.

The waste products of coal-driven power stations (sulphur dioxide and nitrogen oxides) are released into the atmosphere and react with moisture, oxygen and sunlight to form sulphuric acid and saltpetre which are then precipitated as acid rain (Tyson *et al.*, 1988). Such rain leaches oxides of silicon, aluminium, sulphur, iron, magnesium, calcium and potassium out of old coal waste dumps and introduces concentrated amounts of these into rivers (United States Department of the Interior, 1978).

ECOLOGICAL SYSTEMS

The water requirements of riverine ecological systems and nature reserves are predominantly non-consumptive but very significant when considering water resource development and management. The Water Act (Act No. 54 of 1956) states that South African water resources should be equitably

divided between human users and that their chemical, biological and aesthetic quality should be protected. Aquatic ecosystems are, however, largely ignored. Fortunately, the Department of Water Affairs and Forestry recognised the riverine ecology as a water user in its own right with its own water quality and quantity requirements. Therefore, the new approach regarding the management of the water resources of the Olifants River catchment, is to determine the water quantity and quality requirements of each water user sector, after which water could be allocated accordingly to the different water users (Theron *et al.*, 1991d).

"The water requirements of the natural environment may be defined in broad terms as being that quantity of water, and its temporal and spatial distribution necessary to maintain water-dependent ecosystems as a renewable resource. This means that the resource can recover from a stressed situation to its original unstressed condition without loss of any of its components or species diversity" (Theron *et al.*, 1991d). Attempts to assess the water requirements for conservation of the riverine ecosystems in the Olifants River catchment have essentially been confined to the portion of the Olifants River within the Kruger National Park. The amount of water in the river, its variability and the periods of no flow are of importance in the maintenance of riverine ecosystems. It is estimated by the Department of Water Affairs and Forestry (1986) that the minimum annual inflow to the Park should be approximately 220 million m^3/a. The average flow rates over a month during critical dry periods should be an absolute minimum of one m^3/s in winter and 10 m^3/s during summer (Theron *et al.*, 1991b).

Except for the maintenance of riverine ecosystems, the Kruger National Park also requires fresh water for domestic use, game watering and to supply downstream users in Mozambique (Department of Water Affairs, 1986; Moore, 1990). Discreet water management and future development upstream are thus of the utmost importance.

2.2 The Study Area

The study area comprises the lower part of the Selati River (from Namakgale to the confluence with the Olifants River) and the Lower Olifants River from Phalaborwa Barrage to the confluence with the Letaba River (Fig. 2.8). The Selati River drains the area to the west of Phalaborwa, flowing eastward to join the Olifants River approximately 10 km south-southeast of Phalaborwa. The western boundary of the Kruger National Park is located about six kilometres downstream of the Selati-Olifants confluence, which means that the water quality of the Selati River will have an influence on the water quality of the Olifants River flowing through the KNP.

The water quality of the Selati and Olifants Rivers has, unfortunately, been deteriorating since 1983 due to point and non-point sources of pollution (Theron *et al.*, 1992). At point sources of pollution, the origin, volume and concentration of an effluent can normally be quantified, which is not the case with non-point sources.

The Phalaborwa area has many point sources of pollution from both sewage treatment works and from mining and industrial effluent. Sewage treatment plants (in this case Lulekane, Namakgale and Phalaborwa) are point sources of nutrients, *i.e.* nitrates and phosphates, which can lead to eutrophication problems. The mines and industries in the area (especially Foskor, Palabora Mining Company and Fedmis) are point sources of pollution containing constituents such as fluorides, calcium, magnesium, sulphates, potassium, sodium, phosphates and heavy metals (Theron *et al.*, 1992). Palabora Mining Company (PMC) and Foskor are two large mining companies which utilise the same ore body to extract mainly copper and phosphorus but also small quantities of rare metals. PMC discharges approximately four to five million m^3/a into the Loole Creek that flows into the Selati River, while Foskor discharges 7 to 11 million m^3/a into the Selati River. Fedmis, a phosphoric acid plant discharges no effluent directly into the Selati River (Theron *et al.*, 1992).

Non-point sources of importance in the Selati River catchment are agriculture, atmospheric deposition, rural and urban runoff, leakage from evaporation ponds and seepage from tailings dams,

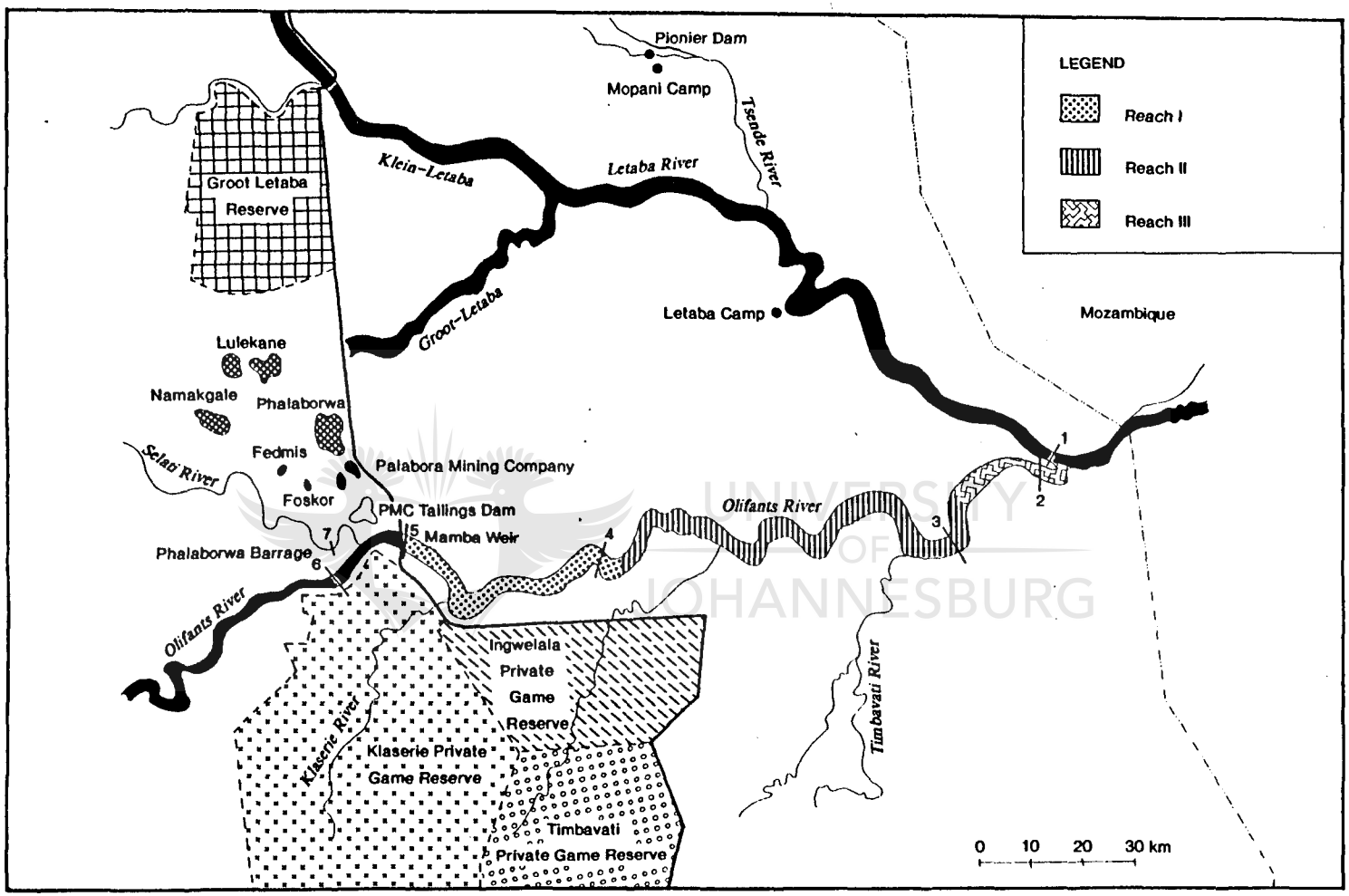

Figure 2.8
The study area in the Lower Olifants River Catchment, indicating the sampling localities 1 to 7

sand dumps and rock dumps. Polluted ground water seeping to surface streams is a sub-surface diffuse source of water pollution and has been recorded as far as below Mamba weir in the Kruger National Park (Bekker, pers. comm.).

As a result of the pollution, the CSIR (1990) found that the electrical conductivity values (and thereby TDS concentrations) in the Lower Selati River and at Mamba weir, are higher than the limits permitted by the Department of Water Affairs and Forestry (Kempster *et al.*, 1982). According to a survey done by the CSIR (1990), the major contributors of TDS are the Foskor effluent discharge and the seepage from the Foskor tailings dams. Moderate TDS loads are contributed by the PMC storm water overflow *via* Loole Creek and seepage from the magnetite tailings dam. Total dissolved salts contributed by the discharge of treated sewage effluent are very small. The water quantity of the Selati River, however, also plays a role in the water quality, for during dry periods the low river flow may consist mainly of effluent from the three sewage works and from Foskor.

The KNP is, in the first instance, a nature reserve, but is dependent on upstream water management for its water resources, having no control over the water quality and quantity flowing into the Park. It is therefore imperative to determine the actual influence of upstream development in the Olifants and Selati Rivers basins on the water resources of the KNP.

As stated in the introduction (Chapter 1), one of the main objectives of this study was to determine the extent (if any) of metal pollution in the Olifants River flowing through the Park. Seven study sites, five inside the KNP and two just outside the Park, were selected (Fig. 2.8). Locality 1 was situated in the Letaba River, while the location of the other sites inside the KNP was chosen in such a way as to represent the three different reaches (based on geology, geomorphology and rainfall) identified by Venter (1991).

The first reach (Fig. 2.8), which included localities 4 and 5, has a single channel with mostly a flat river bed and shallow stream. Short rapids occur over firm or rounded rock, with deep pools only occurring occasionally. The river bed consists of sand and gravel, alternating with small rocky places. No vegetation occurs on the river bed, except for small patches of reed on rocky places. Riparian vegetation is moderately dense with trees such as *Ficus sycomorus, Trichilia emetica, Lonchocarpus capassa, Acacia robusta* and *Diospyros mespiliformes.* Hanging reeds (*Phragmytes* spp.) are limited to isolated small patches.

The channel of the second reach (including locality 3) is mostly irregular and branches off to form small (5 - 10 m) and sometimes deep channels between the islands. The river bed of this reach consists of irregular deposits of silt and sand on firm rock or islands. Dense reed beds occur on islands and sometimes also trees, such as *F. sycomorus* and *Breonadia salicina.* The riparian vegetation is scattered to moderately dense with trees such as *F. sycomorus, T. emetica, L. capassa, Colophospermum mopane, A. robusta* and *D. mespiliformes.* Hanging reeds (*Phragmytes* spp.) are very dense in some locations.

The third reach included locality 2 and has a V- to U-shaped single channel with deep pools and short rapids. At the Olifants rest camp and hiking trail the channel is deeply cut into the rock to form a series of low waterfalls and deep, narrow little ravines. The river bed consists of rock with relatively thick depositions of red silt in deep pools and rounded loose cobble-stones in rapids. Virtually no vegetation occurs on the river bed and reeds are limited to rocky places or alluvial islets. The river banks are open with trees such as *F. sycomorus, L. capassa, A. robusta* and *A. xanthophloea.* Alternatively, hanging reeds (*Phragmytes* spp.) occur (Venter, 1991).

Localities 6 and 7 were selected in order to study the possible effect that the Selati River might have on metal pollution in the Olifants River. Locality 6 was located below the Phalaborwa Barrage, which is before the Selati-Olifants confluence, while locality 7 was located in the Selati River (Fig. 2.8). The channel of the Selati River is single, with large deep pools and small rapids occurring there. Black, smelly silt depositions cover the rocky river bed. Trees such as *Phoenix reclinata, Trichelia emetica* and *Ficus sycamorus* grow on the river banks, while hanging reeds (*Phragmytes* spp.) occur in dense spots along the banks.

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CHAPTER 3

Chapter 3

WATER AND SEDIMENT

3.1 Introduction

In South Africa, which is a developing country, it can be expected that large-scale development will take place. Unfortunately, increasing mining, industrial, agricultural and domestic activities may lead to water pollution unless certain precautions are taken. These precautions can, however, be very costly and are therefore not always enforced. This is partly why the water quality of many South African rivers has been deteriorating over the last few years.

There are five major types of toxic pollutants, namely:

1) Metals (such as zinc, copper, nickel and lead), arising from industrial processes and some agricultural applications.
2) Organic compounds (such as organochlorine pesticides, herbicides, PCB's, chlorinated aliphatic hydrocarbons, organometallic compounds and phenols), originating from industrial, agricultural and some domestic sources.
3) Gases, such as chlorine and ammonia.
4) Anions, such as cyanides, fluorides, sulphides and sulphites.
5) Acids and alkalis. (Mason, 1991)

These pollutants can have a direct or indirect effect on aquatic species, for instance a reduction in the survival, growth and reproduction of the species, an unacceptable level of avoidance behaviour towards the pollutant and an unacceptable percentage of gross deformities or visible tumours in organisms (Stephan, 1986). It is, however, very difficult to relate specific effects to specific pollutants, for the stage of the organism's development, the physical and chemical quality of the environment (e.g. temperature, pH, water hardness), the chemical species and complexes present, and the interactions between pollutants all play a role in the toxicity of a substance (Hellawell, 1986). Interactions between pollutants can be additive (a combined effect), antagonistic (interfering with one another) or synergistic (the overall effect is greater than when each one acts alone). These interrelated pollution problems might be better perceived by using methods such as the famous three-dimensional graph of McLeese (1956) (Fig. 3.1) or the response curve method (Fig. 3.2) as was applied by Costlow *et al.* (1960) and Alderdice (1965), among others.

Bearing in mind that the toxicity of a pollutant to an organism is not always the same due to external influencing factors, one can understand that there are some difficulties in the establishment of water quality guidelines and, eventually, water quality standards. A water quality standard is defined as that concentration, level or value of a particular water quality variable that has been promulgated as a legally enforceable limit (Department of Water Affairs, 1986). In South Africa, water quality standards only apply to effluents discharged into river courses. A water quality guideline, on the other hand, is that concentration, level or value of a particular water quality variable that meets the needs of all water users in a specified river reach (Moore *et al.*, 1991) and has no legal connotations. Water quality guidelines in South Africa are presently being developed, and are based primarily on values from overseas literature, as well as on the limited data available in South Africa.

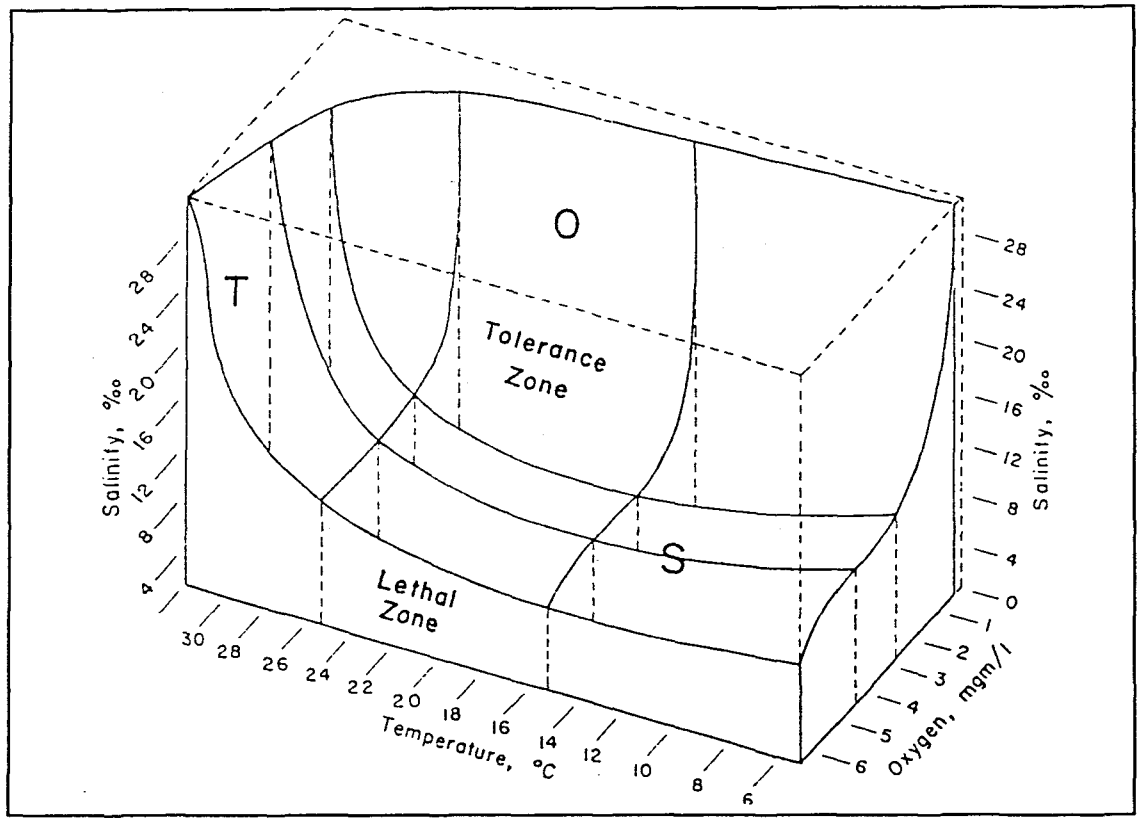

Figure 3.1

A response surface defining 48-h LC50's for combinations of three lethal entities. The example is for the lobster. (From McLeese, 1956)

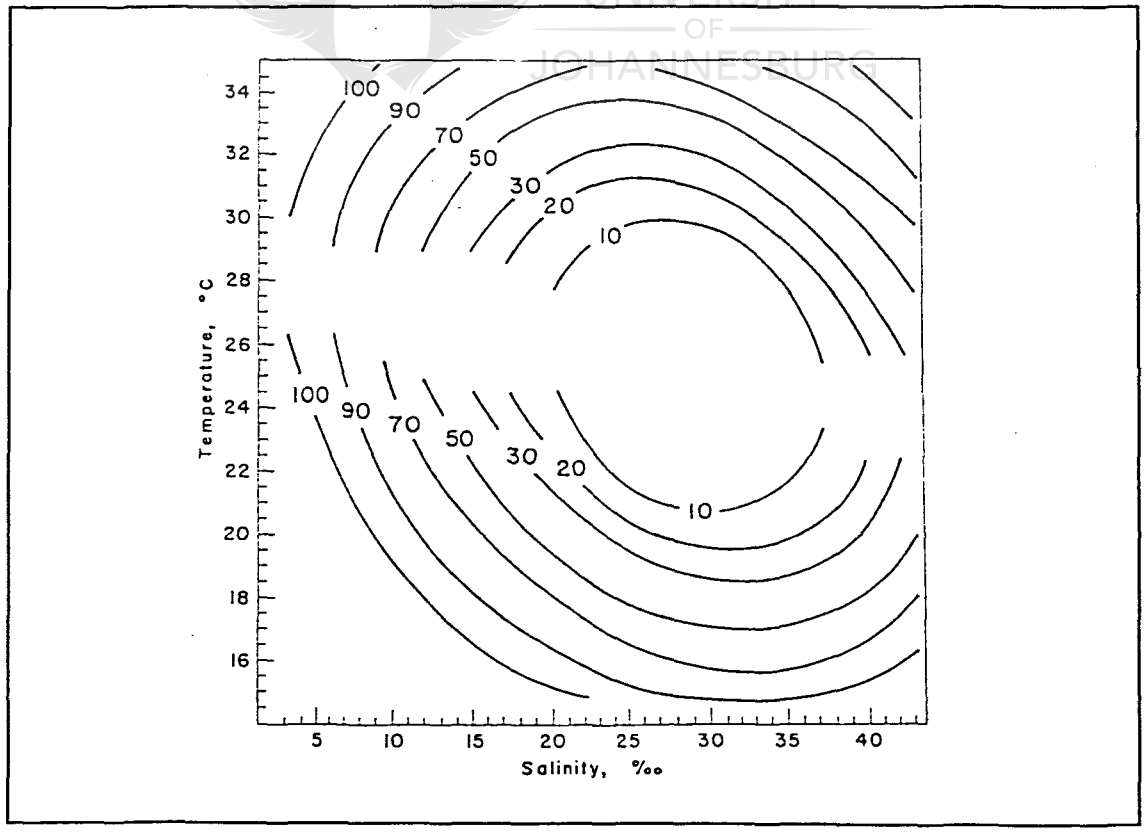

Figure 3.2

Response surfaces fitted to observed percentage mortality of crab larvae at various combinations of temperature and salinity. (From Costlow et al., *1960)*

Because of increasing mining and industrial activities in the Phalaborwa area, it was deemed necessary to determine the effect of these activities on the water and sediment quality of the Lower Olifants River, as well as the extent to which fish in the river accumulate toxicants, especially the metals. The Lower Olifants River flows through the Kruger National Park, which is a nature reserve, and therefore polluted water and sediment are undesirable. In this section of the study, the metal concentrations (Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn) in the water and sediment, as well as the physical and chemical characteristics of the water, were investigated.

3.2 Materials and methods

Water and sediment were sampled every alternate month from April 1990 to February 1992 at six sampling sites (Fig. 2.8) along the Lower Olifants River and one (locality 7) in the Selati River. In February 1992 sampling was also performed at Pionier Dam. This dam is situated in the Tsende River (Kruger National Park), receiving no effluents from outside the Park, and was therefore used as a natural reference point in the study.

WATER

The following variables of surface water were determined on site at each locality: pH (ORION, Model SA250), water temperature (WTW microprocessor, Model OXT 96), dissolved and percentage saturation oxygen (WTW microprocessor, Model OXT 96), turbidity (Secci-disc) and conductivity (Jenway, Model 4070). During the first year these variables were determined once a day in the afternoon. However, in order to determine whether there would be any difference between readings taken in the morning and readings taken in the afternoon, these parameters were determined twice a day at localities 3, 4, 5 and 7 during the second year. Readings were taken between 7:00 and 9:00 in the morning and between 11:00 and 17:00 in the afternoon. At localities 1,2 and 6, as well as the Pionier Dam, the variables were only determined once a day between 11:00 and 17:00.

Two surface water samples were collected at each locality. One sample was preserved with mercuric chloride ($HgCl_2$) and was refrigerated until the Hydrological Research Institute analysed it for sodium (Na), magnesium (Mg), calcium (Ca), fluoride (F^-), chloride (Cl^-), nitrate and nitrite (NO_3+NO_2-N), sulphates (SO_4), phosphates (PO_4-P), total alkalinity (as $CaCO_3$), silicon (Si), potassium (K), ammonia (NH_4-N) and total dissolved salts (TDS) concentrations. The other sample was frozen, until it could be subjected to metal concentration analysis in the laboratory.

After the water samples were thawed in the laboratory, 50 ml of well-mixed river water was measured into a 100 ml Erlenmeyer flask. Ten ml concentrated nitric acid (55%) and five ml concentrated perchloric acid (70%) were added and the mixture was evaporated to 2 to 5 ml on a hot plate until clear (Standard Methods, 1989). Each sample was then made up to 50 ml with doubly distilled water and stored in clean storage glass bottles for metal analysis. Prior to use, all glassware was soaked in a 2% Contrad soap solution (Merck chemicals) for 24h, rinsed in doubly distilled water, acid-washed in 1M HCl for 24h and rinsed again in doubly distilled water (Giesy and Wiener, 1977).

A Varian atomic absorption spectrophotometer (Spectra AA-10) was used to determine the total metal concentrations (dissolved plus suspended) of selected metals in the river water. Analytical standards for Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn were prepared from Holpro stock solutions. For the analysis of strontium, 0.5 ml of a 2.682M potassium chloride (KCl) solution (200 g KCl per litre distilled water) was added to the 50 ml sample in order to suppress ionisation of strontium (Varian, 1989).

The metal concentrations in the river water were calculated as follows:
Metal concentration (µg/l) = AAS reading (µg/ml) x 1000

SEDIMENT

Sediment samples were taken with a pole-operated Ekman grab or by hand, using a plastic bottle (when the underlying substratum was a rock). The samples were frozen until further metal analysis in the laboratory. In the laboratory the samples were thawed and dried in an oven at 90°C for a period of 48 hours. After cooling, one gram of sediment was weighed into a 100 ml Erlenmeyer flask. Ten ml concentrated nitric acid (55%) and five ml concentrated perchloric acid (70%) were added, after which digestion was performed on a hot plate (200 to 250°C) for at least four hours, until the solutions were clear. Each solution was then filtered using a acid resistant 0.45 µm paper filter and a vacuum pump. After filtration the filter system was rinsed with doubly distilled water and the sample was made up to 50 ml with doubly distilled water. The samples were then stored in clean acid-washed glass bottles for the analysis of the different metals. The same procedure was followed as for the water metal analysis. The metal concentrations in the sediment were calculated as follows:

$$\text{Metal concentration } (\mu g/g) = \frac{\text{AAS reading } (\mu g/ml)}{\text{Sample mass } (g)} \times \text{Sample volume } (ml)$$

3.3 Results

Reference will be made to results of the first year and results of the second year. The first year refers to the period April 1990 to February 1991, while the second year refers to the period April 1991 to February 1992. Both years include the seasons autumn (month April), winter (months June and August), spring (month October) and summer (months December - February).

WATER

The selected physical and chemical variables of the Lower Olifants River are summarised in Tables 3.1, 3.2 and 3.4. In general, the readings were found to be slightly higher during the afternoon, except for the conductivity, which was slightly lower (Table 3.1b). The pH of localities 1 to 6 ranged from 8.3 to 8.7 on average over the two year period, while the pH of Pionier Dam and locality 7 (in the Selati River) were slightly lower, namely 8.1 and 7.8 to 7.9 respectively. As can be expected, the temperatures were the lowest during winter time (on average 19.2°C ± 1.4°C in the afternoon for the first year and 20.4°C ± 2.2° C in the afternoon for the second year) and the highest during spring and summer (on average 26.7°C ± 2.3°C in the afternoon for the first year and 30.6°C ± 1.5°C in the afternoon for the second year). The overall temperatures were higher in the second year than in the first (Table 3.4), as a result of the low river flow during the drought. The Olifants River and Pionier Dam seemed to be very well oxygenated, ranging from 8.2 ± 1.8 mg/l to 12.0 ± 1.9 mg/l on average over the two year period. Locality 7, however, had a low dissolved oxygen concentration of 5.6 ± 1.5 to 5.7 ± 0.3 mg/l. Turbidity was not always an easy parameter to determine because of the secci-disc. At times, especially during the drier second year, the river was too shallow in order to take a measurement and so, where possible, values were indicated as "greater than (>)" in Table 3.1b. In winter the water seemed to be the least turbid, while the highest turbidity occurred in summer, especially in December 1990, when values of 1 to 3 cm were measured (Table 3.1a). During this month, heavy rainfall occurred and the entire length of the river flowing through the Park was flooded. Due to this, locality 4 was inaccessible, and no readings could be taken for pH, temperature, oxygen and turbidity. Conductivity shows a different pattern for each year, but in each case locality 7 had the highest conductivity (224.8 ± 39.5 mS/m and 230.3 ± 11.7 mS/m for years 1 and 2 respectively) and locality 1 the lowest (32.5 ± 9.3 mS/m and 37.3 ± 11.5 mS/m for years 1 and 2 respectively). In the first year the conductivity decreased as the river flows eastwards after the Selati-Olifants confluence, but an increase to 95.0 ± 64.2 mS/m (Table 3.4a) was recorded at locality 3 (near Balule). This can mainly be attributed to the high value of 230 mS/m recorded in December 1990 (Table 3.1a). Except for localities 3 and 7, the highest conductivity for each locality was recorded in August or

October 1990, with the lowest being in February 1991. Locality 7 had the highest value in June 1990 and the lowest in April 1990. In the second year there was also a decrease in conductivity as the river flows eastwards, but no increase in conductivity was observed at locality 3 (Table 3.4b). The seasonal pattern showed two peaks in October 1991 and February 1992, with the lowest conductivity values in April 1991. Locality 7 was different, with the highest conductivity being measured in February 1992 and the lowest in June 1990. At the Pionier Dam the conductivity was recorded to be 82 mS/m (Table 3.1b).

The variables Na, Mg, Ca, F, Cl, SO_4 and K, as well as the total alkalinity and TDS were the highest in concentration at locality 7 (in the Selati River) and the lowest at localities 1 (in the Letaba River) and 6 (located before the Selati-Olifants confluence) for both years (Table 3.4). Although no values were available for locality 1 in the first year, the general trends seemed to follow the same pattern as for the second year. The concentrations of these variables decreased from localities 7 to 1 (excluding locality 6). However, during the first year, the concentrations of Na, Mg, F, Cl, SO_4 and K slightly increased at locality 3 (near Balule) and Ca, along with the total alkalinity, increased slightly at locality 4. In the second year the total alkalinity also increased slightly at localities 3 and 4. Noticeable was the low sulphate concentration at Pionier Dam (7 mg/l) in comparison with the concentrations at localities 2 to 7 during February 1992, ranging from 29 mg/l to 969 mg/l (Table 3.1b). For the first year, the general seasonal pattern observed for these variables indicated that the highest concentrations occurred during August and October at localities 2 to 6, and the lowest in February. At locality 7 two peaks of high concentrations were recorded in June/August and February, with the lowest concentrations in April. In the second year, the highest concentrations occurred in October, and another peak was formed in February. The lowest concentrations occurred in April (which is two months later than was the case for the first year). For locality 7 the highest concentrations were recorded in January/February, with the lowest being in June. Comparing the two years, the concentrations were higher in the second than in the first year, with the exception of fluoride at localities 2 and 7, and sulphate at locality 2.

Nitrite, nitrate and ammonia concentrations were determined as nitrogen. The highest concentrations occurred at locality 7 and the lowest varied between localities 2, 3 and 4 (Table 3.4). In the first year, the concentrations decreased from localities 7 to 2, with a slight increase in concentration at locality 4. This increase, especially of nitrite and nitrate, can be attributed mainly to the concentration of 1.19 mg/l NO_3+NO_2-N recorded in December 1990 (Table 3.1a). In the second year, the concentrations also decreased from localities 7 to 1, but nitrite and nitrate increased slightly at locality 1 (due to 0.66 mg/l recorded in February 1992), while ammonia increased slightly at locality 3 (due to 0.62 mg/l recorded in October 1991). Seasonal variations were not clear. The concentrations of the second year were generally higher than those of the first year, with the exception of nitrite and nitrate at locality 4.

The phosphate concentrations (PO_4-P) ranged from 0.052 ± 0.038 mg/l at locality 7 to 0.009 ± 0.002 mg/l at locality 2 in the first year, and from 0.136 ± 0.167 mg/l at locality 7 to 0.022 ± 0.007 mg/l at localities 3 and 4 in the second year (Table 3.4). The silicon concentrations ranged from 6.18 ± 1.10 mg/l at locality 2 to 14.56 ± 2.13 mg/l at locality 7 in the first year. In the second year the concentrations decreased from 14.93 ± 0.58 mg/l at locality 7 to 7.33 ± 1.46 mg/l at locality 2, whereafter it increased to 9.50 ± 0.96 mg/l at locality 1 (Table 3.4).

The metal concentrations of the surface water are summarised in Tables 3.2 and 3.4. Pronounced variations in the metal concentrations precluded unambiguous interpretation of the results. In the first year Cr, Fe and Ni had the highest concentrations at locality 3; Cu, Pb, Sr and Zn at locality 7 and Mn at locality 6. All the metals were the lowest in concentration at locality 1 (Table 3.4a). The iron concentration seemed to increase tremendously at most localities during December 1990 after the heavy rainfalls. These increased concentrations varied from 5680 µg/l at locality 5 to 129240 µg/l at locality 3 (Table 3.2a). In the second year the highest concentrations of Cr and Cu were recorded at locality 1, and the lowest at localities 5 and 4 respectively (Table 3.4b). In October 1991 very low concentrations of chromium were recorded (Table 3.2b), falling below the minimum detection limit of 6 µg/l. The iron concentrations ranged from 1743.3 ± 1376.1 µg/l at locality 7 (which is similar to the concentration found

in Pionier Dam) to 18045.0 ± 35156.5 µg/l at locality 3, while the zinc concentrations ranged from 44.0 ± 19.1 µg/l at locality 7 to 181.8 ± 295.3 µg/l at locality 2. The concentrations of nickel, lead, strontium and manganese were the highest at locality 7 and the lowest at localities 4, 6, 6 and 2 for each metal respectively (Table 3.4b). In October 1991 and January 1992 the nickel concentrations were below the minimum detection limit (Table 3.2b), which is 10 µg/l. The concentrations of iron (1710 µg/l), manganese (43 µg/l) and lead (74 µg/l) in the Pionier Dam were lower than the concentrations recorded at the other localities during February 1992 (Table 3.2b). In general, the metal concentrations were lower in the second than in the first year, except for copper at locality 1; iron at localities 1, 4 and 5; manganese at localities 1, 5 and 7; and strontium at localities 1 to 5 and 7. The trends regarding strontium should, however, be treated with caution, as there is insufficient data for this metal.

SEDIMENT

The sediment metal concentrations showed a high variation (Table 3.3), similar to that found for the water metal concentrations. In the first year chromium and manganese were the highest in concentration at locality 5, while copper, nickel and strontium were the highest in concentration at locality 7. The highest mean concentration of iron was recorded at locality 6 (24069.0 ± 17087.6 µg/g), that of lead at locality 3 (32.0 ± 11.7 µg/g) and that of zinc at locality 1 (248.6 ± 448.1 µg/g). All the metals, except for zinc, were the lowest in concentration at locality 1 (Table 3.4a). In the second year manganese and zinc were the highest in concentration at locality 3, copper and strontium at locality 7 and chromium and lead at locality 6. The manganese concentration recorded at Pionier Dam (53.7 µg/g) was much lower than the concentrations recorded at the other localities (Table 3.3b). The highest mean concentrations of nickel and iron were recorded at localities 5 (58.4 ± 33.6 µg/g) and 4 (27723.7 ± 8596.6 µg/g) respectively (Table 3.4b). The lowest mean concentrations of all the metals were recorded at locality 1. In general, the metal concentrations were lower in the second year than in the first, except for chromium at localities 4 and 6; copper at localities 1 and 3 to 6; iron at localities 2 to 4; manganese at localities 2 to 4 and 6 to 7; nickel at localities 4 and 6; and strontium at localities 1 to 4 and 6 to 7. Again it should be mentioned that the trends found for strontium should be treated with caution, as there is insufficient data for this metal.

TABLE3.1a

PHYSICAL AND CHEMICAL VARIABLES OF SURFACE WATER FROM THE OLIFANTS **RIVER,** KRUGER NATIONAL PARK (APRIL 1990 - FEBRUARY 1991)

Loeallty	Month	pH	Temp ·C	Dissolved Oxygen mg/l	Oxygen Saturtlon /.	Turbidity cm	• EC @25°C mSlm	Na mg/l	Mg mgll	Ca mgll	FI mgll	CI mgll	NO) + N01-N mgll	SO_4 mgll	PO_4 mgll	Alkalinity IICaCO) mg/l	Slle. mgll	K mgll	NH_4-N mg/l	TDS mgll
I	Apr 1990	8.3	24.0	N//\	NI\	17.5	NI\	N//\	N//\	NI/\	N//\	NI\	N//\	N//\	N//\	N//\	N//\	N//\	N//\	N//\
	June 1990	8.4	20.0	N//\	N//\	N//\	N//\	N//\	N//\	N//\	NI/\	NI\	N//\	N//\	N//\	N//\	N//\	N//\	N//\	N//\
	Aug 1990	8.6	18.2	11.8	129	. 32.0	44	N//\	N//\	N//\	N//\	NI\	N//\	NI/\	N//\	N//\	NI\	N//\	N//\	N//\
	Oct 1990	8.8	28.0	10.8	130	N//\	33	N//\	N//\	N//\	N//\	NIA	N/A	NI/\	N/A	N/A	NIA	N//\	N//\	N//\
	Dec \990	8.3	25.9	6.8	86	140	35	N/A	N//\	N//\	N//\	NIA	N/A	N/A	N//\	N/A	N/A	N//\	N/A	N/A
	Feb 1991	8.0	29.8	7.4	113	14.0	18	N//\	N//\	NI/\	N//\	NI\	N//\	N//\	NI/\	N//\	NI\	N//\	N//\	N//\
2	Apr 1990	8.9	23.0	NI\	N//\	14.5	48	35.6	21.6	26.3	0.58	45.0	0.03	57.5	0.009	129.8	6.87	4.76	0.05	357
	June 1990	8.5	19.0	N//\	N//\	2.0	67	52.8	35.8	34.5	0.77	62.9	0.01	110.6	0.008	165.2	4.49	8.75	0.01	512
	Aug \990	8.5	19.6	9.9	105	60.0	91	74.5	56.7	43.3	1.21	87.8	0.01	170.7	0.009	215.0	5.59	15.17	0.02	717
	Oct 1990	8.6	29.6	9.5	120	N//\	92	60.5	51.1	41.0	1.17	69.4	0.02	182.7	0.011	174.8	7.73	17.03	0.04	644
	Dec 1990	8.1	26.3	5.3	74	1.0	44	N//\	N//\	N//\	N//\	NI/\	N//\	N//\	N//\	100.0	N//\	NI/\	NI\	N//\
	Feb 1991	8.3	29.5	7.9	124	15.5	29	208	12.6	23.7	0.33	20.4	0.19	53.3	0.006	71.6	6.22	3.78	0.03	229
3	ADr 1990	9.1	22.0 .	N//\	N//\	17.0	50	35.6	21.1	26.2	0.41	44.4	0.01	59.2	0.007	127.8	6.86	4.58	0.04	354
	June 1990	8.5	19.0	N//\	N//\	46.0	80	57.5	41.8	38.5	0.91	67.1	0.02	148.0	0.003	165.6	4.49	11.34	0.03	572
	Aug 1990	8.4	18.9	\0.6	114	60.0	99	76.9	59.5	43.4	1.23	89.4	0.01	186.3	0.009	210.4	5.73	15.87	0.04	735
	Oct 1990	8.4	27.0	11.3	148	31.5	79	59.0	46.7	36.3	0.76	79.2	0.03	142.9	0.022	174.3	7.69	15.92	0.04	601
	Dec 1990	7.8	23.7	6.4	76	1.0	230	NI/\	N//\	N//\	N//\	N//\	N//\	N//\	N//\	150.0	N//\	N//\	NI/\	N//\
	Feb 1991	8.4	28.5	7.2	107	11.0	32	19.8	10	23.8	0.40	21.7	0.21	52.1	0.017	78.2	6.68	3.83	0.01	235
4	Apr 1990	9.0	25.0	N//\	N//\	18.0	43	32.3	21.3	27.9	0.42	28.2	0.02	50.7	0.015	132.0	7.87	5.49	0.02	335
	June 1990	8.6	19.0	N//\	N//\	45.0	65	54.8	36.8	33.5	0.78	66.4	0.02	103.1	0.005	171.6	4.36	8.60	0.05	518
	Aug 1990	8.6	17.3	9.3	99	80.0	90	72.4	51.7	37.4	1.03	84.7	0.01	158.0	0.013	196.1	4.77	12.83	0.03	662
	Oct 1990	8.7	25.5	11.4	134	33.0	109	72.7	64.2	50.1	1.15	91.9	0.03	262.4	0.021	168.7	9.49	22.94	0.05	781
	Dec 1990	N//\	N//\	N//\	NI\	N//\	45	17.7	23.7	41.0	0.33	24.1	1.19	13.7	0.038	182.1	5.68	3.84	0.12	358
	Feb 1991	8.3	28.6	N//\	125	11.0	32	21.0	II	24.5	0.44	22.6	0.26	61.0	0.016	75.1	6.59	4.28	0.02	244
5	Apr 1990	8.7	24.0	NI/\	N//\	16.5	55	37.1	23.3	27.8	0.44	40.7	0.28	59.4	0.012	123.8	7.71	4.97	0.05	354
	June \990	8.6	18.0	NI/\	N//\	43.0	63	56.6	30.9	32.9	0.66	76.7	0.10	133.7	0.011	174.0	5.13	10.60	.0.05	582
	. Aug 1990	8.2	22.3	13.8	150	700	99	77.9	58,7	41.1	1.12	89.9	0.12	173.6	0.016	204.0	6.32	13.91	0.06	7\2
	Oct 1990	8.6	24.1	1(',1	121	60.0	117	95.0	74.9	48.6	1.21	109.5	0.02	309.6	0.023	195.6	9.84	27.13	0.06	9\4
	Dec 1990	8.1	28.0	N//\	82	1.0	51	42.1	18.7	28.5	0.35	45.4	0.02	45.4	0.021	132.1	9.60	4.71	0.03·	356
	Feb 1991	8.2	28.0	N//\	109	11.0	29	20.2	10.9	24.5	0.44	16.8	0.05	56.0	0.014	82.7	6.58	3.68	0.01	240
6	Apr 1990	8.6	21.0	N//\	N//\	19.5	34	31.0	16.5	23.3	0.31	37.4	0.30	22.3	0.010	116.6	8.29	2.10	0.05	285
	June 1990	8.5	17.0	N//\	N/A·	44.0	48	47.3	24.5	28.8	0.40	52.5	0.07	39.8	0.013	177.9	5.23 ..	2.39	0.04	418
	Aug 1990	8.5	19.4	10.4	117	34.5 .	57	56.8	32.5	26.0	0.32	68.3	0.09	33.3	0.015	193.7	5.92	1.95	0.06	462
	Oct 1990	8.6	22.7	9.2	103	26.5	53	48.6	23.6	26.8	0.33	55.1	0.01	25.3	0.007	182.8	9.21	2.50	0.05	414
	Dec 1990	8.2	26.3	N//\	88	3.0	N//\	36.3	15.9	26.3	0.30	40.4	0.46	20.4	0.008	130.2	9.18	2.36	0.05	303
	Feb 1991	8.4	27.5	N//\	129	17.5	30	N//\	N//\	N//\	N//\	N//\	N//\	N//\	N//\	N//\	N//\	N//\	N//\	NI/\
7	Apr 1990	7.9	23.0	N//\	N//\	47.5	164	121.4	106.9	52.5	2.82	129.8	0.38	564.0	0.014	178.4	10.55	60.17	0.06	1267
	June 1990	7.8	21.0	N//\	N//\	23.0	287	191.3	176.7	96.5	7.70	215.2	1.02	905.7	0.012	250.8	14.36	72.54	0.07	1990
	Aug 1990	7.6	20.7	5.4	64	42.5	244	171.5	192.8	106.9	4.28	210.5	0.49	1005.5	0.111	203.7	16.02	87.87	0.06	2046
	Oct 1990	7.9	21.7	5.9	67	33.5	225	170.3	158.1	82.2	4.34	196.9	0.76	789.0	0.077	232.6	15.15	71.85	0.09	1775
	Dec 1990	7.9	26.5	N//\	64	2.5	190	145.1	119.6	71.0	3.32	176.1	0.70	615.1	0.076	215.3	13.87	62.12	0.19	. 1472
	Feb 1991	7.9	26.7	N//\	71	60.0	239	160.2	179.2	107.1	4.50	197.6	0.98	902.6	0.020	200.1	17.40	82.67	N//\	1528

• EC • Electrical Conductivity N/A - Not available

TABLE 3.1b
PHYSICAL AND CHEMICAL VARIABLES OF SURFACE WATER FROM THE OLIFANTS RIVER, KRUGER NATIONAL PARK (APRIL 1991 - FEBRUARY 1992)

Locality	Month	pH	Temp °C	Dissolved Oxygen mg/l	Oxygen Saturation %	Turbidity cm	* EC @25°C mS/m	Na mg/l	Mg mg/l	Ca mg/l	Fl mg/l	Cl mg/l	NO_3 + NO_2-N mg/l	SO_4 mg/l	PO_4 mg/l	Alkalinity as $CaCO_3$ mg/l	Silica mg/l	K mg/l	NH_4-N mg/l	TDS mg/l
		Sampling times:7:00 - 9:00/ 11:00 - 17:00																		
1	Apr 1991	-/8.4	-/25.7	-/7.2	-/89	-/18	-/22	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	June 1991	-/8.4	-/21.5	-/9.8	-/112	N/A	-/31	22	11	23	0.2	28	0.04	6	0.059	122	9.3	5.5	0.05	243
	Aug 1991	-/8.7	-/23.0	-/11.8	-/140	N/A	-/33	27	14	22	0.2	32	0.04	4	0.021	129	8.1	6.0	0.04	262
	Oct 1991	-/8.5	-/29.2	-/5.4	-/73	N/A	-/49	57	20	21	0.4	66	0.04	6	0.014	170	10.8	11.3	0.06	389
	Jan 1992	-/8.8	-/31.0	-/8.1	-/115	N/A	-/56	47	29	31	0.5	49	0.04	56	0.023	165	9.0	7.3	0.05	421
	Feb 1992	-/8.5	-/30.6	-/8.3	-/112	N/A	-/33	27	10	21	0.3	27	0.66	4	0.036	115	10.3	7.0	0.07	237
2	Apr 1991	-/8.6	-/24.3	-/9.0	-/107	-/37	-/38	32	20	26	0.3	34	0.09	48	0.030	122	7.8	3.9	0.03	314
	June 1991	-/8.8	-/20.4	-/10.7	-/118	-/>49	-/56	41	26	32	0.3	47	0.15	59	0.021	164	7.0	5.0	0.04	409
	Aug 1991	-/8.8	-/24.1	-/10.0	-/124	N/A	-/80	64	44	36	0.8	71	0.04	133	0.019	198	4.9	12.2	0.04	602
	Oct 1991	-/8.6	-/31.3	-/8.7	-/121	N/A	-/118	105	64	44	1.3	125	0.04	220	0.015	241	7.2	20.5	0.06	872
	Jan 1992	-/8.7	-/31.5	-/7.4	-/101	-/22	-/57	65	20	26	0.4	75	0.06	14	0.050	172	9.9	9.9	0.13	420
	Feb 1992	-/8.8	-/32.0	-/10.7	-/139	N/A	-/90	75	48	38	0.8	84	0.04	121	0.024	224	7.2	16.1	0.06	655
3	Apr 1991	8.6/8.7	21.4/24.8	7.6/ 8.7	87/100	29/33	43/ 38	33	21	27	0.4	36	0.23	50	0.020	122	8.1	4.2	0.04	320
	June 1991	8.6/8.5	18.6/20.3	8.6/ 8.6	94/ 94	67/67	55/ 55	42	26	31	0.3	48	0.22	69	0.030	157	6.8	5.1	0.04	412
	Aug 1991	8.7/8.7	19.3/22.0	7.9/ 9.8	93/119	>76/ >76	83/ 82	64	45	36	0.8	73	0.04	139	0.014	191	4.1	11.9	0.04	601
	Oct 1991	8.2/8.5	26.8/33.3	4.8/ 8.2	84/130	>62/ >62	132/132	114	84	49	1.4	135	0.04	298	0.013	254	8.5	24.3	0.62	1016
	Jan 1992	8.5/8.7	28.7/32.4	7.1/ 8.1	93/114	19/23	67/ 68	55	36	36	0.7	57	0.04	96	0.030	166	9.1	9.7	0.06	493
	Feb 1992	8.1/8.5	27.5/31.8	2.4/10.0	32/140	37/37	114/111	92	66	52	1.0	112	0.04	177	0.027	280	10.0	21.0	0.04	862
4	Apr 1991	8.6/8.7	22.2/25.7	8.3/8.7	93/108	36/36	44/ 40	30	20	28	0.3	33	0.24	50	0.020	117	7.8	3.7	0.04	309
	June 1991	8.6/8.6	18.1/20.2	8.7/9.6	95/108	63/63	53/ 54	39	25	32	0.4	45	0.31	60	0.027	156	7.3	5.4	0.04	395
	Aug 1991	9.0/8.9	18.8/22.4	7.7/8.8	91/107	>105/>105	96/ 93	72	54	37	1.1	82	0.04	183	0.010	189	3.4	16.5	0.04	675
	Oct 1991	8.6/8.2	27.2/33.2	6.6/9.7	80/141	>62/ >62	140/141	122	96	49	1.9	140	0.04	343	0.017	236	8.2	30.9	0.05	1068
	Jan 1992	8.6/8.7	27.5/30.6	6.9/8.0	90/112	29/23	66/ 66	53	35	35	1.0	56	0.04	96	0.030	158	8.9	9.6	0.04	477
	Feb 1992	8.2/8.3	29.5/31.5	3.3/6.2	46/ 86	20/21	111/108	89	64	47	1.1	103	0.04	209	0.028	237	9.4	19.4	0.04	821
5	Apr 1991	8.6/8.6	22.2/24.4	7.7/ 8.1	89/ 92	41/41	41/ 39	31	20	25	0.4	36	0.37	53	0.010	113	7.6	3.7	0.04	284
	June 1991	8.6/8.5	15.1/17.7	9.3/10.5	94/113	22/26	56/ 55	41	27	31	0.4	48	0.41	77	0.085	154	7.8	7.0	0.11	420
	Aug 1991	9.1/9.0	18.3/20.7	9.7/10.4	115/128	75/>114	99/ 98	76	57	38	1.2	86	0.06	203	0.027	199	6.4	19.1	0.04	722
	Oct 1991	8.8/8.8	25.0/28.7	9.3/10.2	125/126	76/74	145/145	122	101	56	1.9	145	0.04	389	0.044	222	8.0	34.1	0.04	1117
	Jan 1992	8.8/8.9	28.1/30.4	7.2/ 8.2	95/117	27/30	74/ 76	58	42	35	1.0	60	0.04	145	0.033	147	9.1	14.3	0.07	534
	Feb 1992	8.5/8.6	28.9/31.2	5.4/ 6.9	75/102	17/18	154/153	118	99	62	2.2	132	0.04	467	0.016	222	6.9	39.0	0.04	1187
6	Apr 1991	-/8.5	-/22.8	-/7.9	-/95	-/47	-/34	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	June 1991	-/8.7	-/16.3	-/12.3	-/127	-/40	-/40	33	18	26	0.2	37	0.40	19	0.039	151	7.8	1.5	0.05	319
	Aug 1991	-/8.5	-/17.8	-/10.1	-/114	-/72	-/59	48	31	32	0.5	53	0.06	29	0.011	215	7.7	2.2	0.04	456
	Oct 1991	-/8.5	-/28.2	-/8.1	-/110	-/42	-/72	71	39	33	0.6	81	0.04	45	0.016	225	10.1	3.6	0.04	547
	Jan 1992	-/8.6	-/29.0	-/14.3	-/143	-/30	-/42	36	19	27	0.4	37	0.29	22	0.053	136	9.5	2.7	0.06	311
	Feb 1992	-/8.6	-/30.5	-/7.5	-/103	-/46	-/59	47	30	32	0.6	51	0.04	29	0.015	221	10.1	3.7	0.04	463
7	Apr 1991	7.8/7.8	21.8/23.4	5.0/ 5.8	58/ 75	38/54	244/230	150	179	97	4.2	201	1.60	888	0.020	204	14.9	85.9	N/A	1638
	June 1991	7.6/7.7	18.1/18.9	4.7/ 4.9	54/ 54	112/ 112	224/220	156	165	87	3.4	188	0.39	733	0.439	235	16.0	77.6	0.61	1694
	Aug 1991	8.0/8.0	19.0/20.1	3.9/ 6.3	47/ 77	50/53	231/215	157	165	97	3.8	204	1.13	795	0.045	220	14.0	79.9	0.09	1766
	Oct 1991	7.8/7.8	25.5/27.7	1.8/ 3.5	23/ 46	70/72	234/232	191	182	76	4.1	201	0.38	818	0.016	269	15.0	79.2	0.27	1877
	Jan 1992	7.9/8.0	27.1/29.7	5.8/ 8.2	76/112	67/86	240/233	180	179	75	4.6	203	0.60	821	0.287	235	14.8	78.8	0.22	1824
	Feb 1992	7.9/7.9	27.7/29.5	3.5/ 4.8	49/ 67	57/63	248/252	189	190	98	4.5	219	0.78	969	0.011	246	14.9	81.5	0.15	2048
Pionier Dam	Feb 1992	8.1/8.8	28.2/32.3	4.2/ 9.3	56/133	37/29	84/ 82	120	26	22	0.8	84	0.04	7	0.033	330	6.4	21.8	0.04	683

* EC - Electrical Conductivity N/A - Not available

TABLE 3.2a
CONCENTRATIONS (µg/l) OF SELECTED HEAVY METALS IN THE WATER OF THE OLIFANTS RIVER, KRUGER NATIONAL PARK
(APRIL 1990 - FEBRUARY 1991)

Locality	Month	Chromium	Copper	Iron	Manganese	Nickel	Lead	Strontium	Zinc
1	Apr 1990	190	40	1270	20	210	160	N/A	900
	June 1990	310	60	1880	60	220	210	N/A	1020
	Aug 1990	540	70	1260	60	140	410	N/A	230
	Oct 1990	610	60	1260	50	160	270	80	240
	Dec 1990	120	10	1140	5	80	40	120	20
	Feb 1991	20	20	2230	10	110	90	150	20
2	Apr 1990	210	60	3230	60	230	230	N/A	1740
	June 1990	300	40	1940	60	240	440	N/A	1220
	Aug 1990	620	60	2140	30	140	370	N/A	360
	Oct 1990	800	120	1830	300	130	380	420	970
	Dec 1990	410	60	29760	760	220	60	240	170
	Feb 1991	20	30	2550	40	100	100	340	40
3	Apr 1990	220	70	1510	30	230	190	N/A	1540
	June 1990	530	100	25940	460	340	480	N/A	1220
	Aug 1990	630	30	780	20	160	390	N/A	160
	Oct 1990	810	40	780	200	170	360	450	290
	Dec 1990	1120	180	129240	3500	690	130	420	410
	Feb 1991	120	20	2520	10	80	80	290	20
4	Apr 1990	220	80	8990	180	270	210	N/A	600
	June 1990	560	100	3460	40	360	550	N/A	1420
	Aug 1990	690	20	1140	20	160	380	N/A	260
	Oct 1990	800	40	920	50	190	280	410	410
	Dec 1990	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Feb 1991	270	40	15240	250	150	50	270	90
5	Apr 1990	200	70	1430	220	230	170	N/A	400
	June 1990	720	100	3160	40	420	600	N/A	3840
	Aug 1990	680	30	960	30	190	380	N/A	300
	Oct 1990	360	40	710	20	180	270	240	270
	Dec 1990	250	20	5680	140	130	70	120	30
	Feb 1991	180	30	4020	80	120	80	250	40
6	Apr 1990	220	50	2380	40	240	210	N/A	1970
	June 1990	750	80	2880	50	380	640	N/A	1180
	Aug 1990	590	10	780	20	120	310	N/A	100
	Oct 1990	470	30	1050	30	170	270	120	260
	Dec 1990	850	120	103900	2370	500	130	480	230
	Feb 1991	230	40	2850	16500	160	90	180	210
7	Apr 1990	230	120	1260	120	250	230	N/A	1450
	June 1990	810	160	14420	160	580	840	N/A	3600
	Aug 1990	980	60	400	80	210	440	N/A	340
	Oct 1990	620	40	1060	90	160	320	780	440
	Dec 1990	60	130	740	50	50	60	2510	30
	Feb 1991	170	70	2780	240	120	150	3870	40

N/A - Not available

TABLE 3.2b
CONCENTRATIONS (µg/l) OF SELECTED HEAVY METALS IN THE WATER OF THE OLIFANTS RIVER, KRUGER NATIONAL PARK
(APRIL 1991 - FEBRUARY 1992)

Locality	Month	Chromium	Copper	Iron	Manganese	Nickel	Lead	Strontium	Zinc
1	Apr 1991	190	90	8570	100	100	140	200	110
	June 1991	170	40	3240	70	100	50	190	40
	Aug 1991	152	10	1680	30	120	120	N/A	4
	Oct 1991	<6*	63	310	39	<10*	157	110	457
	Jan 1992	<6	36	450	26	<10	160	620	94
	Feb 1992	358	56	7780	87	30	139	N/A	340
2	Apr 1991	220	30	3050	20	90	120	380	80
	June 1991	100	30	1100	20	50	40	240	100
	*Aug 1991	28	10	1500	21	140	130	N/A	1
	Oct 1991	6	24	1110	52	<10	94	490	27
	Jan 1992	63	88	720	103	<10	97	300	838
	Feb 1992	37	36	3050	62	20	169	N/A	45
3	Apr 1991	210	40	4840	60	90	170	280	110
	June 1991	210	60	2020	30	120	80	330	240
	Aug 1991	125	20	2720	80	80	40	N/A	292
	Oct 1991	<6	35	360	60	<10	185	860	84
	Jan 1992	69	32	1730	82	<10	120	390	162
	Feb 1992	62	48	96600	736	40	184	N/A	43
4	Apr 1991	200	30	2070	50	80	150	210	160
	June 1991	30	30	350	<2*	20	10	30	230
	Aug 1991	28	10	1270	<2	140	160	N/A	24
	Oct 1991	<6	18	170	43	<10	162	840	65
	Jan 1992	7	20	140	18	<10	164	930	79
	Feb 1992	218	44	44000	335	20	153	N/A	70
5	Apr 1991	110	30	1240	30	50	100	200	30
	June 1991	200	40	1230	20	110	90	270	90
	Aug 1991	42	20	2320	21	100	100	N/A	28
	Oct 1991	18	20	150	74	40	154	495	36
	Jan 1992	16	13	20	9	<10	81	570	49
	Feb 1992	53	46	88800	588	40	186	N/A	37
6	Apr 1991	210	30	3130	80	100	120	210	130
	June 1991	170	40	1920	30	80	100	150	20
	Aug 1991	114	10	1270	49	110	70	N/A	14
	Oct 1991	<6	32	230	3	<10	157	80	50
	Jan 1992	18	13	190	22	<10	72	130	8
	Feb 1992	54	46	65400	419	30	95	N/A	75
7	Apr 1991	170	40	1390	110	70	130	2970	40
	June 1991	140	50	1850	740	90	80	3700	30
	Aug 1991	49	30	1510	127	150	210	N/A	25
	Oct 1991	<6	18	320	319	<10	179	2060	50
	Jan 1992	<6	31	780	231	<10	178	2190	83
	Feb 1992	99	47	4610	228	50	158	N/A	36
Pionier Dam	Feb 1992	53	53	1710	43	30	74	N/A	57

* Detection limit of AAS N/A - Not available

TABLE 3.3a
CONCENTRATIONS (µg/g) OF SELECTED HEAVY METALS IN THE SEDIMENT OF THE OLIFANTS RIVER, KRUGER NATIONAL PARK
(APRIL 1990 - FEBRUARY 1991)

Locality	Month	Chromium	Copper	Iron	Manganese	Nickel	Lead	Strontium	Zinc
1	Apr 1990	55.5	12.0	18150	151.5	38.0	25.7	N/A	22.5
	June 1990	51.0	11.0	10241	126.0	38.0	12.0	N/A	17.0
	Aug 1990	44.0	9.0	N/A	55.0	19.0	18.0	9.0	74.0
	Oct 1990	46.0	13.0	N/A	66.0	27.0	16.0	19.0	25.0
	Dec 1990	40.0	6.5	2460	42.8	17.5	45.5	6.0	1248.0
	Feb 1991	33.0	8.0	9436	85.5	18.8	7.5	15.0	105.0
2	Apr 1990	71.5	14.5	32200	181.0	41.0	41.0	N/A	54.5
	June 1990	74.0	13.0	18130	162.0	57.0	23.0	N/A	37.0
	Aug 1990	64.0	15.0	N/A	106.0	35.0	17.0	33.0	30.0
	Oct 1990	56.0	19.0	N/A	96.0	37.0	20.0	35.0	25.0
	Dec 1990	38.6	11.5	8664	142.4	25.3	10.0	24.5	15.5
	Feb 1991	17.2	8.5	3495	73.2	27.2	48.0	6.0	10.5
3	Apr 1990	389.0	39.5	49200	551.7	151.5	38.0	N/A	70.5
	June 1990	170.0	33.0	19010	305.0	94.0	30.0	N/A	91.0
	Aug 1990	93.0	38.0	N/A	253.0	72.0	25.0	88.0	53.0
	Oct 1990	54.0	11.0	N/A	110.0	29.0	15.0	32.0	30.0
	Dec 1990	30.3	19.0	2508	116.5	46.6	53.0	11.0	17.5
	Feb 1991	48.1	27.0	15120	207.3	60.4	31.0	13.5	33.5
4	Apr 1990	36.0	7.5	14800	151.5	43.5	35.5	N/A	27.5
	June 1990	86.0	8.0	18246	157.0	47.0	30.0	N/A	44.0
	Aug 1990	131.0	20.0	N/A	220.0	55.0	25.0	59.0	48.0
	Oct 1990	100.0	20.0	N/A	242.0	54.0	29.0	32.0	56.0
	Dec 1990	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Feb 1991	32.5	9.5	13921	28.3	16.5	10.5	4.0	14.5
5	Apr 1990	100.5	25.5	30050	346.0	83.5	46.0	N/A	34.5
	June 1990	910.0	21.0	19835	422.0	100.0	34.0	N/A	101.0
	Aug 1990	175.0	35.0	N/A	428.0	98.0	27.0	100.0	47.0
	Oct 1990	82.0	13.0	N/A	110.0	39.0	21.0	69.0	29.0
	Dec 1990	81.5	18.5	8835	243.0	53.7	11.0	38.0	23.5
	Feb 1991	87.7	18.5	29400	251.7	53.2	10.5	39.0	25.0
6	Apr 1990	40.5	6.5	50876	189.5	45.0	36.5	N/A	84.0
	June 1990	838.0	24.0	19352	399.0	92.0	23.0	N/A	65.0
	Aug 1990	86.0	15.0	N/A	210.0	43.0	25.0	43.0	43.0
	Oct 1990	85.0	12.0	N/A	194.0	36.0	22.0	41.0	42.0
	Dec 1990	69.1	13.0	3456	254.6	40.8	11.5	34.5	18.0
	Feb 1991	80.2	14.0	22592	253.2	45.1	10.0	36.5	18.0
7	Apr 1990	157.0	441.0	39650	422.0	113.5	62.5	N/A	105.2
	June 1990	173.0	237.0	12043	124.0	78.0	36.0	N/A	37.0
	Aug 1990	196.0	467.0	N/A	430.0	156.0	34.0	275.0	60.0
	Oct 1990	120.0	33.0	N/A	149.0	60.0	19.0	45.0	32.0
	Dec 1990	77.1	40.0	6075	89.5	36.5	7.5	79.5	10.5
	Feb 1991	35.0	37.5	8575	71.3	27.2	15.5	56.0	8.5

N/A - Not available

TABLE 3.3b
CONCENTRATIONS (µg/g) OF SELECTED HEAVY METALS IN THE SEDIMENT OF THE OLIFANTS RIVER, KRUGER NATIONAL PARK
(APRIL 1991 - FEBRUARY 1992)

Locality	Month	Chromium	Copper	Iron	Manganese	Nickel	Lead	Strontium	Zinc
1	Apr 1991	22.2	6.5	5737	41.4	10.2	6.0	7.5	5.5
	June 1991	33.7	9.0	9724	78.1	27.6	8.0	14.5	9.5
	Aug 1991	25.2	6.0	5992	36.9	16.5	6.5	11.5	4.5
	Oct 1991	13.0	8.7	6025	65.8	4.5	3.0	10.5	8.5
	Jan 1992	37.8	15.2	14937	133.2	21.0	2.7	24.0	15.3
	Feb 1992	16.1	16.2	10655	159.2	20.5	14.5	N/A	47.4
2	Apr 1991	28.5	6.5	11583	95.7	18.4	8.0	19.5	8.5
	June 1991	38.3	10.0	17314	143.2	26.4	11.0	29.0	14.0
	Aug 1991	96.6	10.5	33285	189.4	45.3	15.0	28.0	19.0
	Oct 1991	32.8	11.7	20745	171.9	21.0	1.4	30.5	23.6
	Jan 1992	19.9	17.2	14880	262.0	20.5	2.0	19.0	23.1
	Feb 1992	26.8	12.4	20538	182.5	28.0	14.0	N/A	32.8
3	Apr 1991	506.9	38.0	65331	505.4	84.6	34.0	83.0	51.0
	June 1991	30.2	69.5	2034	1078.2	83.6	14.5	61.0	59.0
	Aug 1991	60.4	21.5	35681	208.4	55.4	10.5	36.0	38.0
	Oct 1991	25.7	8.9	18525	153.8	16.5	2.2	16.0	19.0
	Jan 1992	55.4	25.0	19267	45.4	41.5	3.9	61.0	32.0
	Feb 1992	12.0	11.0	9380	116.2	15.1	11.8	N/A	38.2
4	Apr 1991	49.3	12.5	17892	246.6	47.0	13.0	40.5	14.5
	June 1991	53.9	11.5	37634	172.2	39.5	12.0	25.0	20.5
	Aug 1991	1057.8	23.0	40982	381.4	75.4	24.0	46.0	43.0
	Oct 1991	32.0	12.0	20400	167.7	17.5	1.6	25.5	18.3
	Jan 1992	114.3	39.0	25434	109.1	81.5	5.1	62.5	66.3
	Feb 1992	71.4	19.5	24000	267.5	46.0	15.5	N/A	51.2
5	Apr 1991	24.1	5.5	8223	119.2	18.2	9.0	34.0	6.5
	June 1991	63.6	14.5	19688	261.9	57.6	16.0	49.5	14.5
	Aug 1991	92.7	20.5	21116	261.0	67.1	14.0	45.5	17.5
	Oct 1991	48.5	32.8	14610	285.8	38.0	2.0	62.0	27.2
	Jan 1992	56.1	16.9	16650	56.8	44.5	2.0	64.5	27.1
	Feb 1992	216.0	53.6	9480	535.5	125.2	23.1	N/A	8.1
6	Apr 1991	76.0	9.5	14157	118.3	44.0	12.0	41.0	11.5
	June 1991	1457.4	10.0	44919	359.1	45.9	24.0	63.0	39.0
	Aug 1991	50.1	10.0	166	297.6	37.6	11.5	32.5	16.0
	Oct 1991	77.7	18.5	18090	475.8	34.0	2.6	45.0	27.3
	Jan 1992	80.3	2.3	21528	63.2	49.5	4.9	30.5	19.4
	Feb 1992	182.6	69.2	17670	333.0	111.7	53.5	N/A	105.8
7	Apr 1991	54.0	265.0	15131	334.2	49.2	14.5	238.0	18.0
	June 1991	47.3	48.0	8652	598.2	26.0	7.0	68.0	7.5
	Aug 1991	47.1	80.5	9335	129.5	35.2	11.5	78.0	22.0
	Oct 1991	78.2	19.1	19770	303.5	44.0	1.7	53.0	21.9
	Jan 1992	61.9	700.5	25992	131.4	62.0	11.0	618.0	44.0
	Feb 1992	121.5	105.7	14350	119.9	43.9	11.5	N/A	37.5
Pionier Dam	Feb 1992	16.9	14.0	19785	53.7	23.8	9.3	N/A	41.4

N/A - Not available

TABLE 3.4a

MEAN VALUES (±SE*) OF SELECTED VARIABLES FROM THE OLIFANTS RIVER (APR 1990 - FEB 1991) COMPARED TO GUIDELINE VALUES BY KEMPSTER *et al.* (1982), KüHN (1991) AND CANADA (1987)

Variable	Locality							Guideline values			
	1	2	3	4	5	6	7	Kempster *et al.*		Kühn	Canada
								(min-max)	median		
Water											
ΘpH	8.3±0.3	8.4±0.3	8.3±0.4	8.6±0.3	8.3±0.03	8.4±0.2	7.8±0.1	6.0-9.0	6.5-9.0		6.5-9.0
Temperature (°C)	24.3±4.1	24.5±4.3	23.1±3.7	23.1±4.2	24.1±3.4	22.3±3.7	23.3±2.5		a	b	
Dissolved O_2 (mg/l)	9.2±2.1	8.2±1.8	8.9±2.1	10.4±1.1	12.0±1.9	9.8±0.6	5.7±0.3	>4->5.8	>5		>5
O_2 saturation (%)	114.5±17.8	105.8±19.7	111.3±25.6	119.3±14.8	115.5±24.4	109.3±15.3	66.5±2.9				
Turbidity (cm)	19.4±7.4	18.6±21.6	27.8±20.4	37.4±24.4	33.6±25.7	24.2±13.0	34.8±18.4				
Conductivity (mS/m)	32.5±9.3	61.8±23.7	95.0±64.2	64.0±27.5	69.0±29.9	44.4±10.6	224.8±39.5		a		
Na (mg/l)	N/A	48.8±18.8	49.8±19.9	45.2±22.7	54.8±25.2	44.0±9.2	160.0±22.1		500	100	
Mg (mg/l)	N/A	35.6±16.8	35.8±17.9	34.8±18.4	36.2±22.9	22.6±6.1	155.6±31.9		1500		
Ca (mg/l)	N/A	33.8±7.8	33.6±7.5	35.7±8.5	33.9±8.4	26.2±1.8	86.0±19.8		1000		
F (mg/l)	N/A	0.81±0.34	0.74±0.31	0.69±0.32	0.70±0.34	0.33±0.04	4.49±1.56	1.5-1.5	1.5	1.5	
Cl (mg/l)	N/A	57.1±22.9	60.4±24.5	53.0±29.1	63.2±31.7	50.7±11.1	187.7±28.7	50-400		100	
NO_3+NO_2-N (mg/l)	N/A	0.05±0.07	0.06±0.08	0.25±0.43	0.10±0.09	0.19±0.17	0.72±0.23			c 6	f 0.06
SO_4 (mg/l)	N/A	115.0±54.4	117.7±52.9	108.2±82.4	129.6±92.9	28.2±7.3	797.0±160.2		1400	250	
PO_4-P (mg/l)	N/A	0.009±0.002	0.012±0.007	0.018±0.010	0.016±0.004	0.011±0.003	0.052±0.038		0.1		
Alkalinity($CaCO_3$) (mg/l)	N/A	142.7±48.0	151.1±41.1	154.3±40.4	152.0±43.0	160.2±30.8	213.5±23.3	>20->20	>20		
Silica (mg/l)	N/A	6.18±1.10	6.29±1.09	6.46±1.78	7.53±1.72	7.57±1.67	14.56±2.13		50		
K (mg/l)	N/A	9.90±5.36	10.31±5.26	9.66±6.68	10.83±8.15	2.26±0.20	72.87±9.99		50	50	
NH_4-N (mg/l)	N/A	0.03±0.01	0.03±0.01	0.05±0.03	0.04±0.02	0.05±0.01	0.09±0.05	0.016-124	0.016	d 0.01+	d/g 1.37-2.2
TDS (mg/l)	N/A	491.8±179.7	499.4±180.1	483.0±190.1	526.3±234.0	376.4±69.6	1679.7±281.7			800	
Chromium (µg/l)	298.3±214.7	393.3±257.8	571.7±339.2	508.0±228.3	398.3±221.1	518.3±239.2	478.3±344.8	10-100	50		2
Copper (µg/l)	43.3±22.1	61.7±28.5	73.3±54.7	56.0±29.4	48.3±27.9	55.0±35.9	96.7±42.7	5-200	5	50	h 2-4
Iron (µg/l)	1506.7±403.1	6908.3±10230.1	26795.0±46686.7	5950.0±5480.6	2660.0±1799.3	18973.3±37989.2	3443.3±4965.6	200-1000	200	300	300
Manganese (µg/l)	34.2±23.2	208.3±263.6	703.3±1260.6	108.0±90.6	88.3±71.3	3168.3±6022.8	123.3±62.4	100-1000		50	
Nickel (µg/l)	153.3±50.2	176.7±55.0	278.3±200.3	226.0±79.2	211.7±100.2	261.7±135.5	228.3±169.7	25-50	50	50	h 25-150
Lead (µg/l)	196.7±121.3	263.3±144.5	271.7±146.5	294.0±167.2	261.7±185.6	275.0±179.8	340.0±254.0	20-100	30	2	h 1-7
Strontium (µg/l)	116.7±28.7	333.3±73.6	386.7±69.4	340.0±70.0	203.3±59.1	260.0±157.5	2386.7±1264.5		200000	e 10000	
Zinc (µg/l)	405.0±403.6	750.0±611.3	606.7±567.2	556.0±463.6	813.3±1360.3	658.3±688.9	983.3±1263.1	30-100	100	50	30
Sediment											
Chromium (µg/g)	44.9±7.3	53.5±20.0	130.7±124.1	77.1±37.9	239.4±301.6	199.8±285.8	126.3±55.9				
Copper (µg/g)	9.9±2.3	13.6±3.2	27.9±10.2	13.0±5.8	21.9±6.9	14.1±5.2	209.2±187.1				
Iron (µg/g)	10071.7±5559.4	15622.2±10915.6	21459.5±17138.3	15655.7±1866.5	22030.0±8625.0	24069.0±17087.6	16585.7±13483.7				
Manganese (µg/g)	87.8±38.9	126.8±38.0	257.2±148.9	159.8±74.5	300.1±111.9	250.0±71.4	214.3±151.7				
Nickel (µg/g)	26.4±8.8	37.1±10.4	75.6±39.5	43.2±14.0	71.2±23.7	50.3±18.9	78.5±44.7				
Lead (µg/g)	20.8±12.4	26.5±13.5	32.0±11.7	26.0±8.4	24.9±12.6	21.3±8.9	29.1±18.0				
Strontium (µg/g)	12.2±5.1	24.6±11.5	36.1±31.0	31.7±22.5	61.5±25.5	38.8±3.4	113.9±93.9				
Zinc (µg/g)	248.6±448.1	28.8±14.5	49.2±25.3	38.0±15.0	43.3±26.9	45.0±23.8	42.2±33.1				

* Standard Error Θ -log [H^+] N/A - Not available a Depend on local conditions and life species present b Within 5°C of background temperature (99.9% of the time)
c Nitrate d Depend on pH, [Ca^{2+}] and DO e ^{90}Sr f Nitrite g Ammonia h Dependent on hardness

TABLE 3.4b

MEAN VALUES (±SE*) OF SELECTED VARIABLES FROM THE OLIFANTS RIVER (APR 1991 - FEB 1992) COMPARED TO GUIDELINE VALUES BY KEMPSTER *et al.* (1982), KüHN (1991) AND CANADA (1987)

Variable	Locality								Guideline values			
	1	2	3	4	5	6	7	•Pionier Dam	Kempster *et al.*		Kühn	Canada
									(min-max)	median		
Water												
Θ pH	8.5±0.2	8.7±0.1	8.6±0.1	8.5±0.3	8.7±0.2	8.6±0.1	7.9±0.1	8.1	6.0-9.0	6.5-9.0		6.5-9.0
Temperature (°C)	26.8±3.7	27.3±4.5	27.4±5.3	27.3±4.8	25.5±5.0	24.1±5.5	24.9±4.4	32.3		[a]	[b]	
Dissolved O_2 (mg/l)	8.4±2.0	9.4±1.2	8.9±0.7	8.5±1.2	9.1±1.4	10.0±2.5	5.6±1.5	9.3	>4->5.8	>5		>5
O_2 saturation (%)	106.8±21.2	118.3±12.2	116.2±15.9	110.3±16.1	113.0±12.7	115.3±15.8	71.8±21.1	133				
Turbidity (cm)	18.0±0.0	36.0±11.1	49.7±19.6	51.7±29.1	50.5±33.5	46.2±12.8	73.3±20.6	29				
Conductivity (mS/m)	37.3±11.5	73.2±26.3	81.0±32.1	83.7±34.3	94.3±42.8	51.0±13.3	230.3±11.7	82		[a]		
Na (mg/l)	36.0±13.6	63.7±23.7	66.7±28.2	67.5±31.3	74.3±35.2	47.0±13.4	170.5±16.7	120		500	100	
Mg (mg/l)	16.8±7.0	37.0±16.3	46.3±22.3	49.0±26.1	57.7±32.1	27.4±7.9	176.7±9.0	26		1500		
Ca (mg/l)	23.6±3.8	33.7±6.5	38.5±9.1	38.0±7.6	41.2±13.3	30.0±2.9	88.3±9.8	22		1000		
F (mg/l)	0.32±0.12	0.65±0.36	0.77±0.37	0.97±0.53	1.18±0.68	0.46±0.15	4.10±0.41	0.8	1.5-1.5	1.5	1.5	
Cl (mg/l)	40.4±15.1	72.7±29.0	76.8±35.4	76.5±36.7	84.5±41.2	51.8±16.1	202.7±9.0	84	50-400		100	
NO_3+NO_2-N (mg/l)	0.16±0.25	0.07±0.04	0.10±0.09	0.12±0.11	0.16±0.16	0.17±0.15	0.81±0.43	0.04			[c] 6	[f] 0.06
SO_4 (mg/l)	15.2±20.4	99.2±68.0	138.2±83.1	156.8±102.1	222.3±154.8	28.8±9.0	837.3±74.4	7		1400	250	
PO_4-P (mg/l)	0.031±0.016	0.026±0.011	0.022±0.007	0.022±0.007	0.036±0.025	0.027±0.016	0.136±0.167	0.033		0.1		
Alkalinity($CaCO_3$) (mg/l)	140.2±22.8	186.8±39.6	195.0±55.3	182.2±43.7	176.2±40.9	189.6±38.1	234.8±20.3	330	>20->20	>20		
Silica (mg/l)	9.50±0.96	7.33±1.46	7.77±1.91	7.50±1.96	7.63±0.85	9.04±1.08	14.93±0.58	6.4		50		
K (mg/l)	7.42±2.05	11.27±5.84	12.70±7.56	14.25±9.31	19.53±13.08	2.74±0.84	80.48±2.69	21.8		50	50	
NH_4-N (mg/l)	0.05±0.01	0.06±0.03	0.14±0.21	0.04±0.00	0.06±0.03	0.05±0.01	0.27±0.18	0.04	0.016-124	0.016	[d] 0.01+	[d/g] 1.37-2.2
TDS (mg/l)	310.4±78.3	545.3±187.0	617.3±246.7	624.2±262.0	710.7±339.1	419.2±90.9	1807.8±133.1	683			800	
Chromium (µg/l)	147.0±120.2	75.7±71.0	113.7±76.3	81.5±90.8	73.2±64.7	95.3±76.1	78.3±63.2	53	10-100	50		2
Copper (µg/l)	49.2±24.8	36.3±24.5	39.2±12.6	25.3±10.9	28.2±11.7	28.5±13.1	36.0±10.9	53	5-200	5	50	[h] 2-4
Iron (µg/l)	3671.7±3334.3	1755.0±943.0	18045.0±35156.5	8000.0±16114.5	15626.7±32733.1	12023.3±23892.1	1743.3±1376.1	1710	200-1000	200	300	300
Manganese (µg/l)	58.7±28.6	46.3±30.3	174.7±251.6	75.0±117.7	123.7±208.7	100.5±144.4	292.5±212.0	43	100-1000		50	
Nickel (µg/l)	61.7±46.0	53.3±47.8	58.3±41.4	46.7±48.2	58.3±35.3	56.7±41.5	63.3±48.5	30	25-50	50	50	[h] 25-150
Lead (µg/l)	127.7±37.1	108.3±39.4	129.8±55.1	133.2±55.3	118.5±38.1	102.3±29.8	155.8±41.6	74	20-100	30	2	[h] 1-7
Strontium (µg/l)	280.0±199.4	352.5±93.6	465.0±231.4	502.5±389.1	383.8±153.1	142.5±46.6	2730.0±659.4	n/a		200000	[e] 10000	
Zinc (µg/l)	174.2±165.8	181.8±295.3	155.2±87.2	104.7±69.2	45.0±21.2	49.5±42.8	44.0±19.1	57	30-100	100	50	30
Sediment												
Chromium (µg/g)	24.7±8.9	40.5±25.7	115.1±176.0	229.8±371.2	83.5±62.6	320.7±510.1	68.3±26.0	16.9				
Copper (µg/g)	10.3±4.0	11.4±3.2	29.0±20.5	19.6±9.7	24.0±15.5	19.9±22.5	203.1±235.8	14.0				
Iron (µg/g)	8845.0±3339.5	19724.2±6845.4	25036.3±20764.1	27723.7±8596.6	14961.2±4807.1	19421.7±13278.4	15538.3±5981.5	19785.0				
Manganese (µg/g)	85.8±45.6	174.1±50.2	351.2±356.0	224.1±87.8	253.4±151.3	274.5±141.8	269.5±170.5	53.7				
Nickel (µg/g)	16.7±7.6	26.6±9.0	49.5±28.2	51.2±21.7	58.4±33.6	53.8±26.4	43.4±11.2	23.8				
Lead (µg/g)	6.8±3.9	8.6±5.4	12.8±10.4	11.9±7.2	11.0±7.6	18.1±17.2	9.5±4.1	9.3				
Strontium (µg/g)	13.6±5.7	25.2±4.9	51.4±23.1	39.9±14.0	51.1±11.2	42.4±11.6	211.0±214.2	n/a				
Zinc (µg/g)	15.1±14.9	20.2±7.7	39.5±12.9	35.6±19.2	16.8±8.2	36.5±32.2	25.2±12.2	41.4				

* Standard Error • Only one value available Θ -log [H^+] N/A - Not available [a] Depend on local conditions and life species present [b] Within 5°C of back ground temperature (99.9% of the time)
[c] Nitrate [d] Depend on pH, [Ca^{2+}] and DO [e] ^{90}Sr [f] Nitrite [g] Ammonia [h] Dependent on hardness

3.4 Discussion

In evaluating the water quality of the study area, three sets of guidelines were used: those of Kempster *et al.* (1982), those proposed by Kühn (1991) specifically for the Olifants River and the Canadian guidelines (Environment Canada, 1987). According to these guidelines, there were chemical constituents in the water of the study area that exceeded the guideline limits (Table 3.4), especially in the Selati River (a tributary of the Olifants River). Variables of special concern are sodium, fluoride, chloride, sulphate, potassium, the total dissolved salts and the metal concentrations (except strontium). This situation would render the Selati River at locality 7 unfit for aquatic life and might be one of the reasons why *Barbus marequensis,* the fish species used in this study, was only occasionally captured there. Furthermore, the Selati River has a negative influence on the water quality of the Olifants River after their confluence. The concentrations of most parameters detected at localities 2 to 5 were higher than the concentrations detected at locality 6 (located before the Selati-Olifants confluence). In most cases (except for the metal concentrations), the concentrations of the variables decreased from the western side of the KNP to the eastern side. This phenomenon can be attributed to the dilution of the water, caused by the tributaries of the Olifants River. At locality 3 (near Balule) an increase in concentration could sometimes be detected, especially during the first year. The explanation for this might lie in the frequent occurrence of reed beds in that part of the river. Reed beds are known for their cumulative capacity of chemical substances or toxicants (like metals), but during a flood reeds may get deposited on the bottom of the river, from where the toxicants (the metals) may eventually be released again into the river water during the decay process (De Wet *et al.*, 1990). The toxicant concentration in the river water will therefore increase again.

The mean sodium, fluoride, sulphate, chloride, potassium and total dissolved salt concentrations detected at Mamba during April 1990 to February 1991 were compared to the mean concentrations detected in the previous six years (October 1983 - October 1989) and a decrease in concentrations was found. On the other hand, a slight increase in the mean concentrations was detected during April 1991 to February 1992 when compared to the existing six-year record of Van Veelen (1990). The most probable explanation for the decrease and increase of the mean concentrations in the first and second year respectively, is the difference in rainfall pattern of the two years. In the first year the floods contributed to the dilution of the chemical constituent concentrations, but because of the drought in the second year, no dilution could take place and the concentrations have therefore increased.

The TDS (total dissolved salts) concentration gives an indication of the degree of salinity of a water sample. It can be calculated by the summing of the cation and anion concentrations (in mg/l) which are being analysed. Because of the electrical conductivity (EC) exerted by the dissolved salts, the following rule-of-thumb relationship exists between the TDS and the EC: EC (mS/m) x 6.5 $\approx$ TDS (mg/l). The exact conversion factor depends, however, on the composition of the water, especially the pH and the bicarbonate content (Kempster *et al.*, 1982).

Conductivity has an influence on the growth rate and life expectancy of fish, depending on the species sensitivity and conductivity level present (Hellawell, 1986). The effects that TDS concentrations have on aquatic species are, however, due to sudden changes in the concentrations, rather than absolute values of the determinants. Some macrophytes sensitive to changes will, for instance, be replaced by less sensitive species at high TDS concentrations of 1500 - 3000 mg/l (Theron *et al.*, 1991). Such high concentrations were detected at locality 7 (1679.7 $\pm$ 281.7 mg/l and 1807.8 $\pm$ 133.1 mg/l for years 1 and 2 respectively), exceeding the guideline limits of 800 mg/l (Kühn, 1991) and 350 - 550 mg/l TDS (Department of Water Affairs, 1986) by far. Therefore the macrophyte species status in the Selati River needs further investigation.

At Pionier Dam, a fairly high TDS concentration of 683 mg/l was recorded, which is higher than the recommended limit of 350 - 550 mg/l TDS (Department of Water Affairs, 1986). One of the reasons might be evaporation, leading to increased concentrations of dissolved mineral salts (Department of Water Affairs, 1986). The ionic composition seemed to be dominated by sodium, chloride, potassium,

carbonate and bicarbonate. The mean TDS concentrations at localities 2 to 5 ranged from 545.3 ± 187.0 mg/l to 710.7 ± 339.1 mg/l in the second year (April 1991 - February 1992), which were slightly higher than the TDS concentrations recorded for 1983 to 1989 in the Olifants River (Van Veelen, 1990). As already mentioned, this increase can be attributed to the fact that April 1991 to February 1992 was a very dry period. During dry periods, which is also the case in winter time, the lower flows recorded at the barrage, combined with the almost continuous effluent flow in the Selati River, result in poorer water quality in the Lower Olifants River (CSIR, 1990). The major sources responsible for the high TDS concentrations are the effluents (1660 mg/l) and seepage (1660 mg/l) from a phosphorus extraction mining company (CSIR, 1990). Moderate TDS loads are contributed by the storm water overflow of a copper extraction mining company *via* Loole Creek (1250 mg/l) and seepage from a magnetite tailing dam (1200 mg/l). Upstream inflow also contributes heavily to the daily TDS load in the Lower Selati River (1280 mg/l).

Sulphate is the anionic component mainly responsible for the high TDS concentrations in the Olifants River (Moore *et al.*, 1991). The data presented in Figure 3.3 illustrates the relationship between the TDS and sulphate concentrations. The sulphate concentrations recorded at locality 7 exceeded one of the proposed guideline values, namely 250 mg/l by Kühn (1991). As the concentrations were above 600 mg/l, the water should be considered unfit for household purposes. Sulphates may give rise to gastro-intestinal irritation (Department of Water Affairs, 1986). The mean sulphate concentrations at localities 1 to 5 were fortunately well below 600 mg/l, for the main use of the Lower Olifants River after entering the KNP is for game watering, aquatic ecosystem maintenance and the supply of domestic water to the Olifants, Satara and Balule rest camps. Further downstream, the Massingir Dam inside Mozambique also supplies some water for domestic use and game watering (CSIR, 1990). High sulphate concentrations have a definite effect on fish (Burnham & Peterka, 1975). The increased mortality of fathead minnows (*Pimephales promelas*) was attributed to water being high in sodium and sulphate concentrations. This might be one of the reasons why only a few fish species were detected in the Lower Selati River.

The mean fluoride concentrations at locality 7 (4.49 ± 1.56 mg/l and 4.10 ± 0.41 mg/l for years 1 and 2 respectively) were much higher than the concentrations recorded at the other localities and exceeded the limit of 1.5 mg/l. Studies on the ecological significance of exposure of aquatic animals to fluoride are limited (Rose & Marier, 1977). However, when fry of *Catla catla* were exposed to different fluoride concentrations for 96 hours, protein synthesis was inhibited from 1.2 ppm fluoride upwards, glycogen and iron decreased from 4.3 ppm fluoride upwards and the lipid metabolism was altered from 7.2 ppm fluoride upwards (Pillai & Mane, 1984). Fluoride toxicity is influenced, however, by water hardness. High calcium concentrations suppress fluoride concentrations by precipitating insoluble calcium fluoride (Smith *et al.*, 1985). LC50 values (96-hour) for fluoride toxicity do exist, ranging from 51 to 460 mg/l - depending on the species and conditions (Smith *et al.*, 1985). However, the available data suggests that a consensus about the maximum safe level of fluoride concentration for fish in natural waters of varying hardness has not yet been achieved.

Chlorine (a gas) is a highly toxic substance and is more toxic than the chloride ion. Chlorine gas forms hypochlorous acid (HOCl) or its conjugated base (OCl^-) in water, which are commonly called "free chlorine" (Heath, 1987). In the presence of ammonia, some or all of the free chlorine is converted into monochloramine (NH_2Cl) which is known as "combined chlorine". Free chlorine is more toxic, but combined chlorine is more stable and therefore remains active longer (Heath, 1987). The toxicity of chlorine depends on the total amount of chlorine present whether complexed or not (Merkens, 1958). Chlorine causes the epithelium of fish gills to slough off, which leads to mucus production and the eventual clogging of the gill lamellae (Cairns *et al.*, 1975).

However, chlorides occur in all natural soil and water. As salinity increases, the chloride concentrations also increase (Hahne & Kroontje, 1973). At all the localities the chloride concentrations were above 35 mg/l (ppm), which means that the MCl^+ species of Zn(II), Cd(II) and Pb(II) will then appear (Hahne & Kroontje, 1973). However, at a pH of 8.5 (which is the case at some localities), competition between the hydroxyl and chloride complexes will arise, depending on the chloride concentrations. Therefore, in order to determine exact distributions of metals, all other

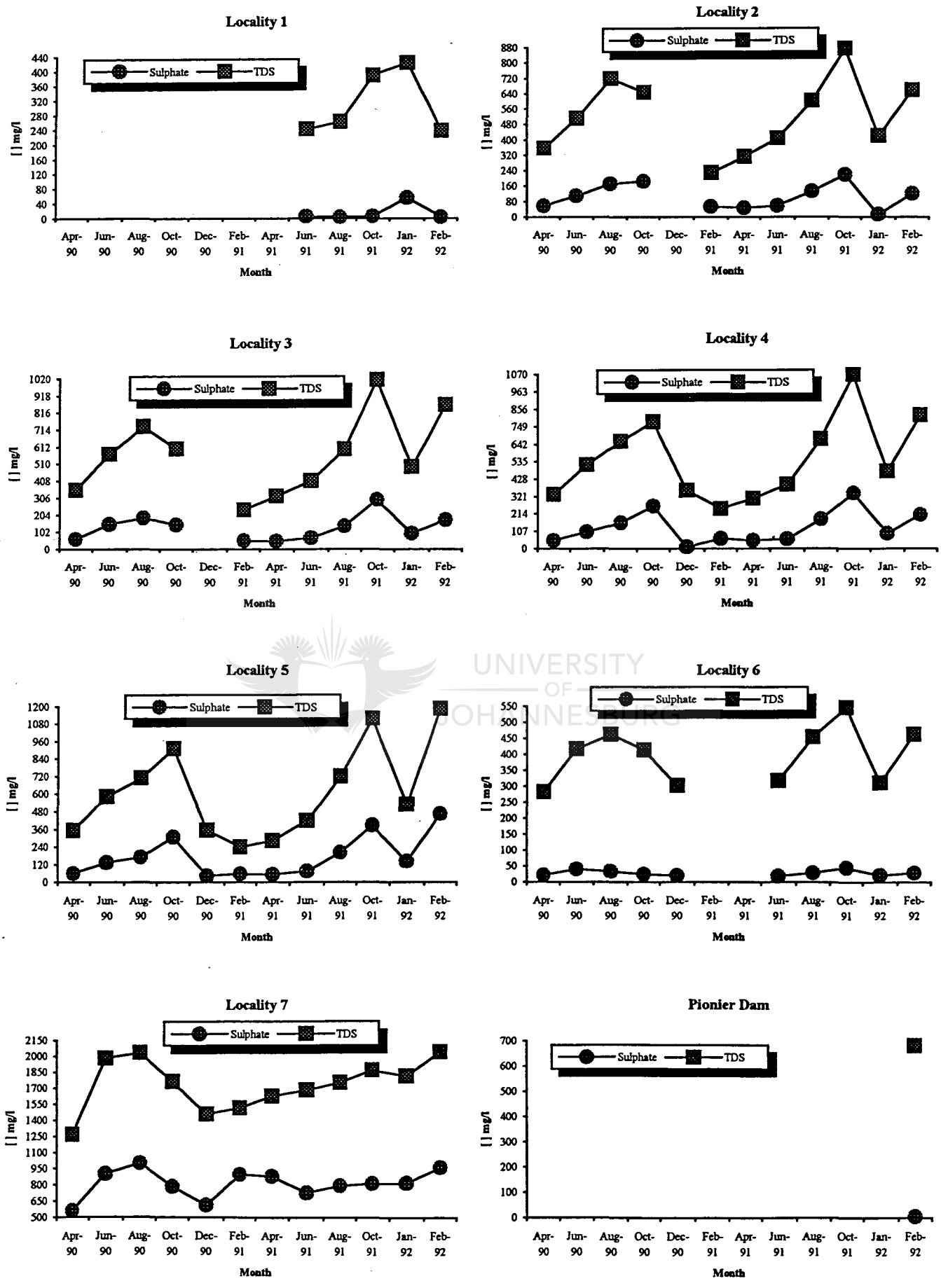

Figure 3.3
Sulphate and Total Dissolved Salt concentrations (mg/l) at the different localities

reactions such as organic complexes, carbonate formations and pH ranges should be considered. Chloride can, however, be regarded as one of the most mobile and persistent complexing agents with regard to metals and may, under certain circumstances, be of great significance in determining metal distribution in the environment (Hahne & Kroontje, 1973).

Although the sodium and potassium concentrations at locality 7 were higher than the guideline values and were also fairly high at Pionier Dam, the lack of sufficient research data on the effects of elevated sodium and potassium concentrations on aquatic life precludes discussion thereof. However, fish mortalities in the Olifants River have previously been associated with high levels of K, Cl, SO_4, Mg and Na. Elevated potassium levels are thought to have been the actual cause of death (Moore, 1990). Potassium and sodium seemed to follow the same trend: at localities 2 to 6, a sudden increase in concentration was detected during October - especially in the second year (Fig. 3.4). At locality 7, however, no sudden increase in concentration could be detected; the changes were more gradual throughout the year. These findings might be explained by the fact that 1991 was a very dry year and only in October 1991 did the first rains fall in the catchment area. The result was an increase in flow during that time, accompanied by the leaching of salts from areas adjacent to the catchment into the river water. Except for potassium and sodium - magnesium, chloride, sulphate, alkalinity and TDS also showed a similar trend.

Ammonia is produced as a metabolite from the natural degradation of nitrogenous organic material present in all surface waters (Ellis, 1989). However, high levels reach waters as fertiliser components and through effluents from industries and sewage works. Ammonia can exist in two forms in water, namely as the ammonium cation (NH_4^+) or as free ammonia (NH_3). The equilibrium existing between the ammonium cation and ammonia ($NH_4^+ + OH^- \Leftrightarrow NH_3 + H_2O$) depends on pH and temperature (Boyd, 1982). The less toxic ammonium ion (NH_4^+) exists at lower pH values, while the more toxic ammonia (NH_3) is present in more alkaline conditions. Therefore as the temperature and pH increase, the percentage toxic free ammonia increases. Even a small increase in pH, from 7 to 8, will increase the toxicity of ammonia approximately 10 fold. In order to obtain the free ammonia concentration, the percentage free ammonia for the specific temperature and pH (Table 2.12 in Boyd, 1982) are multiplied by the total ammonia nitrogen concentration. In the study area, the pH tended to be more alkaline and the temperatures were high. Therefore the ammonia concentrations should be carefully monitored. In addition to its toxicity, ammonia may also impose an additional oxygen demand on the receiving stream as a result of its potential to be oxidised by autotrophic bacteria to nitrite and then to nitrate (Ellis, 1989).

Nitrosomonas		*Nitrobacter*		
Ammonia (NH_3)	$\rightarrow$	nitrite (NO_2^-)	$\rightarrow$	nitrate (NO_3^-)
O_2		O_2		

In order for nitrification to occur, the climate should be warm, a sufficient number of nitrifying organisms should be present and the retention time must be sufficient (Ellis, 1989).

Freshwater plants are more resistant to ammonia than are invertebrates, and invertebrates are in turn more resistant than fish. Fish exposed to sublethal ammonia concentrations experience reduction in growth rate and morphological development, pathological changes in the tissue of kidneys, livers and gills and reduction in the proportion of successful hatchings (Ellis, 1989). A more notable effect is a diuretic response whereby the fish increases its urine production as a result of its increased permeability , in other words more water permeates the body (Lloyd & Orr, 1969). An indication of sublethal concentrations might be 0.006 - 0.34 mg/l NH_3, for Smith & Piper (1975) detected histological effects at these concentrations. This means that the calculated concentration of 0.1782 mg/l NH_3 at locality 7 in the second year might have been sublethal. However, in addition to pH and temperature, there are other factors affecting the toxicity of ammonia. A decrease in dissolved oxygen will increase the toxicity of ammonia, but an increase in [CO_2] in water up to a level of approximately 30 mg/l appears to decrease the toxicity (Ellis, 1989). Copper salts apparently combine additively with ammonia in their toxic effects (Herbert & Van Dyke, 1964), while calcium reduces the toxicity of ammonia.

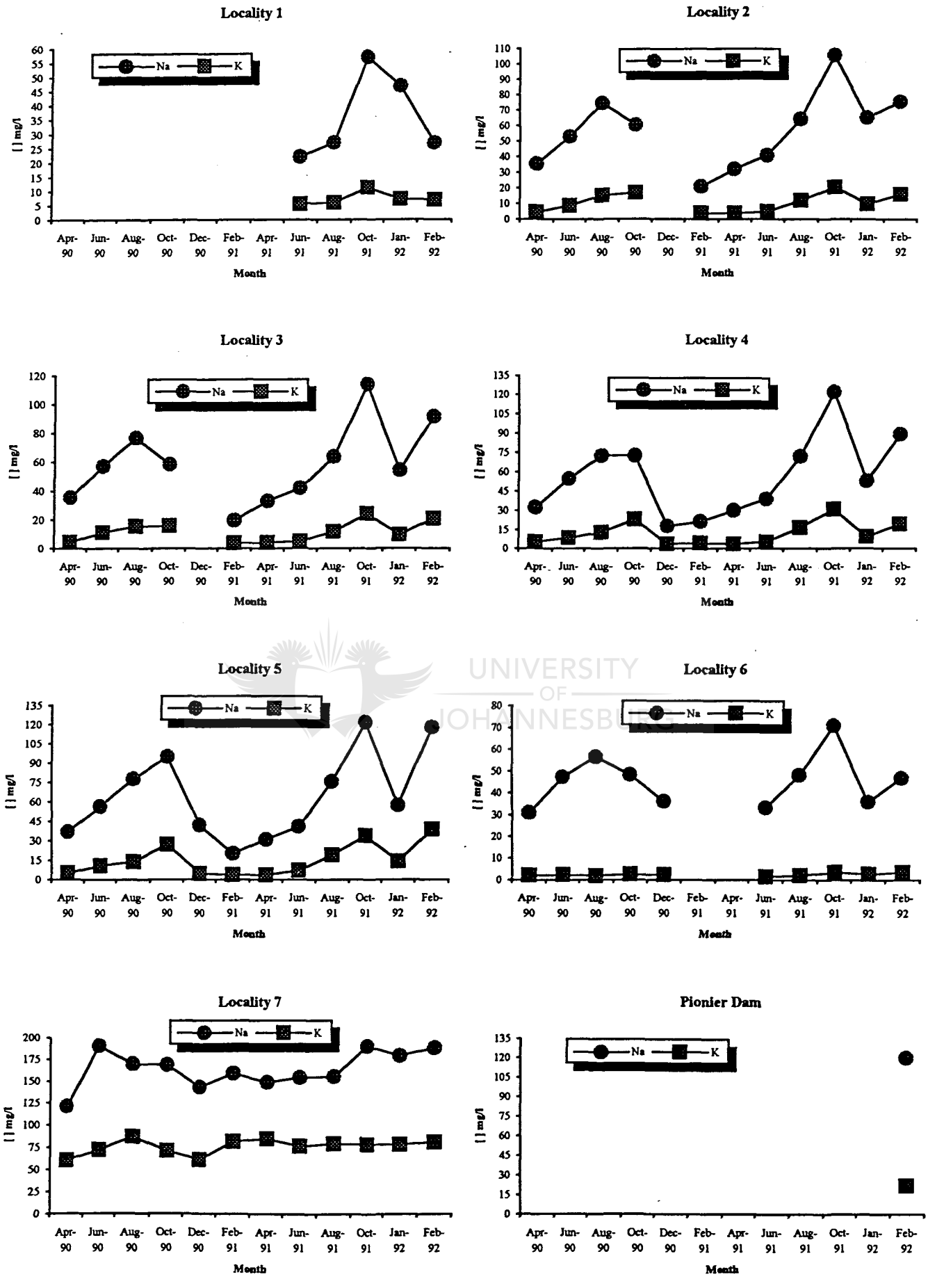

Figure 3.4
Sodium and Potassium concentrations (mg/l) at the different localities

Nitrite (NO_2^-) and nitrate (NO_3^-) are two forms of total oxidised nitrogen (TON). An imbalance in the nitrification reaction can lead to the accumulation of nitrite. However, organisms that oxidise ammonia to nitrite and those that oxidise nitrite to nitrate coexist; nitrite therefore, does not accumulate in natural environments as a result of nitrification (Boyd, 1982). The reduction of nitrate by bacteria in anaerobic sediments or water can also produce nitrite. In addition to nitrates being present as a result of nitrification, it can also be present in treated effluents being discharged into the river, or in the run-off from agricultural land containing fertiliser (Ellis, 1989). According to Ellis (1989) the concentrations of TON in drinking waters should be restricted to less than 11.0 mg/l (as N). In the study area TON concentrations were less than 1.0 mg/l (as N), and therefore comply with the acceptable standards for drinking water. The low TON values can be ascribed to the abundance of phytoplankton occurring in the river during the course of this study (Seymore, *pers. obs.*). Phytoplankton represents the main factor responsible for a decrease in nitrate and nitrite concentrations (Saad, 1987).

Nitrite poisoning in fish is referred to as "brown blood disease", for nitrite absorbed by fish reacts with haemoglobin to form methaemoglobin (a brown substance). This disease can lead to hypoxia and cyanosis, since methaemoglobin is not an effective oxygen carrier (Boyd, 1982). The toxicity of nitrite to fish can be reduced by the addition of calcium (Wedemeyer & Yasutake, 1978) and chloride (Perrone & Meade, 1977; Tomasso *et al.*, 1979). These substances do occur in moderate to high concentrations in the study area, with the result that nitrite toxicity will be reduced if elevated nitrite levels should occur.

Phosphorus in surface water will mostly be present either as orthophosphates or as polyphosphates. All polyphosphates in water will, however, revert in time to orthophosphates (Ellis, 1989). The phosphate levels in the Lower Olifants River were generally around 0.02 mg/l. Only at locality 7 (in the Selati River) higher levels of 0.136 ± 0.167 mg/l on average were detected in the second year. Although phosphates are non-toxic, they are indicative of pollution from detergents, fertilisers, sewage, etc. (Kempster *et al.*, 1982). According to a survey done by the CSIR (1990), orthophosphate (PO_4-P) concentrations in the seepage and effluent discharged into the Selati River by a phosphorus extraction mining company were sufficiently high to cause moderate eutrophication problems. This statement can be confirmed by personal observations, for during the course of the study the aquatic plants and algae seemed to increase, especially at localities 5 (Mamba weir) and 4.

Calcium is an integral part of bone and is non-toxic (Kempster *et al.*, 1982). It is relevant to this study because of the influence it has on metal toxicity. Calcium reduces the toxicity of metals to fish by hindering their adsorption. According to Mason (1991), calcium is antagonistic to lead, zinc and aluminium. The calcium ion competes with other metal cations for binding sites on the gill surface, thereby decreasing the direct uptake of cationic metals by fish. In contradiction to this, Giesy & Alberts (1984) pointed out that although Ca^{2+} may occupy sites on the organic ligand, the binding strengths are low compared to transition metals. Therefore, Ca^{2+} is not capable of blocking sites in the presence of other metal ions and will be exchanged for by the other metals on the organic ligands.

Alkalinity in water represents its ability to neutralise strong acids. It is caused mainly by the presence of bicarbonates, carbonates and hydroxyl ions which are formed as a result of the interaction of carbon dioxide in water with basic materials such as the calcium carbonate of chalk or limestone in soils and rocks (Ellis, 1989):

$$\underset{}{CO_2} + \underset{\text{calcium carbonate}}{CaCO_3} + \underset{}{H_2O} \rightarrow \underset{\text{calcium bicarbonate}}{Ca(HCO_3)_2}$$

The buffering capacity of the study area seemed to be fairly good, as the alkalinity ranged between 140.2 ± 22.8 and 234.8 ± 20.3 mg/l $CaCO_3$. The alkalinity of natural water is rarely more than 500 mg/l as $CaCO_3$ (Kempster *et al.*, 1982). Total alkalinity is sometimes confused with total hardness. Total hardness refers to the concentration of divalent metal ions in water, expressed as milligrams per litre of equivalent calcium carbonate (Boyd, 1982). Fortunately, total hardness and total alkalinity have similar concentrations in most waters (Boyd, 1982). The water of the Lower Olifants River

would be considered hard and most metals are less toxic in hard water than in soft water (Hellawell, 1986).

Temperature changes can have a major impact on fish life. One example is the low temperature discharges from impoundments that may trigger spawning (Theron *et al.*, 1991). According to the guidelines proposed by Kühn (1991), the temperature of the water being discharged into the Olifants River at Phalaborwa Barrage, for instance, should be within 5°C of the background water temperature. Another example of fish being affected by temperature changes, happened on the 25th of October 1989, when a hail storm caused a sudden decline in temperature. This incident was thought to have been the actual reason for fish mortalities in the Olifants River (Deacon, pers. comm.). It is therefore not the temperature itself that causes concern, but the rate of change of water temperature. Although a sudden temperature change was detected in the study area from August to October, it is of no value, since information like this should be recorded on a daily basis.

The effect of temperature on toxicity is complex. Elevated temperatures do not always increase toxicity of substances. The toxicity of some is increased and that of others decreased by an increase in temperature (Alabaster *et al.*, 1972). Temperature influences the rate of metabolic processes, including the uptake, metabolism and excretion of poisons. Increased temperature will increase the oxygen requirements of aquatic organisms, while decreasing the solubility of oxygen in water. The properties of the poison itself may also be directly influenced by temperature (Abel, 1989). In the literature contradictory results are reported on toxicity effects, especially on the effect temperature has on zinc toxicity. It would therefore be presumptuous to draw conclusions about temperature effects on toxicity.

Dissolved oxygen (DO) is essential to all aquatic life. For warm water species the target guideline value is >5 mg/l (Kempster *et al.*, 1982). At locality 7 the mean DO concentration was just above 5 mg/l, namely 5.7 ± 0.3 mg/l and 5.6 ± 1.5 mg/l for years 1 and 2 respectively. However, as temperature increases, the DO decreases (Fig. 3.5). This effect could clearly be seen at locality 7 in August 1991 and October 1991 when the DO decreased from 3.9 mg/l to 1.8 mg/l in the morning, with an increase in temperature from 19.0°C to 25.5°C (Table 3.1b). Although 3.9 and 1.8 mg/l DO concentrations are very low, time is the deciding factor in the survival of fish species. Warm water species would survive 3 - 5 mg/l DO if they are not exposed to it for more than eight hours out of any 24-hour period, and some species would survive 1 - 3 mg/l DO if they are not exposed to it for more than a few hours (Train, 1979). Species not able to resist low DO concentrations would therefore not occur in the Selati River at locality 7, which might be another reason why only a few fish species were detected there. The mean DO concentrations of the other localities ranged from 8 - 12 mg/l. According to Ellis (1989) it is rare to find more than 8 - 10 mg/l of oxygen, even under optimum conditions, since the amount of oxygen dissolved from the air into water is small. Higher oxygen concentrations can, however, occur, due to photosynthetic oxygen produced under the influence of sunlight by algae and other aquatic plants, as was observed for the locality at Mamba.

The effects of dissolved oxygen on toxicity have been less widely investigated, but in general low dissolved oxygen concentrations appear to cause an increase in the toxicity of poisons (Abel, 1989). For instance, the American Petroleum Institute (1983) established that chromium concentrations increased in the gills and kidneys of the bluegill sunfish (*Lepomis macrochiris*) as the dissolved oxygen decreased. The growth of fish is extremely sensitive to reduced oxygen levels and fish eggs develop more slowly with the lowering of oxygen concentrations (Sprague, 1971).

The pH of the water in the study area seemed to be very stable and well within the target guideline range of 6 - 9. A slight decrease in pH was observed in December 1990. The reason is that under high rainfall conditions, leaching is more pronounced and systems usually have lower pH values (Hahne & Kroontje, 1973). Aqueous pH can greatly influence the toxicity and bioavailability of cationic metals to fish. At low pH, hydrogen ion can compete for metal binding sites on particle surfaces and solution ligands (thereby increasing metal bioavailability) and on biological membranes such as the gill surface (potentially reducing metal uptake and toxicity). Hydrogen ion can also act as a stress factor, depleting gill calcium and causing ionoregulatory stress (Spry & Wiener, 1991). The toxic action of hydrogen ions on goldfish has been ascribed by several authors to the precipitation of mucus on the gill epithelium causing death by suffocation, or by precipitation of proteins within the

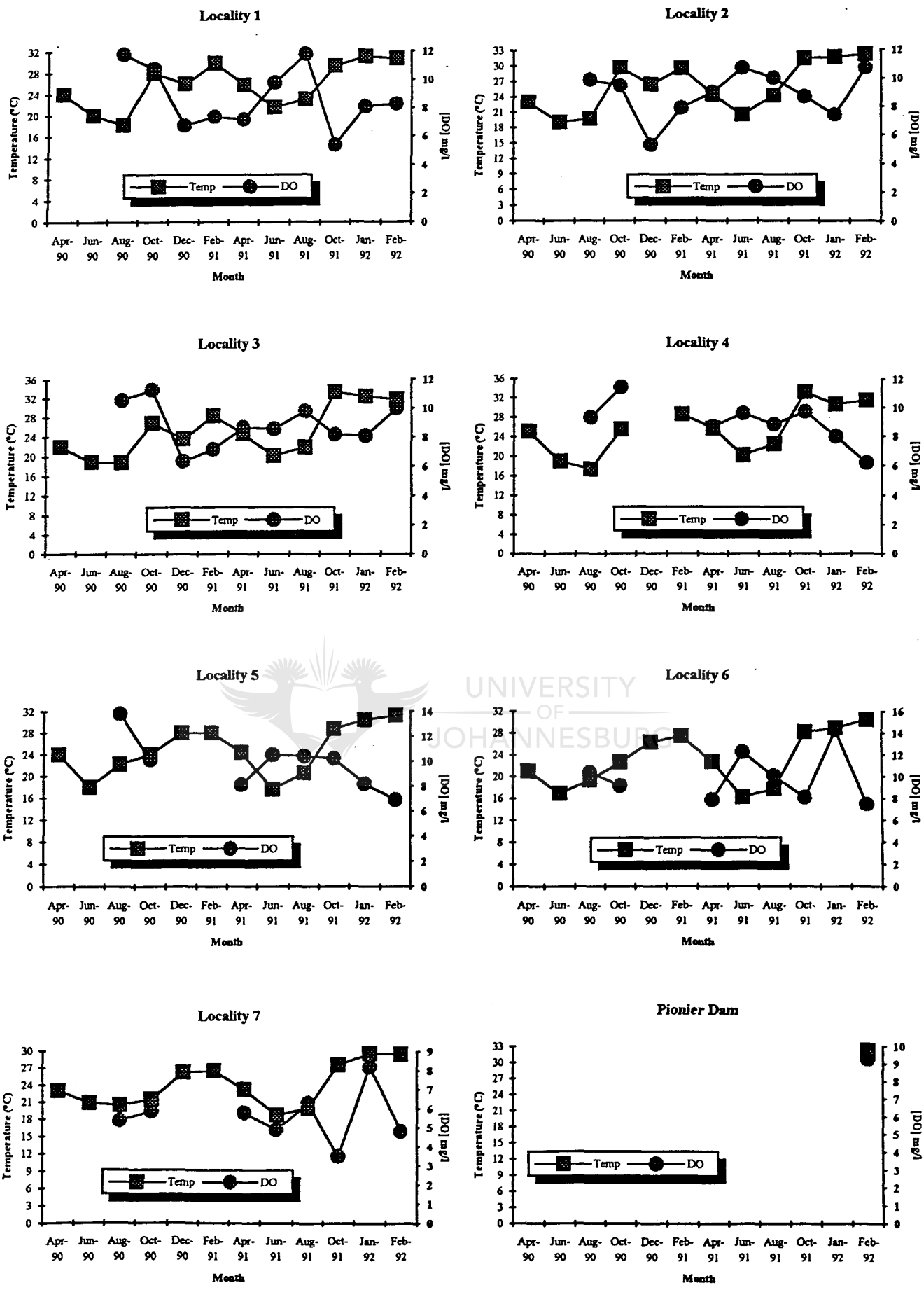

Figure 3.5
Temperature (°C) compared to dissolved oxygen concentrations (mg/l) at the different localities (The afternoon readings were taken for the second year)

epithelial cells (Ellis, 1937; Westfall, 1945). If waters are more acidic than pH 6.5 or more alkaline than pH 9 - 9.5 for long periods, reproduction and growth of fish will diminish (Swingle, 1961; Mount, 1973).

METAL CONCENTRATIONS IN THE WATER AND SEDIMENT

Mining and industrial effluents are the general sources of elevated metal concentrations in river water. It is usually the ionic forms that produce the immediate fish mortalities, while complexed metal compounds tend to act by accumulation in the body tissue over a considerably longer period (Ellis, 1989). The approximate order of the toxicity of metals, which is based on published data, is given in Table 3.5 (Hellawell, 1986). Several factors can influence their toxicity, for instance: their concentration in the water, the form in which they are present (ionic, complexed or organic), the difference in species sensitivity and life stage sensitivity to toxicants, the type and concentration of other toxicants present (the effect being additive, antagonistic or synergistic) or the condition and quality of the water itself (factors such as dissolved oxygen, water hardness, temperature and pH). Generally toxicity increases with decreasing dissolved oxygen and pH and declines with increasing hardness (Ellis, 1989). There are, however, a few exceptions, like zinc, for which the effects of certain parameters are uncertain. The effects that elevated metal concentrations have on fish will be discussed in the following chapters.

TABLE 3.5
TENTATIVE TABLE OF THE APPROXIMATE ORDER OF TOXICITY OF METALS
(From Hellawell, 1986)

Highly toxic					Decreasing toxicity →						
Hg											
	Cu	Cd Zn	Au?	Ag?	Pt?						
			Sn	Al Ni	Fe^{3+} Fe^{2+}						
						Ba					
							Mn Co	Li K	Ca	Sr Mg	Na

Bottom sediments play an important role in the distribution of metals in the aquatic environment. They can act as reservoirs and release metals into the water through resuspension or leaching (Salomons, 1985; Salomons *et al.*, 1987). The organisms that would especially be affected by sediment contamination, are the bottom-dwellers. Investigations on the toxicity of sediments are, however, limited by the complexity of sediment-water column and sediment-biota interactions, as well as the unavailability of an adequate number of soil ecotoxicity test guidelines. The latter limitation is one of the reasons why an integrated soil research program is being carried out in the Netherlands (ISRP, 1989).

If the factors influencing metal toxicity are excluded for the moment, it is clear from Table 3.4 that the metal concentrations of the selected metals in the water of the study area are mostly higher than the recommended guideline values (except for strontium). The assumption was made that the authors of the guidelines refer to total metal concentrations and not bioavailable or soluble metal concentrations. In this study, much higher concentrations were detected in the sediment (µg/g x 1000) than in the water (µg/l), due to the adsorption of metals on sediment particles. It is also an indication of the chronic nature of pollution in the area (Dallinger & Kautzky, 1985; Mac & Schmitt, 1992). There is, however, a continuous interaction between the water and the sediment columns, depending on factors such as the water pH. When the pH is alkaline, in other words more hydroxyl ions (OH^-) are present than hydrogen ions (H^+), insoluble metal hydroxyl complexes will form. However, when rainfall occurs, as was the case in December 1990, the hydrogen ion concentration will increase. The solubility of the metals will increase slightly and an increase in the water metal concentrations may be detected (Table 3.2a). The iron concentrations in the water increased

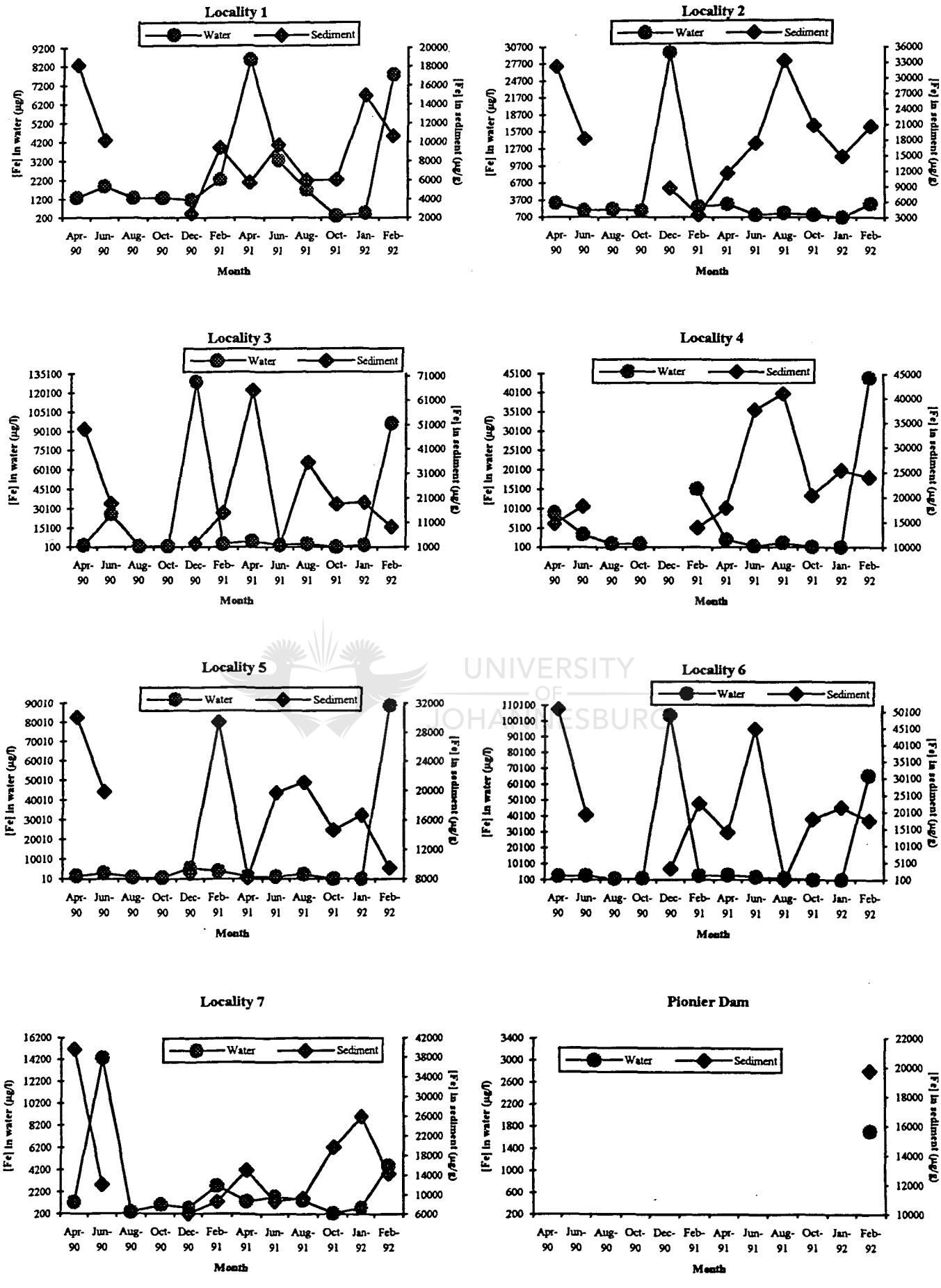

Figure 3.6
Iron concentrations in the water (µg/l) and sediment (µg/g) of the different localities

considerably in December 1990 (Fig. 3.6), but increasing solubility was not the only reason for this phenomenon. Weathering of underlying rock formations, especially basalt, will produce iron (Dury, 1981). As locality 3 (near Balule) is underlain by basalt, the highest iron concentrations were detected there. Iron is also a highly abundant element and therefore, of all the metals investigated, iron was found to occur in the highest concentrations. The copper and strontium concentrations in the Selati River, especially in the sediment, were much higher than the concentrations in the Olifants River. This indicates that these two metals originate from a local source which is not connected to the Kruger National Park.

A factor playing a major role in metal distribution is, as mentioned earlier, rainfall. A noticeable difference could be seen between the wetter first year and the drier second year. In the first year peaks of the metal concentrations in the water occurred at localities 7 and 3 (Fig. 3.7a). Peaks at locality 7 can mainly be attributed to mining and industrial effluents, while peaks at locality 3 might be attributed to the frequent occurrence of reed beds, accumulating the metals and releasing them again when decaying. In the second year, peaks also occurred at localities 7 and 3, but with the addition of locality 1 (in the Letaba River) (Fig. 3.7b). It might be that because of the drought, the river flow in the Olifants River was very low and therefore the carrying capacity of the water volume for metals decreased. By contrast, the Letaba River might have had a stronger flow, thus rendering higher solubility and concentrations of metals.

3.5 Conclusion

The mining and industrial activities in the Phalaborwa complex definitely have an influence on the water quality of the lower Selati River. The sodium, fluoride, chloride, sulphate, potassium, TDS and metal concentrations (except for strontium) were higher than the guideline values of Kempster *et al.* (1982), Kühn (1991) and Canada (Environment Canada, 1987). The water quality of the Lower Olifants River after the Selati-Olifants confluence was also influenced by activities upstream of the Selati River, especially localities 5 (Mamba weir) and 3 (near Balule). At Mamba the mean TDS, potassium, chloride, sulphate, fluoride and sodium concentrations reported for 1991/1992 were very similar or slightly higher than the mean concentrations reported for 1983 to 1989 by Van Veelen (1990). However, dilution caused by smaller tributaries decreased the toxicant concentrations to levels that, with the exception of the metal concentrations, comply with the recommended guideline values. The mean metal concentrations (excluding strontium) were higher than the guideline values at all the localities. The large variance detected in the metal concentrations of the water and sediment points to the need for more frequent monitoring of this area.

It is recommended that a more intensive study should be undertaken specifically on the water and sediment quality of the study area. The metal levels in particular should be studied, as well as the effect thereof on aquatic life. It will be necessary to combine the field study with experimental work, in order to determine the effects of the physical and chemical environment on the metal toxicity. This is very important, for the water in the Lower Olifants River is hard and alkaline and will definitely have an influence on the metal toxicity. Monitoring can be limited to localities 2, 3, 5, 6 and 7. Special attention should be given to locality 3, in order to determine the role of the reed beds. The interaction between water and sediment with regard to metal distribution should be investigated, as well as seasonal effects on toxicity and metal distribution.

For future management it is recommended that drastic measurements should be taken in order to reduce the impact of mining activities on the water quality of the Selati River, because it is not only the water quality of the Selati River that is being influenced, but also the water quality of the Lower Olifants River (especially during low flow periods). If, for some or other reason, the water quality of the Selati River cannot be improved, it should at least be maintained at its present status. A further degradation in water quality cannot be afforded.

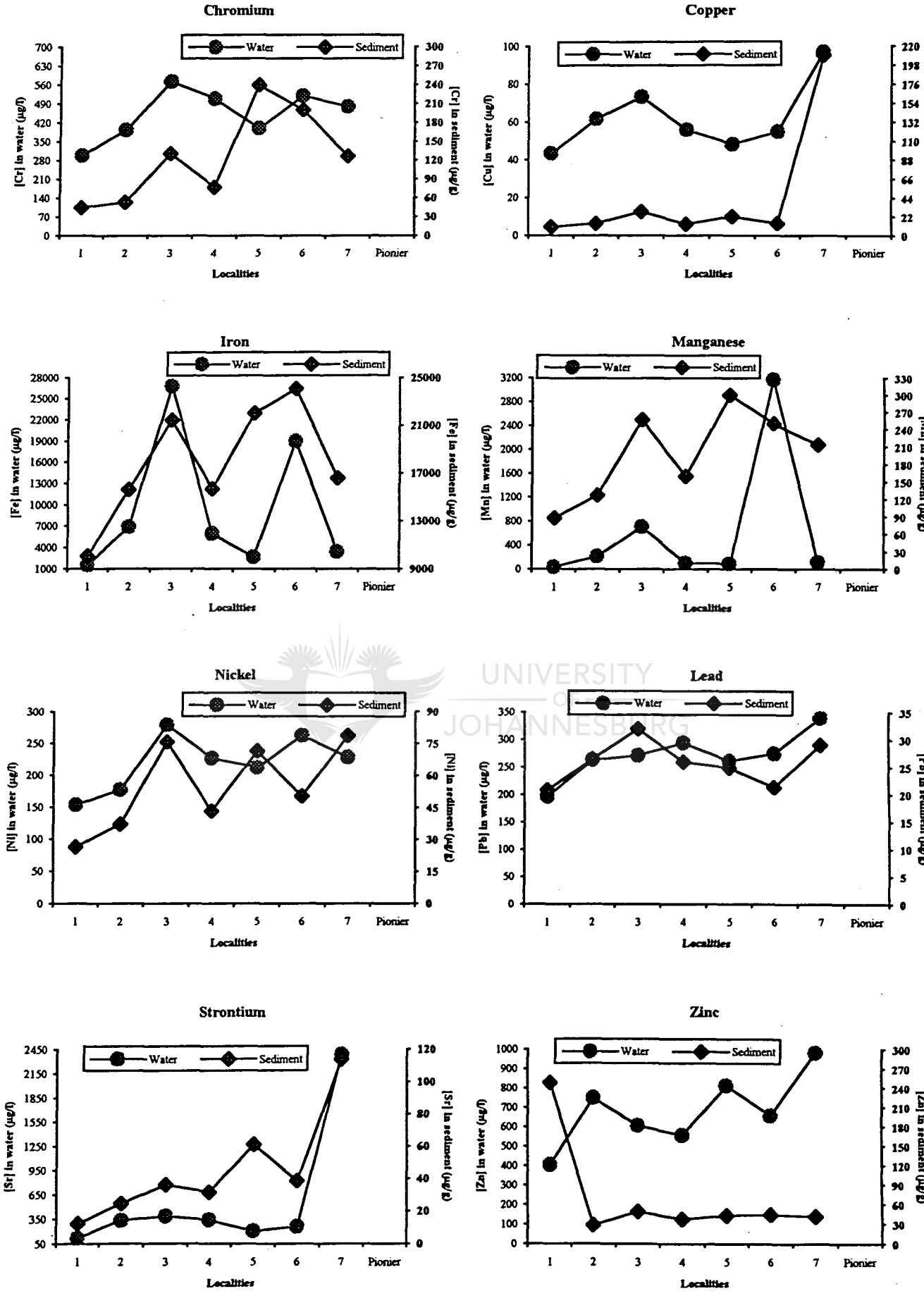

Figure 3.7a
Mean metal concentrations in the water (µg/l) and sediment (µg/g) for the period April 1990 - February 1991

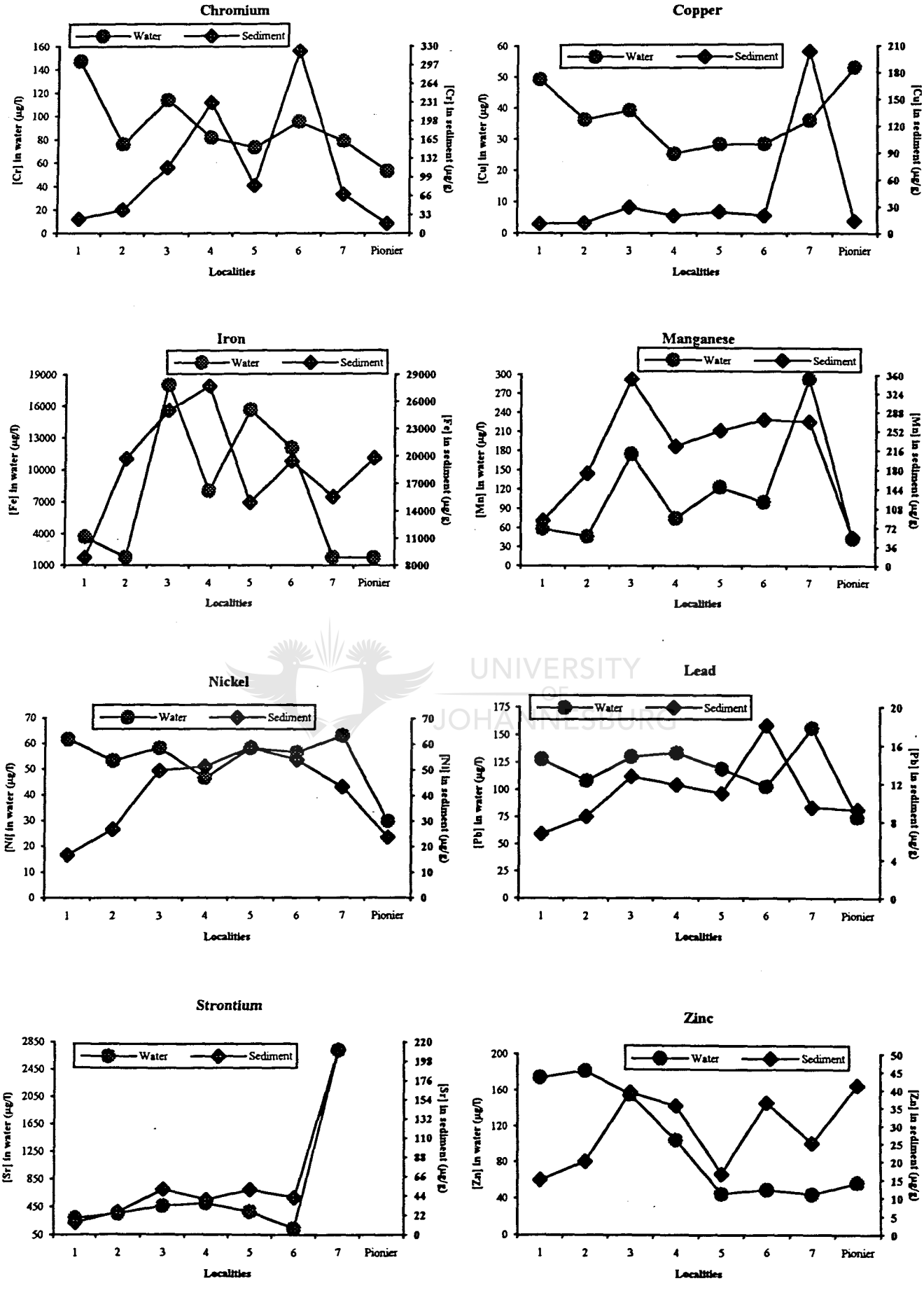

Figure 3.7b
Mean metal concentrations in the water (µg/l) and sediment (µg/g) for the period April 1991 - February 1992

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CHAPTER 4

Chapter 4

ZINC BIOACCUMULATION IN THE ORGANS AND TISSUES OF *BARBUS MAREQUENSIS*

4.1 Introduction

Zinc is relatively rare in nature, comprising approximately 120 grams per ton of the earth's crust (Hale, 1977). In nature it is predominantly found in the sulfide form. Zinc is, however, a common pollutant of surface fresh waters in many industrial areas. Elevated levels in the aquatic environment can be caused by atmospheric deposition, liquid effluent discharge, the leaching of metal bearing minerals and domestic sewage (Van Loon & Beamish, 1977; Weatherly *et al.*, 1980). The main anthropogenic sources of zinc include processes of galvanising, plating, rubber processing, rayon manufacturing and the production of iron and steel (Hellawell, 1986).

The toxicity of zinc to fish has been the subject of interest to many researchers worldwide. Acutely toxic zinc concentrations result in gill damage, which interferes with respiration, leading to hypoxia (Skidmore & Tovell, 1972; Burton *et al.*, 1972; Heath, 1987). Chronically toxic concentrations, on the other hand, do not affect the gills, but cause general enfeeblement and extensive deterioration of the liver, kidneys, heart, skeletal muscles, gonads and spleen (Crandall & Goodnight, 1962 & 1963; Wong *et al.*, 1977). Chronic sub-lethal zinc concentrations can also delay or inhibit the growth, sexual maturity and reproduction of the fish (Pierson, 1981; Brungs, 1969).

Sub-lethal effects on fish due to zinc exposure have been shown to occur over the range of approximately 30 - 200 µg/l Zn (Brungs, 1969; Eaton, 1973), while the 96-hour LC50 value can range from less than 0.14 mg/l Zn (Everall *et al.*, 1989) to 41 mg/l Zn (Pickering & Henderson, 1966), depending on the fish species and the physico-chemical characteristics of the water. The most important factor influencing the toxicity of zinc, is water hardness. Reports indicate that increased hardness decreases zinc toxicity (Lloyd, 1960; Wang, 1987; Solbé, 1974; Farmer *et al.*, 1979). The two possible mechanisms involved, are (i) the complexation of the metal ion with carbonates, thereby decreasing the bioavailibility of zinc and (2) the competition between the metal ion and Ca and/or Mg ions at the gill epithelium sites (Wang, 1987; Zitko & Carson, 1976). The effect of temperature on zinc toxicity is contradictory and species dependent (Cairns *et al.*, 1975). The bulk of the evidence indicates, however, that zinc toxicity increases as temperature increases (Wang, 1987; Skidmore, 1964). The pH is known to affect both the solubility and speciation of many metals (Campbell & Stokes, 1985). Several reports indicate that the toxicity of zinc increases with increasing pH, especially from pH 4 to 7 (Wang, 1987), which is the pH range where dissolved zinc predominates (NRCC, 1981). Zinc toxicity can also be affected by organic substances (Wang, 1987; Hellawell, 1986), inorganic ligands such as Cl^-, OH^- and PO_4^{3-} (Wang, 1987), metal interactions (Skidmore, 1964; Heath, 1987), sediments (Wang, 1987) and the dissolved oxygen content (Hale, 1977). With regard to the fish itself, acclimated organisms are generally more tolerant than unacclimated organisms, early life stages are usually more sensitive to toxicants and different species may respond differently to toxicants (Heath, 1987; Skidmore, 1964; Wang, 1987).

Although zinc can be toxic to fish at elevated levels, it is an essential trace element and is presumably homeostatically controlled in the fish. The bioaccumulation of zinc in fish tissues seems to be species-dependent, but, according to literature, zinc mainly accumulates in the skin, bone, liver, gill, kidney and gut of the fish (Mount, 1964; Skidmore, 1964). When assessing the extent of zinc accumulation in a fish, it is therefore essential to consider both the species involved, as well as the tissues of individual species. In this section of the study, the concentrations of zinc in a benthic feeder, *Barbus marequensis*, from the Olifants River (Kruger National Park) were investigated. The information was then used to determine the extent and pattern of zinc bioaccumulation in the tissues of the species.

4.2 Materials and methods

FIELD SAMPLING

Largescaled yellowfish (*Barbus marequensis*) were sampled with gill nets (70 - 120 mm stretched mesh size) and throw 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 (Fig. 2.8). In February 1992 ten fish were also collected at Pionier Dam (Kruger National Park), the natural reference point used in this study. After capture, the weight 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 & 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.

LABORATORY PROCEDURES

After the tissue samples were thawed, all the organs and tissues (except for the bile and blood) were dried in an oven at 60°C for a period of 48 hours. The wet and dry weights of the samples were recorded in order to calculate the percentage of moisture of each sample. Ten ml concentrated nitric acid (55%) and 5 ml perchloric acid (70%) were added to one gram dry tissue in a 100 ml Erlenmeyer flask. Digestion was performed on a hot plate (200 to 250°C) for at least four hours, until the solutions were clear (Van Loon, 1980). The bile was digested in a similar manner, except that it had not been dried. For the blood digestion, 5 ml each of concentrated nitric (55%) and perchloric acid (70%) were added to 0.5 ml blood in a 100 ml Erlenmeyer flask and digestion similar to the other samples was then performed.

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 ml each with doubly distilled water. The samples were stored in clean glass bottles, until the zinc concentrations could be determined. Prior to use, all glassware was soaked in a 2% Contrad soap solution (Merck chemicals) for 24 hours, rinsed in doubly distilled water, acid-washed in 1M HCl for 24 hours and rinsed again in doubly distilled water (Giesy & Wiener, 1977).

A Varian atomic absorption spectrophotometer (Spectra AA-10) was used to determine the zinc concentrations in the tissue samples of the fish. Analytical standards were prepared from Holpro stock solutions. The metal concentrations in the tissue samples were calculated as follows:

$$\text{Metal concentration } (\mu g/g) = \frac{\text{AAS reading } (\mu g/ml)}{\text{Sample mass } (g)} \times \text{Sample volume (50 ml)}$$

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

$$BFw \text{ or } BFs = \frac{[Zn] \text{ in organ } (\mu g / g \text{ dry wt.})}{[Zn] \text{ in water } (\mu g / ml) \text{ or sediment } (\mu g / g)}$$

AGE DETERMINATION

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 & Johnson, 1983).

STATISTICAL PROCEDURES

Statistical differences between the different organs and tissues were determined by grouping together the localities inside the KNP (3, 4 and 5). Comparisons were made for winter 1991 (June and August 1991), spring 1991 (October 1991) and summer 1992 (January and February 1992) by means of the Scheffe statistical test. The significant level was $p \leq 0.05$.

Variation in capture success limited the statistical comparisons of the localities. Only a few organs, sampled in months when the number of fish caught at each locality was three or more, were used. For the first year, localities 3 to 5 were compared using the zinc concentrations in the gill, liver and muscle tissues of October 1990. In the second year, the zinc concentrations in the fat, muscle, vertebrae and blood were used to compare localities 3, 4, 5 and 7 in January 1992 and localities 3 to 5 in June 1991, October 1991, January 1992 and February 1992. Pionier Dam was also compared to localities 3 to 5 in February 1992. The Hotelling T^2 and Scheffe tests of the BMDP 2V statistical program were used ($p \leq 0.05$).

Seasonal differences were determined for males and females, as well as for the sexes combined. The data collected at localities 3, 4 and 5 were grouped into seasons as follows: autumn 1990 (April 1990), winter 1990 (June and August 1990), spring 1990 (October 1990), summer 1990/91 (December 1990 and February 1991), autumn 1991 (April 1991), winter 1991 (June and August 1991), spring 1991 (October 1991) and summer 1992 (January and February 1992). The seasons were statistically compared using the zinc concentrations in the muscle, gill (excluding autumn 1991), liver (excluding autumn 1990 and summer 1990/91), blood (excluding the seasons of the first year), skin and vertebrae (excluding the seasons of the first year, as well as autumn 1991). The Scheffe and Hotelling T^2 tests (BMDP 2V program) were used ($p \leq 0.05$).

Using the Hotelling T^2 test (BMDP 2V program), the first year (April 1990 - February 1991) and second year (April 1991 - February 1992) were statistically compared with respect to the gill, gonad, liver and muscle zinc concentrations. In order to obtain a reliable comparison with a large *N* value, the data of localities 3 to 5 were grouped together.

4.3 Results

FISH SIZE AND AGE

The mean weight and length of the fish that were caught at the different localities for each month are presented in Table 4.1A for the first year and Table 4.1B for the second year. In general, the female fish were larger than the male fish at each locality. The 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.

The 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. Nevertheless, the data indicated that the fish were 1 to 2 years of age at a forklength of 14 to 20 cm, 2 to 3 years at 20 to 30 cm forklength, 3 to 4 years at 30 to 34 cm forklength and 4 to 6 years at 34 to 40 cm forklength.

TABLE 4.1A
LENGHTS AND WEIGHTS OF *BARBUS MAREQUENSIS* CAUGHT IN THE OLIFANTS RIVER (KNP) DURING THE PERIOD APR. 1990 - FEB. 1991

Month	Locality	N	Weight (g)		Length (cm)	
			Range	X ± SD	Range	X ± SD
Apr. 1990	3	1 F#	800		35.5	
	4	7 F	186 - 701	353.1 ± 169.3	23.8 - 36.5	28.8 ± 3.9
		2 M*	102 - 199	150.5 ± 68.6	19.1 - 23.9	21.5 ± 3.4
	5	0	-	-	-	-
	7	10 F/M•	46 - 134	64.8 ± 27.5	15.0 - 21.4	16.4 ± 2.0
June 1990	3	2 F	216 - 222	219.0 ± 4.2	26.5 - 28.0	27.3 ± 1.1
	4	0	-	-	-	-
	5	0	-	-	-	-
	7	0	-	-	-	-
Aug. 1990	3	4 F	81 - 509	254.8 ± 181.4	17.3 - 32.5	24.8 ± 6.2
		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
		3 M	391 - 592	507.7 ± 104.3	30.5 - 35.5	33.0 ± 2.5
	5	2 F	262 - 356	309.0 ± 66.5	26.0 - 28.5	27.3 ± 1.8
		1 M	573		32.0	
		3 F/M	227 - 246	237.0 ± 9.5	25.0 - 25.9	25.5 ± 0.5
	7	5 F/M	24 - 44	34.2 ± 9.6	12.5 - 15.2	14.0 ± 1.3
Oct. 1990	3	2 F	383 - 545	464.0 ± 114.6	28.3 - 30.9	29.6 ± 1.8
		1 M	1000		35.9	
		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
		7 M	550 - 800	636.3 ± 98.0	27.5 - 33.7	30.2 ± 2.5
	5	1 F	592.0		31.2	
		6 M	166 - 1050	477.3 ± 323.8	20.3 - 38.7	30.6 ± 6.9
		3 F/M	169 - 272	235.7 ± 57.8	21.5 - 23.5	22.8 ± 1.1
	7	1 F	900		34.0	
Dec. 1990	3	4 F	171 - 549	302.0 ± 175.0	22.0 - 32.3	26.0 ± 4.7
		1 M	254		24.2	
		2 F/M	70 - 80	75.0 ± 7.1	16.6 - 18.6	17.6 ± 1.4
	4	0	-	-	-	-
	5	1 F/M	80		17	
	7	0	-	-	-	-
Feb. 1991	3	0	-	-	-	-
	4	0	-	-	-	-
	5	1 M	220		24.0	
	7	4 F/M	71 - 225	125.0 ± 69.8	16.9 - 23.5	19.5 ± 3.0

Female * Male • Female or male (fish immature)

TABLE 4.1B
LENGHTS AND WEIGHTS OF *BARBUS MAREQUENSIS* CAUGHT IN THE OLIFANTS RIVER (KNP) DURING THE PERIOD APR. 1991 - FEB. 1992

Month	Locality	N	Weight (g)		Length (cm)	
			Range	X ± SD	Range	X ± SD
Apr. 1991	3	6 F#	205 - 470	304.3 ± 94.2	23.6 - 29.0	25.9 ± 1.9
		2 M*	125 - 193	159.0 ± 48.1	20.0 - 22.4	21.2 ± 1.7
	4	9 F	33 - 470	161.4 ± 154.7	13.5 - 30.4	20.1 ± 6.3
		1 M	220		24.9	
	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	1 M	240		25.0	
		1 F/M	45		14.5	
June 1991	3	9 F	215 - 720	319.4 ± 154.6	23.0 - 33.3	25.7 ± 3.1
	4	1 F	475		28.7	
		6 M	230 - 360	309.2 ± 52.7	23.1 - 27.9	25.6 ± 2.0
	5	3 M	200 - 330	261.7 ± 65.3	22.7 - 26.3	24.5 ± 1.8
	7	0	-	-	-	-
Aug. 1991	3	1 F	400		27.2	
	4	8 F	290 - 550	404.8 ± 76.6	24.5 - 31.1	28.0 ± 1.9
	5	11 F	610 - 1110	872.7 ± 178.5	30.6 - 40.0	35.4 ± 3.0
		1 M	510		27.5	
	7	1 F/M	120		20.6	
Oct. 1991	3	4 F	390 - 793	540.5 ± 177.0	28.0 - 34.6	30.8 ± 2.8
		2 M	269 - 400	334.5 ± 92.6	25.0 - 27.2	26.1 ± 1.6
	4	9 F	155 - 889	603.9 ± 206.3	21.0 - 36.0	31.1 ± 4.4
		2 M	400 - 459	429.5 ± 41.7	26.8 - 28.5	27.7 ± 1.2
	5	12 F	474 - 800	655.9 ± 114.8	27.9 - 34.0	31.0 ± 1.9
		3 M	400 - 617	502.3 ± 109.0	28.5 - 31.0	29.7 ± 1.3
	7	1 F	188		23.0	
Jan. 1992	3	4 F	451 - 641	567.3 ± 84.2	29.1 - 32.0	30.7 ± 1.3
		2 F/M	117 - 148	132.5 ± 21.9	19.3 - 20.8	20.1 ± 1.1
	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	8 F	439 - 944	699.5 ± 163.9	29.2 - 34.7	32.4 ± 1.7
		4 M	368 - 520	456.0 ± 63.6	27.0 - 29.9	28.7 ± 1.3
	7	8 F/M	46 - 245	90.3 ± 69.1	14.1 - 25.5	17.8 ± 3.8
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	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	10 F	399 - 1211	659.9 ± 242.6	29.0 - 40.5	33.1 ± 3.6
	7	0	-	-	-	-
	Pionier	5 F	1035 - 1679	1408 ± 300.7	35.4 - 43.5	40.2 ± 3.5
	Dam	5 M	710 - 845	806.6 ± 55.9	33.3 - 34.6	34.2 ± 0.5

Female * Male • Female or male (fish immature)

ZINC BIOACCUMULATION IN THE DIFFERENT ORGANS AND TISSUES

The moisture content in the tissues differed, with the mean percentage of moisture being 79 ± 2% 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 zinc concentrations in the different organs and tissues (Table 4.2) were calculated on a dry weight basis. Large variation was detected between the tissue zinc concentrations of individuals at the same locality, e.g. zinc concentrations in the female gonads ranged from 107.4 µg/g to 483.6 µg/g Zn at locality 4 in October 1991 (Table 4.2). Variation was also detected between the zinc concentrations of the different tissues, but the bioaccumulation pattern of zinc in *B. marequensis* was determined to be: skin > gonads (F) > liver > hindgut contents > vertebrae > gills > kidney > hindgut ≈ foregut > gonads (M) > foregut contents > muscle > blood > fat > bile. Zinc concentrations in the skin and female gonads differed significantly ($p \leq 0.05$) from the zinc concentrations in all the other organs and tissues. However, no significant difference ($p > 0.05$) was detected between the various zinc concentrations in the muscle, blood, fat and bile (Table 4.3).

The bioconcentration factors between the tissues and water were mostly very high (Table 4.2), ranging from 2.2 for the bile (June 1991) to 15 760 for the skin (August 1991). The BFs between the tissues and sediment, on the other hand, were much lower and ranged from 0.02 for the bile (June 1991) to 32.7 for the skin (February 1992).

LOCALITY DIFFERENCES

In the first year (October 1990), locality 3 differed significantly from both localities 4 (with respect to liver and muscle zinc concentrations) and 5 (with respect to gill, liver and muscle zinc concentrations). In the second year locality 3 differed significantly from locality 4 in January 1992 (with respect to the blood zinc concentrations) and February 1992 (with respect to the muscle, blood fat and vertebrae zinc concentrations). Localities 3 and 5 differed significantly only in February 1992 with respect to the muscle, fat and vertebrae zinc concentrations, while localities 3 and 7 differed significantly in January 1992 with respect to the blood zinc concentrations. No differences occurred in June 1991, while in October 1991 only localities 4 and 5 differed significantly with respect to the muscle zinc concentrations. Localities 4 and 5 also differed in January 1992 with respect to the muscle and fat zinc concentrations. In February 1992, Pionier Dam differed significantly from all three localities: from locality 3 with respect to the muscle, from locality 4 with respect to the blood and from locality 5 with respect to the fat, muscle and vertebrae zinc concentrations.

SEASONAL DIFFERENCES

Generally, significant seasonal differences were detected (Table 4.4), but it was not always the same organs that indicated these seasonal differences. For instance, winter 1990 differed from summer 1992 with respect to the muscle, gill and liver zinc concentrations, while spring 1991 and summer 1992 only differed with respect to the liver zinc concentrations. No differences occurred, however, between spring 1990 and summer 1990/91, as well as between autumn 1991 and each of winter 1990, summer 1990/91 and winter 1991. Comparing the zinc concentrations in the organs of the males and females seasonally, a difference was noticed in some organs. The females had higher zinc concentrations than the males in the gonads, liver, hindgut, kidney and bile, while the males had higher zinc concentrations in the vertebrae (Figures 4.1 and 4.2). The zinc concentrations in the skin were higher in the females in winter 1991 and summer 1992, but in spring 1991 the males had a concentration of 295 µg/g Zn (dry weight) compared to the 213 µg/g Zn (dry weight) of the females (Fig. 4.1).

ANNUAL DIFFERENCES

Not all the organs and tissues were sampled during the first year, but by comparing the mean zinc concentrations in the organs and tissues, as well as in the gut contents, of the second year (Fig. 4.3), the bioaccumulation pattern was as follows: skin > gonads (F) > liver > gills > vertebrae > gonads (M) > hindgut > foregut contents > kidney ≈ foregut > hindgut contents > muscle > blood > fat > bile.

TABLE 4.2

MEAN ZINC CONCENTRATIONS (µg/g dry wt) IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* (BF_W AND BF_S = BIOCONCENTRATION FACTORS OF THE WATER AND SEDIMENT RESPECTIVELY)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle
Apr. '90	3	n®						1
		Range						252.2
		Median						
		Mean	N/A	N/A	N/A	N/A	N/A	
		SD^Δ						
		BFw						163.8
		BFs						3.6
	4	n	4	3				5
		Range	138.5-188.5	285.7-471.4				95.6-178.3
		Median	151.9	357.1				113.0
		Mean	157.7	371.4	N/A	N/A	N/A	131.3
		SD	21.5	93.7				34.6
		BFw	262.8	619.0				218.8
		BFs	5.7	13.5				4.8
	7	n	7				4	9
		Range	119.2-265.4				107.4-281.5	73.9-165.2
		Median	176.9				231.5	113.0
		Mean	186.3	N/A	N/A	N/A	213.0	115.9
		SD	53.2				74.6	27.0
		BFw	128.5				146.9	79.9
		BFs	1.8				2.0	1.1
June '90	3	n	2					2
		Range	73.1-103.9					100.0-113.0
		Median	88.5					106.5
		Mean	88.5	N/A	N/A	N/A	N/A	106.5
		SD	21.8					9.2
		BFw	72.5					87.3
		BFs	1.0					1.2
Aug. '90	3	n	8	2	1	1	2	9
		Range	115.4-153.9	392.9-428.6	142.9	22.5	111.1-125.9	43.5-87.0
		Median	115.4	410.7			118.5	87.0
		Mean	124.5	410.7			118.5	77.3
		SD	15.9	25.3			10.5	19.1
		BFw	778.1	158.1	893.1	140.6	740.6	483.1
		BFs	2.3	0.5	2.7	0.4	2.2	1.5
	4	n	9	4	3	4	6	9
		Range	107.7-153.9	292.9-500.0	147.6-190.5	16.3-28.6	74.1-114.8	43.5-130.4
		Median	115.4	410.7	190.5	20.4	79.6	82.6
		Mean	125.6	403.6	176.2	21.4	88.9	72.0
		SD	18.0	86.2	24.7	5.1	19.2	30.5
		BFw	483.1	1552.3	677.7	82.3	341.9	276.9
		BFs	2.6	8.4	3.7	0.4	1.9	1.5
	5	n	7	1	2	3	7	6
		Range	76.9-307.7	778.6	95.2-142.9	20.4-61.2	74.1-333.3	43.5-130.4
		Median	115.4		119.1	20.4	74.1	87.0
		Mean	148.4		119.1	34.0	116.4	87.0
		SD	75.1		33.7	23.6	96.6	27.5
		BFw	494.7	2595.3	397.0	113.3	388.0	290.0
		BFs	3.2	16.6	2.5	0.7	2.5	1.9
	7	n						5
		Range						69.6-100.0
		Median						87.0
		Mean	N/A	N/A	N/A	N/A	N/A	86.1
		SD						10.8
		BFw						253.2
		BFs						1.4

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 4.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Gut	Gut cont	Blood
Oct. '90	3	n[Φ]	7	2	1	2	6	7			
		Range	146.2-219.2	178.6-200.0	181.0	59.2-79.6	129.6-192.6	121.7-243.5			
		Median	173.1	189.3		69.4	168.5	173.9			
		Mean	178.0	189.3		69.4	166.7	180.7	N/A	N/A	N/A
		SD[Δ]	26.1	15.2		14.4	22.5	37.5			
		BFw	613.8	652.8	624.1	239.3	574.8	623.1			
		BFs	5.9	6.3	6.0	2.3	5.6	6.0			
	4	n	10	1	7	6	9	10			
		Range	96.2-192.3	314.3	90.5-171.4	26.5-79.6	29.6-155.6	26.1-134.8			
		Median	138.5		142.9	55.1	70.4	80.4			
		Mean	141.2		134.7	55.4	81.9	79.6	N/A	N/A	N/A
		SD	31.9		31.8	21.1	40.0	32.7			
		BFw	344.4	766.6	328.5	135.1	199.8	194.1			
		BFs	2.5	5.6	2.4	1.0	1.5	1.4			
	5	n	9	1	6	9	9	10			
		Range	111.5-165.4	210.7	90.5-171.4	16.3-44.9	59.3-118.5	47.8-117.4			
		Median	134.6		159.5	26.5	81.5	76.1			
		Mean	133.8		139.7	29.5	86.4	81.7	N/A	N/A	N/A
		SD	15.2		38.4	11.7	16.9	22.1			
		BFw	495.6	780.4	517.4	109.3	320.0	302.6			
		BFs	4.6	7.3	4.8	1.0	3.0	2.8			
	7	n	1	1		1	1	1			
		Range	196.2	181.0		55.1	118.5	130.4			
		Median									
		Mean			N/A				N/A	N/A	N/A
		SD									
		BFw	445.9	411.4		125.2	269.3	296.4			
		BFs	6.1	5.7		1.7	3.7	4.1			
Dec. '90	3	n	7	3	1	3	2	7			
		Range	65.4-207.7	107.1-235.7	90.5	10.2-44.9	92.6-159.3	43.5-104.4			
		Median	180.8	132.1		30.6	125.9	69.6			
		Mean	165.9	158.3		28.6	125.9	72.1	N/A	N/A	N/A
		SD	47.4	68.2		17.4	47.1	25.3			
		BFw	404.6	386.1	220.7	69.8	307.1	175.9			
		BFs	9.5	9.0	5.2	1.6	7.2	4.1			
	5	n	1					1			
		Range	250.0					104.4			
		Median									
		Mean		N/A	N/A	N/A	N/A		N/A	N/A	N/A
		SD									
		BFw	8333.3					3480.0			
		BFs	10.6					4.4			
Feb. '91	5	n	1		1			1	1	1	1
		Range	134.7		65.5			51.6	56.8	73.3	11.5
		Median									
		Mean		N/A		N/A	N/A				
		SD									
		BFw	3367.5		1637.5			1290.0	1420.0		287.5
		BFs	5.4		2.6			2.1	2.3		0.5
	7	n	2					2		1	6
		Range	93.5-100.0					32.2-48.3		85.4	14.8-21.6
		Median	96.7					40.3			16.3
		Mean	96.7	N/A	N/A	N/A	N/A	40.3	N/A		17.4
		SD	4.6					11.4			3.0
		BFw	2417.5					1007.5			435.0
		BFs	11.4					4.7			2.0

Φ Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 4.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	Gut cont.	Vertebrae	Bile	Blood
Apr. '91	3	n[①]	3	4	1	1	3	6		2	1	3			8
		Range	145.0-284.7	90.6-150.4	93.4	47.1	96.8-103.4	40.2-70.8		63.0-108.2	73.1	120.5-131.7			12.8-62.3
		Median	165.5	120.5			97.9	55.8		85.6		128.5			15.2
		Mean	198.4	120.5			99.4	55.5	N/A	85.6		126.9	N/A	N/A	23.3
		SD[Δ]	75.4	27.1			3.6	11.2		32.0		5.8			17.7
		BFw	1803.6	1095.5	849.1	428.2	903.6	504.5		778.2	664.5				211.8
		BFs	3.9	2.4	1.8	0.9	1.9	1.1		1.7	1.4				0.5
	4	n	3		1	2	2	6		1		2			10
		Range	151.1-203.3		98.3	26.0-35.0	98.5-214.4	40.2-68.4		90.0		126.1-176.6			10.4-53.0
		Median	177.2			30.5	156.4	62.6				151.3			15.5
		Mean	177.2	N/A		30.5	156.4	60.1	N/A		N/A	151.3	N/A	N/A	20.1
		SD	26.1			6.3	81.9	10.1				35.8			12.4
		BFw	1107.5		614.4	190.6	977.5	375.6		562.5					125.6
		BFs	12.2		6.8	2.1	10.8	4.1		6.2					1.4
	5	n	4	5		3	3	7		3	2	3			10
		Range	116.9-165.9	91.0-128.8		21.9-25.6	98.3-132.6	48.3-80.9		79.8-112.7	81.7-111.8	100.5-140.3			11.8-18.1
		Median	150.4	104.6		24.2	118.1	59.6		92.8	96.7	132.5			14.2
		Mean	145.9	109.5	N/A	23.9	116.8	61.7	N/A	95.1	96.7	124.4	N/A	N/A	14.4
		SD	23.3	15.9		1.9	16.4	10.1		16.6	21.3	21.1			2.2
		BFw	4863.3	3650.0		796.7	3893.3	2056.7		3170.0	3223.3				480.0
		BFs	22.4	16.8		3.7	18.0	9.5		14.6	14.9				2.2
	7	n	1		1	1	1	1				1			1
		Range	120.6		58.4	14.9	103.8	51.6				111.7			26.8
		Median													
		Mean		N/A					N/A	N/A	N/A		N/A	N/A	
		SD													
		BFw	3015.0		1460.0	372.5	2595.0	1290.0							670.0
		BFs	6.7		3.2	0.8	5.8	2.9							1.5
June '91	3	n	9	3		9	9	9	9	8	2	8	9		9
		Range	82.6-121.4	92.7-172.3		6.1-23.8	51.4-87.5	27.8-42.9	67.0-127.8	64.6-84.8	69.1-76.5	69.4-178.1	85.6-133.8		11.1-54.7
		Median	91.9	98.5		8.5	75.4	31.7	87.6	73.8	72.8	108.9	103.2		14.7
		Mean	93.9	121.2	N/A	12.7	74.2	34.2	95.3	73.8	72.8	117.3	108.9	N/A	19.2
		SD	11.6	44.4		7.1	11.3	5.5	23.7	6.5	5.3	39.5	17.5		13.5
		BFw	391.2	505.0		52.9	309.2	142.5	397.1	307.5	303.3		453.7		80.0
		BFs	1.6	2.1		0.2	1.3	0.6	1.6	1.3	1.2		1.8		0.3
	4	n	7	1	6	6	7	7	4	7		7	7	1	7
		Range	76.1-110.0	106.6	49.5-138.3	1.4-28.8	47.2-76.1	19.2-38.7	105.9-263.4	67.7-93.5		43.5-161.5	91.3-115.3	0.5	13.0-28.7
		Median	84.8		87.5	3.9	61.7	25.9	129.5	72.6		125.0	98.5		14.5
		Mean	87.8		91.0	7.8	62.9	27.6	157.1	74.7	N/A	115.9	100.7		16.3
		SD	12.9		37.7	10.5	10.0	6.1	72.1	8.7		39.7	7.9		5.6
		BFw	381.7	463.5	395.7	33.9	273.5	120.0	683.0	324.8			437.8	2.2	70.9
		BFs	4.3	5.2	4.4	0.4	3.1	1.3	7.7	3.6			4.9	0.02	0.8
	5	n	3		3	3	3	3	3	3	1	2	3		3
		Range	85.0-125.2		71.9-94.1	6.6-14.6	62.4-68.8	29.8-43.3	86.8-105.8	73.9-76.7	78.3	100.4-150.6	97.1-124.7		11.6-13.8
		Median	95.6		73.2	8.8	65.4	40.1	97.4	74.8		125.5	109.9		12.2
		Mean	102.0	N/A	79.7	10.0	65.5	37.7	96.7	75.1		125.5	110.6	N/A	12.5
		SD	20.8		12.5	4.1	3.2	7.1	9.5	1.4		35.5	13.8		1.1
		BFw	1133.3		885.6	111.1	727.8	418.9	1074.4	834.4	870.0		1228.9		138.9
		BFs	7.0		5.5	0.7	4.5	2.6	6.7	5.2	5.4		7.6		0.9

① Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 4.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont	HGut cont	Vertebrae	Kidney	Bile	Blood
Aug. '91	3	n®	1	1		1	1	1	1	1		1		1			1
		Range	100.5	395.2		15.3	105.2	30.4	196.7	88.1		73.9		94.6			15.1
		Median															
		Mean			N/A						N/A		N/A		N/A	N/A	
		SD^Δ															
		BFw	344.2	1353.4		52.4	360.3	104.1	673.6	301.7				324.0			51.7
		BFs	2.6	10.4		0.4	2.8	0.8	5.2	2.3				2.5			0.4
	4	n	8	5		8	8	8	7	7		5	5	8	2		8
		Range	87.0-118.6	102.6-365.7		9.2-42.7	82.7-128.0	25.8-90.8	119.0-250.0	70.1-91.0		37.6-243.5	32.5-420.4	84.0-150.6	58.9-70.7		14.6-18.3
		Median	95.1	213.1		19.0	108.0	33.9	172.2	84.3		70.0	79.7	96.5	64.8		15.8
		Mean	99.3	224.6	N/A	21.8	106.5	42.7	176.9	83.0	N/A	96.1	139.5	102.0	64.8	N/A	16.0
		SD	9.8	110.1		12.1	15.7	20.9	44.6	6.5		84.5	160.6	20.7	8.3		1.2
		BFw	4137.5	9358.3		908.3	4437.5	1779.2	7370.8	3458.3				4250.0	2700.0		666.7
		BFs	2.3	5.2		0.5	2.5	1.0	4.1	1.9				2.4	1.5		0.4
	5	n	12	11	1	12	12	12	12	10	4	8	8	12	7	4	12
		Range	70.4-238.7	79.8-162.8	397.8	4.4-17.4	38.5-116.9	22.6-80.7	109.5-269.4	63.1-238.8	87.6-96.0	25.4-88.2	42.9-104.3	79.8-109.1	72.7-114.7	5.0-6.8	12.2-20.0
		Median	86.3	92.5		9.5	86.2	33.6	162.4	79.7	90.7	70.1	78.3	90.8	94.3	6.5	17.6
		Mean	97.0	98.3		10.3	86.0	38.1	173.0	95.9	91.3	67.3	79.9	92.3	91.5	6.2	17.5
		SD	45.4	23.2		3.9	21.8	16.1	56.8	51.6	3.5	19.3	21.4	7.8	14.2	0.8	2.1
		BFw	3464.3	3510.7		367.9	3071.4	1360.7	6178.6	3425.0	3260.7			3296.4	3267.9	221.4	625.0
		BFs	5.5	5.6		0.6	4.9	2.2	9.9	5.5	5.2			5.3	5.2	0.4	1.0
	7	n	1			1	1	1	1	1				1		1	1
		Range	111.2			73.5	87.0	41.5	394.0	82.7				119.8		18.3	26.8
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A		N/A		
		SD															
		BFw	4448.0			2940.0	3480.0	1660.0	15760.0	3308.0				4792.0		732.0	1072.0
		BFs	5.1			3.3	4.0	1.9	17.9	3.8				5.4		0.8	1.2
Oct. '91	3	n	6	2	1	6	5	6	6	5	3	2	3	6	3	5	6
		Range	69.6-94.5	390.9-548.1	81.1	3.7-18.2	83.8-197.8	20.2-29.2	178.1-356.3	79.7-89.8	73.1-97.8	68.7-80.9	79.0-143.9	79.4-118.5	75.4-87.3	1.8-6.6	12.2-15.2
		Median	79.3	469.5		6.4	112.0	22.4	254.6	88.5	87.6	74.8	112.2	87.0	81.8	4.4	13.1
		Mean	80.6	469.5		8.1	121.9	23.2	257.9	86.8	86.1	74.8	111.7	90.9	81.5	4.3	13.5
		SD	9.1	111.2		5.2	44.2	3.1	64.7	4.1	12.4	8.6	32.4	14.0	5.9	1.8	1.4
		BFw	959.5	5589.3	965.5	96.4	1451.2	276.2	3070.2	1033.3	1025.0			1082.1	970.2	51.2	160.7
		BFs	4.2	24.7	4.3	0.4	6.4	1.2	13.6	4.6	4.5			4.8	4.3	0.2	0.7
	4	n	11	7	1	11	8	11	11	5	2	9	4	11	3	6	10
		Range	70.2-100.7	107.4-483.6	67.0	3.9-46.0	80.4-150.2	19.7-40.1	164.7-326.7	72.3-89.4	70.7-87.4	24.9-87.0	28.5-53.3	76.0-108.9	75.9-96.0	2.4-16.6	13.1-21.5
		Median	81.9	113.8		6.7	95.7	24.6	232.5	74.4	79.1	40.4	40.5	78.6	76.9	7.2	16.5
		Mean	83.6	185.8		10.5	107.1	27.2	238.8	76.9	79.1	49.6	40.8	85.6	82.9	8.9	16.2
		SD	10.0	136.3		12.0	26.7	7.3	44.9	7.0	11.8	22.7	9.0	12.0	11.3	6.3	2.4
		BFw	1286.2	2858.5	1030.8	161.5	1647.7	418.5	3673.8	1183.1	1216.9			1316.9	1275.4	136.9	249.2
		BFs	4.6	10.2	3.7	0.6	5.9	1.5	13.0	4.2	4.3			4.7	4.5	0.5	0.9
	5	n	14	9	4	15	13	15	14	3	3	10	4	15	5	9	10
		Range	77.9-129.7	110.2-512.6	33.4-130.0	2.6-8.8	78.5-122.0	17.9-28.1	146.0-292.1	75.8-90.9	72.1-93.5	34.0-88.5	27.7-80.2	77.0-97.3	51.4-116.8	3.3-17.7	13.3-20.6
		Median	91.7	144.1	43.3	4.3	96.1	19.8	205.3	82.4	76.4	40.2	39.5	82.2	84.6	4.9	15.7
		Mean	95.6	196.0	62.5	4.5	100.2	20.9	209.2	83.0	80.7	49.7	46.7	82.7	83.6	6.5	16.1
		SD	14.2	128.4	45.7	1.8	14.8	2.9	45.9	7.6	11.4	18.3	23.6	5.4	25.9	4.5	2.3
		BFw	2655.6	5444.4	1736.1	125.0	2783.3	580.6	5811.1	2305.6	2241.7			2297.2	2322.2	180.6	447.2
		BFs	3.5	7.2	2.3	0.2	3.7	0.8	7.7	3.1	3.0			3.0	3.1	0.2	0.6
	7	n	1			1	1	1	1	1				1	1	1	1
		Range	92.3			20.2	75.9	38.7	464.6	83.8				122.6	125.3	2.7	13.0
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A				
		SD															
		BFw	1846.0			404.0	1518.0	774.0	9292.0	1676.0				2452.0	2506.0	54.0	260.0
		BFs	4.2			0.9	3.5	1.8	21.2	3.8				5.6	5.7	0.1	0.6

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 4.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont.	HGut cont.	Vertebrae	Kidney	Bile	Blood
Jan. '92	3	n[Φ]	5	4		5	3	6	2	2	2	2	2	6	1	5	6
		Range	85.0-128.3	96.5-337.5		5.1-18.5	93.9-150.0	21.7-34.3	280.3-390.0	91.0-98.2	94.3-102.0	70.3-112.4	65.5-86.9	75.1-101.1	76.8	10.8-84.4	12.0-14.2
		Median	86.1	181.8		8.9	115.5	22.6	335.1	94.6	98.1	91.4	76.2	84.3		22.6	13.2
		Mean	94.8	199.4	N/A	10.4	119.8	24.8	335.1	94.6	98.1	91.4	76.2	87.1		32.0	13.1
		SD[Δ]	18.8	102.6		5.1	28.3	4.9	77.6	5.0	5.4	29.8	5.2	10.0		30.5	0.9
		BFw	585.2	1230.9		64.2	739.5	153.1	2068.5	584.0	605.6			537.7	474.1	197.5	80.9
		BFs	3.0	6.2		0.3	3.7	0.8	10.5	3.0	3.1			2.7	2.4	1.0	0.4
	4	n	4	3		10	2	11	3	1	1	1	3	9	1	2	11
		Range	80.2-105.0	98.6-121.6		9.5-25.6	133.1-147.2	23.0-34.9	216.8-360.0	96.2	103.8	60.9	70.4-100.1	73.4-123.7	90.8	11.8-23.8	14.8-17.7
		Median	92.5	107.7		15.7	140.1	30.5	228.0				99.8	101.7		17.8	16.4
		Mean	92.5	109.3	N/A	16.1	140.1	29.7	268.3				90.1	103.1		17.8	16.2
		SD	13.1	11.6		4.8	9.9	3.7	79.6				17.0	14.2		8.5	1.0
		BFw	1170.9	1383.5		203.8	1773.4	375.9	3396.2	1217.7	1313.9			1305.1	1149.4	225.3	205.1
		BFs	1.4	1.6		0.2	2.1	0.4	4.0	1.5	1.6			1.6	1.4	0.3	0.2
	5	n	12	8	4	12	9	12	11	3	3	2	1	12	3	9	12
		Range	72.9-113.6	80.5-197.0	52.1-59.4	4.2-14.4	97.6-165.6	15.8-32.5	160.9-301.0	79.8-101.0	85.4-114.6	33.8-97.1	131.2	70.6-108.8	52.7-109.2	3.5-17.1	12.1-19.6
		Median	91.7	112.3	54.5	6.6	118.9	19.6	225.2	80.7	102.4	65.5		84.1	62.7	8.7	14.9
		Mean	92.7	123.9	55.1	7.8	124.7	22.1	219.6	87.1	100.8	65.5		85.1	74.8	9.3	15.1
		SD	13.1	36.4	3.1	3.1	24.2	5.4	39.6	12.0	14.7	44.8		11.8	30.1	4.5	2.6
		BFw	1891.8	2528.6	1124.5	159.2	2544.9	451.0	4481.6	1777.6	2057.1			1736.7	1526.5	189.8	308.2
		BFs	3.4	4.6	2.0	0.3	4.6	0.8	8.1	3.2	3.7			3.1	2.8	0.3	0.6
	7	n	1			5		5		1				5			5
		Range	149.0			10.6-22.1		25.6-39.9		97.7				98.9-132.6			14.5-21.8
		Median				10.9		39.1						114.3			17.5
		Mean		N/A	N/A	13.8	N/A	34.3	N/A		N/A	N/A	N/A	115.5	N/A	N/A	18.0
		SD				5.0		7.3						14.2			2.9
		BFw	1795.2			166.3		413.3		1177.1				1391.6			216.9
		BFs	3.4			0.3		0.8		2.2				2.6			0.4
Feb. '92	3	n	2			6		6	3					6			6
		Range	113.5-120.8			7.9-16.0		28.5-41.3	285.3-343.8					97.2-112.3			13.5-26.6
		Median	117.1			11.8		35.0	291.6					108.6			17.7
		Mean	117.1	N/A	N/A	11.9	N/A	35.7	306.9	N/A	N/A	N/A	N/A	107.2	N/A	N/A	18.6
		SD	5.1			2.9		4.7	32.1					5.6			4.7
		BFw	2723.3			276.7		830.2	7137.2					2493.0			432.6
		BFs	3.1			0.3		0.9	8.0					2.8			0.5
	4	n	6	4		10	7	10	8	1	1	1	1	10	1	9	10
		Range	75.2-99.2	106.0-149.7		4.7-13.4	134.6-205.0	21.3-34.6	167.0-325.4	96.8	94.8	75.0	61.3	78.6-112.5	115.1	1.4-15.3	10.5-14.6
		Median	87.1	134.8		6.6	157.8	22.8	258.5					93.2		6.6	12.9
		Mean	86.9	131.3	N/A	7.6	163.2	24.6	247.8					93.1		6.5	12.6
		SD	8.9	20.5		2.5	28.8	4.0	53.4					9.3		4.9	1.4
		BFw	1241.4	1875.7		108.6	2331.4	351.4	3540.0	1382.9	1354.3			1330.0	1644.3	92.9	180.0
		BFs	1.7	2.6		0.1	3.2	0.5	4.8	1.9	1.9			1.8	2.2	0.1	0.2
	5	n	10	10		10	8	10	10	2	2			10	2	10	10
		Range	72.3-92.3	92.4-312.3		5.0-14.1	115.8-186.5	18.9-28.1	213.2-337.6	97.6-99.0	83.5-101.5			73.3-94.0	79.3-119.4	3.2-27.8	13.7-21.0
		Median	82.8	149.4		6.5	140.2	21.4	257.9	98.3	92.5			86.6	99.3	5.4	15.1
		Mean	83.0	168.9	N/A	7.2	148.1	22.0	264.8	98.3	92.5	N/A	N/A	86.5	99.3	8.4	16.0
		SD	5.6	72.7		2.7	27.1	2.7	45.5	1.0	12.7			6.6	28.3	7.4	2.7
		BFw	2243.2	4564.9		194.6	4002.7	594.6	7156.8	2656.8	2500.0			2337.8	2683.8	227.0	432.4
		BFs	10.2	20.9		0.9	18.3	2.7	32.7	12.1	11.4			10.7	12.3	1.0	2.0
	Pionier Dam	n	10	5	5	10	9	10	10	6	2	6		10	4	5	10
		Range	70.1-102.4	111.2-171.2	44.7-57.8	6.6-13.1	72.1-130.2	20.6-30.2	100.3-263.1	80.2-102.6	96.1-105.3	23.9-118.4		82.7-120.0	96.4-109.5	1.9-3.6	14.4-20.6
		Median	88.4	137.8	51.2	9.1	112.3	26.1	194.7	83.2	100.7	66.3		96.3	107.6	3.0	17.1
		Mean	88.9	137.4	50.6	9.5	106.3	25.6	195.9	85.7	100.7	66.9	N/A	99.1	105.2	2.7	16.8
		SD	10.0	22.8	5.2	2.5	23.0	3.3	45.2	8.5	6.6	30.2		12.8	6.0	0.7	2.1
		BFw	1559.6	2410.5	887.7	166.7	1864.9	449.1	3436.8	1503.5	1766.7			1738.6	1845.6	47.4	294.7
		BFs	2.1	3.3	1.2	0.2	2.6	0.6	4.7	2.1	2.4			2.4	2.5	0.1	0.4

Φ Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 4.3

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE ZINC 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	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	SP2, S2												
Gonad (Males)		SP2, S2											
Fat	W2, SP2, S2	W2, SP2, S2	W2										
Liver	S2	W2, SP2	SP2, S2	W2, SP2, S2									
Muscle	W2, SP2, S2	W2, SP2, S2	W2		W2, SP2, S2								
Skin	W2, SP2, S2	SP2, S2	SP2, S2	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2							
Gut		W2, SP2		W2, SP2, S2		W2, SP2	W2, SP2, S2						
Gut cont.	SP2	SP2		W2, SP2	SP2, S2	W2	SP2, S2						
Vertebrae		SP2, S2		W2, SP2, S2	S2	W2, SP2, S2	W2, SP2, S2		SP2				
Kidney		SP2					S2						
Bile	SP2, S2	SP2, S2			SP2, S2		SP2, S2	SP2, S2	S2	S2	S2		
Blood	W2, SP2, S2	W2, SP2, S2	W2		W2, SP2, S2		W2, SP2, S2	W2, SP2	W2, SP2	W2, SP2, S2			

TABLE 4.4

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN ZINC CONCENTRATIONS IN THE MUSCLE (M), GILL (G), LIVER (L), VERTEBRAE (V), SKIN (S) AND BLOOD (B) OF *BARBUS MAREQUENSIS* FOR THE SEXES COMBINED (*), AS WELL AS FOR MALES AND FEMALES SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Autumn 1990	Winter 1990	Spring 1990	Summer 1990/91	Autumn 1991	Winter 1991	Spring 1991	Summer 1992
Autumn 1990	Female →	M	M	M	M	M, G	M, G	M, G
	Male →							
Winter 1990	M*	Female →	L			M, G	M, G	M, G, L
		Male →				M	M	M
Spring 1990	M*	M*	Female →			M, G, L	M, G	M, G
			Male →			M, G	M	M
Summer 1990/91	M*	G*		Female →		G	G	G
				Male →				
Autumn 1991	M*		M*		Female →		M	M
					Male →			
Winter 1991	M*,G*	M*,G*	M*,G*	M*,G*		Female →	S, V	L, S, V
						Male →	S	S
Spring 1991	M*,G*	M*,G*	M*,G*	M*,G*	M*,G*	S*,V*	Female →	L
							Male →	
Summer 1992	M*,G*	M*,G*,L*	M*,G*	M*,G*	M*,G*	L*.S*,V*	L*	

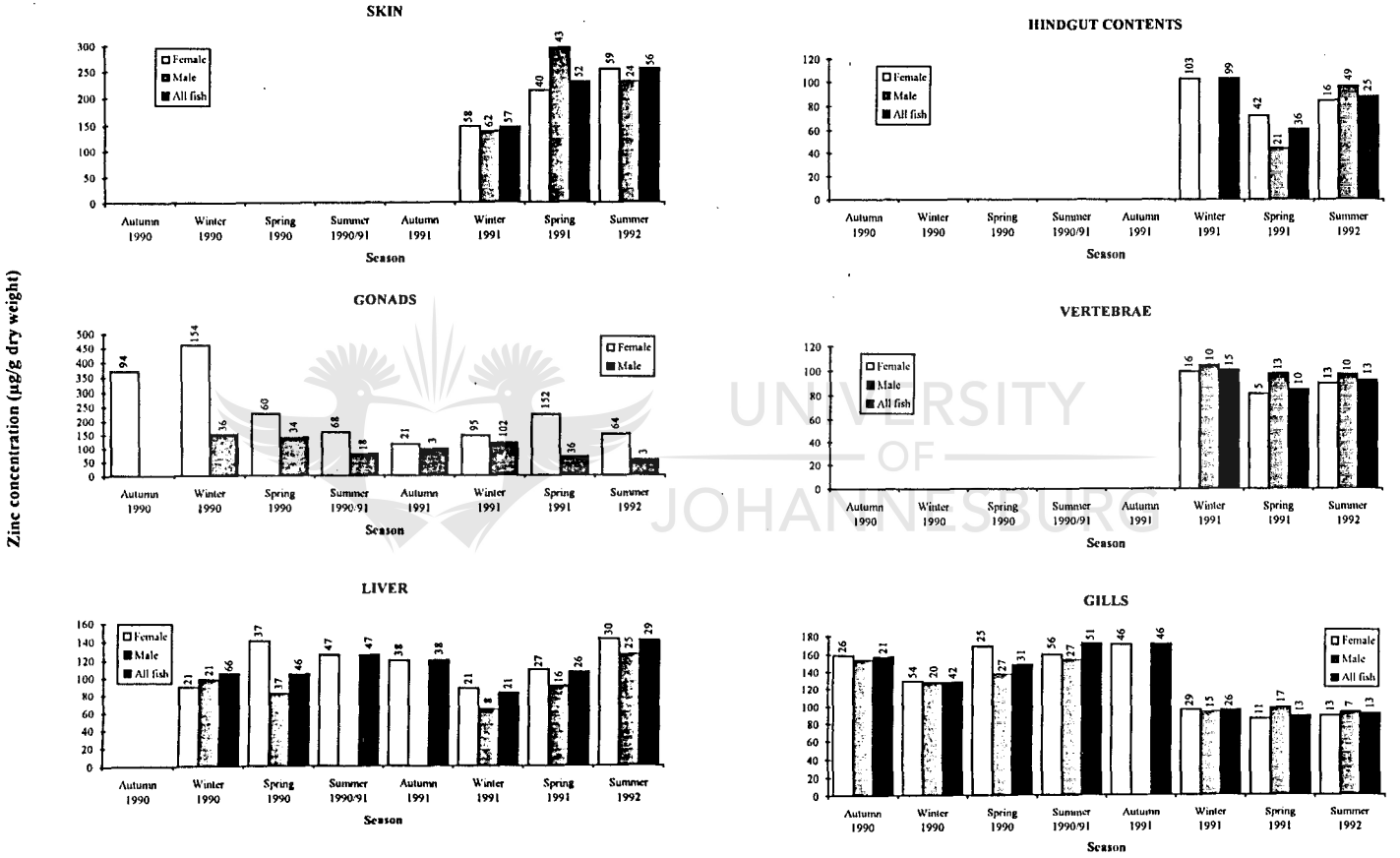

Figure 4.1

Mean seasonal zinc concentrations (µg/g dry wt.) in the skin, gonads, liver, hindgut contents, vertebrae and gills of Barbus marequensis *for males and females seperately, as well as the sexes combined. (Standard deviations are indicated above each bar)*

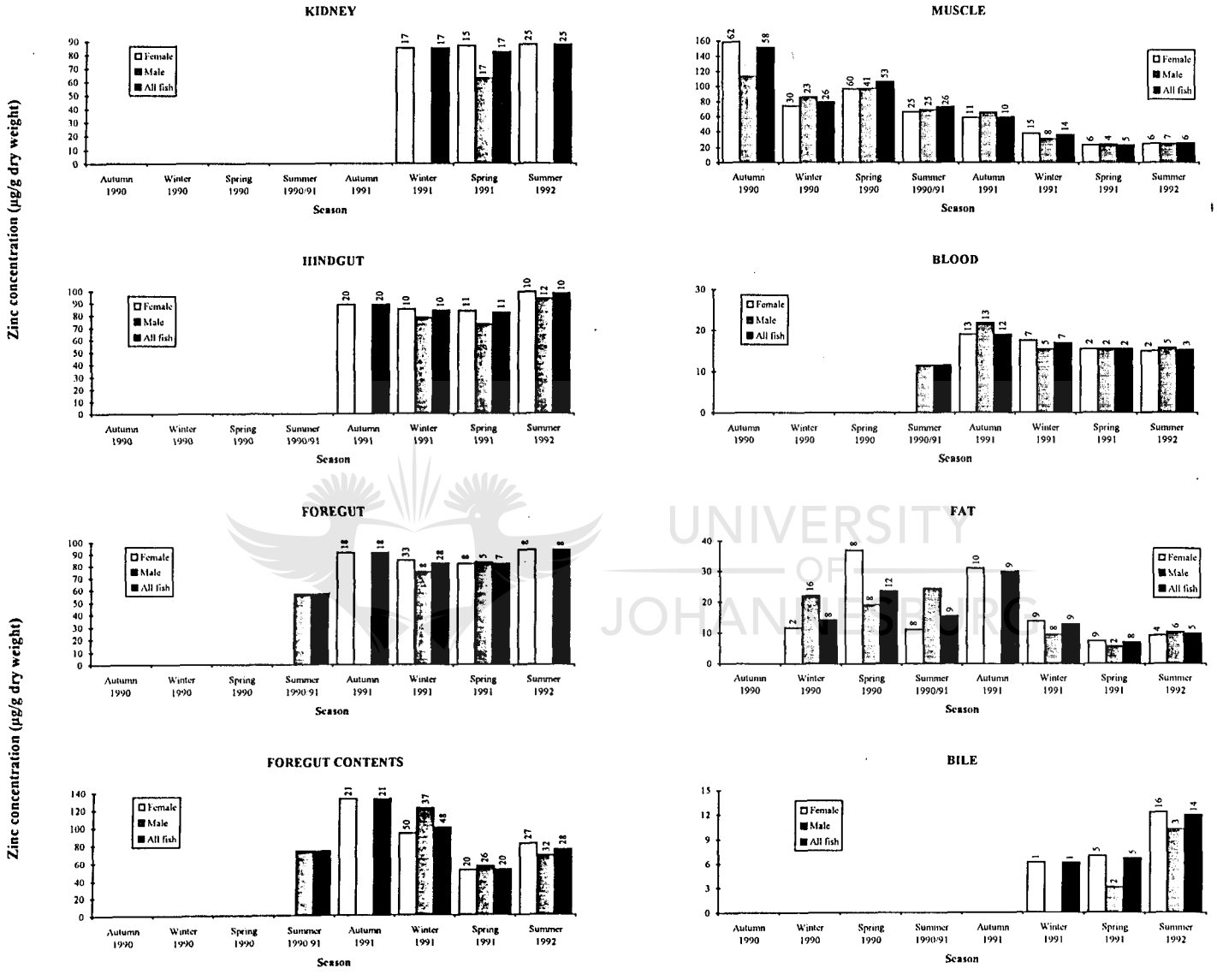

Figure 4.2

Mean seasonal zinc concentrations (µg/g dry wt.) in the kidney, hindgut, foregut, foregut contents, muscle, blood, fat and bile of Barbus marequensis *for males and females seperately, as well as the sexes combined. (Standard deviations are indicated above each bar)*

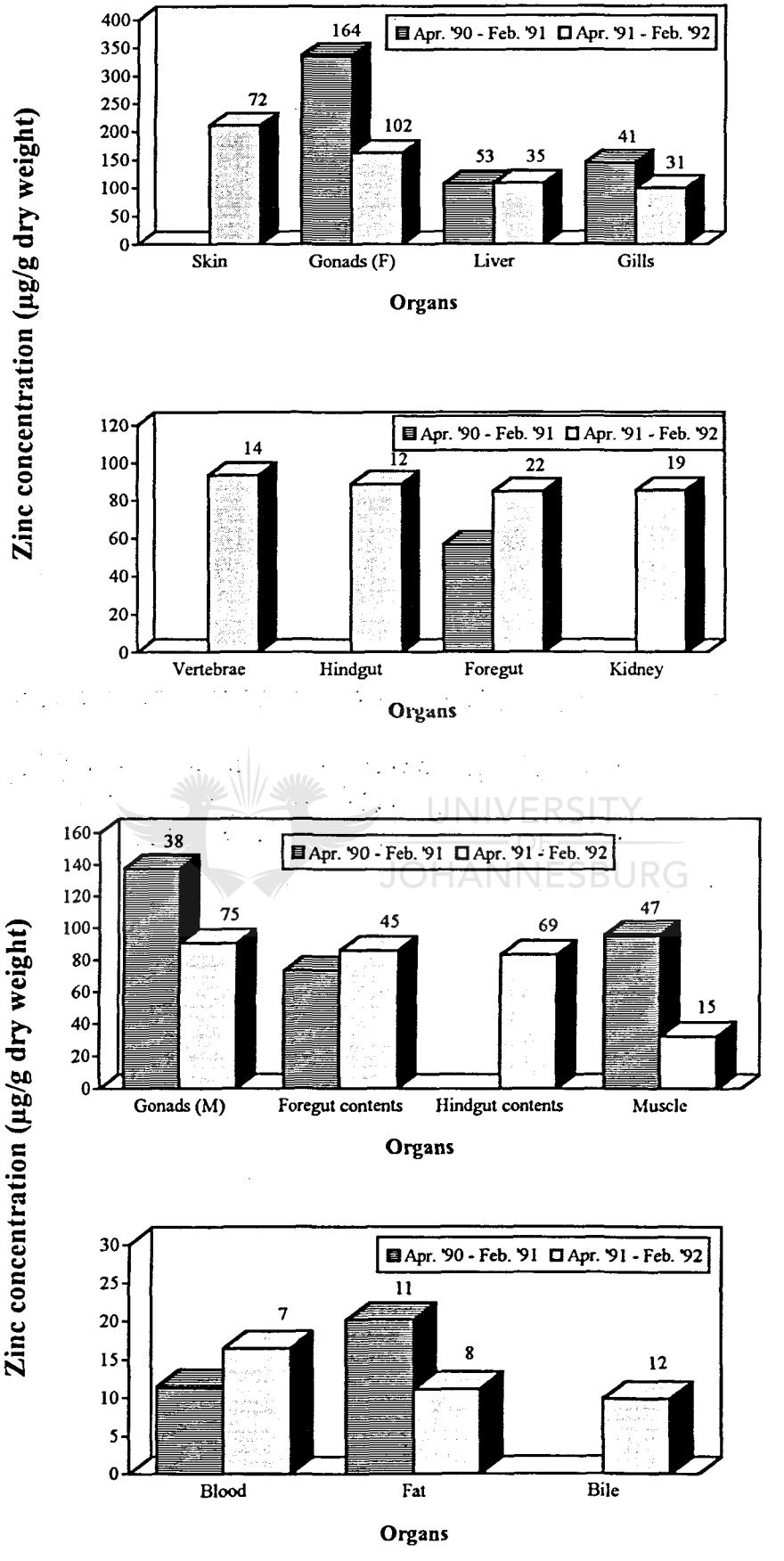

Figure 4.3

Mean zinc 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)*

This pattern is slightly different from the one already mentioned (based on the monthly data), but the skin, female gonads and liver still accumulated the highest zinc concentrations, while the muscle, blood, fat and bile accumulated the lowest.

The first and second year differed significantly with respect to the zinc concentrations in the gills, gonads and muscle, but not with respect to the liver zinc concentrations (Fig. 4.3).

4.4. Discussion

ZINC BIOACCUMULATION IN THE DIFFERENT ORGANS AND TISSUES

The large variation in zinc concentration between the individual fish suggests that the number of fish sampled at each locality should be increased to at least 20 to 30 individuals, but in order to still conserve the fish species, the number of sampling sites will need to be decreased. The size of the fish is also important, for one gram of dried tissue is necessary for accurate and reliable metal analysis with the atomic absorption spectrophotometer. The removal of external "surface" water from wet tissues will affect the determination of the actual metal concentration in the tissue, thereby increasing the experimental error. Furthermore, since the moisture contents of individual tissues differ from one another, as well as from one individual to the next, it is suggested that working on a dry weight basis instead of a wet weight basis would decrease variation.

The zinc concentrations in the tissues of *B. marequensis* (recorded in summer 1992 for the Olifants River, KNP) were generally lower than the summer 1988/89 zinc concentrations in the tissues of *Clarias gariepinus*, recorded by Bezuidenhout *et al.* (1990) for the industrial and mine polluted Germiston lake in the Transvaal. The only tissues of *B. marequensis* that had similar or higher zinc concentrations than *C. gariepinus* had, were the liver, gonads and vertebrae. Ignoring for a moment species differences, it seems that *C. gariepinus* was exposed to higher zinc levels than *B. marequensis*, although the higher liver and vertebrae zinc concentrations of *B. marequensis* might suggest chronic zinc exposure at a lower level.

Bioconcentration factors are not readily available in literature, making it difficult to compare data on this basis. Saltes & Bailey (1984) did, however, record factors of 9708X and 3835X for the gill and liver tissues respectively, which is higher than or similar to the factors determined in this study. On the other hand, the BFs recorded by Du Preez & Steyn (1992) were lower than a hundred, which are much lower than the BFs determined in this study. However, the BFs recorded by Du Preez & Steyn (1992) were based on wet tissue zinc concentrations and not dry tissue zinc concentrations. The high water bioconcentration factors (BF_W) determined in this study suggest a high degree of zinc bioavailibility to the fish. But these factors only represent the ratio of the metal concentration in the fish to the total (not bioavailable) concentration in the water. In hard water systems, as in the case of the Olifants River, metals will be less available for uptake by the fish. This aspect, as well as the fact that zinc is being regulated in the fish and therefore mostly independent of concentrations in the water (Wiener & Giesy, 1979), are not taken into consideration in the BF formula. Therefore, in this discussion more emphasis will be placed on the actual concentrations in the organs than on the BFs.

Zinc is primarily taken up by the intestine of the fish *via* the food (Pentreath, 1973; Willis & Sunda, 1984). Because not all the fish feed on the same food at the same time in nature, a high standard deviation can be expected for the zinc concentrations in the gut contents (Table 4.2). However, when the dietary supply of zinc is low (Spry *et al.*, 1988) and/or the zinc levels in the water are elevated, as was the case in the study area, zinc can also be taken up through the gills and maybe even the skin (Skidmore, 1964; Handy & Eddy, 1990; Hogstrand & Haux, 1991; Heath, 1987). In the first year, the mean zinc concentration in the river water was higher than the mean zinc concentration in the water of the second year (see Table 3.4). One would therefore expect that the zinc concentrations in the gill would be higher than the zinc concentrations in the gut for the first year. Unfortunately, only a pilot study was conducted in the first year (sampling only the basic organs) in order to determine whether considerable zinc levels would be detected in the fish. No gut tissue was therefore sampled until the

second year, when the study was expanded. In February 1991, however, the one fish that was caught at locality 5 did show the expected trend (Table 4.2). From April 1991 to August 1991 the gill still seemed to be the main route of uptake (Table 4.2), but as the mean zinc concentration in the water decreased, the gill as an uptake route became less pronounced, until in January and February 1992 the gut was the main route of uptake (Table 4.2), as usual. Zinc uptake was mostly higher in the hindgut than in the foregut, but at times it was also the reverse.

After absorption, zinc is distributed *via* the blood to accumulate in both soft (skin, liver, kidney, muscle and fat) and skeletal tissues (scales and vertebrae). The data showed that high zinc concentrations occurred in the skin, which is similar to the findings of Mount (1964) and Khalaf *et al.* (1985). This may suggest that zinc is primarily distributed to this tissue (Hogstrand & Haux, 1991). It can also be that the skin plays a role in the uptake and/or excretion of zinc. The liver is also a site of high zinc bioaccumulation (Table 4.2), reflecting its multifunctional role in the detoxification (through metallothionein binding) and storage processes (Carpenè *et al.*, 1990). The exact role of the kidney in the regulation of zinc is not yet known, especially because zinc excretion through the kidneys is minimal (Romanenko *et al.*, 1985; Klaassen, 1976). Good regulation takes place in the muscle and therefore low zinc concentrations were detected in this tissue (Table 4.2). The muscle zinc concentrations were well below the set standard for food by the National Health and Medical Research Council, which is 1000 µg/g Zn wet weight or in this case 4000 µg/g Zn dry weight (Anon., 1974). Scales and bone are regarded as significant storage sites (Sauer & Watabe, 1984) and therefore a substantial amount of zinc accumulated in the vertebrae (Table 4.2). It appears that the zinc content of fish scales is closely correlated to the concentration of zinc in environmental water (Sauer & Watabe, 1984), making it a sensitive environmental indicator when zinc levels increase. In future monitoring programmes, scales should therefore be included in the tissues that are being sampled for zinc analysis.

Zinc is necessary for gonad development and, consequently, the concentrations in the gonads will increase until the fish are sexually mature. Dietary zinc sources are, however, not adequate during this time and therefore internal sources, such as the liver, skin, muscle, vertebrae and scales are utilised (Fletcher & King, 1978; O'Grady, 1981). It was noted in this study, that when the zinc concentrations in the gonads (especially female gonads) decreased in spring 1990 and summer 1992, the zinc concentrations increased in the internal zinc sources (e.g. liver, skin, vertebrae) and also the other way around (Fig. 4.1). The breeding season stretches from October to April (Bell-Cross & Minshull, 1988) and in the first year the female gonads were fully developed by winter, but in the second year it was developed only later in spring (Fig. 4.1), probably due to the prolonged drought period. The standard deviations of the zinc concentrations in the gonads were very high, because the gonads of individuals were in different stages of development at the same time. The males were sexually mature by winter in the second year, which is one season earlier than the females were (Fig. 4.1). Because growth is retarded by sexual development (Love, 1980), the male fish were smaller than the female fish of the same age (Tables 4.1A & B). Presumably the zinc deposited in the gonads during their development was lost from the fish at spawning (spring 1990 to autumn 1991 in Fig. 4.1). This suggests that female fish would require greater amounts of zinc each year than the male fish would (Fletcher & King, 1978), as is illustrated in Figure 4.1. This might possibly be a reason why the zinc concentrations in the male vertebrae were higher than the zinc concentrations in the female vertebrae. Females need to utilise all possible sources for gonad development, but this is not the case with males, and skeletal sources would most likely be utilised after the soft tissue sources have been utilised.

After the storage and transformation processes in the different soft and skeletal tissues have taken place, excessive zinc is excreted. The major excretion route for zinc is faecal, with little being excreted by the kidneys and gills. The bile may (Romanenko *et al.*, 1985) or may not (Klaassen, 1976) play a role in zinc elimination; however, the low zinc concentrations detected in the bile of *B. marequensis* support the findings of Klaassen (1976). The role of the skin in zinc excretion has not yet been ascertained.

LOCALITY DIFFERENCES

The differences in localities did not seem to be correlated to the different zinc concentrations in the water of each locality (Table 3.2). This may be attributed to the fact that too few water samples were taken, so that no realistic and reliable correlation could be obtained. It is possible that the differences in localities were related rather to the type of food taken in by the fish at each locality. Sometimes the fish caught at locality 3 bioaccumulated the highest zinc levels (e.g. October 1990, October 1991 and February 1992), while in June 1991 it was the fish at locality 5 and in January 1992 the fish at locality 7.

An aspect to consider, is whether regulating organs (e.g. the muscle and liver) are reliable for use in statistical comparisons. Zinc levels in these organs will be regulated to a physiological acceptable level which is similar in all fish of the same species. As the zinc concentration in the environmental water increases, regulation will take place on a higher level than it does normally. In other words, if there is no distinct difference between the uptake of zinc concentrations by the fish at the different localities, the zinc concentrations in the fish will be similar at all the localities. Therefore, it might be better to use a storage organ, such as the vertebrae, where transformation and regulation are slow.

SEASONAL DIFFERENCES

The seasons in the first year differed significantly from most of the seasons in the second year (Table 4.4), with the zinc concentrations in the gill and muscle tissues being higher in the first than in the second year (Figures 4.1 and 4.2). These findings may be attributed to the difference in climatic conditions between the two years. Autumn 1990 also differed significantly from the other seasons in the first year with respect to the zinc concentrations in the muscle. This might not have been realistic, however, due to the fact that a value of 252.2 µg/g Zn at locality 3 in April 1990 (Table 4.2) increased the mean muscle zinc concentration in autumn 1990 to a value of 151 µg/g Zn (Fig. 4.2).

Females showed greater seasonal differences in zinc concentrations than males. This can be attributed to female gonad development, for no significant differences were recorded in the males with respect to liver and vertebrae zinc concentrations. However, differences were detected in the females (Table 4.4).

ANNUAL DIFFERENCES

As mentioned before, the two years did differ significantly, mostly due to the rain and floods in the first year compared to the continuous drought in the second year. No significant difference was, however, recorded between the zinc concentrations in the liver tissues of the two years (Fig. 4.3), indicating the good regulation and detoxification of zinc that takes place in this organ.

4.5 Conclusion

The skin and female gonads of *B. marequensis* accumulated the highest zinc concentrations, while the fat and bile accumulated the lowest. The zinc concentrations detected in all the organs and tissues suggest no serious zinc pollution problem in the study area, although the zinc levels detected in the liver and vertebrae might indicate chronic zinc exposure of the fish, causing possible sub-lethal effects. However, the latter statement needs to be further investigated in future monitoring programmes and also through experimental work. Suggested organs to sample for analysis of zinc pollution in fish, are: skin, vertebrae, scales, gonads (within a season) and muscle tissue (to test its fitness for human consumption). The gill and liver tissues will only be of value during acute exposures, unless histopathological studies are performed in addition to the zinc analysis.

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CHAPTER 5

Chapter 5

COPPER AND IRON BIOACCUMULATION IN THE ORGANS AND TISSUES OF *BARBUS MAREQUENSIS*

5.1 Introduction

Although copper is an essential trace element, elevated copper levels can be toxic to freshwater fish, even more toxic than any other metal, with the exception of mercury. Anthropogenic sources of copper include effluents from industries such as non-ferrous foundries, basic steel works, pulp and paper mills, electroplating, metal processing and petroleum refining (Nisha & Pandey, 1982), as well as compounds that are being applied as algaecides (Felts & Heath, 1984). In polluted fresh water copper may be present in a particulate and/or colloidal state, associated with suspended solids, as well as in different soluble chemical states (Stiff, 1971). Soluble matter is defined here as that which passes through a 0.45-µm membrane filter and includes copper both as free cupric ion (Cu^{2+}) and as soluble complexes (with carbonate, cyanide, amino acids, polypeptides and humic substances). The toxicity of copper appears to be related to the soluble copper present, with the cupric (Cu^{2+}) and to some extent the copper hydroxyl (Cu(OH)n) ions being the toxic forms (Stiff, 1971; Andrew *et al.*, 1977). Copper is, however, relatively insoluble in natural waters (Hale, 1977), as more than 90% of total copper in freshwater are bound to humic materials (Mantoura *et al.*, 1978). Even the soluble copper in river waters consists almost entirely of complexed forms, of which most complexes are "non-toxic" (Stiff, 1971). The free ion, therefore, rarely occurs in river waters, except in pure acidic soft waters. Factors which influence copper toxicity, other than pH and the presence of organic matter, include alkalinity (rather than hardness), temperature and dissolved oxygen. A problem encountered in assessing the toxicity of copper, is that the toxicity of copper in natural waters is usually less than that predicted from laboratory tests in clean water, except perhaps in very soft water free from organic matter or inorganic solids (Alabaster & Lloyd, 1980). The reason is the presence of non-toxic complexes and insoluble precipitates in natural waters. It is therefore imperative to include sufficient information as to the physical and chemical characteristics of the test water that was being used in the laboratory.

High iron concentrations are present in the aquatic environment due to the element's abundance in the earth's crust. Under aerobic and alkaline conditions, iron is mainly present as colloidal material in river waters due to its precipitation as $Fe(OH)_3$ (Håkanson & Jannson, 1983) or as FeOOH (Förstner & Wittmann, 1983) in the absence of organic chelating agents. When organics are present in surface waters, however, iron is always associated with them (Pitwell, 1974). Subterranean waters often contain high concentrations of dissolved iron. When these are discharged at the surface, for example as a result of mining operations, the iron precipitates as the hydroxide and ochreous deposits form on the bed of the receiving watercourse (Hellawell, 1986). Similar effects may be observed when rainfall seeps through coal mine spoil heaps and enters rivers. Iron is considered to be of moderate toxicity to aquatic life (Kempster *et al.*, 1982), but in general ferro-compounds are more toxic than ferric-

compounds. Iron pentacarbonyl is highly toxic, probably because of its potential to release carbon monoxide (Anon., 1977).

Both copper and iron have been reported to accumulate mostly in the liver, kidney and gill tissues of fish (Buckley *et al.*, 1982; Vorob'yev & Zaytsev, 1975). Effects on fish resulting from sub-lethal chronic exposure to copper (0.02 - 0.2 mg/l), include a reduction in survival, growth and reproduction rate, a loss of appetite and also behavioural changes, such as decreased concealment and ability to orientate (Moore & Ramamoorthy, 1984). The fish organs that are mainly damaged by acute copper exposure are the liver, gills, skin (Wong *et al.*, 1977), kidney (Moore & Ramamoorthy, 1984), stomach and gut (Singh, 1985). LC50's (96-h) range from 0.017 to 1.0 mg/l Cu, depending on the fish species and the water chemistry. Unusually high water hardness may even increase the 96-h LC50 to 3.0 mg/l Cu (Moore & Ramamoorthy, 1984). Limited research has been done on the toxic effects of iron on fish, although a slight reduction in growth of brook trout (*Salvelinus fontinalis*) was observed at 12 mg/l Fe and even more so at 50 mg/l Fe (Hellawell, 1986). Iron compounds appear to affect fish more indirectly than directly by destroying benthic food resources and by precipitating on the gills and gill filaments of fish, probably causing mechanical obstruction (Hellawell, 1986). Furthermore, precipitation of iron deposits on the leaves of macrophytes or the surfaces of algae may inhibit photosynthesis and, if severe enough, may ultimately lead to the disappearance of the flora. Ochreous deposits affect the stream environment in much the same way as other suspended solids do, but the effluents may have more serious direct consequences if the iron precipitates on the gills or other respiratory surfaces of fish or invertebrates (Hellawell, 1986).

In this section of the study, the extent of copper and iron bioaccumulation in the organs and tissues of *Barbus marequensis* was determined, as well as the organs that accumulated the highest and lowest metal levels respectively.

5.2 Materials and methods

Barbus marequensis was sampled and dissected as described in Chapter 4. Laboratory procedures for copper and iron analysis of the fish samples were the same as the procedures described for zinc analysis. Statistical procedures were also the same as described in Chapter 4.

5.3 Results

FISH SIZE AND AGE

The size and age data are summarised in Table 4.1 (see Chapter 4).

BIOACCUMULATION OF COPPER AND IRON IN THE DIFFERENT ORGANS AND TISSUES.

The order of bioaccumulation of copper and iron in the different organs and tissues of *B. marequensis* differed slightly, but both metals accumulated mostly in the liver, kidney and gut. High copper and iron concentrations were also detected in the gut contents (Tables 5.1 and 5.2). The general order of bioaccumulation for copper, was: liver > hindgut contents > foregut contents > hindgut > foregut > kidney > gill > bile > female gonads > vertebrae > blood > male gonads > skin > muscle > fat. The largest variation in copper accumulation was detected in the liver concentrations (Table 5.1), but the overall variation in copper concentration was much less than the variation in iron concentration. Statistically the copper concentrations in the liver differed significantly ($p \leq 0.05$) from the copper concentrations in all the other organs (Table 5.3). Additionally the gut contents differed significantly from the gills, fat, muscle, skin, vertebrae and blood with respect to the accumulated copper concentrations, but only during the winter of 1991 (Table 5.3). The general order of bioaccumulation for iron was: hindgut contents > foregut contents > hindgut > liver ≈ kidney > blood > foregut > gill > skin ≈ female gonads > male gonads > bile ≈ muscle > fat > vertebrae. From April 1990 to August

TABLE 5.1
MEAN COPPER CONCENTRATIONS (µg/g dry wt.) IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* (BF_W AND BF_s = BIOCONCENTRATION FACTORS OF THE WATER AND SEDIMENT RESPECTIVELY)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle
Apr. '90	3	n[①]						1
		Range						17.4
		Median						
		Mean	N/A	N/A	N/A	N/A	N/A	
		SD[Δ]						
		BFw						248.6
		BFs						0.44
	4	n	4	3				5
		Range	3.9-11.5	3.6-7.1				4.4-4.4
		Median	7.7	7.1				4.4
		Mean	7.7	6.0	N/A	N/A	N/A	4.4
		SD	3.1	2.1				0.0
		BFw	96.2	75.0				55.0
		BFs	1.03	0.80				0.59
	7	n	7				4	9
		Range	3.9-7.7				11.1-59.3	4.4-26.1
		Median	7.7				31.5	4.4
		Mean	7.1	N/A	N/A	N/A	33.3	8.7
		SD	1.5				22.4	8.7
		BFw	59.2				277.5	72.5
		BFs	0.02				0.08	0.02
June '90	3	n	2					2
		Range	3.9-7.7					4.4-4.4
		Median	5.8					4.4
		Mean	5.8	N/A	N/A	N/A	N/A	4.4
		SD	2.7					0.0
		BFw	58.0					44.0
		BFs	0.18					0.13
Aug. '90	3	n	8	2	1	1	2	9
		Range	7.7-11.5	7.1-7.1	4.8	1.1	29.6-33.3	4.4-4.4
		Median	7.7	7.1			31.5	4.4
		Mean	8.2	7.1			31.5	4.4
		SD	1.4	0.0			2.6	0.0
		BFw	273.3	236.7	160.0	36.7	1050.0	146.7
		BFs	0.22	0.19	0.13	0.03	0.83	0.12
	4	n	9	4	3	4	6	9
		Range	7.7-15.4	3.6-7.1	4.8-9.5	1.1-2.2	7.4-25.9	4.4-8.7
		Median	7.7	3.6	9.5	1.1	16.7	4.4
		Mean	9.8	4.5	7.9	1.4	17.9	6.3
		SD	2.8	1.8	2.7	0.6	7.2	2.3
		BFw	490.0	225.0	395.0	70.0	895.0	315.0
		BFs	0.49	0.22	0.39	0.07	0.89	0.31
	5	n	7	1	2	3	7	6
		Range	3.9-15.4	14.3	9.5-19.1	1.1-3.3	11.1-92.6	4.4-21.7
		Median	11.5		14.3	2.2	14.8	8.7
		Mean	10.4		14.3	2.2	31.2	10.1
		SD	4.3		6.7	1.1	29.9	6.5
		BFw	346.7	476.7	476.7	73.3	1040.0	336.7
		BFs	0.30	0.41	0.41	0.06	0.89	0.29
	7	n						5
		Range						8.7-13.0
		Median						8.7
		Mean	N/A	N/A	N/A	N/A	N/A	9.6
		SD						1.9
		BFw						160.0
		BFs						0.02

① Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 5.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Gut	Gut cont	Blood
Oct. '90	3	n®	7	2	1	2	6	7			
		Range	3.9-11.5	3.6-7.1	9.5	1.1-1.1	3.7-18.5	4.4-8.7			
		Median	3.9	5.4		1.1	7.4	4.4			
		Mean	6.0	5.4		1.1	10.5	5.0	N/A	N/A	N/A
		SD^Δ	3.0	2.5		0.0	6.4	1.6			
		BFw	150.0	135.0	237.5	27.5	262.5	125.0			
		BFs	0.55	0.49	0.86	0.10	0.95	0.45			
	4	n	10	1	7	6	9	10			
		Range	3.9-15.4	21.4	4.8-19.1	1.1-4.4	3.7-100.0	4.4-8.7			
		Median	3.9		4.8	2.2	3.7	4.4			
		Mean	7.3		8.2	2.4	19.3	5.2	N/A	N/A	N/A
		SD	4.6		5.3	1.3	31.5	1.8			
		BFw	182.5	535.0	205.0	60.0	482.5	130.0			
		BFs	0.36	1.07	0.41	0.12	0.96	0.26			
	5	n	9	1	6	9	9	10			
		Range	3.9-11.5	10.7	4.8-47.6	1.1-2.2	11.1-251.9	4.4-91.3			
		Median	7.7		14.3	1.1	29.6	8.7			
		Mean	8.5		18.3	1.5	49.0	15.2	N/A	N/A	N/A
		SD	2.6		15.2	0.6	76.6	26.8			
		BFw	212.5	267.5	457.5	37.5	1225.0	380.0			
		BFs	0.65	0.82	1.41	0.12	3.77	1.17			
	7	n	1	1		1	1	1			
		Range	15.4	19.1		6.6	25.9	17.4			
		Median									
		Mean			N/A				N/A	N/A	N/A
		SD									
		BFw	385.0	477.5		165.0	647.5	435.0			
		BFs	0.47	0.58		0.20	0.78	0.53			
Dec. '90	3	n	7	3	1	3	2	7			
		Range	11.5-19.2	14.3-14.3	9.5	1.1-2.2	33.3-40.7	4.4-13.0			
		Median	15.4	14.3		2.2	37.0	8.7			
		Mean	15.4	14.3		1.8	37.0	9.3	N/A	N/A	N/A
		SD	2.2	0.0		0.6	5.2	3.9			
		BFw	85.6	79.4	52.8	10.0	205.6	51.7			
		BFs	0.81	0.75	0.50	0.09	1.95	0.49			
	5	n	1					1			
		Range	15.4					8.7			
		Median									
		Mean		N/A	N/A	N/A	N/A		N/A	N/A	N/A
		SD									
		BFw	770.0					435.0			
		BFs	0.83					0.47			
Feb. '91	5	n	1		1			1	1	1	1
		Range	9.7		10.0			10.9	13.6	27.9	1.3
		Median									
		Mean		N/A		N/A	N/A				
		SD									
		BFw	323.3		333.3			363.3	453.3		43.3
		BFs	0.52		0.54			0.59	0.74		0.07
	7	n	2					2		1	6
		Range	15.2-21.4					4.6-5.9		254.2	1.1-1.7
		Median	18.3					5.3			1.3
		Mean	18.3	N/A	N/A	N/A	N/A	5.3	N/A		1.3
		SD	4.4					0.9			0.2
		BFw	261.4					75.7			18.6
		BFs	0.49					0.14			0.03

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 5.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	Gut cont.	Vertebrae	Bile	Blood
Apr. '91	3	n[@]	3	4	1	1	3	6		2	1	3			8
		Range	9.9-14.4	8.2-14.4	13.8	7.9	15.9-36.7	8.3-16.1		10.5-13.7	20.8	35.3-41.4			1.1-1.7
		Median	10.9	10.5			23.3	9.9		12.1		38.3			1.2
		Mean	11.7	10.9			25.3	10.7	N/A	12.1		38.3	N/A	N/A	1.4
		SD[Δ]	2.3	2.6			10.5	3.0		2.3		3.0			0.3
		BFw	292.5	272.5	345.0	197.5	632.5	267.5		302.5	520.0				35.0
		BFs	0.31	0.29	0.36	0.21	0.67	0.28		0.32	0.55				0.04
	4	n	3		1	2	2	6		1		2			10
		Range	9.3-11.4		14.9	4.7-7.8	24.5-26.1	6.8-17.1		12.2		32.5-58.9			1.3-1.6
		Median	10.3			6.2	25.3	8.7				45.7			1.5
		Mean	10.3	N/A		6.2	25.3	10.3	N/A		N/A	45.7	N/A	N/A	1.5
		SD	1.0			2.2	1.2	3.9				18.7			0.1
		BFw	343.3		496.7	206.7	843.3	343.3		406.7					50.0
		BFs	0.82		1.19	0.50	2.02	0.82		0.98					0.12
	5	n	4	5		3	4	7		3	2	3			10
		Range	7.4-12.1	6.5-18.9		2.3-4.8	14.7-61.0	4.6-15.5		12.1-27.1	10.7-16.0	30.0-57.5			1.3-2.4
		Median	10.4	8.4		3.3	26.7	5.8		13.3	13.3	33.1			1.5
		Mean	10.1	10.7	N/A	3.4	32.3	7.9	N/A	17.5	13.3	40.2	N/A	N/A	1.6
		SD	2.1	4.9		1.2	21.3	4.0		8.3	3.7	15.1			0.3
		BFw	336.7	356.7		113.3	1076.7	263.3		583.3	443.3				53.3
		BFs	1.84	1.95		0.62	5.87	1.44		3.18	2.42				0.29
	7	n	1		1	1	1	1				1			1
		Range	14.7		4.6	4.5	21.5	7.4				152.7			1.6
		Median													
		Mean		N/A					N/A	N/A	N/A		N/A	N/A	
		SD													
		BFw	367.5		115.0	112.5	537.5	185.0							40.0
		BFs	0.06		0.02	0.02	0.08	0.03							0.01
June '91	3	n	9	3		9	9	9	9	8	2	8	9		9
		Range	5.3-8.9	1.4-8.0		1.0-3.9	23.5-44.5	4.9-8.9	5.0-8.6	14.3-23.1	16.3-23.3	28.3-62.2	4.5-7.3		1.1-1.6
		Median	6.7	2.4		1.7	36.6	6.6	6.0	19.1	19.8	40.6	5.4		1.4
		Mean	6.8	3.9	N/A	1.9	34.5	6.8	6.2	19.0	19.8	40.4	5.6	N/A	1.4
		SD	1.1	3.6		0.9	7.2	1.3	1.1	2.9	4.9	10.7	0.9		0.2
		BFw	113.3	65.0		31.7	575.0	113.3	103.3	316.7	330.0		93.3		23.3
		BFs	0.10	0.06		0.03	0.50	0.10	0.09	0.27	0.28		0.08		0.02
	4	n	7	1	6	6	7	7	4	7		7	7	1	7
		Range	7.2-13.0	7.6	4.3-12.1	1.0-5.1	16.3-103.6	4.5-8.6	3.2-23.8	14.4-35.4		25.3-54.5	6.4-10.9	7.0	1.3-1.9
		Median	9.1		7.5	1.4	23.3	5.0	5.2	25.5		51.2	6.9		1.6
		Mean	9.3		7.6	2.1	34.1	5.4	9.3	25.1	N/A	47.2	7.5		1.6
		SD	2.0		2.9	1.6	31.2	1.4	9.7	7.4		10.2	1.6		0.2
		BFw	310.0	253.3	253.3	70.0	1136.7	180.0	310.0	836.7			250.0	233.3	53.3
		BFs	0.81	0.66	0.66	0.18	2.97	0.47	0.81	2.18			0.65	0.61	0.14
	5	n	3		3	3	3	3	3	3	1	2	3		3
		Range	6.6-9.8		3.0-4.3	1.3-2.4	16.8-32.6	4.8-6.6	5.5-6.0	12.8-16.5	26.5	52.5-60.4	5.0-6.4		1.1-1.4
		Median	6.7		3.7	1.5	26.7	6.3	5.7	16.2		56.5	5.4		1.3
		Mean	7.7	N/A	3.7	1.7	25.3	5.9	5.7	15.2		56.5	5.6	N/A	1.3
		SD	1.8		0.6	0.6	8.0	1.0	0.3	2.1		5.6	0.7		0.2
		BFw	192.5		92.5	42.5	632.5	147.5	142.5	380.0	662.5		140.0		32.5
		BFs	0.53		0.26	0.12	1.74	0.41	0.39	1.05	1.83		0.39		0.09

[@] Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 5.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont	HGut cont	Vertebrae	Kidney	Bile	Blood
Aug. '91	3	n[Φ]	1	1		1	1	1	1	1		1		1			1
		Range	3.7	2.7		0.4	39.1	2.4	3.5	6.5		12.7		3.2			2.9
		Median															
		Mean			N/A						N/A		N/A		N/A	N/A	
		SD[Δ]															
		BFw	185.0	135.0		20.2	1955.0	120.0	175.0	325.0				160.0			145.0
		BFs	0.17	0.13		0.02	1.82	0.11	0.16	0.30				0.15			0.13
	4	n	8	5		8	8	8	7	7		5	5	8	2		8
		Range	4.1-6.8	3.9-8.9		0.5-1.8	47.7-470.1	4.4-41.8	7.2-34.6	8.3-12.0		14.5-34.0	11.0-35.7	2.9-5.1	5.9-7.5		2.3-3.3
		Median	5.1	4.8		1.1	158.9	10.4	11.2	10.4		18.8	16.2	4.5	6.7		2.8
		Mean	5.3	5.5	N/A	1.2	180.9	14.3	14.4	10.1	N/A	20.6	20.3	4.3	6.7	N/A	2.7
		SD	0.9	1.9		0.4	135.8	12.4	9.4	1.2		7.8	10.0	0.6	1.2		0.3
		BFw	530.0	550.0		120.0	18090.0	1430.0	1440.0	1010.0				430.0	670.0		270.0
		BFs	0.23	0.24		0.05	7.87	0.62	0.63	0.44				0.19	0.29		0.12
	5	n	12	11	1	12	12	12	12	10	4	8	8	12	7	4	12
		Range	4.1-7.3	4.2-6.3	4.3	0.3-1.5	19.1-102.0	2.8-8.6	1.6-5.9	8.4-24.2	21.5-52.9	11.2-46.0	24.8-97.3	3.0-4.9	5.7-13.5	4.8-10.3	2.7-4.2
		Median	5.4	5.4		0.6	35.9	4.3	4.3	12.7	35.9	27.6	41.8	4.4	7.7	6.3	3.3
		Mean	5.6	5.1		0.7	39.4	4.7	4.0	13.7	36.5	28.2	50.5	4.2	8.7	6.9	3.3
		SD	1.0	0.7		0.4	21.9	1.7	1.1	4.6	14.9	12.0	23.6	0.6	3.1	2.3	0.4
		BFw	280.0	255.0	215.0	35.0	1970.0	235.0	200.0	685.0	1825.0			210.0	435.0	345.0	165.0
		BFs	0.27	0.25	0.21	0.03	1.92	0.23	0.20	0.67	1.78			0.20	0.42	0.34	0.16
	7	n	1			1	1	1	1	1				1		1	1
		Range	4.7			3.1	16.3	3.4	1.5	11.2				4.1		1.9	5.4
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A		N/A		
		SD															
		BFw	156.7			103.3	543.3	113.3	50.0	373.3				136.7		63.3	180.0
		BFs	0.06			0.04	0.20	0.04	0.02	0.14				0.05		0.02	0.07
Oct. '91	3	n	6	2	1	6	5	6	6	5	3	2	3	6	3	5	6
		Range	3.7-5.9	4.3-4.8	3.4	0.5-1.1	32.2-63.3	1.3-1.8	1.9-2.7	10.2-13.0	12.6-17.1	19.2-23.1	22.3-23.8	3.3-3.6	6.3-7.1	0.9-2.9	3.1-3.7
		Median	4.5	4.6		0.7	33.3	1.5	2.1	11.0	14.6	21.2	23.4	3.5	7.0	2.0	3.5
		Mean	4.6	4.6		0.8	40.1	1.5	2.2	11.5	14.7	21.2	23.1	3.5	6.8	2.0	3.4
		SD	0.8	0.3		0.2	13.3	0.2	0.3	1.2	2.3	2.8	0.8	0.1	0.5	0.8	0.2
		BFw	131.4	131.4	97.1	22.9	1145.7	42.9	62.9	328.6	420.0			100.0	194.3	57.1	97.1
		BFs	0.52	0.52	0.38	0.09	4.51	0.17	0.25	1.29	1.65			0.39	0.76	0.22	0.38
	4	n	11	7	1	11	8	11	11	5	2	9	5	11	3	6	10
		Range	3.2-12.3	3.0-8.0	1.8	0.7-1.9	13.3-530.3	1.2-2.5	1.6-2.6	12.4-40.3	14.9-41.5	8.3-24.1	10.4-21.7	3.1-3.8	7.9-8.0	1.5-5.0	2.7-3.7
		Median	6.4	4.4		0.9	50.4	1.8	2.0	16.4	28.2	16.3	17.7	3.3	7.9	2.1	3.0
		Mean	7.1	4.9		1.2	123.2	1.8	2.0	22.6	28.2	16.2	16.3	3.4	7.9	2.5	3.1
		SD	2.7	1.8		0.4	175.5	0.4	0.3	12.6	18.8	4.7	4.3	0.2	0.1	1.3	0.3
		BFw	394.4	272.2	100.0	66.7	6844.4	100.0	111.1	1255.6	1566.7			188.9	438.9	138.9	172.2
		BFs	0.59	0.41	0.15	0.10	10.27	0.15	0.17	1.88	2.35			0.28	0.66	0.21	0.26
	5	n	14	9	4	15	13	15	14	3	3	10	4	15	5	9	10
		Range	6.1-19.9	4.7-6.7	2.5-3.3	0.8-2.8	24.0-313.0	1.5-3.0	1.5-2.7	24.4-73.1	26.0-50.5	14.3-142.4	22.8-35.1	3.6-5.1	4.1-21.3	3.1-12.5	2.5-3.4
		Median	8.2	5.2	2.8	1.2	92.5	1.9	1.8	27.0	30.8	27.0	27.8	3.8	5.0	6.6	3.0
		Mean	10.3	5.4	2.8	1.4	114.3	1.9	1.9	41.5	35.8	50.4	28.4	3.9	7.9	7.3	2.9
		SD	4.6	0.6	0.4	0.5	93.0	0.4	0.3	27.4	12.9	47.1	5.5	0.4	7.5	3.8	0.3
		BFw	515.0	270.0	140.0	70.0	5715.0	95.0	95.0	2075.0	1790.0			195.0	395.0	365.0	145.0
		BFs	0.31	0.16	0.09	0.04	3.48	0.06	0.06	1.27	1.09			0.12	0.24	0.22	0.09
	7	n	1			1	1	1	1	1				1	1	1	1
		Range	5.4			1.3	19.9	2.6	3.6	30.8				4.3	7.8	8.0	3.0
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A				
		SD															
		BFw	300.0			72.2	1105.6	144.4	200.0	1711.1				238.9	433.3	444.4	166.7
		BFs	0.28			0.07	1.04	0.14	0.19	1.61				0.23	0.41	0.42	0.16

Φ Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 5.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont.	HGut cont.	Vertebrae	Kidney	Bile	Blood
Jan. '92	3	n[①]	5	4		5	3	6	2	2	2	2	2	6	1	5	6
		Range	4.7-12.5	3.7-6.0		0.5-3.4	22.6-107.6	1.2-2.8	1.6-2.2	12.5-18.7	16.9-19.5	17.4-27.0	14.0-18.0	3.8-4.8	7.1	1.5-4.2	2.0-3.5
		Median	5.8	4.8		0.9	48.6	1.8	1.9	15.6	18.2	22.2	16.0	4.0		2.2	2.3
		Mean	7.0	4.8	N/A	1.4	59.6	1.9	1.9	15.6	18.2	22.2	16.0	4.2		2.4	2.6
		SD[Δ]	3.1	0.9		1.2	43.6	0.6	0.4	4.4	1.8	6.8	2.9	0.4		1.1	0.6
		BFw	218.8	150.0		43.8	1862.5	59.4	59.4	487.5	568.7			131.2	221.9	75.0	81.2
		BFs	0.28	0.19		0.06	2.38	0.08	0.08	0.62	0.73			0.17	0.28	0.10	0.10
	4	n	4	3		10	2	11	3	1	1	1	3	9	1	2	11
		Range	6.3-6.5	5.0-5.4		1.0-2.2	65.9-73.7	1.6-2.9	2.3-3.2	19.2	21.7	22.6	27.6-38.9	4.0-4.8	8.0	3.2-4.6	1.6-3.4
		Median	6.5	5.3		1.4	69.8	2.0	2.7				32.0	4.2		3.9	2.7
		Mean	6.4	5.2	N/A	1.4	69.8	2.1	2.8				32.8	4.3		3.9	2.5
		SD	0.1	0.2		0.4	5.5	0.4	0.5				5.7	0.3		1.0	0.7
		BFw	320.0	260.0		70.0	3490.0	105.0	140.0	960.0	1085.0			215.0	400.0	195.0	125.0
		BFs	0.16	0.13		0.04	1.79	0.05	0.07	0.49	0.56			0.11	0.21	0.10	0.06
	5	n	12	8	4	12	9	12	11	3	3	2	1	12	3	9	12
		Range	6.9-21.5	4.0-5.8	2.6-3.7	0.5-1.3	24.2-500.5	1.0-2.1	1.5-2.7	9.3-12.8	11.0-29.8	11.0-36.1	66.4	3.5-5.9	5.6-10.2	2.7-14.4	1.8-6.3
		Median	8.0	5.0	3.4	0.9	92.2	1.5	1.9	9.9	13.7	23.6		4.0	6.1	4.4	3.3
		Mean	9.7	5.0	3.3	0.9	166.0	1.6	2.0	10.7	18.2	23.6		4.1	7.3	5.4	3.6
		SD	4.1	0.6	0.5	0.2	186.9	0.3	0.4	1.9	10.2	17.8		0.6	2.5	3.7	1.2
		BFw	746.2	384.6	253.8	69.2	12769.2	123.1	153.8	823.1	1400.0			315.4	561.5	415.4	276.9
		BFs	0.57	0.30	0.20	0.05	9.82	0.09	0.12	0.63	1.08			0.24	0.43	0.32	0.21
	7	n	1			5		5		1				5			5
		Range	9.9			2.1-6.0		2.5-4.2		21.4				4.4-6.7			1.6-2.9
		Median				4.0		2.8						5.6			2.7
		Mean		N/A	N/A	4.0	N/A	3.1	N/A		N/A	N/A	N/A	5.7	N/A	N/A	2.4
		SD				1.4		0.7						1.0			0.6
		BFw	319.4			129.0		100.0		690.3				183.9			77.4
		BFs	0.01			0.01		0.004		0.03				0.01			0.003
Feb. '92	3	n	2			6		6	3					6			6
		Range	9.3-9.7			1.2-2.2		2.4-3.0	2.5-2.7					4.8-5.7			2.2-4.0
		Median	9.5			1.6		2.6	2.6					5.0			3.4
		Mean	9.5	N/A	N/A	1.6	N/A	2.6	2.6	N/A	N/A	N/A	N/A	5.1	N/A	N/A	3.3
		SD	0.3			0.4		0.3	0.1					0.3			0.6
		BFw	197.9			33.3		54.2	54.2					106.3			68.8
		BFs	0.86			0.15		0.24	0.24					0.46			0.30
	4	n	6	4		10	7	10	8	1	1	1	1	10	1	9	10
		Range	5.3-6.0	4.9-6.7		0.6-1.8	50.7-134.0	1.4-3.3	1.7-2.4	17.9	16.9	31.3	33.3	3.9-5.3	13.7	2.5-16.5	1.7-3.5
		Median	5.5	5.7		0.9	106.9	1.8	2.1					4.2		9.3	2.9
		Mean	5.6	5.7	N/A	1.0	101.2	1.9	2.1					4.3		8.9	2.6
		SD	0.3	0.8		0.4	30.2	0.5	0.3					0.4		5.4	0.7
		BFw	127.3	129.5		22.7	2300.0	43.2	47.7	406.8	384.1			97.7	311.4	202.3	59.1
		BFs	0.29	0.29		0.05	5.19	0.10	0.11	0.92	0.87			0.22	0.70	0.46	0.13
	5	n	10	10		10	8	10	10	2	2			10	2	10	10
		Range	4.7-8.5	3.5-7.1		0.5-2.2	50.8-139.2	1.2-2.0	1.5-3.8	20.9-24.4	18.1-22.7			4.2-4.8	7.1-8.2	2.8-18.9	2.4-4.1
		Median	6.2	5.6		0.8	96.5	1.7	1.9	22.7	20.4			4.4	7.7	5.6	3.6
		Mean	6.5	5.5	N/A	1.0	97.5	1.6	2.2	22.7	20.4	N/A	N/A	4.4	7.7	7.8	3.3
		SD	1.1	1.0		0.5	29.7	0.3	0.7	2.5	3.2			0.2	0.7	5.5	0.7
		BFw	141.3	119.6		21.7	2119.6	34.8	47.8	493.5	443.5			95.7	167.4	169.6	71.7
		BFs	0.12	0.10		0.02	1.82	0.03	0.04	0.42	0.38			0.08	0.14	0.15	0.06
	Pionier Dam	n	10	5	5	10	9	10	10	6	2	6		10	4	5	10
		Range	5.6-9.2	3.5-4.9	2.4-3.3	0.8-2.0	32.3-70.5	1.0-2.3	1.3-2.7	11.2-18.2	20.0-20.8	7.4-21.4		3.6-4.4	8.6-10.8	0.5-4.0	2.5-3.2
		Median	7.6	4.2	2.5	1.0	46.8	1.3	1.8	17.3	20.4	17.6		4.0	10.2	1.3	2.8
		Mean	7.4	4.1	2.6	1.2	49.3	1.4	1.8	16.4	20.4	16.3	N/A	4.0	10.0	1.8	2.8
		SD	1.1	0.6	0.3	0.4	13.4	0.4	0.4	2.7	0.6	4.9		0.2	0.9	1.4	0.2
		BFw	139.6	77.4	49.1	22.6	930.2	26.4	34.0	309.4	384.9			75.5	188.7	34.0	52.8
		BFs	0.53	0.29	0.19	0.09	3.52	0.10	0.13	1.17	1.46			0.29	0.71	0.13	0.20

[①] Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 5.2
MEAN IRON CONCENTRATIONS (µg/g dry wt.) IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS*
(BF_w AND BF_s = BIOCONCENTRATION FACTORS OF THE WATER AND SEDIMENT RESPECTIVELY)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle
Apr. '90	3	n®						1
		Range						413.0
		Median						
		Mean	N/A	N/A	N/A	N/A	N/A	
		SD$^{\Delta}$						
		BFw						273.5
		BFs						0.01
	4	n	4	3				4
		Range	776.9-4776.9	207.1-1325.0				208.7-478.3
		Median	2207.7	450.0				284.8
		Mean	2492.3	660.7	N/A	N/A	N/A	314.1
		SD	2009.6	588.0				117.4
		BFw	277.2	73.5				34.9
		BFs	0.17	0.04				0.02
	7	n	7				4	9
		Range	596.2-1111.5				277.8-548.2	117.4-247.8
		Median	746.2				455.6	147.8
		Mean	791.2	N/A	N/A	N/A	434.3	161.4
		SD	196.0				117.9	38.7
		BFw	627.9				344.7	128.1
		BFs	0.02				0.01	0.004
June '90	3	n	2					2
		Range	476.9-734.6					156.5-287.0
		Median	605.8					221.7
		Mean	605.8	N/A	N/A	N/A	N/A	221.7
		SD	182.2					92.2
		BFw	23.4					8.5
		BFs	0.03					0.01
Aug. '90	3	n	8	2	1	1	2	9
		Range	334.6-734.6	221.4-289.3	114.3	82.2	344.4-563.0	108.7-691.3
		Median	463.5	255.4			453.7	139.1
		Mean	500.5	255.4			453.7	207.2
		SD	145.0	48.0			154.5	184.2
		BFw	641.7	327.4	146.5	105.4	581.7	265.6
		BFs	N/A	N/A	N/A	N/A	N/A	N/A
	4	n	9	4	3	4	6	9
		Range	546.2-1334.6	221.4-267.9	261.9-357.1	81.1-410.0	274.1-740.7	160.9-743.5
		Median	834.6	244.6	328.6	237.2	403.7	217.4
		Mean	894.0	244.6	315.9	241.4	447.5	284.5
		SD	256.6	19.0	48.9	151.6	162.5	186.4
		BFw	784.2	214.6	277.1	211.8	392.5	249.6
		BFs	N/A	N/A	N/A	N/A	N/A	N/A
	5	n	7	1	2	3	7	6
		Range	238.5-946.2	350.0	257.1-352.4	35.6-70.0	170.4-3218.5	139.1-287.0
		Median	619.2		304.8	46.7	329.6	206.5
		Mean	557.7		304.8	50.8	797.9	205.1
		SD	242.5		67.3	17.6	1102.0	60.6
		BFw	580.9	364.6	317.5	52.9	831.1	213.6
		BFs	N/A	N/A	N/A	N/A	N/A	N/A
	7	n						5
		Range						113.0-226.1
		Median						126.1
		Mean	N/A	N/A	N/A	N/A	N/A	144.3
		SD						46.4
		BFw						360.7
		BFs						N/A

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 5.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Gut	Gut cont	Blood
Oct. '90	3	n[Φ]	7	2	1	2	6	7			
		Range	234.6-546.2	196.4-310.7	204.8	47.8-63.3	181.5-392.6	156.5-269.6			
		Median	361.5	253.6		55.6	222.2	182.6			
		Mean	355.5	253.6		55.6	245.1	195.7	N/A	N/A	N/A
		SD[Δ]	118.6	80.8		11.0	79.1	40.4			
		BFw	455.8	325.1	262.6	71.3	314.2	250.9			
		BFs	N/A	N/A	N/A	N/A	N/A	N/A			
	4	n	10	1	7	6	9	10			
		Range	338.5-1769.2	703.6	147.6-890.5	136.7-265.6	259.3-751.9	130.4-491.3			
		Median	1140.4		395.2	157.2	614.8	258.7			
		Mean	1129.6		455.8	177.1	569.1	273.0	N/A	N/A	N/A
		SD	457.8		261.4	50.9	175.7	118.5			
		BFw	1227.8	764.8	495.4	192.5	618.6	296.7			
		BFs	N/A	N/A	N/A	N/A	N/A	N/A			
	5	n	9	1	6	9	9	10			
		Range	215.4-669.2	175.0	181.0-390.5	30.0-83.3	177.8-485.2	113.0-160.9			
		Median	296.2		242.9	46.7	240.7	132.6			
		Mean	353.8		249.2	53.9	277.0	136.1	N/A	N/A	N/A
		SD	140.0		77.2	18.9	99.5	15.6			
		BFw	498.3	246.5	351.0	75.9	390.1	191.7			
		BFs	N/A	N/A	N/A	N/A	N/A	N/A			
	7	n	1	1		1	1	1			
		Range	223.1	190.5		105.6	296.3	139.1			
		Median									
		Mean			N/A				N/A	N/A	N/A
		SD									
		BFw	210.5	179.7		99.6	279.5	131.2			
		BFs	N/A	N/A		N/A	N/A	N/A			
Dec. '90	3	n	7	3	1	3	2	7			
		Range	1219.2-3600.0	382.1-482.1	547.6	80.0-177.8	488.9-759.3	208.7-387.0			
		Median	2961.5	439.3		148.9	624.1	273.9			
		Mean	2776.9	434.5		135.6	624.1	273.3	N/A	N/A	N/A
		SD	821.3	50.2		50.2	191.2	62.8			
		BFw	21.5	3.4	4.2	1.0	4.8	2.1			
		BFs	1.11	0.17	0.22	0.05	0.25	0.11			
	5	n	1					1			
		Range	1588.5					343.5			
		Median									
		Mean		N/A	N/A	N/A	N/A		N/A	N/A	N/A
		SD									
		BFw	279.7					60.5			
		BFs	0.18					0.04			
Feb. '91	5	n	1		1			1	1	1	1
		Range	887.5		268.2			375.0	1279.6	7241.9	404.0
		Median									
		Mean		N/A		N/A	N/A				
		SD									
		BFw	220.8		66.7			93.3	318.3		100.5
		BFs	0.03		0.01			0.01	0.04		0.01
	7	n	2					2		1	6
		Range	782.6-970.0					210.5-252.5		12650.0	277.0-421.0
		Median	876.3					231.5			306.0
		Mean	876.3	N/A	N/A	N/A	N/A	231.5	N/A		321.5
		SD	132.5					29.7			50.6
		BFw	315.2					83.3			115.6
		BFs	0.10					0.03			0.04

Φ Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 5.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	Gut cont.	Vertebrae	Bile	Blood
Apr. '91	3	n[Φ]	3	4	1	1	3	6		2	1	3			8
		Range	687.9-804.3	162.5-491.2	295.1	558.6	305.1-416.7	196.4-493.1		848.7-963.6	6853.9	7097.1-15090.2			253.0-346.0
		Median	723.5	309.9			403.3	314.8		906.1		13406.3			317.0
		Mean	738.5	318.4			375.0	324.0	N/A	906.1		11864.5	N/A	N/A	309.3
		SD[Δ]	59.6	134.6			60.9	112.3		81.3		4213.7			33.8
		BFw	152.6	65.8	61.0	115.4	77.5	66.9		187.2	1416.1				63.9
		BFs	0.01	0.005	0.005	0.01	0.01	0.005		0.01	0.10				0.005
	4	n	3		1	2	2	6		1		2			10
		Range	616.0-954.9		388.9	272.1-493.1	558.0-606.4	253.5-401.2		1618.0		16942.9-31838.6			134.0-362.0
		Median	950.7			382.6	582.2	342.1				24390.7			323.0
		Mean	840.2	N/A		382.6	582.2	326.3	N/A		N/A	24390.7	N/A	N/A	300.6
		SD	195.0			156.3	34.3	60.8				10532.9			65.7
		BFw	405.9		187.9	184.8	281.3	157.6		781.6					145.2
		BFs	0.05		0.02	0.02	0.03	0.02		0.09					0.02
	5	n	4	5		3	4	7		3	2	3			10
		Range	470.2-926.3	124.2-694.7		128.6-813.9	230.5-2267.1	126.5-171.9		1853.3-5179.6	3264.3-10227.0	6436.5-14784.6			185.0-346.0
		Median	652.9	239.0		276.3	432.8	160.8		5106.3	6745.7	14148.3			309.5
		Mean	675.6	330.9	N/A	406.3	840.8	154.7	N/A	4046.4	6745.7	11789.8	N/A	N/A	300.3
		SD	202.1	239.9		360.7	957.9	17.0		1899.6	4923.4	4647.0			45.8
		BFw	544.8	266.9		327.7	678.1	124.8		3263.2	5440.1				242.2
		BFs	0.08	0.04		0.05	0.10	0.02		0.49	0.82				0.04
	7	n	1		1	1	1	1				1			1
		Range	651.3		248.1	283.1	581.9	224.4				14611.7			216.0
		Median													
		Mean		N/A					N/A	N/A	N/A		N/A	N/A	
		SD													
		BFw	468.6		178.5	203.7	418.6	161.4							155.4
		BFs	0.04		0.02	0.02	0.04	0.01							0.01
June '91	3	n	9	3		9	9	9	9	8	2	8	9		9
		Range	270.6-889.5	119.9-285.6		68.8-479.2	233.7-508.7	125.0-302.2	164.5-546.0	374.1-927.8	1405.0-2318.5	7915.0-29385.8	70.1-161.2		283.0-355.0
		Median	465.5	258.0		105.6	382.5	193.4	337.5	702.5	1861.8	15441.6	124.8		328.0
		Mean	508.6	221.2	N/A	156.8	385.3	191.1	340.3	664.8	1861.8	17501.9	120.7	N/A	324.8
		SD	181.1	88.8		129.8	83.4	55.2	142.5	209.4	646.0	7665.8	36.6		26.0
		BFw	251.8	109.5		77.6	190.7	94.6	168.5	329.1	921.7		59.8		160.8
		BFs	0.25	0.11		0.08	0.19	0.09	0.17	0.33	0.92		0.06		0.16
	4	n	7	1	6	6	7	7	4	7		7	7	1	7
		Range	351.5-746.8	136.1	60.6-298.4	40.9-86.4	137.0-501.6	54.3-105.5	82.9-173.7	325.5-566.0		4680.0-23897.6	53.4-93.3	221.7	326.0-425.0
		Median	541.0		198.0	65.4	168.0	62.1	149.5	425.7		13237.5	64.6		360.0
		Mean	525.0		183.6	65.0	223.1	75.5	138.9	438.4	N/A	15440.4	67.1		365.9
		SD	142.9		96.8	18.1	131.3	23.0	39.3	93.9		7382.6	12.9		35.7
		BFw	1500.0	388.9	524.6	185.7	637.4	215.7	396.9	1252.6			191.7	633.4	1045.4
		BFs	0.01	0.004	0.005	0.002	0.01	0.002	0.004	0.01			0.002	0.01	0.01
	5	n	3		3	3	3	3	3	3	1	2	3		3
		Range	296.4-509.1		123.1-143.1	49.5-253.6	136.3-158.8	89.4-117.1	128.3-321.4	204.0-522.2	525.0	12214.3-13591.7	50.0-87.2		222.0-317.0
		Median	336.2		136.4	79.1	150.0	105.3	129.0	424.2		12903.0	68.3		239.0
		Mean	380.6	N/A	134.2	127.4	148.4	103.9	192.9	383.5		12903.0	68.5	N/A	259.3
		SD	113.1		10.2	110.3	11.4	13.9	111.3	163.0		974.0	18.6		50.7
		BFw	309.4		109.1	103.6	120.7	84.5	156.8	311.8	426.8		55.7		210.8
		BFs	0.02		0.01	0.01	0.01	0.01	0.01	0.02	0.03		0.003		0.01

[Φ] Number of samples analyzed [Δ] Standard deviation N/A Not available

TABLE 5.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont	HGut cont	Vertebrae	Kidney	Bile	Blood
Aug. '91	3	n[①]	1	1		1	1	1	1	1		1		1			1
		Range	286.4	153.9		38.0	251.0	47.4	414.2	625.7		5963.7		48.4			292.0
		Median															
		Mean			N/A						N/A		N/A		N/A	N/A	
		SD[Δ]															
		BFw	105.3	56.6		14.0	92.3	17.4	152.3	230.0				17.8			107.4
		BFs	0.01	0.004		0.001	0.01	0.001	0.01	0.02				0.001			0.01
	4	n	8	5		8	8	8	7	7		5	5	8	2		8
		Range	128.5-494.4	58.3-168.2		9.2-258.6	146.6-329.4	40.7-81.1	52.7-107.2	198.2-590.0		5179.2-20498.7	11841.2-60802.0	29.9-50.5	248.8-312.0		315.0-490.0
		Median	336.1	115.6		25.3	265.2	62.7	79.0	248.7		12650.0	12908.3	39.1	280.4		392.5
		Mean	312.0	109.8	N/A	52.7	251.4	61.5	79.7	287.3	N/A	13583.5	27534.0	39.5	280.4	N/A	390.4
		SD	122.3	46.3		83.7	60.9	15.6	19.5	136.0		6352.5	22044.1	6.4	44.7		52.3
		BFw	245.7	86.5		41.5	198.0	48.4	62.8	226.2				31.1	220.8		307.4
		BFs	0.01	0.003		0.001	0.01	0.002	0.002	0.01				0.001	0.01		0.01
	5	n	12	11	1	12	12	12	12	10	4	7	8	12	7	4	12
		Range	81.0-189.6	44.9-146.9	310.0	22.6-185.1	86.8-169.8	29.5-89.2	39.6-107.4	120.3-312.4	319.6-522.8	1390.7-6338.4	5838.8-72167.1	28.1-77.0	342.9-705.5	18.1-107.6	238.0-366.0
		Median	134.4	73.9		65.4	113.5	48.2	62.6	242.3	422.8	2893.0	12036.9	35.2	406.9	71.7	305.5
		Mean	129.9	77.1		67.6	121.7	51.1	64.8	230.8	422.0	3438.3	19575.1	46.2	459.7	67.3	299.6
		SD	33.4	29.7		46.3	28.2	16.7	21.2	68.4	83.0	1725.3	21973.3	20.0	129.5	44.3	43.8
		BFw	56.0	33.2	133.6	29.1	52.5	22.0	27.9	99.5	181.9			19.9	198.1	29.0	129.1
		BFs	0.01	0.004	0.01	0.003	0.01	0.002	0.003	0.01	0.02			0.002	0.02	0.003	0.01
	7	n	1			1	1	1	1	1				1		1	1
		Range	165.0			116.0	148.2	34.0	81.3	315.4				54.9		45.2	438.0
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A		N/A		
		SD															
		BFw	109.3			76.8	98.1	22.5	53.8	208.9				36.4		29.9	290.1
		BFs	0.02			0.01	0.02	0.004	0.01	0.03				0.01		0.005	0.05
Oct. '91	3	n	6	2	1	6	5	6	6	5	3	2	3	6	3	5	6
		Range	147.2-200.0	144.6-187.7	80.2	19.6-75.3	265.1-610.0	36.5-66.0	51.5-156.9	205.7-411.9	534.6-1311.8	11928.1-30498.8	15362.1-26065.8	24.5-38.2	296.1-440.7	10.6-22.2	266.0-368.0
		Median	190.5	166.1		32.6	480.7	42.0	90.0	281.9	781.4	21213.5	15914.5	31.2	405.9	19.5	316.0
		Mean	181.1	166.1		38.9	462.8	47.3	99.2	295.5	875.9	21213.5	19114.1	30.5	380.9	18.4	315.0
		SD	21.0	30.5		20.8	124.4	12.2	44.5	75.3	397.1	13131.5	6026.7	5.0	75.6	4.6	33.9
		BFw	503.1	461.4	222.8	108.1	1285.6	131.4	275.6	820.8	2433.1			84.7	1058.1	51.1	875.0
		BFs	0.01	0.01	0.004	0.002	0.02	0.003	0.01	0.02	0.05			0.002	0.02	0.001	0.02
	4	n	11	7	1	11	8	11	11	5	2	9	5	11	3	6	10
		Range	130.1-513.4	53.5-256.7	61.3	29.3-125.6	262.1-565.2	32.7-96.1	87.9-341.7	195.6-464.4	816.2-1101.4	1524.3-22758.6	1563.2-15792.9	25.0-50.7	355.7-468.9	27.1-154.7	281.0-447.0
		Median	214.4	105.0		37.2	390.1	45.0	121.3	316.7	958.8	7086.1	6531.3	31.5	459.7	97.9	321.5
		Mean	253.7	130.0		49.8	399.8	49.5	150.9	311.2	958.8	8677.5	7080.2	33.6	428.1	93.3	347.9
		SD	120.2	69.7		29.9	119.9	18.1	81.8	105.1	201.7	6951.8	5297.2	8.0	62.8	62.1	64.5
		BFw	1492.4	764.7	360.6	292.9	2351.8	291.2	887.6	1830.6	5640.0			197.6	2518.2	548.8	2046.5
		BFs	0.01	0.01	0.003	0.002	0.02	0.002	0.01	0.02	0.05			0.002	0.02	0.005	0.02
	5	n	14	9	4	15	13	15	14	3	3	10	4	15	5	9	10
		Range	141.6-580.7	53.9-143.5	28.0-62.1	17.5-47.1	200.0-393.3	24.4-61.7	39.7-105.7	306.3-330.9	468.8-582.8	1997.8-10033.8	8273.4-13280.9	23.1-56.3	177.7-427.0	16.1-81.3	299.0-470.0
		Median	218.1	83.0	51.3	24.5	241.0	35.4	52.9	326.3	538.6	3306.6	10761.6	26.6	292.1	23.0	379.0
		Mean	257.1	91.1	48.2	26.4	263.1	36.0	61.6	321.2	530.1	4553.8	10769.4	29.6	307.9	29.8	379.3
		SD	108.7	27.6	14.5	7.9	59.3	8.8	21.5	13.1	57.5	2879.8	2570.8	8.1	115.9	20.4	46.1
		BFw	1714.0	607.3	321.3	176.0	1754.0	240.0	410.7	2141.3	3534.0			197.3	2052.7	198.7	2528.7
		BFs	0.02	0.01	0.003	0.002	0.02	0.002	0.004	0.02	0.04			0.002	0.02	0.002	0.03
	7	n	1			1	1	1	1	1				1	1	1	1
		Range	189.0			63.8	114.3	41.8	101.3	242.9				37.3	215.0	60.7	345.0
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A				
		SD															
		BFw	590.6			199.4	357.2	130.6	316.6	759.1				116.6	671.9	189.7	1078.1
		BFs	0.01			0.003	0.01	0.002	0.01	0.01				0.002	0.01	0.003	0.02

① Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 5.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont.	HGut cont.	Vertebrae	Kidney	Bile	Blood
Jan. '92	3	n[®]	5	4		5	3	6	2	2	2	2	2	6	1	5	6
		Range	251.4-468.8	55.9-144.3		19.0-193.4	432.6-660.8	23.9-41.8	72.6-519.4	461.1-638.6	1600.0-1662.7	15840.7-22786.5	19448.9-24709.4	20.6-74.1	713.5	44.2-284.9	255.0-351.0
		Median	291.5	104.7		44.4	557.3	30.7	296.0	549.9	1631.4	19313.6	22079.1	32.1		150.0	284.5
		Mean	362.1	102.4	N/A	82.9	550.3	31.2	296.0	549.9	1631.4	19313.6	22079.1	37.1		150.7	290.7
		SD[Δ]	119.3	46.9		75.4	114.3	6.3	316.0	125.5	44.3	4911.4	3719.8	19.0		95.3	32.7
		BFw	209.3	59.2		47.9	318.1	18.0	171.1	317.9	943.0			21.4	412.4	87.1	168.0
		BFs	0.02	0.01		0.004	0.03	0.002	0.02	0.03	0.08			0.002	0.04	0.01	0.02
	4	n	4	3		10	2	11	3	1	1	1	3	9	1	2	11
		Range	238.2-395.7	50.9-84.2		38.7-241.3	548.3-619.8	27.2-116.3	86.5-122.6	306.8	884.2	9343.2	14765.4-22778.8	22.9-62.5	483.3	20.5-40.8	293.0-429.0
		Median	269.0	62.2		82.6	584.0	34.1	88.7				20390.2	28.7		30.7	356.0
		Mean	293.0	65.7	N/A	97.0	584.0	45.7	99.3				19311.5	32.1		30.7	353.4
		SD	70.1	17.0		61.3	50.6	27.4	20.3				4114.1	11.9		14.3	38.7
		BFw	2092.9	469.3		692.9	4171.4	326.4	709.3	2191.4	6315.7			229.3	3452.1	219.3	2524.3
		BFs	0.01	0.003		0.004	0.02	0.002	0.004	0.01	0.03			0.001	0.02	0.001	0.01
	5	n	12	8	4	12	9	11	11	3	3	2	1	12	3	9	12
		Range	203.4-398.3	53.4-84.7	50.0-127.6	22.1-248.9	366.2-779.1	22.8-79.8	49.7-340.9	132.1-285.8	726.7-1505.6	1592.3-27921.4	29020.7	19.8-73.9	314.1-500.7	11.9-182.7	261.0-405.0
		Median	259.9	76.0	67.5	33.4	510.5	42.5	68.4	173.2	750.0	14756.9		32.7	327.7	43.1	304.0
		Mean	281.1	71.4	78.2	53.8	507.8	45.3	97.0	197.0	994.1	14756.9		38.1	380.8	60.7	324.6
		SD	75.9	11.3	34.2	62.7	125.5	17.5	83.5	79.6	443.1	18617.5		17.5	104.0	51.2	43.7
		BFw	14055.0	3570.0	3910.0	2690.0	25390.0	2265.0	4850.0	9850.0	49705.0			1905.0	19040.0	3035.0	16230.0
		BFs	0.02	0.004	0.005	0.003	0.03	0.003	0.01	0.01	0.06			0.002	0.02	0.004	0.02
	7	n	1			5		5		1				5			5
		Range	289.2			106.1-833.7		45.8-109.3		564.3				44.0-193.5			300.0-350.0
		Median				137.6		50.0						67.1			324.0
		Mean		N/A	N/A	272.7	N/A	62.9	N/A		N/A	N/A	N/A	107.3	N/A	N/A	323.8
		SD				314.6		26.5						67.8			22.5
		BFw	370.8			349.6		80.6		723.5				137.6			415.1
		BFs	0.01			0.01		0.002		0.02				0.004			0.01
Feb. '92	3	n	2			6		6	3					6			6
		Range	230.6-237.7			29.4-96.1		25.6-60.6	38.5-73.3					21.4-42.4			296.0-430.0
		Median	234.2			47.1		27.2	48.0					28.1			349.0
		Mean	234.2	N/A	N/A	51.5	N/A	33.9	53.3	N/A	N/A	N/A	N/A	29.2	N/A	N/A	349.7
		SD	5.1			23.4		13.7	17.9					7.2			46.9
		BFw	2.4			0.5		0.4	0.6					0.3			3.6
		BFs	0.02			0.01		0.004	0.01					0.003			0.04
	4	n	6	4		10	7	10	8	1	1	1	1	10	1	9	10
		Range	134.5-216.2	50.0-125.3		17.6-50.0	382.4-1063.3	32.3-75.5	50.5-390.0	284.9	370.4	17457.4	26568.4	17.4-50.0	455.2	6.0-21.6	256.0-337.0
		Median	171.9	72.3		30.9	800.0	46.8	106.8					25.2		12.7	273.0
		Mean	173.3	80.0	N/A	33.4	745.9	48.1	153.0					28.3		13.5	287.3
		SD	29.7	35.7		12.1	226.7	13.3	120.1					11.0		6.2	31.6
		BFw	3.9	1.8		0.8	17.0	1.1	3.5	6.5	8.4			0.6	10.3	0.3	6.5
		BFs	0.01	0.003		0.001	0.03	0.002	0.01	0.01	0.02			0.001	0.02	0.001	0.01
	5	n	10	10		10	8	10	10	2	2			10	2	10	10
		Range	140.2-296.2	53.5-192.5		11.6-109.0	501.9-834.4	18.7-66.8	38.6-97.8	236.3-240.0	412.5-502.9			19.7-83.9	350.8-373.4	9.6-36.5	195.0-414.0
		Median	195.3	74.5		41.9	580.2	26.6	52.3	238.1	457.7			26.2	362.1	16.9	302.0
		Mean	205.3	92.8	N/A	51.5	610.1	31.1	59.4	238.1	457.7	N/A	N/A	39.3	362.1	18.5	289.2
		SD	57.6	44.8		36.8	119.5	14.4	20.0	2.6	64.0			25.6	16.0	7.6	61.0
		BFw	2.3	1.0		0.6	6.9	0.4	0.7	2.7	5.2			0.4	4.1	0.2	3.3
		BFs	0.02	0.01		0.01	0.06	0.003	0.01	0.03	0.05			0.004	0.04	0.002	0.03
	Pionier Dam	n	10	5	5	10	9	10	10	6	2	6		10	4	5	10
		Range	170.5-480.2	86.7-150.7	32.8-83.3	33.8-315.9	473.6-1220.8	21.0-91.6	22.9-134.9	424.6-696.8	1796.6-2003.2	4342.9-10656.3		16.0-53.0	419.1-746.1	34.6-95.7	201.0-339.0
		Median	270.7	122.4	43.8	105.3	641.4	34.3	46.4	557.4	1899.9	6363.8		19.5	598.0	75.0	283.0
		Mean	274.6	123.7	53.5	110.3	681.7	46.0	63.7	554.0	1899.9	6820.8	N/A	24.9	590.3	73.6	278.5
		SD	89.6	26.0	22.8	82.6	226.5	26.4	44.0	105.1	146.1	2603.3		13.7	136.2	24.6	45.0
		BFw	160.6	72.3	31.3	64.5	398.7	26.9	37.3	324.0	1111.1			14.6	345.2	43.0	162.9
		BFs	0.01	0.01	0.003	0.01	0.03	0.002	0.003	0.03	0.10			0.001	0.03	0.004	0.01

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 5.3

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE COPPER 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	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)													
Gonad (Males)													
Fat													
Liver	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2	W2, SP2, S2									
Muscle					W2, SP2, S2								
Skin					W2, SP2, S2								
Gut					W2, SP2, S2								
Gut cont.	W2			W2	SP2, S2	W2	W2						
Vertebrae					W2, SP2, S2				W2				
Kidney					SP2, S2								
Bile					SP2, S2								
Blood					W2, SP2, S2				W2				

1991, however, the gills accumulated higher iron concentrations than the liver (Table 5.2). A large variation in iron concentration was detected between the different organs and tissues, as well as between individuals, especially with regard to the gut contents (Table 5.2). The concentrations in the gut contents differed significantly from the iron concentrations in all the other organs (Table 5.4). The iron concentrations in the gills, liver, skin and blood also differed significantly from the concentrations in a few other organs, but only during the summer of 1992 (Table 5.4).

The copper and iron bioconcentration factors between the water and the organs (BF_w) were much higher than the bioconcentration factors between the sediment and the organs (BF_s). The copper BF_w values ranged from 10 (calculated for fat tissue in December 1990) to 18 090.0 (calculated for the liver in August 1991), while the BF_s values ranged from 0.01 (calculated for tissues in January 1992) to 10.27 (calculated for the liver in October 1991) (Table 5.1). In the case of iron, the BF_w values ranged from 0.2 (calculated for bile in February 1992) to 49 705.0 (calculated for the hindgut in January 1992), while the BF_s values ranged from 0.001 (calculated for tissues from August 1991 to February 1992) to 1.11 (calculated for the gill in December 1990) (Table 5.2).

LOCALITY DIFFERENCES

No locality differences had occurred in the first year (October 1990) with respect to the copper concentrations in the gill, liver and muscle tissues. In the second year locality 3 differed significantly ($p \leq 0.05$) from locality 4 during the months of June 1991 (with respect to the vertebrae copper concentrations) and February 1992 (with respect to the fat, muscle and vertebrae copper concentrations). Localities 3 and 5 differed significantly in October 1991 (with respect to the fat, vertebrae and blood copper concentrations) and in February 1992 (with respect to the muscle, fat and vertebrae copper concentrations), while localities 4 and 5 only differed significantly with respect to the vertebrae copper concentrations in October 1991 and also the blood copper concentrations in January 1992. In general most of the organs collected at locality 7 had accumulated higher copper concentrations than the collected organs at the other localities (Table 5.1). In January 1992 locality 7 differed significantly from localities 3 (with respect to the fat, muscle and vertebrae concentrations), 4 (with respect to the fat and vertebrae concentrations) and 5 (with respect to the fat, muscle and vertebrae concentrations). Most of the organs collected at Pionier Dam in February 1992 had accumulated lower copper concentrations than the collected organs at the other localities (Table 5.1) and differed significantly from localities 3 (with respect to the fat, muscle, vertebrae and blood concentrations), 4 (with respect to the muscle and vertebrae concentrations) and 5 (with respect to the vertebrae concentrations).

In the case of iron, locality 4 differed significantly from both localities 3 (with respect to the gill and liver iron concentrations) and 5 (with respect to the gill, liver, muscle and fat iron concentrations) in October 1990, the first year of this study. In June 1991, the second year, locality 3 differed significantly from localities 4 (with respect to the muscle and vertebrae iron concentrations) and 5 (with respect to the muscle, vertebrae and blood iron concentrations), but in January 1992 it only differed significantly from locality 4 (with respect to the blood iron concentrations). Locality 4 differed significantly from locality 5 in June 1991 (with respect to the blood iron concentrations), in October 1991 (with respect to the fat iron concentrations) and in February 1992 (with respect to the muscle iron concentrations). In January 1992 locality 7 differed significantly from all the other localities (3, 4 and 5) with respect to the iron concentrations in the fat and vertebrae. Pionier Dam differed significantly from localities 3 (with respect to the blood iron concentrations) and 5 (with respect to the fat iron concentrations).

SEASONAL DIFFERENCES

Significant seasonal differences ($p \leq 0.05$) were detected, but it was not always the same organs that indicated these differences (Tables 5.5 and 5.6). Using the data for both sexes combined, the summer of 1990/91 differed significantly from all the other seasons (except autumn 1990 and autumn 1991) with respect to the copper and iron concentrations in the gills. This trend was also found for the females, but to a lesser extent than for the males (except in the case of iron). The iron concentrations in the muscle also indicated significant differences between the summer of 1990/91 and the seasons of the second year, except autumn 1991 (Table 5.6). Autumn and winter of 1990 differed significantly

TABLE 5.4
SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE IRON 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	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	S2												
Gonad (Males)	S2												
Fat	S2												
Liver	S2	S2	S2	S2									
Muscle	S2				S2								
Skin	S2				S2	S2							
Gut													
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				S2		S2		W2, SP2, S2				
Kidney									SP2, S2				
Bile	S2				S2				SP2, S2				
Blood	S2	S2	S2	S2	S2	S2	S2		W2, SP2	S2		S2	

TABLE 5.5
SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN COPPER 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 SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

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

TABLE 5.6
SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN IRON 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 SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

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

from most of the other seasons (especially in the case of the female fish), but only with respect to the iron concentrations in the organs (Table 5.6) and not with respect to the copper concentrations (Table 5.5). Furthermore, all the seasons in the second year differed significantly from one another with respect to the copper and iron concentrations in various organs (Tables 5.5 and 5.6).

Comparing the metal concentrations in the organs and tissues of the males and females seasonally (Figures 5.1-5.4), a difference was noticed in some organs. The copper concentrations in the gonads, muscle, vertebrae and fat of the males were mostly higher than that of the females, while the females had higher copper concentrations in the blood and bile (Figure 5.2). The iron concentrations were mostly higher in the gills, gonads, muscle and vertebrae of the males (Figure 5.4), while the females had higher hindgut, liver, skin and bile iron concentrations (Figures 5.3 and 5.4).

ANNUAL DIFFERENCES

The first and second year differed significantly with respect to the gill, liver, muscle and male gonad copper concentrations (Figure 5.5) and also with respect to the iron concentrations in the gill, muscle and gonads of both sexes (Figure 5.6). The mean metal concentrations in the organs and gut contents during the second year (Figures 5.5 and 5.6) were also used to determine the order of bioaccumulation and it differed slightly from the order based on the monthly data. For copper it was: liver > foregut contents > hindgut contents > hindgut > foregut > kidney > gill > bile ≈ female gonads > male gonads > vertebrae ≈ muscle > skin > blood > fat; and for iron, hindgut contents > foregut contents > hindgut > foregut > kidney > liver > blood > gill > male gonads > female gonads > skin > muscle > fat > bile > vertebrae.

5.4 Discussion

BIOACCUMULATION OF COPPER AND IRON IN THE DIFFERENT ORGANS AND TISSUES

Copper and iron were found to have accumulated in all the tissues and organs of *Barbus marequensis*. Of all the organs, the liver accumulated the highest copper concentrations, thereby confirming the view that the liver of freshwater fishes is a copper storage organ. Elevated copper levels in the liver can be ascribed to the binding of copper to metallothionein (MT), which serves as a detoxification mechanism (Hogstrand & Haux, 1991). Copper is also part of the liver proteins hemocuprein and hepatocuprein (Voynar, 1960) and several oxidative enzymes. The activity of the liver enzyme, xanthine oxidase, can be used as an indicator of sub-lethal copper exposure, because copper increases the activity in exposed fish whereas lead, mercury, silver and cadmium inhibit it (Jackim *et al.*, 1970). A large variation was detected in the liver copper concentrations (Table 5.1), which might be partly due to the rate of erythrocyte maturation differing from individual to individual. Copper is essential for this maturation process (Vorob'yev & Zaytsev, 1975). The liver of *B. marequensis* also accumulated significant levels of iron. These elevated iron levels can be ascribed to the ferritin content (Vorob'yev & Zaytsev, 1975), iron-containing enzymes (Voynar, 1960) and the extensive vascular system of the liver. The haemoglobin in the blood binds approximately three-quarters of the iron present in the body (Voynar, 1960), explaining the accumulation of iron by the liver and kidneys. Copper is required for the synthesis of haemoglobin (Heath, 1987), but it is transported in the blood by the protein ceruloplasmin, which is believed to be the link between copper and iron in the vertebrates (Moore & Ramamoorthy, 1984).

Food seems to be a more important source of copper than water to fish (Moore & Ramamoorthy, 1984). Presumably this is also the case for iron, because higher iron concentrations were detected in the gut than in the gills (Table 5.2). The large variation in iron concentration that was detected in the gut contents of *B. marequensis* can largely be explained by its feeding habits. *Barbus marequensis* is a benthic feeder and, in addition to the benthic organisms, sediment rich in iron, the amount of which will differ from individual to individual, will be ingested by the fish. Furthermore, the mouth form of *B. marequensis* is highly variable (Pienaar, 1978), resulting in varied foraging habits in a population. As manifested by the order of bioaccumulation, the gut wall was a major site of deposition. Increased

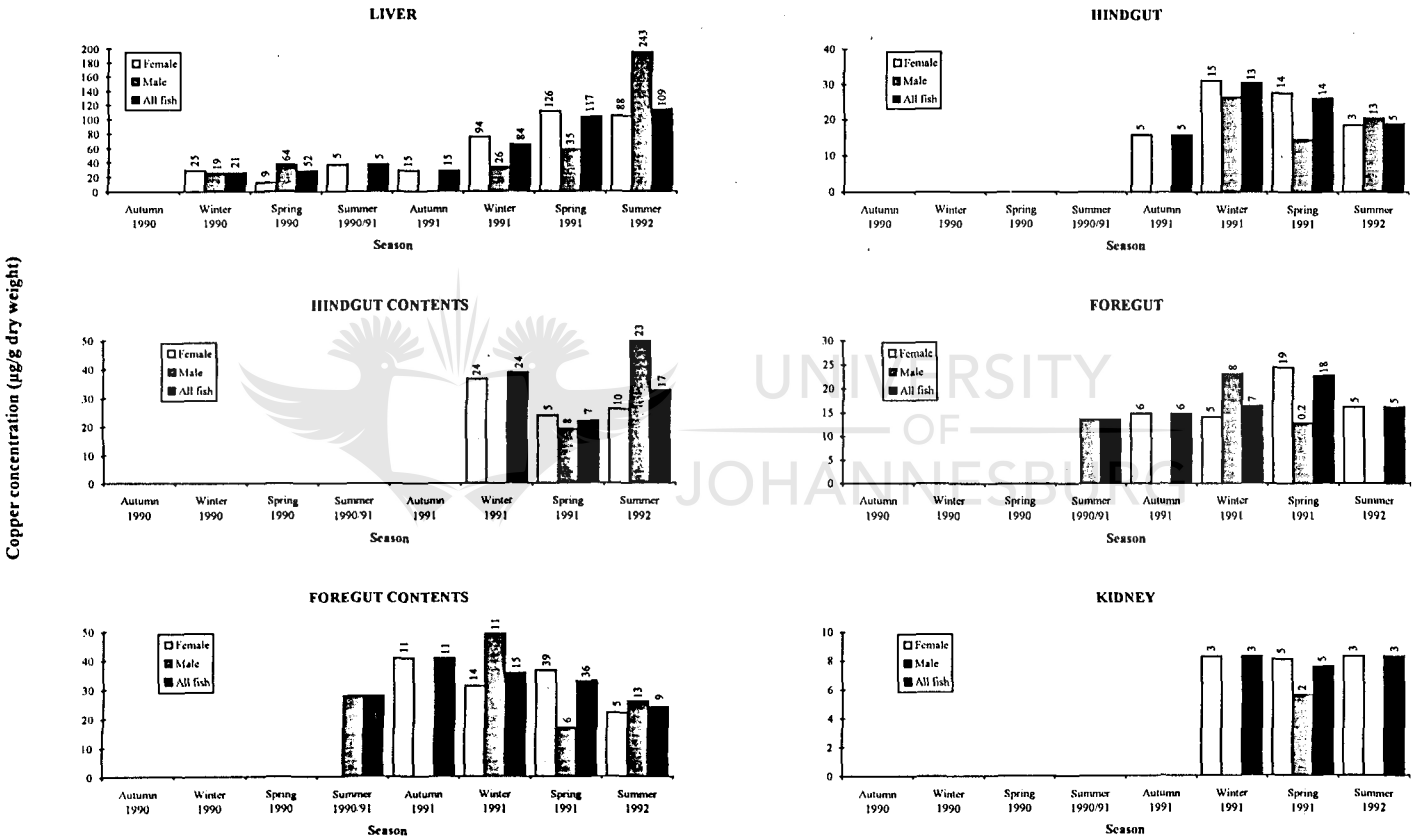

Figure 5.1

Mean seasonal copper concentrations (µg/g dry wt.) in the liver, hindgut contents, foregut contents, hindgut, foregut and kidney of Barbus marequensis *for males and females seperately, as well as the sexes combined. (Standard deviations are indicated above each bar)*

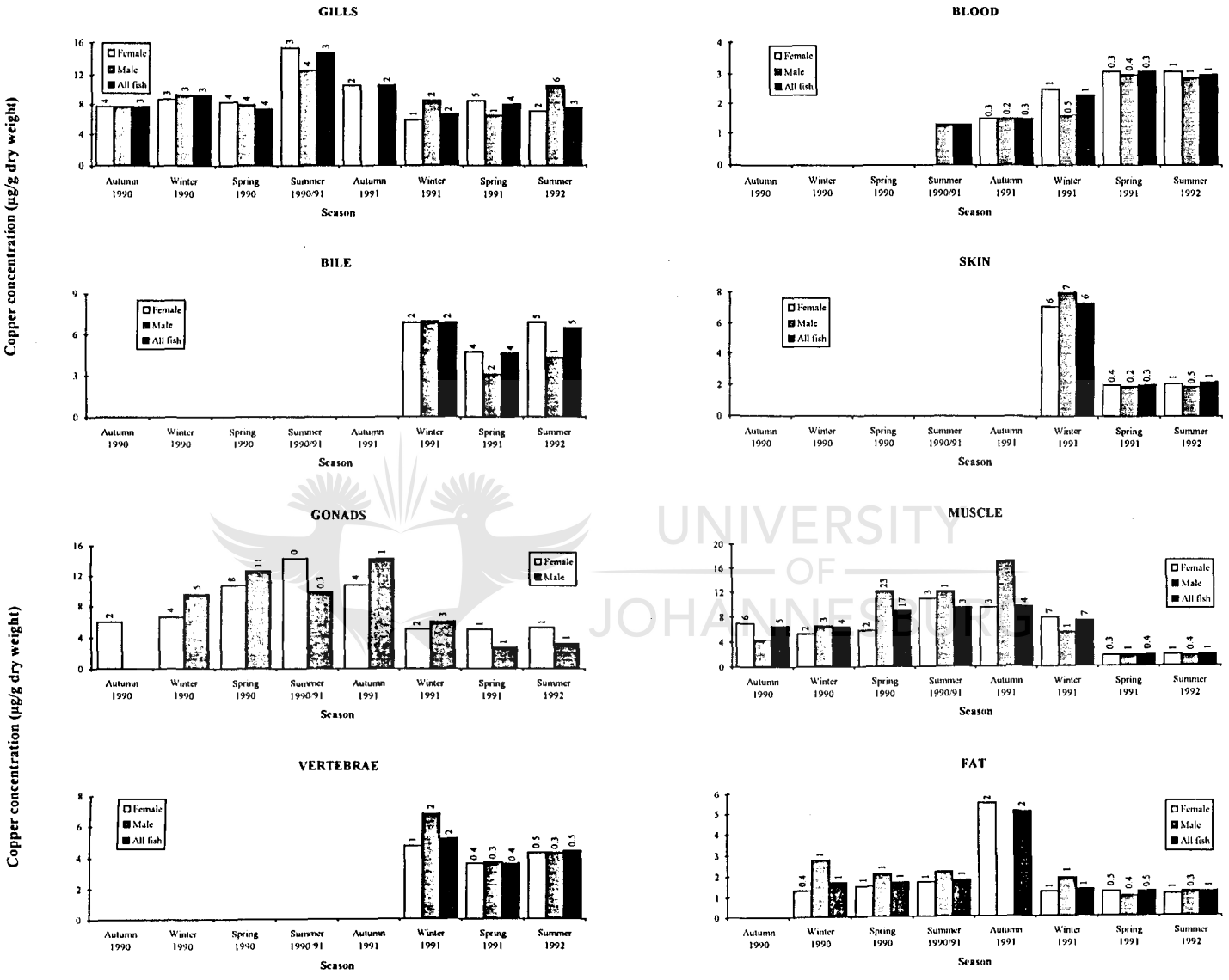

Figure 5.2

Mean seasonal copper concentrations (µg/g dry wt.) in the gills, bile, gonads, vertebrae, blood, skin, muscle and fat of Barbus marequensis *for males and females seperately, as well as the sexes combined. (Standard deviations are indicated above each bar)*

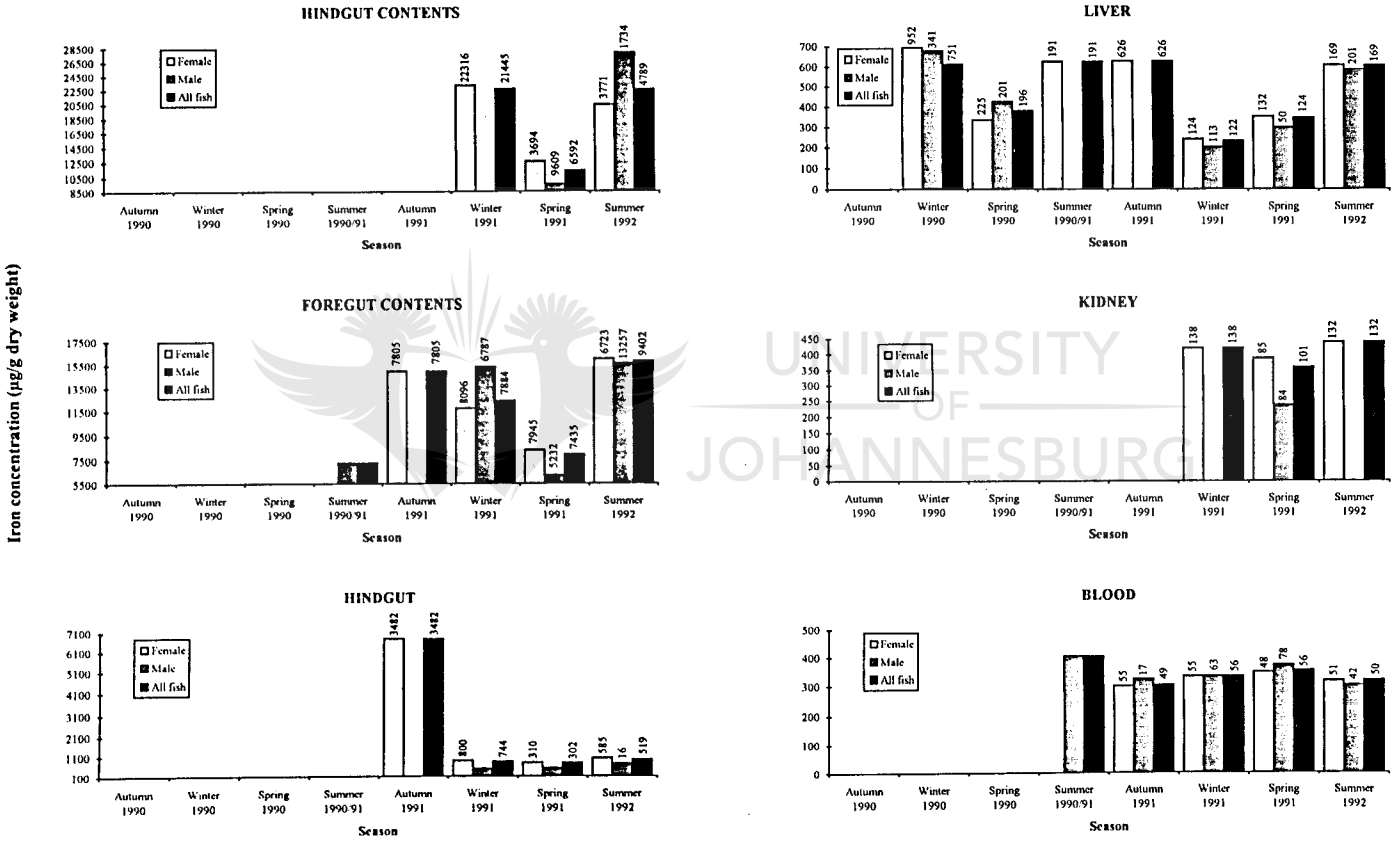

Figure 5.3

Mean seasonal iron concentrations (µg/g dry wt.) in the hindgut contents, foregut contents, hindgut, liver, kidney and blood of Barbus marequensis *for males and females seperately, as well as the sexes combined. (Standard deviations are indicated above each bar)*

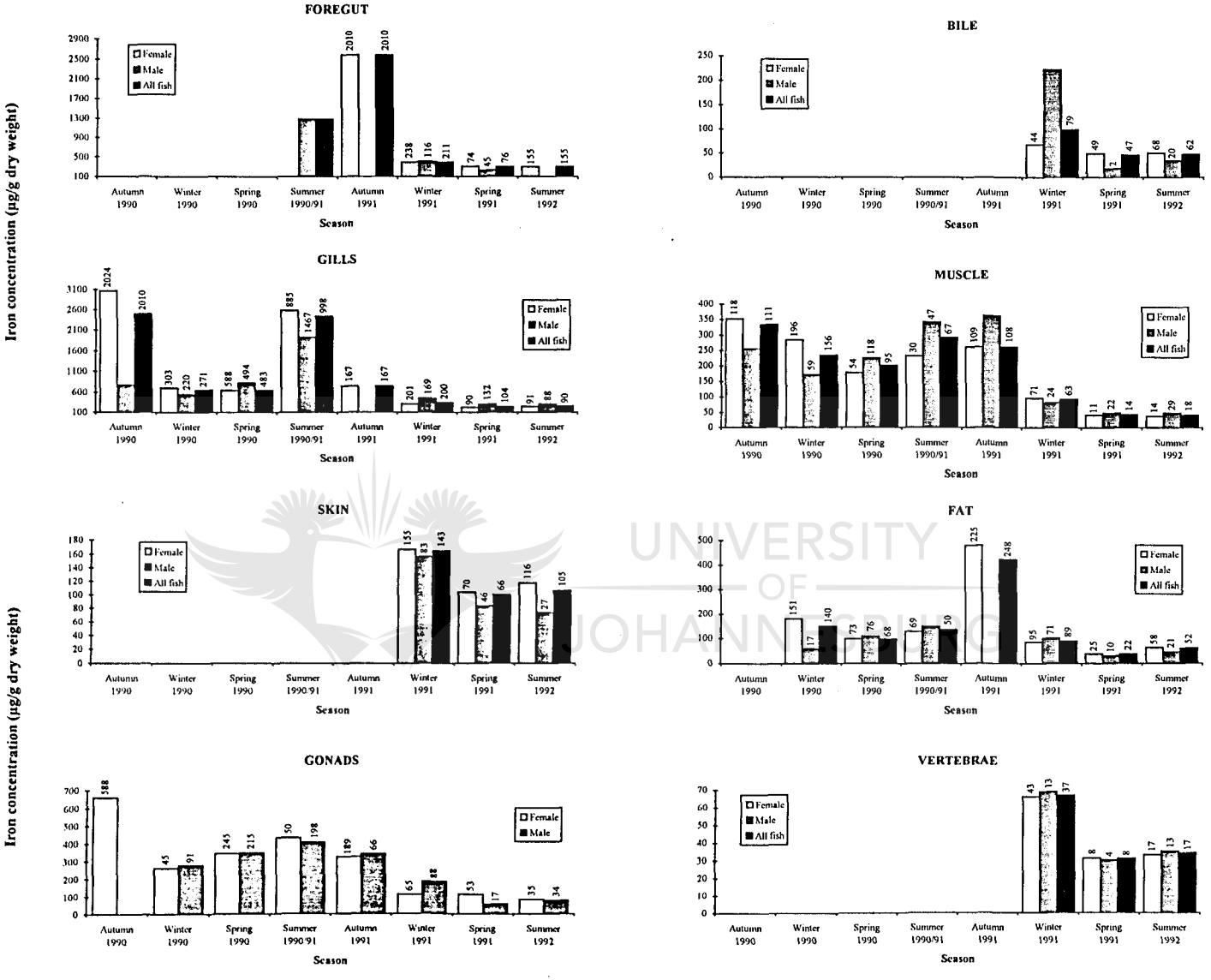

Figure 5.4

Mean seasonal iron concentrations (µg/g dry wt.) in the foregut, gills, skin, gonads, bile, muscle, fat and vertebrae of Barbus marequensis *for males and females seperately, as well as the sexes combined. (Standard deviations are indicated above each bar)*

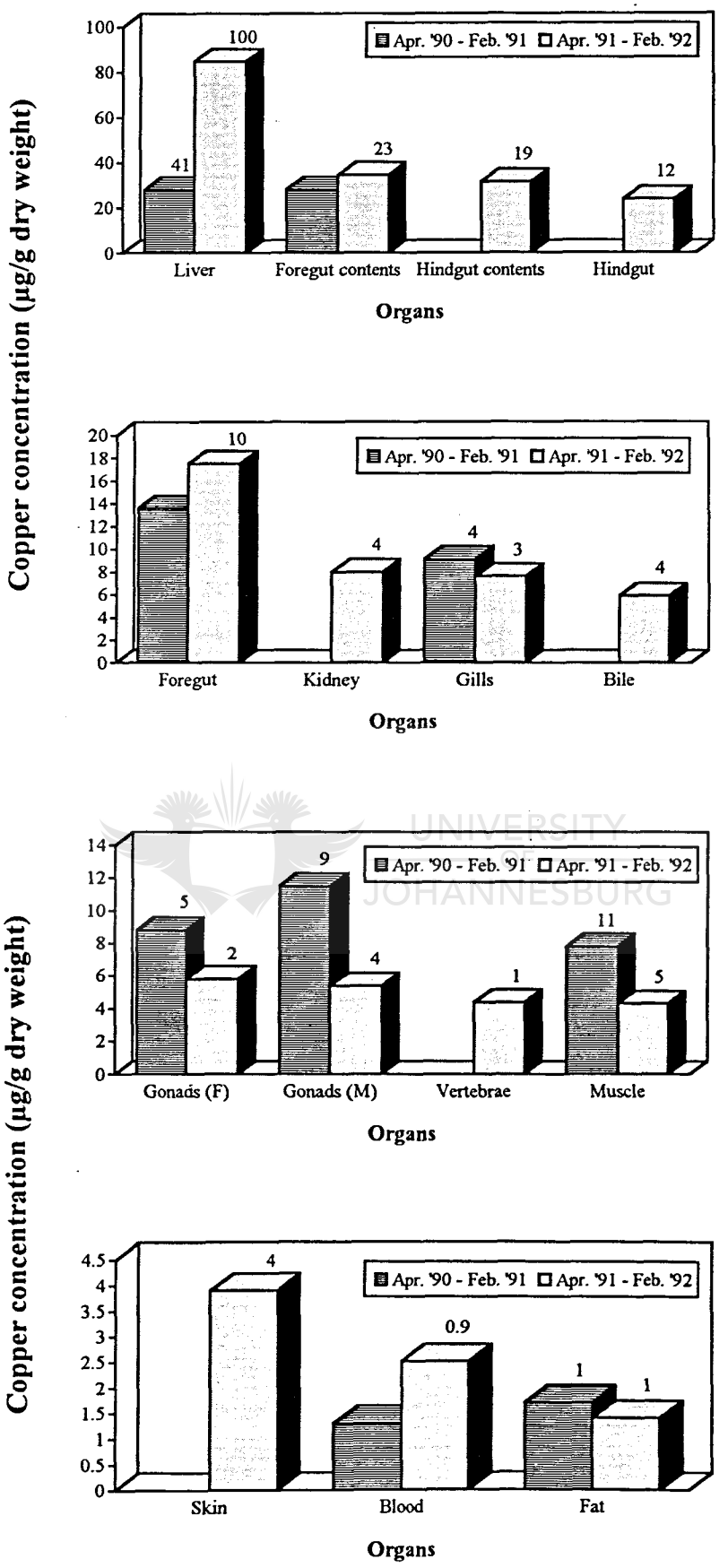

Figure 5.5

Mean copper 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)*

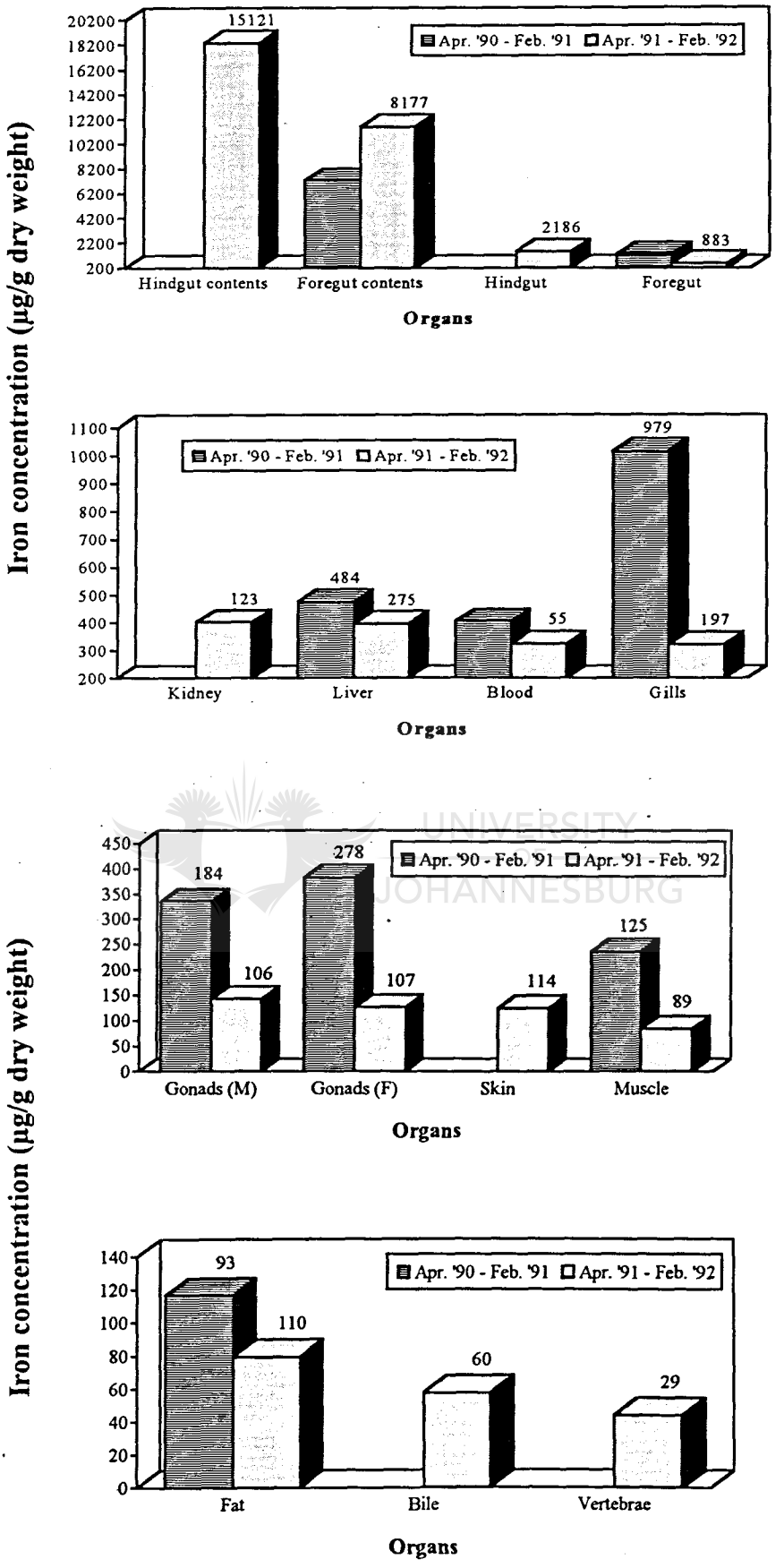

Figure 5.6

Mean iron 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)*

metal levels, especially of iron, in the hindgut (Tables 5.1 and 5.2) suggested, however, that much of the ingested copper and iron was not assimilated (Vidal, 1978). Unfortunately the extent of excretion could not be quantified.

Accumulation in the gills is related to the copper concentration in the water (Benedetti *et al.*, 1989) and presumably also the iron concentration in the water. This was illustrated in December 1990 (Tables 5.1 and 5.2) when elevated copper and iron levels in the water, mainly caused by the floods, led to significant accumulation of these metals in the gills. Elevated copper and iron concentrations in the gills could be due to the metals complexing with the mucus (Heath, 1987), while the extensive vascular network in the gill would have ensured that the blood-borne metals were in intimate contact with the gill tissue (Laurent & Dunel, 1980). Gills have been shown to produce a Cu-binding MT, but in contrast to the liver MT, gill MT only binds very small amounts of copper (Noël-Lambot *et al.*, 1978).

The female gonads accumulated less copper and iron than the male gonads did, except in summer 1990/91, spring 1991 and summer 1992 (Figures 5.2 and 5.4). It was noted that the copper and iron concentrations in the gonads followed a seasonal trend that differed from the trend regarding the zinc concentrations in the gonads. The highest copper concentration in the female gonads occurred in summer 1990/91 (Fig. 5.2), whereas the highest zinc concentration occurred in winter 1990 (Fig. 4.1). The specific role, if any, of copper and iron in gonad development is not certain, but from this study it seemed that if copper and iron were required for certain stages of gonad development, zinc was required for others.

Unlike copper, iron accumulated more in the skin than in the vertebrae (Tables 5.1 and 5.2). The low copper levels in the muscle tissues were well below the set standard for food by the National Health and Medical Research Council, which is 30 µg/g Cu wet weight (Anon., 1972) or in this case 120 µg/g Cu dry weight (the moisture percentage of the muscle was 75%). No comparable standard was available for the iron concentration in the muscle. The copper concentration in the muscle did, however, exceed 4 µg/g Cu dry weight (or 1 µg/g Cu wet weight) from April 1990 to August 1991 (Table 5.1), which is seldom the level of concentration in fish from polluted fresh water (Moore & Ramamoorthy, 1984). Metals that tend to concentrate in the liver may be excreted by the bile (Heath, 1987), which, following the metal concentrations in Tables 5.1 and 5.2, might be the case for copper but not for iron. Little is known about excretion routes in teleosts, but excretion of iron is presumably faecal and/or urinary. The main route for excretion of copper in mammals is *via* the faeces (Klaassen, 1976) and it might also be the case in fish. There are, however, indications that at least some urinary and biliary excretion of copper occurs (Dixon & Sprague, 1981; Heath, 1987).

The low calculated bioconcentration factors (BFs) between the fish organs and the sediment indicated that very little to no copper and iron in the sediment were bioavailable to the fish for uptake. The higher BFs that were calculated between the fish organs and the water suggested a higher degree of metal bioavailability to the fish through the water, although factors such as the water chemistry and regulating processes of copper and iron in the fish (as discussed in Chapter 4) should also be considered in determining the actual degree of metal bioavailability to the fish. The BFs recorded for *Barbus marequensis* in October 1990 at locality 3 in this study, were generally higher than the BFs recorded for *Hydrocynus vittatus* in October 1990 at the same locality (Du Preez & Steyn, 1992), which were generally lower than a hundred. It was only the BFs regarding the copper concentrations in the fat and liver, as well as the iron concentrations in the liver of *B. marequensis* that were lower than the BFs recorded for *H. vittatus*. The BFs recorded by Du Preez & Steyn (1992) were, however, calculated on a wet weight basis and not a dry weight basis, making direct comparisons between the two studies difficult.

The concentrations of copper and iron in the organs and tissues of *B. marequensis* (recorded in summer 1992 in the Olifants River, KNP) were generally lower than the recorded 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). Although the copper concentration in the water of Germiston lake was higher than that in the Olifants River, the liver of *B. marequensis* accumulated more copper than the liver of *C. gariepinus*. It therefore appears that the detected copper concentration in the liver of *B. marequensis* was still below the toxic level and was thus accumulated

rather than regulated. The iron concentration in the water of Germiston lake was either lower or higher than that in the Olifants River, depending on the locality. The higher accumulation of iron by *C. gariepinus* suggested, however, that iron was more available for uptake in Germiston lake than it was in the Olifants River, except at locality 7 in the Selati River (a tributary of the Olifants River), where the vertebrae, fat and gut of *B. marequensis* accumulated more iron than did the same organs of *C. gariepinus*. The gut of *B. marequensis* must therefore have been a more important uptake route of iron than it was for *C. gariepinus*.

LOCALITY DIFFERENCES

No definite trend as to where the highest bioaccumulation had occurred could be established, especially with regard to copper. In general, the fish at locality 7 did, however, accumulate more copper than the fish at the other localities, while the fish at Pionier Dam accumulated the least. The highest iron concentrations were accumulated by the fish at localities 3 and 4, as well as at Pionier Dam and this is probably due to underlying rock formations that produce iron through weathering processes.

The differences that occurred between the localities with regard to the accumulated copper and iron concentrations in the fish organs, did not seem to be correlated to the copper and iron concentrations in the water (Table 3.2), but rather to the concentrations in the food. In October 1991 (Table 5.1), for instance, the fish at locality 5 biomagnified more copper than the fish at the other localities did. In the first year (April 1990 to February 1991), however, there was a correlation between the iron concentrations in the water at each locality (Table 3.2) and the iron concentrations in the gills of the fish at each locality (Table 5.2). This might be due to the fact that the fish were exposed to higher iron concentrations in the first year, because the stronger river flow caused more iron to be available from the underlying substratum through weathering processes.

SEASONAL DIFFERENCES

The summer of 1990/91 differed from the other seasons with respect to the copper and iron concentrations in the fish gills (Figures 5.2 and 5.4). This was due to the higher metal levels in the water after the heavy rainfall in December 1990. The other fish organs did not necessarily accumulate the highest copper and iron concentrations in December 1990, because these metals are biomagnified (accumulated through food) by the fish rather than bioconcentrated (accumulated through water). No definite seasonal trend could therefore be established for most of the organs.

The gonads accumulated the highest copper and iron concentrations in summer 1990/91 (Figures 5.2 and 5.4), but high iron concentrations were also accumulated in autumn 1990 (Fig. 5.4). It is not sure what role, if any, copper and iron played in the gonad development, but there did not seem to be a relationship between the concentrations in the gonads and the concentrations in the liver (Figures 5.1-5.4) to prove that these metals were actually being taken from the liver for gonad development, as was the case with zinc (Chapter 4). Instead it was noted that the seasonal trend in the muscle copper and iron concentrations were similar to the trend in the gonad concentrations, being more pronounced for the copper concentrations (Fig. 5.2). It is not certain why the sexual differences in accumulation had occurred, but these differences are similar to the findings of Vorob'yev & Zaytsev (1975) and De Wet (1990).

ANNUAL DIFFERENCES

The iron concentrations in the organs of *B. marequensis* were higher in the first year than in the second year. More iron was therefore taken up by the fish in the first year, as was illustrated by the foregut iron concentrations (Fig. 5.6). The high accumulation of iron by the gills in the first year, compared to the accumulation in the second year, occurred because the fish were exposed to high iron concentrations in the summer of 1990/91 as a result of the heavy rainfall during that time.

The copper concentrations in the fish organs also seemed to be higher in the first than in the second year (Fig. 5.5), suggesting that more copper must have been ingested by the fish in the first year. Although the foregut showed lower instead of higher copper accumulation in the first year (Fig. 5.5),

it was based on only one sample, which was collected in February 1991 (Table 5.1). It can therefore be assumed that the copper concentrations in the foregut were actually higher in the first than in the second year. Contradicting this was the low copper concentrations in the blood and liver tissues in the first year (Fig. 5.5) compared to that in the second year. A reason for this might be that the fish were actually confronted with higher copper levels in the first year and therefore regulation and detoxification had to take place in the liver. In the second year, however, the copper levels to accumulate were much lower and copper could accordingly be stored in the liver instead of being regulated or detoxified. Low copper levels in the liver could therefore actually indicate high copper intake by the fish. The lower blood copper concentrations in the first year (Fig. 5.5) can be explained by the findings of Grobler-Van Heerden *et al.* (1991), where a decreased bioconcentration in the blood occurred with an increased exposure concentration. Fish, therefore, have a mechanism to prevent excess bioconcentration of copper in the blood.

5.5 Conclusion

The liver accumulated the highest copper concentrations, followed by the gut and kidney, while the fat accumulated the lowest. The detected concentrations in the fish organs suggested no serious copper pollution problem in the study area, although, according to the liver concentrations the fish were exposed to higher copper levels in the first year than in the second year. Suggested organs to sample for copper analysis in fish, are: liver, gut, kidney, bile and muscle tissue (to test its fitness for human consumption). The gills can also be of value in the case of acute copper exposure, especially if histopathological studies are performed in addition to the copper analysis.

Iron mainly accumulated in the gut, followed by the kidney and liver, while the lowest iron concentrations occurred in the vertebrae. Very high iron concentrations had occurred in the study area, but it was mostly unavailable for uptake by the fish. Heavy rainfall can, however, increase iron levels in the water, leading to higher accumulation thereof in the gills. In serious cases the iron can precipitate on the gills, thereby causing a mechanical obstruction that will impair oxygen exchange. Suggested organs to sample for iron analysis in fish, are: the gut, muscle tissue (to test its fitness for human consumption), gills (coupled with histopathological studies) and maybe the skin.

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CHAPTER 6

Chapter 6

CHROMIUM AND NICKEL BIOACCUMULATION IN THE ORGANS AND TISSUES OF *BARBUS MAREQUENSIS*

6.1 Introduction

Chromium and nickel are regarded as essential elements, but, apart from the fact that chromium is found in RNA of a few organisms and also is involved in the glucose tolerance factor, these elements are virtually absent from living organisms (Moore & Ramamoorthy, 1984; Vos *et al.*, 1986). This can probably be ascribed to the lower stability of their protein complexes, which results from the irregular geometry of the protein chelating sites compared to the octahedral sites provided by soil silicates (Moore & Ramamoorthy, 1984). 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. Chromium (VI) is more toxic than chromium (III) and exists only as oxy species, of which hydrochromate ($HCrO_4^-$) and chromate (CrO_4^{2-}) are the most common species (Van der Putte *et al.*, 1981). Interconversions between Cr (VI) and Cr (III) do occur, but most of the time anthropogenically introduced soluble Cr (VI) is reduced to Cr (III). Chromium (III) is kinetically stable, binding to naturally occurring solids and therefore, the dominating fraction of chromium in freshwaters 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 (halides, sulphates, phosphates, carbonates and carbonyls) and organic (oxygen, nitrogen and sulfur donor atoms) 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 *et al.*, 1982) and lethally when exposed to concentrations ranging from 3.5 to 280 mg/l Cr (Moore & Ramamoorthy, 1984; Van der Putte *et al.*, 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 depend 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 toxic action of hexavalent chromium at higher pH values are therefore quite different from that of most other metals, in that ionic Cr (VI) penetrates the gill membrane without binding to it and accumulates in various internal organs (Doudoroff & Katz, 1953; Knoll & Fromm, 1960). 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 *et al.*, 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 *et al.*, 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 *et al.*, 1979; Nath & Kumar, 1990). Lethal concentrations range from 4.4 to 118 mg/l Ni (Pickering & Henderson, 1966) 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).

In this section of the study, the extent of chromium and nickel bioaccumulation in the organs and tissues of *Barbus marequensis* was determined, as well as the organs that accumulated the highest and lowest metal levels respectively.

6.2 Materials and methods

Barbus marequensis was sampled and dissected as described in Chapter 4. Laboratory procedures for chromium and nickel analysis of the fish samples were the same as the procedures described for zinc analysis. Statistical procedures were also the same as described in Chapter 4.

6.3 Results

FISH SIZE AND AGE

The size and age data are summarised in Table 4.1 (see Chapter 4).

BIOACCUMULATION OF CHROMIUM AND NICKEL IN THE DIFFERENT ORGANS AND TISSUES

The order of bioaccumulation of chromium and nickel in the different organs and tissues of *B. marequensis* was not clear to distinguish, 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 (Tables 6.1 and 6.2). 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 > male gonads ≈ fat > female gonads ≈ muscle > skin. Statistically the gut contents differed significantly ($p \leq 0.05$) from all the organs 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 6.3). In the case of nickel, the general order of bioaccumulation was: hindgut contents > foregut contents ≈ blood > vertebrae > gill > hindgut > bile > kidney > foregut > liver > muscle ≈ female gonads > male goads ≈ skin > fat. Statistically the gills, 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 6.4).

The calculated bioconcentration factors between the water and the organs (BF_w) were higher than the biocencentration factors between the sediment and the organs (BF_s). The chromium BF_w values ranged from 4.6 (calculated for female gonads in February 1992) to 2314.3 (calculated for blood in

TABLE 6.1
MEAN CHROMIUM CONCENTRATIONS (µg/g dry wt.) IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS*
(BF_W AND BF_S = BIOCONCENTRATION FACTORS OF THE WATER AND SEDIMENT RESPECTIVELY)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle
Apr. '90	3	n®						1
		Range						17.4
		Median						
		Mean	N/A	N/A	N/A	N/A	N/A	
		SD$^{\Delta}$						
		BFw						79.1
		BFs						0.04
	4	n	4	3				5
		Range	15.4-57.7	10.7-28.6				8.7-34.8
		Median	34.6	25.0				21.7
		Mean	35.6	21.4	N/A	N/A	N/A	20.9
		SD	23.7	9.5				10.4
		BFw	161.8	97.3				95.0
		BFs	0.99	0.59				0.58
	7	n	7				4	9
		Range	23.1-53.9				18.5-29.6	13.0-69.6
		Median	30.8				25.9	39.1
		Mean	33.0	N/A	N/A	N/A	25.0	38.2
		SD	10.8				4.7	16.2
		BFw	143.5				108.7	166.1
		BFs	0.21				0.16	0.24
June '90	3	n	2					2
		Range	26.9-26.9					17.4-34.8
		Median	26.9					26.1
		Mean	26.9	N/A	N/A	N/A	N/A	26.1
		SD	0.0					12.3
		BFw	50.8					49.2
		BFs	0.16					0.15
Aug. '90	3	n	8	2	1	1	2	9
		Range	19.2-30.8	17.9-50.0	23.8	5.6	18.5-25.9	17.4-65.2
		Median	23.1	33.9			22.2	21.7
		Mean	24.0	33.9			22.2	25.1
		SD	4.0	22.7			5.2	15.2
		BFw	38.1	53.8	37.8	8.9	35.2	39.8
		BFs	0.26	0.36	0.26	0.06	0.24	0.27
	4	n	9	4	3	4	6	9
		Range	15.4-69.2	14.3-60.7	19.1-23.8	4.4-8.9	14.8-66.7	13.0-73.9
		Median	26.9	19.6	23.8	6.7	18.5	17.4
		Mean	30.8	28.6	22.2	6.7	25.9	24.6
		SD	16.3	21.6	2.7	2.0	20.2	18.7
		BFw	44.6	41.4	32.2	9.7	37.5	35.7
		BFs	0.24	0.22	0.17	0.05	0.20	0.19
	5	n	7	1	2	3	7	6
		Range	15.4-23.1	17.9	19.1-23.8	3.3-5.6	11.1-14.8	13.0-21.7
		Median	19.2		21.4	4.4	14.8	15.2
		Mean	19.2		21.4	4.4	13.2	15.9
		SD	2.2		3.4	1.2	2.0	3.6
		BFw	28.2	26.3	31.5	6.5	19.4	23.4
		BFs	0.11	0.10	0.12	0.03	0.08	0.09
	7	n						5
		Range						17.4-17.4
		Median						17.4
		Mean	N/A	N/A	N/A	N/A	N/A	17.4
		SD						0.0
		BFw						17.8
		BFs						0.09

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 6.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Gut	Gut cont	Blood
Oct. '90	3	n[①]	7	2	1	2	6	7			
		Range	19.2-26.9	14.3-17.9	19.1	5.6-5.6	14.8-22.2	17.4-30.4			
		Median	23.1	16.1		5.6	18.5	17.4			
		Mean	22.0	16.1		5.6	18.5	21.1	N/A	N/A	N/A
		SD[Δ]	2.9	2.5		0.0	2.3	5.3			
		BFw	27.2	19.9	23.6	6.9	22.8	26.0			
		BFs	0.41	0.30	0.35	0.10	0.34	0.39			
	4	n	10	1	7	6	9	10			
		Range	19.2-80.8	25.0	9.5-33.3	3.3-8.9	11.1-22.2	8.7-26.1			
		Median	38.5		23.8	6.7	18.5	17.4			
		Mean	39.6		21.1	6.5	17.3	17.8	N/A	N/A	N/A
		SD	17.9		8.6	1.8	3.2	5.2			
		BFw	49.5	31.3	26.4	8.1	21.6	22.2			
		BFs	0.40	0.25	0.21	0.06	0.17	0.18			
	5	n	9	1	6	9	9	10			
		Range	11.5-19.2	10.7	14.3-19.1	3.3-8.9	11.1-14.8	8.7-17.4			
		Median	15.4		14.3	4.4	11.1	13.0			
		Mean	15.4		15.1	5.0	12.3	13.9	N/A	N/A	N/A
		SD	2.7		1.9	1.6	1.9	2.7			
		BFw	42.8	29.7	41.9	13.9	34.2	38.6			
		BFs	0.19	0.13	0.18	0.06	0.15	0.17			
	7	n	1	1		1	1	1			
		Range	26.9	33.3		6.7	18.5	26.1			
		Median									
		Mean			N/A				N/A	N/A	N/A
		SD									
		BFw	43.4	53.7		10.8	29.8	42.1			
		BFs	0.22	0.28		0.06	0.15	0.22			
Dec. '90	3	n	7	3	1	3	2	7			
		Range	38.5-142.3	32.1-57.1	42.9	8.9-11.1	29.6-37.0	30.4-43.5			
		Median	119.2	53.6		10.0	33.3	34.8			
		Mean	104.4	47.6		10.0	33.3	35.4	N/A	N/A	N/A
		SD	34.9	13.5		1.1	5.2	5.3			
		BFw	93.2	42.5	38.3	8.9	29.7	31.6			
		BFs	3.45	1.57	1.42	0.33	1.10	1.17			
	5	n	1					1			
		Range	88.5					26.1			
		Median									
		Mean		N/A	N/A	N/A	N/A		N/A	N/A	N/A
		SD									
		BFw	354.0					104.4			
		BFs	1.09					0.32			
Feb. '91	5	n	1		1			1	1	1	1
		Range	45.7		25.6			43.3	66.4	115.9	17.9
		Median									
		Mean		N/A		N/A	N/A				
		SD									
		BFw	253.9		142.2			240.6	368.9		99.4
		BFs	0.52		0.29			0.49	0.76		0.20
	7	n	2					2		1	6
		Range	35.2-35.6					15.4-17.7		123.3	16.4-26.1
		Median	35.4					16.6			17.2
		Mean	35.4	N/A	N/A	N/A	N/A	16.6	N/A		18.6
		SD	0.2					1.6			3.7
		BFw	208.2					97.6			109.4
		BFs	1.01					0.47			0.53

① Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 6.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	Gut cont.	Vertebrae	Bile	Blood
Apr. '91	3	n[®]	3	4	1	1	3	6		2	1	3			8
		Range	17.9-23.9	7.5-29.6	17.1	27.2	13.9-27.1	11.5-25.4		18.6-22.7	94.6	67.3-115.4			15.7-18.2
		Median	18.4	19.0			20.3	15.8		20.6		114.4			18.1
		Mean	20.1	18.8			20.5	17.3	N/A	20.6		99.0	N/A	N/A	17.6
		SD[Δ]	3.4	9.0			6.6	6.3		2.9		27.5			0.9
		BFw	95.7	89.5	81.4	129.5	97.6	82.4		98.1	450.5				83.8
		BFs	0.04	0.04	0.03	0.05	0.04	0.03		0.04	0.19				0.03
	4	n	3		1	2	1	6		1		2			10
		Range	23.2-32.3		34.7	18.4-39.0	26.4	15.0-33.0		48.6		251.8-895.3			13.1-16.9
		Median	29.0			28.7		21.2				573.5			15.6
		Mean	28.2	N/A		28.7		22.6	N/A		N/A	573.5	N/A	N/A	15.4
		SD	4.6			14.6		6.1				455.0			1.2
		BFw	141.0		173.5	143.5	132.0	113.0		243.0					77.0
		BFs	0.57		0.70	0.58	0.54	0.46		0.99					0.31
	5	n	4	5		3	3	7		3	2	3			10
		Range	9.4-14.6	2.6-16.2		7.4-14.2	8.4-13.3	7.1-40.0		28.7-60.8	34.6-48.8	79.0-435.8			11.1-19.1
		Median	12.0	13.1		7.4	11.6	7.6		40.1	41.7	192.4			17.8
		Mean	12.0	10.2	N/A	9.7	11.1	12.7	N/A	43.2	41.7	235.7	N/A	N/A	17.0
		SD	2.9	6.2		3.9	2.5	12.1		16.3	10.1	182.3			2.5
		BFw	109.1	92.7		88.2	100.9	115.5		392.7	379.1				154.5
		BFs	0.50	0.42		0.40	0.46	0.53		1.79	1.73				0.71
	7	n	1		1	1	1	1				1			1
		Range	34.0		23.9	14.3	34.7	14.0				59.7			16.3
		Median													
		Mean		N/A					N/A	N/A	N/A		N/A	N/A	
		SD													
		BFw	200.0		140.6	84.1	204.1	82.4							95.9
		BFs	0.63		0.44	0.26	0.64	0.26							0.30
June '91	3	n	9	3		9	9	9	9	8	2	8	9		9
		Range	12.3-25.7	14.5-22.5		3.3-14.2	14.5-43.2	13.6-26.2	22.1-54.0	22.7-64.7	40.2-57.3	100.2-558.5	8.8-22.4		18.1-20.2
		Median	19.8	22.1		7.4	24.8	20.2	36.7	40.3	48.7	254.2	15.3		19.3
		Mean	19.3	19.7	N/A	7.5	26.2	18.8	37.3	38.3	48.7	251.6	16.0	N/A	19.3
		SD	4.2	4.5		3.1	8.5	4.7	13.2	13.7	12.1	142.5	5.1		0.8
		BFw	91.9	93.8		35.7	124.8	89.5	177.6	182.4	231.9		76.2		91.9
		BFs	0.64	0.65		0.25	0.87	0.62	1.24	1.27	1.61		0.53		0.64
	4	n	7	1	6	6	7	7	4	7		7	7	1	7
		Range	8.3-20.7	3.9	6.2-32.1	3.6-7.8	7.4-12.3	3.2-8.2	8.4-18.7	12.8-31.9		34.7-239.8	6.3-11.0	40.0	16.0-19.8
		Median	13.4		13.5	5.6	10.0	5.3	14.6	21.3		144.1	9.8		18.6
		Mean	14.5		17.1	5.8	10.0	5.7	14.1	20.9	N/A	140.8	9.6		18.4
		SD	4.7		10.0	1.6	1.6	1.7	5.0	5.9		65.2	1.6		1.2
		BFw	483.3	130.0	570.0	193.3	333.3	190.0	470.0	696.7			320.0	1333.3	613.3
		BFs	0.27	0.07	0.32	0.11	0.19	0.11	0.26	0.39			0.18	0.74	0.34
	5	n	3		3	3	3	3	3	3	1	2	3		3
		Range	11.4-14.7		17.2-20.2	6.0-7.4	10.2-11.0	9.9-13.2	14.6-22.6	18.6-20.3	28.3	101.0-124.3	8.5-13.3		18.8-19.5
		Median	14.6		19.6	7.1	10.3	12.8	19.2	19.2		112.6	9.4		19.0
		Mean	13.6	N/A	19.0	6.8	10.5	11.9	18.8	19.4		112.6	10.4	N/A	19.1
		SD	1.9		1.6	0.7	0.4	1.8	4.0	0.8		16.5	2.5		0.4
		BFw	68.0		95.0	34.0	52.5	59.5	94.0	97.0	141.5		52.0		95.5
		BFs	0.21		0.30	0.11	0.17	0.19	0.30	0.31	0.44		0.16		0.30

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 6.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont	HGut cont	Vertebrae	Kidney	Bile	Blood
Aug. '91	3	n①	1	1		1	1	1	1	1		1		1			1
		Range	4.1	3.7		1.7	2.5	1.1	2.6	2.7		22.0		5.0			14.2
		Median															
		Mean			N/A						N/A		N/A		N/A	N/A	
		SD△															
		BFw	32.8	29.6		13.6	20.0	8.8	20.8	21.6				40.0			113.6
		BFs	0.07	0.06		0.03	0.04	0.02	0.04	0.04				0.08			0.24
	4	n	8	5		8	8	8	7	7		5	5	8	2		8
		Range	2.5-4.9	0.4-4.8		0.2-1.4	0.8-3.3	0.5-1.0	0.7-1.3	1.2-7.5		45.2-519.5	109.1-1462.6	4.3-5.2	2.1-3.0		14.0-15.7
		Median	2.8	0.9		0.9	1.2	0.6	1.0	1.8		118.5	416.2	4.5	2.6		15.0
		Mean	3.1	1.6	N/A	0.8	1.4	0.7	1.0	2.6	N/A	195.0	671.6	4.6	2.6	N/A	14.9
		SD	0.8	1.8		0.3	0.8	0.2	0.2	2.2		195.8	540.6	0.3	0.7		0.5
		BFw	110.7	57.1		28.6	50.0	25.0	35.7	92.9				164.3	92.9		532.1
		BFs	0.003	0.002		0.001	0.001	0.001	0.001	0.002				0.004	0.002		0.01
	5	n	12	11	1	12	12	12	12	10	4	8	8	12	7	4	12
		Range	2.3-8.5	0.5-5.2	26.0	0.9-6.8	1.0-7.6	1.1-5.0	1.0-4.6	1.5-7.4	1.4-3.7	10.8-306.6	23.6-204.0	4.7-10.5	2.7-7.8	1.3-10.7	13.4-15.3
		Median	2.8	1.2		1.9	1.6	1.5	1.6	2.5	2.3	30.1	64.7	5.6	4.8	6.4	14.5
		Mean	3.8	1.7		3.0	2.9	2.2	2.1	3.0	2.4	94.3	95.5	6.7	4.7	6.2	14.5
		SD	1.9	1.5		2.1	2.4	1.4	1.2	1.9	1.1	106.8	67.4	2.2	1.7	4.6	0.6
		BFw	90.5	40.5	619.0	71.4	69.0	52.4	50.0	71.4	57.1			159.5	111.9	147.6	345.2
		BFs	0.04	0.02	0.28	0.03	0.03	0.02	0.02	0.03	0.03			0.07	0.05	0.07	0.16
	7	n	1			1	1	1	1	1				1		1	1
		Range	5.2			10.1	4.1	1.2	5.2	5.2				7.7		6.2	28.2
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A		N/A		
		SD															
		BFw	106.1			206.1	83.7	24.5	106.1	106.1				157.1		126.5	575.5
		BFs	0.11			0.21	0.09	0.03	0.11	0.11				0.16		0.13	0.60
Oct. '91	3	n	6	2	1	6	5	6	6	5	3	2	3	6	3	5	6
		Range	3.6-9.4	2.8-15.1	12.3	2.4-6.5	3.5-13.1	1.0-5.8	1.4-5.6	2.1-95.6	5.0-20.6	117.3-462.9	55.6-421.5	5.8-9.8	3.4-10.1	1.0-23.3	13.7-15.8
		Median	7.3	9.0		4.0	6.4	3.0	2.7	5.2	6.6	290.1	236.1	6.9	9.5	8.0	14.6
		Mean	6.7	9.0		4.2	7.0	3.3	3.3	24.2	10.8	290.1	237.7	7.3	7.7	10.6	14.6
		SD	2.6	8.7		1.4	3.6	1.9	1.6	40.2	8.6	244.4	182.9	1.5	3.7	9.5	0.7
		BFw	*	*	*	*	*	*	*	*	*			*	*	*	*
		BFs	0.26	0.35	0.48	0.16	0.27	0.13	0.13	0.94	0.42			0.28	0.30	0.41	0.57
	4	n	11	7	1	11	8	11	11	5	2	9	4	11	3	6	10
		Range	3.5-5.6	1.3-2.8	1.8	2.2-3.7	2.6-5.3	0.9-4.5	1.6-4.3	1.4-2.4	4.2-7.0	18.5-1003.5	15.2-496.6	5.6-8.4	4.1-6.7	2.5-9.6	12.5-17.2
		Median	4.0	1.8		2.8	3.6	1.5	2.1	1.7	5.6	47.0	156.0	6.6	6.3	8.1	14.1
		Mean	4.3	1.9		2.8	3.8	2.0	2.5	1.8	5.6	173.1	205.9	6.7	5.7	6.8	14.2
		SD	0.7	0.5		0.4	1.0	1.2	1.0	0.4	2.0	317.7	222.4	1.0	1.4	3.1	1.4
		BFw	*	*	*	*	*	*	*	*	*			*	*	*	*
		BFs	0.13	0.06	0.06	0.09	0.12	0.06	0.08	0.06	0.17			0.21	0.18	0.21	0.44
	5	n	14	9	4	15	13	15	14	3	3	10	4	15	5	9	10
		Range	3.8-7.0	1.2-4.8	1.4-1.9	1.3-7.7	1.8-5.9	0.5-3.5	0.7-2.7	1.8-4.8	4.4-5.0	8.8-195.4	48.0-96.8	5.8-8.9	1.6-11.3	0.9-6.2	12.1-19.3
		Median	4.2	1.6	1.5	1.8	2.6	1.3	1.2	2.6	4.8	50.7	85.1	7.1	2.7	2.3	15.0
		Mean	4.5	1.9	1.6	2.2	3.0	1.4	1.3	3.1	4.7	69.0	78.7	7.0	4.3	2.9	14.7
		SD	1.0	1.1	0.3	1.5	1.0	0.8	0.6	1.6	0.3	70.3	21.3	0.9	4.1	1.9	2.0
		BFw	250.0	105.6	88.9	122.2	166.7	77.8	72.2	172.2	261.1			388.9	238.9	161.1	816.7
		BFs	0.09	0.04	0.03	0.05	0.06	0.03	0.03	0.06	0.10			0.14	0.09	0.06	0.30
	7	n	1			1	1	1	1	1				1	1	1	1
		Range	6.8			3.9	3.3	1.4	4.1	5.3				8.6	12.8	10.7	12.7
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A				
		SD															
		BFw	*			*	*	*	*	*				*	*	*	*
		BFs	0.09			0.05	0.04	0.02	0.05	0.07				0.11	0.16	0.14	0.16

① Number of samples analyzed △ Standard deviation N/A Not available * [Cr] in water below AAS detection limit

TABLE 6.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont.	HGut cont.	Vertebrae	Kidney	Bile	Blood
Jan. '92	3	n[Φ]	5	4		5	3	6	2	2	2	2	2	6	1	5	6
		Range	4.1-7.5	1.4-2.8		1.6-37.7	2.5-2.7	0.9-1.8	2.3-3.2	2.4-6.4	8.1-16.0	416.3-1259.2	457.2-648.7	5.3-20.1	4.1	7.3-46.8	14.6-17.1
		Median	4.3	1.9		2.4	2.6	1.4	2.7	4.4	12.1	837.7	552.9	5.9		23.4	15.9
		Mean	5.0	2.0	N/A	9.3	2.6	1.4	2.7	4.4	12.1	837.7	552.9	8.3		22.5	15.8
		SD[Δ]	1.4	0.7		15.9	0.1	0.3	0.6	2.8	5.6	596.0	135.4	5.8		16.2	0.9
		BFw	72.5	29.0		134.8	37.7	20.3	39.1	63.8	175.4			120.3	59.4	326.1	229.0
		BFs	0.09	0.04		0.17	0.05	0.03	0.05	0.08	0.22			0.15	0.07	0.41	0.29
	4	n	4	3		10	2	11	3	1	1	1	3	9	1	2	11
		Range	5.3-6.0	1.5-2.9		2.2-4.9	2.1-3.6	1.4-3.0	2.2-4.9	2.5	7.6	84.3	101.7-995.6	5.9-16.3	3.7	1.4-4.3	13.5-25.5
		Median	5.6	2.3		3.3	2.9	2.1	2.9				727.4	6.9		2.9	15.3
		Mean	5.6	2.2	N/A	3.4	2.9	2.2	3.3				608.2	7.9		2.9	16.2
		SD	0.3	0.7		1.0	1.1	0.5	1.4				458.7	3.2		2.1	3.3
		BFw	800.0	314.3		485.7	414.3	314.3	471.4	357.1	1085.7			1128.6	528.6	414.3	2314.3
		BFs	0.05	0.02		0.03	0.03	0.02	0.03	0.02	0.07			0.07	0.03	0.03	0.14
	5	n	12	8	4	12	9	12	11	3	3	2	1	12	3	9	12
		Range	4.1-8.7	2.1-3.3	2.1-2.5	1.3-3.7	2.5-8.6	1.2-15.5	1.3-3.6	3.4-7.8	5.4-16.9	15.5-671.6	341.6	5.8-8.9	3.4-5.3	1.0-62.5	13.0-29.0
		Median	6.1	2.3	2.2	3.1	4.2	2.0	2.0	5.0	6.3	343.6		6.7	4.0	4.8	14.5
		Mean	6.2	2.4	2.2	2.7	4.6	3.7	2.5	5.4	9.5	343.6		6.7	4.2	12.1	15.7
		SD	1.6	0.4	0.2	0.9	1.8	4.3	0.9	2.2	6.4	463.9		0.8	1.0	19.4	4.3
		BFw	387.5	150.0	137.5	168.7	287.5	231.2	156.3	337.5	593.8			418.8	262.5	756.2	981.2
		BFs	0.11	0.04	0.04	0.05	0.08	0.07	0.04	0.10	0.17			0.12	0.07	0.22	0.28
	7	n	1			5		5		1				5			5
		Range	4.8			1.1-6.9		1.1-5.3		4.6				6.5-28.4			14.2-17.0
		Median				3.8		1.5						8.3			15.7
		Mean		N/A	N/A	4.0	N/A	2.3	N/A		N/A	N/A	N/A	14.7	N/A	N/A	15.7
		SD				2.4		1.7						10.5			1.1
		BFw	*			*		*		*				*			*
		BFs	0.08			0.06		0.04		0.07				0.24			0.25
Feb. '92	3	n	2			6		6	3					6			6
		Range	3.2-4.3			0.3-1.1		0.3-1.4	0.8-1.3					5.2-6.3			13.6-23.8
		Median	3.7			0.8		0.9	0.9					5.9			15.6
		Mean	3.7	N/A	N/A	0.8	N/A	0.9	1.0	N/A	N/A	N/A	N/A	5.8	N/A	N/A	16.7
		SD	0.8			0.3		0.4	0.3					0.4			3.8
		BFw	59.7			12.9		14.5	16.1					93.5			269.4
		BFs	0.31			0.07		0.07	0.08					0.48			1.39
	4	n	6	4		10	7	10	8	1	1	1	1	10	1	9	10
		Range	3.1-4.1	0.7-1.2		0.6-1.6	1.9-3.6	0.4-1.7	0.3-7.2	21.1	2.9	118.3	439.7	5.1-6.9	2.1	0.1-3.4	13.4-16.2
		Median	3.5	1.0		0.9	3.0	1.1	2.0					5.8		1.4	14.7
		Mean	3.6	1.0	N/A	1.1	2.8	1.1	2.2					5.8		1.4	14.7
		SD	0.5	0.3		0.4	0.6	0.4	2.2					0.6		1.0	0.8
		BFw	16.5	4.6		5.0	12.8	5.0	10.1	96.8	13.3			26.6	9.6	6.4	67.4
		BFs	0.05	0.01		0.02	0.04	0.02	0.03	0.30	0.04			0.08	0.03	0.02	0.21
	5	n	10	10		10	8	10	10	2	2			10	2	10	10
		Range	2.8-21.3	0.5-5.2		0.3-20.5	1.5-3.0	0.4-10.5	0.7-8.8	2.3-2.9	1.5-2.8			4.8-22.0	2.3-2.3	0.3-1.7	13.3-26.2
		Median	3.7	0.8		1.0	2.2	0.7	1.0	2.6	2.1			5.8	2.3	0.9	15.7
		Mean	5.4	1.2	N/A	7.2	2.2	2.1	2.4	2.6	2.1	N/A	N/A	9.5	2.3	0.9	17.4
		SD	5.6	1.4		9.1	0.5	3.4	3.0	0.4	0.9			6.7	0.0	0.4	4.4
		BFw	101.9	22.6		135.8	41.5	39.6	45.3	49.1	39.6			179.2	43.4	17.0	328.3
		BFs	0.02	0.01		0.03	0.01	0.01	0.01	0.01	0.01			0.04	0.01	0.004	0.08
	Pionier Dam	n	10	5	5	10	9	10	10	6	2	6		10	4	5	10
		Range	3.2-15.5	1.6-13.8	0.9-1.5	0.7-31.4	1.1-19.8	0.5-12.8	0.5-22.4	2.8-27.3	5.0-25.7	17.0-83.0		4.8-12.9	1.6-26.4	10.0-18.0	10.3-12.6
		Median	4.2	13.3	1.1	4.3	10.3	1.0	1.7	13.1	15.3	49.1		5.5	18.9	12.8	12.1
		Mean	7.4	9.9	1.1	9.8	9.6	4.0	7.3	14.3	15.3	47.7	N/A	6.8	16.5	13.3	11.7
		SD	5.1	5.3	0.3	11.6	7.7	4.4	8.4	11.9	14.6	26.3		3.0	10.7	3.4	0.8
		BFw	139.6	186.8	20.8	184.9	181.1	75.5	137.7	269.8	288.7			128.3	311.3	250.9	220.8
		BFs	0.44	0.59	0.07	0.58	0.57	0.24	0.43	0.85	0.91			0.40	0.98	0.79	0.69

Φ Number of samples analyzed Δ Standard deviation N/A Not available * [Cr] in water below AAS detection limit

TABLE 6.2
MEAN NICKEL CONCENTRATIONS (µg/g dry wt.) IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS*
(BF_w AND BF_s = BIOCONCENTRATION FACTORS IN THE WATER AND SEDIMENT RESPECTIVELY)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle
Apr. '90	3	n®						1
		Range						26.1
		Median						
		Mean	N/A	N/A	N/A	N/A	N/A	
		SD^Δ						
		BFw						113.5
		BFs						0.17
	4	n	4	3				5
		Range	11.5-26.9	7.1-21.4				8.7-34.8
		Median	19.2	10.7				13.0
		Mean	19.2	13.1	N/A	N/A	N/A	16.5
		SD	7.0	7.4				10.8
		BFw	71.1	48.5				61.1
		BFs	0.44	0.30				0.38
	7	n	7				4	9
		Range	15.4-38.5				7.4-18.5	4.4-26.1
		Median	23.1				13.0	17.4
		Mean	22.5	N/A	N/A	N/A	13.0	17.4
		SD	7.8				6.4	6.1
		BFw	90.0				52.0	69.6
		BFs	0.20				0.11	0.15
June '90	3	n	2					2
		Range	19.2-19.2					13.0-17.4
		Median	19.2					15.2
		Mean	19.2	N/A	N/A	N/A	N/A	15.2
		SD	0.0					3.1
		BFw	56.5					44.7
		BFs	0.20					0.16
Aug. '90	3	n	8	2	1	1	2	9
		Range	15.4-30.8	17.9-21.4	23.8	3.3	22.2-22.2	8.7-34.8
		Median	23.1	19.6			22.2	21.7
		Mean	23.6	19.6			22.2	22.7
		SD	5.6	2.5			0.0	9.4
		BFw	147.5	122.5	148.7	20.6	138.7	141.9
		BFs	0.33	0.27	0.33	0.05	0.31	0.32
	4	n	9	4	3	4	6	9
		Range	15.4-34.6	7.1-28.6	19.1-33.3	1.1-8.9	7.4-33.3	8.7-39.1
		Median	19.2	14.3	33.3	3.3	14.8	17.4
		Mean	21.8	16.1	28.6	4.2	17.9	18.8
		SD	5.4	9.0	8.2	3.3	9.8	9.5
		BFw	136.2	100.6	178.7	26.3	111.9	117.5
		BFs	0.40	0.29	0.52	0.08	0.33	0.34
	5	n	7	1	2	3	7	6
		Range	11.5-19.2	14.3	4.8-9.5	1.1-2.2	3.7-14.8	4.4-13.0
		Median	15.4		7.1	1.1	7.4	6.5
		Mean	14.8		7.1	1.5	9.0	8.0
		SD	3.5		3.4	0.6	4.7	4.3
		BFw	77.9	75.3	37.4	7.9	47.4	42.1
		BFs	0.15	0.15	0.07	0.02	0.09	0.08
	7	n						5
		Range						4.4-8.7
		Median						4.4
		Mean	N/A	N/A	N/A	N/A	N/A	6.1
		SD						2.4
		BFw						29.0
		BFs						0.04

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 6.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Gut	Gut cont	Blood
Oct. '90	3	n®	7	2	1	2	6	7			
		Range	7.7-15.4	7.1-10.7	9.5	2.2-3.3	3.7-14.8	4.4-13.0			
		Median	11.5	8.9		2.8	7.4	8.7			
		Mean	11.0	8.9		2.8	8.6	9.3	N/A	N/A	N/A
		SD$^{\Delta}$	3.5	2.5		0.8	3.8	3.0			
		BFw	64.7	52.4	55.9	16.5	50.6	54.7			
		BFs	0.38	0.31	0.33	0.10	0.30	0.32			
	4	n	10	1	7	6	9	10			
		Range	3.9-19.2	3.6	4.8-23.8	1.1-6.7	3.7-18.5	4.4-13.0			
		Median	13.5		9.5	2.2	7.4	8.7			
		Mean	13.1		10.2	3.3	8.6	8.7	N/A	N/A	N/A
		SD	6.6		7.0	2.7	4.5	4.1			
		BFw	68.9	18.9	53.7	17.4	45.3	45.8			
		BFs	0.24	0.07	0.19	0.06	0.16	0.16			
	5	n	9	1	6	9	9	10			
		Range	3.9-26.9	14.3	4.8-19.1	2.2-7.8	3.7-14.8	4.4-26.1			
		Median	11.5		9.5	4.4	7.4	10.9			
		Mean	12.8		11.1	4.3	8.2	13.0	N/A	N/A	N/A
		SD	6.9		4.9	1.8	3.6	6.8			
		BFw	71.1	79.4	61.7	23.9	45.6	72.2			
		BFs	0.33	0.37	0.28	0.11	0.21	0.33			
	7	n	1	1		1	1	1			
		Range	19.2	28.6		7.8	22.2	26.1			
		Median									
		Mean			N/A				N/A	N/A	N/A
		SD									
		BFw	120.0	178.7		48.8	138.7	163.1			
		BFs	0.32	0.48		0.13	0.37	0.43			
Dec. '90	3	n	7	3	1	3	2	7			
		Range	15.4-46.2	7.1-17.9	14.3	2.2-6.7	18.5-22.2	4.4-17.4			
		Median	38.5	10.7		4.4	20.4	8.7			
		Mean	34.6	11.9		4.4	20.4	10.6	N/A	N/A	N/A
		SD	12.6	5.5		2.3	2.6	4.2			
		BFw	50.1	17.2	20.7	6.4	29.6	15.4			
		BFs	0.74	0.26	0.31	0.09	0.44	0.23			
	5	n	1					1			
		Range	38.5					21.7			
		Median									
		Mean		N/A	N/A	N/A	N/A		N/A	N/A	N/A
		SD									
		BFw	296.2					166.9			
		BFs	0.72					0.40			
Feb. '91	5	n	1		1			1	1	1	1
		Range	31.9		15.5			26.6	38.6	61.6	16.4
		Median									
		Mean		N/A		N/A	N/A				
		SD									
		BFw	265.8		129.2			221.7	321.7		136.7
		BFs	0.60		0.29			0.50	0.73		0.31
	7	n	2					2		1	6
		Range	31.4-43.5					13.2-14.4		116.7	16.7-27.9
		Median	37.5					13.8			18.3
		Mean	37.5	N/A	N/A	N/A	N/A	13.8	N/A		19.6
		SD	8.5					0.9			4.2
		BFw	312.5					115.0			163.3
		BFs	1.38					0.51			0.72

® Number of samples analyzed $^{\Delta}$ Standard deviation N/A Not available

TABLE 6.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	Gut cont.	Vertebrae	Bile	Blood
Apr. '91	3	n[Φ]	3	4	1	1	3	6		2	1	3			8
		Range	19.4-22.3	5.6-26.5	13.9	29.3	8.7-19.7	10.1-23.7		17.0-17.6	42.3	36.5-63.7			14.5-17.0
		Median	21.4	14.4			15.6	16.5		17.3		42.2			15.7
		Mean	21.0	15.2			14.7	16.6	N/A	17.3		47.5	N/A	N/A	15.8
		SD[Δ]	1.5	8.9			5.6	5.7		0.4		14.3			0.8
		BFw	233.3	168.9	154.4	325.6	163.3	184.4		192.2	470.0				175.6
		BFs	0.25	0.18	0.16	0.35	0.17	0.20		0.20	0.50				0.19
	4	n	3		1	2	2	6		1		2			10
		Range	20.3-26.3		25.0	14.0-27.6	19.3-25.6	14.3-23.7		24.4		73.2-79.8			14.8-23.5
		Median	25.5			20.8	22.5	17.1				76.5			16.2
		Mean	24.0	N/A		20.8	22.5	18.0	N/A		N/A	76.5	N/A	N/A	16.9
		SD	3.3			9.6	4.5	3.4				4.7			2.5
		BFw	300.0		312.5	260.0	281.3	225.0		305.0					211.2
		BFs	0.51		0.53	0.44	0.48	0.38		0.52					0.36
	5	n	4	5		3	3	7		3	2	3			10
		Range	10.1-15.3	2.6-11.8		4.0-7.6	5.4-22.9	5.1-7.9		9.6-15.9	21.6-28.6	29.3-46.5			15.0-17.4
		Median	12.0	8.0		4.6	7.3	5.9		13.3	25.1	34.7			16.3
		Mean	12.4	7.4	N/A	5.4	10.7	6.1	N/A	12.9	25.1	36.8	N/A	N/A	16.3
		SD	2.2	4.5		2.0	8.2	0.9		3.2	4.9	8.8			0.7
		BFw	248.0	148.0		108.0	214.0	122.0		258.0	502.0				326.0
		BFs	0.68	0.41		0.30	0.59	0.34		0.71	1.38				0.90
	7	n	1		1	1	1	1				1			1
		Range	29.5		17.3	11.5	25.0	11.3				53.3			15.5
		Median													
		Mean		N/A					N/A	N/A	N/A		N/A	N/A	
		SD													
		BFw	421.4		247.1	164.3	357.1	161.4							221.4
		BFs	0.60		0.35	0.23	0.51	0.23							0.32
June '91	3	n	9	3		9	9	9	9	8	2	8	9		9
		Range	11.9-23.2	11.9-19.0		4.5-13.5	10.9-24.4	10.2-16.7	16.1-42.0	17.2-55.6	38.9-47.5	30.8-69.6	10.8-21.1		14.9-17.6
		Median	17.4	17.3		5.8	17.0	16.0	27.8	31.3	43.2	63.8	14.9		16.2
		Mean	17.0	16.1	N/A	7.1	18.5	14.7	27.4	31.0	43.2	60.3	15.4	N/A	16.4
		SD	3.4	3.7		2.8	4.4	2.3	9.8	11.9	6.1	12.7	3.2		0.9
		BFw	141.7	134.2		59.2	154.2	122.5	228.3	258.3	360.0		128.3		136.7
		BFs	0.20	0.19		0.08	0.22	0.18	0.33	0.37	0.52		0.18		0.20
	4	n	7	1	6	6	7	7	4	7		7	7	1	7
		Range	6.9-21.3	3.9	4.7-26.1	1.8-5.5	3.2-12.9	3.8-9.0	7.9-13.8	7.6-14.6		27.0-82.9	10.8-12.5	17.4	15.0-18.6
		Median	11.0		7.9	2.9	5.8	4.4	10.2	10.9		51.7	11.7		17.0
		Mean	11.4		12.0	3.2	6.6	5.5	10.5	11.2	N/A	54.7	11.7		16.9
		SD	4.9		9.2	1.3	3.2	2.0	3.1	2.9		19.6	0.6		1.2
		BFw	570.0	195.0	600.0	160.0	330.0	275.0	525.0	560.0			585.0	870.0	845.0
		BFs	0.29	0.10	0.30	0.08	0.17	0.14	0.27	0.28			0.30	0.44	0.43
	5	n	3		3	3	3	3	3	3	1	2	3		3
		Range	6.9-20.5		10.3-17.3	2.8-4.4	5.9-9.3	4.9-9.2	10.0-19.1	13.6-20.0	20.8	44.6-59.7	8.6-16.0		15.1-16.4
		Median	12.7		15.2	3.2	7.8	5.8	10.5	13.9		52.2	10.0		15.4
		Mean	13.4	N/A	14.3	3.4	7.7	6.6	13.2	15.8		52.2	11.5	N/A	15.6
		SD	6.8		3.6	0.8	1.7	2.3	5.1	3.6		10.7	3.9		0.7
		BFw	121.8		130.0	30.9	70.0	60.0	120.0	143.6	189.1		104.5		141.8
		BFs	0.23		0.25	0.06	0.13	0.11	0.23	0.27	0.36		0.20		0.27

Φ Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 6.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont	HGut cont	Vertebrae	Kidney	Bile	Blood
Aug. '91	3	n[®]	1	1		1	1	1	1	1		1		1			1
		Range	10.0	1.9		0.5	2.1	0.9	1.9	2.7		22.6		15.4			20.0
		Median															
		Mean			N/A						N/A		N/A		N/A	N/A	
		SD[Δ]															
		BFw	125.0	23.8		6.3	26.3	11.3	23.8	33.7				192.5			250.0
		BFs	0.18	0.03		0.01	0.04	0.02	0.03	0.05				0.28			0.36
	4	n	8	5		8	8	8	7	7		5	5	8	2		8
		Range	4.8-7.3	0.5-4.6		0.3-0.7	0.5-2.2	0.4-1.8	0.5-2.5	0.9-3.3		15.0-59.6	20.8-61.0	11.0-13.4	1.1-3.8		19.9-21.0
		Median	6.4	0.8		0.4	1.1	1.0	1.7	2.2		37.8	41.3	12.3	2.4		20.5
		Mean	6.3	1.6	N/A	0.4	1.2	1.0	1.5	2.1	N/A	36.2	38.3	12.2	2.4	N/A	20.5
		SD	0.9	1.7		0.1	0.5	0.5	0.8	0.8		17.6	16.1	0.9	1.9		0.4
		BFw	45.0	11.4		2.9	8.6	7.1	10.7	15.0				87.1	17.1		146.4
		BFs	0.08	0.02		0.01	0.02	0.01	0.02	0.03				0.16	0.03		0.27
	5	n	12	11	1	12	12	12	12	10	4	8	8	12	7	4	12
		Range	5.9-9.9	0.3-4.6	12.5	0.4-3.6	0.4-4.8	0.9-4.2	0.5-3.7	0.8-5.9	2.6-3.6	8.1-39.9	17.2-44.4	6.6-16.7	2.9-6.3	1.2-8.9	19.2-23.6
		Median	6.8	0.7		1.0	1.1	1.7	1.3	2.6	2.9	17.5	31.6	14.3	4.7	4.5	20.8
		Mean	7.3	1.2		1.6	1.8	2.0	1.7	2.9	3.0	19.9	31.5	13.9	4.7	4.8	20.9
		SD	1.3	1.3		1.2	1.5	1.0	1.0	1.5	0.4	9.7	8.6	2.7	1.2	3.5	1.3
		BFw	73.0	12.0	125.0	16.0	18.0	20.0	17.0	29.0	30.0			139.0	47.0	48.0	209.0
		BFs	0.11	0.02	0.19	0.02	0.03	0.03	0.03	0.04	0.04			0.21	0.07	0.07	0.31
	7	n	1			1	1	1	1	1				1		1	1
		Range	5.0			3.0	1.9	0.8	2.1	3.9				8.8		4.8	38.8
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A		N/A		
		SD															
		BFw	33.3			20.0	12.7	5.3	14.0	26.0				58.7		32.0	258.7
		BFs	0.14			0.09	0.05	0.02	0.06	0.11				0.25		0.14	1.10
Oct. '91	3	n	6	2	1	6	5	6	6	5	3	2	3	6	3	5	6
		Range	6.9-10.9	0.8-2.3	0.6	0.2-1.2	0.5-1.3	0.3-2.1	0.4-0.8	1.2-1.9	1.9-3.2	36.3-59.3	44.2-55.5	12.1-15.3	1.8-2.6	0.4-1.2	19.9-21.8
		Median	8.7	1.6		0.5	0.8	1.0	0.6	1.3	2.9	47.8	45.2	13.7	2.1	0.7	20.5
		Mean	8.8	1.6		0.6	0.9	1.1	0.6	1.4	2.7	47.8	48.3	13.8	2.2	0.8	20.7
		SD	1.5	1.1		0.4	0.3	0.6	0.1	0.3	0.7	16.3	6.3	1.3	0.4	0.3	0.8
		BFw	*	*	*	*	*	*	*	*	*			*	*	*	*
		BFs	0.53	0.10	0.04	0.04	0.05	0.07	0.04	0.08	0.16			0.84	0.13	0.05	1.25
	4	n	11	7	1	11	8	11	11	5	2	9	5	11	3	6	10
		Range	6.5-9.4	0.4-1.8	1.2	0.6-2.1	0.8-1.8	0.6-3.2	0.4-3.2	0.9-2.4	1.5-4.6	8.6-53.2	10.2-48.2	12.0-15.2	2.9-4.0	0.6-20.5	20.1-46.2
		Median	7.3	0.9		1.0	1.3	1.3	1.1	1.7	3.0	31.4	39.4	13.3	3.2	8.4	27.7
		Mean	7.6	1.0		1.2	1.2	1.4	1.3	1.6	3.0	29.6	35.9	13.3	3.4	9.6	29.6
		SD	0.9	0.5		0.5	0.4	0.8	0.8	0.7	2.2	14.8	15.3	1.0	0.6	9.5	8.7
		BFw	*	*	*	*	*	*	*	*	*			*	*	*	*
		BFs	0.43	0.06	0.07	0.07	0.07	0.08	0.07	0.09	0.17			0.76	0.19	0.55	1.69
	5	n	14	9	4	15	13	15	14	3	3	10	4	15	5	9	10
		Range	6.8-9.1	0.6-4.6	1.0-1.2	0.6-3.5	1.2-2.6	0.7-1.5	0.4-1.0	2.2-3.3	3.2-5.7	9.1-42.1	36.0-79.8	9.4-15.3	1.3-2.9	0.8-4.4	21.4-45.8
		Median	7.7	1.0	1.1	0.9	1.7	1.1	0.7	2.4	3.8	16.6	59.6	13.6	2.1	1.0	34.7
		Mean	7.8	1.3	1.1	1.1	1.7	1.1	0.7	2.6	4.2	20.9	58.7	13.5	2.1	1.4	31.8
		SD	0.7	1.3	0.1	0.7	0.4	0.2	0.2	0.6	1.3	10.7	20.4	1.3	0.6	1.1	8.7
		BFw	195.0	32.5	27.5	27.5	42.5	27.5	17.5	65.0	105.0			337.5	52.5	35.0	795.0
		BFs	0.21	0.03	0.03	0.03	0.04	0.03	0.02	0.07	0.11			0.36	0.06	0.04	0.84
	7	n	1			1	1	1	1	1				1	1	1	1
		Range	10.6			1.8	2.1	2.2	2.8	2.8				12.4	4.3	3.6	20.2
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A				
		SD															
		BFw	*			*	*	*	*	*				*	*	*	*
		BFs	0.24			0.04	0.05	0.05	0.06	0.06				0.28	0.10	0.08	0.46

® Number of samples analyzed Δ Standard deviation N/A Not available * [Ni] in water below AAS detection limit

TABLE 6.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont.	HGut cont.	Vertebrae	Kidney	Bile	Blood
Jan. '92	3	n[Φ]	5	4		5	3	6	2	2	2	2	2	6	1	5	6
		Range	8.6-11.3	1.8-3.3		2.0-2.9	3.5-3.5	1.3-3.3	2.9-4.3	3.6-11.4	7.3-15.0	44.1-72.9	37.6-54.8	13.5-17.1	5.2	8.4-32.1	21.4-22.4
		Median	9.1	2.8		2.5	3.5	2.3	3.6	7.5	11.1	58.5	46.2	15.6		19.0	21.8
		Mean	9.5	2.7	N/A	2.5	3.5	2.3	3.6	7.5	11.1	58.5	46.2	15.5		17.6	21.9
		SD[Δ]	1.1	0.8		0.3	0.0	0.7	0.9	5.5	5.5	20.4	12.2	1.5		9.6	0.4
		BFw	*	*		*	*	*	*	*	*			*	*	*	*
		BFs	0.23	0.07		0.06	0.08	0.06	0.09	0.18	0.27			0.37	0.13	0.42	0.53
	4	n	4	3		10	2	11	3	1	1	1	3	9	1	2	11
		Range	8.9-10.7	2.7-3.8		1.8-8.3	4.9-6.3	1.0-6.0	1.6-11.2	8.0	19.8	46.3	48.9-66.0	13.2-22.4	11.2	3.1-18.4	20.0-39.5
		Median	9.5	3.1		4.5	5.6	4.0	3.9				49.0	18.5		10.8	21.0
		Mean	9.6	3.2	N/A	4.6	5.6	3.4	5.5				54.6	18.3		10.8	22.6
		SD	0.8	0.5		2.1	1.0	1.6	5.0				9.8	3.2		10.8	5.6
		BFw	*	*		*	*	*	*	*	*			*	*	*	*
		BFs	0.12	0.04		0.06	0.07	0.04	0.07	0.10	0.24			0.22	0.14	0.13	0.28
	5	n	12	8	4	12	9	12	11	3	3	2	1	12	3	9	12
		Range	7.6-11.8	1.3-4.6	1.9-4.3	2.0-3.7	2.4-6.6	1.7-11.5	1.9-7.1	6.0-8.5	7.1-18.3	16.1-94.7	170.7	14.0-19.8	5.1-9.6	2.9-30.4	19.3-39.6
		Median	8.5	2.3	3.6	2.9	4.5	3.2	3.2	6.8	15.4	55.4		16.8	6.6	6.8	20.6
		Mean	9.0	2.4	3.4	2.9	4.9	4.1	4.1	7.1	13.6	55.4		16.9	7.1	10.6	22.3
		SD	1.3	1.0	1.1	0.4	1.5	2.7	1.8	1.3	5.8	55.6		1.7	2.3	8.9	5.6
		BFw	*	*	*	*	*	*	*	*	*			*	*	*	*
		BFs	0.20	0.05	0.08	0.07	0.11	0.09	0.09	0.16	0.31			0.38	0.16	0.24	0.50
	7	n	1			5		5		1				5			5
		Range	9.7			2.9-7.4		1.3-5.9		8.8				17.9-36.2			20.8-23.2
		Median				4.4		3.0						27.0			21.6
		Mean		N/A	N/A	4.7	N/A	3.2	N/A		N/A	N/A	N/A	27.1	N/A	N/A	21.9
		SD				1.7		1.7						6.5			0.9
		BFw	*			*		*		*				*			*
		BFs	0.16			0.08		0.05		0.14				0.44			0.35
Feb. '92	3	n	2			6		6	3					6			6
		Range	9.3-9.6			0.4-1.5		0.8-1.3	0.4-0.6					13.4-15.1			19.7-40.6
		Median	9.4			0.7		1.0	0.6					14.7			21.3
		Mean	9.4	N/A	N/A	0.8	N/A	1.0	0.5	N/A	N/A	N/A	N/A	14.5	N/A	N/A	24.2
		SD	0.3			0.4		0.2	0.1					0.6			8.1
		BFw	235.0			20.0		25.0	12.5					362.5			605.0
		BFs	0.62			0.05		0.07	0.03					0.96			1.60
	4	n	6	4		10	7	10	8	1	1	1	1	10	1	9	10
		Range	7.2-8.4	0.4-1.3		0.3-1.5	1.2-3.5	0.6-1.9	0.7-2.2	11.6	3.8	69.1	88.2	13.3-17.3	4.4	0.4-2.4	20.4-23.2
		Median	8.3	0.8		0.7	2.2	1.5	1.4					14.6		1.3	21.8
		Mean	8.1	0.8	N/A	0.8	2.2	1.4	1.5					14.8		1.3	21.8
		SD	0.5	0.4		0.4	0.8	0.4	0.6					1.2		0.6	0.9
		BFw	405.0	40.0		40.0	110.0	70.0	75.0	580.0	190.0			740.0	220.0	65.0	1090.0
		BFs	0.18	0.02		0.02	0.05	0.03	0.03	0.25	0.08			0.32	0.10	0.03	0.47
	5	n	10	10		10	8	10	10	2	2			10	2	10	10
		Range	6.8-18.6	0.2-3.2		0.2-13.5	0.5-3.4	0.8-6.3	1.0-5.0	3.3-5.9	7.5-10.4			12.8-26.2	4.2-5.3	0.7-2.8	19.5-39.5
		Median	8.3	0.9		1.4	2.2	1.4	1.6	4.6	9.0			15.7	4.7	1.3	21.9
		Mean	9.3	1.1	N/A	4.8	2.2	2.0	2.3	4.6	9.0	N/A	N/A	17.4	4.7	1.5	24.1
		SD	3.3	0.9		5.6	0.9	1.7	1.5	1.8	2.1			4.6	0.8	0.7	6.8
		BFw	232.5	27.5		120.0	55.0	50.0	57.5	115.0	225.0			435.0	117.5	37.5	602.5
		BFs	0.07	0.01		0.04	0.02	0.02	0.02	0.04	0.07			0.14	0.04	0.01	0.19
	Pionier Dam	n	10	5	5	10	9	10	10	6	2	6		10	4	5	10
		Range	7.8-15.6	1.6-8.1	0.7-3.2	0.2-22.9	0.7-12.9	0.6-7.5	1.2-14.7	1.6-16.9	8.1-18.3	15.8-54.7		13.5-19.9	4.3-17.4	7.1-11.7	20.6-22.7
		Median	9.3	7.7	1.3	3.1	5.8	1.9	2.2	9.7	13.2	33.6		15.1	14.6	9.3	21.9
		Mean	10.8	5.9	1.6	6.3	6.1	3.2	5.1	9.6	13.2	33.9	N/A	15.8	12.7	9.2	21.6
		SD	3.1	2.9	1.0	7.4	4.6	2.4	5.0	7.1	7.2	16.1		2.0	6.1	1.9	0.7
		BFw	360.0	196.7	53.3	210.0	203.3	106.7	170.0	320.0	440.0			526.7	423.3	306.7	720.0
		BFs	0.45	0.25	0.07	0.26	0.26	0.13	0.21	0.40	0.55			0.66	0.53	0.39	0.91

Φ Number of samples analyzed Δ Standard deviation N/A Not available * [Ni] in water below detection limit

TABLE 6.3

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE CHROMIUM 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	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)													
Gonad (Males)													
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	

TABLE 6.4

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE 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	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	S2												
Gonad (Males)													
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	

January 1992), while the BF_s values ranged from 0.001 (calculated for various tissues in August 1991) to 3.45 (calculated for the gills in December 1990) (Table 6.1). Nickel BF_w values ranged from 2.9 (calculated for fat tissue in August 1991) to 1090 (calculated for blood in February 1992), while the BF_s values ranged from 0.01 (calculated for various tissues in August 1991 and February 1992) to 1.69 (calculated for blood in October 1991) (Table 6.2).

LOCALITY DIFFERENCES

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. In the first year (October 1990) locality 3 differed significantly from localities 4 (with respect to the gill chromium concentrations) and 5 (with respect to the liver and muscle chromium concentrations), as did locality 4 from locality 5 (with respect to the gill and liver chromium concentrations). Similarly, in June 1991 (the second year) locality 3 differed significantly from localities 4 (with respect to the muscle and vertebrae chromium concentrations) and 5 (with respect to the muscle chromium concentrations), but in October 1991 it only differed significantly from locality 5 (with respect to the fat and muscle chromium concentrations). Locality 7 differed significantly from localities 3 (with respect to the fat chromium concentrations) and 5 (with respect to the fat and vertebrae chromium concentrations) in January 1992 and in February 1992. Pionier Dam differed significantly from localities 3 (with respect to the fat, muscle and blood chromium concentrations), 4 (with respect to the fat and blood chromium concentrations) and 5 (with respect to the blood chromium concentrations). The chromium concentrations in the blood of the fish at Pionier Dam were lower than at the other localities, but in the other organs the chromium concentrations were higher (Table 6.1).

Nickel concentrations detected at locality 7 differed significantly from concentrations detected at localities 3 and 4 (with respect to the muscle), as well as from concentrations detected at locality 5 (with respect to the liver) in October 1990. Locality 3 differed significantly from localities 4 (with respect to the fat, muscle and vertebrae nickel concentrations) and 5 (with respect to the muscle nickel concentrations) in June 1991, but only from locality 5 (with respect to the blood nickel concentrations) in October 1991. In January 1992 locality 7 differed significantly from localities 5 and 4 (with respect to the vertebrae nickel concentrations), as well as from locality 3 (with respect to the vertebrae and fat nickel concentrations). Pionier Dam differed significantly from localities 3 and 4 with respect to the fat and muscle nickel concentrations, for more nickel accumulated in the fish tissues at Pionier Dam than it did at the other localities (Table 6.2).

SEASONAL DIFFERENCES

Significant seasonal differences ($p \leq 0.05$) with regard to the mean chromium and nickel concentrations in various organs were detected. Chromium concentrations in the summer of 1990/91 and winter of 1991 differed significantly from all the other seasons, as indicated in Table 6.5. The spring of 1991 and summer of 1992 also differed significantly from the other seasons, but not from each other (Table 6.5). As for the nickel concentrations, the winter of 1990 and summer of 1990/91 differed significantly from all the seasons but the autumn periods, while the winter of 1991 differed significantly from all the seasons but the spring of 1990 (Table 6.6). In addition, the spring of 1991 and summer of 1992 differed significantly from all the other seasons (Table 6.6).

Comparing the seasonal chromium and nickel concentrations in the organs, tissues and gut contents of the males and females separately, a difference was noticed in the gut contents and some organs (Figures 6.1 - 6.4). The chromium concentrations in the gut contents of the females were higher than that of the males, while the males generally had higher chromium concentrations in the bile, vertebrae, hindgut, skin and gonads (Figures 6.1 and 6.2). Differences were not so obvious for nickel, but the males did have higher nickel concentrations in the foregut contents and gonads than the females did (Figures 6.3 and 6.4).

ANNUAL DIFFERENCES

The first and second year differed significantly with respect to the chromium concentrations in the gill, liver, muscle and male and female gonads (Fig. 6.5), and also with respect to the nickel

TABLE 6.5
SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN CHROMIUM 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 SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

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

TABLE 6.6
SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN 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 SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

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

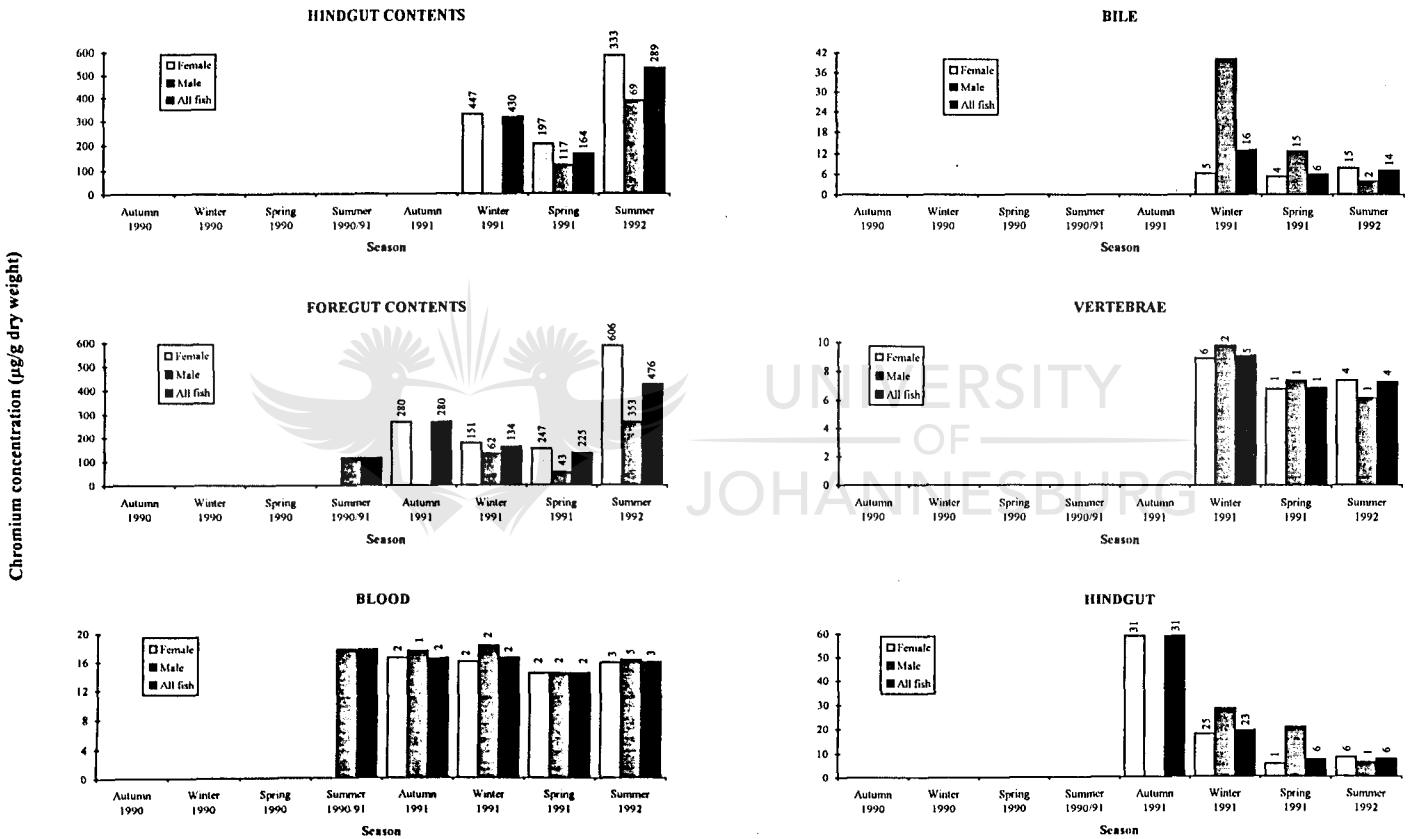

Figure 6.1

Mean seasonal chromium concentrations (µg/g dry wt.) in the hindgut contents, foregut contents, blood, bile, vertebrae and hindgut of Barbus marequensis *for males and females seperately, as well as the sexes combined. (Standard deviations are indicated above each bar)*

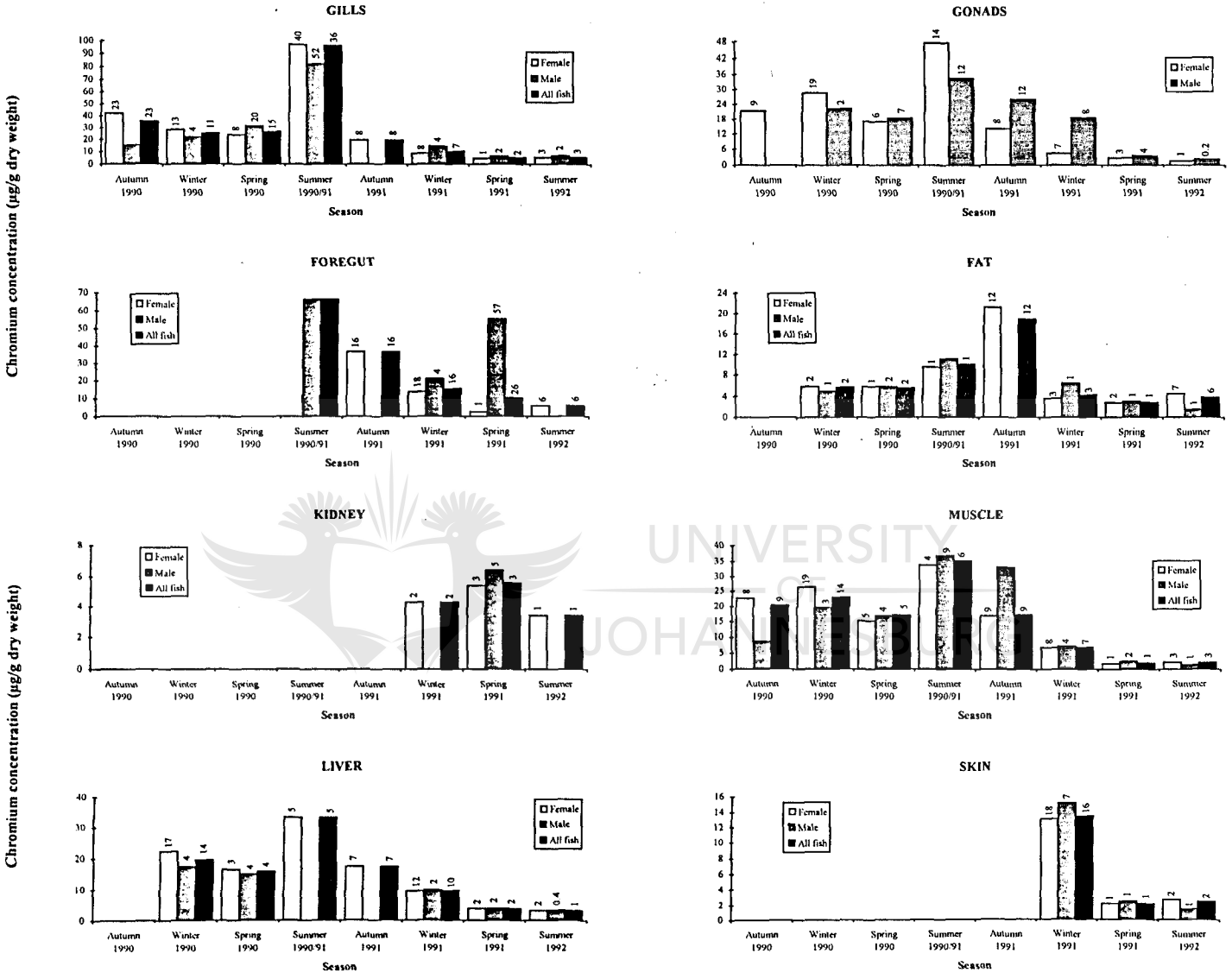

Figure 6.2

Mean seasonal chromium concentrations (µg/g dry wt.) in the gills, foregut, kidney, liver, gonads, fat, muscle and skin of Barbus marequensis *for males and females seperately, as well as the sexes combined. (Standard deviations are indicated above each bar)*

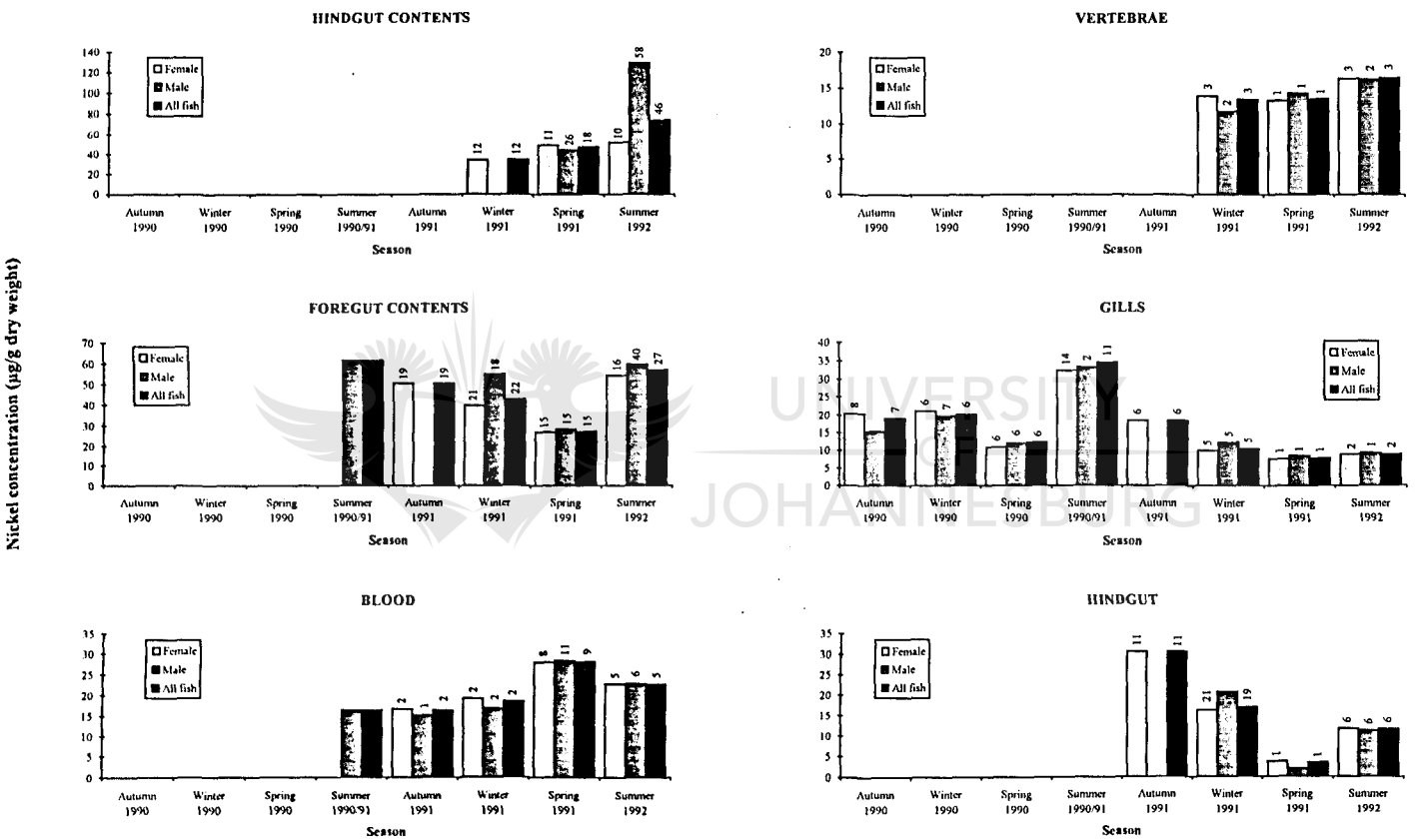

Figure 6.3

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

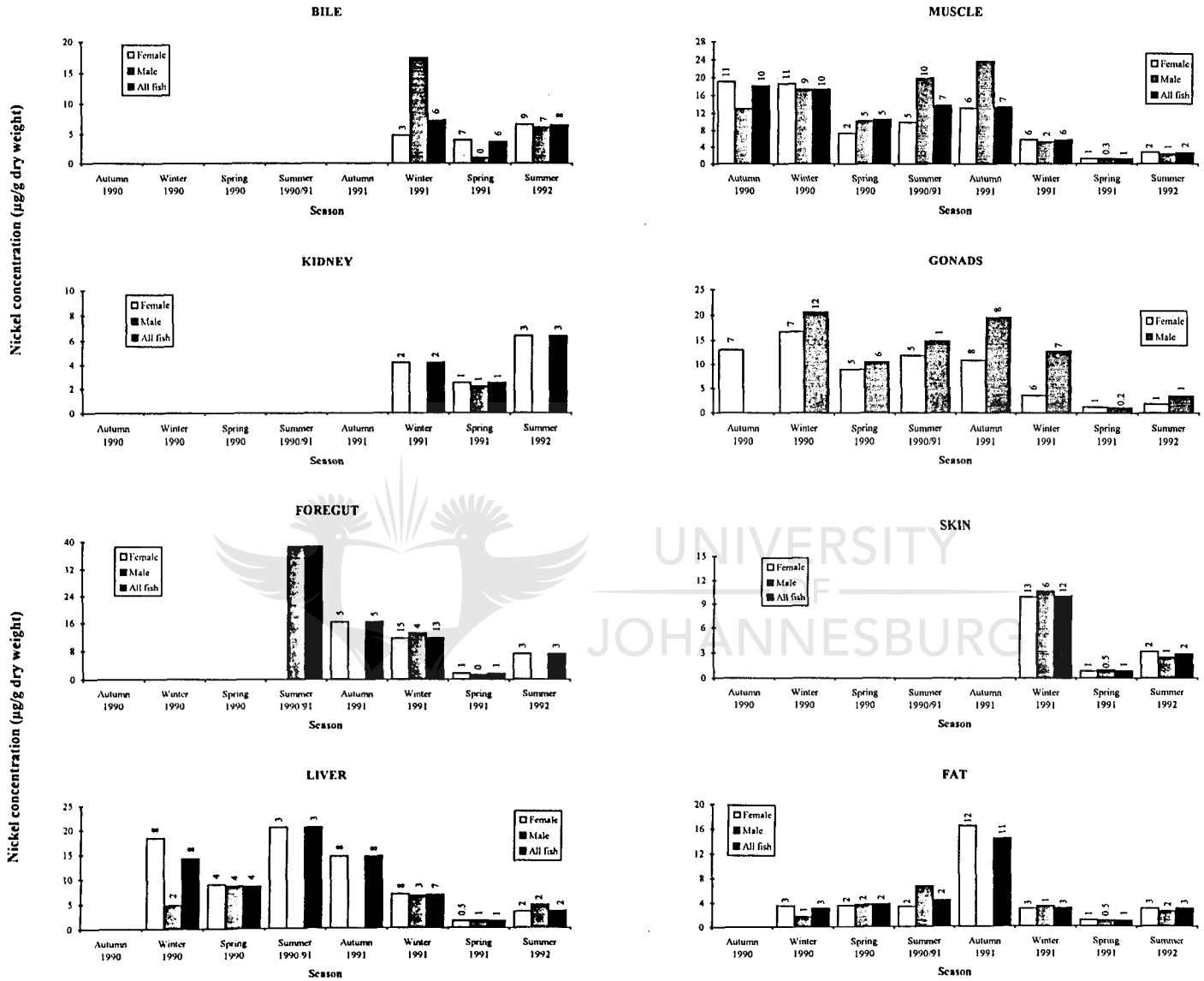

Figure 6.4

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

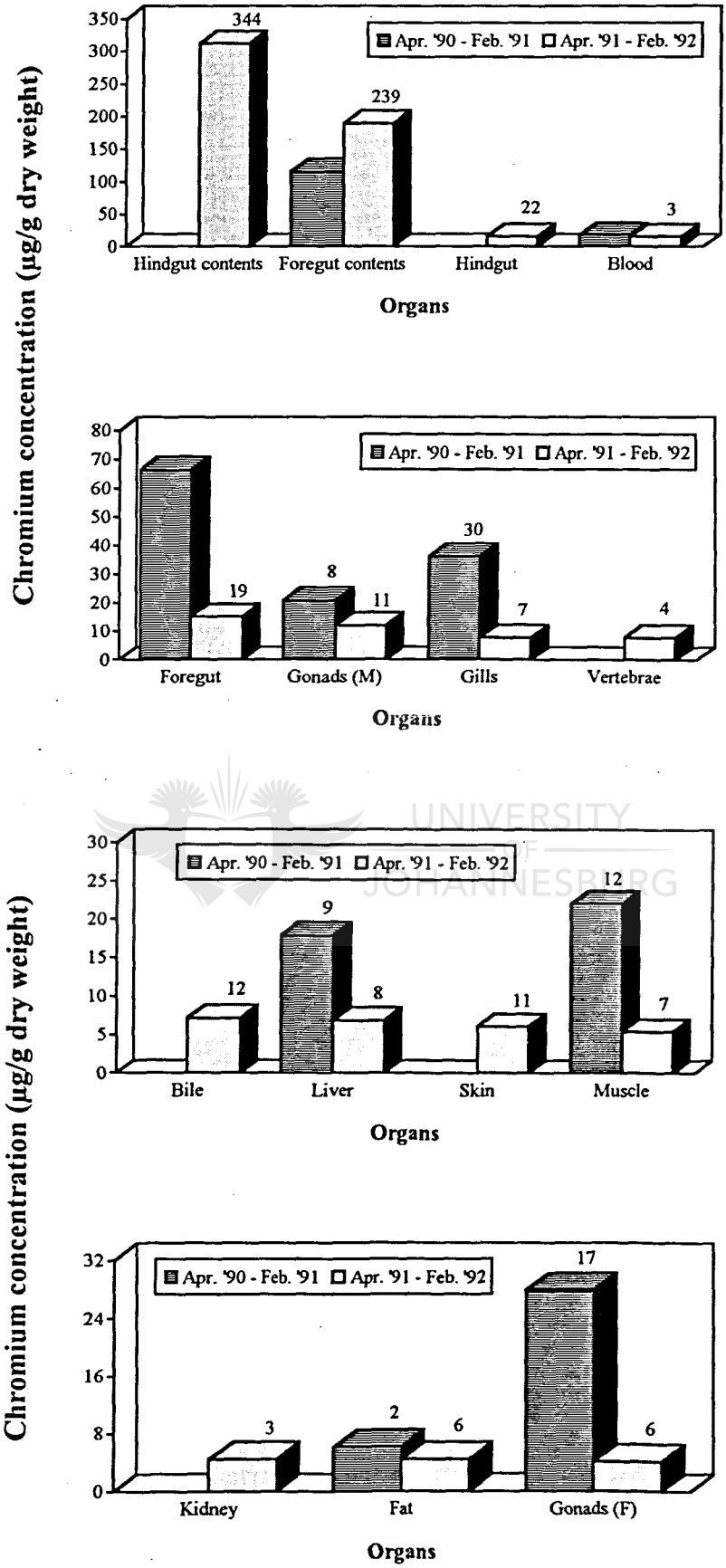

Figure 6.5
Mean chromium concentrations (µg/g dry wt) for the two years in the different organs and tissues of Barbus marequensis. *(Standard deviations are indicated above each bar)*

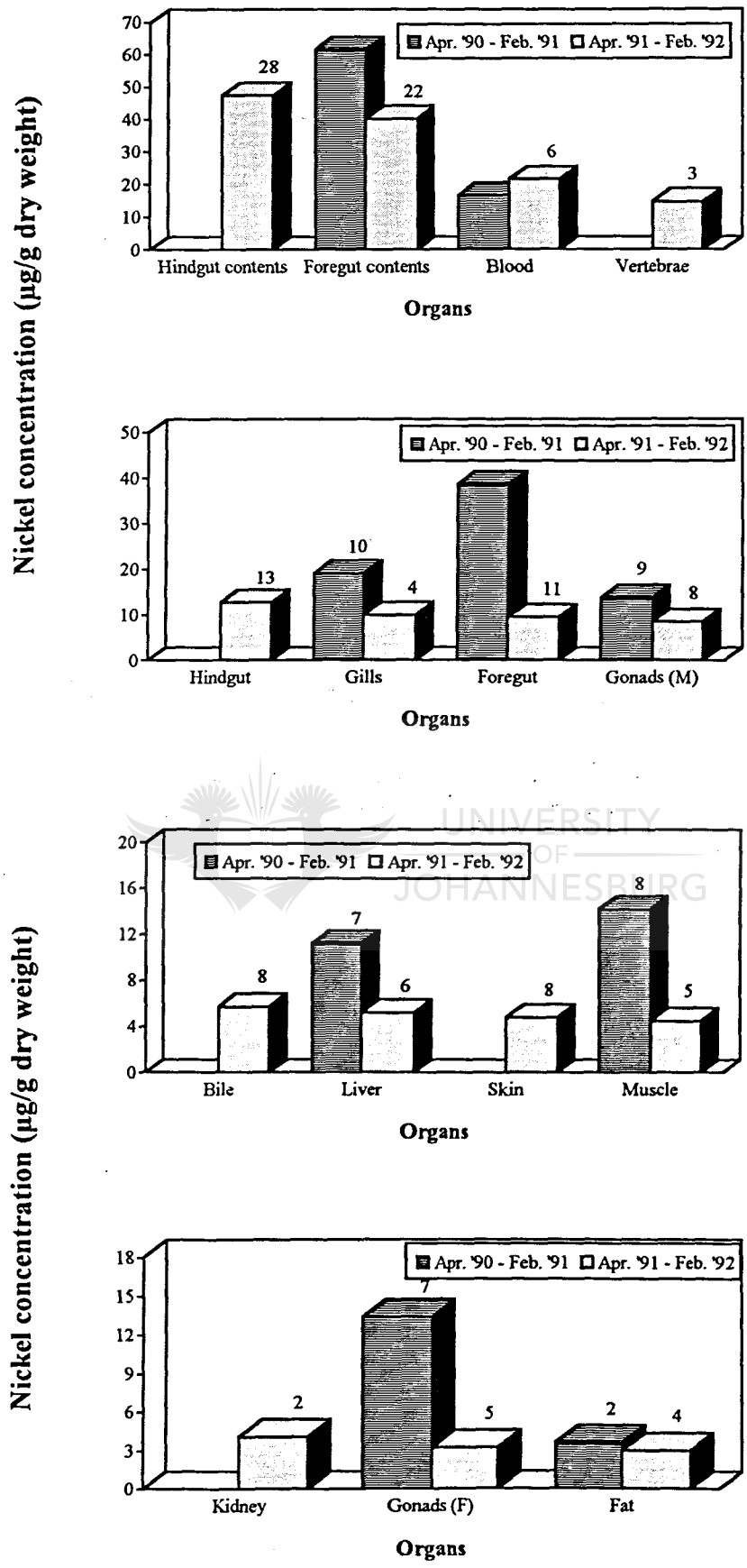

Figure 6.6
Mean nickel concentrations (µg/g dry wt) for the two years in the different organs and tissues of Barbus marequensis. *(Standard deviations are indicated above each bar)*

concentrations in the gill, liver, muscle and female gonads (Fig. 6.6). The mean metal concentrations in the fish organs during the second year (Figures 6.5 and 6.6) 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 > male gonads > vertebrae ≈ gills > bile > liver > skin > muscle > kidney ≈ fat > female gonads; and for nickel, hindgut contents > foregut contents > blood > vertebrae > hindgut > gills > foregut > male gonads > bile > liver > skin > muscle > kidney > female gonads > fat.

6.4 Discussion

BIOACCUMULATION OF CHROMIUM AND NICKEL IN THE DIFFERENT ORGANS AND TISSUES

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. More chromium and nickel would probably have concentrated in the gills, however, if the water pH were more acidic (as discussed in the "Introduction" of this chapter). 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 *et al.*, 1983). A large variation in the chromium and nickel concentrations of the gut contents (Table 6.1) can be expected, because of the differing foraging habits of *B. marequensis* (see also Chapter 5). Excretion was mainly biliary, especially in the case of chromium. It has been suggested by Flos *et al.* (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 (see Table 3.2 in Chapter 3). 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 (Tables 6.1 and 6.2) and October 1991 (Table 6.2). 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 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 sparmanii* in such a way that they were potentially hazardous (Wepener *et al.*, 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 *et al.*, 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 and, other than that, accumulation was preferentially by the kidneys rather than the liver (Tables 6.1 and 6.2), 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 (Tables 6.1 and 6.2). The suggested chromium concentrations in the muscle of freshwater fish from industrialised parts 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 (Table 6.1) when the chromium concentrations in the muscle ranged from 5.7 (June 1991) to 43.3 (February 1991) µg/g Cr dry weight. Suggested nickel concentrations in the muscle of freshwater fish were not available. From April 1990 to June 1991 the chromium and nickel concentrations in the water of the study area were higher than the water concentrations from

August 1991 to February 1992 (Table 3.2 in Chapter 3), which might explain the higher accumulation of these metals during the first period.

The nickel BFs recorded for *B. marequensis* in October 1990 at locality 3 in this study, were mostly higher than the nickel BFs recorded for *Hydrocynus vittatus* in October 1990 at the same locality (Du Preez & Steyn, 1992), which ranged from 17.8 to 54.1. It was only the BFs regarding the nickel concentrations in the fat of *B. marequensis* that were lower than the BFs recorded for *H. vittatus*. It is important to remember, however, that the BFs for *H. vittatus* were calculated on a wet weight basis, while the BFs for *B. marequensis* were calculated on a dry weight basis, making direct comparisons difficult.

The chromium and nickel 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). *Barbus marequensis* (collected at all the localities in the study area) did, however, accumulate more nickel than *C. gariepinus* in their vertebrae, while only the fish collected at locality 7 accumulated more chromium in their vertebrae than *C. gariepinus* did. This suggests chronic exposure of *B. marequensis* to sub-lethal concentrations of these metals at the relevant localities. Furthermore, *B. marequensis* collected at Pionier Dam, accumulated more chromium than *C. gariepinus* in their kidneys and gut, while the livers of both species accumulated similar chromium concentrations. More chromium was therefore taken up by the gut of *B. marequensis* than was the case with *C. gariepinus*.

LOCALITY DIFFERENCES

The localities did not differ that much from each other 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 (Tables 6.1 and 6.2). 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.

SEASONAL DIFFERENCES

The high chromium and nickel concentrations in the gills of *B. marequensis* during the summer of 1990/91 (Figures 6.2 and 6.3) 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 (Table 3.2 in Chapter 3). 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 6.1 - 6.4). The seasonal trends regarding the chromium and nickel concentrations in the gonads (Figures 6.2 and 6.4) of *B. marequensis* differed slightly from the trends regarding the zinc, copper and iron concentrations (Figures 4.1, 5.2 and 5.4) in the gonads and it is therefore not certain what role, if any, chromium and nickel played in the gonad development. The highest nickel concentrations in the gonads did, however, occur in the winter of 1990 (Figure 6.4), which is the period when the gonads were fairly well-developed. No relationship seemed to have existed between the chromium and nickel concentrations in the liver and gonads, although it has been observed by Shearer (1984) that the chromium levels in the liver of *Oncorhynchus mykiss* decreased significantly during sexual maturation, while the levels in the female gonads increased. The observed seasonal sexual differences in accumulation cannot be explained readily, but they might be related to female gonad development, seeing that higher chromium levels were detected in the gonads and vertebrae of the male fish (Figures 6.1 and 6.2).

Higher chromium and nickel concentrations were detected in the water of the study area in the first year than in the second year (Table 3.2 in Chapter 3), 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 (Figures 6.5 and 6.6). 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 6.6). This can be explained by assuming that fish have a mechanism to prevent excess bioconcentration of nickel in blood (Grobler-Van Heerden *et al.*, 1991), as was found with copper (see Chapter 5).

6.5 Conclusion

According to the monthly data, the blood accumulated the highest chromium concentrations, followed by the bile and vertebrae, while the skin accumulated the lowest. Nickel mainly accumulated in the blood, followed by the vertebrae and gills, while the lowest nickel concentrations occurred in the fat tissue. 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. 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.

6.6 References

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CHAPTER 7

Chapter 7

MANGANESE, LEAD AND STRONTIUM BIOACCUMULATION IN THE ORGANS AND TISSUES OF *BARBUS MAREQUENSIS*

7.1 Introduction

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

In the natural environment, water manganese concentrations rarely exceed one mg/l (Hellawell, 1986), while concentrations of soluble lead are generally less than or equal to three µg/l (Förstner & Wittman, 1979). Values for naturally occurring strontium concentrations in the water are at present not available. The forms in which manganese and lead occur in fresh water are mainly particulate or complexed forms (Seenayya & Prahalad, 1987; Moore & 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 become more prevalent and toxicity increases (Wang, 1987). Strontium, on the other hand, is found in water in solution rather than in particulate form (Carraça *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 & 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 fallout is usually the most important source of lead in fresh waters (Moore & Ramamoorthy, 1984). Fish can be affected sub-lethally when they are chronically exposed to lead concentrations ranging from 5 to 500 µg/l inorganic lead (Haux *et al.*, 1986). Two distinctive characteristics of chronic lead poisoning in fish are black tails, which is an early symptom of spinal deformities (Hodson *et al.*, 1979), and a strong inhibition of the δ-aminolevulinic acid dehydratase (ALA-D) activity in erythrocytes (Haux *et al.*, 1986). The 96-hour LC50 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 & Ramamoorthy, 1984; Pickering & Henderson, 1966). Manganese and strontium can also affect fish adversely at elevated levels, but limited research has been done in this field. Sub-lethal effects can

occur at a manganese concentration of 0.278 g/l (see Chapter 8), while the 96-h LC50 value can vary from 1.723 (see Chapter 8) to 3.230 g/l Mn (Nath & Kumar, 1987). For strontium the 96-h LC50 value for fish has previously 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. Influencing factors, such as environmental conditions, should however be taken into consideration when assessing the toxicity of these metals to fish.

In this section of the study, the extent of metal bioaccumulation with regard to manganese, lead and strontium in the organs and tissues of *Barbus marequensis* was determined, as well as the organs that accumulated the highest and lowest metal levels respectively.

7.2 Materials and methods

Barbus marequensis was sampled and dissected as described in Chapter 4. Laboratory procedures for manganese, lead and strontium analysis of the fish samples were the same as the procedures described for zinc analysis. For the analysis of strontium an additional 0.5 ml of a 2.682M potassium chloride solution (200 g KCl per litre distilled water) was added to the digested 50 ml samples in order to suppress the ionisation of strontium (Varian, 1989). Statistical calculations were also the same as described in Chapter 4.

7.3 Results

FISH SIZE AND AGE

The size and age data are summarised in Table 4.1 (see Chapter 4).

BIOACCUMULATION OF MANGANESE, LEAD AND STRONTIUM IN THE DIFFERENT ORGANS AND TISSUES

Manganese, lead and strontium accumulated mostly in the vertebrae and gills of *B. marequensis*. High metal concentrations were also detected in the gut contents of the fish (Tables 7.1 - 7.3). 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. 977.6 - 4575.5 µg/g Mn at locality 5 in October 1991) and, in the first year, also in the gills (e.g. 23.1 - 123.1 µg/g Mn at locality 4 in April 1990) (Table 7.1). For strontium, the largest variation was detected in the vertebrae (e.g. 1403.0 - 3924.9 µg/g Sr at locality 7 in January 1992), gills (e.g. 600.6 - 2115.7 µg/g Sr at locality 5 in January 1992) and gut contents (e.g. 132.2 - 1325.6 µg/g Sr at locality 4 in August 1991) (Table 7.3). The general order of bioaccumulation for manganese was: hindgut contents > foregut contents > gills > vertebrae > hindgut > foregut > liver > kidney > blood > female gonads > fat ≈ bile > skin > muscle > male gonads. For lead the order was: foregut contents > hindgut contents ≈ vertebrae > hindgut > gills > foregut > blood > bile > male gonads > kidney ≈ liver > fat > female gonads > skin > muscle; and for strontium it was vertebrae > gills > foregut contents > hindgut contents > hindgut > muscle > foregut > liver > female gonads > bile > kidney > male gonads > 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 Tables 7.4 to 7.6. In addition, the liver and blood differed significantly from some organs with respect to the manganese and lead concentrations respectively (Tables 7.4 and 7.5), but only during the summer of 1992.

The calculated bioconcentration factors between water and organs (BF_w) were higher than the bioconcentration factors between sediment and organs (BF_s). Manganese BF_w values ranged from 0.7 (calculated for bile in February 1992) to 3593.3 (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) (Table 7.1). Lead BF_w values ranged from 10.8 (calculated for fat in October 1990) to 2610.0 (calculated for bile in June 1991), while the BF_s values ranged from 0.08 (calculated for fat

TABLE 7.1

MEAN MANGANESE CONCENTRATIONS (µg/g dry wt.) IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* (BF_w AND BF_s = BIOCONCENTRATION FACTORS OF THE WATER AND SEDIMENT RESPECTIVELY)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle
Apr. '90	3	n®						1
		Range						8.7
		Median						
		Mean	N/A	N/A	N/A	N/A	N/A	
		SD$^{\Delta}$						
		BFw						290.0
		BFs						0.02
	4	n	4	3				5
		Range	23.1-123.1	3.6-17.9				4.4-26.1
		Median	73.1	7.1				8.7
		Mean	73.1	9.5	N/A	N/A	N/A	11.3
		SD	55.6	7.4				9.0
		BFw	406.1	52.8				62.8
		BFs	0.48	0.06				0.07
	7	n	7				4	8
		Range	26.9-42.3				3.7-7.4	4.4-4.4
		Median	38.5				7.4	4.4
		Mean	36.8	N/A	N/A	N/A	6.5	4.4
		SD	4.9				1.9	0.0
		BFw	306.7				54.2	36.7
		BFs	0.09				0.02	0.01
June '90	3	n	2					2
		Range	19.2-80.8					4.4-4.4
		Median	50.0					4.4
		Mean	50.0	N/A	N/A	N/A	N/A	4.4
		SD	43.6					0.0
		BFw	108.7					9.6
		BFs	0.16					0.01
Aug. '90	3	n	8	2	1	1	2	9
		Range	20.4-33.5	4.3-5.7	6.2	3.7	10.4-14.8	4.8-10.9
		Median	25.8	5.0			12.6	7.8
		Mean	26.2	5.0			12.6	7.2
		SD	4.5	1.0			3.1	1.9
		BFw	1310.0	250.0	310.0	185.0	630.0	360.0
		BFs	0.10	0.02	0.02	0.01	0.05	0.03
	4	n	9	4	3	4	6	9
		Range	20.4-44.6	6.1-9.6	4.8-6.7	3.2-8.0	5.6-10.0	3.9-12.6
		Median	31.5	6.6	6.2	4.2	7.4	8.3
		Mean	33.7	7.2	5.9	4.9	7.8	8.3
		SD	8.4	1.6	1.0	2.2	1.7	2.4
		BFw	1685.0	360.0	295.0	245.0	390.0	415.0
		BFs	0.15	0.03	0.03	0.02	0.04	0.04
	5	n	7	1	2	3	7	6
		Range	26.5-82.3	10.4	4.3-6.2	1.3-1.7	4.1-8.5	4.4-23.9
		Median	43.9		5.2	1.4	7.0	8.7
		Mean	45.4		5.2	1.5	6.6	10.6
		SD	18.8		1.3	0.2	1.6	7.2
		BFw	1513.3	346.7	173.3	50.0	220.0	353.3
		BFs	0.11	0.02	0.01	0.004	0.02	0.02
	7	n						5
		Range						3.9-7.8
		Median						4.8
		Mean	N/A	N/A	N/A	N/A	N/A	5.5
		SD						1.6
		BFw						68.8
		BFs						0.01

® Number of samples analyzed $^{\Delta}$ Standard deviation N/A Not available

TABLE 7.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Gut	Gut cont	Blood
Oct. '90	3	n[Φ]	7	2	1	2	6	7			
		Range	19.2-57.3	5.7-7.1	3.8	1.4-2.1	4.4-10.7	5.7-11.7			
		Median	25.4	6.4		1.8	5.9	8.7			
		Mean	32.6	6.4		1.8	6.6	8.4	N/A	N/A	N/A
		SD[Δ]	14.0	1.0		0.5	2.3	2.4			
		BFw	163.0	32.0	19.0	9.0	33.0	42.0			
		BFs	0.30	0.06	0.03	0.02	0.06	0.08			
	4	n	10	1	7	6	9	10			
		Range	24.6-83.9	11.8	8.1-21.0	2.4-4.9	6.7-13.7	6.5-13.9			
		Median	52.3		9.1	3.3	8.9	7.8			
		Mean	54.1		11.0	3.4	9.3	8.5	N/A	N/A	N/A
		SD	16.8		4.5	0.9	2.6	2.4			
		BFw	1082.0	236.0	220.0	68.0	186.0	170.0			
		BFs	0.22	0.05	0.05	0.01	0.04	0.04			
	5	n	9	1	6	9	9	10			
		Range	30.0-68.9	6.1	4.3-14.3	0.7-2.1	3.7-19.3	3.9-34.4			
		Median	39.6		7.6	1.1	5.6	7.6			
		Mean	43.3		8.4	1.3	7.6	9.9	N/A	N/A	N/A
		SD	12.1		3.3	0.5	5.0	8.9			
		BFw	2165.0	305.0	420.0	65.0	380.0	495.0			
		BFs	0.39	0.06	0.08	0.01	0.07	0.09			
	7	n	1	1		1	1	1			
		Range	91.9	18.6		6.1	19.6	31.7			
		Median									
		Mean			N/A				N/A	N/A	N/A
		SD									
		BFw	1021.1	206.7		67.8	217.8	352.2			
		BFs	0.62	0.12		0.04	0.13	0.21			
Dec. '90	3	n	7	3	1	3	2	7			
		Range	86.5-216.2	11.1-28.9	19.5	3.4-4.7	9.6-18.9	9.1-11.7			
		Median	196.2	15.0		4.3	14.3	10.4			
		Mean	176.4	18.3		4.1	14.3	10.5	N/A	N/A	N/A
		SD	47.9	9.4		0.7	6.5	1.0			
		BFw	50.4	5.2	5.6	1.2	4.1	3.0			
		BFs	1.51	0.16	0.17	0.04	0.12	0.09			
	5	n	1					1			
		Range	168.1					14.8			
		Median									
		Mean		N/A	N/A	N/A	N/A		N/A	N/A	N/A
		SD									
		BFw	1200.7					105.7			
		BFs	0.69					0.06			
Feb. '91	5	n	1		1			1	1	1	1
		Range	46.3		4.9			8.0	17.3	198.0	2.5
		Median									
		Mean		N/A		N/A	N/A				
		SD									
		BFw	578.7		61.2			100.0	216.2		31.3
		BFs	0.18		0.02			0.03	0.07		0.01
	7	n	2					2		1	6
		Range	40.4-48.4					4.8-4.8		405.4	2.2-2.9
		Median	44.4					4.8			2.4
		Mean	44.4	N/A	N/A	N/A	N/A	4.8	N/A		2.4
		SD	5.7					0.0			0.3
		BFw	185.0					20.0			10.0
		BFs	0.62					0.07			0.03

Φ Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 7.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	Gut cont.	Vertebrae	Bile	Blood
Apr. '91	3	n®	3	4	1	1	3	6		2	1	3			8
		Range	25.8-33.8	2.1-8.7	4.3	5.3	2.7-6.4	2.7-6.8		42.5-44.5	68.5	125.9-310.0			2.7-3.2
		Median	32.2	3.0			4.8	4.5		43.5		155.0			2.8
		Mean	30.6	4.2			4.6	4.6	N/A	43.5		197.0	N/A	N/A	2.9
		SDΔ	4.2	3.0			1.8	1.7		1.4		99.0			0.2
		BFw	510.0	70.0	71.7	88.3	76.7	76.7		725.0	1141.7				48.3
		BFs	0.06	0.01	0.01	0.01	0.01	0.01		0.09	0.14				0.01
	4	n	3		1	2	2	6		1		2			10
		Range	29.4-35.5		4.3	3.1-6.0	5.6-9.9	3.5-5.1		77.8		272.1-475.8			1.9-3.6
		Median	35.4			4.6	7.7	4.5				374.0			2.3
		Mean	33.4	N/A		4.6	7.7	4.4	N/A		N/A	374.0	N/A	N/A	2.4
		SD	3.5			2.1	3.0	0.6				144.0			0.5
		BFw	668.0		86.0	92.0	154.0	88.0		1556.0					48.0
		BFs	0.14		0.02	0.02	0.03	0.02		0.32					0.01
	5	n	4	5		3	4	7		3	2	3			10
		Range	25.0-56.0	2.5-29.8		2.2-11.0	3.5-57.9	2.2-5.3		25.0-49.9	103.6-112.0	221.0-628.7			1.8-3.3
		Median	35.3	3.6		3.1	5.1	3.7		25.4	107.8	275.1			2.1
		Mean	37.9	8.6	N/A	5.5	17.9	3.8	N/A	33.4	107.8	374.9	N/A	N/A	2.3
		SD	13.9	11.9		4.8	26.7	1.2		14.3	6.0	221.4			0.5
		BFw	1263.3	286.7		183.3	596.7	126.7		1113.3	3593.3				76.7
		BFs	0.32	0.07		0.05	0.15	0.03		0.28	0.90				0.02
	7	n	1		1	1	1	1				1			1
		Range	53.6		3.4	66.9	8.8	7.6				1296.0			2.7
		Median													
		Mean		N/A					N/A	N/A	N/A		N/A	N/A	
		SD													
		BFw	487.3		30.9	608.2	80.0	69.1							24.5
		BFs	0.16		0.01	0.20	0.03	0.02							0.01
June '91	3	n	9	3		9	9	9	9	8	2	8	9		9
		Range	14.5-26.3	1.4-2.8		0.9-5.7	5.9-9.1	2.9-4.9	1.9-5.8	9.0-14.7	11.7-11.7	155.6-487.9	10.9-18.5		1.7-2.4
		Median	17.1	2.7		1.8	8.4	3.2	3.8	11.4	11.7	338.3	13.6		2.0
		Mean	18.2	2.3	N/A	2.1	8.0	3.4	3.8	11.7	11.7	345.9	13.4	N/A	2.0
		SD	3.7	0.8		1.5	1.0	0.6	1.2	1.8	0.0	106.0	2.4		0.2
		BFw	606.7	76.7		70.0	266.7	113.3	126.7	390.0	390.0		446.7		66.7
		BFs	0.02	0.002		0.002	0.01	0.003	0.004	0.01	0.01		0.01		0.002
	4	n	7	1	6	6	7	7	4	7		7	7	1	7
		Range	13.8-20.6	2.3	1.6-6.3	0.6-1.8	4.8-8.7	1.7-3.1	1.2-3.1	9.1-14.7		147.7-605.9	9.9-14.6	2.2	2.1-2.4
		Median	16.6		2.8	1.3	5.9	1.9	1.9	10.4		383.8	11.9		2.3
		Mean	17.0		3.1	1.3	6.2	2.2	2.0	10.7	N/A	393.7	12.0		2.3
		SD	2.7		1.7	0.4	1.3	0.6	0.8	1.9		165.3	1.7		0.1
		BFw	*	*	*	*	*	*	*	*			*	*	*
		BFs	0.10	0.01	0.02	0.01	0.04	0.01	0.01	0.06			0.07	0.01	0.01
	5	n	3		3	3	3	3	3	3	1	2	3		3
		Range	16.9-24.8		3.1-3.6	1.4-4.7	5.4-7.0	3.0-4.9	3.0-5.0	6.0-12.0	10.6	316.6-388.5	10.4-22.2		2.0-2.2
		Median	18.5		3.2	2.2	6.5	4.0	3.7	11.0		352.5	18.6		2.0
		Mean	20.0	N/A	3.3	2.7	6.3	3.9	3.9	9.6		352.5	17.1	N/A	2.1
		SD	4.2		0.3	1.7	0.8	1.0	1.0	3.2		50.8	6.0		0.1
		BFw	1000.0		165.0	135.0	315.0	195.0	195.0	480.0	530.0		855.0		105.0
		BFs	0.08		0.01	0.01	0.02	0.01	0.01	0.04	0.04		0.07		0.01

® Number of samples analyzed Δ Standard deviation N/A Not available * [Mn] in water below AAS detection limit

TABLE 7.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont	HGut cont	Vertebrae	Kidney	Bile	Blood
Aug. '91	3	n®	1	1		1	1	1	1	1		1		1			1
		Range	27.6	3.3		1.3	8.1	1.6	5.8	11.2		313.6		10.7			2.2
		Median															
		Mean			N/A						N/A		N/A		N/A	N/A	
		SD^Δ															
		BFw	345.0	41.3		16.3	101.2	20.0	72.5	140.0				133.7			27.5
		BFs	0.13	0.02		0.01	0.04	0.01	0.03	0.05				0.05			0.01
	4	n	8	5		8	8	8	7	7		5	5	8	2		8
		Range	17.1-38.3	1.0-2.0		0.03-2.2	5.1-11.3	1.1-2.8	0.5-2.9	5.0-10.1		119.8-688.1	136.4-822.5	10.1-14.5	3.5-4.8		1.8-2.5
		Median	20.1	1.2		0.3	6.1	1.6	1.3	7.1		283.2	554.6	11.7	4.1		2.2
		Mean	22.0	1.4	N/A	0.7	6.7	1.7	1.6	7.5	N/A	322.4	543.0	12.0	4.1	N/A	2.1
		SD	6.8	0.5		0.8	2.0	0.6	0.8	1.9		215.5	254.7	1.7	0.9		0.2
		BFw	*	*		*	*	*	*	*				*	*		*
		BFs	0.06	0.004		0.002	0.02	0.004	0.004	0.02				0.03	0.01		0.01
	5	n	12	11	1	12	12	12	12	10	4	8	8	12	7	4	12
		Range	15.9-32.1	0.9-2.6	1.5	0.5-3.0	4.0-8.2	1.4-2.8	0.9-2.5	5.3-14.1	4.9-11.7	62.8-465.1	158.5-544.9	13.0-20.4	5.1-11.3	0.9-2.5	1.7-2.2
		Median	22.3	1.5		1.7	6.9	1.9	1.7	7.9	8.9	120.3	297.2	14.9	8.5	1.7	2.1
		Mean	22.2	1.5		1.6	6.6	2.0	1.7	8.5	8.6	172.2	321.3	15.7	8.0	1.7	2.1
		SD	4.6	0.5		0.7	1.2	0.4	0.5	2.8	2.8	132.1	130.5	2.4	2.0	0.8	0.2
		BFw	1057.1	71.4	71.4	76.2	314.3	95.2	81.0	404.8	409.5			747.6	381.0	81.0	100.0
		BFs	0.09	0.01	0.01	0.01	0.03	0.01	0.01	0.03	0.03			0.06	0.03	0.01	0.01
	7	n	1			1	1	1	1	1				1		1	1
		Range	37.8			9.6	8.0	2.2	4.0	37.7				36.0		2.9	3.8
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A		N/A		
		SD															
		BFw	297.6			75.6	63.0	17.3	31.5	296.9				283.5		22.8	29.9
		BFs	0.29			0.07	0.06	0.02	0.03	0.29				0.28		0.02	0.03
Oct. '91	3	n	6	2	1	6	5	6	6	5	3	2	3	6	3	5	6
		Range	14.4-47.1	1.9-2.0	0.6	0.6-5.7	6.0-7.6	0.6-1.8	0.2-2.3	11.7-14.3	16.5-18.4	644.7-1175.6	903.6-1503.7	9.7-21.0	4.0-6.9	0.2-0.5	2.1-2.4
		Median	22.8	2.0		1.0	7.0	1.1	1.3	12.3	16.5	910.1	957.1	12.5	6.1	0.3	2.2
		Mean	25.7	2.0		1.8	6.8	1.2	1.3	12.7	17.1	910.1	1121.5	13.5	5.6	0.3	2.3
		SD	12.1	0.1		2.0	0.6	0.4	0.8	1.0	1.1	375.4	332.1	4.4	1.5	0.1	0.1
		BFw	428.3	33.3	10.0	30.0	113.3	20.0	21.7	211.7	285.0			225.0	93.3	5.0	38.3
		BFs	0.17	0.01	0.004	0.01	0.04	0.01	0.01	0.08	0.11			0.09	0.04	0.002	0.01
	4	n	11	7	1	11	8	11	11	5	2	9	4	11	3	6	10
		Range	12.5-73.5	1.7-3.1	1.3	0.7-8.7	4.5-23.7	1.0-2.2	1.0-3.5	5.0-12.5	8.2-17.0	43.3-1337.1	130.4-1415.3	10.1-21.2	7.1-10.9	1.5-3.3	2.0-3.6
		Median	24.0	2.6		1.3	6.6	1.5	2.1	8.7	12.6	309.9	675.0	12.9	7.7	2.3	2.6
		Mean	27.5	2.4		2.7	9.0	1.6	2.0	8.7	12.6	414.5	816.4	14.5	8.6	2.3	2.7
		SD	17.3	0.5		2.6	6.4	0.4	0.7	2.7	6.2	411.0	509.6	4.0	2.0	0.7	0.6
		BFw	639.5	55.8	30.2	62.8	209.3	37.2	46.5	202.3	293.0			337.2	200.0	53.5	62.8
		BFs	0.16	0.01	0.01	0.02	0.05	0.01	0.01	0.05	0.08			0.09	0.05	0.01	0.02
	5	n	14	9	4	15	13	15	14	3	3	10	4	15	5	9	10
		Range	27.3-51.0	2.1-3.5	1.1-2.0	0.6-4.9	6.7-12.5	0.8-2.1	1.0-4.0	9.3-14.2	10.3-15.7	88.6-1897.5	977.6-4575.5	11.2-49.0	3.0-5.1	0.6-5.0	2.2-3.5
		Median	36.3	2.8	1.7	1.5	8.9	1.5	1.5	11.8	12.2	185.0	1737.5	14.3	4.1	1.6	2.9
		Mean	35.4	2.7	1.6	1.6	9.2	1.5	1.7	11.8	12.7	383.4	2257.0	17.1	4.1	1.9	2.8
		SD	7.2	0.4	0.4	1.0	2.1	0.4	0.7	2.5	2.7	545.7	1641.7	9.3	1.0	1.4	0.4
		BFw	478.4	36.5	21.6	21.6	124.3	20.3	23.0	159.5	171.6			231.1	55.4	25.7	37.8
		BFs	0.12	0.01	0.01	0.01	0.03	0.01	0.01	0.04	0.04			0.06	0.01	0.01	0.01
	7	n	1			1	1	1	1	1				1	1	1	1
		Range	36.0			2.7	4.6	1.9	2.4	11.2				19.2	8.0	1.1	1.6
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A				
		SD															
		BFw	112.9			8.5	14.4	6.0	7.5	35.1				60.2	25.1	3.4	5.0
		BFs	0.12			0.01	0.02	0.01	0.01	0.04				0.06	0.03	0.004	0.01

® Number of samples analyzed Δ Standard deviation N/A Not available * [Mn] in water below AAS detection limit

TABLE 7.1 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont.	HGut cont.	Vertebrae	Kidney	Bile	Blood
Jan. '92	3	n[Φ]	5	4		5	3	6	2	2	2	2	2	6	1	5	6
		Range	15.6-52.0	1.8-2.7		0.6-4.3	4.3-6.2	1.3-1.8	1.5-3.3	6.8-9.1	13.4-14.3	274.6-573.6	283.2-311.9	9.6-23.1	5.6	1.4-5.8	1.9-2.2
		Median	19.6	2.4		1.2	5.7	1.4	2.4	8.0	13.8	424.1	297.6	14.7		3.5	2.1
		Mean	27.7	2.3	N/A	1.8	5.4	1.5	2.4	8.0	13.8	424.1	297.6	15.3		3.3	2.1
		SD[Δ]	15.0	0.4		1.5	1.0	0.2	1.3	1.6	0.6	211.5	20.3	4.6		1.8	0.1
		BFw	337.8	28.0		22.0	65.9	18.3	29.3	97.6	168.3			186.6	68.3	40.2	25.6
		BFs	0.61	0.05		0.04	0.12	0.03	0.05	0.18	0.30			0.34	0.12	0.07	0.05
	4	n	4	3		10	2	11	3	1	1	1	3	9	1	2	11
		Range	17.8-27.8	1.7-2.8		1.3-4.2	6.4-7.1	1.6-3.9	1.3-3.8	8.5	14.6	211.2	331.9-584.6	10.6-22.1	4.7	1.4-2.6	1.9-3.6
		Median	24.0	2.0		2.5	6.7	1.8	1.7				335.2	15.5		2.0	2.2
		Mean	23.4	2.2	N/A	2.6	6.7	2.0	2.2				417.2	16.3		2.0	2.2
		SD	4.2	0.6		1.0	0.5	0.6	1.4				145.0	3.9		0.9	0.5
		BFw	1300.0	122.2		144.4	372.2	111.1	122.2	472.2	811.1			905.6	261.1	111.1	122.2
		BFs	0.21	0.02		0.02	0.06	0.02	0.02	0.08	0.13			0.15	0.04	0.02	0.02
	5	n	12	8	4	12	9	12	11	3	3	2	1	12	3	9	12
		Range	12.1-70.8	2.2-3.8	1.8-3.2	0.7-3.5	5.2-13.6	1.2-7.3	1.1-3.5	9.8-13.6	11.3-51.7	51.5-795.3	852.4	8.7-35.3	3.4-6.5	0.9-6.2	1.7-3.2
		Median	23.0	2.6	2.3	1.1	6.6	1.8	1.4	11.9	21.4	423.4		13.9	4.7	2.2	2.4
		Mean	28.8	2.8	2.4	1.6	8.1	2.2	1.8	1.8	28.1	423.4		16.6	4.9	2.6	2.3
		SD	15.9	0.6	0.6	1.0	2.9	1.6	0.8	1.9	21.0	526.0		7.4	1.5	1.6	0.4
		BFw	3200.0	311.1	266.7	177.8	900.0	244.4	200.0	200.0	3122.2			1844.4	544.4	288.9	255.6
		BFs	0.51	0.05	0.04	0.03	0.14	0.04	0.03	0.03	0.49			0.29	0.09	0.05	0.04
	7	n	1			5		5		1				5			5
		Range	55.5			5.3-38.3		2.3-15.7		25.0				20.6-32.2			2.0-2.4
		Median				18.3		2.9						26.8			2.2
		Mean		N/A	N/A	18.0	N/A	5.6	N/A		N/A	N/A	N/A	25.8	N/A	N/A	2.2
		SD				13.4		5.7						4.5			0.1
		BFw	240.3			77.9		24.2		108.2				111.7			9.5
		BFs	0.42			0.14		0.04		0.19				0.20			0.02
Feb. '92	3	n	2			6		6	3					6			6
		Range	29.9-32.6			1.1-5.7		1.0-1.6	0.6-1.2					10.7-18.1			2.1-3.2
		Median	31.3			1.8		1.3	0.9					12.2			2.4
		Mean	31.3	N/A	N/A	2.4	N/A	1.3	0.9	N/A	N/A	N/A	N/A	13.7	N/A	N/A	2.4
		SD	1.9			1.7		0.2	0.3					3.3			0.4
		BFw	42.5			3.3		1.8	1.2					18.6			3.3
		BFs	0.27			0.02		0.01	0.01					0.12			0.02
	4	n	6	4		10	7	10	8	1	1	1	1	10	1	9	10
		Range	12.9-28.2	2.1-3.0		0.6-4.7	5.4-16.4	1.1-2.3	1.0-3.8	8.2	6.5	1345.6	2441.8	11.6-20.2	3.6	0.2-1.8	2.0-2.4
		Median	17.1	2.4		1.6	7.5	1.5	1.8					13.2		0.6	2.1
		Mean	18.5	2.5	N/A	2.0	9.4	1.6	2.0					14.8		0.7	2.2
		SD	5.5	0.4		1.2	4.3	0.4	1.0					3.5		0.6	0.1
		BFw	55.2	7.5		6.0	28.1	4.8	6.0	24.5	19.4			44.2	10.7	2.1	6.6
		BFs	0.07	0.01		0.01	0.04	0.01	0.01	0.03	0.02			0.06	0.01	0.003	0.01
	5	n	10	10		10	8	10	10	2	2			10	2	10	10
		Range	13.3-30.1	1.6-3.2		0.6-2.2	4.9-10.7	0.6-1.6	0.5-1.6	6.0-7.3	5.7-7.7			8.9-18.3	3.0-4.0	0.3-0.5	2.2-3.7
		Median	22.9	2.0		1.5	5.9	1.0	0.8	6.6	6.7			13.4	3.5	0.4	2.5
		Mean	22.7	2.1	N/A	1.4	6.6	1.0	0.9	6.6	6.7	N/A	N/A	13.5	3.5	0.4	2.7
		SD	5.0	0.6		0.6	1.8	0.3	0.4	0.9	1.4			3.0	0.7	0.1	0.5
		BFw	38.6	3.6		2.4	11.2	1.7	1.5	11.2	11.4			23.0	6.0	0.7	4.6
		BFs	0.04	0.004		0.003	0.01	0.002	0.002	0.01	0.01			0.03	0.01	0.001	0.01
	Pionier Dam	n	10	5	5	10	9	10	10	6	2	6		10	4	5	10
		Range	13.1-32.3	2.7-3.7	1.2-3.3	0.9-5.9	2.7-7.5	0.8-1.6	0.4-2.7	7.9-10.9	6.9-15.6	119.1-278.8		13.9-22.2	6.7-8.9	1.1-1.9	2.0-2.6
		Median	22.4	3.1	1.6	2.5	5.4	1.1	1.0	8.5	11.2	217.9		15.9	7.5	1.4	2.3
		Mean	22.4	3.2	1.8	2.8	5.3	1.1	1.2	9.1	11.2	206.5	N/A	16.5	7.7	1.5	2.3
		SD	5.2	0.4	0.8	1.7	1.6	0.3	0.8	1.4	6.1	74.1		2.6	0.9	0.4	0.2
		BFw	520.9	74.4	41.9	65.1	123.3	25.6	27.9	211.6	260.5			383.7	179.1	34.9	53.5
		BFs	0.42	0.06	0.03	0.05	0.10	0.02	0.02	0.17	0.21			0.31	0.14	0.03	0.04

Φ Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 7.2
MEAN LEAD CONCENTRATIONS (µg/g dry wt.) IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* (BF_W AND BF_S = BIOCONCENTRATION FACTORS OF THE WATER AND SEDIMENT RESPECTIVELY)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle
Apr. '90	3	n®						1
		Range						56.5
		Median						
		Mean	N/A	N/A	N/A	N/A	N/A	
		SD^Δ						
		BFw						297.4
		BFs						1.49
	4	n	4	3				5
		Range	38.5-46.2	17.9-32.1				21.7-47.8
		Median	44.2	28.6				34.8
		Mean	43.3	26.2	N/A	N/A	N/A	34.8
		SD	3.7	7.4				9.7
		BFw	206.2	124.8				165.7
		BFs	1.22	0.74				0.98
	7	n	7				4	9
		Range	26.9-50.0				18.5-25.9	17.4-30.4
		Median	38.5				22.2	26.1
		Mean	37.9	N/A	N/A	N/A	22.2	25.6
		SD	7.5				3.0	4.0
		BFw	164.8				96.5	111.3
		BFs	0.61				0.36	0.41
June '90	3	n	2					2
		Range	26.9-42.3					17.4-21.7
		Median	34.6					19.6
		Mean	34.6	N/A	N/A	N/A	N/A	19.6
		SD	10.9					3.1
		BFw	72.1					40.8
		BFs	1.15					0.65
Aug. '90	3	n	8	2	1	1	2	9
		Range	46.2-69.2	28.6-46.4	47.6	12.2	51.9-51.9	39.1-69.6
		Median	59.6	37.5			51.9	47.8
		Mean	58.2	37.5			51.9	50.2
		SD	7.8	12.6			0.0	9.0
		BFw	149.2	96.2	122.1	31.3	133.1	128.7
		BFs	2.33	1.50	1.90	0.49	2.08	2.01
	4	n	9	4	3	4	6	9
		Range	42.3-57.7	21.4-32.1	42.9-47.6	6.7-11.1	22.2-37.0	34.8-47.8
		Median	46.2	26.8	42.9	8.9	29.6	39.1
		Mean	47.4	26.8	44.4	8.9	30.2	39.6
		SD	6.1	4.6	2.7	2.0	6.4	4.0
		BFw	124.7	70.5	116.8	23.4	79.5	104.2
		BFs	1.90	1.07	1.78	0.36	1.21	1.58
	5	n	7	1	2	3	7	6
		Range	42.3-61.5	71.4	33.3-57.1	7.8-11.1	25.9-48.2	34.8-56.5
		Median	46.2		45.2	8.9	33.3	45.7
		Mean	49.5		45.2	9.3	34.9	45.7
		SD	8.1		16.8	1.7	7.7	8.1
		BFw	130.3	187.9	118.9	24.5	91.8	120.3
		BFs	1.83	2.64	1.67	0.34	1.29	1.69
	7	n						5
		Range						34.8-56.5
		Median						43.5
		Mean	N/A	N/A	N/A	N/A	N/A	44.3
		SD						7.8
		BFw						100.7
		BFs						1.30

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 7.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Gut	Gut cont	Blood
Oct. '90	3	n[Φ]	7	2	1	2	6	7			
		Range	26.9-38.5	17.9-17.9	38.1	3.3-4.4	14.8-29.6	13.0-34.8			
		Median	34.6	17.9		3.9	18.5	30.4			
		Mean	34.1	17.9		3.9	19.8	27.3	N/A	N/A	N/A
		SD[Δ]	6.1	0.0		0.8	5.6	8.6			
		BFw	94.7	49.7	105.8	10.8	55.0	75.8			
		BFs	2.27	1.19	2.54	0.26	1.32	1.82			
	4	n	10	1	7	6	9	10			
		Range	19.2-34.6	21.4	19.1-47.6	4.4-10.0	14.8-25.9	13.0-30.4			
		Median	25.0		23.8	5.6	18.5	19.6			
		Mean	26.5		27.2	6.5	18.5	20.4	N/A	N/A	N/A
		SD	6.1		10.5	2.0	3.7	6.5			
		BFw	94.6	76.4	97.1	23.2	66.1	72.9			
		BFs	0.91	0.74	0.94	0.22	0.64	0.70			
	5	n	9	1	6	9	9	10			
		Range	30.8-46.2	28.6	23.8-38.1	3.3-8.7	22.2-33.3	21.7-39.1			
		Median	38.5		35.7	5.6	29.6	30.4			
		Mean	37.2		34.1	5.8	28.8	31.3	N/A	N/A	N/A
		SD	4.7		5.6	1.8	4.0	4.9			
		BFw	137.8	105.9	126.3	21.5	106.7	115.9			
		BFs	1.77	1.36	1.62	0.28	1.37	1.49			
	7	n	1	1		1	1	1			
		Range	30.8	33.3		4.4	18.5	39.1			
		Median									
		Mean			N/A				N/A	N/A	N/A
		SD									
		BFw	96.2	104.1		13.7	57.8	122.2			
		BFs	1.62	1.75		0.23	0.97	2.06			
Dec. '90	3	n	7	3	1	3	2	7			
		Range	15.4-50.0	28.6-42.9	38.1	3.3-5.6	25.9-33.3	21.7-34.8			
		Median	34.6	35.7		4.4	29.6	26.1			
		Mean	31.9	35.7		4.4	29.6	28.0	N/A	N/A	N/A
		SD	11.3	7.1		1.2	5.2	5.5			
		BFw	245.4	274.6	293.1	33.8	227.7	215.4			
		BFs	0.60	0.67	0.72	0.08	0.56	0.53			
	5	n	1					1			
		Range	46.2					13.0			
		Median									
		Mean		N/A	N/A	N/A	N/A		N/A	N/A	N/A
		SD									
		BFw	660.0					185.7			
		BFs	4.20					1.18			
Feb. '91	5	n	1		1			1	1	1	1
		Range	38.8		7.6			9.1	16.4	21.2	9.8
		Median									
		Mean		N/A		N/A	N/A				
		SD									
		BFw	485.0		95.0			113.7	205.0		122.5
		BFs	3.70		0.72			0.87	1.56		0.93
	7	n	2					2		1	6
		Range	6.0-21.5					3.8-5.8		25.6	4.7-18.0
		Median	13.8					4.8			10.5
		Mean	13.8	N/A	N/A	N/A	N/A	4.8	N/A		10.6
		SD	11.0					1.4			4.6
		BFw	92.0					32.0			70.7
		BFs	0.89					0.31			0.68

[Φ] Number of samples analyzed [Δ] Standard deviation N/A Not available

TABLE 7.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	Gut cont.	Vertebrae	Bile	Blood
Apr. '91	3	n[Φ]	3	4	1	1	3	6		2	1	3			8
		Range	2.6-11.6	2.8-11.1	5.2	4.1	4.8-6.4	2.4-7.3		6.9-7.5	16.4	2.0-19.8			5.2-14.0
		Median	7.2	7.3			5.9	5.1		7.2		5.7			10.0
		Mean	7.1	7.1			5.7	5.0	N/A	7.2		9.2	N/A	N/A	9.7
		SD[Δ]	4.5	3.4			0.8	2.0		0.4		9.4			3.3
		BFw	41.8	41.8	30.6	24.1	33.5	29.4		42.4	96.5				57.1
		BFs	0.21	0.21	0.15	0.12	0.17	0.15		0.21	0.48				0.29
	4	n	3		1	2	2	6		1		2			10
		Range	6.0-7.5		12.4	4.7-15.0	4.1-6.0	2.9-11.8		9.7		8.4-12.1			2.1-10.4
		Median	6.3			9.9	5.1	8.8				10.3			5.1
		Mean	6.6	N/A		9.9	5.1	8.1	N/A		N/A	10.3	N/A	N/A	5.5
		SD	0.8			7.3	1.4	3.2				2.6			2.9
		BFw	44.0		82.7	66.0	34.0	54.0		64.7					36.7
		BFs	0.51		0.95	0.76	0.39	0.62		0.75					0.42
	5	n	4	5		3	3	7		3	2	3			10
		Range	1.6-7.2	0.3-15.4		2.2-5.7	2.4-7.5	1.8-8.7		2.3-11.7	5.1-15.7	7.3-11.6			2.4-11.6
		Median	5.7	1.1		2.3	4.9	3.3		3.7	10.4	9.2			6.6
		Mean	5.1	4.9	N/A	3.4	4.9	4.2	N/A	5.9	10.4	9.4	N/A	N/A	6.3
		SD	2.4	6.5		2.0	2.5	2.3		5.1	7.5	2.1			3.1
		BFw	51.0	49.0		34.0	49.0	42.0		59.0	104.0				63.0
		BFs	0.57	0.54		0.38	0.54	0.47		0.66	1.16				0.70
	7	n	1		1	1	1	1				1			1
		Range	10.8		3.6	2.1	9.3	7.1				10.0			7.7
		Median													
		Mean		N/A					N/A	N/A	N/A		N/A	N/A	
		SD													
		BFw	83.1		27.7	16.2	71.5	54.6							59.2
		BFs	0.74		0.25	0.14	0.64	0.49							0.53
June '91	3	n	9	3		9	9	9	9	8	2	8	9		9
		Range	15.9-30.0	8.7-15.3		1.0-15.2	7.5-17.1	7.9-14.8	11.5-22.9	10.5-30.6	22.2-32.5	19.2-52.0	20.1-25.7		5.9-16.3
		Median	19.1	10.0		7.8	14.0	10.2	18.1	20.5	27.4	43.8	24.0		9.7
		Mean	21.0	11.3	N/A	7.6	13.3	10.7	17.9	20.4	27.4	39.2	23.3	N/A	9.7
		SD	4.5	3.5		4.3	3.0	2.5	3.7	6.2	7.3	12.0	2.1		3.5
		BFw	262.5	141.2		95.0	166.2	133.7	223.7	255.0	342.5		291.2		121.2
		BFs	1.45	0.78		0.52	0.92	0.74	1.23	1.41	1.89		1.61		0.67
	4	n	7	1	6	6	7	7	4	7		7	7	1	7
		Range	11.0-25.5	5.8	4.7-26.1	1.8-3.6	3.9-8.1	2.1-7.5	5.3-20.7	6.4-18.9		31.3-50.0	20.8-25.5	26.1	4.6-11.8
		Median	16.4		9.0	2.5	6.4	5.2	11.5	10.9		36.6	22.9		6.5
		Mean	17.5		12.6	2.6	6.1	4.8	12.2	12.1	N/A	38.9	22.6		7.8
		SD	5.1		8.8	0.8	1.6	1.9	6.4	4.8		7.0	1.6		3.0
		BFw	1750.0	580.0	1260.0	260.0	610.0	480.0	1220.0	1210.0			2260.0	2610.0	780.0
		BFs	1.46	0.48	1.05	0.22	0.51	0.40	1.02	1.01			1.88	2.17	0.65
	5	n	3		3	3	3	3	3	3	1	2	3		3
		Range	20.7-22.7		16.7-26.9	2.5-4.4	9.8-10.8	7.7-15.8	15.8-33.3	11.1-20.0	12.5	32.1-34.7	22.6-31.9		8.1-10.4
		Median	21.8		25.9	2.7	10.2	12.2	31.7	16.7		33.4	24.7		8.7
		Mean	21.7	N/A	23.2	3.2	10.3	11.9	26.9	15.9		33.4	26.4	N/A	9.1
		SD	1.0		5.6	1.0	0.5	4.1	9.7	4.5		1.8	4.9		1.2
		BFw	241.1		257.8	35.6	114.4	132.2	298.9	176.7	138.9		293.3		101.1
		BFs	1.36		1.45	0.20	0.64	0.74	1.68	0.99	0.78		1.65		0.57

Φ Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 7.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont	HGut cont	Vertebrae	Kidney	Bile	Blood
Aug. '91	3	n®	1	1		1	1	1	1	1		1		1			1
		Range	7.7	8.7		3.2	9.3	8.5	17.3	15.3		19.5		16.3			10.1
		Median															
		Mean			N/A						N/A		N/A		N/A	N/A	
		SD^Δ															
		BFw	192.5	217.5		80.0	232.5	212.5	432.5	382.5				407.5			252.5
		BFs	0.73	0.83		0.30	0.89	0.81	1.65	1.46				1.55			0.96
	4	n	8	5		8	8	8	7	7		5	5	8	2		8
		Range	0.4-5.8	1.1-11.6		0.5-4.8	1.2-11.2	0.6-5.7	2.2-7.7	2.4-15.9		1.9-7.4	2.9-14.6	1.9-7.6	7.4-15.4		11.3-14.5
		Median	2.6	1.3		2.8	3.3	2.3	4.5	5.3		6.3	9.8	4.4	11.4		12.1
		Mean	2.8	3.9	N/A	2.7	3.9	2.7	4.1	6.7	N/A	5.0	8.3	4.6	11.4	N/A	12.4
		SD	1.9	4.5		1.4	3.0	1.5	2.0	4.9		2.4	5.0	1.8	5.6		1.1
		BFw	17.5	24.4		16.9	24.4	16.9	25.6	41.9				28.8	71.2		77.5
		BFs	0.12	0.16		0.11	0.16	0.11	0.17	0.28				0.19	0.47		0.52
	5	n	12	11	1	12	12	12	12	10	4	8	8	12	7	4	12
		Range	0.1-5.9	1.0-2.6	16.3	0.3-5.7	0.5-4.9	0.5-8.1	0.8-10.1	1.0-6.7	2.8-5.7	1.8-15.0	2.7-13.3	2.4-10.4	1.6-27.0	2.8-6.7	7.2-12.6
		Median	1.5	2.0		2.3	1.9	3.0	2.6	3.4	3.8	4.9	5.1	3.3	6.7	3.3	10.9
		Mean	1.9	1.8		2.7	2.3	3.3	3.3	3.5	4.0	7.1	6.6	4.3	7.9	4.0	10.7
		SD	1.6	0.5		2.1	1.4	2.0	2.5	1.6	1.3	5.2	4.0	2.4	8.8	1.8	1.5
		BFw	19.0	18.0	163.0	27.0	23.0	33.0	33.0	35.0	40.0			43.0	79.0	40.0	107.0
		BFs	0.14	0.13	1.16	0.19	0.16	0.24	0.24	0.25	0.29			0.31	0.56	0.29	0.76
	7	n	1			1	1	1	1	1				1		1	1
		Range	15.0			6.4	19.3	3.4	38.3	19.6				6.8		21.4	22.0
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A		N/A		
		SD															
		BFw	71.4			30.5	91.9	16.2	182.4	93.3				32.4		101.9	104.8
		BFs	1.30			0.56	1.68	0.30	3.33	1.70				0.59		1.86	1.91
Oct. '91	3	n	6	2	1	6	5	6	6	5	3	2	3	6	3	5	6
		Range	9.7-20.0	10.4-17.8	13.5	1.6-6.7	6.0-9.3	3.1-8.6	4.8-8.4	6.7-16.3	8.3-21.0	19.1-24.2	16.6-23.6	20.1-23.2	7.0-18.0	4.1-9.4	3.9-13.9
		Median	12.9	14.1		3.8	8.3	6.2	5.8	11.6	10.0	21.6	17.5	20.4	14.7	7.9	11.2
		Mean	13.3	14.1		4.1	8.1	6.1	6.2	11.4	13.1	21.6	19.2	20.9	13.2	7.1	10.4
		SD	3.6	5.3		1.8	1.3	1.8	1.3	3.7	6.9	3.6	3.8	1.2	5.7	2.3	3.4
		BFw	71.9	76.2	73.0	22.2	43.8	33.0	33.5	61.6	70.8			113.0	71.4	38.4	56.2
		BFs	6.05	6.41	6.14	1.86	3.68	2.77	2.82	5.18	5.95			9.50	6.00	3.23	4.73
	4	n	11	7	1	11	8	11	11	5	2	9	5	11	3	6	10
		Range	9.5-13.9	2.9-10.0	4.3	2.2-11.5	5.0-11.0	2.8-8.4	1.5-8.9	6.0-10.6	8.1-17.0	15.9-70.2	10.1-34.0	17.5-22.8	9.8-20.3	4.5-12.5	6.0-13.4
		Median	11.1	5.0		5.9	7.3	5.0	6.1	8.9	12.6	28.3	16.7	19.4	12.0	6.9	9.6
		Mean	11.5	5.5		6.6	7.6	5.3	5.9	8.3	12.6	31.7	19.0	19.6	14.0	7.7	9.6
		SD	1.6	2.3		3.1	1.8	1.6	2.4	1.8	6.3	16.8	9.0	1.8	5.5	2.7	2.7
		BFw	71.0	34.0	26.5	40.7	46.9	32.7	36.4	51.2	77.8			121.0	86.4	47.5	59.3
		BFs	7.19	3.44	2.69	4.13	4.75	3.31	3.69	5.19	7.88			12.25	8.75	4.81	6.00
	5	n	14	9	4	15	13	15	14	3	3	10	4	15	5	9	10
		Range	10.2-15.1	3.7-19.1	5.2-9.2	4.7-11.1	5.9-22.6	3.0-8.8	0.9-6.2	5.9-19.7	2.8-31.4	8.5-48.6	16.2-45.7	15.8-24.7	2.6-10.2	5.4-30.4	3.8-14.1
		Median	11.8	6.1	7.6	7.3	8.4	4.7	4.6	9.7	15.0	29.8	27.6	20.6	5.6	10.3	8.2
		Mean	11.8	7.3	7.4	7.8	9.4	4.9	4.4	11.8	16.4	27.6	29.3	20.3	6.8	12.5	8.6
		SD	1.4	4.6	1.8	1.9	4.4	1.5	1.4	7.1	14.3	11.0	12.3	2.5	3.3	7.7	3.1
		BFw	76.6	47.4	48.1	50.6	61.0	31.8	28.6	76.6	106.5			131.8	44.2	81.2	55.8
		BFs	5.90	3.65	3.70	3.90	4.70	2.45	2.20	5.90	8.20			10.15	3.40	6.25	4.30
	7	n	1			1	1	1	1	1				1	1	1	1
		Range	21.2			2.3	7.5	6.8	5.5	4.9				9.5	20.5	17.9	14.2
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A				
		SD															
		BFw	118.4			12.8	41.9	38.0	30.7	27.4				53.1	114.5	100.0	79.3
		BFs	12.47			1.35	4.41	4.00	3.24	2.88				5.59	12.06	10.53	8.35

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 7.2 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont.	HGut cont.	Vertebrae	Kidney	Bile	Blood
Jan. '92	3	n[Φ]	5	4		5	3	6	2	2	2	2	2	6	1	5	6
		Range	10.3-18.1	4.1-7.2		3.6-8.0	4.6-6.2	3.6-7.2	5.4-5.4	7.4-13.6	7.1-25.5	15.9-16.6	26.0-28.1	16.2-25.7	4.6	2.6-11.1	4.1-10.4
		Median	10.9	4.9		5.2	6.1	4.8	5.4	10.5	16.3	16.2	27.1	20.3		8.6	7.5
		Mean	12.1	5.3	N/A	5.5	5.7	5.2	5.4	10.5	16.3	16.2	27.1	20.4		6.8	7.3
		SD[Δ]	3.4	1.3		1.6	0.9	1.6	0.0	4.4	13.0	0.5	1.5	3.1		3.7	2.7
		BFw	100.8	44.2		45.8	47.5	43.3	45.0	87.5	135.8			170.0	38.3	56.7	60.8
		BFs	3.10	1.36		1.41	1.46	1.33	1.38	2.69	4.18			5.23	1.18	1.74	1.87
	4	n	4	3		10	2	11	3	1	1	1	3	9	1	2	11
		Range	12.1-15.1	2.9-3.3		4.1-18.5	4.2-6.6	2.5-8.8	1.4-21.7	8.5	11.1	26.2	22.1-25.8	14.8-27.3	6.1	5.6-19.6	2.9-12.2
		Median	13.2	3.1		8.1	5.4	6.5	5.4				22.3	21.5		12.6	7.2
		Mean	13.4	3.1	N/A	8.5	5.4	6.4	9.5				23.4	22.3		12.6	7.9
		SD	1.2	0.2		4.0	1.7	1.6	10.8				2.1	3.7		9.9	3.1
		BFw	81.7	18.9		51.8	32.9	39.0	57.9	51.8	67.7			136.0	37.2	76.8	48.2
		BFs	2.63	0.61		1.67	1.06	1.25	1.86	1.67	2.18			4.37	1.20	2.47	1.55
	5	n	12	8	4	12	9	12	11	3	3	2	1	12	3	9	12
		Range	8.3-14.7	3.3-9.0	6.2-8.9	1.0-8.6	3.0-10.7	1.4-5.7	1.7-9.9	9.4-10.5	12.8-18.5	19.0-28.7	20.0	15.4-20.7	1.6-6.6	2.9-18.3	3.7-13.6
		Median	11.0	4.7	7.7	5.8	6.0	3.8	3.2	10.0	13.9	23.8		18.2	5.8	6.2	9.8
		Mean	11.4	5.2	7.6	5.5	5.9	3.6	4.6	10.0	15.1	23.8		17.8	4.7	7.7	9.1
		SD	2.3	1.8	1.1	2.3	2.2	1.4	2.7	0.5	3.0	6.9		1.7	2.7	5.1	3.2
		BFw	140.7	64.2	93.8	67.9	72.8	44.4	56.8	123.5	186.4			219.8	58.0	95.1	112.3
		BFs	5.70	2.60	3.80	2.75	2.95	1.80	2.30	5.00	7.55			8.90	2.35	3.85	4.55
	7	n	1			5		5		1				5			5
		Range	10.5			0.6-10.1		2.3-4.4		19.6				16.6-28.4			5.3-7.9
		Median				4.6		3.0						24.9			6.7
		Mean		N/A	N/A	4.4	N/A	3.2	N/A		N/A	N/A	N/A	22.9	N/A	N/A	6.6
		SD				3.6		0.9						5.0			1.0
		BFw	59.0			24.7		18.0		110.1				128.7			37.1
		BFs	0.95			0.40		0.29		1.78				2.08			0.60
Feb. '92	3	n	2			6		6	3					6			6
		Range	16.9-17.3			4.4-11.6		4.0-7.2	3.3-8.2					21.5-27.9			2.1-12.2
		Median	17.1			8.6		6.1	5.5					25.7			7.1
		Mean	17.1	N/A	N/A	8.4	N/A	5.9	5.7	N/A	N/A	N/A	N/A	25.3	N/A	N/A	7.0
		SD	0.3			2.5		1.2	2.5					2.1			5.0
		BFw	92.9			45.7		32.1	31.0					137.5			38.0
		BFs	1.45			0.71		0.50	0.48					2.14			0.59
	4	n	6	4		10	7	10	8	1	1	1	1	10	1	9	10
		Range	11.2-14.0	2.8-6.9		5.2-13.8	1.0-14.9	4.0-7.2	1.6-6.9	4.7	5.0	25.7	25.4	19.2-25.7	9.2	1.8-12.5	1.2-11.2
		Median	12.3	4.7		7.3	6.8	5.5	4.6					21.1		5.2	9.2
		Mean	12.4	4.8	N/A	8.4	6.8	5.6	4.5					21.1		5.8	7.7
		SD	0.9	1.7		2.8	4.1	1.2	1.9					1.9		3.6	3.6
		BFw	81.0	31.4		54.9	44.4	36.6	29.4	30.7	32.7			137.9	60.1	37.9	50.3
		BFs	0.80	0.31		0.54	0.44	0.36	0.29	0.30	0.32			1.36	0.59	0.37	0.50
	5	n	10	10		10	8	10	10	2	2			10	2	10	10
		Range	11.3-17.6	1.4-8.3		1.1-15.1	1.6-13.6	2.0-7.4	0.8-8.4	3.9-13.9	6.5-26.7			16.2-25.9	3.0-9.1	3.2-9.8	3.1-11.1
		Median	14.7	4.8		3.5	5.6	4.3	5.1	8.9	16.6			20.7	6.1	5.8	9.3
		Mean	14.7	4.9	N/A	4.8	5.9	4.3	4.2	8.9	16.6	N/A	N/A	21.0	6.1	5.9	8.5
		SD	2.0	1.8		4.1	3.9	1.5	2.6	7.1	14.3			3.8	4.3	2.2	2.5
		BFw	79.0	26.3		25.8	31.7	23.1	22.6	47.8	89.2			112.9	32.8	31.7	45.7
		BFs	0.64	0.21		0.21	0.26	0.19	0.18	0.39	0.72			0.91	0.26	0.26	0.37
	Pionier Dam	n	10	5	5	10	9	10	10	6	2	6		10	4	5	10
		Range	10.4-18.2	3.7-5.6	5.8-9.8	1.8-13.3	2.2-10.8	2.2-6.8	2.8-8.0	6.7-21.3	5.5-9.6	5.8-20.9		17.3-22.0	5.3-7.8	1.5-6.0	5.8-10.5
		Median	14.0	5.2	8.5	8.2	3.4	4.6	4.5	8.8	7.6	10.0		19.2	6.8	3.7	8.7
		Mean	13.8	4.9	8.0	7.6	4.5	4.7	4.7	10.4	7.6	11.9	N/A	19.6	6.7	3.8	8.5
		SD	2.2	0.8	1.6	3.3	2.7	1.3	1.8	5.5	2.9	5.7		1.7	1.0	1.7	1.7
		BFw	186.5	66.2	108.1	102.7	60.8	63.5	63.5	140.5	102.7			264.9	90.5	51.4	114.9
		BFs	1.48	0.53	0.86	0.82	0.48	0.51	0.51	1.12	0.82			2.11	0.72	0.41	0.91

[Φ] Number of samples analyzed [Δ] Standard deviation N/A Not available

TABLE 7.3

MEAN STRONTIUM CONCENTRATIONS (µg/g dry wt.) IN THE ORGANS, TISSUES AND GUT CONTENTS OF *BARBUS MAREQUENSIS* (BF_w AND BF_s = BIOCONCENTRATION FACTORS OF THE WATER AND SEDIMENT RESPECTIVELY)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle
Apr. '90	3	n®						
		Range						
		Median						
		Mean	N/A	N/A	N/A	N/A	N/A	N/A
		SD$^{\Delta}$						
		BFw						
		BFs						
	4	n						
		Range						
		Median						
		Mean	N/A	N/A	N/A	N/A	N/A	N/A
		SD						
		BFw						
		BFs						
	7	n						
		Range						
		Median						
		Mean	N/A	N/A	N/A	N/A	N/A	N/A
		SD						
		BFw						
		BFs						
June '90	3	n						
		Range						
		Median						
		Mean	N/A	N/A	N/A	N/A	N/A	N/A
		SD						
		BFw						
		BFs						
Aug. '90	3	n	8	2	1	1	2	8
		Range	419.2-1126.9	17.9-32.1	23.8	14.4	18.5-22.2	21.7-39.1
		Median	540.4	25.0			20.4	32.6
		Mean	588.9	25.0			20.4	31.0
		SD	229.7	10.1			2.6	5.9
		BFw	N/A	N/A	N/A	N/A	N/A	N/A
		BFs	6.69	0.28	0.27	0.16	0.23	0.35
	4	n	9	4	3	4	6	9
		Range	423.1-1046.2	17.9-32.1	28.6-33.3	5.6-14.4	18.5-107.4	21.7-69.6
		Median	603.9	23.2	28.6	8.4	31.5	34.8
		Mean	641.0	24.1	30.2	9.2	41.4	42.0
		SD	185.7	6.1	2.7	3.7	33.1	18.1
		BFw	N/A	N/A	N/A	N/A	N/A	N/A
		BFs	10.86	0.41	0.51	0.16	0.70	0.71
	5	n	7	1	2	3	7	6
		Range	550.0-1411.5	64.3	47.6-71.4	7.8-22.2	25.9-81.5	26.1-108.7
		Median	746.2		59.5	13.3	48.2	76.1
		Mean	830.8		59.5	14.4	52.9	75.4
		SD	279.3		16.8	7.3	21.1	28.3
		BFw	N/A	N/A	N/A	N/A	N/A	N/A
		BFs	8.31	0.64	0.59	0.14	0.53	0.75
	7	n						4
		Range						43.5-82.6
		Median						47.8
		Mean	N/A	N/A	N/A	N/A	N/A	55.4
		SD						18.2
		BFw						N/A
		BFs						0.20

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 7.3 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Gut	Gut cont	Blood
Oct. '90	3	n[Φ]	7	2	1	2	6	7			
		Range	530.8-711.5	46.4-50.0	66.7	13.3-20.0	59.3-92.6	69.6-95.7			
		Median	596.2	48.2		16.7	70.4	78.3			
		Mean	595.6	48.2		16.7	72.8	80.7	N/A	N/A	N/A
		SD[Δ]	59.1	2.5		4.7	11.4	10.6			
		BFw	1323.6	107.1	148.2	37.1	161.8	179.3			
		BFs	18.61	1.51	2.08	0.52	2.27	2.52			
	4	n	10	1	7	6	9	10			
		Range	376.9-830.7	32.1	28.6-90.5	8.9-17.8	33.3-63.0	34.8-91.3			
		Median	580.8		57.1	13.9	37.0	50.0			
		Mean	580.8		55.1	13.3	42.8	56.1	N/A	N/A	N/A
		SD	131.4		21.8	3.1	10.7	19.5			
		BFw	1416.6	78.3	134.4	32.4	104.4	136.8			
		BFs	18.15	1.00	1.72	0.42	1.34	1.75			
	5	n	9	1	6	9	9	10			
		Range	546.2-1096.2	39.3	33.3-76.2	7.8-15.6	22.2-51.9	21.7-78.3			
		Median	715.4		35.7	10.0	40.7	58.7			
		Mean	762.0		44.4	10.9	39.9	51.7	N/A	N/A	N/A
		SD	171.8		17.2	2.5	9.6	17.2			
		BFw	3175.0	163.7	185.0	45.4	166.2	215.4			
		BFs	11.04	0.57	0.64	0.16	0.58	0.75			
	7	n	1	1		1	1	1			
		Range	676.9	66.7		22.2	63.0	95.7			
		Median									
		Mean			N/A				N/A	N/A	N/A
		SD									
		BFw	867.8	85.5		28.5	80.8	122.7			
		BFs	15.04	1.48		0.49	1.40	2.13			
Dec. '90	3	n	7	3	1	3	2	7			
		Range	692.3-969.2	28.6-60.7	33.3	7.8-10.0	29.6-40.7	30.4-47.8			
		Median	776.9	50.0		8.9	35.2	39.1			
		Mean	780.8	46.4		8.9	35.2	37.9	N/A	N/A	N/A
		SD	94.4	16.4		1.1	7.9	6.5			
		BFw	1859.0	110.5	79.3	21.2	83.8	90.2			
		BFs	70.98	4.22	3.03	0.81	3.20	3.45			
	5	n	1					1			
		Range	1234.6					60.9			
		Median									
		Mean		N/A	N/A	N/A	N/A		N/A	N/A	N/A
		SD									
		BFw	10288.3					507.5			
		BFs	32.49					1.60			
Feb. '91	5	n	1		1			1	1	1	1
		Range	751.9		19.3			18.9	66.6	47.0	4.0
		Median									
		Mean		N/A		N/A	N/A				
		SD									
		BFw	3007.6		77.2			75.6	266.4		16.0
		BFs	19.28		0.49			0.48	1.71		0.10
	7	n	2					2		1	6
		Range	860.7-1269.7					63.8-77.0		469.2	2.0-15.2
		Median	1065.2					70.4			7.6
		Mean	1065.2	N/A	N/A	N/A	N/A	70.4	N/A		7.9
		SD	289.2					9.4			4.4
		BFw	275.2					18.2			2.0
		BFs	19.02					1.26			0.14

Φ Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 7.3 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	Gut cont.	Vertebrae	Bile	Blood
Apr. '91	3	n®	3	4	1	1	3	6		2	1	3			8
		Range	439.0-502.9	6.3-17.8	16.6	34.8	13.5-24.5	12.4-23.9		10.3-10.9	15.5	30.0-56.4			1.0-5.1
		Median	454.5	13.7			15.4	14.1		10.6		40.8			4.5
		Mean	465.5	12.9			17.8	15.8	N/A	10.6		42.4	N/A	N/A	3.9
		SD^Δ	33.3	4.8			5.9	4.4		0.5		13.3			1.6
		BFw	1662.5	46.1	59.3	124.3	63.6	56.4		37.9	55.4				13.9
		BFs	5.61	0.16	0.20	0.42	0.21	0.19		0.13	0.19				0.05
	4	n	3		1	2	2	6		1		2			10
		Range	454.5-702.9		21.0	10.4-19.2	16.1-16.8	10.4-20.0		14.2		27.1-79.7			1.0-5.1
		Median	610.0			14.8	16.5	14.6				53.4			4.0
		Mean	589.1	N/A		14.8	16.5	14.2	N/A		N/A	53.4	N/A	N/A	3.9
		SD	125.5			6.2	0.5	3.5				37.3			1.2
		BFw	2805.2		100.0	70.5	78.6	67.6		67.6					18.6
		BFs	14.55		0.52	0.37	0.41	0.35		0.35					0.10
	5	n	4	5		3	3	7		3	2	3			10
		Range	447.2-816.2	3.5-16.1		2.7-49.9	11.1-28.9	13.7-131.2		9.7-23.6	21.6-24.6	38.3-232.1			1.0-5.1
		Median	578.5	7.8		10.5	18.8	22.7		11.5	23.1	46.4			4.5
		Mean	605.1	8.8	N/A	21.0	19.4	46.5	N/A	14.9	23.1	105.6	N/A	N/A	3.6
		SD	155.4	4.6		25.3	7.4	46.4		7.6	2.1	109.6			1.7
		BFw	3025.5	44.0		105.0	97.0	232.5		74.5	115.5				18.0
		BFs	17.80	0.26		0.62	0.57	1.37		0.44	0.68				0.11
	7	n	1		1	1	1	1				1			1
		Range	1266.4		11.7	12.2	56.1	37.9				525.2			12.1
		Median													
		Mean		N/A					N/A	N/A	N/A		N/A	N/A	
		SD													
		BFw	426.4		3.9	4.1	18.9	12.8							4.1
		BFs	5.32		0.05	0.05	0.24	0.16							0.05
June '91	3	n	9	3		9	9	9	9	8	2	8	9		9
		Range	281.8-490.2	5.8-14.0		0.3-9.5	2.9-45.0	3.5-45.0	1.8-25.8	5.2-27.8	29.6-32.5	55.4-475.0	255.6-784.6		1.0-5.1
		Median	331.6	8.0		3.0	11.4	10.5	6.9	12.2	31.1	188.7	672.9		3.0
		Mean	357.8	9.2	N/A	3.9	14.7	14.6	9.7	14.5	31.1	193.8	567.5	N/A	3.1
		SD	80.0	4.3		3.3	13.5	12.9	7.7	9.6	2.0	129.1	204.5		1.5
		BFw	1084.2	27.9		11.8	44.5	44.2	29.4	43.9	94.2		1719.7		9.4
		BFs	5.87	0.15		0.06	0.24	0.24	0.16	0.24	0.51		9.30		0.05
	4	n	7	1	6	6	7	7	4	7		7	7	1	7
		Range	339.7-575.5	7.7	3.2-34.8	1.3-6.4	2.9-12.9	9.3-26.9	7.9-27.6	8.6-19.5		108.3-300.0	336.1-1252.9	21.7	1.0-5.1
		Median	389.7		9.8	3.0	6.2	14.9	15.5	12.8		178.1	809.2		2.0
		Mean	439.2		15.4	3.2	6.3	15.6	16.6	13.6	N/A	197.0	766.0		2.9
		SD	93.1		13.2	1.8	3.3	5.8	8.5	4.5		71.1	285.2		1.8
		BFw	14640.0	256.7	513.3	106.7	210.0	520.0	553.3	453.3			25533.3	723.3	96.7
		BFs	17.57	0.31	0.62	0.13	0.25	0.62	0.66	0.54			30.64	0.87	0.12
	5	n	3		3	3	3	3	3	3	1	2	3		3
		Range	355.2-469.1		12.1-17.2	0.6-13.1	7.8-9.8	9.8-26.9	6.7-14.5	10.6-16.0	20.8	25.0-91.7	533.7-890.3		1.0-4.0
		Median	468.2		13.5	0.9	8.3	19.7	11.9	12.5		58.3	746.8		1.0
		Mean	430.8	N/A	14.3	4.9	8.7	18.8	11.0	13.0		58.3	723.6	N/A	2.0
		SD	65.5		2.7	7.1	1.0	8.6	4.0	2.7		47.1	179.4		1.7
		BFw	1595.6		53.0	18.1	32.2	69.6	40.7	48.1	77.0		2680.0		7.4
		BFs	8.70		0.29	0.10	0.18	0.38	0.22	0.26	0.42		14.62		0.04

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 7.3 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont	HGut cont	Vertebrae	Kidney	Bile	Blood
Aug. '91	3	n[®]	1	1		1	1		1	1				1			1
		Range	340.5	9.7		3.3	5.3		4.8	17.7				552.2			1.0
		Median															
		Mean			N/A			N/A			N/A	N/A	N/A		N/A	N/A	
		SD[Δ]															
		BFw	N/A	N/A		N/A	N/A		N/A	N/A				N/A			N/A
		BFs	9.46	0.27		0.09	0.15		0.13	0.49				15.34			0.03
	4	n	8	5		8	8	8	7	7		5	5	8	2		8
		Range	374.5-574.1	2.1-6.9		0.3-2.0	0.9-6.6	1.8-14.2	0.5-5.9	8.4-23.6		549.6-703.1	132.2-1325.6	459.5-1300.9	6.3-6.6		1.0-3.0
		Median	442.0	4.0		0.8	2.3	9.3	2.8	15.8		641.4	408.6	725.2	6.5		2.0
		Mean	458.1	3.9	N/A	0.8	2.7	8.0	2.8	15.8	N/A	627.7	618.9	778.4	6.5	N/A	1.8
		SD	71.2	1.9		0.6	1.7	4.4	1.6	6.1		62.0	520.4	280.4	0.2		0.7
		BFw	N/A	N/A		N/A	N/A	N/A	N/A	N/A				N/A	N/A		N/A
		BFs	9.96	0.08		0.02	0.06	0.17	0.06	0.34				16.92	0.14		0.04
	5	n	12	11	1	12	12	12	12	10	4	8	8	12	7	4	12
		Range	374.3-713.2	2.6-5.3	7.6	1.1-4.1	1.7-4.8	2.9-20.5	1.2-5.4	9.1-74.8	9.5-19.5	17.2-69.9	37.4-320.0	244.0-1694.9	4.9-29.0	3.1-8.9	1.0-3.0
		Median	500.8	3.9		2.8	2.2	9.2	2.9	15.6	12.8	37.6	85.0	781.9	16.8	5.8	2.0
		Mean	509.8	4.1		2.6	2.5	9.8	2.9	20.5	13.7	40.7	121.3	824.9	18.1	5.9	2.2
		SD	101.9	0.8		0.9	0.9	4.7	1.1	19.4	4.2	19.9	98.3	437.2	8.7	3.2	0.7
		BFw	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A			N/A	N/A	N/A	N/A
		BFs	11.20	0.09	0.17	0.06	0.05	0.22	0.06	0.45	0.30			18.13	0.40	0.13	0.05
	7	n	1			1	1	1	1	1				1		1	1
		Range	1360.1			8.1	15.0	20.4	27.4	25.3				2311.1		19.2	10.1
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A		N/A		
		SD															
		BFw	N/A			N/A	N/A	N/A	N/A	N/A				N/A		N/A	N/A
		BFs	17.44			0.10	0.19	0.26	0.35	0.32				29.63		0.25	0.13
Oct. '91	3	n	6	2	1	6	5	6	6	5	3	2	3	6	3	5	6
		Range	401.9-640.0	5.2-5.5	4.7	0.8-1.9	4.1-6.2	8.0-33.3	2.8-4.6	6.4-18.8	5.9-13.0	96.3-662.8	90.4-177.6	590.4-771.3	4.7-5.4	4.0-5.1	2.0-2.0
		Median	451.0	5.3		1.1	4.7	14.2	3.9	9.2	13.0	379.6	120.6	673.5	5.3	4.5	2.0
		Mean	504.0	5.3		1.3	5.0	16.0	3.9	10.5	10.6	379.6	129.5	678.2	5.1	4.5	2.0
		SD	106.7	0.2		0.5	0.8	9.3	0.8	5.0	4.0	400.6	44.3	58.1	0.4	0.4	0.0
		BFw	586.0	6.2	5.5	1.5	5.8	18.6	4.5	12.2	12.3			788.6	5.9	5.2	2.3
		BFs	31.50	0.33	0.29	0.08	0.31	1.00	0.24	0.66	0.66			42.39	0.32	0.28	0.13
	4	n	11	7	1	11	8	11	11	5	2	9	5	11	3	6	10
		Range	416.2-683.8	3.1-20.9	7.1	0.9-4.1	4.0-15.7	5.1-24.1	3.1-7.8	9.0-16.3	20.5-21.6	193.6-998.3	297.5-950.5	637.2-925.5	5.8-7.9	5.6-10.1	2.0-3.0
		Median	563.6	4.7		1.2	7.5	14.4	4.5	11.2	21.1	362.3	644.4	798.5	6.2	7.0	3.0
		Mean	555.0	8.3		1.6	8.4	14.0	4.7	11.6	21.1	521.4	610.9	791.3	6.6	7.2	2.6
		SD	64.8	7.0		1.0	4.0	6.2	1.3	3.0	0.8	294.5	288.3	73.4	1.1	1.7	0.5
		BFw	660.7	9.9	8.5	1.9	10.0	16.7	5.6	13.8	25.1			942.0	7.9	8.6	3.1
		BFs	21.76	0.33	0.28	0.06	0.33	0.55	0.18	0.45	0.83			31.03	0.26	0.28	0.10
	5	n	14	9	4	15	13	15	14	3	3	10	4	15	5	9	10
		Range	534.0-857.1	3.5-16.6	2.5-4.9	0.4-7.9	7.3-31.0	3.3-25.0	2.2-5.0	23.7-53.2	28.4-45.9	102.2-1120.9	355.7-454.5	654.2-1710.9	3.0-10.4	5.1-10.5	2.0-3.0
		Median	688.8	6.2	2.9	1.5	12.8	10.3	3.4	24.6	41.8	569.2	384.1	945.5	4.5	5.8	2.5
		Mean	685.5	8.6	3.3	2.2	14.1	12.9	3.5	33.8	38.7	581.0	394.6	958.0	5.4	6.6	2.5
		SD	95.5	4.7	1.1	1.9	6.1	7.3	0.8	16.8	9.1	320.8	43.1	237.3	2.9	1.8	0.5
		BFw	1384.8	17.4	6.7	4.4	28.5	26.1	7.1	68.3	78.2			1935.4	10.9	13.3	5.1
		BFs	11.06	0.14	0.05	0.04	0.23	0.21	0.06	0.55	0.62			15.45	0.09	0.11	0.04
	7	n	1			1	1	1	1	1				1	1	1	1
		Range	1343.8			13.1	9.2	54.4	22.0	20.7				2192.6	22.7	21.6	15.2
		Median															
		Mean		N/A	N/A						N/A	N/A	N/A				
		SD															
		BFw	652.3			6.4	4.5	26.4	10.7	10.0				1064.4	11.0	10.5	7.4
		BFs	25.35			0.25	0.17	1.03	0.42	0.39				41.37	0.43	0.41	0.29

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 7.3 (Continued)

Month	Locality		Gill	Gonad (F)	Gonad (M)	Fat	Liver	Muscle	Skin	Foregut	Hindgut	FGut cont.	HGut cont.	Vertebrae	Kidney	Bile	Blood
Jan. '92	3	n®	5	4		5	3	6	2	2	2	2	2	6	1	5	6
		Range	495.9-853.2	4.4-14.0		0.8-11.7	3.7-4.8	6.2-22.4	2.8-6.6	10.5-13.8	26.5-31.7	127.0-274.9	364.9-488.4	618.6-1256.0	6.4	3.4-24.5	1.0-2.0
		Median	536.8	8.7		5.9	4.0	14.3	4.7	12.1	29.1	201.0	426.6	827.1		6.7	2.0
		Mean	642.9	9.0	N/A	5.7	4.2	14.0	4.7	12.1	29.1	201.0	426.6	909.6		9.7	1.7
		SD^Δ	167.4	4.6		4.5	0.6	5.6	2.7	2.3	3.7	104.6	87.3	247.6		8.5	0.5
		BFw	1648.5	23.1		14.6	10.8	35.9	12.1	31.0	74.6			2332.3	16.4	24.9	4.4
		BFs	10.54	0.15		0.09	0.07	0.23	0.08	0.20	0.48			14.91	0.10	0.16	0.03
	4	n	4	3		10	2	11	3	1	1	1	3	9	1	2	11
		Range	479.0-805.7	3.3-4.9		1.1-6.9	3.3-5.2	5.3-17.3	3.0-7.8	5.7	12.3	494.4	233.8-396.1	621.8-1195.4	4.9	3.7-4.0	1.0-2.0
		Median	632.4	3.5		2.1	4.3	11.8	4.8				325.4	1039.5		3.9	1.0
		Mean	637.4	3.9	N/A	3.1	4.3	11.6	5.2				318.4	994.4		3.9	1.5
		SD	139.4	0.9		2.0	1.3	3.6	2.4				81.4	175.2		0.2	0.5
		BFw	685.4	4.2		3.3	4.6	12.5	5.6	6.1	13.2			1069.2	5.3	4.2	1.6
		BFs	10.20	0.06		0.05	0.07	0.19	0.08	0.09	0.20			15.91	0.08	0.06	0.02
	5	n	12	8	4	12	9	12	11	3	3	2	1	12	3	9	12
		Range	600.6-2115.7	2.9-11.3	2.4-6.2	0.4-8.7	3.8-11.0	7.5-37.0	1.6-5.5	7.4-10.5	15.7-24.3	127.8-671.7	127.1	608.8-1212.0	2.6-3.9	3.6-19.4	2.0-2.0
		Median	821.1	4.1	3.3	2.1	7.5	15.5	3.3	9.3	16.8	399.7		930.6	3.3	4.4	2.0
		Mean	944.5	5.2	3.8	2.3	7.6	17.0	3.5	9.0	19.0	399.7		967.1	3.2	6.6	2.0
		SD	424.4	2.9	1.7	2.2	2.6	8.8	1.2	1.6	4.7	384.6		165.5	0.7	5.1	0.0
		BFw	1657.0	9.1	6.7	4.0	13.3	29.8	6.1	15.8	33.3			1696.7	5.6	11.6	3.5
		BFs	14.64	0.08	0.06	0.04	0.12	0.26	0.05	0.14	0.29			14.99	0.05	0.10	0.03
	7	n	1			5		5		1				5			5
		Range	1463.4			3.8-15.3		17.2-45.4		43.3				1403.0-3924.9			2.0-4.0
		Median				8.8		25.5						2548.0			3.0
		Mean		N/A	N/A	8.8	N/A	29.8	N/A		N/A	N/A	N/A	2519.8	N/A	N/A	3.0
		SD				4.6		12.8						936.8			0.7
		BFw	668.2			4.0		13.6		19.8				1150.6			1.4
		BFs	2.37			0.01		0.05		0.07				4.08			0.005
Feb. '92	3	n	2			6		5	3					6			6
		Range	650.9-829.0			0.9-6.8		9.3-19.2	2.6-4.9					694.2-1201.8			2.0-3.0
		Median	739.9			3.4		15.7	3.3					814.7			2.0
		Mean	739.9	N/A	N/A	3.4	N/A	15.0	3.6	N/A	N/A	N/A	N/A	855.2	N/A	N/A	2.2
		SD	125.9			2.1		3.7	1.1					182.7			0.4
		BFw	N/A			N/A		N/A	N/A					N/A			N/A
		BFs	N/A			N/A		N/A	N/A					N/A			N/A
	4	n	6	4		10	7	10	8	1	1	1	1	10	1	9	10
		Range	448.4-890.8	2.6-9.5		0.5-6.4	2.6-14.0	5.4-25.8	3.1-11.3	5.3	6.6	211.4	211.3	717.3-1343.9	5.3	3.4-10.9	1.0-2.0
		Median	711.6	5.1		1.2	8.1	14.7	3.7					956.8		5.6	1.0
		Mean	674.8	5.6	N/A	2.0	8.2	14.6	4.5					962.5		6.2	1.3
		SD	178.4	3.4		1.9	4.8	7.1	2.8					215.0		2.6	0.5
		BFw	N/A	N/A		N/A	N/A	N/A	N/A	N/A	N/A			N/A	N/A	N/A	N/A
		BFs	N/A	N/A		N/A	N/A	N/A	N/A	N/A	N/A			N/A	N/A	N/A	N/A
	5	n	10	10		10	8	10	10	2	2			10	2	10	10
		Range	678.9-1201.1	3.4-12.7		0.5-7.7	4.5-10.8	2.3-29.3	2.6-11.4	9.0-10.1	16.8-17.8			781.9-1527.8	4.7-4.8	4.3-12.4	2.0-3.0
		Median	821.7	7.1		1.0	5.6	8.4	3.5	9.5	17.3			1241.6	4.8	7.7	3.0
		Mean	860.3	7.1	N/A	1.8	6.7	9.3	4.4	9.5	17.3	N/A	N/A	1176.7	4.8	8.0	2.6
		SD	168.1	2.8		2.2	2.2	8.1	2.6	0.8	0.7			253.7	0.1	2.5	0.5
		BFw	N/A	N/A		N/A	N/A	N/A	N/A	N/A	N/A			N/A	N/A	N/A	N/A
		BFs	N/A	N/A		N/A	N/A	N/A	N/A	N/A	N/A			N/A	N/A	N/A	N/A
	Pionier Dam	n	10	5	5	10	9	10	10	6	2	6		10	4	5	10
		Range	262.0-431.6	2.1-3.3	2.1-4.0	0.4-3.0	1.1-3.8	0.8-5.8	1.0-3.3	3.5-6.5	6.5-8.7	9.1-25.3		279.9-522.5	3.9-5.6	2.0-3.4	1.0-2.0
		Median	371.9	2.9	2.3	1.2	1.5	1.4	1.7	4.5	7.6	18.1		439.5	5.4	2.7	1.5
		Mean	357.7	2.9	2.6	1.5	1.7	2.2	1.9	4.7	7.6	17.7	N/A	413.0	5.1	2.6	1.5
		SD	58.6	0.5	0.8	0.9	0.8	1.8	0.7	1.1	1.5	5.6		81.2	0.8	0.6	0.5
		BFw	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A			N/A	N/A	N/A	N/A
		BFs	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A			N/A	N/A	N/A	N/A

® Number of samples analyzed Δ Standard deviation N/A Not available

TABLE 7.4

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE MANGANESE 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	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	S2												
Gonad (Males)	S2												
Fat	S2												
Liver	S2			S2									
Muscle	S2				S2								
Skin	S2				S2								
Gut													
Gut cont.	W2, SP2, S2	W2, SP2	W2	W2, SP2, S2	W2, SP2, S2	W2, SP2	W2, 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			

TABLE 7.5

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE LEAD 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	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	SP2, S2												
Gonad (Males)													
Fat	SP2, S2												
Liver	S2												
Muscle	SP2, S2												
Skin	SP2, S2												
Gut													
Gut cont.	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			

TABLE 7.6

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE 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	Gonad (Females)	Gonad (Males)	Fat	Liver	Muscle	Skin	Gut	Gut cont.	Vertebrae	Kidney	Bile	Blood
Gill													
Gonad (Females)	W2, SP2, S2												
Gonad (Males)	W2, SP2, S2												
Fat	W2, SP2, S2												
Liver	W2, SP2, S2												
Muscle	W2, SP2, S2												
Skin	W2, SP2, S2												
Gut	W2, SP2, S2												
Gut cont.	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	W2, 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	W2, SP2, S2								W2, SP2	W2, SP2, S2			

in December 1990) to 12.47 (calculated for the gills in October 1991) (Table 7.2). Strontium BF_w values ranged from 1.4 (calculated for blood in January 1992) to 25533.3 (calculated for the vertebrae in June 1991), while the BF_s values ranged from 0.005 (calculated for blood in January 1992) to 70.98 (calculated for the gills in December 1990) (Table 7.3).

LOCALITY DIFFERENCES

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

In October 1990 (the first year) locality 7 differed significantly from localities 3 (with respect to the gill, liver and muscle manganese concentrations), 4 (with respect to the muscle manganese concentrations) and 5 (with respect to the gill and muscle manganese concentrations). Lead concentrations detected at locality 5 differed significantly from those at localities 3 (with respect to the liver) and 4 (with respect to the gill, liver and muscle), while strontium concentrations detected at locality 3 differed significantly from those at localities 4 (with respect to the liver) and 5 (with respect to the muscle and liver) in October 1990. In June 1991 (the second year) locality 4 differed significantly from localities 3 and 5 with respect to the manganese concentrations in the muscle tissue, as well as the lead concentrations in the muscle and fat tissues. Locality 5 differed significantly from locality 3 in October 1991 with respect to the lead concentrations in the fat and the strontium concentrations in the vertebrae. In January 1992 locality 7 differed significantly from localities 3, 4 and 5 with respect to the strontium concentrations in the blood and vertebrae and the manganese concentrations in the fat, but it only differed significantly from locality 5 with respect to the lead concentrations in the vertebrae. Locality 5 differed significantly from locality 4 with respect to the muscle and vertebrae lead concentrations in January 1992, the muscle and blood manganese concentrations in February 1992 and the blood strontium concentrations in January and February 1992. Furthermore in February 1992, locality 3 differed significantly from localities 4 (with respect to the lead and strontium concentrations in the vertebrae and blood respectively) and 5 (with respect to the lead and strontium concentrations in the vertebrae). The Pionier Dam differed significantly from locality 3 with respect to the lead concentrations in the vertebrae, as well as the strontium concentrations in the fat, muscle, vertebrae and blood. It also differed significantly from locality 4 with respect to the manganese concentrations in the muscle and blood, as well as the strontium concentrations in the muscle and vertebrae, and from locality 5 with respect to the manganese concentrations in the fat, vertebrae and blood, as well as the strontium concentrations in the muscle, vertebrae and blood.

SEASONAL DIFFERENCES

Significant seasonal differences ($p \leq 0.05$) with regard to the mean manganese, lead and strontium concentrations in various organs were detected, but no distinguished trend could be established. 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 7.7. Nearly all the seasons differed from each other with respect to the mean lead concentrations detected in various organs (Table 7.8), but not with respect to the mean strontium concentrations. The seasonal differences regarding the mean strontium concentrations are indicated in Table 7.9.

The mean seasonal manganese, lead and strontium concentrations, as determined separately for male and female organs and tissues, are indicated in Figures 7.1 to 7.6. 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 (Figures 7.1 and 7.3).

TABLE 7.7
SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN MANGANESE 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 SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

	Autumn 1990	Winter 1990	Spring 1990	Summer 1990/91	Autumn 1991	Winter 1991	Spring 1991	Summer 1992
Autumn 1990	Female →	G, M	G, M	G	M	G, M	G, M	G, M
	Male →							
Winter 1990		Female →		G		M	M	M
		Male →		G				
Spring 1990			Female →	G		M	M	M
			Male →	G		M	M	M
Summer 1990/91	G*	G*	G*	Female →	M	G, M	G, M	G, M
				Male →		G	G	G
Autumn 1991	M*		M*	M*	Female →	B	M	M
					Male →			
Winter 1991	G*,M*	M*	G*,M*	G*,M*	B*	Female →	B	B, S
						Male →		
Spring 1991	G*,M*	M*	M*	G*,M*		S*,B*	Female →	
							Male →	
Summer 1992	G*,M*	M*	G*,M*	G*,M*		S*,B*	B*	

TABLE 7.8
SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN LEAD 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 SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

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

TABLE 7.9

SUMMARY OF STATISTICAL DIFFERENCES ($P \leq 0.05$) BETWEEN THE VARIOUS SEASONS WITH RESPECT TO THE MEAN 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 SEPERATELY. (BLANK SPACES INDICATE NO SIGNIFICANT DIFFERENCE)

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

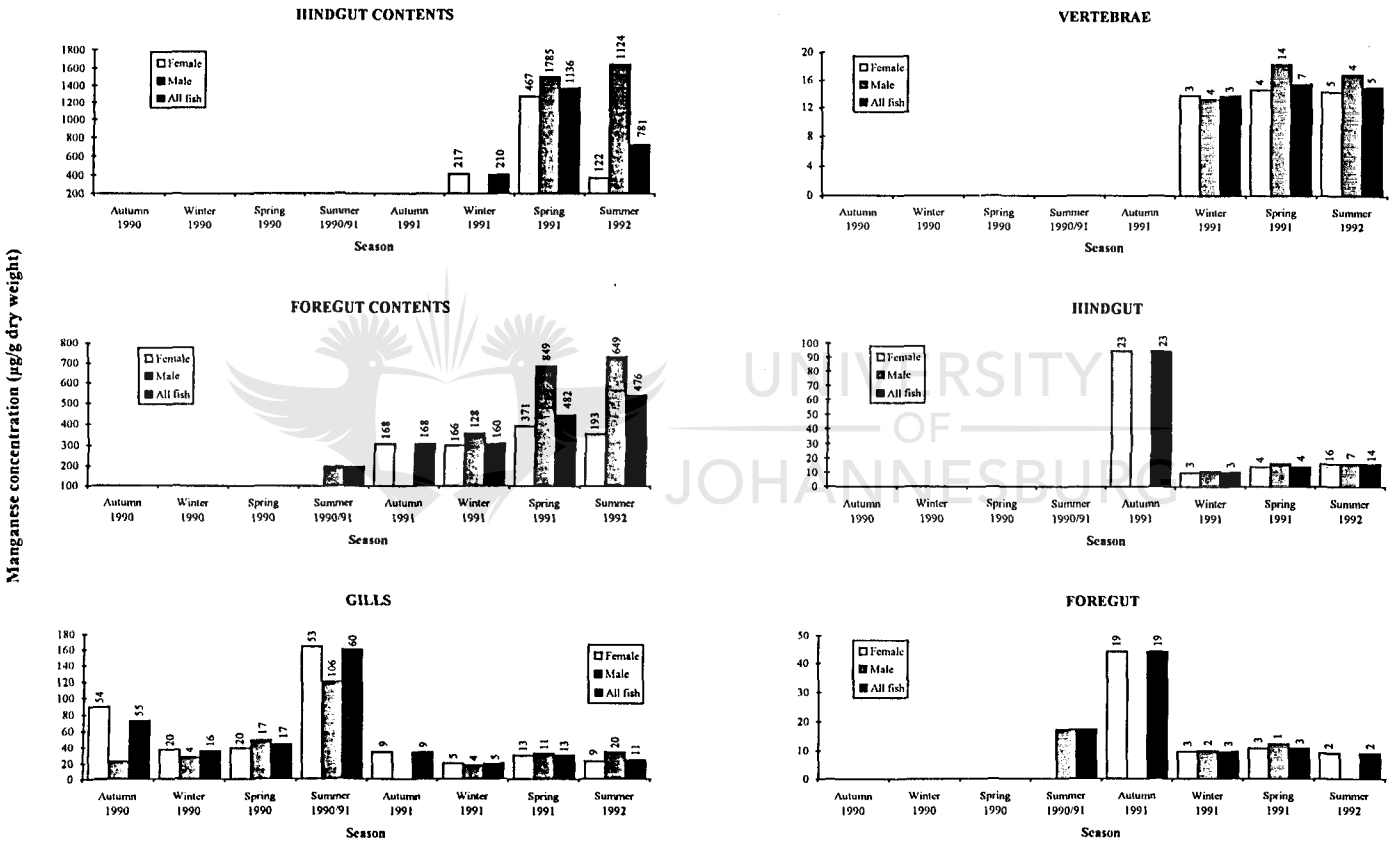

Figure 7.1

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

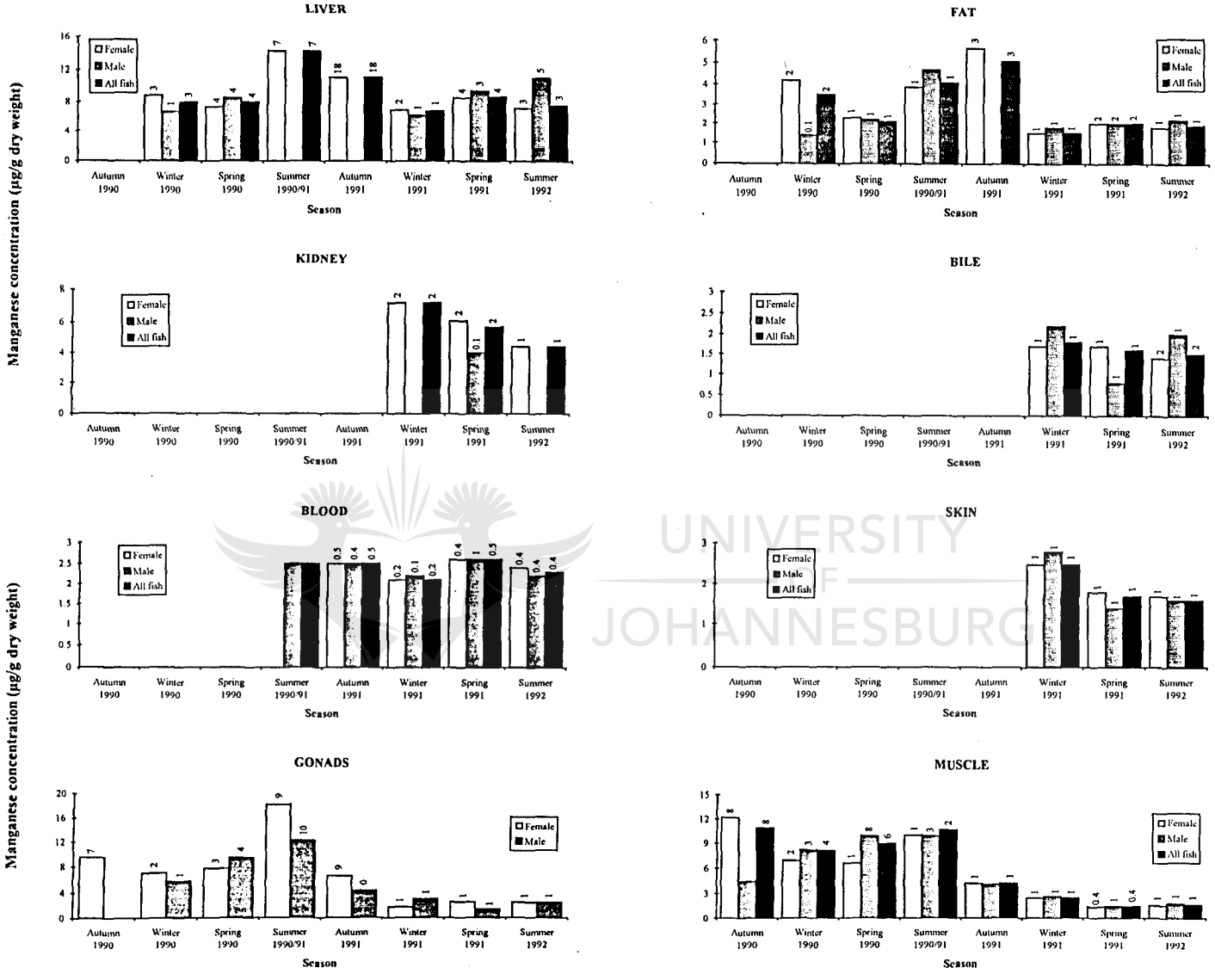

Figure 7.2

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

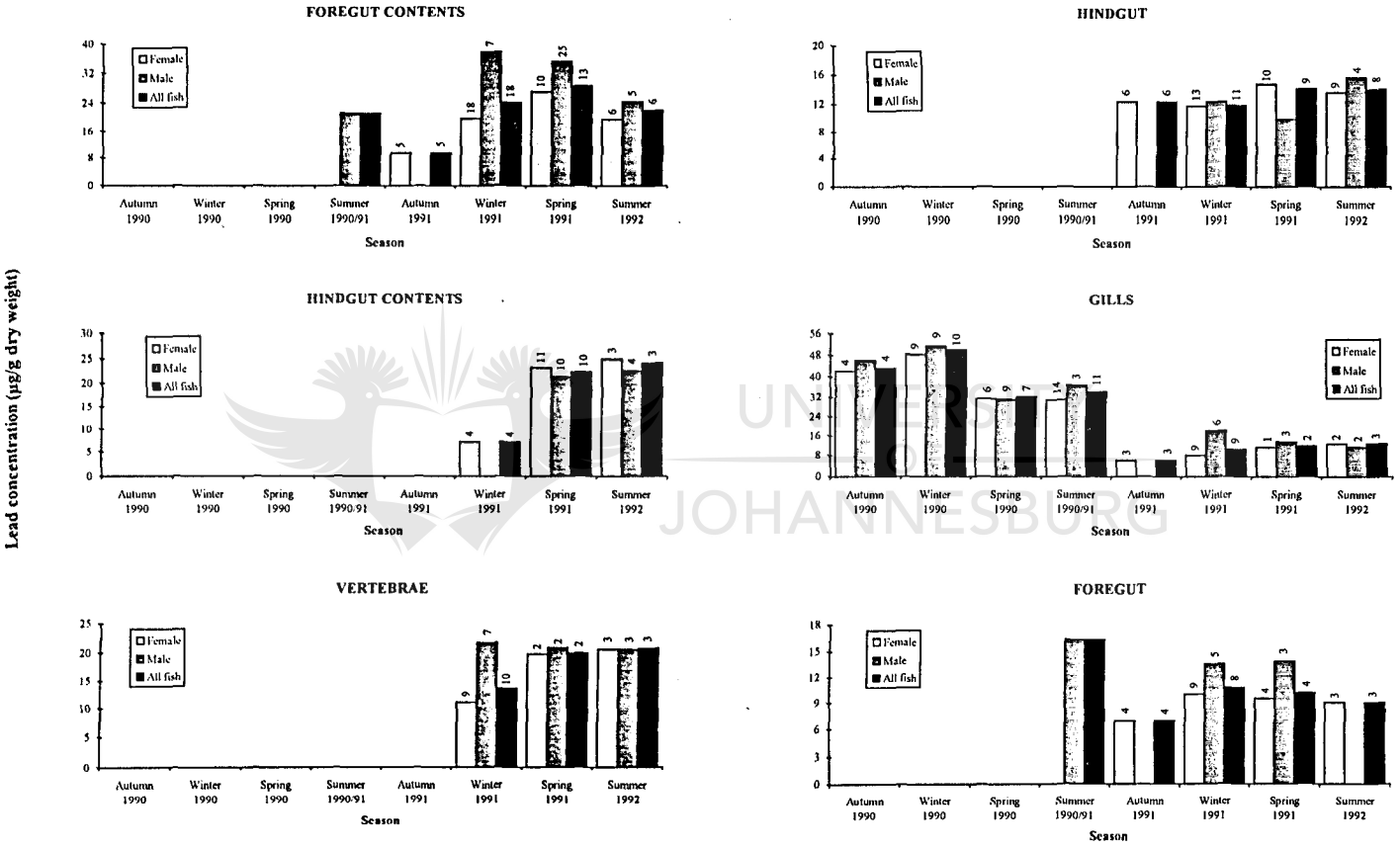

Figure 7.3

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

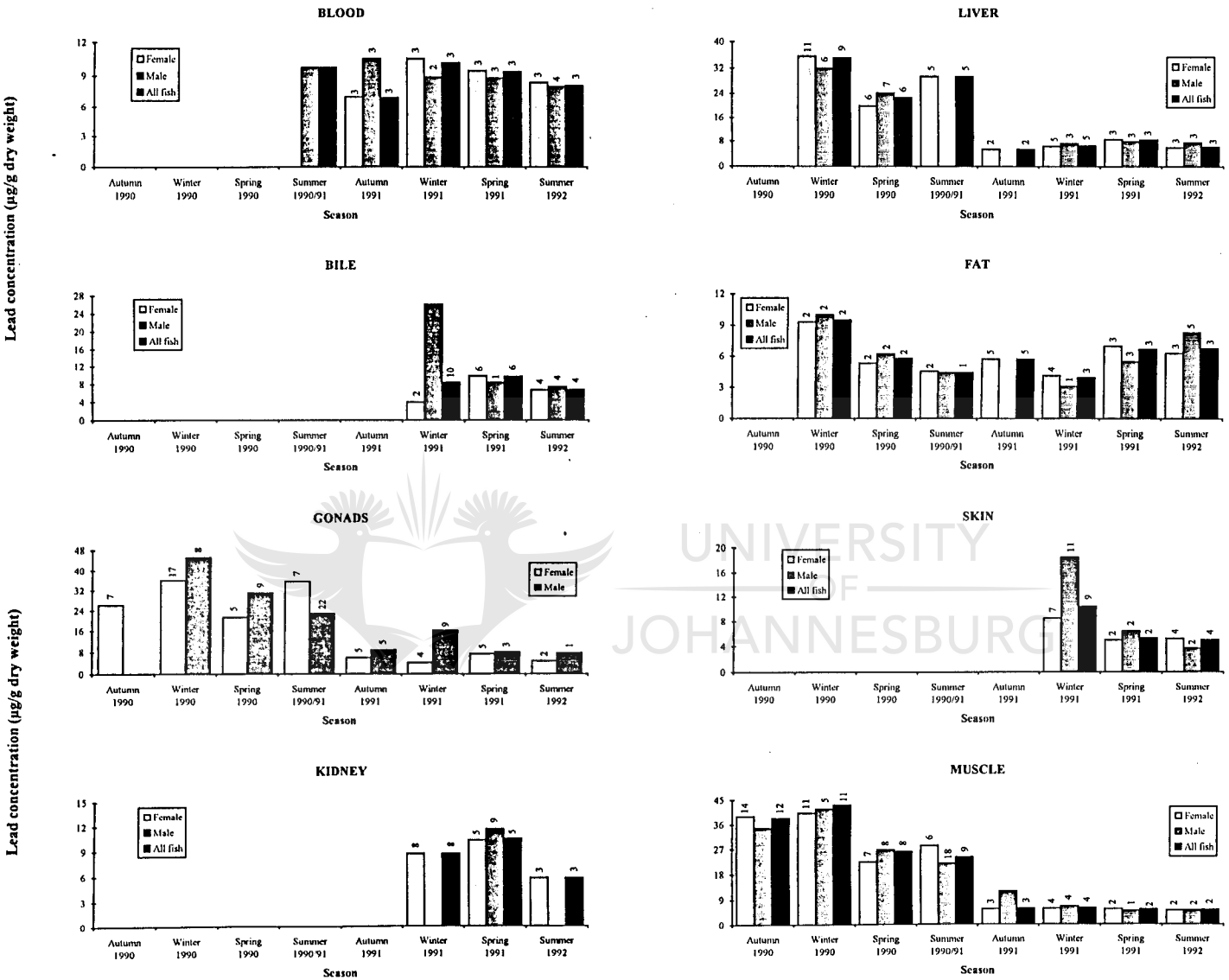

Figure 7.4

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

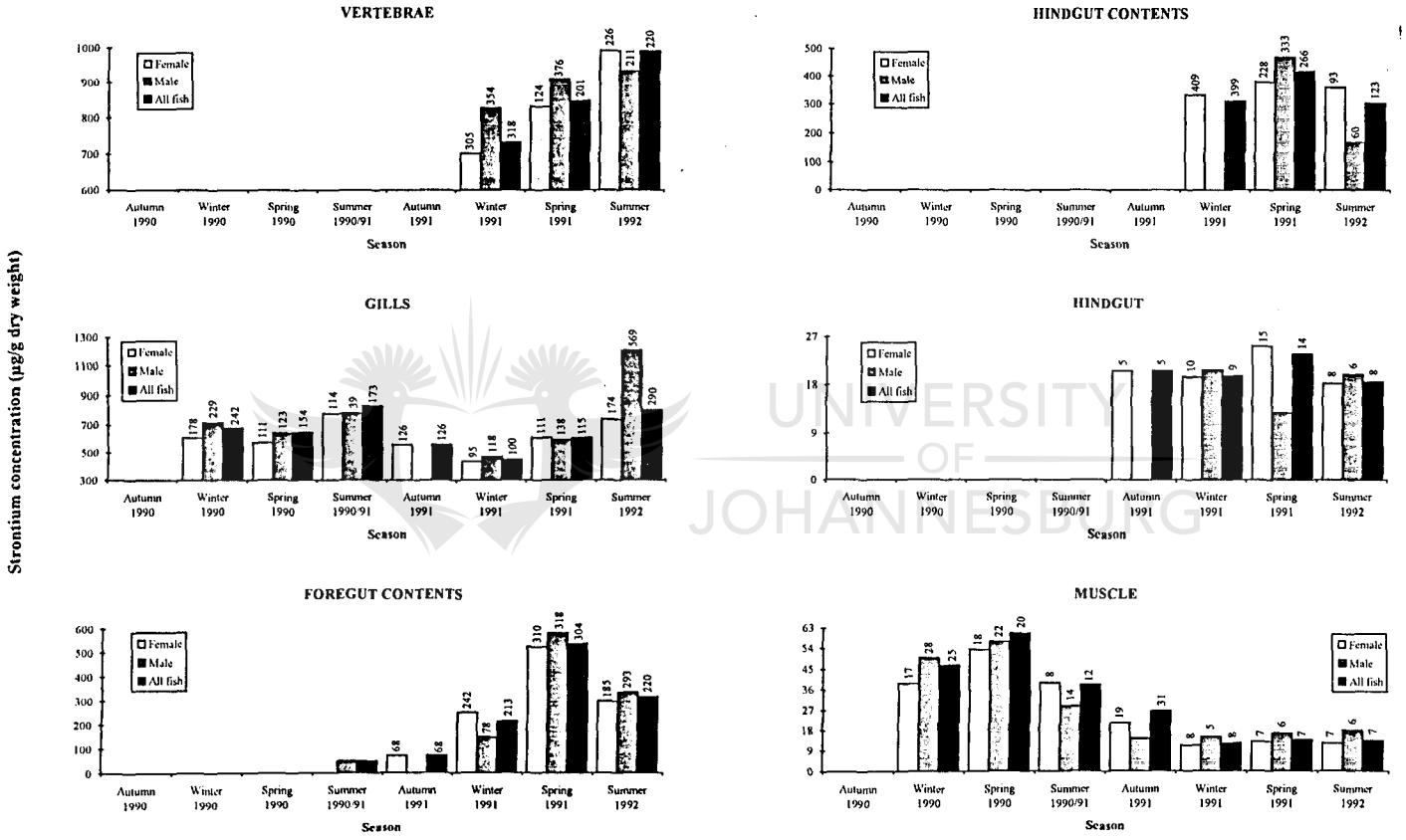

Figure 7.5

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

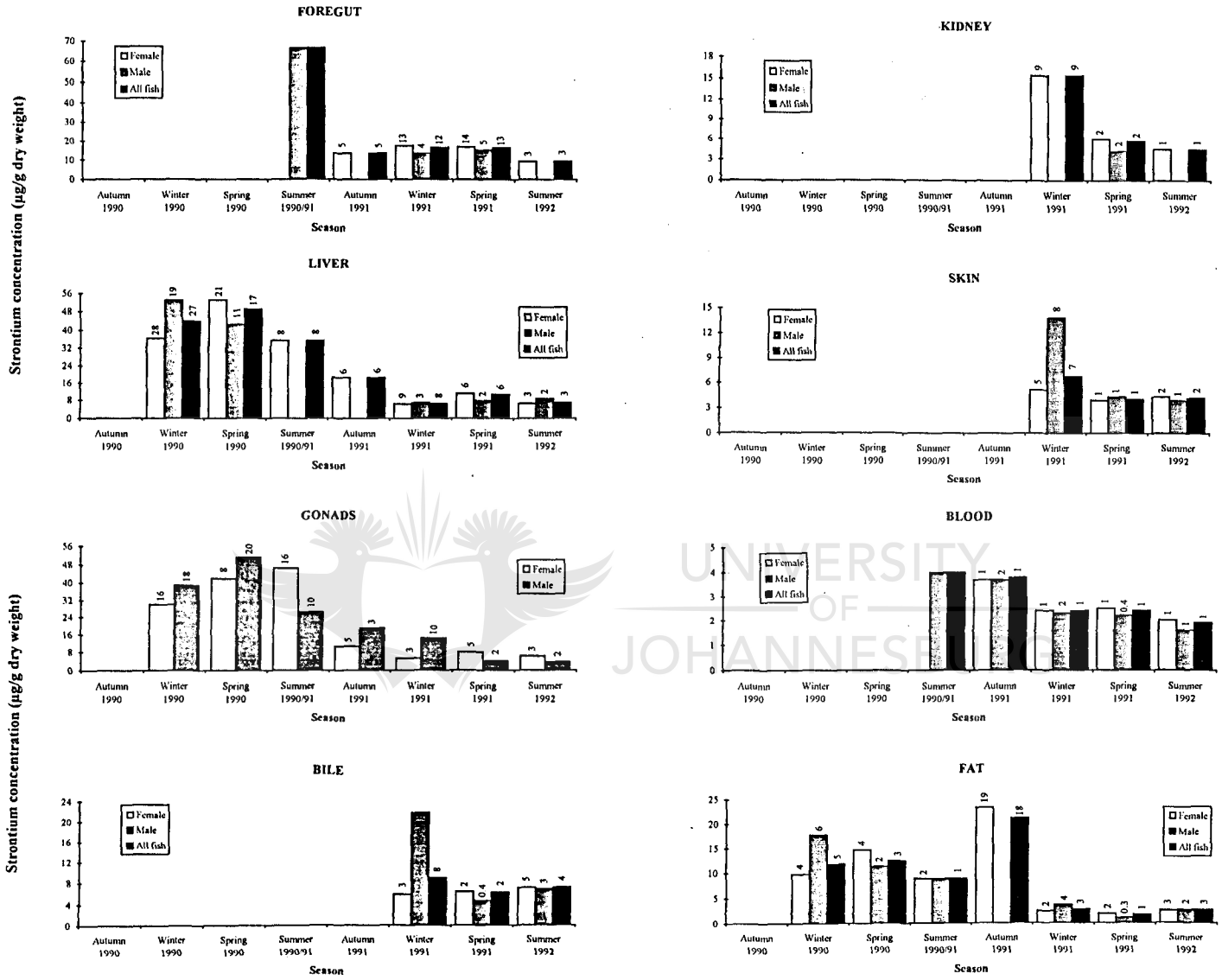

Figure 7.6

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

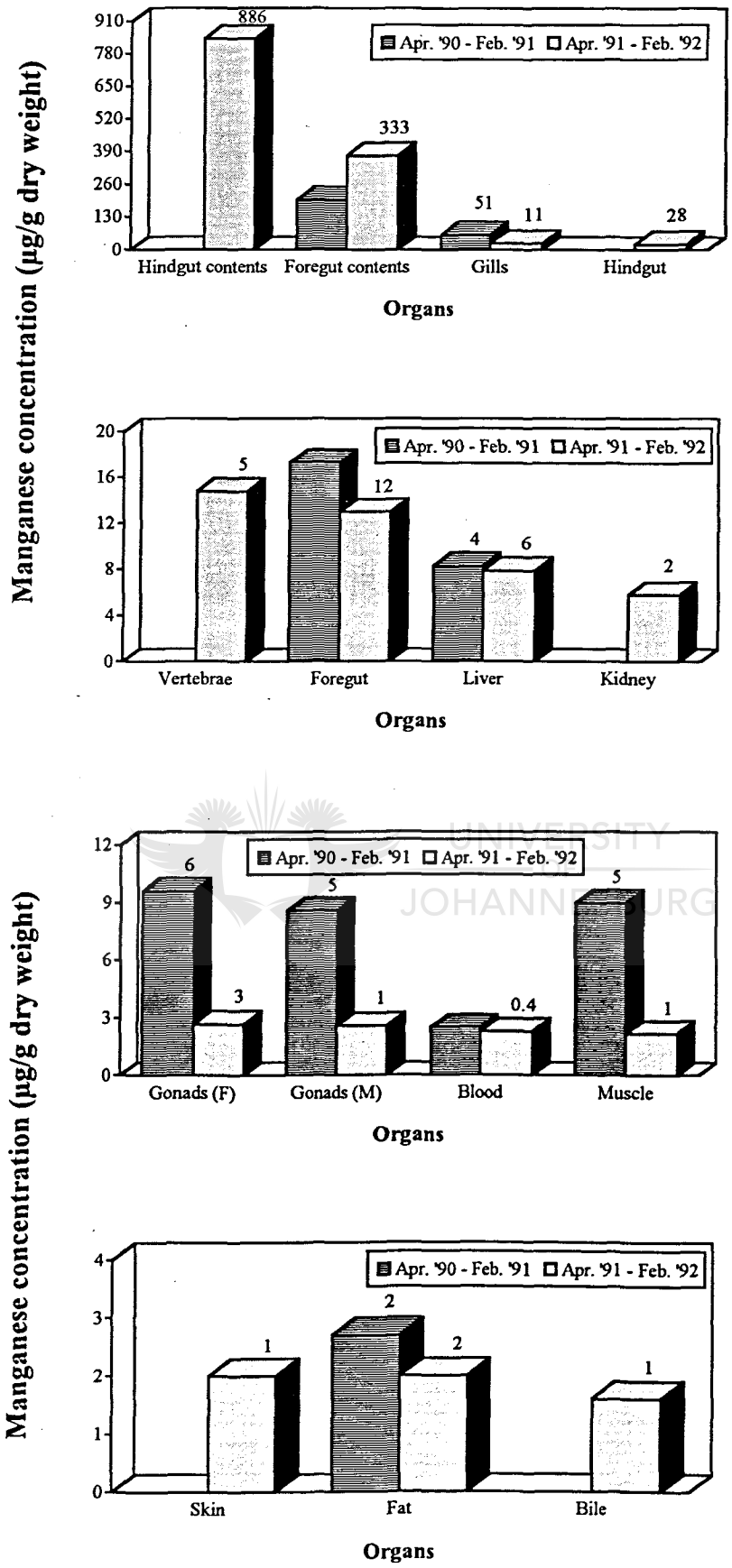

Figure 7.7

Mean manganese 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)*

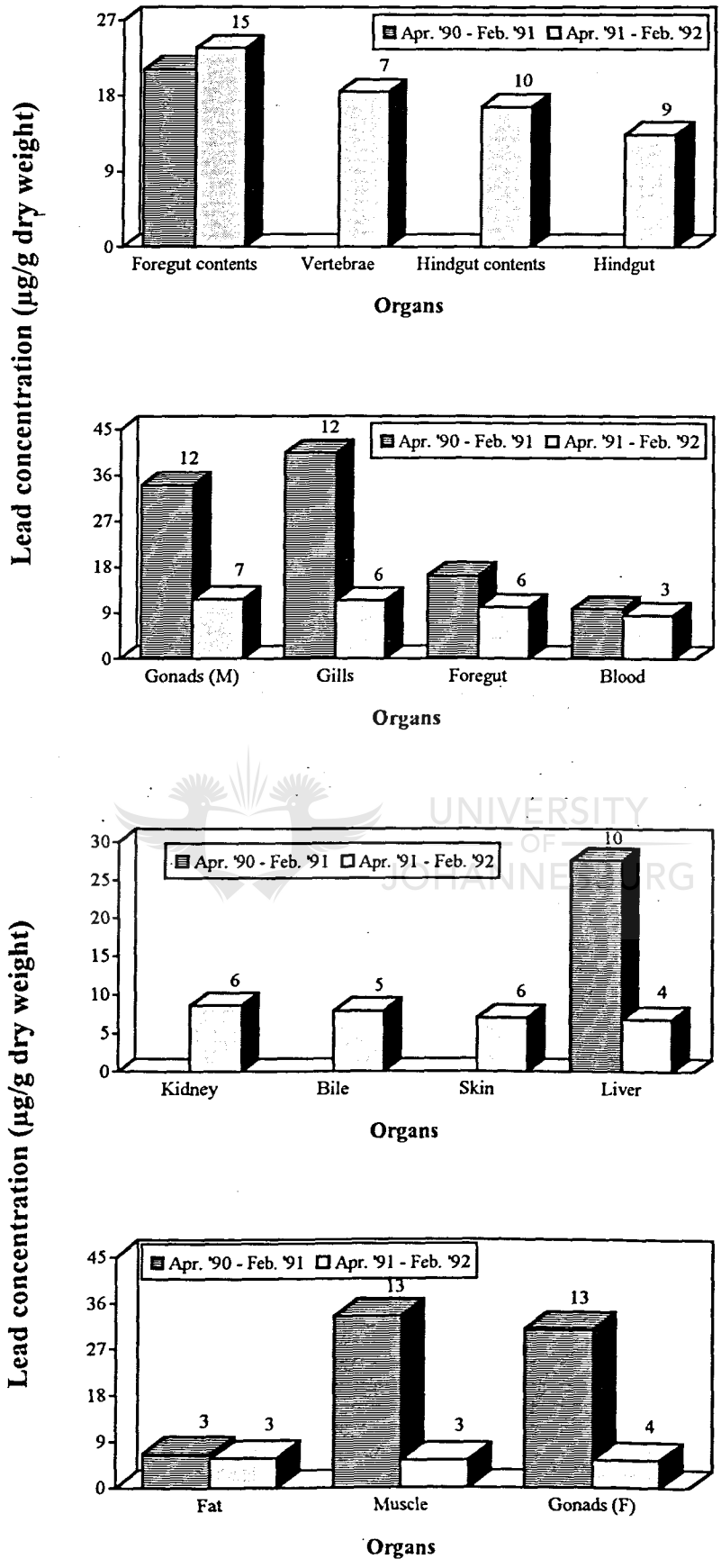

Figure 7.8
Mean lead concentrations (µg/g dry wt) for the two years in the different organs and tissues of Barbus marequensis. *(Standard deviations are indicated above each bar)*

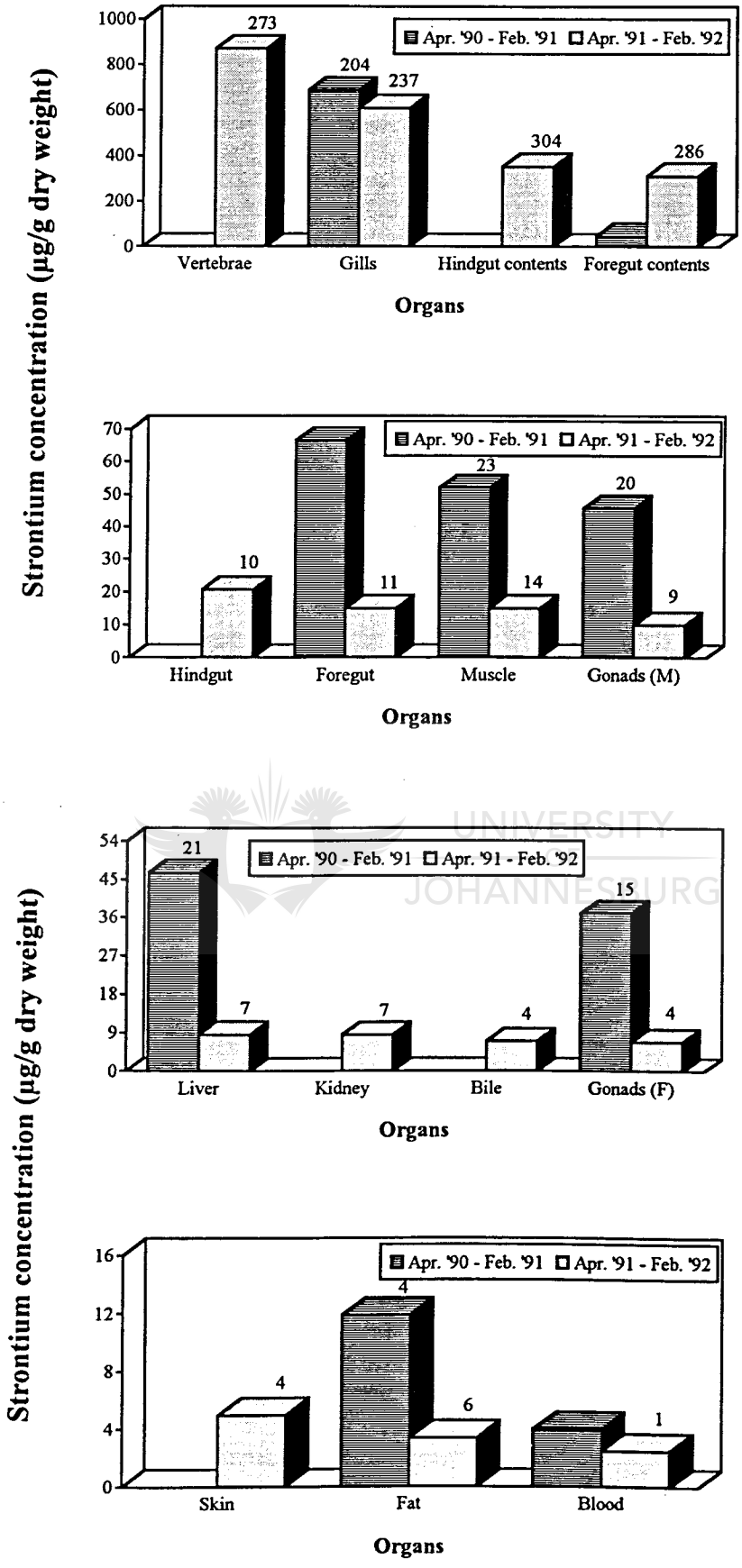

Figure 7.9

Mean strontium 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)*

ANNUAL DIFFERENCES

The first and second year differed significantly ($p \leq 0.05$) with respect to the manganese concentrations in the gills, muscle and gonads (Fig. 7.7); and also with respect to the lead and strontium concentrations in the gills, liver, muscle and gonads (Figures 7.8 and 7.9). Using the mean manganese, lead and strontium concentrations detected in the fish organs during the second year (Figures 7.7 - 7.9), the order of metal accumulation in *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 > female gonads ≈ male gonads > blood ≈ muscle > skin ≈ fat > bile; for lead, foregut contents > vertebrae > hindgut contents > hindgut > male gonads > gills > foregut > blood ≈ kidney > bile > skin > liver > fat > muscle ≈ female gonads; and for strontium, vertebrae > gills > hindgut contents > foregut contents > hindgut > foregut ≈ muscle > male gonads > liver ≈ kidney > bile ≈ female gonads > skin > fat > blood.

7.4 Discussion

BIOACCUMULATION OF MANGANESE, LEAD AND STRONTIUM IN THE DIFFERENT ORGANS AND TISSUES

The uptake and excretion of metals by fish is a subject of interest to many researchers, but little is known about the exact routes of these processes 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 concentrations of dissolved metals *via* the gills (Bendell-Young & Harvey, 1986; Hodson *et al.*, 1978; Carraça *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 resorption of these two metals occurs through the gut from the food (Katz *et al.*, 1972). These were also the findings in the present study, because higher manganese and strontium concentrations were detected in the gills than in the gut (Tables 7.1 and 7.3). It has been demonstrated, though, that water-borne lead was readily taken up by fish resulting in subtle sub-lethal 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, they 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 & Wiener, 1991). Lead concentrations were very similar in the gills and in the gut of *B. marequensis* (Table 7.2), 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 of 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 & 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* (Table 7.1) suggested urinary excretion of manganese rather than biliary excretion. On the other hand, excretion of lead and strontium seemed to be biliary and urinary (Tables 7.2 and 7.3), although 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* (Tables 7.1-7.3) 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 mineralization process (Guggenheim & 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 resemblances to calcium (Moore & Ramamoorthy, 1984; Phillips & Russo, 1978). The retention of strontium can be sufficiently long, because it interchanges with calcium (Radtke, 1989). Older fish will therefore have higher strontium concentrations in their bony tissues than the younger ones. This might explain the

large variation that was detected in the vertebrae strontium concentrations of *B. marequensis* (Table 7.3), for the age of the fish that were caught during the study varied from one to six years (see Chapter 4). Scales have also been reported to be major storage sites of manganese, lead and strontium (Sauer & Watabe, 1989). Bony tissues of fish (e.g. vertebrae, scales and opercular bone) will therefore be good indicators of sub-lethal manganese, lead and strontium exposures.

Other tissues in *B. marequensis* also accumulated manganese, lead and strontium, although to a much lesser degree than the skeletal tissues (Tables 7.1-7.3). 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 (Table 7.3), which would probably render this tissue a good indicator of strontium exposure. Lead concentrations in the muscle differed only slightly from the lead concentrations in some other tissues, such as the liver (Table 7.2). 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 & Ramamoorthy, 1984). In the first year the muscle lead concentrations ranged from 13 to 56.5 µg/g Pb dry weight (Table 7.2), exceeding the maximum allowable concentration of lead in fish flesh, which is 2 µg/g Pb wet weight or 8 µg/g Pb dry weight (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, as is evident from Table 3.4 (Chapter 3), and these were probably sub-lethal concentrations. No "normal" or allowable values are available for manganese and strontium concentrations in fish flesh. The detected concentrations of these two metals in the muscle tissues during the first year were, however, also higher than the muscle concentrations in the second year (Tables 7.1 and 7.3). Fish were therefore exposed to higher manganese and strontium concentrations in the first year, which is also evident from Table 3.4 (Chapter 3).

The manganese and lead BFs recorded for *Barbus marequensis* in October 1990 at locality 3 in this study, were mostly higher than the manganese and lead BFs recorded for *Hydrocynus vittatus* in October 1990 at the same locality (Du Preez & Steyn, 1992), which ranged from 28.9 to 156.6 and 20.7 to 41.4 respectively. It was only the BFs 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 BFs recorded for *H. vittatus*. It is important to remember, however, that the BFs for *H. vittatus* were calculated on a wet weight basis, while the BFs for *B. marequensis* were calculated on a dry weight 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 Olifants River (*B. marequensis*) did, however, accumulate more manganese in their organs than *C. gariepinus* did and the average water manganese concentration at locality 7 (229.5 ± 2.1 µg/l Mn) was, in fact, higher than the average manganese concentration at Germiston lake (35.6 ± 31.0 µg/l Mn). This proves the Selati River to be more polluted with manganese than Germiston lake. In general, *B. marequensis* accumulated more manganese in their gut than *C. gariepinus* did. This suggests that conditions in the Olifants River 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 that much 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, accumulate the lowest strontium levels (Table 7.3). 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 (Table 2.4 in Chapter 2). Indications are, therefore, that manganese and strontium originated from a source close to locality 7, which was not connected to the KNP.

SEASONAL DIFFERENCES

The high manganese concentrations in the organs of *B. marequensis* during the summer of 1990/91 (Figures 7.1 and 7.2) might have been due to the heavy rainfall in December 1990. Under high rainfall conditions, leaching is more pronounced and systems usually have lower pH values (Hahne & 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 (Figures 7.3 to 7.6), but were rather mediated by the lead and strontium concentrations in the water (Table 3.2 in Chapter 3). The seasonal trend regarding manganese accumulation in the gonads (Fig. 7.2) was similar to that of iron (Fig. 5.4 in Chapter 5), lead accumulation (Fig. 7.4) was similar to that of chromium (Fig 6.2 in Chapter 6) and strontium accumulation (Fig. 7.6) was similar to that of copper and iron in the gonads (Figures 5.2 and 5.4 in Chapter 5). 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 (as was the case with zinc in Chapter 4). Strontium has, however, been reported to increase in concentration in the ovary 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 was observed to decrease significantly during the sexual maturation of *O. mykiss*.

Seasonal differences that occurred between the males and females in the accumulation of manganese, lead and strontium in their organs were such that no definite pattern could be established to relate the differences to processes taking place in the bodies of the fish. 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 genders at times.

ANNUAL DIFFERENCES

As mentioned before, 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 than in the second year (Table 3.2 in Chapter 3), more manganese, lead and strontium were accumulated by *B. marequensis* in the first year (Figures 7.7 to 7.9). It was only the gut contents that did not necessarily accumulate higher manganese, lead and strontium levels in the first year (Figures 7.7 to 7.9), for there would be no direct relation between the gut contents concentrations and the water concentrations.

7.5 Conclusion

Barbus marequensis bioaccumulated 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 high strontium levels. Sub-lethal and lethal levels of strontium to fish are, however, not known, because strontium is regarded as a non-toxic metal and, therefore, limited research is being done on this metal. The detected lead and manganese concentrations in the fish organs suggested no serious lead and manganese pollution problem in the study area, although the fish did seem to have been chronically exposed to sub-lethal lead concentrations in the first year. In addition, the fish at locality 7 might have been exposed to sub-lethal manganese concentrations. The source of these metals needs to be identified in future monitoring programmes and, if necessary, measures should be taken in order to reduce the levels thereof. 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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CHAPTER 8

Chapter 8

ACUTE TOXICITY TEST OF MANGANESE ON JUVENILE *OREOCHROMIS MOSSAMBICUS*

8.1 Introduction

A number of chemical substances in industrial, agricultural and domestic effluents, as well as in effluents resulting from mining activities, are likely to contaminate watercourses. These toxicants have a definite effect on all aquatic life, but it is not always known at what concentrations effects will start to occur and to what degree the aquatic life will be affected. It is therefore essential to determine the toxicity of a substance, in order to derive water quality standards. The first step in determining the toxicity of a substance, as well as the adverse effects it will have on aquatic life, is to perform acute toxicity tests, especially when time is of the essence. Chronic tests are the second step and provide a reference point closer to the actual no-effect level at the ecosystem level (Van Leeuwen, 1988a).

Acute toxicity can be defined as the severe effects suffered by organisms from short-term exposure to toxic chemicals (Van Leeuwen, 1988a). Usually the objective of such a test is to determine the median lethal concentration (LC50), which is defined as the concentration of the test material that will kill or immobilise 50% of the test organisms in a predetermined length of time - usually 24 to 96 hours (Rand & Petrocelli, 1985). The criteria for death in such a test are usually lack of movement (especially of the operculum) and lack of reaction to gentle prodding (Parrish, 1985). The incipient LC50 (the point at which the toxicity curve becomes asymptotic to the time axis) is the concentration at which 50% of the test population can live for an indefinite time, or the lethal concentration for 50% of the test organisms in long-term exposure (Rand & Petrocelli, 1985). The quotient of the incipient LC50 and the LC50 values is used as a safety factor in order to determine "acceptable" toxicant levels in the natural environment (Van Leeuwen, 1990). These safety factors (also called application or extrapolation factors) can also be used to estimate the incipient LC50 value of species A if the value is known for species B, providing the lethal dose for species A is known (Hellawell, 1986).

Chronic tests extend over longer periods than acute tests and often involve life-cycle toxicity tests. The objective of chronic toxicity testing is to determine if prolonged exposure to the concentrations of a chemical expected to be present in the aquatic environment, will have significant adverse effects on aquatic ecosystems (Van Leeuwen, 1988b). Apart from lethality, chronic toxicity studies comprise endpoints like individual growth, abnormal development, hatching time, reproduction, and behavioural aspects. Statistical analyses of these data then determine the lowest tested concentration of which the mean response significantly differs from the control (Van Leeuwen, 1988c).

Manganese is considered to be of moderate toxicity to aquatic life (Kempster *et al.*, 1982). High manganese concentrations can, however, have toxic effects on fish such as altered liver glycogen and blood glucose levels (Nath & Kumar, 1987). Nevertheless, it is evident from the literature that relatively few studies on the lethal and sub-lethal effects of manganese on fish have been undertaken. The objective of this experiment was therefore to determine the LC50 and incipient LC50 values of Mn for juvenile *Oreochromis mossambicus*, a fish species indigenous to south-east Africa which is

widely distributed from the lower Zambezi system southwards to the Bushmans river of the eastern Cape in South Africa (Bruton *et al.*, 1982). *Oreochromis mossambicus* was used as a test organism instead of *B. marequensis*, because *O. mossambicus* is more easily kept in the laboratory than *B. marequensis*, it occurs in the Olifants River system and it is a fish species widely used in South African experimental work, making direct comparison of results easier.

8.2 Materials and methods

Juvenile *Oreochromis mossambicus* were obtained from a hatchery in the Brits district of the Transvaal province, South Africa. At the aquarium they were kept in a recirculating system, consisting mainly of a 1000 litre reservoir and a biological filter. Borehole water circulated from the reservoir through the biological filter and was pumped back again to the reservoir. On arrival, the fish underwent a week-long infection treatment, by daily dissolving two handfuls of coarse salt and one teaspoon per seven kilograms of body mass Terravit (a pfizer antibiotic product) in the water. The healthy fish were then allowed to acclimatise in the recirculating system to borehole water with physico-chemical characteristics as given in Table 8.1 for three months. During this period they were fed daily on commercial trout pellets with a 50% protein content.

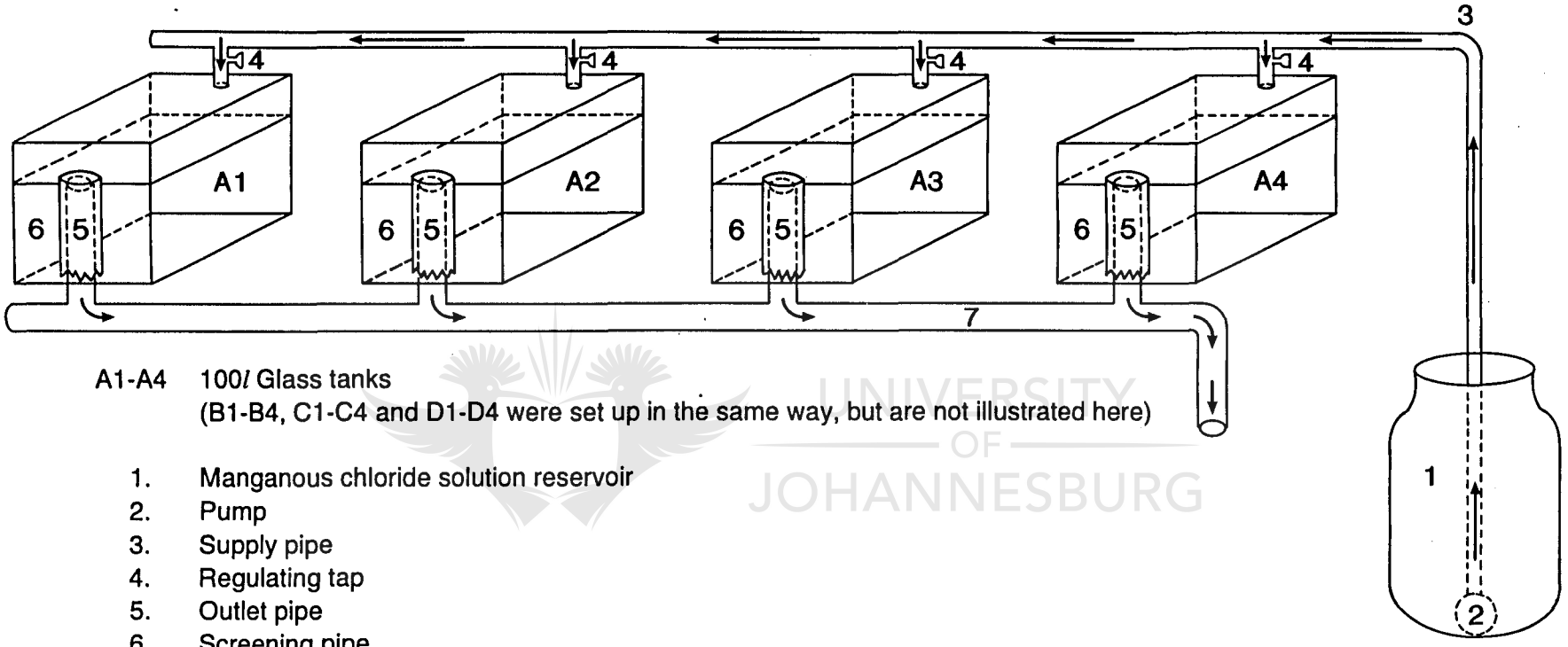
For the purpose of performing the toxicity test, the fish were transferred to a flow-through system (Fig. 8.1). The system consisted of four series of glass tanks, each series consisting of four tanks, of which series A (illustrated) was used for the control fish groups and series B, C and D (not illustrated) were used for the exposure fish groups. To operate the system, the test solutions were added directly to the glass tanks containing the fish, after which a continuous supply of the specified concentrations was maintained by pumping the test solutions from each of the four 200 litre reservoirs (Fig. 8.1) to each series of glass tanks. The volume and depth of the tanks are given in Table 8.2. Excess water that was being replaced in the tanks, left the system through the outlet pipe. The rate of flow was regulated to be 1.5 litre/hour to each tank.

In performing the toxicity test, 160 fish were divided among the 16 glass tanks (10 per tank), where they were allowed to acclimatise for a week. During this time they were fed daily on Wardley Cichlid Ten medium floating pellets (2% of their body mass). Feeding was ended 40 hours prior to the start of the toxicity test. In order to determine the range enclosing the Mn 96-hour LC50 value, a trial test was performed in which the fish were exposed to 0.0 (control), 0.1, 1.0 and 10.0 g/l manganous chloride tetrahydrate for 96 hours. The test solutions were made up by dissolving $MnCl_2.4H_2O$ (MW = 197.91 g), supplied by Associated Chemical Enterprises CC, in the borehole water to which the fish were acclimatised to. After the trial test, the system was decontaminated using a dilute hydrochloric acid solution. The toxicity test was repeated using manganous chloride tetrahydrate concentrations of 1.5, 2.0, 2.5, 3.0, 4.0, 4.5, 5.0, 5.5, 5.8 and 6.0 g/l. The ranges and mean weight and length of the fish used for each concentration in the toxicity test are given in Table 8.3. The temperature was kept at 27 ± 1°C, the mean dissolved oxygen concentration at 5 mg/l and the ammonium concentration at 0.01 mg/l. Visible sub-lethal effects, mortalities and pH were monitored for each tank after 2, 6, 24, 30, 48, 54, 72, 78 and 96 hours.

Water samples (50 ml) were taken daily in order to determine the real manganese concentrations present in the water. In the laboratory the water samples were acidified using 5 ml concentrated perchloric acid (70%) and 10 ml concentrated nitric acid (55%). The mixture was then concentrated on a hot plate to 25 ml, whereafter it was made up to 100 ml with doubly distilled water. A Varian atomic absorption spectrophotometer (Spectra AA-10) was used to determine the total Mn concentrations. Analytical standards for Mn were prepared from Holpro stock solutions. The Mn concentrations in the samples were calculated as follows:

$$\text{Mn concentration (mg/l)} = \frac{\text{AAS reading (mg/l)}}{\text{Initial volume (50ml)}} \times \text{Final volume (100ml)}$$

The LC50 values were obtained by plotting dosage-survival curves at 24, 48, 72 and 96 hours. Percentage survival of fish was plotted on the ordinate and the Mn concentrations on the abscissa



A1-A4 100*l* Glass tanks
(B1-B4, C1-C4 and D1-D4 were set up in the same way, but are not illustrated here)

1. Manganous chloride solution reservoir
2. Pump
3. Supply pipe
4. Regulating tap
5. Outlet pipe
6. Screening pipe
7. Drainage pipe

Figure 8.1
Diagram of the experimental flow-through system used in the Mn toxicity test

TABLE 8.1
WATER QUALITY OF THE BOREHOLE WATER DURING THE MANGANESE TOXICITY TEST

pH	7.95
Temperature (°C)	26.80
Conductivity (µS/cm)	166
Total alkalinity as $CaCO_3$(mg/l)	76.00
Total hardness as $CaCO_3$ (mg/l)	79.00
Calcium (mg/l)	26.00
Magnesium (mg/l)	3.00
Sodium (mg/l)	7.00
Chloride (mg/l)	7.00
Sulphate (mg/l)	11.00
Nitrate (mg/l)	0.67
Fluoride (mg/l)	0.20

TABLE 8.2
VOLUMES OF TEST TANKS USED IN THE TOXICITY TEST

Tank no.	Volume water (l)	Depth of water (cm)
A1	74.28	30.60
A2	74.41	30.65
A3	50.62	20.85
A4	74.36	30.63
B1	50.74	20.90
B2	50.88	20.96
B3	50.74	20.90
B4	74.58	30.72
C1	74.43	30.66
C2	74.43	30.66
C3	74.43	30.66
C4	74.43	30.66
D1	74.28	30.60
D2	74.45	30.67
D3	74.45	30.67
D4	74.28	30.60

TABLE 8.3
MEAN WEIGHT AND LENGTH OF THE EXPOSED LIVE AND DEAD FISH AT EACH CONCENTRATION DURING THE MN TOXICITY TEST

[$MnCl_2$. $4H_2O$] g/l	Tank no.	Live fish			Dead fish		
		N	Weight g	Length cm	N	Weight g	Length cm
0.0	A1-A4	40	8.1±3.3(3.5-18.3)	7.9±1.1(3.0-9.8)	0	-	-
0.1	B1	10	6.7±1.7(4.7-10.2)	7.8±0.7(6.9-9.1)	0	-	-
0.1	B2	10	6.3±1.4(4.1-8.7)	7.7±0.6(6.7-8.5)	0	-	-
0.1	B3	10	6.4±1.3(4.1-8.3)	7.7±0.4(7.2-8.4)	0	-	-
0.1	B4	10	8.9±2.3(6.8-13.3)	8.4±0.8(7.5-10.0)	0	-	-
0.0	A1-A4	40	8.1±3.3(3.5-18.3)	7.9±1.1(3.0-9.8)	0	-	-
1.0	C1	10	9.4±2.5(6.2-13.7)	8.3±0.7(7.1-9.4)	0	-	-
1.0	C2	10	8.1±2.7(5.8-13.3)	8.0±0.8(7.1-9.4)	0	-	-
1.0	C3	7	9.1±2.0(6.9-12.4)	8.2±0.7(7.3-9.2)	0		
1.0	C4	8	8.3±2.4(4.8-11.7)	8.0±0.9(6.5-9.4)	0		
0.0	A1-A4	40	7.1±1.9(3.9-11.7)	8.1±0.7(6.7-9.7)	0	-	-
1.5	B1	10	7.7±3.8(3.9-16.4)	7.8±0.9(6.6-9.2)	0	-	-
1.5	B2	10	7.9±1.7(5.6-10.8)	8.2±0.5(7.7-9.2)	0	-	-
1.5	B3	10	6.9±3.0(4.2-14.7)	8.1±1.0(7.3-10.7)	0	-	-
1.5	B4	10	8.2±2.0(5.8-11.4)	8.5±0.7(7.5-9.5)	0	-	-
0.0	A1-A4	40	7.4±2.1(3.8-12.4)	8.0±0.9(6.2-9.8)	0	-	-
2.0	B1	10	6.5±2.2(3.7-10.7)	7.5±0.9(6.3-9.0)	0	-	-
2.0	B2	10	6.6±2.0(4.1-10.0)	7.6±0.8(6.2-8.7)	0	-	-
2.0	B3	10	6.4±1.5(3.6-8.1)	7.6±0.5(6.5-8.3)	0	-	-
2.0	B4	10	5.2±1.8(3.9-9.7)	7.0±0.7(6.1-8.6)	1	3.9	6.1
0.0	A1-A4	40	7.1±1.9(3.9-11.7)	8.1±0.7(6.7-9.7)	0	-	-
2.5	C1	10	8.1±3.7(4.9-16.3)	8.4±0.8(7.5-9.8)	0	-	-
2.5	C2	10	8.0±2.6(4.5-13.0)	7.9±0.6(6.7-8.7)	0	-	-
2.5	C3	10	6.2±2.2(3.4-11.1)	7.5±0.8(6.3-9.2)	0	-	-
2.5	C4	10	8.9±2.9(5.2-13.5)	8.3±1.2(6.7-10.1)	0	-	-
0.0	A1-A4	40	7.1±1.9(3.9-11.7)	8.1±0.7(6.7-9.7)	0	-	-
3.0	D1	10	8.5±6.0(4.2-25.3)	8.0±0.9(6.3-9.8)	0	-	-
3.0	D2	10	6.7±2.1(4.0-10.5)	7.7±0.6(6.5-8.6)	0	-	-
3.0	D3	10	6.5±1.7(4.7-9.8)	7.8±0.6(7.2-8.8)	0	-	-
3.0	D4	10	8.9±2.3(4.6-12.4)	8.5±0.9(6.9-9.9)	0	-	-
0.0	A1-A4	40	7.9±2.8(2.9-15.9)	8.0±1.0(6.0-10.3)	0	-	-
4.0	B1	10	9.2±2.8(5.8-14.6)	8.5±0.8(7.4-10.0)	0	-	-
4.0	B2	10	8.4±2.8(5.5-14.5)	8.2±0.8(7.4-10.0)	0	-	-
4.0	B3	10	8.4±2.3(5.2-13.7)	8.3±0.8(7.0-10.0)	0	-	-
4.0	B4	10	8.3±3.5(4.8-14.5)	8.3±1.1(7.0-10.0)	0	-	-
0.0	A1-A4	40	7.9±2.8(2.9-15.9)	8.0±1.0(6.0-10.3)	0	-	-
4.5	C1	10	7.7±2.0(4.1-11.1)	8.1±0.8(6.6-9.1)	1	9.1	8.5
4.5	C2	10	8.3±2.9(5.1-13.0)	8.2±0.9(7.1-9.6)	0	-	-
4.5	C3	10	6.4±1.7(4.3-9.5)	7.5±0.7(6.6-8.8)	0	-	-
4.5	C4	10	6.5±1.7(4.0-9.5)	7.6±1.0(6.5-9.5)	0	-	-
0.0	A1-A4	40	8.2±2.4(4.4-13.7)	8.2±0.8(6.5-10.0)	0	-	-
5.0	C1	10	5.9±1.5(2.3-7.4)	7.3±0.8(5.4-8.0)	3	5.3±2.6(2.3-7.1)	6.9±1.3(5.4-7.9)
5.0	C2	10	7.1±1.5(5.1-10.2)	7.8±0.6(7.0-9.1)	2	7.6±0.4(7.3-7.8)	8.1±0.2(7.9-8.2)
5.0	C3	10	8.5±2.4(5.1-12.1)	8.2±0.8(7.0-9.5)	2	9.6±1.9(8.2-10.9)	8.4±0.8(7.8-9.0)
5.0	C4	10	8.4±2.6(4.5-12.3)	8.2±0.8(7.0-9.1)	3	10.2±1.8(8.8-12.3)	8.6±0.4(8.4-9.1)
0.0	A1-A4	40	8.2±2.4(4.4-13.7)	8.2±0.8(6.5-10.0)	0	-	-
5.5	D1	10	7.2±2.8(3.5-12.1)	7.7±1.0(6.2-9.1)	6	8.4±2.8(5.6-12.1)	8.2±0.9(7.3-9.1)
5.5	D2	10	8.8±2.1(5.0-11.8)	8.4±0.6(7.1-9.4)	3	8.5±3.2(5.0-11.4)	8.1±0.9(7.1-8.8)
5.5	D3	10	8.1±2.8(5.6-14.3)	8.1±0.9(7.2-10.2)	0	-	-
5.5	D4	10	8.4±1.9(4.7-11.3)	8.3±0.7(7.0-9.2)	5	8.4±1.7(6.7-11.3)	8.3±0.5(7.8-9.0)
0.0	A1-A4	40	7.9±2.8(2.9-15.9)	8.0±1.0(6.0-10.3)	0	-	-
5.8	D1	10	7.2±2.9(3.2-13.6)	7.8±1.1(5.9-9.9)	2	7.2±0.6(6.7-7.6)	8.1±0.7(7.6-8.6)
5.8	D2	10	9.2±2.7(5.2-12.1)	8.5±0.9(6.9-9.4)	3	9.8±4.0(5.2-12.1)	8.5±1.4(6.9-9.4)
5.8	D3	10	7.4±2.4(4.0-11.1)	7.8±0.8(6.4-8.8)	9	7.2±2.5(4.0-11.1)	7.7±0.8(6.4-8.8)
5.8	D4	10	9.2±3.6(4.4-14.5)	8.5±1.1(6.8-10.0)	4	10.7±3.8(5.5-14.5)	8.8±1.1(7.4-10.0)
0.0	A1-A4	40	7.4±2.1(3.8-12.4)	8.0±0.9(6.2-9.8)	0	-	-
6.0	D1	10	6.7±1.3(5.1-8.7)	7.6±0.5(7.0-8.4)	10	6.7±1.3(5.1-8.7)	7.6±0.5(7.0-8.4)
6.0	D2	10	8.7±3.1(4.5-15.3)	8.1±1.0(6.6-10.0)	10	8.7±3.1(4.5-15.3)	8.1±1.0(6.6-10.0)
6.0	D3	10	6.4±1.7(3.7-9.1)	7.4±0.7(6.4-8.5)	10	6.4±1.7(3.7-9.1)	7.4±0.7(6.4-8.5)
6.0	D4	10	7.3±1.8(4.4-10.8)	7.7±0.7(6.4-8.7)	9	7.3±1.9(4.4-10.8)	7.7±0.7(6.4-8.7)
0.0	A1-A4	40	8.1±3.3(3.5-18.3)	7.9±1.1(3.0-9.8)	0	-	-
10.0	D1	10	10.3±2.8(7.0-16.0)	8.6±0.8(7.5-10.1)	10	10.3±2.8(7.0-16.0)	8.6±0.8(7.5-10.1)
10.0	D2	10	9.3±2.3(6.0-13.0)	8.1±0.7(7.4-9.5)	10	9.3±2.3(6.0-13.0)	8.1±0.7(7.4-9.5)
10.0	D3	10	10.4±3.1(7.0-15.0)	8.5±0.8(7.6-9.6)	10	10.4±3.1(7.0-15.0)	8.5±0.8(7.6-9.6)
10.0	D4	10	10.8±1.7(8.0-13.0)	8.7±0.4(8.0-9.4)	10	10.8±1.7(8.0-13.0)	8.7±0.4(8.0-9.4)

(Gopal & Misra, 1988). Median survival times (LT50 values) were obtained from time-survival curves, which were plotted using the results obtained at 0.555, 1.249, 1.388, 1.527, 1.610, 1.666 and 2.776 g/l Mn concentrations. Both the LT50 and LC50 values were used to construct a toxicity curve, leading to the calculation of the incipient LC50 value. In both cases the toxicity curves were plotted using a log scale, as well as a linear scale. The 95% confidence limits of the LC50 and LT50 values were calculated using the statistical methods of Zar (1984).

8.3 Results and Discussion

In performing a toxicity test it is essential to determine the actual toxicant concentration present in the water during exposure and to compare it with the toxicant concentration that was originally added to the water. More often than not, it is found that on average, the measured toxicant concentration is lower (or even higher) than the original concentration. This trend can clearly be seen in Table 8.4, where the Mn concentrations that were originally added to the water as manganous chloride tetrahydrate are higher (and sometimes lower) than the measured Mn concentrations. A decrease in toxicant concentration can be attributed to the apparent adsorption onto the test container material (Sprague, 1969). In this study, the unstable background Mn levels in the borehole water could have contributed to the variation in toxicant concentration. However, the variation could also have been due to the absorption and metabolism of Mn by the fish (Abel, 1989). When the fish were exposed to 0.028 g/l Mn, the mean measured Mn concentration was 0.027 g/l Mn (Table 8.4), indicating good regulation by the fish or perhaps that no absorption took place. At 0.278 and 0.416 g/l Mn exposure, the initial Mn concentration measured lower than expected - possibly indicating absorption - and increased thereafter in the water until it stabilised at a certain level, indicating that a steady state or equilibrium had been reached. Therefore, good regulation took place. From 0.555 g/l Mn to 2.776 g/l Mn exposure, it seemed, however, that the fish had some difficulty in regulating the Mn levels. The Mn concentrations at first measurements were always lower than the original concentrations as made up by dissolving the correct calculated masses of $MnCl_2.4H_2O$ per volume of water, thus indicating immediate absorption by the fish. As time progressed, the Mn concentrations increased and reached levels that exceeded the exposure concentrations. This could possibly be attributed to the fish trying to excrete excessive Mn from the inside of their bodies, but failed to do so - indicating that no equilibrium or steady state had been reached.

An important aspect in the performance of a toxicity test, is a pH change after the test solution has been introduced and the consequent adjustment thereof. In this study the water pH of the control groups was 7.95 ± 0.15 (7.4 - 8.2), while the water pH of the experimental groups ranged from 7.0 to 7.8. No drastic pH change took place and it was therefore not necessary to adjust the pH. The pH did, however, decrease slightly when the test solutions were added. When manganous chloride is dissolved in water, the following equilibrium is established:

$$MnCl_2 + 2H_2O \rightarrow \underset{\text{weak base}}{Mn(OH)_2} + \underset{\text{strong acid}}{(2H^+ + 2Cl^-)}$$

Dissolving $MnCl_2$ in water, creates an equilibrium in which the conjugated acid-base pair $Mn(OH)_2/HCl$ is formed. $Mn(OH)_2$ is a weak base thus only partially dissociating in water to form Mn^{2+} and OH^-. Strong mineral acids such as HCl dissociate fully and predominate the acidity of a solution containing the above mentioned acid/base pair. It is therefore expected that the pH of a $MnCl_2$ solution would be more acidic than the solute (the water).

The effects of pollutants on individuals may range from rapid death through sub-lethal effects to no effects at all (Moriarty, 1990). The most important responses, however, are death, disturbed physiology, reproductive impairment and aberrant behaviour (Hellawell, 1986). The visible sub-lethal effects that Mn had on juvenile *O. mossambicus* in this study, were opaque eyes and haemorrhaging at the pectoral fins and nose (0.278 - 2.776 g/l Mn), excessive mucus production (0.694 - 2.776 g/l Mn), white burnt fins (1.666 - 2.776 g/l Mn) and "turnover" (1.388 - 2.776 g/l Mn), which is a common response of fish to toxicants indicating a loss of balance and the inability to control their normal swimming position (Hellawell, 1986). Most of the time the fish remained at the bottom of the tank,

TABLE 8.4
EXPERIMENTAL DATA FROM THE Mn TOXICITY TEST ON JUVENILE *OREOCHROMIS MOSSAMBICUS*

[$MnCl_2.4H_2O$] g/l	[Mn] g/l	Measured [Mn] g/l	Total No. of Fish N	% Survival of fish 2hr	6hr	24hr	30hr	48hr	54hr	72hr	78hr	96hr	Time-survival curve: For Y = a + bx a	b	LT50 (hours) estim. from graph	95% Confidence limits
0.0		0.001±0.002	200	100	100	100	100	100	100	100	100	100				
		(0.000-0.011)														
0.1	0.028	0.027±0.006	40	100	100	100	100	100	100	100	100	100				
		(0.020-0.042)														
1.0	0.278	0.265±0.010	40	100	100	100	100	100	100	100	100	100				
		(0.242-0.280)														
1.5	0.416	0.395±0.029	40	100	100	100	100	100	100	100	100	100				
		(0.330-0.432)														
2.0	0.555	0.530±0.037	40	100	100	100	100	100	100	100	100	97.5	100.4	-0.01	5040	
		(0.436-0.574)														
2.5	0.694	0.679±0.021	40	100	100	100	100	100	100	100	100	100				
		(0.630-0.706)														
3.0	0.833	0.818±0.029	40	100	100	100	100	100	100	100	100	100				
		(0.772-0.870)														
4.0	1.110	1.042±0.117	40	100	100	100	100	100	100	100	100	100				
		(0.840-1.202)														
4.5	1.249	1.240±0.050	40	100	100	100	100	100	100	97.5	97.5	97.5	100.6	-0.03	1686.7	1152.5/2541.2
		(1.132-1.316)														
5.0	1.388	1.295±0.050	40	100	100	97.5	97.5	92.5	92.5	85.0	82.5	75.0	103.2	-0.26	204.6	186.2/226.7
		(1.216-1.396)														
5.5	1.527	1.451±0.062	40	100	100	100	97.5	95.0	92.5	85.0	77.5	65.0	105.9	-0.34	164.4	144.0/184.2
		(1.324-1.518)														
5.8	1.610	1.646±0.062	40	100	100	100	100	97.5	95.0	87.5	82.5	55.0	108.0	-0.38	152.6	132.0/178.8
		(1.526-1.726)														
6.0	1.666	1.585±0.047	40	100	100	92.5	90.0	52.5	35.0	17.5	10.0	2.5	110.8	-1.21	50.2	43.7/56.7
		(1.498-1.674)														
10.0	2.776	2.625±0.470	40	100	100	77.5	45.0	7.5	0.0	0.0	0.0	0.0	93.5	-1.25	34.8	26.0/43.7
		(2.038-3.348)														

Dosage-survival curve: For Y = a + bx:											
a	118.6	147.0	174.2	178.3	169.9	165.9	155.6				
b	-14.37	-35.23	-59.59	-64.75	-63.33	-62.81	-61.30				
LC50 (g/l Mn) estimated from graph	4.774	2.753	2.084	1.981	1.893	1.845	1.723				
95% Confidence limits	4.381	2.548	1.904	1.790	1.678	1.620	1.489				
	5.198	2.963	2.268	2.183	2.112	2.072	1.957				

but occasionally, at the higher Mn concentrations (1.388 - 2.776 g/l Mn), they would suddenly surge upwards and immediately sink to the bottom again.

Mortalities started to occur at 1.249 g/l Mn (Table 8.4) and continued to occur until 2.776 g/l Mn, at which concentration all the fish were dead. No mortalities occurred in the control tanks. The single deaths that occurred at 0.555 and 1.249 g/l Mn are natural phenomena, since every population has those individuals which are weaker and more susceptible to environmental stress factors or pollutants than others and are thus not truly representative of the whole population. In the test population (size range 3.0 - 10.7 cm) both small and large fish died (Table 8.3). The weaker individuals were therefore not necessarily the smallest fish of the test population.

As illustrated in Table 8.4, no gradual fish mortality pattern was observed. All the fish survived the lower dosage range, while a rapid onset of mortalities occurred at the upper dosage range. The data were consequently handled as two separate sets when dosage-survival curves were drawn for the determination of LC50 values (Fig. 8.2). The slope of a dosage-survival curve is indicative of the sensitivity range to the chemical within the fish test sample (Rand & Petrocelli, 1985). Therefore, the steep slopes of the curves at 48h, 72h and 96h indicated that large increases in mortality were associated with relatively small increases in concentration. It was also an indication of rapid absorption and rapid onset of effects. By contrast, a flat slope (e.g. the 24h-curve) indicated that mortality increased by small increments as the concentration increased and may also have been indicative of slow absorption, rapid excretion or detoxification, or delayed toxification (Rand & Petrocelli, 1985). The LC50 values were calculated to be 4.774 g/l Mn at 24 hours, 2.084 g/l Mn at 48 hours, 1.893 g/l Mn at 72 hours and 1.723 g/l Mn at 96 hours (Fig. 8.2; Table 8.4). The 96-hour LC50 value determined in this study (1.723 g/l Mn), was lower than the 96-hour LC50 value of 3.230 g/l Mn determined by Nath and Kumar (1987). This difference in value can be attributed to several factors. Nath and Kumar performed a static bioassay, exposing the freshwater perch, *Colisa fasciatus*, to different concentrations of $MnSO_4.H_2O$. The mean weight and length of the adult specimens were 5.74 ± 0.28 g and 5.93 ± 0.28 cm. On average the fish were therefore smaller than the ones used in the performance of this toxicity test, and it was also a different fish species. Furthermore, $MnSO_4$ is known to be less toxic than $MnCl_2$ and a higher LC50 value for $MnSO_4$ can be expected. *Colisa fasciatus* was kept in tap water with a mean temperature of 24.33 ± 1.69°C and a mean hardness of 165.33 ± 6.17 mg/l as $CaCO_3$. On the other hand, *Oreochromis mossambicus* was kept in borehole water with a mean temperature of 26.8 ± 1.3°C and a hardness of 61.0 mg/l as $CaCO_3$. The higher temperature and softer water in the case of *O. mossambicus* could thus have increased the Mn toxicity (Hellawell, 1986)

The median survival time (LT50) is the time required for half the fish to die at a specific toxicant concentration (Abel, 1989). In this study, LT50 values were calculated at 0.555, 1.249, 1.388, 1.527, 1.610, 1.666 and 2.776 g/l Mn from the time-survival curves (Fig. 8.3) and are given in Table 8.4. Three LT50 fish exposure groupings can be distinguished in Figure 8.3. The first group were exposed to the highest Mn concentrations during the toxicity test, namely 2.776 and 1.666 g/l Mn. At these concentrations the LT50's were only one to two days. The second group of fish were exposed to 1.610, 1.527 and 1.388 g/l Mn, with the LT50's being six to nine days. The third group of fish were exposed to the lowest Mn concentrations during the toxicity test (1.249 to 0.028 g/l Mn) and the resulting LT50's were 70 to 210 days. There thus seemed to be a noticeable difference between the LT50 at 1.388 g/l Mn (8.5 days) and the LT50 at 1.249 g/l Mn (70 days) (Table 8.4). The relatively flat slopes of the curves in Figure 8.3 (especially of the last two groupings), indicated that mortalities due to Mn exposure increased slowly with time. It is therefore recommended that the extent and degree of sub-lethal effects experienced by fish exposed to Mn concentrations of 0.028 g/l to 1.610 g/l Mn should be investigated by means of chronic toxicity tests.

The incipient LC50 value in this study was calculated to be 1.99 g/l Mn (Fig. 8.4a) or 1.46 g/l Mn (Fig. 8.5a), using LC50 and LT50 values respectively. The application factor for Mn (Incipient LC50/96-h LC50) would therefore be 1.155 or 0.847. For certain applications, where the test forms part of a research programme designed to establish water quality standards, it would most probably be preferable to use the concentration-response approach (e.g. Fig. 8.4) rather than the time-response approach (e.g. Fig. 8.5) (Abel, 1989). Therefore the toxicity curve based on LC50 values would be used. However, Gaddum (1953) estimates that approximately half the information will be lost when

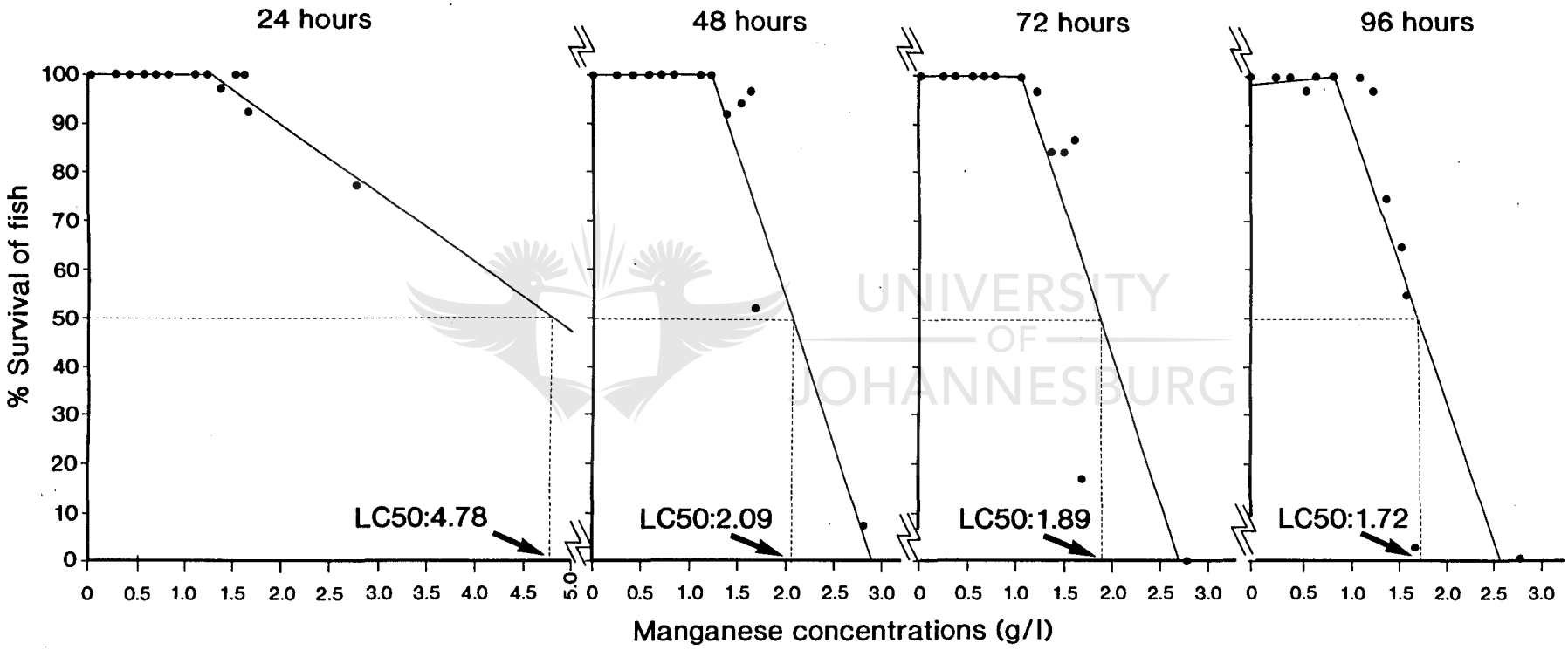


Figure 8.2
Dosage survival curves of Mn for juvenile O. mossambicus

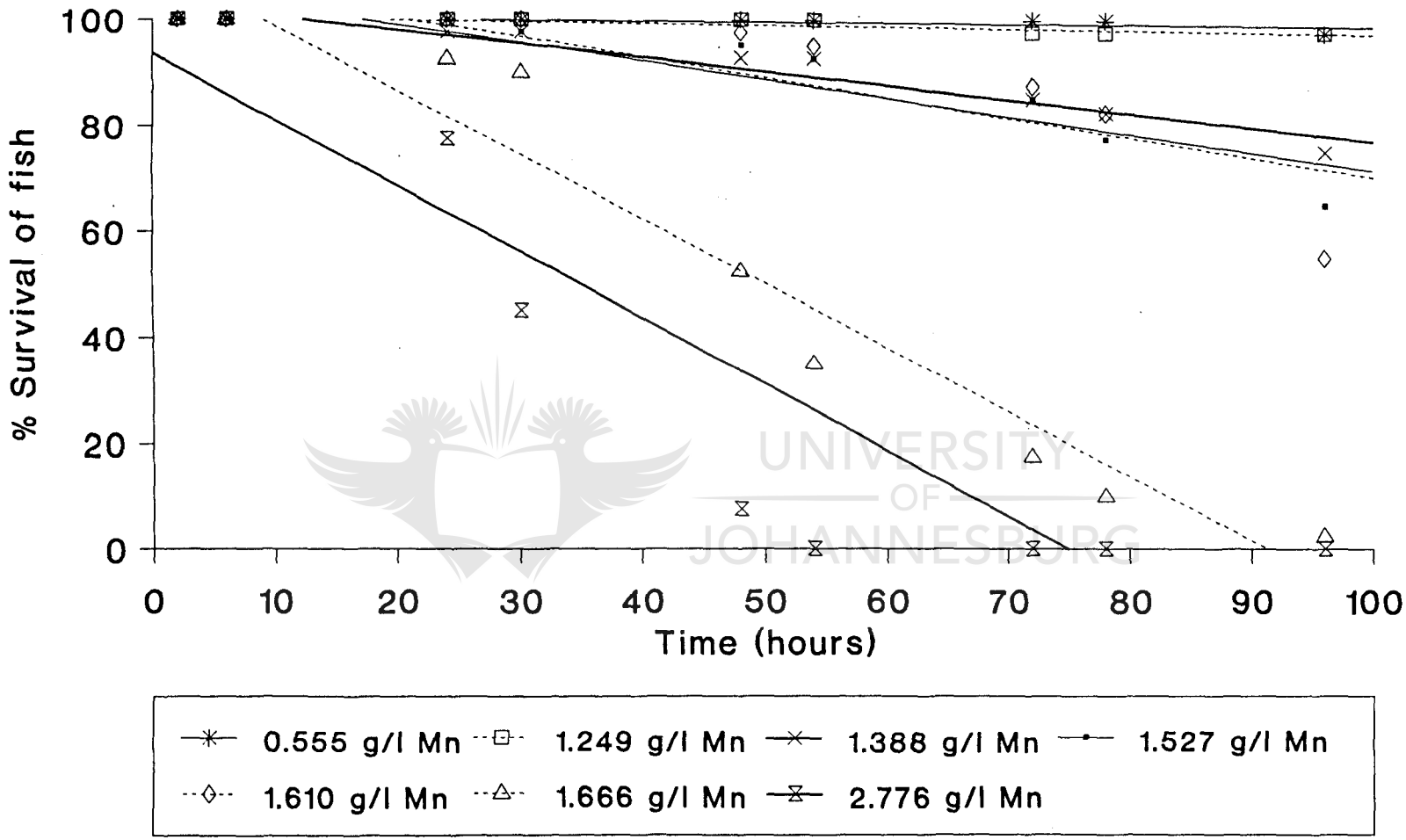


Figure 8.3
Time-survival curves of Mn for juvenile O. mossambicus

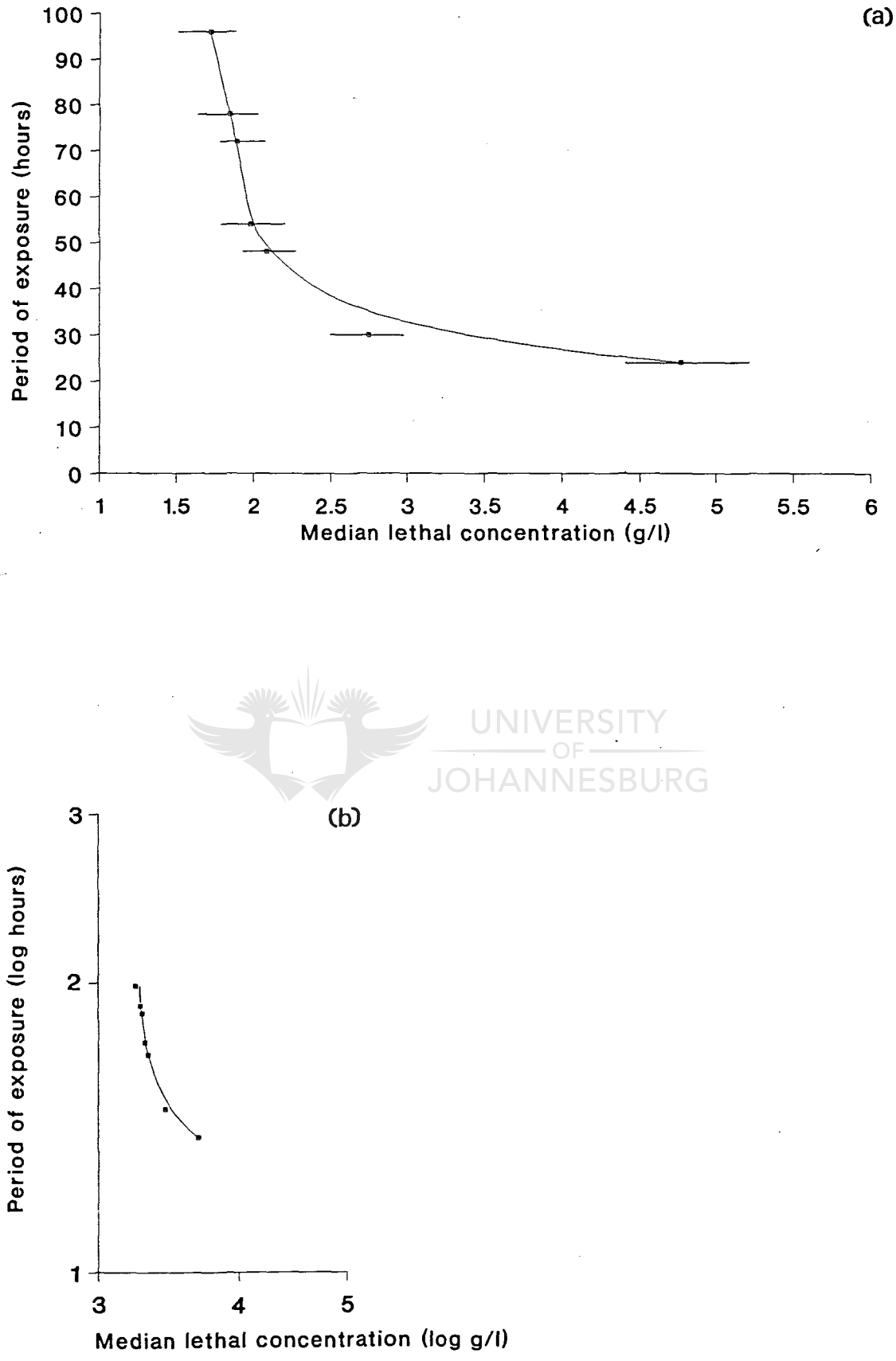
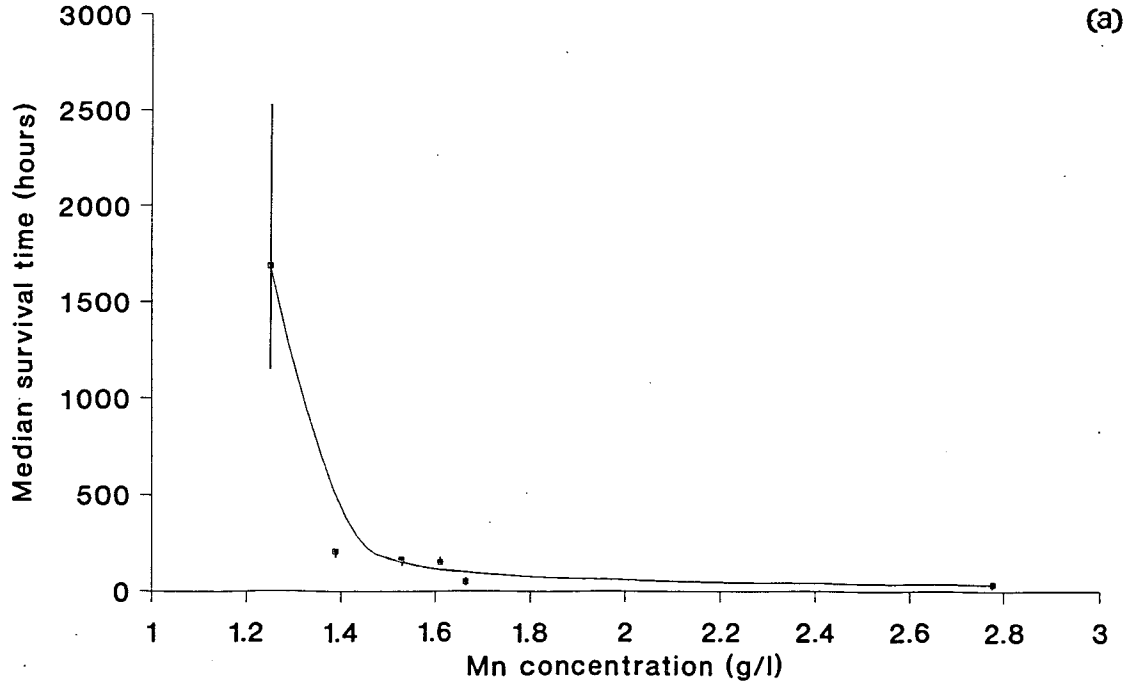


Figure 8.4
Toxicity curve of Mn for juvenile O. mossambicus *based on the determined LC50 values. a) Linear scale b) Log scale*



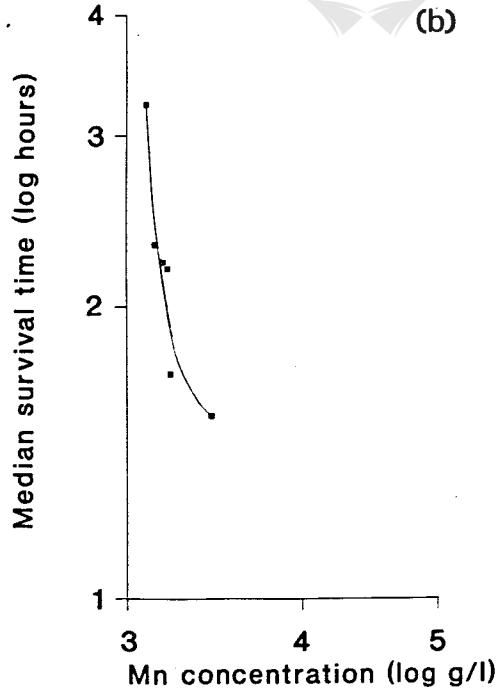


Figure 8.5
Toxicity curve of Mn for juvenile O. mossambicus *based on the determined LT50 values: a) Linear scale b) Log scale*

using the dosage-response approach, so that twice as many observations will be needed for any given degree of accuracy. An added advantage of the time-response approach, is that events during the different test exposures can be observed separately (Fig. 8.3). Therefore, the reactions of the exposed fish can be monitored carefully with time (Sprague, 1969). It would thus depend on the goal set for the toxicity test in order to decide whether the concentration-response or the time-response approach should be used.

8.4 Conclusions

The determined 96-hour LC50 value (1.723 g/l Mn) and also the incipient LC50 value (1.46 g/l Mn) were much higher than the naturally occurring Mn concentrations in the environment, which rarely exceeds one mg/l (Hellawell, 1986). The values were also higher than the Mn concentration of 0.206 g/l that was detected in the West Wits Gold field mine effluent (Whitman & Förstner, 1977). However, 0.206 g/l Mn is a concentration level whereby fish might be affected sub-lethally, since in this study visible sub-lethal effects started to occur at a Mn concentration of 0.278 g/l. Attention must therefore be given to the performance of chronic Mn toxicity tests in the future, in order to determine the lowest Mn concentration whereby sub-lethal effects will still occur. In this way the existing water quality guideline of one mg/l Mn as a maximum concentration for the protection of aquatic life (Kempster *et al.*, 1982) could be verified.

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CHAPTER 9

Chapter 9

SUMMARY AND RECOMMENDATIONS

9.1 Summary

WATER AND SEDIMENT

The water quality of the Selati River at locality 7 was found to be stressful to the aquatic life due to chemical constituents that exceeded the recommended guideline limits. Variables of special concern were: sodium, fluoride, chloride, sulphate, potassium, the total dissolved salts and the metal concentrations (except strontium). Effluents of a phosphorus extraction mining company and copper extraction mining company in the Phalaborwa area, as well as upstream inflow into the Selati River contributed to the high TDS concentrations in this river. The anionic component mainly responsible for the high TDS concentrations was sulphate. Furthermore, the Selati River had a negative influence on the water quality of the Olifants River after the confluence of the two rivers. This was clearly illustrated by the concentrations of some chemical constituents detected in the water at Mamba Weir. The negative influence of the Selati River was more pronounced during low flow periods (e.g. droughts or winter months) when limited water releases from the Phalaborwa Barrage reduced the dilution effect of the water on chemical constituent levels. Most of the chemical constituent concentrations (not the metal concentrations) did, however, decrease from the western side of the KNP to the eastern side, due to the dilution of the water through the tributaries of the Olifants River. At locality 3 (near Balule) some chemical constituents increased again in concentration, especially from April 1990 to February 1991. The frequent occurrence of reed beds in that part of the river was the possible explanation to this. Most of the time, the water quality of the Olifants River in the KNP complied with the recommended guideline limits, except for the metal concentrations at most localities. The high metal concentrations in the water did not, however, necessarily indicate toxic conditions to aquatic life. The water of the Olifants River is, amongst other features, hard (as $CaCO_3$), decreasing the bioavailability of the metals to aquatic life and therefore decreasing the toxicity of the metals. Higher metal concentrations were detected in the sediment than in the water, due to the adsorption of metals on sediment particles. This indicated the chronic nature of metal pollution in the area. A large variation was detected in the metal concentrations of the water and sediment, making it difficult to establish the order of metal occurrence in the study area. According to the sediment metal concentrations (which fluctuated less than the water), the general order from April 1990 to February 1991 for localities 1 to 6 was: Fe > Mn > Cr > Ni > Zn > Sr > Pb > Cu. For locality 7 in the Selati River it was: Fe > Mn > Cu > Cr > Sr > Ni > Zn > Pb. From April 1991 to February 1992 the general order of occurrence for localities 1 to 6 was Fe > Mn > Cr > Ni > Sr > Zn > Cu > Pb, and for locality 7 it was Fe > Mn > Sr > Cu > Cr > Ni > Zn > Pb. The sediment at Pionier Dam had an occurrence pattern of metals similar to that of localities 1 to 6, except that more zinc than chromium was detected in the sediment. In the Selati River (at locality 7) much higher copper and strontium concentrations were detected in the sediment than in the Olifants River (at localities 1 to 6). This indicated that these two metals originated from a local source which was not connected to the KNP.

BIOACCUMULATION OF THE SELECTED METALS IN THE ORGANS AND TISSUES OF *BARBUS MAREQUENSIS*

The accumulated metals (Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn) in the organs and tissues of *Barbus marequensis* gave a good indication of the metal levels to which the fish were exposed, especially when compared with the metal concentrations of a fish species from a polluted system (Germiston lake). *Barbus marequensis* seemed to have been chronically exposed to zinc, copper, lead and nickel, probably at sub-lethal levels. In addition, the fish at locality 7 seemed to have been chronically exposed to iron, chromium and manganese, also probably sub-lethally.

Metals were usually taken up *via* the gut and/or *via* the gills. The high metal concentrations in the gut contents of *B. marequensis* were not only due to the food ingested by the fish, but also to the metal-rich sediment associated with the food (*B. marequensis* is a benthic feeder). In the summer of 1990/91 the heavy rainfall increased the solubility of the metals and therefore metals could be taken up *via* the gills, and maybe even the skin, more easily, leading to a higher accumulation of metals in the fish. The various metals were distributed differently in the organs and tissues of *B. marequensis*, indicating that it is not necessarily the same organs that should be sampled for the analysis of different metals. It is therefore possible that, in using the wrong organs, an incorrect conclusion can be drawn in the assessment of the extent of metal pollution in an area. The suggested organs and tissues that should be sampled for the analysis of Cr, Cu, Fe, Mn, Ni, Pb, Sr and Zn in fish, as well as the organs and tissues of *B. marequensis* that accumulated the highest concentrations of these metals, are indicated in Table 9.1. Muscle tissue should always be sampled to test its fitness for human consumption. Apart from this, the gills, gut, liver and bony tissues seem to be good representative organs and tissues in general metal pollution surveys. If, however, surveys are being done on specific metals, organs and tissues as illustrated in Table 9.1, should be sampled. Seasonal differences in the bioaccumulation of the metals in the organs and tissues of *B. marequensis* did occur. Zinc is known to be essential for gonad development, especially for females, and therefore displayed a seasonal trend. The role of the other metals in gonad development (if any) is, however, not certain and cannot be related to this process as yet. Moreover, seasonal differences were related to the available metal concentrations that were taken up during a season.

TABLE 9.1
SUMMARY OF FISH ORGANS IMPORTANT IN METAL POLLUTION SURVEYS

	Zn	Cu	Fe	Cr	Ni	Mn	Pb	Sr
Bile		*		*	*			
Blood				*	*		*	
Gill	*	*	*	*	*	*	*	*
Gonads (F)	*							
Gonads (M)	*							
Gut		*	*	*	*			
Kidney		*		*	*			
Liver	*	*		*	*	*	*	*
Muscle	*	*	*	*	*	*	*	*
Opercular bone	*					*	*	*
Scales	*					*	*	*
Skin	*		*					
Vertebrae	*			*	*	*	*	*

* Fish organs to sample for metal analysis
Organs of *B. marequensis* with highest metal concentrations
Histopathological studies should be done in addition to metal analysis

ACUTE TOXICITY TEST OF MANGANESE ON JUVENILE *OREOCHROMIS MOSSAMBICUS*

The 96-hour LC50 value of manganese for juvenile *O. mossambicus* was determined to be 1.723 g/l Mn, while the incipient LC50 value was 1.46 g/l Mn. These concentrations are much higher than the manganese concentrations occurring in the environment, which rarely exceeds one mg/l. Effluents of

mines can, however, contain manganese concentrations that will have sub-lethal effects on fish. The highest manganese concentration detected in the water of the study area, was 16.5 mg/l Mn. Attention should therefore be given to the performance of chronic manganese toxicity tests in the future, in order to verify the existing water quality guideline of one mg/l Mn as a maximum concentration for the protection of aquatic life.

9.2 Recommendations

It is recommended that a more intensive study on the water and sediment quality of the study area should be undertaken. The interaction between the water and the sediment with regard to metal distribution should be investigated, as well as the bioavailability of the metals to the fish. This can best be achieved by combining the field study with experimental work, in order to determine the effects of the physical and chemical environment on the metal toxicity. Water and sediment samples should be increased to at least ten per locality, thereby decreasing the variation in metal concentrations. Monitoring can be limited to localities 2, 3, 5, 6 and 7, giving special attention to locality 3 to determine the role of the reed beds. Sampling should also be performed higher up in the Olifants River catchment, in order to determine the influence of those mining, industrial and agricultural activities. Biological monitoring should not only include a sensitive fish species, but also sensitive plant and invertebrate species. All the biological species need only be sampled at localities 5, 6 and 7, as well as higher up in the catchment, and only the fish organs as suggested in Table 9.1. The number of fish should, however, be increased to 20 - 30 individuals and the fish size should be large enough so that one gram of dried tissue will be available. Working on a dry weight basis, as well as the large *N*-value, will decrease the large variation in metal concentrations.

For future management it is recommended that drastic measures should be taken in order to reduce the impact of mining activities on the water quality of the Selati River and also, indirectly, the Lower Olifants River (especially during low flow periods). It is important for enough water to be released into the Olifants River from Phalaborwa Barrage in order to dilute the Selati River water, especially during low flow periods (e.g. droughts and winter periods). If the water quality of the Selati River cannot be improved, it should at least be maintained at its present status, for a further degradation in water quality cannot be afforded.